VARIATION AND AVAILABILITY OF NUTRIENTS IN CO-PRODUCTS FROM BIO-ETHANOL PRODUCTION FED TO RUMINANTS

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

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ABSTRACT

The main objective of this project was to investigate the effects of the type of dried distillers grains with solubles (wheat DDGS, corn DDGS, and blend DDGS (eg. wheat:corn = 70:30)) and bio-ethanol plant origin on the nutrient variation and availability in ruminants. In addition, DDGS products were studied as opposed to their parental grains. The project was divided into the several following studies.

In Study 1, we studied the nutritive value of DDGS products in terms of (1) chemical profiles, (2) protein and carbohydrate sub-fractions associated with different degradation rates, and (3) digestible component nutrients and energy values using the NRC 2001-chemical approach and the *in situ* assay-biological approach. Also, we tested the validity of acid detergent insoluble crude protein (ADICP) and acid detergent lignin (ADL) to predict the potential degradability of DDGS. Due to starch fermentation in the ethanol process, the chemical components in DDGS became approximately threefold more concentrated than in feedstock grains. Slowly degraded protein (PB3) and unavailable protein (PC) increased in DDGS, indicating a decrease in the overall protein degradability in the rumen. Intermediately degraded protein (PB2) was higher for corn DDGS than for wheat DDGS and blend DDGS (54.2 vs. 27.7 vs. 30.8 %CP), while PB3 was higher for wheat DDGS and blend DDGS (29.9 vs. 51.2 vs. 53.2 %CP). Mainly as a result of differing heat conditions, PC differed significantly between wheat DDGS originated at different bio-ethanol plants (0.7 vs. 7.6 %CP). The prediction of truly digestible CP (tdCP) and NDF (tdNDF) differed between the NRC 2001-chemical approach and the *in situ* assay-biological approach; however, both approaches reported similar energy values. These values were the highest for corn DDGS (DE_{3X}: 3.9 Mcal kg^{-1}), followed by blend DDGS (DE_{3X}: 3.6 Mcal kg^{-1}), and wheat DDGS (DE_{3X}: 3.4 Mcal kg^{-1}). Corn DDGS was superior to corn, wheat DDGS was similar to wheat and corn, and blend DDGS was similar to corn. No significant differences in energy values were reported between bio-ethanol plants. ADICP was not an accurate indicator of the potential degradability of protein in DDGS samples, while ADL seemed to be an acceptable indicator of the potential degradability of DM ($r = -0.87$; $P<0.01$), CP ($r = -0.89$; $P<0.01$), and NDF ($r = -0.82$; $P<0.01$) in wheat DDGS samples incubated in rumen during 48 h.

In Study 2, we studied the ruminal and intestinal digestion profiles and the hourly effective rumen degradation ratios between nitrogen (N) and energy. The results showed a reduction in the effective degradability of DM (EDDM), OM (EDOM) and CP (EDCP) of wheat DDGS relative to wheat; however, corn DDGS remained the same as corn. The effective degradability of NDF (EDNDF) did not vary between the DDGS samples and feedstock grains. Among DDGS types, EDDM ranged from 52.4 to 57.7 %, EDOM from 46.4 to 53.5 %DM, and EDCP from 34.0 to 45.6 %CP, being higher as the proportion of wheat in feedstock increased. No significant differences in EDDM, EDOM, EDCP and EDNDF for wheat DDGS were detected between the different bio-ethanol plants. The hourly effective degradability ratios between N and energy indicated a potential excess of N in rumen when DDGS samples were evaluated as single ingredient. This excess increased as the proportion of wheat in feedstock increased. Estimated intestinal digestibility of rumen bypass protein (IDP) was similar between wheat and wheat DDGS, but higher in corn DDGS than in corn. Blend DDGS had the highest IDP (93.9 %RUP). Due to the significantly different PC sub-fraction found in wheat DDGS originated at the different bio-ethanol plants, a large but numerical difference was detected in IDP (89.4 vs. 75.9 %RUP).

In Study 3, we used both the DVE/OEB System and the NRC 2001 Model to reveal the metabolic characteristics of DDGS protein and predict the protein supply to dairy cattle. The two models showed higher protein values (DVE or MP) for DDGS samples than for feedstock grains. The higher IDP for blend DDGS largely contributed to the higher protein value relative to wheat DDGS and corn DDGS (MP: 277 vs. 242 vs. 250 g kg^{-1} DM). Similarly, protein values differed significantly between the bio-ethanol plants mainly as a result of the numerical but large difference in IDP (MP: 272 vs. 223 g kg^{-1} DM). According to the two models, the degraded protein balance for DDGS products was higher than in the parental grains. Wheat DDGS showed the highest potential N excess (DBP^{NRC}: 78 g kg^{-1} DM). For corn DDGS, however, the DVE/OEB System suggested a potential N excess (11 g kg^{-1} DM) while the NRC 2001 Model exhibited a potential N deficiency (-12 g kg^{-1} DM). The degraded protein balance for wheat DDGS was similar between the different bio-ethanol plants.

In conclusion, the chemical and biological characteristics of DDGS varied among types and between wheat DDGS samples manufactured at the different bio-ethanol plants. Thus, it is inappropriate to assume fixed values for the nutritive value of DDGS without considering factors

such as type of grain used and bio-ethanol plant origin. Further research with higher number of samples will help to clarify the use of the chemical profile to predict energy values and the potential degradability of DDGS.

ACKNOWLEDGMENTS

This thesis has been a wonderful journey and I owe many thanks to those who helped me along the way.

First and foremost, I would like to dedicate this thesis to my Mom, for her never ending support and encouragement. I know that my goals are also hers.

I wish to express my appreciation to Dr. Peiqiang Yu for his support, guidance and encouragement throughout the program. You have been a great non-bossy boss. I am also thankful to my supervisory committee, Dr. D. Christensen, Dr. J. McKinnon, Dr. B. Laarveld, Dr. T. Mutsvangwa, Dr. F. Buchanan, and Dr. S. Wright, for their assistance. Special thanks to Dr. D. Christensen for his advice and as well for sharing his invaluable knowledge. Acknowledgment also goes to Zhiyuan Niu for helping me during the endless hours of laboratory work.

This project has been possible thanks to the funding body: Agriculture Development Fund, ABIP-FOBI, and Feed Chair. Also, my appreciation to the organizations that awarded my work: Fundación Caja Madrid, DDGS Council, Purina Land O' Lakes Feeds, and the Hugh Nicholson Memorial Scholarship. Thanks for the motivation and for allowing me to show my research internationally.

I am also thankful to other graduate students and faculty members that have contributed to this fulfilling experience; Lynne, Lee-Anne, Ravindra, Gwinyai, Ravinder, Dr. M. Drew and Dr. C. Christensen. Thanks for the listening, advice and laugh.

To my friends in Spain; Javi, Manuel, Pachi, David, Chemake, Pedro, Sergio, Jesius, Markitos, Ramón, Marco, Pableras, Oscar y Gamu. Each time I came back home was priceless.

And last but not least, this thesis is also dedicated to my people in Canada; Diana, Felipe, Ari, Jose, Michelle, Pati, Manu, Isa, Alex, Joe, Ana, Beto, Manena, Vicky, Pato, Sonia, Carlos, Zafer, Elias, Clare, Thiago, Vitor and Luisao. I am lucky to have such an international family.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
AECP	Truly absorbable endogenous protein
AMCP	Truly absorbable microbial protein synthesized in the rumen
ARUP	Truly absorbed bypass feed protein in small intestine
Ca	Calcium
CA	Rapidly fermented carbohydrate sub-fraction as per CNCPS (Kd = 200 - 350 $\%h^{-1}$)
CB1	Intermediately degraded carbohydrate sub-fraction as per CNCPS (Kd = 20 -50 $\%h^{-1}$)
CB2	Slowly degraded carbohydrate sub-fraction as per CNCPS (Kd = 2 - 10 $\%h^{-1}$)
CC	Unavailable cell wall as per CNCPS
CDS	Condensed distillers solubles
Cfat	Crude fat
CHO	Total carbohydrate
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
D	Potentially degradable fraction during *in situ* ruminal incubation
DDG	Dried distillers grains
DDGS	Dried distillers grains with solubles
DE	Digestible energy
DE_{1X}	Digestible energy at maintenance level
DE_{3X}	Digestible energy at production level when intake is 3 times maintenance intake
DM	Dry matter
DOM_{120}	Digestible organic matter at 120 h rumen incubation
DPB	Degraded protein balance
DVE	Truly absorbable protein in small intestine as per DVE/OEB System
ECP	Correction for endogenous protein losses during digestion process
ED	Effective degradability

ENDP	Endogenous protein losses in the small intestine
FC	Fiber carbohydrates
FOM	Fermentable organic matter
FP	Fermentation products that are assumed to be zero for concentrates
h	Hours
HEDN	Hourly effective degradability of nitrogen
HEDOM	Hourly effective degradability of organic matter
IADP	Estimated intestinally absorbable feed protein
IDP	Estimated intestinal digestibility of rumen bypass protein
ISCPD	In situ degradability of crude protein after 48 h incubation
ISFAD	In situ degradability of fatty acids after 48 h incubation
ISNDFnD	In situ degradability of neutral detergent fiber (corrected for NDICP) after 48 h incubation
ISNFCD	In situ degradability of non-fiber carbohydrates after 48 h incubation
Kd	Rate constant for in situ rumen degradation of D fraction
Kp	Rate of passage
MCP_{FOM}	Microbial protein synthesized from energy available from rumen fermented organic matter
MCP_{RDP}	Microbial protein synthesized from rumen degraded protein
ME_{3X}	Metabolizable energy at production level when intake is 3 times maintenance intake
MP	Metabolizable protein
MWDGS	Modified wet distillers grains with solubles
N	Nitrogen
NDF	Neutral detergent fiber
NDFn	Neutral detergent fiber adjusted for protein (NDFn = NDF – NDICP)
NDICP	Neutral detergent insoluble crude protein
NE_{L3X}	Net energy for lactation when intake is 3 times maintenance intake
NE_m	Net energy for maintenance in growing animals
NE_g	Energy retention or gain
NFC	Non fiber carbohydrates
NPN	Non-protein nitrogen

OM	Organic matter
P	Phosphorus
PA	Rapidly degradable protein sub-fraction as per CNCPS (Kd = assumed to be infinity)
PB1	Rapidly degradable protein sub-fraction as per CNCPS (Kd = 120 - 400 $\%h^{-1}$)
PB2	Intermediately degradable protein sub-fraction as per CNCPS (Kd = 3 - 16 $\%h^{-1}$)
PB3	Slowly degradable protein sub-fraction as per CNCPS (Kd = 0.06 - 0.55 $\%h^{-1}$)
PC	Undegradable protein sub-fraction as per CNCPS
PEM	Polioencephalomalacia
peNDF	Physically effective neutral detergent fiber
PUFA	Polyunsaturated fatty acids
r	Pearson correlation coefficient
R^2	Coefficient of determination
RDP	Rumen degraded protein
R(t)	Residue present at a specific time t during *in situ* ruminal incubation
RUP	Rumen by-pass protein
RUSt	Rumen undegraded starch
RSD	Residual standard deviation
S	Potentially soluble fraction during *in situ* ruminal incubation
SCP	Buffer soluble protein
SEM	Standard error of the mean
SFA	Saturated fatty acids
tdCP	Truly digestible crude protein
tdFA	Truly digestible fatty acid
TDN	Total digestible nutrients
TDN_{1X}	Total digestible nutrients at a maintenance level
TDN_{3X}	Total digestible nutrients at three times maintenance
tdNDF	Truly digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrates
TDP	Total digestible feed protein
T_0	Lag time
TP	True protein

TS	Thin stillage
U	Potentially undegradable fraction during *in situ* ruminal incubation
UDM	Undigested dry matter
WDG	Wet distillers grains
WDGS	Wet distillers grains with solubles

1. GENERAL INTRODUCTION

The development and rapid expansion of the fuel ethanol industry in North America has increased the availability of distillers grains to livestock producers. Due to the ease of storage, dried distillers grains with solubles (DDGS) are the most commonly produced type of bio-ethanol co-products. As wheat is readily available in western Canada, it is the primary substrate for bio-ethanol production; however, corn can be relatively cheap and may be used in combination with wheat. As a result, DDGS from pure wheat and from blends containing different proportions of wheat and corn are currently manufactured. In 2010, the DDGS production capacity of western Canada will be greater than 400,000 tonnes (CRFA 2009).

The high protein, high fiber, high fat, and low starch contents make DDGS a very attractive ingredient to be used in ruminant diets; however, the nutrient profile of DDGS varies significantly among plants and over time within the same plant (Cromwell et al. 1993; Spiehs et al. 2002; Shurson 2005; Kleinschmit et al. 2007). This variability prevents DDGS from being totally accepted as livestock feed, as it may result in inaccurate ration formulation and diminished animal performance. Thus, it is important to identify the factors causing this inconsistency so that processing conditions can be enhanced, top quality DDGS can be produced, and accuracy in ration formulation can be increased.

While research has been conducted to evaluate the nutritive characteristics of corn DDGS (Cromwell et al. 1993; Spiehs et al. 2002; Shurson 2005; Kleinschmit et al. 2006; Rosentrater and Muthukumarappan 2006; Kleinschmit et al. 2007; Martinez-Amezcua et al. 2007; Stein and Shurson 2009), information on the nutritive value of wheat DDGS and blend (wheat/corn) DDGS is less available (Dong et al. 1987b; Boila and Ingalls 1994a, 1994b; Nyachoty et al. 2005; Widyaratne and Zijlstra 2006; Gibb et al. 2008). Similarly, plant to plant inconsistencies have been reported for corn DDGS (Cromwell et al. 1993; Spiehs et al. 2002; Shurson 2005; Kleinschmit et al. 2007) but not for wheat DDGS. Assumed fixed values for the amount and digestibility of rumen undegraded protein (RUP) of DDGS are commonly assumed in current ration formulation; however, the rumen availability of protein from DDGS may vary among DDGS types and bio-ethanol plants as the chemical profile does. Thus, the knowledge of the chemical profile, protein and carbohydrate fractions, energy values, digestibility characteristics, and the protein metabolic characteristics and predicted protein supply to small intestine of each type of DDGS is essential in order to achieve accurate ration formulation.

Therefore, the objectives of the literature review that follows are 1) to review the production process of bio-ethanol and related co-products, 2) to provide detailed information on the nutrient characteristics of DDGS as affected by type and processing conditions, and 3) to introduce the feed evaluation methods that will be utilized to conduct a complete feed evaluation of different types of DDGS currently fed to ruminants in western Canada.

2. LITERATURE REVIEW

2. 1. Benefits of bio-ethanol production

The energy market is now being influenced by the concerns associated with global warming and environmental degradation. Ethanol is a renewable fuel because the energy is derived from plants that can be replenished. It contains a greater proportion of oxygen than gasoline, consequently reducing polluting emissions (Drapcho et al. 2008). In addition, the plant biomass used to produce ethanol absorbs carbon dioxide (CO_2) as it grows, thus reducing the total greenhouse gas emissions. According to Farrell et al. (2006), the switch from gasoline to corn ethanol reduces greenhouse gas emissions moderately, by around 13%, and petroleum use by around 95% on an energetic basis. The benefits of bio-ethanol production are also social and economical, as this industry contributes to the economic growth of rural areas by opening new markets for Canadian agriculture and forestry and by creating jobs at bio-ethanol production plants (Government of Alberta 2008).

Ethanol production from cereal grains generates the co-products collectively known as distillers grains. These co-products are normally sold as livestock feed ingredients; however, substantial research is currently being undertaken to study the potential use of distillers grains as human food ingredients and other value-added applications (Rosentrater 2007). The economic viability of the bio-ethanol plant is significantly enhanced by the sale of distillers grains (approximately 0.1 US$/L of ethanol produced from corn), thus these co-products represent a vital element to bio-ethanol production (Rosentrater 2007). The net energy gain generated by corn ethanol and its derived co-products has been reported to be about 4 MJ/ L to 9 MJ/L of ethanol (Farrell et al. 2006).

2.2. Feedstocks used for the production of bio-ethanol and related co-products

Ethanol produced from agricultural and forestry feedstocks results from the fermentation of starch, sugars, or lignocellulose.

2.2.1. Starch and sugar-based feedstocks

Most of today's ethanol is generated from starch- and sugar- based feedstocks

(Burden 2009). Starch-based feedstocks include the processing of grains such as corn (Spiehs et al. 2002), wheat (Ojowi et al. 1997; Mustafa et al. 2000a, 2000b; Nyachoty et al. 2005), sorghum (Lodge et al. 1997; Al-Suwaiegh 2002) and barley (Mustafa et al. 2000b), while sugar-based feedstocks include plants such as sugar cane or sugar beets (McKendrick et al. 2003). The former feedstocks include starches or chains of sugars that have to be broken down before fermentation, while the latter feedstocks contain simple sugars that are rapidly extracted and fermented.

Starch-based feedstocks are expensive because they are in demand for other applications; however, the high costs are compensated for by the commercialization of distillers grains (Government of Alberta 2008; Burden 2009). Each kg of wheat yields approximately 0.37 liters of ethanol and 0.29 kg of wheat dried distillers grains with solubles (DDGS) (Nichols et al. 1998; CRFA 2009), while each kg of corn produces 0.40 liters of ethanol and 0.32 kg of corn DDGS (Pimentel and Patzek 2005). Corn has a higher starch content than wheat, but wheat is higher in protein and lysine (NRC 2001). The drawback of wheat protein is its water insolubility that may cause problems in the downstream processing of bio-ethanol production (Drapcho et al. 2008). For this reason, low protein varieties of wheat such as Canadian Prairie Spring (Red and White), Canadian Western Red Winter, and Canadian Western Soft White are the most suitable for bio-ethanol production (Drapcho et al. 2008).

2.2.2. Lignocellulose-based feedstocks

Lignocellulosic ethanol is produced from a wide range of biomass feedstocks, such as agricultural residuals (cereal straws, leaves, husks), forestry residues (wood chips, sawdust), fast growing trees (poplar, willow), plant waste from industrial processes (paper pulp, distillers grains) and grasses grown specifically for fuel production (switchgrass) (Anonymus 2009; Burden 2009). The main challenge is that these feedstocks contain cellulose and hemicellulose that are more difficult than starch-based feedstocks to biochemically break down into their component sugars and convert into ethanol (Drapcho et al. 2008). This is largely attributed to the inefficiency of the current commercial preparations of cellulases as well as the higher percentage of pentoses contained in hemicellulose, which unlike hexoses, are not easily fermented (Burden 2009; Singhania 2009).

Lignocellulosic feedstocks are more abundant than starch- and sugar- based feedstocks, thus they can be used to produce larger amounts of ethanol. Furthermore, the co-products derived

from processing (lignin, ash, and hard-to-process proteins) can replace fossil fuels as source of heat and power in the ethanol production facility, while the non-combustible ash can be marketed as fertilizer (Anonymus 2009). Although the production of ethanol from lignocellulose is of growing interest worldwide, development of lignocellulose-to-ethanol technology is still underway (Purwadi et al. 2007).

2.3. Bio-ethanol production in Canada

Canada has the cropland and forest resources required to support an significant ethanol production. With this in mind, the Federal Government proclaimed the intention to implement a Federal Renewable Fuels Standard (RFS) in December 2006. This mandates an average of 5% renewable fuel content in gasoline by 2010 and 2% in diesel and home heating fuels no later than 2012 (O'Connor 2007). Earlier in 2005, the government of Saskatchewan, Manitoba and Ontario also announced the mandatory use of ethanol-blends in those provinces (O'Connor 2007). As a result of these federal and provincial initiatives, 15 bio-ethanol plants are currently in operation and four are under construction (CRFA 2009). According to the Canadian Renewable Fuel Association (CRFA), the Canadian bio-ethanol production will reach 1,700 million liters in 2010. Only 2.5 % of this bio-ethanol production will be generated from lignocellulosic feedstocks; the remainder 97.5 % will originate from starchy cereal grains, primarily wheat and corn (CRFA 2009). The amount of distillers grains, predominantly DDGS, generated as co-products of this starch-based bio-ethanol production will exceed 1.4 million tones (Government of Alberta 2008).

The use of wheat or corn for ethanol production mainly depends on geographical area and market value of the commodity. Although wheat is normally processed in the west and corn in the east, the relatively cheap price of corn has recently forced western Canadian bio-ethanol plants to include corn in combination with wheat in the processing. As a result, DDGS from pure wheat and from blends containing different proportions of wheat and corn are presently produced in western Canada. In 2010, the production capacity of western Canada from starch based-feedstocks is estimated to be 512 million liters of ethanol and more than 400,000 tones of DDGS (CRFA 2009).

2.4. Production process of bio-ethanol and related co-products

Most of Canada's ethanol is generated from starch-based feedstocks. The production of ethanol from starch is similar for all grains: starch is enzymatically broken down to glucose, followed by the fermentation of glucose to ethanol. This conversion can be carried out by three different commercial processes: dry grinding, dry milling, and wet milling. The dry milling and wet milling processes have been thoroughly reviewed by Rausch and Belyea (2006). The term dry milling may erroneously be used to describe the dry grinding process. In dry milling, the processing is started by increasing the kernel moisture in order to facilitate the separation of the different grain components (pericarp, germ and endosperm); however, these steps are lacking in dry grinding (Rausch and Belyea 2006). In western Canada, ethanol is produced primarily from wheat via dry grind processing (Government of Alberta 2008; CRFA 2009). A schematic of the dry grinding process is illustrated in Figure 2.1.

2.4.1. Initial handling of grain

In the dry grinding process (Figure 2.1), feedstock is directly cleaned and ground by hammer mills or roller mills (Rausch and Belyea 2006). Grinding allows water penetration and maximizes the accessibility of enzymes to starch molecules, and allows separation of unfermented particles from liquid at the end of the process (Nichols and Bothast 2008). Facilities that process both wheat and corn feedstocks grind the grains together. Before the addition of enzymes, the ground material is mixed with water to form a slurry (Nichols and Bothast 2008).

2.4.2. Hydrolyzation of starch to fermentable sugars

Starch consists of two main structural components; amylose and amylopectin. Amylose is a linear polymer of glucose in which the glucose residues are connected by α-1,4 linkages, while amylopectin is a larger and branched polymer with both α-1,4 and α-1,6 linkages (Rausch and Belyea 2006). Although the ratio amylose:amylopectin varies among starch sources, starch from wheat and corn usually contains approximately 25% amylose and 75% amylopectin (Nichols and Bothast 2008).

The process of converting starch to simple sugars is termed saccharification and uses a combination of heat and enzymes (Power 2003). In an initial step (Figure 2.1), referred as to liquefaction, the slurry is mixed with alpha-amylase enzyme and cooked by pressurized steam a

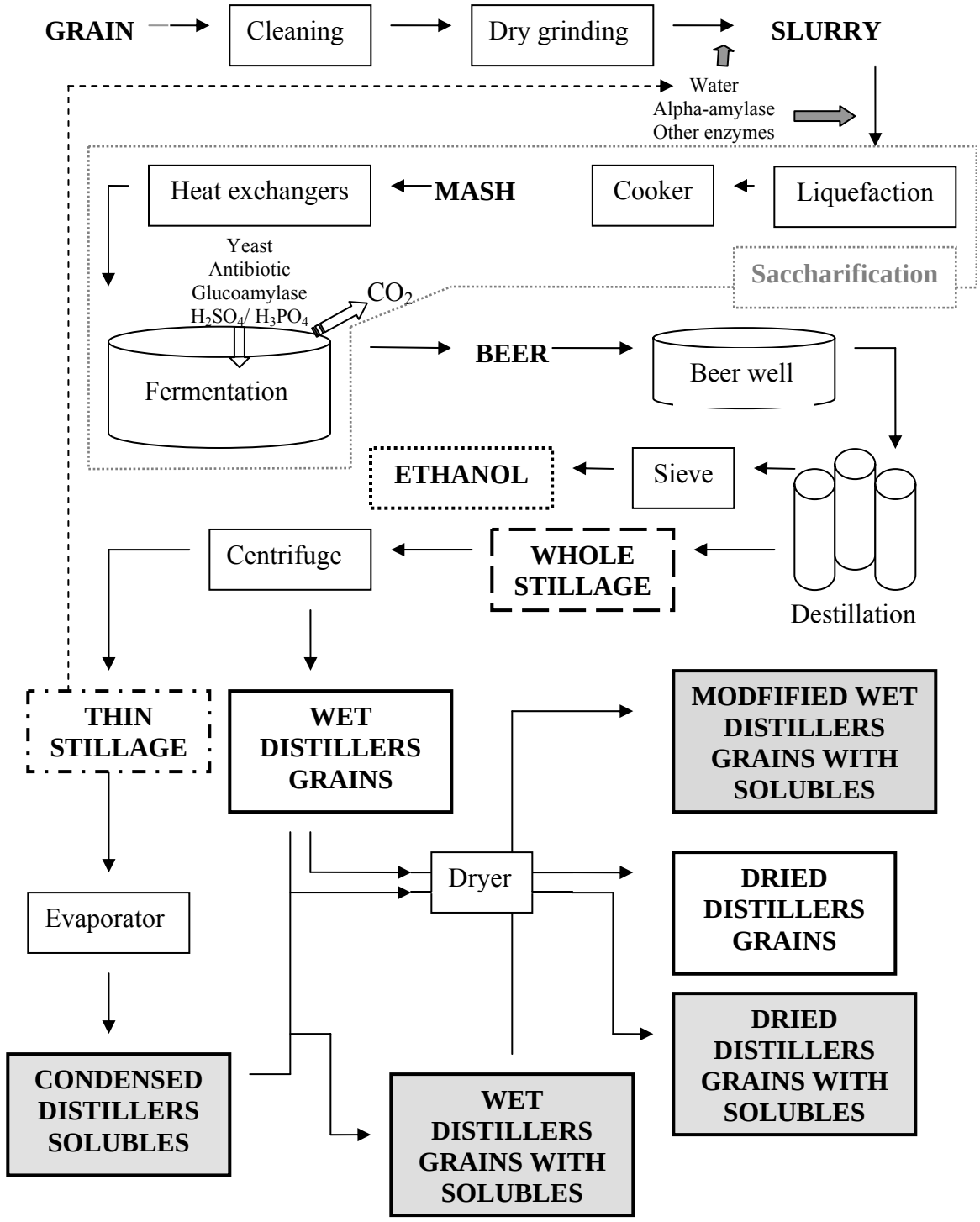

Figure 2.1. The dry grinding process of ethanol production. Adapted from Rausch and Belyea (2006).

110 °C. Starch granules swell and gelatinize creating a thick mash. As the mash reaches the enzyme's optimum temperature, the enzyme chemically breaks down the starch into short chain molecules (dextrins) by hydrolyzing internal α-1,4 glucosidic linkages (Nichols and Bothast 2008). In some plants, additional alpha-amylase as well as other enzymes, such as proteases, xylanases and cellulases, can be used to enhance starch conversion and reduce viscosity of the mash (Ingledew et al. 1999). The mash is then cooked (~70°C) for a short period of time to reduce the level of lactic acid producing bacteria (Nichols and Bothast 2008). These bacteria decrease the efficiency of ethanol fermentation by two mechanisms: 1) by competing with yeast for glucose, and 2) by producing lactic and acetic acids that inhibit yeast growth and metabolism (Bayrock et al. 2003).

In a final step, saccharification and fermentation occur simultaneously. The mash is firstly cooled (~32°C) by heat exchangers and transferred into a fermentation vessel where glucoamylase enzyme, yeast species (*Saccharomyces cerevisiae*), and urea are added. Glucoamylase cleaves α-1,4 glucosidic linkages at non-reducing chain ends, and also has the ability to act on α-1,6 glucosidic linkages at amylopectin branch points (Nichols and Bothast 2008). The production of ethanol by yeast occurs as quickly as glucose is released from dextrins by glucoamylase (Russell 2003). Urea, along with recycled water (thin stillage), is used by yeasts as a major source of nitrogen (Davis 2001). During fermentation, two methods are normally used in order to suppress the growth of contaminant bacteria: the addition of antibiotics such as penicillin or virginiamycin, and the addition of sulfuric acid (H_2SO_4) to maintain pH to acidic levels (4-5) (Bayrock et al. 2003; Nichols and Bothast 2008). Due to the problems associated with high sulfur levels in distillers grains, phosphoric acid (H_3PO_4) has been tested as an alternative; however, H_2SO_4 is the most economical option (Vaness et al. 2009).

2.4.3. Ethanol fermentation and recovery

Fermentation is completed in 40-60 hours (Nichols and Bothast 2008). The conversion pathway from glucose to ethanol is explained in detail by Rusell (2003). Yeast function is inhibited by ethanol, which increases the fluidity and permeability of the membrane, and by organic acids produced by the yeast itself and the contaminant bacteria (Drapcho et al. 2008). Approximately, 0.51 g of ethanol and 0.49 g of CO_2 are produced per g of glucose (Nichols and Bothast 2008). As illustrated in Figure 2.1, the resulting beer is sent to the beer well, from which it is transferred

to a distillation area for ethanol removal. Two products are collected in the distillation area; an ethanol-water mixture and the whole stillage. The ethanol-water mixture is further dehydrated by molecular sieves, typically zeolites, to obtain near-anhydrous (99.5%) ethanol (Bibb Swain 2003). The whole stillage, which contains water, fiber, oil, protein, yeast cells, and unfermented portions of grains, is further processed to generate the bio-ethanol co-products.

2.4.4. Stillage processing

The whole stillage is separated into liquid and coarse solids by centrifugation (Figure 2.1). The liquid fraction, referred to as thin stillage (TS), is normally higher in fat and minerals (Belyea et al. 1998; Rausch and Belyea 2006; Kalscheur et al. 2008; Cao et al. 2009). TS also contains water soluble components, mostly fermentation by-products (i.e. glycerol), soluble sugars, soluble proteins and organic acids (i.e. lactid acid, acetic acid) (Kim et al. 2008). TS can be concentrated by evaporation to become condensed distillers solubles (CDS), or can be recycled back (15% or more) into the process as water added to form the slurry (Rausch and Belyea 2006; Nichols and Bothast 2008). If evaporated, most of the soluble sugars and a small portion of the soluble proteins are extracted (Kim et al. 2008).

The solid fraction, termed wet distillers grains (WDG) or wet cake, is higher than TS in fiber (Kim et al. 2008). WDG can be blended with CDS resulting in wet distillers grains with solubles (WDGS), or can be blended and dried with CDS resulting in dry distillers grains with solubles (DDGS). In rotatory drum dryers commonly used in old-generation plants, the blending process to obtain DDGS with a dry matter (DM) content of 82 – 100 % consists of WDG at 25 - 55 %DM, CDS at 35 - 40 solid content and freshly dried DDGS at 90 %DM (Ileleji and Rosentrater 2008). The ratio of WDG, CDS and DDGS should be such that the DM content of the blend entering the dryer must be about 65% (Ileleji and Rosentrater 2008). Once the product is dried, approximately 70% is routed to the storage pad while the rest is blended with more WDG and CDS. In some plants, WDGS are partially dried to produce a co-product called modified wet distillers grains with solubles (MWDGS) (De Mello Jr et al. 2009). These co-products are utilized as livestock feed; however, each stream has a specific nutritional profile and logistics that must be evaluated carefully in order to include them in ruminant rations. When the bio-ethanol plant is in close proximity to the farm, wet products are an excellent alternative for

ruminant rations; however, due to the enhanced ease of handling, storage and shipping DDGS, these are the most commonly utilized ruminant rations (Lardy 2007).

2.5. Nutrient characteristics of DDGS

Due to the starch removal, the remaining chemical components in the distillers grains products become concentrated, approximately threefold compared to the parent grain (Weigel et al. 1997). Thus, these co-products are generally characterized by high crude protein (CP), fat, neutral detergent fiber (NDF), ash, and low starch. This profile makes them highly attractive to be used in ruminant diets; however, the nutritional properties of distillers grains can be very variable (Spiehs et al. 2002). Table 2.1 is a compilation of data that demonstrates the variability in the nutrient composition of different corn- and wheat-based bio-ethanol co-products.

Regarding DDGS, Shurson (2005) found that the nutrient composition fluctuates between old- and new-generation plants. This explains the higher protein and energy values of DDGS available today compared to the older values reported by the NRC 2001 (Birkelo et al. 2004). This variation can be attributed the overall improvement in the bio-ethanol industry processing as well as by the lack of standard compositional analysis procedures (Kim et al. 2008). Of more interest is the nutrient variability detected among new-generation plants (Cromwell et al. 1993; Spiehs et al. 2002; Kleinschmit et al. 2007) and over time within the same plant (Belyea et al. 2004; Shurson 2005), which is attributed to differing feedstocks and processing conditions. This inconsistency is one of the biggest issues preventing DDGS acceptance as livestock feed since it results in inaccurate ration formulation. A complete chemical analysis conducted at least once yearly is recommended to account for this variability (Spiehs et al. 2002).

2.5.1. Protein

Dried distillers grains with solubles are a very good source of CP for ruminants. On a dry matter basis, reported values vary from 26.8 to 33.7 % for corn DDGS and from 30.5 to 45.8 %CP for wheat DDGS (Table 2.1). The variation within DDGS type is attributed to grain varieties, which in turn depends on geographical area and soil fertility (Dong et al. 1987b), and processing methods. Some bio-ethanol plants remove the germ or pericarp prior to fermentation, resulting in reduced fat and fiber and augmented protein in the DDGS product (Martinez-

Table 2.1. Chemical profile of corn- and wheat-based ethanol co-products

Items	Corn grain [1 - 3]	Corn DDGS[z] [1 -9]	Corn WDG[y] [1, 2, 3, 4, 10]	Corn TS[x] [4, 11, 12]	Wheat grain [1, 13]	Wheat DDGS [2, 13 - 17]	Wheat WDG [18 - 20]	Wheat TS [18, 21, 22]
DM[w]	88.1	86.2 - 93.0	30.9 - 35.5	5.0 - 6.2	87.1 - 90.0	91.6 - 95.6	27.2 - 31.6	6.3
CP[v] (%DM)	9.4 - 10.8	26.8 - 33.7	25.0 - 39.5	1.3 - 16.8	11.1 - 16.3	30.5 - 45.8	26.0 - 26.5	36.6 - 46.6
SCP[u] (%CP)	14.6 - 25.3	5.3 - 10.7	8.3		20.2 - 35.7		4.0	23.7
NPN[t] (%SCP)	70	77.3	65.8		25		90.0	84.4
Lysine (%CP)	2.6 -2.8	1.9 - 3.3	3.2	0.1	2.0 - 3.0	1.6 - 2.8	1.0	0.8
Methionine (%CP)	1.6 - 2.0	1.7 - 2.2	1.7	0.1	1.3 - 1.6	1.4 - 1.5	0.7	0.5
RUP[s] (%CP)	44.6	47.0 - 69.0	49.9	20.0	25.6 - 41.2	51.3	31.0 - 46.1	45.6
ADICP[r] (%CP)	0.3 - 1.2	7.5 - 23.1	1.5 - 5.7		0.1 - 0.9	7.4	5.9 - 16.7	16.7
CFat[q] (%DM)	4.2	3.5 - 12.8	8.5 - 14.5	8.1 - 20.9	1.4 - 2.7	3.1 - 9.9	7.4 - 14.0	5.9 - 11.2
Starch (%DM)	65.0 - 75.1	3.8 - 11.4	4.6 - 9.0	0.5 - 2.2	54.3 - 70.0		2.0 - 15.0	2.0
NDF[p] (%DM)	9.5 - 12.9	25.0 - 51.3	39.4 - 58.1	11.7	6.9 - 20.2	28.9 - 57.0	55.5 - 74.0	35.2 - 38.4
ADF[n] (%DM)	2.1 - 5.1	8.0 - 21.0	23.4 - 25.3	0.1	1.2 - 8.6	11.1 - 24.3	20. 2 - 22.0	2.0 - 8.5
Lignin (%DM)	0.8 - 1.5	3.5 - 6.8	3.0 - 7.4		1.1 - 2.4			1.6
Ash (%DM)	0.2 - 0.8	2.0 - 9.8	3.9 - 7.2	5.9 - 8.7	0.9 - 2.9	2.1	4.0	6.4 - 9.4
Calcium	0.0 - 0.1	0.0 - 0.5	0.0 - 0.2	0.0 - 0.1	0.0 - 0.8	0.2 - 0.3		
Phosphorus	0.2 - 0.4	0.4 – 1.0	0.7 - 1.0	1.2 - 1.4	0.0 - 1.0	1.0 - 1.1		
Sulfur	0.0 - 0.2	0.3 - 1.1	0.4 - 0.7	0.1 -1.0	0.1 - 0.2	0.5 - 0.6		
TDN_{1X} (%)	88.7	85.0 - 90.0	79.5 - 90.2		81.2 - 86.5			
NE_{L3X} (Mcal kg^{-1})	2.0 - 2.2	2.3	2.0 - 2.3		1.9 - 2.0			
NE_m (Mcal kg^{-1})	2.2 - 2.3	1.9 - 2.0	2.1 - 2.4	1.9 - 2.3	2.0 - 2.2			
NE_g (Mcal kg^{-1})	1.5 - 1.6	1.3 - 1.4	1.4 - 1.7	1.6 - 1.8	1.3 - 1.5			

Source: [1]Dairy One (2009); [2]NRC 2001; [3]Sniffen et al. (1992); [4]Rosentrater and Muthukumarappan (2006); [5]Sphies et al. 2002; [6]Kleinschmit et al. 2006; [7]Kleinschmit et al. 2007; [8]Martinez-Amezcua et al. (2007); [9]Stein and Shurson (2009); [10]Cao et al. (2009); [11]Kim et al. (2008); [12]Lardy (2007); [13]Dong et al. (1987b); [14]Gibb et al. (2008); [15]Widyaratne and Zijlstra (2006); [16]Boila and Ingalls (1994a); [17]Nyachoti et al. (2005); [18]Mustafa et al. (2000a); [19]Ojowi et al. (1997); [20]F. Reveco, personal communication; [21]Iwanchysko et al. (1999);[22]Ojowi et al. (1996)

[z]Dried distillers grains with solubles; [y]Wet distillers grains with solubles; [x]Thin stillage; [w]Dry matter; [v]Crude protein; [u]Soluble crude protein; [t]Non-protein nitrogen; [s]Rumen undegraded protein; [f]Acid detergent insoluble crude protein; [q]Crude fat; [p]Neutral detergent fiber; [n]Acid detergent fiber

Amezcua et al. 2007). Similarly, the protein content of DDGS can increase with the amount of yeast utilized. The growth of yeast during fermentation creates a cell mass that is highly rich in protein and substantially contributes to the protein content in the DDGS product (Belyea et al. 2004).

The protein content of the solubles is lower than in WDG, thus a decrease in the CP concentration of DDGS is observed as the amount of solubles added back increases (Noll et al. 2007; Cao et al. 2009). Normally the ratio of distillers grains to solubles is 67:33 (as is); however, variations in this ratio as well as the variability in the CP of the solubles will affect the CP of the DDGS product (Belyea et al. 1998; Martinez-Amezcua et al. 2007).

When comparing wheat DDGS with corn DDGS (Table 2.1), the higher CP values for wheat DDGS can be largely attributed to the higher CP content of wheat relative to corn. Several reports (Boila and Ingalls 1994a; University of Saskatchewan 2009) have showed that the CP content of DDGS increases as the wheat:corn ratio increases in the mixture.

A good reason to include DDGS in ruminant rations is because of its significant amount and intestinal digestible rumen undegraded protein (RUP) (Ingalls 1995; Stern et al. 1995; O'Mara et al. 1997; Kleinschmit et al. 2007; Cao et al. 2009). Due to the degradation of a large part of the readily degradable protein during fermentation, and due to the reduced solubility of protein as a result of heat during liquefaction and drying, the remaining protein in DDGS has a higher proportion of RUP (Firkins et al. 1985; Arieli et al. 1989). While a substantial number of studies (Ingalls 1995; Stern et al. 1995; O'Mara et al. 1997; Kleinschmit et al. 2007; Cao et al. 2009) have reported large variation in the RUP content for corn DDGS, ranging from 40.0 to 76.0 %CP, less information (NRC 2001; Gibb et al. 2008) is available for wheat DDGS, in which RUP values range from 51.3 to 59.5 %CP. This variability between and within DDGS types can be explained by the grain type and variety as well as by differing processing conditions. The content of RUP in corn is greater than in wheat due to the resistance of zein, the major corn protein source, to ruminal degradation (Little et al. 1968). Thus, RUP in blend (wheat/corn) DDGS increased as the content of corn in feedstock increased relative to wheat (Boila and Ingalls 1994a). The degradability and solubility of CP in the rumen decreases as temperature and time of drying increases (Arieli et al. 1989; McKinnon et al. 1995). Heat facilitates the Maillard reaction, through which sugar residues condensate with amino acids, rendering proteins indigestible (Firkins et al. 1985; Van Soest 1994). These indigestible proteins are recovered in

the lignin and acid detergent fiber fraction (ADF). Thus, an indication of the severity of the drying conditions can be provided by the content of acid detergent insoluble crude protein (ADICP) (Goering et al. 1972; Kleinschmit et al. 2007). A negative relationship between ADICP and the ruminal and intestinal availability of DDGS protein has been reported; however, ADICP levels must be higher than 13 %CP (Harty et al. 1998). Heat also denatures yeast, rendering them resistant to rumen degradation (Bruning and Yokoyama 1988). While most of the protein content in the solubles is heated yeast (Belyea et al. 2004; Klopfenstein et al. 2008), only 20 % is ruminally degradable (Herold 1999). Additionally, the solubles contribute to the RUP content in DDGS by providing simple sugars that increase the susceptibility to Maillard reaction during drying (Martinez-Amezcua et al. 2007). The effect of heat and solubles on RUP can be verified by previous studies, in which higher RUP values reported in DDGS compared to WDG (Firkins et al. 1985), and in DDGS compared to WDG (Ojowi et al. 1997; Mustafa et al. 2000a; Gibb et al. 2008). Contrary to these results, Cao et al. (2009) showed that increasing the soluble portion augmented the overall ruminal degradability of protein due to the increasing soluble protein fraction; however, drying conditions in this experiment differed from those performed in the bio-ethanol plant, likely resulting in less severe reduction of the soluble protein fraction.

Knowledge of differences in the levels and availability of amino acids between and within DDGS types is required for accurate diet formulation. The reported lysine contents range from 1.9 to 3.3 of %CP in corn DDGS and from 1.6 to 2.8 of %CP for wheat DDGS (Table 2.1). Since the amino acid profile of distillers grains is similar to that of the feedstock grain, differences in the grain type and variety are reflected in the amino acid composition of the DDGS (Dong et al. 1987b). On a dry matter basis, the lysine and methionine content in wheat are higher than in corn, as they are in wheat DDGS compared to corn DDGS. Belyea et al. (1998) found differences in the content and digestibility of essential amino acids in condensed distillers solubles, which suggests that the amino acid profile and availability can vary depending on the solubles added back. Lysine is the most susceptible amino acid to Maillard reaction due to the free amino group at the epsilon carbon unit (Warnick and Anderson 1968), and this susceptibility is increased by the sugars contained in the solubles; therefore, lysine digestibility for DDGS is lower than that for parental grain, solubles, and wet DG (Martinez-Amezcua et al. 2007).

Inconsistencies in the protein content of DDGS may lead to excessive dietary nitrogen and increased nitrogen in manure, which increases the manure's agronomic value but also results

in increased ammonia emissions (Hao et al. 2009). Cole et al (2005) reported that *in vitro* daily ammonia emissions increased 60 to 200% when protein content of the diet varied from 11.5 to 13% DM, primarly as a result of increased urinary nitrogen excretion. Several studies have shown that the nitrogen concentration in manure increases with increasing levels of distillers grains (Hao et al. 2009; Spiehs and Varel 2009). Normally, up to 30% and 20% of DDGS as dry matter can be included in dairy and beef rations respectively without detecting a negative effect nitrogen excretion (Janicek et al. 2008; Hao et al. 2009) .

2.5.2. Energy

Dried distillers grains with solubles are an excellent source of energy for ruminants. Normally, the energy values for DDGS are higher than those for parent grains (Table 2.1). In finishing beef cattle, improved gains and feed efficiency have been shown when replacing corn grain with corn DDGS (Ham et al. 1994). Barley, whose net energy content for gain (NEg) is 1.4 Mcal kg^{-1} DM (NRC 2001), is the most common grain utilized in ruminant rations in western Canada. No effect on cattle performance of finishing steers was observed when barley was replaced with wheat DDGS up to 32 %DM (Beliveau 2008). When replacement levels were higher than 47 %DM, NEg and feed conversion were reduced, but this was attributed to a reduction in the digestibility of the diet (Gibb et al. 2008). The similar or increased energy value of DDGS relative to cereal grains is attributed to the approximately threefold concentration of fat, and the readily digestible fiber (Klopfenstein et al. 2008; Schingoethe et al. 2009). Therefore, knowledge of the differences in the nutritional characteristics of fat and fiber among and within DDGS types is crucial to provide accurate levels of energy in the ration.

2.5.3. Fat

Reported fat values vary from 3.5 to 12.8 %DM for corn DDGS and from 3.1 to 9.9 %DM for wheat DDGS (Table 2.1). Fat content in the solubles can be as high as 34 %DM, thus increasing fat levels in DDGS are observed as the amount of solubles blended back increases (Noll et al. 2007; Cao et al. 2009). As there exists a high variability in the fat concentration among solubles collected within the same and from different plants (Belyea et al. 1998; Knott et al. 2004), they are a determining factor in the fat composition variability of DDGS. Differences in the fat content within the same type of DDGS can also be explained by the method of analysis utilized.

Cao et al. (2009) showed that the official method (AOAC 954.02), which uses diethyl ether as the solvent, resulted in higher fat values than the use of petroleum ether, indicating that the energy content of the DDGS is defined by the method of analaysis.

Despite the high and variable fat content in the solubles, several studies (Lodge et al. 1997; F. Reveco, personal communication; Kim et al. 2008) have reported similar fat concentrations between DDGS and WDG. This discrepancy can be explained by differences in the fat levels of the solubles as a result of differing processing techniques among bio-ethanol plants. For instance, the germ of the grain, where the highest fat concentration is located, is sometimes removed prior to processing in corn based-ethanol plants, resulting in reduced lipid levels in DDGS relative to those produced by conventional processing (Martinez-Amezcua et al. 2007; Tedeschi et al. 2009).

When comparing corn DDGS and wheat DDGS, the fat content is higher for corn DDGS largely as a result of the higher fat content of corn relative to wheat (Dong et al. 1987b).

Fat contains more energy than starch (9 vs. 4.5 kcal g^{-1}), and the gross energy content of fat increases with the degree of saturation (Van Soest 1994). In DDGS, fat is primarily in the unsaturated form (Schingoethe et al. 2009). Although the degree of unsaturation is slightly higher in CDS than in WDG, fat concentration on a dry matter basis is twofold greater in CDS compared to WDG (Cao et al. 2009). Thus, increasing the level of solubles augments the gross energy content of DDGS and enhances the fatty acid profile.

Fat composition, as well as fat availability, is important from a meat composition and quality standpoint (Klopfenstein et al. 2008). The fatty acid profile of corn DDGS is similar to corn grain; however, small amounts of docosahexaenoic acid (DHA) are only present in DDGS samples (Martinez-Amezcua et al. 2007). This fatty acid may be provided by the yeast, which is able to modify the fatty acid composition depending on fermentation conditions (Torija et al. 2003). Compared to feeding corn oil, the quantity and digestibility of unsaturated fat in duodenum was higher in steers fed corn DDGS, suggesting that some of the fat in corn DDGS can be protected from rumen hydrogenation (Vander Pol et al. 2007). In addition, Depenbusch et al. (2009) has recently showed that the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) in beef meat increases with increasing levels of corn DDGS. Despite the human health benefits associated with PUFA consumption, a greater ratio of PUFA:SFA in meat

increases lipid oxidation and the subsequent production of off-flavors (Melton 1983; Depenbusch et al. 2009).

2.5.4. Fiber

DDGS contain a large amount of NDF, which can range from 25.0 to 51.3 %DM for corn DDGS and from 28.9 to 57.0 %DM for wheat DDGS (Table 2.1). This large variability is attributable to grain type and variety, bio-ethanol processing methods, and NDF analytical procedures. Dong and Rasco (1987a) showed that the NDF content of wheat was affected by variety. Some corn-based plants increase the nutritional value of DDGS for non-ruminant animals by recovering the pericarp fiber at the beginning of the dry grind process, consequently reducing the NDF content in the final co-product (Martinez-Amezcua et al. 2007). The solubles have less NDF fiber compared to distillers grains, thus the fiber concentration in DDGS decline as the inclusion of solubles increases (Cao et al. 2009).

Rasco et al. (1989) found that drying renders some of the protein in distillers grains insoluble in the neutral detergent solution, increasing the NDF concentration of the dried product due to the augmented NDIN content. In distillers grains, NDIN content may represent 40% of the NDF (Krishnamoorthy et al. 1982); therefore, the reported NDF value must be corrected for NDIN for balancing diets. The AOAC procedure for NDF analysis, referred to as amylase treated NDF (Mertens 2002), uses sodium sulfite and heat-stable amylase to remove protein and starch contamination in NDF, respectively. However, other variants of this method, such as the absence of sodium sulfite and posterior NDIN correction, have been reported in studies with distillers grains (Boila and Ingalls 1994a; Mustafa et al. 2000a). In a comparison made by Dong and Rasco (1987a), the NDF value corrected for NDIN was higher in the absence of sodium sulfite than the NDIN-corrected NDF value in the presence of sodium sulfite. In addition, the use of fine paper filtration or centrifugation to perform the amylase treated NDF procedure adds variability to the NDF value. In DDGS samples, higher NDF values were observed by using the filtration method (Udén 2006).

Lignin content in DDGS is low, which explains the high NDF digestibility (from 62 to 71 %) (Birkelo et al. 2004; Vander Pol et al. 2009). A first factor causing variation in the NDF digestibility among DDGS samples can be the degree of fat removal, as fat inhibits microbial growth and reduces fiber digestibility (Nagaraja et al. 1997). Moreover, the determination of the

degradation characteristics of NDF is affected by the physical form of the feed, as well as by the fermentation method (*in situ* vs. *in vitro*) utilized in analysis.

In feedlot diets, DDGS can replace grains such as barley or corn providing energy in the form of readily digestible fiber. This fiber is digested at a slower rate and less lactic acid is produced relative to starch (U.S. Grains Council 2007b). Although a reduced incidence of rumen acidosis is presumed when DDGS are fed to beef cattle (Ham et al. 1994; Klopfenstein et al. 2001), it has not been demonstrated yet. This can be attributed to the small particle size of DDGS, which contributes to the low physically effective NDF (peNDF) (3.4 – 19.8 %) (Kleinschmit et al. 2007). In lactating dairy cows, this low peNDF is an important consideration, as dietary fiber may not be adequate to prevent milk fat depression (Cyriac et al. 2005). For this reason, the use of DDGS in lactating dairy rations is recommended to replace concentrate ingredients, not forage ingredients (Schingoethe et al. 2009). In a recent study (Penner et al. 2009), it was concluded that dried distillers grains can replace 10% of dietary concentrate without affecting milk yield, milk composition, and chewing activity in dairy cows.

2.5.5. Residual starch

Although most of the starch in the parent grain is converted to ethanol during the fermentation process, some residual starch is present in DDGS. In corn DDGS, the amount of residual starch among studies varies from 3.8 to 11.9 % DM (Table 2.1). The amount of unconverted starch may be dependent on the type of raw starch in the parent grain and the processing conditions (Sharma et al. 2009).

Some raw starch is resistant to enzymes and has characteristics of crude fiber (Xie et al. 2006). Differences in starch resistance among parent grains have been related to differences in starch granule structure (Stevnebø et al. 2009). Amylose levels in grain starch showed a negative correlation with starch degradability (Berry 1986; Stevnebø et al. 2006), while small starch granules had a faster degradation rate than large granules due to the relatively larger surface area (Stevnebø et al. 2006).

Cooking starch results in considerable amounts of resistant starch and therefore starch residue (Berry 1986). Temperature and pH during liquefaction may affect the yield of resistant starch (Sharma et al. 2009). After gelatinization, starch is cooled and retrogradaion occurs, converting linear dextrines, mostly amylase, in an insoluble precipitate (Jameson et al. 2001).

This crystallization process is encouraged by low temperature (50°C), pH between 5 and 7, and high concentration of starch with long chain lenghts (Jameson et al. 2001; Sharma et al. 2009). Other factors influencing the yield of resistant starch during cooking are the formation of amylase-lipid complexes, the proportion of water and starch in the mash, cooking temperature, and the number of cooking/cooling cycles (Jameson et al. 2001). Also, a possible explanation for the presence of residual starch in DDGS products is the ineffectiveness of the processing conditions, since the full conversion of starch to ethanol requires an optimal combination of different factors (temperature, pH, time, enzymes, and yeast). It has been speculated that large protein molecules and the presence of unknown proteasas could inhibit the fermentation process (Bahdra et al. 2007). The improvements carried out by the bio-ethanol industry during the past ten years have reduced the residual starch of DDGS manufactured in new-generation plants relative to those originated in less efficient old-generation plants (Schingoethe et al. 2009). Further studies investigating the residual starch degradability of DDGS samples in both ruminants and monogastrics will help to clarify the nature of this residual starch.

2.5.6. Minerals

DDGS are low in calcium (Ca) but high in phosphorus (P) and sulfur relative to cattle nutrient needs (NRC 2001). These minerals affect not only animal performance but also animal health and the environment.

Table 2.1 shows that Ca levels found in the literature range from <0.1 to 0.5 %DM for corn DDGS and from 0.2 to 0.3 for wheat DDGS. For P, levels range from 0.4 to 1.0 %DM for corn DDGS and from 1.0 to 1.1 %DM for wheat DDGS. These variations between and within DDGS types can be explained by differing grain types and varieties as well as by the solubles blended back. Tabular values indicate higher Ca and P levels for wheat relative to corn (Table 2.1); while Cao et al. (2009) showed that Ca and P levels were higher in CDS than in corn WDG, resulting in increasing levels in DDGS as the inclusion of solubles increased.

The dietary Ca and P requirements for a feedlot steer are approximately 0.6 %DM and 0.3 %DM respectively, while the concentration in a concentrate-based diet containing 60 % DDGS and not supplemented with Ca is approximately 0.8 %DM for Ca and 0.7 %DM for P (NRC 1996; Gibb et al. 2008). Thus, finishing rations containing a high inclusion of DDGS can easily result in low Ca:P ratios if not properly supplemented. This in turn may cause metabolic

disorders (i.e. bone abnormalities) as well as decreased performance (NRC 2001). A diet containing 60% DDGS and supplemented with limestone was fed to feedlot cattle; however, no positive effect on daily gain and feed efficiency was detected (Gibb et al. 2008). The high P intake associated with DDGS feeding is also a concern, as an excess of P is excreted in feces and urine (Luebbe et al. 2008; Spiehs and Varel 2009). A high P concentration in livestock manure requires a greater landbase for manure application and promotes a risk for P runoff (Bremer et al. 2008; Spiehs and Varel 2009).

Sulfur in DDGS results from two primarly sources: the addition of sulfuric acid during fermentation and S-containing amino acids (Nichols and Bothast 2008; Spiehs and Varel 2009). Yeast also contributes to the total sulfur in DDGS, since yeast creates some sulfites during fermentation and sulfur makes up 3.9 g/kg of the yeast composition (Snider 2004). Solubles are more abundant in sulfur than WDG, thus sulfur content in DDGS increases with increasing levels of solubles (Cao et al. 2009; Schingoethe et al. 2009; Stein and Shurson 2009). The maximum tolerable sulfur concentration is 0.30 and 0.40 % of the diet DM for beef and dairy cattle, respectively (NRC 2001). As shown in Table 2.1, sulfur values greater than 1% have been reported for both wheat DDGS and corn DDGS. If a high sulfur content in DDGS is coupled with a high intake of the product, polioencephalomalacia (PEM) may occur (Schingoethe et al. 2009). This disease is characterized by disturbances of the central nervous system (Sheep Industry Development Program 1988). Several studies (T. McAllister, personal communication; Vaness et al. 2009) have observed some strange behaviors and depressed mood in feedlot cattle fed 20 - 60% DDGS of the diet, which may be attributed to high dietary sulfur content. Moreover, an increased sulfur excretion contributes to the production of odorous compounds from manure (Spiehs and Varel 2009).

2.5.7. Particle size and density

Inconsistency in the physical properties between new- and old-generation plants, among new-generation plants, and within the same plant over time, has been shown in several studies of corn DDGS (Shurson 2005; Rosentrater and Muthukumarappan 2006; Ileleji et al. 2007; Kingsly et al. 2009), and represents one of the major market barriers to the use of DDGS as a livestock feed. A major issue is the poor flowability of DDGS as a result of the caking phenomenon. Due to this caking, particles tend to stick to each other forming unwanted agglomerates, which are

cause of troublesome storage and shipping of the product (Bahdra et al. 2009b). These agglomerates, also termed "syrup balls", vary in size and density. Thus, when the DDGS product is incorporated in the animal ration they may result in segregation, leading to uneven nutrient distribution, and consequent inconsistencies in diet formulation (Ileleji et al. 2007). The mean retention time in rumen of particles with higher density and smaller particle size is shorter (desBordes and Welch 1984; Ehle and Stern 1985; Kaske and Engelhardt 1990). In fact, alterations in density created a greater magnitude of response than for particle size (Ehle and Stern 1985). This suggests that the digestibility of DDGS products may be affected by variations in the density and size of the particles; however, information on the effect of DDGS particle size and density on ruminal digestibility has not been published yet.

Several factors affecting the physical properties of DDGS have been described. The amount of CDS added during drying seems to have the greatest effect on the resulting DDGS particle size and bulk density. Increasing the inclusion of solubles increased inter-particle affinity and induced particle agglomeration during drying (Kingsly et al. 2009). Another study (Ileleji and Rosentrater 2008) has shown that the use of two rotary drum dryers results in DDGS with smaller particle size than those resulted from the use of a high-capacity rotary drum steam tube dryer. The reduced presence of syrup balls is due to the addition of the solubles in two steps in each of the two dryers (Ileleji and Rosentrater 2008). Other chemical properties of DDGS, such as the fat, have als been shown to cause stickiness among DDGS particles giving rise to larger agglomerates, contributing to reduced flow conditions and irregular nutrient distribution in the ration (Bahdra et al. 2009a).

2.6. Feed evaluation

2.6.1. The Cornell Net Carbohydrate and Protein System (CNCPS)

The CNCPS (Fox et al. 1992; Russell et al. 1992; O'Connor et al. 1993; Fox et al. 2004) is a mathematical model that was developed to evaluate requirements, feed utilization, animal performance and nutrient excretion for ruminants based on the accumulated knowledge about feed composition, feed digestion, feed passage and physiological status (Fox et al. 2004). The CNCPS uses different sub-models: maintenance, growth, pregnancy, lactation, body reserves,

feed intake and composition, rumen fermentation, intestinal digestion, metabolism, and nutrient excretion. The original feed composition sub-model was described in detail by Sniffen et al. (1992). It uses information on chemical profile to partition feed protein and carbohydrate pools into different fractions, each of them having a different rate of degradation. A schematic of the CNCPS is illustrated in Figure 2.2.

The protein pool is divided into three fractions: non-protein nitrogen (PA), true protein (PB), and unavailable protein (PC). Fraction PA is buffer soluble protein consisting of ammonia, peptides and amino acids. Fraction PB is further divided into three sub-fractions based on their inherent rates of degradation. PB1 is represented by buffer soluble protein which is rapidly degraded in the rumen (Kd = 120 - 400 $\%h^{-1}$). PB2 is made up of protein not bound to NDF that is insoluble in buffer but soluble in neutral detergent. While some PB2 is intermediately fermented in the rumen (Kd = 3 - 16 $\%h^{-1}$), some escapes to the small intestine. PB3 consists of protein insoluble in neutral detergent but soluble in acid detergent. PB3 is associated with the cell wall, thus it is slowly degraded in the rumen (Kd = 0.06 - 0.55 $\%h^{-1}$) and most of it escapes to the small intestine. Fraction PC represents protein insoluble in the acid detergent and ends up being unavailable to the ruminant. PC is made up of protein associated with lignin, tannin-protein complexes, and Maillard products.

The carbohydrate pool is categorized into four fractions: CA, CB1, CB2, and CC. Fraction CA contains sugars and organic acids that are water soluble and rapidly fermented (Kd = 200 - 350 $\%h^{-1}$). Fraction CB1 consists of starch and pectins that are intermediately degraded in the rumen, as its degradation rate is lower than CA (Kd = 20 - 50 $\%h^{-1}$). Fraction CB2 represents fiber carbohydrates (FC) that are available and slowly degraded in the rumen (Kd = 2 - 10 $\%h^{-1}$). Fraction CC is undegradable FC associated with lignin and resistant starch. In order to account for the variability in the non-fiber carbohydrate (NFC) digestibility when various processing treatments are applied, and in order to accurately predict volatile fatty acid production and pH, a new division of the NFC fractions has been published by Lanzas et al. (2007); however, the original feed composition sub-model is widely utilized.

2.6.2. NRC 2001 Model for feed energy estimation

An accurate knowledge of the energy content of each feed included in the ration is crucial to accurate formulation. The traditional approach to estimate the energy value of feeds is to

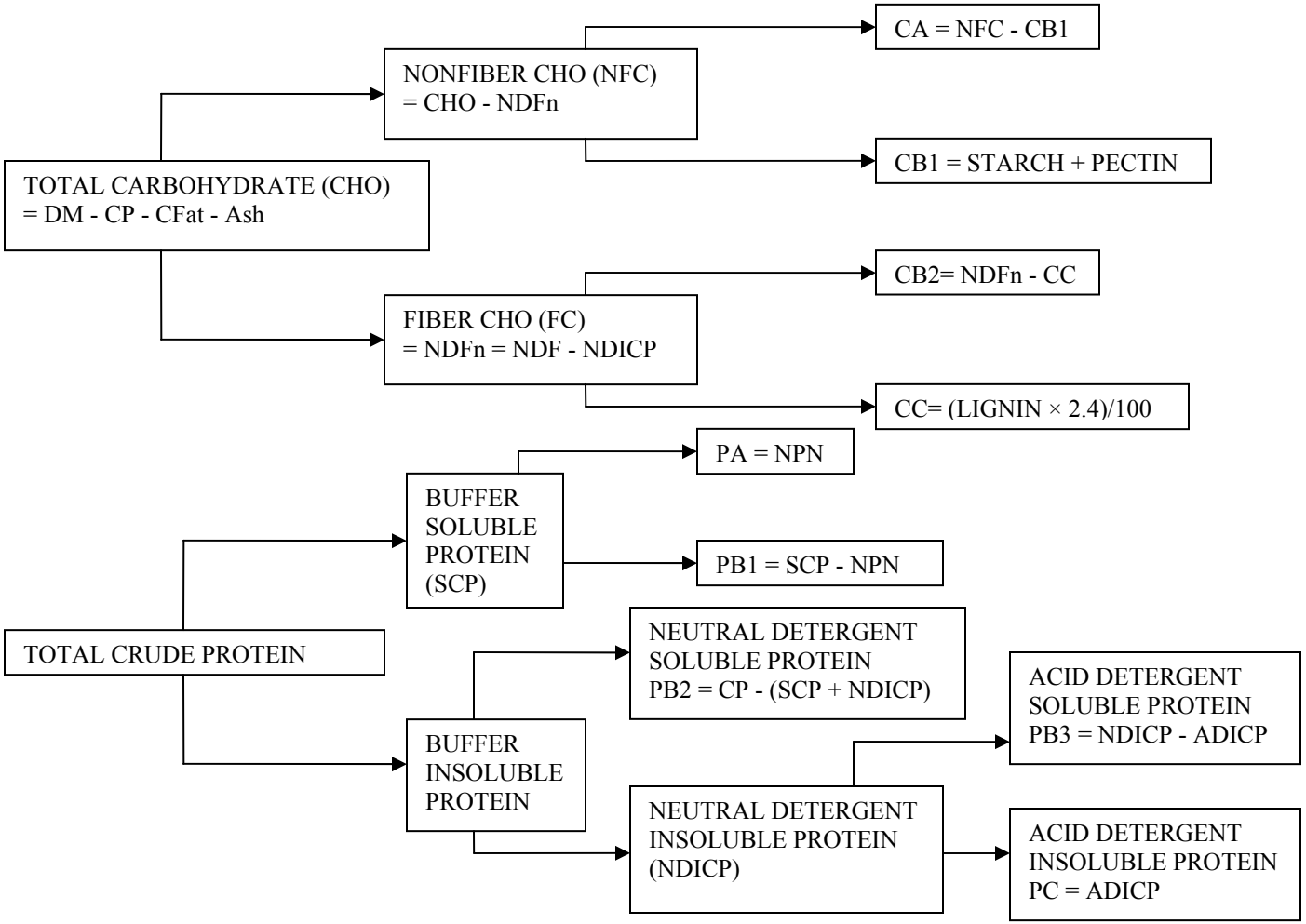

Figure 2.2. The Cornell Net Carbohydrate and Protein System (CNCPS). Adapted from Fox et al. (2004)

calculate its total digestible nutrient level (TDN) using a summative approach based on the chemical components. In this approach, the concentration of truly digestible NFC (tdNFC), CP (tdCP), fatty acids (tdFA) and NDF (tdNDF) of each feed is calculated as follows (Weiss et al. 1992):

$$\text{tdNFC (\%DM)} = 0.98 \times (100 - [(\text{NDF} - \text{NDICP}) + \text{CP} + \text{EE} + \text{Ash}]) \times \text{PAF};$$

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

$$\text{tdCP (\%DM) for concentrates} = [\,1 - (0.4 \times (\text{ADICP/CP}))] \times \text{CP}$$

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

$$\text{tdFA (\%DM)} = \text{FA};$$

where, FA represents the fatty acid fraction that is estimated as CFat -1.

$$\text{tdNDF (\%DM)} = 0.75 \times (\text{NDFn} - \text{ADL}) \times [1 - (\text{ADL/NDFn})^{0.667}];$$

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

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

$$TDN_{1X} = \text{tdNFC} + \text{tdCP} + (\text{tdFA} \times 2.25) + \text{tdNDF} - 7;$$

where, 7 represents the metabolic fecal TDN, allowing the formula to account for the apparent digestibility.

The above TDN_{1X} formula is only valid for feeds of plant origin. Due to the uncharacteristic chemical composition of animal protein meals and fat supplements, NRC 2001 provides modified versions of the TDN_{1X} formula for these feeds.

In NRC 1989, the TDN_{1X} was used to estimate the digestible energy (DE) and derived energy values; however, as different nutrients have different heat combustion values, NRC 2001 computes apparent DE as:

$$DE_{1X}\ (\text{Mcal/kg}) = (\text{tdNFC}/100 \times 4.2) + (\text{tdNDF}/100 \times 4.2) + (\text{tdCP}/100 \times 5.6) + (\text{FA}/100 \times 9.4) - 0.3;$$

where, 0.3 results from multiplying metabolic fecal TDN value of 7 by its assumed heat combustion value.

Similar to TDN_{1X} value, different DE equations for animal protein meals and fat supplements are suggested by NRC 2001.

However, the energy content of feeds is not a constant value. As dry matter intake increases, the concentration of digestible energy tends to decrease. Thus, NRC 2001 proposes

a variable discount that uses the TDN_{1X} value and intake level of a diet to account for this change.

$$\text{Discount} = [TDN_{1X} - (0.18 \times TDN_{1X} - 10.3)] \times \text{intake} / TDN_{1X};$$

where, intake is expressed as incremental intake above maintenance.

Based on the DE_{1X} value and the discount variable, the different energy values of a single feed ingredient at different production levels of intake are calculated as follows:

$$DE_p \text{ (Mcal/kg)} = DE \times \text{Discount}$$

$$ME_p \text{ (Mcal/kg)} = (1.01 \times DE_p) - 0.45) + 0.0046 \times (\text{Cfat} - 3), \text{ if Cfat} > 3\%$$

$$ME_p \text{ (Mcal/kg)} = (1.01 \times DE_p) - 0.45, \text{ if Cfat} < 3\%$$

$$NE_{Lp} \text{ (Mcal/kg)} = 0.703 \times ME_p - 0.19 + \{[(0.097 \times ME_p + 0.19)/97] \times [\text{Cfat} - 3]\}$$

$$NE_m \text{ (Mcal/kg)} = (1.37 \times ME) - (0.138 \times ME^2) + (0.0105 \times ME^3) - 1.12;$$

where, $ME = DE_{1X} \times 0.82$.

$$NE_g \text{ (Mcal/kg)} = (1.42 \times ME) - (0.174 \times ME^2) + (0.0122 \times ME^3) - 1.65$$

For fat supplements, NRC 2001 proposes different NE_m and NE_g equations due to different DE-ME conversion efficiencies.

Despite the widely use of the NRC 2001 method to estimate the energy density of a diet, doubts have been raised about the accuracy of the chemical approach as well as the discounting method. One of the problems of the chemical approach is that the digestibility of NDF varies among and within feeds. Moreover, this approach is inaccurate due to the analytical procedure error and poor relationship between lignin levels and feed digestibility (Robinson et al. 2004). Thus, NRC 2001 also proposes a biological approach, in which 48 h *in vitro* incubation is performed to determine NDF digestibility and the subsequent tdNDF. The digestibility of NDF and rest of the feed components can also be estimated by 48h *in situ* incubation. A comparison of the three approaches in forages was done by Yu et al. (2004), concluding that the resulting energy values were the highest for the *in situ* approach, lowest for the chemical approach, and intermediate for the *in vitro* approach. Similarly, Robinson et al (2004) found that ME of distillers grains was higher using the *in vitro* approach than the chemical approach.

Based on the NRC discounts, a high producing dairy cow with an energy output equivalent to nine times maintenance needs to consume 78 kg DM per day, suggesting that a decrease in the energy concentration of the diet as the energy output of the cow increases is not a correct approach (Robinson 2007). Using previous studies with lactating dairy cows, Robinson (2007) observed that the NE_L concentration of a diet decreases as DM intake increases but it increases by

increasing NE_L output. This could be explained by the genetic merit of the cow, as cows with higher NE_L outputs absorb more nutrients from the digestive tract. Therefore, both the anticipated level of DM intake and energy output are required inputs to enhance accuracy in the estimation of the NE_L concentration of a diet (Robinson 2007).

2.6.3. *In situ* technique to estimate rumen degradability and kinetics of feed components

In order to provide a ruminant with all nutrients required to achieve optimal performance, the ruminal availability of the feed components must be known. The *in situ* incubation technique is used to investigate the ruminal degradation characteristics of the different feed components, thus it is a basic procedure in many feed evaluation systems. This technique is simple and allows quick estimations for a large number of feed samples. The methodological factors affecting the repeatability of in situ disappearance, as well as the choice of mathematical models to fit the curves and goodness of fit of the model have been reviewed (Nocek 1988; Huntington and Givens 1995; Nasri et al. 2006).

The most widely used model is the nonlinear model firstly reported by Orskov and McDonald (1979) and later modified by Robinson et al. (1986) and Dhanoa (1988):

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

where, R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); T_0 = lag time (h); and Kd = degradation rate (% h^{-1}).

Based on the nonlinear parameters estimated in the above equation (U, D, Kd), the effective degradability (ED), or extent of degradation, of each nutrient is predicted according to NRC 2001 as:

$$ED\ (\%) = S + (D \times Kd) / (Kp + Kd);$$

where, S = soluble fraction (%); Kp = estimated rate of outflow of digesta from rumen (% h^{-1}) and it is assumed to be 4.5 % h^{-1} for forages and 6 % h^{-1} for concentrates (Tamminga et al. 1994; Yu et al. 2003a; Yu et al. 2003b).

In contrast to the first-order kinetic model, which estimates the disappearance of each nutrient for a given time period, ED equation considers the fractional outflow rate of digesta from the rumen, thus predicting the amount of nutrient that will be truly digested in the rumen

over time. The rumen undegradable fraction of each feed component can be calculated as 100 - ED (%).

2.6.4. Chemical profile: Acid detergent insoluble crude protein (ADICP) and lignin

Ruminant nutritionists have been interested in using ADICP and lignin as indicators of digestibility for many decades. Although ADICP occurs naturally in the plant, it also results from heat damage via Maillard reaction during storage or processing. Some studies have established a negative correlation between ADICP and protein digestibility in forages (Goering et al. 1972; Yu and Thomas 1976; Van Soest and Mason 1991a; Waters et al. 1992) and non-forages (Arieli et al. 1989; Waters et al. 1992; McKinnon et al. 1995); however, it is not clear whether ADICP behavior in DDGS is similar to conventional feeds. Nakamura et al. (1994a) found a negative correlation (r = - 0.49) between ADICP and protein digestibility, while Klopfenstein (1996) did not find any relationship. Harty et al. (1998) tested a high number of samples and observed that the best correlations were observed when ADICP levels were higher than 13 % of CP.

In the cell wall, lignin cements and anchors the cellulosic microfibrils, thereby stiffening it and protecting it from degradation (Hindrichsen et al. 2006). Several reports have shown negative correlations between lignin and both DM and NDF digestibilities in forages (Johnson et al. 1962; Tomlin et al. 1964; Smith et al. 1972; Jung et al. 1997). Less attention, however, has been paid to the reliability of lignin to predict fiber digestibility of concentrates and by-product feeds. The first limitation encountered when quantifiying lignin is the method of analysis, as lignin is higly insoluble and difficult to determine directly with any specific procedure (Hindrichsen et al. 2006). In both concentrates and forages, Klason Lignin (KL) method yielded higher values than the Acid Detergent Lignin (ADL) method (ADL) (Hindrichsen et al. 2006). In concentrate diets, a weak relationship between both KL and ADL and fiber digestibility was reported, suggesting that lignin can be considered a good indicator of fiber digestibility only in diets in which most part of the lignin content is supplied by the forage (Hindrichsen et al. 2006). In distillers grains, Robinson et al. (2004) showed a poor relationship between ADL content and *in vitro* NDF digestibility at 48 h; however, more studies are required in order to confirm this lack of correlation.

2.6.5. Prediction of truly digestible protein supply in small intestine

Nutrition models are able to predict the protein value of a single feed or diet before it is fed to the animal, therefore allowing for optimization of feed utilization, production, farm income as well as avoiding adverse effects to the animal and environment.

Several nutrition models (INRA 1978; ARC 1984; Madsen 1985; NKJ-NJF 1985; NRC 1985; Tamminga et al. 1994) and the corresponding updates (Verité and Peyraud 1989; Madsen et al. 1995; NRC 2001; Tamminga et al. 2007) have been developed during the last forty years with the purpose of predicting the availability of the consumed protein as truly digested and absorbed protein in the small intestine. These models distinguish between the protein incorporated to ruminant microorganisms and protein flowing to the small intestine as undegraded protein. Therefore, the determination of the chemical profiles, the dynamic aspects of the protein degradation in rumen, as well as the intestinal digestibility of rumen bypass protein, are critical points in the prediction of protein supply to small intestine by the most modern models.

Two modern and common protein evaluation systems are the DVE/OEB System (Tamminga et al. 1994), currently being used in Europe, and the NRC 2001 Dairy Model (NRC 2001) used in North America. The frameworks of these two sophisticated models were developed based on the principles of previous models, thus incorporating important elements such as the potential for microbial protein synthesis (Tamminga et al. 1994; Yu et al. 2000; Yu et al. 2003b; Yu 2005).

A detailed description of the DVE/OEB System and the NRC 2001 Model can be found in Tamminga et al. (1994) and NRC 2001, respectively. Two major outputs can be predicted from the two models: 1) the truly digested and absorbed protein in the small intestine and 2) the degraded protein balance. The first output includes the truly absorbable rumen synthesized microbial protein in the small intestine, the truly absorbed bypass feed protein in the small intestine, and endogenous protein losses. The second output reflects the balance between available N and energy in the rumen that is crucial to achieve efficient synthesis of microbial protein (Tamminga et al. 1994). In spite of the similar principles between the two models, some of the concepts and factors used in quantifying calculations differ (Yu et al. 2003a; Yu et al. 2003b). The most important differences are in the determination of endogenous protein losses, microbial protein synthesis and absorbable rumen bypass protein (Yu et al. 2003a; Yu et al. 2003b). Significant differences in the predicted values from the two models were found when evaluating

concentrates and forages; however, correlations of the predicted values were high (Yu et al. 2003a; Yu et al. 2003b). In addition, the DVE/OEB System and the NRC 2001 Model also predict protein requirements, which also differ between the two models (Yu et al. 2003a).

A more complete version of the DVE/OEB System has been recently published and is available for a fee (Tamminga et al. 2007). Compared to the previous version, the new system requires a much more detailed description of the protein and carbohydrate fractions of each feed, and it also provides a deeper insight into the breakdown dynamics of the nutrients in rumen. Therefore, a more precise prediction of the availability of rumen degradable protein, rumen bypass protein, volatile fatty acids, and rumen bypass carbohydrates, can be achieved. This enables an enhanced balance of nitrogen and energy to rumen microorganisms, and ultimately increases accuracy in ration formulation.

2.7. Summary

In western Canada, wheat DDGS, blend (wheat/corn) DDGS and imported corn DDGS are utilized in ruminant diets. Although substantial body of research has been reported for corn DDGS, information on the nutritional characteristics of wheat DDGS and blend DDGS for ruminants is scarce. DDGS is a good source of rumen bypass protein for ruminants. In current ration formulation, it is common to assume fixed values for the amount and digestibility of RUP of DDGS; however, the rumen availability of protein and other feed components may vary among DDGS types as the chemical profile has been shown to. Thus, a database on chemical profile, protein and carbohydrate fractions, energy values, and nutrient availability in the rumen and the small intestine for each type of DDGS needs to be created in order to increase accuracy in feeding formulation as well as to reduce feeding cost and environmental impact.

Literature shows a high inconsistency in the nutritional value among corn DDGS samples collected at different plants. The most important factors affecting this variability are differences in the nutrient content of the parental grain, differences in the ratio of solubles and distillers grains blended, and differences in drying conditions. Plant to plant inconsistencies have not been reported for wheat DDGS yet; however, this information is required to improve processing conditions and product quality consistency of wheat DDGS.

The use of the chemical profile to determine energy values and to predict the potential degradability has been used successfully with many forages and concentrates. In DDGS, scarce information is available on the validity of these methodologies.

Therefore, the purpose of this project was 1) to investigate the effects of DDGS type and bio-ethanol plant on the nutritive value (nutrient profile, protein and carbohydrate fractions and energy values), 2) to evaluate the validity of the NRC 2001 chemical approach in determining energy values of DDGS, 3) to investigate the effects of DDGS type and bio-ethanol plant on the in situ rumen degradation characteristics and intestinal digestibility of bypass protein, 4) to test the relationship between chemical profile and potential rumen degradability of DDGS, and 5) to investigate the effects of DDGS type and bio-ethanol plant on the prediction of the potential protein supply to small intestine. In addition, the comparison of DDGS type with its respective feedstock grain on each of the above parameters will be studied. To produce top quality DDGS suitable for export and marketing and for the livestock industry in western Canada, there is an urgent need for this information.

3. NUTRIENT VARIATION AND AVAILABILITY (PROTEIN, FIBER AND ENERGY) OF CO-PRODUCTS OF BIO-ETHANOL PRODUCTION: COMPARISON AMONG WHEAT DDGS, CORN DDGS AND BLEND DDGS, AND BETWEEN DIFFERENT BIO-ETHANOL PLANTS

3.1. Introduction

Dried distillers grains with solubles (DDGS) are a byproduct of fermentation during bio-ethanol production. As a result of the government policies to stimulate the expansion and consumption of bio-fuels, the number of bio-ethanol plants has increased in Western Canada. Although the bio-ethanol industry in this part of the country is wheat based, the fluctuation in the price of wheat has forced bio-ethanol companies to include corn in the feedstock for bio-ethanol processing, consequently not only pure wheat DDGS but also blend (wheat/corn) DDGS is available.

While the nutritional value of corn DDGS for ruminants has been extensively documented (Cromwell et al. 1993; Spiehs et al. 2002; Shurson 2005; Kleinschmit et al. 2006; Rosentrater and Muthukumarappan 2006; Kleinschmit et al. 2007; Martinez-Amezcua et al. 2007; Stein and Shurson 2009), detailed information on wheat DDGS and wheat/corn blend DDGS is scarce and not recent (Dong and Rasco 1987a; Boila and Ingalls 1994a, 1994b; Nyachoty et al. 2005; Widyaratne and Zijlstra 2006; Penner et al. 2009). Thus, a database on chemical profile, protein and carbohydrate fractions, and energy values needs to be created in order to proceed with feeding recommendations for ruminants. Likewise, it is important to detect plant to plant variation and how this inconsistency in the product affects nutrient supply and consequently animal performance. To produce DDGS suitable for export and marketing, and for livestock industry within Canada, there is an urgent need for this information. So far, little research has been conducted to determine the magnitude of the differences in the nutritive value among wheat DDGS, corn DDGS and blend DDGS (particular blend DDGS), and between wheat DDGS originated at different bio-ethanol plants in Canada.

In addition to product inconsistency, the methodology used in the nutritive characterization is crucial in order to avoid misformulation of the ration and diminished animal productivity. In the estimation of energy values, NRC (2001) describes a chemical approach in which the equations for truly digestible nutrient are based on the chemical composition of natural

feeds (Weiss et al. 1992). Yet, the energy values of a feedstuff are not chemical constituents. Thus, it is a question whether this chemical approach described by NRC 2001 can accurately estimate energy values of DDGS. A biological approach, such as *in vitro* and *in situ* incubation, is considered a superior predictor of truly digestible nutrients (Robinson et al. 2004; Yu et al. 2004). The method preferred for most researchers, and also suggested by NRC 2001, is the 48 h *in vitro* incubation; however, inflated results for feeds with a high content of soluble sugars have been reported (Seker 2002). For this reason, and because of the greater similarity to real animal conditions, an *in situ* assay (48 h incubation) provides the best estimation of the total tract digestion, and consequently truly digestible nutrients and energy values (Tamminga et al. 1994; Yu et al. 2003a; Yu et al. 2004). To date, there is no information on the relationship of the NRC 2001-chemical approach and the *in situ* assay-biological approach that clarifies the accuracy of NRC 2001-chemical approach on the prediction of energy values for DDGS.

The chemical profile has been used as a method to estimate the potential digestibility of feedstuffs. While a strong negative relationship between ADICP content and protein degradability is well established in forages (Goering et al. 1972; Yu and Thomas 1976; Van Soest and Mason 1991a), moderate (Nakamura et al. 1994a) or no correlation (Klopfenstein 1996) have been found in distillers grains. Likewise, NRC (2001) uses acid detergent lignin (ADL) to predict potential neutral detergent fiber (NDF) digestibility. In order to acquire the best DDGS for livestock, more information on the use of chemical profile as predictor of potential digestibility of DDGS is required.

The objectives of this study were 1) to determine the effect of DDGS type and bio-ethanol plant on chemical profile, mineral concentrations, protein and carbohydrate fractions, and energy values; 2) to elucidate the validity of the NRC 2001 chemical approach in the determination of energy values for DDGS; and 3) to determine the effect of DDGS type and bio-ethanol plant on in situ rumen degradability of DDGS components and test the relationship between chemical profile and rumen degradability. In addition, these parameters were studied in feedstock grains as opposed to their derived DDGS product. The final aim was to provide detailed information on the nutritive value of wheat DDGS, corn DDGS and blend DDGS in order to assist nutritionists in designing low cost, efficient feed programs in Canada.

3.2. Materials and methods

3.2.1. Wheat DDGS, corn DDGS and blend DDGS and original cereal grains (corn and wheat)

During May to December in 2007, three to five batches from each of wheat DDGS, blend DDGS (wheat:corn=70:30), and wheat samples were collected from two bio-ethanol plants (SK-Plant 1 and SK-Plant 2) located in western Canada. Both plants used local wheat feedstock for bio-ethanol production. During the same time frame, corn DDGS and corn samples produced by a bio-ethanol plant in North Dakota were obtained through Federated Co-Op Ltd, Saskatoon. Due to cold climate conditions, western Canada does not produce large amounts of corn and corn DDGS; however, corn DDGS and corn samples were used as reference samples for comparison with wheat and wheat DDGS.

3.2.2. Rumen *in situ* assay

A rumen *in situ* assay was performed in order to measure rumen degradability and estimate truly digestible nutrients (tdNDF, tdCP, tdFA, tdNFC). Prior to ruminal incubation, samples were processed using a Sven Roller Mill (Apollo Machine and Products Ltd., Saskatoon, SK). The roller gap was adjusted to a size of 0.203 mm in order to equalize the particle size of all samples. Three Holstein dry cows fitted with a rumen cannula (Bar Diamond Inc, Parma, ID, USA) with an internal diameter of 10 cm were used in this study. The cows were individually fed twice daily at 0800 and 1600 according to the nutrient requirement defined by NRC (2001) at a maintenance level (See Appendix Table 8.1). The animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993). Ruminal degradability of dry matter (DDM), organic matter (DOM), crude protein (DCP) and neutral detergent fiber (DNDF) at 24 and 48 h incubation, as well as ruminal degradability of total fatty acids (DFA), non-fiber carbohydrates (DNFC) and neutral detergent insoluble crude protein (NDICP) at 48 h incubation, were determined by *in situ* method (Yu et al. 2000) using nylon bags with a pore size of 40 microns. The 24 and 48 h incubation times were based on the NRC-2001 suggestion in energy estimation (48 h) and the previous published report (Yu et al. 2000). Each treatment was randomly assigned to the cows for two runs. After incubation, the bags were removed from the rumen and rinsed under a cold stream of tap water to remove excess ruminal contents. The bags

were washed with cool water without detergent and subsequently dried at 55°C for 48 h. Dry samples were stored in a refrigerated room (4°C) until analysis. The residues were ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments (Canada) LTD, Ontario) for chemical analysis.

3.2.3. Chemical analysis

All samples for chemical analysis were ground through a 1 mm screen. Dry matter (AOAC 930.15), ash (AOAC 942.05), crude fat (CFat) (AOAC 954.02) and crude protein (CP, AOAC 984.13) contents were analyzed according to the procedure of the AOAC (1990). For the starch analysis, samples were ground to 0.5 mm and analyzed using the Megazyme Total Starch Assay Kit 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 filtration method (Van Soest et al. 1991b). Alpha-amylase without sodium sulfite was used prior to neutral detergent extraction. The N adjusted NDF (NDFn) was calculated as NDF-NDICP. The acid (ADICP) and neutral detergent insoluble crude protein (NDICP) values were determined (Licitra et al. 1996). The NPN content was analyzed by precipitating of true protein with tungstic acid (samples were soaked into 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 bicarbonate-phosphate buffer and filtering through Whatman #54 filter paper (pore size = 20 - 25 μm) (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-fibre carbohydrates and calculated (Grings et al. 1992). The carbohydrate (CHO) and true protein, hemicellulose, and cellulose were calculated (Van Soest et al. 1991b). Calcium (Ca) (AOAC 927.02) concentrations were determined by atomic absorption spectroscopy (Model Perkin Elmer 2380, Norwalk, CT) and phosphorus (P) (AOAC965.17) concentrations by spectrophotometry (Model Pharmacia LKB Biochrom Ltd, Ultroscope III, Cambridge, UK) according to the procedures of the AOAC (1990). Sulphur (AOAC 935.13) analysis was carried out by inductively couple plasma-optical emission spectrometer (Model Perkin Elmer Optima 4300 DV ICP-OES, Waltham, MA) at ALS Laboratory Group, Saskatoon, SK, Canada

according to the procedures of the AOAC (1990). All samples were analyzed in duplicate and repeated if error was in excess of 5%.

3.2.4. Partitioning protein and carbohydrate fractions

The crude protein and carbohydrate (CHO) subfractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al. 1992; Chalupa and Sniffen 1994). The characterization of the CP fractions as applied in this system is as follows: fraction PA is non-protein N (NPN), fraction PB is true protein (TP), and fraction PC is unavailable protein. Fraction PA is rapidly degradable with an assumed degradation rate to be infinity. Fraction PB is further divided into three fractions (PB1, PB2, and PB3) that are believed to have different rates of degradation in the rumen (Sniffen et al. 1992). Fraction PB1 is represented by buffer soluble protein whis is rapidly degraded in the rumen (120-400 % h^{-1}). Buffer-insoluble protein minus fraction PB3 is used to estimate fraction PB2. Fraction PB2 is insoluble in buffer but soluble in neutral detergent, while fraction PB3 is insoluble in both buffer and neutral detergent but soluble in acid detergent solution. Fraction PB2 is fermented in the rumen at a lower rate (3-16 % h^{-1}) than the buffer-soluble fraction, and some PB2 fraction escapes to the lower gut. Fraction PB3 is believed to be more slowly degraded in the rumen (0.06-0.55% h^{-1}) than fractions PB1 and PB2 because of its association with the plant cell walls, and a large proportion of PB3 is believed to escape the rumen. Fraction PC is ADICP, which is highly resistant to breakdown by microbial and mammalian enzymes, and it is assumed to be unavailable to the animal.

Carbohydrate was partitioned into: rapidly degradable fraction (CA) which is composed of sugars and organic acids that have a rapid degradation rate of 300% h^{-1}, intermediately degradable fraction (CB1) which is starch and pectin with an intermediate degradation rate of 20-50% h^{-1}, slowly degradable fraction (CB2) which is available cell wall with a slow degradation rate of 2-10% h^{-1}, and an unfermentable fraction (CC) which is the unavailable cell wall.

3.2.5. Energy values

Estimated energy contents for truly digestible crude protein (tdCP), fatty acid (tdFA), neutral detergent fiber (tdNDF) and non-fiber carbohydrates (tdNFC) were calculated separately using the two different approaches as follows:

1) Using NRC 2001-chemical approach (values expressed in %DM):

a. tdNFC (%DM) = 0.98 × (100 - [(NDF - NDICP) + CP + EE + Ash]) × PAF;

where, PAF is the processing adjustment factor and was assumed to be 1.00 according to NRC 2001.

b. tdCP (%DM) = [1 - (0.4 × (ADICP/CP))] × CP

c. tdFA (%DM) = FA, where FA = Cfat -1.

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

where, NDFn = NDF – NDICP.

2) Using *in situ* assay – biological approach:

a. tdNFC (%DM) = NFC (%DM) × DNFC48 (%),

where, NFC = 100 - ([NDF - NDICP] + CP + EE + Ash) and DNFC48 stands for *in situ* digestibility of NFC after 48 h incubation.

b. tdCP (%DM) = CP (%DM) × DCP48 (%),

where, DCP48 stands for *in situ* digestibility of CP after 48 h incubation.

c. tdFA (%DM) = FA (%DM) × DFA-48 (%),

where, FA = Cfat - 1 and DFA48 stands for *in situ* digestibility of FA after 48 h incubation.

d. tdNDF (%DM) = NDFn (%DM) × DNDFn48 (%),

where, DNDFn48 stands for *in situ* digestibility of NDF after 48 h incubation and corrected for NDICP.

Based on the values of truly digestible nutrients, the energy contents of total digestible nutrient at maintenance (TDN_{1X}), digestible energy at production level of intake (DE_{3x}), metabolizable energy at production level of intake (ME_{3x}) and net energy for lactation at production level of intake (NE_{L3x}) were determined using a summative approach (Weiss et al. 1992) from the NRC 2001 dairy (NRC 2001), while net energy for maintenance (NE_m), and net energy for growth (NE_g) were determined using the NRC 1996 beef (NRC 1996). Both NRC dairy and NRC beef used the same formula to estimate NEg and NEm.

3.2.6. Statistical analysis

Study on the effect of type of DDGS and feedstock grain on chemical profile, protein and carbohydrate fractions and estimated energy values (chemical and biological approaches).

Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). 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 sources, as a fixed effect; batch as replication for the chemical approach; batch and run as replications for the biological approach; and e_{ij} was the random error associated with the observation ij.

Study on the effect of bio-ethanol plant on chemical profile, protein and carbohydrate fractions and estimated energy values of wheat DDGS. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). The model used for the analysis was: $Y_{ij} = \mu + P_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ij ; μ was the population mean for the variable; P_i was the effect of bio-ethanol plant, as a fixed effect; batch as replications; and e_{ij} was the random error associated with the observation ij.

Study on the comparison of NRC 2001-chemical approach with biological approach (*in situ* assay) in the determination of digestible nutrients and energy values of DDGS. Paired t test procedure of SAS (SAS 2005) and correlation analysis were performed in order to establish the relationship between the NRC 2001-chemical approach and the *in situ* assay-biological approach.

Study on the effect of DDGS type on *in situ* rumen degradability. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). The model used for the analysis was: $Y_{ij} = \mu + F_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ijk; μ was the population mean for the variable; F_i was the effect of feed resources, as a fixed effect; batch and run as replications; and e_{ij} was the random error associated with the observation ijk. The relationship between chemical profile and potential degradability was evaluated by correlation analysis.

Studies on effect of bio-ethanol plant on *in situ* degradability of wheat DDGS. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). The model used for the analysis was: $Y_{ijk} = \mu + P_i + e_{ijk}$, where, Y_{ijk} was an observation of the dependent variable ijk; μ was the population mean for the variable; P_i was the effect of plant, as a fixed effect; batch and run as replicates; 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 $P\leq0.10$. Treatment means were compared using the Fisher's Protected LSD method.

3.3. Results and discussion

3.3.1. Effects of DDGS type (wheat DDGS, corn DDGS vs. blend DDGS) and bio-ethanol plant on sulphur, calcium (Ca), and phosphorus (P)

Dried distillers grains with solubles are low in Ca but high in P and sulfur relative to cattle nutrient needs (NRC 2001). This may have repercussions on animal performance, animal health, and the environment, especially if high levels of DDGS are included in the ration. Sulfur in the ruminant diet is primarily to provide adequate substrate to ensure maximal microbial protein synthesis. In general, the recommended sulfur content in the diet should be directly related to the protein concentration. However, DDGS contains much higher sulfur due to the addition of sulfuric acid during the ethanol production process. The maximum tolerable level of sulfur in dairy cattle diets is less than 0.4% of dietary DM (NRC 2001). In studies with beef cattle, some strange behaviours and "depressed mood" in cattle fed 60% DDGS were observed (T. McAllister, personal communication). One possibility is that excess sulfur in the diet may be contributing to neurologic changes such as polioencephalomalacia (PEM) ("a disease characterized by a disturbance of the central nervous system. The PEM sometimes occurs on high grain diets, and diets that include plants/grain high on sulfur") (Sheep Industry Development Program 1988). Therefore, the DDGS inclusion level in order to keep sulphur level at a safe concentration should be investigated

The results (Table 3.1) showed that mineral profiles were significantly different between wheat and corn with wheat higher in Ca, P and sulfur. The mineral profiles were also different among the three types of DDGS with wheat DDGS lower ($P<0.05$) in sulfur (0.39 vs. 0.72 %DM), higher ($P<0.05$) in Ca (0.18 vs. 0.05 %DM) and P (0.91 vs. 0.77 %DM) than corn DDGS, but similar to blend DDGS. There were no significant differences between the two bio-ethanol plants (SK- Plant 1 vs. Plant 2) (Table 3.1).

3.3.2. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on chemical characteristics and profiles

Effects of DDGS type (wheat DDGS, corn DDGS vs. blend DDGS) and bio-ethanol plant on chemical characteristics and profiles are presented in Table 3.1. Chemical profiles were significantly different among wheat DDGS, corn DDGS and blend DDGS (wheat:corn =70:30).

Table 3.1. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat:corn =70:30) and comparison of different bio-ethanol plants in terms of chemical profile

	Feeds sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
Basic chemical profile									
DM (%)	89.52 bc	88.77 c	93.76 a	91.44 b	91.61 b	0.697	92.43	94.65	1.050
Ash (%DM)	2.12 c	1.73 c	5.12 a	4.32 b	5.09 a	0.203	4.98	5.21	0.391
OM (%DM)	97.88 a	98.26 a	94.88 c	95.67 b	94.91 c	0.203	95.02	94.79	0.391
Cfat (%DM)	1.91 d	4.59 c	4.98 c	16.53 a	8.53 b	0.432	6.18 a	4.18 b	5.277
Structural carbohydrate profile									
NDF (%DM)	17.22 b	14.47 b	48.07 a	49.46 a	51.50 a	1.701	52.76 a	44.94 b	1.505
ADF (%DM)	3.68 c	3.66 c	10.99 b	14.68 a	10.80 b	0.567	10.82	11.11	0.861
ADL (%DM)	0.99 c	0.54 c	4.32 a	2.80 b	3.66 ab	0.445	3.62	4.78	0.871
Hemicellulose (%DM)	13.55 c	10.82 c	37.04 ab	34.78 b	40.70 a	1.673	41.95 a	33.83 b	0.821
Cellulose (%DM)	2.68 c	3.11 c	6.68 b	11.88 a	7.14 b	0.531	7.20	6.33	0.291
Non-structural carbohydrate profile									
Starch (%DM)	60.35 a	63.41 a	6.32 b	4.38 b	3.99 b	1.417	6.16	6.44	1.520
Crude protein profile									
CP (%DM)	14.28 d	10.13 e	39.32 a	32.01 c	36.82 b	0.832	39.99	38.87	1.664
SCP (%CP)	24.56 a	14.71 b	16.29 b	11.44 c	14.86 b	0.920	16.90	15.88	1.243
NPN (%SCP)	89.62 b	96.70 ab	100 a	100 a	100 a	2.522	100	100	0.000
NDICP (%CP)	13.51 c	4.75 d	56.04 a	34.37 b	54.40 a	1.422	59.33 a	53.84 b	1.028
ADICP (%CP)	0.00 c	0.08 c	4.85 ab	6.44 a	1.17 bc	1.412	0.69 b	7.63 a	0.125
Minerals									
Sulfur (%DM)	0.16 c	0.12 d	0.39 b	0.72 a	0.37 b	0.010	0.37	0.40	0.011
Calcium (%DM)	0.07 b	0.02 c	0.18 a	0.05 bc	0.15 a	0.013	0.19	0.18	0.007
Phosphorus (%DM)	0.37 c	0.29 c	0.91 a	0.77 b	0.92 a	0.037	0.90	0.91	0.072

SEM = standard error of mean. Means with different letters in the same row are significantly different (P<0.05)

Wheat DDGS was lower (P<0.05) in Cfat than corn DDGS (5.0 vs. 16.5 %DM) and blend DDGS (5.0 vs. 8.5 %DM), lower (P<0.05) in ADF (11.0 vs. 14.7 %DM) but higher (P<0.05) ADL (4.3 vs. 2.8 %DM) than corn DDGS but not significantly different from blend DDGS (P>0.05). There was still some residual starch content in all DDGS, which indicated that the completeness of the fermentation was not achieved in any of the DDGS samples. However, residual starch contents in DDGS were not significantly different among wheat DDGS, corn DDGS and blend DDGS and averaged 4.9 %DM. Wheat DDGS was higher (P<0.05) in CP than corn DDGS (39.3 vs. 32.0 %DM) and blend DDGS (39.3 vs. 36.8 %DM). There were no significant differences in SCP with average of 13.6 %CP and NPN with average of 100.00 %SCP. Protein solubility could be reduced in DDGS due to the heat applied during processing and/or due to fermentation processing, compared to original grains. It is possible that during fermentation, some soluble proteins were at least partially degraded, leaving a greater proportion of insoluble protein to end up in DDGS. This result is in agreement with previous reports (Firkins et al. 1985; Boila and Ingalls 1994a). Our results showed that SCP in all three types of DDGS was mainly NPN. Wheat DDGS contained similar (P>0.05) ADICP to corn DDGS with average of 5.6 %CP but higher (P<0.05) than blend DDGS (1.2 %CP). Wheat DDGS also contained higher (P<0.05) NDICP than corn DDGS (56.0 vs. 34.4 %CP) but similar (P>0.05) to blend DDGS (54.4 %CP). In general, DDGS samples contained about three times the percentage of most chemical components compared its respective original grain (Spiehs et al. 2002; Nyachoty et al. 2005; Widyaratne and Zijlstra 2006). The amount of CP and Cfat increases while starch decreases as wheat or corn are utilized in DDGS processing (Lee et al. 1991; Widyaratne and Zijlstra 2006). The values shown in the present study are similar to those listed by NRC 2001, with exception of Cfat in corn DDGS (16.5 vs. 10.0 %DM) and CP in wheat DDGS (39.3 vs. 42.3 %DM), and also to those reported by previous studies (Cromwell et al. 1993; Spiehs et al. 2002; Widyaratne and Zijlstra 2006).

There were significant plant effects on chemical characteristics in CFat (6.2 vs. 4.2 %DM), NDF (52.7 vs. 44.9 %DM), NDICP (59.3 vs. 53.8 %CP), and ADICP (0.69 vs. 7.6 %CP) (Table 3.1). These results indicated that the feedstock used, as well as the different bio-ethanol processing methods used in each plant, such as fermentation, distillation, amount of solubles blended back, and DDGS drying, affected chemical profiles. The amount of solubles added back would affect protein solubility and degradability in the rumen by providing simple sugars that

would increase the susceptibility to the Maillard reaction (Belyea et al. 1998). The DDGS from SK-Plant 2 had visually distinguished darker colors due to a higher level of ADICP compared to those from SK-Plant 1.

3.3.3. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on protein and carbohydrate sub-fractions

The CP and carbohydrate sub-fractions, partitioned according to the Cornell Net Carbohydrate and Protein System (Sniffen et al. 1992; Chalupa and Sniffen 1994) included PA, PB1, PB2, PB3 and PC for protein fractions, and CA, CB1, CB2 and CC for carbohydrate fractions. Each fraction has different degradation behavior (degradation rate) in the rumen which is highly related to component nutrient availability in ruminants. The effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on protein and carbohydrate sub-fractions are presented in Table 3.2. Original wheat and corn had significantly different protein sub-fractions but similar carbohydrate fractions (Table 3.2). Wheat was higher ($P<0.05$) than corn in rapidly degradable non-protein nitrogen (PA: 21.9 vs. 14.5 %CP), higher ($P<0.05$) in rapidly degradable CP fraction (PB1: 2.7 vs. 0.2 %CP), lower ($P<0.05$) in intermediately degradable CP fraction (PB2: 61.9 vs. 80.5 %CP), and higher ($P<0.05$) in slowly degradable CP fraction (PB3: 13.5 vs. 4.7 %CP). There were no significant differences in carbohydrate fractions between wheat and corn with averages of rapidly degradable free sugars (CA: 7.9 %CHO), rapidly degradable CHO fraction (CB1: 74.5 %CHO), intermediately degradable CHO fraction (CB2: 15.5 %CHO) and, unavailable CHO fraction (CC: 2.2 %CHO).

Comparing among the three types of DDGS, wheat DDGS and blend DDGS were higher ($P<0.05$) than corn DDGS in PA (16.3 vs. 14.9 vs. 11.4 %CP) and PB3 (51.2 vs. 53.2 vs. 27.9 %CP). While PB1 was 0 %CP for the three types, corn DDGS was significantly higher than wheat DDGS and blend DDGS in PB2 (54.2 vs. 27.7 vs. 30.8 %CP) and numerically higher in PC (6.4 vs. 4.9 vs. 1.2 %CP). Differences in PB2 and PB3 sub-fractions between wheat DDGS and corn DDGS may be explained by differing protein sub-fraction profile between wheat and corn as well as by differing processing conditions. As a result of heating, the major shifts in the protein fractions between feedstock grains and DDGS were observed in PB2 and PB3. Approximately half of PB2 in wheat was allocated into PB3 in wheat DDGS, while one third of PB2 in corn was allocated into PB3 in corn DDGS. PC sub-fraction is protein bound to other

Table 3.2. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of protein and carbohydrate sub-fractions according to CNCPS system

	Feeds sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
Protein sub-fractions									
PA (%CP)	21.86 a	14.51 b	16.29 b	11.44 c	14.86 b	0.718	16.90	15.88	1.242
PB1 (%CP)	2.70 a	0.20 b	0.00 b	0.00 b	0.00 b	0.569	0.00	0.00	0.000
PB2 (%CP)	61.93 b	80.54 a	27.68 d	54.20 c	30.75 d	1.548	23.77 b	30.28 a	0.754
PB3(%CP)	13.51 c	4.66 d	51.18 a	27.93 b	53.23 a	2.524	58.63 a	46.21 b	1.025
PC (%CP)	0.00 c	0.08 c	4.86 ab	6.44 a	1.17 bc	1.412	0.69 b	7.64 a	0.125
True protein[z] (%CP)	78.14 b	85.41 a	78.85 b	82.12 ab	83.97 a	1.571	82.41 a	76.48 b	1.204
Carbohydrate sub-fractions									
CHO[y] (%DM)	81.69 a	83.54 a	50.58 b	47.14 c	49.57 bc	1.174	48.85	51.74	2.191
NFC[x] (%CHO)	81.29 a	83.22 a	48.34 b	18.36 d	36.49 c	2.841	40.55 b	53.53 a	2.661
CA (%CHO)	7.40 b	8.12 b	35.94 a	9.08 b	28.47 a	3.461	27.96 b	41.27 a	2.291
CB1 (%CHO)	73.89 a	75.10 a	12.40 b	9.28 b	8.02 b	1.918	12.60	12.26	2.470
CB2 (%CHO)	15.79 d	15.22 d	31.26 c	67.42 a	45.77 b	3.552	41.67 a	24.33 b	1.675
CC (%CHO)	2.92 c	1.56 c	20.40 a	14.22 b	17.74 ab	2.003	17.78	22.14	4.037

SEM = standard error of mean. Means with different letters in the same row are significantly different ($P<0.05$).

[z]True protein = PB1 + PB2 + PB3

[y]CHO = total carbohydrates = 100 – CP – Cfat – ash

[x]NFC = non-fiber carbohydrates = 100 – (NDF – NDICP) – Cfat – CP - ash

feed components such as lignin and tannins and is considered not degradable in rumen (Sniffen et al. 1992). As a result of the Maillard reaction, PC sub-fraction was higher in DDGS relative to feedstcok grain. In conclusion, the degradability of protein in rumen decreased in DDGS samples relative to feedstock grains. Although ruminal undegradable protein (RUP) is mainly represented by PB3 and PC sub-fractions, some RUP is present in PB2. Thus, an *in situ* degradability study is required in order to estimate more accurately the quantity of RUP in each type of DDGS.

Although original wheat and corn were not different in carbohydrate fractions, wheat DDGS, corn DDGS and blend DDGS differed significantly (Table 3.2). Compared to corn DDGS, wheat DDGS was higher ($P<0.05$) in the non-fiber carbohydrate fraction (NFC: 48.3 vs. 18.4 %CHO), higher ($P<0.05$) in highly degradable free sugars fraction (CA: 35.9 vs. 9.1 %CHO), higher in unavailable CHO (CC: 20.4 vs. 14.2 %CHO), similar ($P>0.05$) in rapidly degradable CHO fraction (average 10.8 %CHO), and lower in intermediately degradable CHO (CB2: 31.3 vs. 67.4 %CHO). An explanation fot the outstanding difference in the CA fraction between wheat DDGS and corn DDGS may be found in the type of raw starch in the feedstock and processing conditions. CA sub-fraction contains sugars and organic acids (Sniffen et al. 1992). Although the starch content of corn is higher than in wheat, the rate and extent of degradation of wheat starch is higher (See Appendix. Table 8.2), suggesting that wheat ethanol is generated more rapidly than corn ethanol. This may imply higher conversion of ethanol to organic acids, such as lactic and acetic acid, when wheat is used as feedstock, and consequently higher CA fraction in wheat DDGS relative to corn DDGS. In addition, wheat DDGS and corn DDGS were manufactured at different bio-ethanol plants, which differed in processing conditions such as amount of yeast utilized and fermentation time.

There were significant bio-ethanol plant effects on protein and carbohydrate sub-fractions between the two plants (Table 3.2). SK-Plant 1 had lower ($P<0.05$) PB2, PC, and CA, higher ($P<0.05$) PB3, CB2 in DDGS than SK-Plant 2, indicating that bio-ethanol plant processing methods may affect protein and carbohydrate sub-fractions. For example, SK-Plant 1 and SK-Plant 2 used different time and temperature of drying. This higher PC fraction which is not degradable is likely due to more heating applied during processing (Larson et al. 1993).

3.3.4. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on energy values as determined from the NRC 2001 formula (chemical approach)

The NRC 2001 formula is the usual method to estimate energy values for feeds. This method is a chemical approach that uses analytical results to estimate the values of truly digestible nutrients. The effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on energy content as determined using the NRC dairy (NRC 2001) and beef (NRC 1996) are presented in Table 3.3. Wheat and corn had similar (P>0.05) tdNFC (average 66.6 %DM) and tdNDF (average 9.0 %DM); however, wheat was higher (P<0.05) in tdCP (14.3 vs. 10.1 %CP) and lower (P<0.05) in tdFA (0.9 vs. 3.4 %DM) than corn. TDN_{1X} was lower (P<0.05) in wheat than in corn (83.4 vs. 88.3 %DM). Wheat was significantly different from corn in energy values of $ME_{3X,}$ and NEL_{3X} (Table 3.3). The estimated energy content for wheat and corn were similar to the tabular values in the NRC 2001, except that TDN_{1X} value, of wheat was lower (83.4 vs. 86.6 %DM).

Wheat DDGS was higher (P<0.05) than corn DDGS in tdNFC (20.1 vs. 8.5 %DM) and tdCP (38.6 vs. 31.2 %DM), but lower (P<0.05) in tdFA (4.0 vs. 15.5 %DM) and tdNDF (11.5 vs. 22.1 %DM). Wheat DDGS had lowest (P<0.05) and corn DDGS had highest (P<0.05) energy values (TDN_{1X}, DE_{3X}, $ME_{3X,}$ NE_{L3X} for dairy; NE_m and NE_g beef cattle) and blend DDGS was in between (Table 3.3). Our results showed that TDN_{1X} in wheat DDGS was lower (P<0.05) than that in original wheat, but TDN_{1X} in corn DDGS was similar (P>0.05) to original corn grain. This is because corn DDGS significantly reduced tdNFC from 68.2 %DM in original corn to 8.5 %DM, although corn DDGS significantly increased tdFA content from 3.6 %DM in corn to 15.5 %DM (Table 3.3). However, some studies showed that corn distiller's grains is higher than corn in terms of energy content (Larson et al. 1993; Ham et al. 1994). TDN_{1X} in corn DDGS was higher than the tabular value in NRC (2001) (89.8 vs. 79.5 %DM).

The DE_{3X}, $ME_{3X,}$ NE_{L3X}, NE_m and NE_g values in wheat DDGS were similar (P>0.05) to wheat and corn, suggesting wheat DDGS as an alternative to wheat and corn in dairy and beef diets. However, the DE_{3X}, $ME_{3X,}$ NE_{L3X}, NE_m and NE_g values in corn DDGS were all higher (P<0.05) than in corn, indicating that corn DDGS are superior to original corn in dairy and beef diets. The energy values (DE_{3X}, $ME_{3X,}$ NE_{L3X} , NE_m and NE_g) in the blend DDGS were higher than in wheat, corn and wheat DDGS, suggesting blend DDGS as an alternative to corn and

Table 3.3. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat:corn =70:30) and comparison of different bio-ethanol plants in terms of truly digestible nutrients, total digestible nutrient content at maintenance level, and energy values using the NRC 2001-chemical approach

	Feeds sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
Component digestible nutrient									
tdNFC (%DM)	65.08 a	68.15 a	20.05 b	8.50 d	17.73 c	1.828	19.42 b	27.14 a	1.698
tdCP (%DM)	14.28 c	10.13 d	38.56 a	31.20 b	36.65 a	0.910	39.88	37.68	1.613
tdFA (%DM)	0.91 d	3.59 c	3.98 c	15.53 a	7.53 b	0.432	5.18 a	3.18 b	0.304
tdNDF (%DM)	8.99 c	8.95 c	11.48 c	22.12 a	15.90 b	1.069	14.32 a	9.58 b	0.467
Total digestible nutrient at maintenance level									
TDN_{1X} (%DM)	83.39 b	88.31 a	76.04 d	89.77 a	80.21 c	0.965	78.26	74.56	1.569
Predicted energy values									
DE_{3X} (Mcal kg^{-1} DM) (Dairy)	3.39 c	3.53 bc	3.42 c	3.85 a	3.56 b	0.044	3.52	3.35	0.069
ME_{3X} (Mcal kg^{-1} DM) (Dairy)	2.97 d	3.12 bc	3.01 cd	3.50 a	3.17 b	0.046	3.12	2.94	0.071
NE_{L3X} (Mcal kg^{-1} DM) (Dairy)	1.89 d	2.01 bc	1.94 cd	2.35 a	2.06 b	0.034	2.02	1.88	0.051
NE_m (Mcal kg^{-1} DM) (Beef)	2.06 c	2.16 bc	2.08 c	2.39 a	2.17 b	0.032	2.15	2.03	0.051
NE_g (Mcal kg^{-1} DM) (Beef)	1.40 c	1.48 bc	1.41 c	1.67 a	1.49 b	0.027	1.48	1.37	0.043

SEM = standard error of mean. Means with different letters in the same row are significantly different ($P<0.05$)

superior to wheat and wheat DDGS in dairy and beef diets. There were no significant effects on TDN_{1X} and energy values (DE_{3X}, $ME_{3X,}$ NE_{L3X}, NE_m and NE_g) between SK-Plant 1 and SK-Plant 2 (Table 3.3).

3.3.5 Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on energy values as determined from the *in situ* assay (biological approach)

The energy values of a feedstuff are not chemical constituents, thus it is questionable whether these values can be accurately estimated by a chemical assay. The *in situ* assay is a more realistic approach for the estimation of truly digestible nutrients. The effects of DDGS type and bio-ethanol plant on energy content are presented on Table 3.4. Wheat DDGS was higher ($P<0.05$) than corn DDGS in tdNFC (23.6 vs. 6.4 %DM) and tdCP (35.5 vs. 22.7 %DM) but lower ($P<0.05$) in tdFA (3.7 vs. 15.1 %DM) and tdNDF (17.3 vs. 33.9 %DM). Blend DDGS was in between for tdNDF and tdFA but was as high as wheat DDGS for tdNFC and tdCP. As a result, TDN_{1X} and energy values (DE_{3X}, $ME_{3X,}$ NE_{L3X}, NE_m and NE_g) were higher ($P<0.05$) in corn DDGS relative to wheat DDGS while blend DDGS was in between.

Regarding the plant effect, SK-Plant 1 was lower ($P<0.05$) in tdNFC (18.5 vs. 27.1 %DM) but higher ($P<0.05$) in tdFA (5.0 vs. 2.9 %DM) and tdNDF (20.2 vs. 15.3 %DM) than SK-Plant 2; however, there was not significant plant effect in terms of TDN_{1X} and energy values (DE_{3X}, $ME_{3X,}$ NE_{L3X} , NE_m and NE_g).

3.3.6. Comparison of NRC 2001-chemical approach with biological approach (*in situ* assay) in the determination of truly digestible nutrients and energy values of DDGS

Both approaches, chemical and biological, detected that DDGS type and bio-ethanol plant had a significant effect on truly digestible nutrients (tdNDF, tdCP, tdFA and tdNFC), TDN_{1X}, and energy values (DE_{3X}, $ME_{3X,}$ NEL_{3X} , NE_m and NE_g). The difference and the correlation analysis between the chemical and the biological approaches for DDGS samples are presented in Table 3.5. The numeric difference between the two approaches was significant for the predicted truly digestible nutrients; the highest difference was found in tdNDF (-7.7 %DM) followed by tdCP (4.8 %DM). Higher predicted tdNDF was found when using the *in situ* assay; however, higher

Table 3.4. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat:corn =70:30) and comparison of different bio-ethanol plants in terms of truly digestible nutrients, total digestible nutrient content at a maintenance level, and energy values at three times maintenance level using the *in situ* assay-biological approach

	Feed sources				Bio-ethanol plant		
Items	Wheat DDGS n = 5	Corn DDGS n =3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
Truly digestible nutrient							
tdNFC (%DM)	23.63 a	6.35 b	16.99 a	2.124	18.49 b	27.06 a	1.782
tdCP (%DM)	35.53 a	22.69 b	33.64 a	1.349	37.37	34.29	2.051
tdFA (%DM)	3.72 c	15.06 a	7.22 b	0.477	4.96 a	2.89 b	0.322
tdNDF (%DM)	17.26 c	33.92 a	22.84 b	1.185	20.15 a	15.34 b	0.736
Total digestible nutrient at a maintenance level							
TDN_{1X} (%DM)	77.77 b	89.85 a	81.73 b	2.068	80.16	76.18	3.505
Predicted energy values							
DE_{3X} (Mcal kg^{-1} DM) (Dairy)	3.47 b	3.79 a	3.59 ab	0.091	3.58	3.40	0.152
ME_{3X} (Mcal kg^{-1} DM) (Dairy)	3.07 b	3.44 a	3.20 ab	0.093	3.18	2.98	0.154
NE_{L3X} (Mcal kg^{-1} DM) (Dairy)	1.98 b	2.30 a	2.09 ab	0.067	2.07	1.91	1.992
NE_m (Mcal kg^{-1} DM) (Beef)	2.11 b	2.34 a	2.20 ab	0.328	2.19	2.06	0.112
NE_g (Mcal kg^{-1} DM) (Beef)	1.44 b	1.63 a	1.51 ab	0.057	1.51	1.39	0.096

SEM = standard error of mean. Means with different letters in the same row are significantly different ($P<0.05$)

In situ assay-biological approach: Truly digestible nutrients (tdNFC, tdCP, tdFA and tdNDF) estimated after 48 h rumen incubation. Energy values (DE_{3X}, ME_{3X}, NE_{L3X}, NE_m, NE_g) calculated according to NRC 2001 formulas.

alysis between NRC-2001-chemical approach and *in situ* assay-biological approach in the determination of truly digestible nutrients, total digestible nutrient content a

Comparison				
NRC-2001 chemical vs. biological approach				
Mean^{biological}	Numeric difference	SED	P value	

23.32	-7.74	0.834	<0.0001
31.24	+4.79	0.901	0.0003
7.77	+0.33	0.036	<0.0001
17.11	+0.98	0.257	0.0034
82.15	-1.23	0.689	0.1054
3.59	-0.0	0.034	0.6797

	1		
3.20	-0.02	0.034	0.6797
2.09	-0.01	0.024	0.6854
2.20	-0.01	0.024	0.6870
1.51	-0.01	0.021	0.6919

correlation coefficient. Comparison method: Paired t test

FC, tdCP, tdFA and tdNDF) and energy values (DE_{3X}, ME_{3X}, NE_{L3X}, NE_m, NE_g) calculated according to NRC 2001 formulas.

nutrients estimated after 48 h rumen incubation. Energy values calculated according to NRC 2001 formulas.

tdCP, tdFA and tdNFC were found when using the NRC 2001 approach. Although no significant differences between the two approaches were detected in terms of TDN_{1X} and energy values, the predicted values were slightly higher for the biological approach.

These results are in agreement with a previous study (Yu et al. 2004), in which the highest difference between the chemical and biological approaches was found in tdNDF, being higher when the biological approach was used. NRC 2001-chemical approach estimates tdNDF based on the acid detergent lignin (ADL) content of the feed. Robinson et al. (2004) showed the poor relationship between ADL content and NDF digestibility in different feedstuffs including distillers grains, and concluded that the formula is not an accurate predictor of tdNDF. In that study, metabolizable energy of distillers grains was 13% higher when tdNDF was predicted *in vitro*. Differences among different feeds in the lignin content as well as in the extent to which lignin is bonded to other components of cell wall might be the reason for the deviation in the NDF digestibility (Chesson and Murison 1989). An independent comparison of the chemical and biological approach within each type of DDGS shows that the difference in tdNDF between the two approaches generally increases as the ruminal availability of NDF increases (See Appendix. Figure 8.1), thus the quantity and digestibility of NDF will determine the accuracy of the NRC 2001-chemical approach when evaluating DDGS products. As NDF availability was lower for wheat DDGS, tdNDF values for wheat DDGS were more accurately predicted by the chemical approach than the other two DDGS types.

In terms of tdCP, the calculation according to NRC 2001 is based on the ADICP content; however, a negative correlation between ADICP and protein digestibility was detected only when ADICP levels were higher than 13% CP (Harty et al. 1998). As shown in Table 3.1, ADICP levels in the current DDGS samples ranged from 1.2 to 6.4%CP. Other studies (Rocha Jr et al. 2001; Detmann et al. 2004; Detmann et al. 2008) have reported that NRC 2001-chemical approach is inaccurate in predicting TDN_{1X} content of feeds under tropical conditions. Of interest for further research would be the use of DDGS samples with higher ADCIP levels to investigate the accuracy of the NRC 2001-chemical approach to predict tdCP.

Correlation analysis between the chemical approach and the biological approach for DDGS samples showed significant and strong relationships for truly digestible nutrients, TDN_{1X}, and energy values.

3.3.7. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plant on *in situ* rumen degradability

The effects of DDGS type and bio-ethanol plant on *in situ* rumen degradability of DM (DDM), OM (DOM), CP (DCP) and NDF (DNDF) after 24 h and 48 h incubations are presented in Table 3.6. There were significant differences (P>0.05) in DDM and DNDF for 24 h incubation between wheat and corn (DDM24: 90.6 vs. 74.9 %; DNDF: 43.8 vs. 26.9 %), but after a 48 h incubation, the difference disappeared, averaging 91.8% (DDM48) and 48.2% (DNDF48). However, protein degradation patterns were different from DM and NDF. At both incubation times, wheat was always higher than corn (DCP24: 93.5 vs. 53.0 %; DCP48: 95.4 vs. 86.6 %).

Wheat DDGS had significantly higher *in situ* CP degradability (DCP: 61.1 vs. 47.1 % at 24 h; 90.0 vs. 68.1% at 48 h) and lower *in situ* NDF degradability (DNDF: 63.5 vs. 79.4% at 48 h) than corn DDGS, but similar *in situ* degradability to blend DDGS. Comparing the bio-ethanol plants, SK-Plant 1 had lower DDM24 than SK-Plant 2, but similar *in situ* degradability of CP and NDF. In the lactating cow with typical levels of intake, little DM, cell wall, or protein remains at 48 h. There may not be significant quantities at the end of 24 h of fermentation. However, in this study, we would like to know relative differences in maximum rumen degradability between the different types of DDGS. The degradability at the longer incubation can be used as an indicator for total tract digestibility.

3.3.8. Correlation analysis between ADICP and ADL and rumen degradability in overall DDGS, wheat DDGS, corn DDGS and blend DDGS

Acid detergent insoluble crude protein and lignin are known for their adverse effects on digestibility in the animal. In DDGS, the high temperature (100 – 600 °C) applied during the drying process usually results in ADICP formation due to the Maillard reaction (Weiss et al. 1986). While some studies have established a negative correlation between ADICP and CP digestibility in forages (Goering et al. 1972; Yu and Thomas 1976; Van Soest and Mason 1991a; Waters et al. 1992) and non-forages (Arieli et al. 1989; Waters et al. 1992; McKinnon et al. 1995), it is not clear whether ADICP behavior in DDGS is similar to conventional feeds. While Nakamura et al. (1994a) found a moderate correlation between ADICP and protein digestibility, Klopfenstein (1996) did not find any relationship. However, Harty et al. (1998) tested a high number of samples and observed a strong relationship between ADICP and *in vitro* protein

Table 3.6. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn =70:30) and comparison of different bio-ethanol plants in terms of *in situ* degradability of dry matter (DDM), organic matter (DOM), crude protein (DCP) and NDF (DNDF) at 24 and 48 h incubations

	Feeds sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS \n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
In situ degradability of DM at 24 and 48 h incubations									
DDM24 (%)	90.62 a	74.86 b	68.44 c	65.81 c	65.44 c	1.557	64.44 b	71.11 a	1.948
DDM48 (%)	92.29 a	91.35 a	85.40 b	81.80 c	84.47 bc	1.219	85.88	85.09	2.164
In situ degradability of OM at 24 and 48 h incubations									
DOM24 (%)	89.62 a	71.47 b	65.30 c	61.60 c	61.13 c	1.805	60.45 b	68.54 a	2.102
DOM48 (%)	91.55 a	90.23 a	84.12 b	79.75 c	82.69 bc	1.322	84.45	83.90	2.325
In situ degradability of CP at 24 and 48 h incubations									
DCP24 (%)	93.52 a	53.04 cd	61.07 b	47.10 d	56.99 bc	2.747	57.07	63.74	3.289
DCP48 (%)	95.36 a	86.63 b	89.95 ab	68.14 c	87.64 b	2.140	93.04	87.89	3.013
In situ degradability of NDF at 24 and 48 h incubations									
DNDF24 (%)	43.75 ab	26.90 c	42.02 ab	50.49 a	38.68 b	3.416	40.45	43.07	2.968
DNDF48 (%)	51.43 c	44.90 c	63.50 b	79.36 a	63.36 b	2.942	63.60	63.44	3.260

SEM = standard error of mean. Means with the different letters in the same row are significantly different (P<0.05)
Samples were rolled to 0.203 mm prior to rumen incubation and ground to 1 mm for chemical analysis

utilization when ADICP levels were greater than 13% CP. Although results from the present study (Table 3.7) indicated a moderate correlation ($r = 0.46$; $P = 0.0327$) between ADICP and DM digestibility (24 h incubation) in the overall DDGS samples; no correlation between the ADICP content and the *in situ* digestibility of protein within each type of DDGS was found. Thus, these results would be in accordance with Klopfenstein (1996) and Harty et al. (1998), as ADICP levels in the present DDGS samples were lower than 13 %CP (Table 3.1). Based on the amount of samples under study, it is concluded that, as long as ADICP levels are below 13 %CP, ADICP may not be utilized as an accurate indicator of protein utilization in DDGS samples. Unlike the intrinsic ADICP of conventional feeds, the added ADICP in by-products created by the Maillard reaction is 60 - 80% digestible (Waters et al. 1992; Van Soest 1994). This would explain that ADICP levels greater than 13 %CP are required to observe a negative effect on protein digestibility.

The amount of lignin as well as extent to which it is bonded to other components of cell wall affects ruminal digestibility (Chesson and Murison 1989). After 48 h incubation, there was a negative correlation between acid detergent lignin (ADL) and *in situ* degradability of DM, CP, and NDF for wheat DDGS ($r < -0.82$, $P = 0.0035$), but not for corn DDGS and blend DDGS, nor was there correlation after 24 h. Hindrichsen et al. (2006) concluded that ADL is not an accurate method to measure lignin and predict fiber digestibility in concentrates, while Jung et al. (1997) observed that correlation between lignin and the digestibility of DM and NDF was only found for legumes and several types of grasses. Also in the study by Jung et al. (1997), and in accordance with the present study, the higher the lignin content the stronger negative correlation. Although a limited amount of samples were utilized in the present study, it was concluded that ADL content can only be used as indicator of digestibility for wheat DGGS. The lack of correlation in corn DDGS and blend DDGS may be explained by the lower ADL content relative to wheat DDGS, differences in the distribution of lignin among the three types of DDGS, and weakness of the ADL method.

Table 3.7. Correlation between ADICP (%CP) and ADL (%DM) and *in situ* digestibility of dry matter (DDM), crude protein (DCP) and neutral detergent fibre (DNDF) at 24 ad 48 h incubations of overall DDGS, wheat DDGS, corn DDGS and blend DDGS (wheat:corn=70:30)

Items	DDGS Overall n = 11	DDGS type: Wheat DDGS n = 5	DDGS type: Corn DDGS n = 3	DDGS type: Blend DDGS (W:C=70:30) n = 3	Bio-ethanol plant: SK-Plant 1 Wheat DDGS n = 2	Bio-ethanol plant: SK-Plant 2 Wheat DDGS n = 3
	----------Pearson Correlation Coefficients: r (P value)----------					
Correlation between ADL and *in situ* degradability						
ADL vs. DDM24	NS	NS	NS	NS	NS	NS
ADL vs. DDM48	NS	r = -0.87 (P = 0.0010)	NS	NS	NS	r = -0.95 (P = 0.0031)
ADL vs. DCP24	NS	NS	NS	NS	NS	NS
ADL vs. DCP48	NS	r =-0.89 (P = 0.0006)	NS	NS	NS	r = -0.88 (P = 0.0204)
ADL vs. DNDF24	r = -0.48 (P = 0.0224)	NS	NS	NS	NS	NS
ADL vs. DNDF48	r = -0.72 (P = 0.0002)	r = -0.82 (P = 0.0035)	NS	NS	NS	r = -0.98 (P = 0.0007)
Correlation between ADICP and *in situ* degradability						
ADICP vs. DDM24	r = 0.46 (P = 0.0327)	NS	NS	NS	NS	NS
ADICP vs. DDM48	NS	NS	NS	NS	NS	NS
ADICP vs. DCP24	NS	NS	NS	NS	NS	NS
ADICP vs. DCP48	NS	NS	NS	NS	NS	NS
ADICP vs. DNDF24	NS	NS	NS	NS	NS	NS
ADICP vs. DNDF48	NS	NS	NS	NS	NS	NS

r = Pearson correlation coefficient

3.4. Conclusions

It was concluded that wheat DDGS, corn DDGS and blend DDGS (wheat:corn=70:30) differed in chemical characterization and profiles, mineral concentration (Sulfur, Ca and P), CNCPS protein and carbohydrate sub-fractions, energy values at production levels for both beef and dairy cattle, and *in situ* degradability. The bio-ethanol plant origin also had significant impact on the nutritive value of DDGS. According to NRC 2001-chemical approach, the estimated energy values for wheat DDGS were similar to those for wheat and corn, suggesting wheat DDGS is an alternative to wheat and corn in dairy and beef diets. The estimated energy values for corn DDGS were significantly higher than those for corn, wheat DDGS, and blend DDGS, indicating that corn DDGS is a superior source of energy in dairy and beef diets. These energy values were similar to those obtained by the *in situ* assay-biological approach; however, the prediction of tdNDF and tdCP differed. While a refinement of the NRC 2001 formula to predict tdNDF in DDGS is required, DDGS samples with higher ADICP levels may be required to investigate the accuracy of the tdCP formula.

Results also revealed that the potential degradability of CP and NDF was higher in wheat DDGS and corn DDGS, respectively. While ADICP content was not an accurate indicator of the potential degradability of CP in DDGS samples, ADL seems to be an acceptable indicator of the potential degradability of wheat DDGS. Further research with more samples may help to clarify these relationships. Despite the nutrient variability between plants and the methodology utilized to determine the nutritive value of DDGS, it is clear that wheat DDGS, blend DDGS and corn DDGS are an excellent source of protein and energy for dairy and beef cattle.

4. *IN SITU* RUMEN DEGRADATION KINETICS, EFFECTIVE DEGRADABILITY AND ESTIMATED INTESTINAL DIGESTIBILITY OF RUMEN UNDEGRADED PROTEIN OF CO-PRODUCTS OF BIO-ETHANOL PRODUCTION: COMPARISON AMONG WHEAT DDGS, CORN DDGS AND BLEND DDGS, AND BETWEEN DIFFERENT BIO-ETHANOL PLANTS

4.1. Introduction

As the price of cereal grains continues to increase, the demand for alternative feed ingredients such as dried distillers grains with solubles (DDGS), which provide sources of energy and protein in livestock diets, will also increase (Goverment of Alberta 2008b).

The market value of DDGS is affected by the inconsistency of their nutritional properties. This inconsistency is one of the challenges in including DDGS in ruminant diets since accurate ration formulation is not consistently achieved. The nutrient composition, the availability of these nutrients in rumen, as well as the utilization of rumen undegraded protein (RUP) vary among DDGS samples derived from different feedstocks (Boila and Ingalls 1994a, 1994b; Lodge et al. 1997; Al-Suwaiegh 2002), and among corn DDGS samples collected at different bio-ethanol plants (Cromwell et al. 1993; Spiehs et al. 2002; Kleinschmit et al. 2007). Factors such as the type and quality of feedstock grain, the extent of fermentation, the amount of solubles blended back, and the extent and temperature of drying contribute to the variability in DDGS properties (Carpenter 1970; Olentine 1986; Spiehs et al. 2002; U.S. Grains Council 2007a).

Currently in western Canada, wheat DDGS and blend (wheat/corn) DDGS manufactured at different bio-ethanol plants as well as corn DDGS imported from the United States are utilized in ruminant diets. In Chapter 3, the detailed chemical profile, protein and carbohydrate fractions, and energy values affected by DDGS type and bio-ethanol plant origin were described; however, information on the degradability of each feed component, the hourly effective rumen degradation ratios, and the intestinal digestibility of RUP was not provided.

Animal performance is partially related to the truly digested and absorbed protein in the small intestine, which is largely determined by microbial protein synthesized in the rumen and RUP. In order to achieve optimum microbial protein synthesis, the degradation of nitrogen (N) in rumen should match that of organic matter (OM), particularly the carbohydrate fraction. Thus,

the ratios between the effective degradability of N and energy should be used in feed formulation to optimize the composition of dairy diets (Tamminga et al. 1990; Tamminga et al. 1994). Good quality RUP must be digestible and available for absorption in the small intestine. Data on the RUP digestibility of DDGS derived from wheat or a blend of wheat and corn is scarce (Boila and Ingalls 1994b). The readily digestible fiber in DDGS is an important factor contributing to the energy content of DDGS (Schingoethe et al. 2009); however, little attention has been paid to the differences in the neutral detergent fiber (NDF) availability among different types of DDGS and among DDGS generated from different plants. This information is required for a detailed description of the nutritive value of these new co-products of bio-ethanol production.

The purpose of this study was to investigate the effects of DDGS type and bio-ethanol plant origin on 1) rumen degradation kinetics and rumen availability of DM, OM, CP, and NDF, 2) the hourly effective degradability ratio between N and energy, and 3) estimated intestinal digestibility of RUP. In addition, these parameters were compared in DDGS as opposed to parental grain. This research may be applied to routine ration formulation procedures that should improve the accuracy of predicting nutrient supply and utilization of animals consuming diets containing DDGS products.

4.2. Materials and methods

4.2.1. Samples

Samples used in this experiment were the same seventeen feeds used in Chapter 3: wheat DDGS (5 batches), corn DDGS (3 batches), blend DDGS (3 batches), wheat (3 batches) and corn (3 bacthes). Prior to ruminal incubation, samples were processed using a Sven Roller Mill (Apollo Machine and Products Ltd., Saskatoon, SK). The roller gap was adjusted to a size of 0.203 mm in order to increase similarity in the particle size of all samples.

4.2.2. Animals and diets

Three non-lactating Holstein cows fitted with a rumen cannula (Bar Diamond Inc, Parma, ID, USA) with an internal diameter of 10 cm were used in this study. The cows were individually fed twice daily at 08:00 and 16:00 h receiving 14 kg (7 kg at each feeding time) on a DM basis of a

totally mixed ration consisting of 56.82 % barley silage, 10.23 % alfalfa hay, 4.54 % dehydrated alfalfa pellets, 21.59 % standard dairy concentrate (containing barley, wheat, oats, dairy supplement pellets and molasses) and 6.82 % fresh cow concentrate (containing barley, oats, canola meal, soybean meal, wheat DDGS, corn gluten meal, molasses, golden flakes, canola oil, minerals and vitamins) (See Appendix, Table 8.1). The animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993).

4.2.3. Rumen incubation procedure

Rumen degradation parameters were determined using the *in situ* method described by Yu et al. (2000). Seven grams of sample were weighed and placed into numbered nylon bags (Nitex 03 - 41/31 monofilament open mesh fabric, Screentec Corp., Mississagua, ON) measuring 10 cm × 20 cm with a pore size of 41 μm. The ratio of sample size to bag surface area was calculated and equal to 17.5 mg/cm^2, which is within the range recommended by previous reports (Ørskov 1982; Nocek 1988). A polyester mesh bag (45 cm × 45 cm with a 90 cm length of rope to be anchored to the cannula) was used to hold the bags in the rumen. Sample bags were added into the polyester mesh bag according to the 'gradual addition/all out' schedule and incubated for 48, 24, 12, 8, 4, 2 and 0 h. Data from Urdl et al. (2006) was used to determine the number of bags incubated from each sample, which increased in relation to incubation time. The maximum number of bags in the rumen at any one time was 30. All treatments for each incubation time were incubated in duplicates (2 runs) and randomly allocated to the three non-lactating cows. After incubation, the bags were removed from the rumen and, together with those representing 0 h, rinsed under cold tap water to remove excess ruminal contents. The bags were washed with cool water without detergent and subsequently dried at 55°C for 48 h. Dry samples were stored in a refrigerated room (4°C) until analysis.

4.2.4. Chemical analysis

Original samples and pooled residues for each treatment, incubation time, and run, were ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments (Canada) LTD, Ontario), and analyzed for DM (AOAC 930.15), ash (AOAC 942.05), CP (Leco protein/N analyzer. Model FP-528, Leco Corp., St. Joseph, MI, USA), and NDF (Ankom A200 Filter Bag Technique (pore size = 25 μm), Ankom Technology, Fairport, NY, USA). In order to prevent the high fat content of

DDGS from giving inaccurately high values for NDF, fat was extracted by 2 h incubation of samples in acetone. Sodium sulfite and heat-stable amylase were used prior to NDF extraction. All samples were analyzed in duplicate and repeated when the error was higher than 5%. The rest of the chemical components were chemically assessed as described in Chapter 3. Nutrient composition of original samples is presented in Table 3.1.

4.2.5. Rumen degradation parameters

Degradation characteristics were determined for DM, OM, CP and NDF. The percentage of each nutrient was fitted to 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:

$$R(t) = U + D \times e^{-Kd \times (t - T_0)};$$

where, R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); T_0 = lag time (h); and Kd = degradation rate (% h^{-1}). The results were calculated using the NLIN (nonlinear) procedure of SAS (2005) via interative least squares regression (Gauss Newton method).

Based on the nonlinear parameters estimated in the above equation (U, D, Kd), the effective degradability (ED), or extent of degradation, of each nutrient was predicted according to NRC 2001 as:

$$ED\ (\%) = S + (D \times Kd) / (Kp + Kd);$$

where, S = soluble fraction (%); Kp = estimated rate of outflow of digesta from rumen (% h^{-1}) and was assumed to be 6 % h^{-1} (Tamminga et al. 1994). In contrast to the first-order kinetic model, which estimates the disappearance of each nutrient for a given time period, the ED equation considers the fractional outflow rate of digesta from the rumen, thus predicting the amount of nutrient that will be truly digested in rumen over time (NRC 2001).

The effective extent of degradation of N and OM was also calculated hourly as outlined by Sinclair et al. (1993) as:

$$\text{Hourly ED (g kg}^{-1}\text{ DM)} = S + [(D \times Kd) / (Kp + Kd)] \times [\ 1 - e^{-t \times (Kd + Kp)}].$$

The difference in cumulative amounts degraded between successive hours was regarded as the quantity degraded per hour. From the quantity of N and OM degraded per hour, an hourly ratio of N to OM was calculated:

$$\text{Hourly ED ratio of N/OM}_t = (HEDN_t - HEDN_{t-1}) / (HEDOM_t - HEDOM_{t-1});$$

where, ratio N/OM_t = ratio of N to OM at time t (g N kg^{-1} OM); $HEDN_t$ = hourly effective degradability of nitrogen at time t (g kg^{-1} DM); $HEDN_{t-1}$ = hourly effective degradability of nitrogen 1 h before than t (g kg^{-1} DM); $HEDOM_t$ = hourly effective degradability of OM at time t (g kg^{-1} DM); and $HEDOM_{t-1}$ = hourly effective degradability of OM at 1 h before than t (g kg^{-1} DM). As reported by Czerkawski (1986), 25 g N kg^{-1} OM truly digested in the rumen is the optimal ratio to maximize microbial protein synthesis efficiency.

4.2.6. *In vitro* estimation of intestinal digestibility of RUP (IDP)

The estimation of intestinal digestibility 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 12 h ruminal incubation were exposed for 1 h in 10 mL of 0.1 N HCl solution containing 1 g/L of pepsin. The pH was neutralized with 0.5 mL of 1 N NaOH and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg of pancreatin that were added to the solution and incubated at 38°C for 24 h. After incubation, 3 mL of a 100% (wt/vol) trichloroacetic acid (TCA) solution were 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). Estimated intestinal digestion of protein is calculated as TCA-soluble N divided by the amount of N in the 12 h residue sample. Although 16 h ruminal incubation period is recommended by the above procedure, no differences in pepsin-pancreatin digestion of N were reported when samples were suspended in rumen for 12 to 18 h (Calsamiglia and Stern 1995).

4.2.7. Statistical analysis

Study on the effect of type of DDGS and feedstock grain on rumen and intestinal degradation parameters. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). 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 sources, as a fixed effect; batch and runs as replications; and e_{ij} was the random error associated with the observation ij.

Study on the effect of bio-ethanol plant on rumen and intestinal degradation parameters. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). The model used for the analysis was: $Y_{ij} = \mu + P_i + e_{ij}$, where, Y_{ij} was an observation of

the dependent variable ij ; μ was the population mean for the variable; P_i was the effect of bio-ethanol plant, as a fixed effect; batch as replications; and e_{ij} was the random error associated with the observation ij.

For all statistical analyses, significance was declared at $P<0.05$ and trends at $P\leq0.10$. Treatment means were compared using the Fisher's Protected LSD method.

4.3. Results and discussion

4.3.1. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on rumen degradation characteristics of DM and OM

The effects of DDGS type and bio-ethanol plant on lag time (T_0), rumen fractions (S, D, U), rate of degradation (Kd), and effective degradability of DM (EDDM) is presented in Table 4.1. Except T_0, which was similar across treatments, the *in situ* rumen degradation characteristics of DM were significantly different between feedstock grain and DDGS products. Wheat was lower ($P<0.05$) than wheat DDGS in the washable fraction S but higher ($P<0.05$) in the degradable fraction D, Kd (36.67 vs. 5.98 % h^{-1}) and EEDM (79.25 vs. 57.67 %). Similarly, corn was lower ($P<0.05$) than corn DDGS in S fraction and higher ($P<0.05$) in D fraction, but no significant differences were detected in terms of Kd (average 5.49 % h^{-1}) and EDDM (average EDDM: 53.80 %). The reduced Kd and EDDM in wheat DDGS relative to wheat can be partly explained by the formation of Maillard products during the drying process; however, due to the slow degradability of corn, the Maillard reaction was not as apparent in the case of corn DDGS degradability.

Herrera-Saldana et al. (1990) reported Kd of DM for wheat and corn of 12.37 % h^{-1} and 4.70 % h^{-1}, respectively. The higher Kd for wheat observed in the current study can be explained by the high degradation rate of starch (43.48 %h^{-1}) (See appendix. Table 8.2). In the production of ethanol, specific genotypes characterized by high content of rapidly fermentable starch are utilized (Bothast and Schlicher 2005).

There was also significant variation in the *in situ* degradation kinetics among the three types of DDGS (Table 4.1). Similar to the trend observed in wheat and corn, wheat DDGS was similar ($P>0.05$) to corn DDGS in S fraction, and lower ($P<0.05$) than corn DDGS in D fraction.

Table 4.1. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of *in situ* rumen characteristics of DM and OM

Items	Feed sources						Bio-ethanol plant		
	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
In situ rumen degradation characteristics of DM									
T_0 (h)	0.14	0.09	0.02	0.17	0.00	0.090	0.00	0.03	0.029
S (%)	19.16 c	12.46 d	29.10 a	26.25 ab	25.37 b	1.060	29.03	29.15	1.187
D (%)	70.44 b	82.20 a	60.11 d	65.01 c	62.46 cd	1.776	64.83 a	56.96 b	1.322
Kd (% h^{-1})	36.67 a	6.86 b	5.98 b	4.11 b	5.55 b	1.673	4.01	7.30	1.036
EDDM (%)	79.25 a	55.21 bc	57.67 b	52.39 c	54.62 bc	1.422	54.89	59.53	1.946
In situ rumen degradation characteristics of OM									
T_0 (h)	0.00	0.10	0.02	0.17	0.00	0.074	0.00	0.03	0.025
S (%OM)	8.70 c	0.85 d	21.72 a	16.88 b	15.93 b	1.110	20.71	22.40	0.884
D (%OM)	79.07 b	93.27 a	66.57 c	73.91 b	71.23 bc	2.286	72.98 a	62.30 b	1.618
Kd (% h^{-1})	34.07 a	6.80 b	6.07 b	4.06 b	5.37 b	1.282	3.97 b	7.48 a	1.009
EDOM (%OM)	75.62 a	49.16 bc	53.47 b	46.36 c	48.72 c	1.665	49.64	56.02	2.183
EDOM (g kg^{-1} DM)	740.4 a	483.0 bc	507.3 b	443.6 c	462.5 c	15.881	471.7	531.0	20.776

SEM = standard error of mean. Means with the different letters in the same row are significantly different ($P<0.05$)

T_0 = Lag time; S = Soluble fraction; D = Degradable fraction; Kd = Rate of degradation; EDDM = Effective degradability of dry matter; EDOM = Effective degradability of organic matter

Blend DDGS was similar (P>0.05) to corn DDGS but lower (P<0.05) than wheat DDGS in S fraction, and intermediate between wheat DDGS and corn DDGS in D fraction. Compared to corn DDGS, wheat DDGS had numerically higher Kd (4.11 vs. 5.98 % h^{-1}) and significantly higher EDDM (52.4 vs. 57.7 %). Although no significant variation was observed between blend DDGS and corn DDGS, blend DDGS was numerically higher in Kd (5.55 vs. 4.11 % h^{-1}) and EDDM (54.6 vs. 52.4 % h^{-1}). Overall, the potential degradability of DM in DDGS increases as the content of wheat in the feedstock increases. This is not in agreement with Urdl et al. (2006), where higher EDDM was reported for corn DDGS relative to wheat DDGS (63.1 vs. 60.1 %). In that study, the S fraction for corn DDGS was significantly higher than that for wheat DDGS while D fraction and Kd were similar between the two types of DDGS. Compared to the current results, Urdl et al. 2006 also showed higher S fractions and lower D fractions for both wheat DDGS and corn DDGS, lower Kd for wheat DDGS (5.98 vs. 4.7 % h^{-1}) and higher Kd for corn DDGS (4.11 vs. 4.80 % h^{-1}). These differences in the rumen degradation kinetics between the two studies may be attributed to factors associated with DDGS samples, such as feedstock grains and bio-ethanol plant processing procedures, and to factors associated to the experimental procedure, such as feed particle size, ratio of sample size to bag surface area, pore size, and assumed passage rates (6.00 vs. 5.00 % h^{-1}).

Wheat DDGS from different plants differed only slightly, though significantly in the D fraction, which was higher (P<0.05) for wheat DDGS from SK-Plant 1 (64.4 vs. 60.0 %) (Table 4.1). Heating promotes the Maillard reaction, which is partially responsible for an increase in acid detergent insoluble crude protein (ADICP) and lignin and a decrease in hemicellulose (Weiss et al. 1986). Wheat DDGS from SK-Plant 2 showed higher ADICP, lower hemicellulose, and numerically higher ADL, pointing towards the idea of reduced DM degradability as a result of greater heat damage. However, wheat DDGS from SK-Plant 2 showed numerically higher Kd (7.30 vs. 4.01 % h^{-1}) and EDDM (59.5 vs. 54.9 %). A possible explanation for this may be found in differences in rates of degradation of different wheat varieties obtained by the two plants and, consequently, in the resulted co-product. Although not yet reported for wheat, a considerable variety effect in the rate of degradation of DM has been reported in barley grown in western Canada (Yu et al. 2009). In addition, other factors related to the operational decisions of each plant, such as the amount of solubles blended back, may affect the degradability of the other feed components, thus affecting the entire DM degradability.

Because ash content differed among different samples were highly parallel to DM content differences, the pattern and changes of OM degradation kinetics were highly related to those of DM (Table 4.1).

4.3.2. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on rumen degradation characteristics of CP

The effects of DDGS type and bio-ethanol plant on mean values of ruminal degradation variables and effective degradability of CP (EDCP) is presented in Table 4.2. Lag time and S fraction were similar (P>0.05) across the treatments. Comparing feedstock grain with DDGS, wheat was similar (P>0.05) to wheat DDGS in D fraction but higher (P<0.05) in EDCP (73.6 vs. 45.6 %CP) due to higher (P<0.05) Kd (18.50 vs. 4.56 % h^{-1}). Corn was higher (P<0.05) than corn DDGS in D fraction, but similar (P>0.05) in EDCP (average 37.0 %CP) because of the numerically lower Kd for corn (3.90 vs. 4.19 % h^{-1}). However, the concentration of CP in DDGS was roughly threefold the concentration in feedstock grain, thus EDCP expressed as g kg^{-1} DM was higher (P<0.05) in wheat DDGS and corn DDGS compared to wheat (185 vs. 126 g kg^{-1} DM) and corn (114 vs. 49 g kg^{-1} DM), respectively.

Compared with the present results, Arieli et al. (1995) reported a lower EDCP for wheat (73.6 vs. 50.5 %CP) but similar levels for corn (40.1 vs. 43.6 %CP), while Herrera Saldana (1990) showed higher Kd and S fraction for wheat (Kd: 18.50 vs. 25.36 % h^{-1}; S: 8.4 vs. 72.5 %CP) and corn (Kd: 3.90 vs. 7.90 % h^{-1}; S: 3.2 vs. 40.9 %CP). Tabular values (NRC 2001) are higher in S fraction and Kd for wheat DDGS (S: 8.1 vs. 39.5 %; Kd: 4.56 vs. 26.10 % h^{-1}) and corn DDGS (S: 3.2 vs. 21.1 %CP; Kd: 4.19 vs. 7.90 % h^{-1}). The observed EDCP for corn DDGS (34.0 %CP) was lower than the range (37.4 - 52.2 %CP) and the value (47.0 %CP) published by Harty et al. (1998) and Ham et al. (1980), respectively. These differences among studies are likely due to differences in the feedstock grains, ethanol plant processing methods, and experimental procedure. Samples in the present study were rolled to 0.203 mm prior to rumen incubation. This may have contributed to the lower S fraction in comparison with the quoted studies in which samples were normally ground to 1 mm. Likewise, the higher values for SCP (%CP) (Table 3.1) relative to the S fractions obtained in the present study are attributable to the differing particle size between the analytical procedure and the *in situ* assay. The S fraction of CP is negatively affected by an increase in temperature and time of drying (Arieli et al. 1989).

Table 4.2. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of *in situ* rumen characteristics of CP and NDF

	Feed sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
In situ rumen degradation kinetics of CP									
T_0 (h)	0.57	0.64	0.32	0.00	0.10	0.221	0.42	0.26	0.208
S (%CP)	8.41	3.19	8.08	3.19	7.18	1.875	12.23 a	5.32 b	1.777
D (%CP)	86.65 b	95.36 a	89.45 b	73.65 c	91.74 ab	2.302	87.77	90.56	2.095
Kd (% h^{-1})	18.50 a	3.90 b	4.56 b	4.19 b	3.75 b	0.641.	3.54	5.24	0.763
EDCP (%CP)	73.63 a	40.13 bc	45.58 b	33.95 c	36.20 c	3.095	44.80	46.10	2.893
EDCP (g kg^{-1} DM)	126.0 b	48.5 c	185.1 a	114.4 b	141.8 b	11.620	188.7	182.7	11.311
RUP (%CP)	26.37 c	59.87 ab	54.42 b	66.05 a	63.80 a	3.029	55.20	53.90	2.893
RUP (g kg^{-1} DM)	45.2 b	72.2 b	222.8 a	222.6 a	246.6 a	11.995	232.5	216.3	16.739
In situ rumen degradation kinetics of NDF									
T_0 (h)	0.75 ab	0.24 ab	0.16 b	0.90 a	0.10 b	0.259	0.05	0.23	0.073
S (%NDF)	5.88 b	19.19 a	0.00 b	0.00 b	0.00 b	2.930	0.00	0.00	0.00
D (%NDF)	46.42 c	40.95 c	68.45 b	98.10 a	74.89 b	6.818	67.55	69.04	3.573
Kd (% h^{-1})	11.58 a	8.99 ab	7.45 ab	3.67 b	5.49 b	2.055	6.13	8.33	1.771
EDNDF (%NDF)	34.72 ab	41.78 a	35.59 ab	37.06 ab	31.65 b	2.509	33.47	37.01	2.795
EDNDF (g kg^{-1} DM)	50.5 c	59.4 c	107.3 ab	116.8 a	97.1 b	6.071	103.4	109.9	9.037

SEM = standard error of mean. Means with different letters in the same row are significantly different (P<0.05)
T_0 = Lag time; S = Soluble fraction; D = Degradable fraction; Kd = Rate of degradation; EDCP = Effective degradability of crude protein; EDNDF = Effective degradability of neutral detergent fiber
CP analyzed by Leco protein/N analyzer
NDF analyzed by Ankom A200 Filter Bag Technique

This is noted when comparing with a study reported by Mustafa et al. (2000a), in which wet distillers grains from wheat showed higher S fraction (15.2 vs. 8.1 %CP), similar D fraction (81.2 vs. 89.5 %CP), similar Kd (4.60 vs. 4.56 % h^{-1}), and higher EDCP (60.8 vs. 45.6 %CP) than the wheat DDGS utilized in the present study. Therefore, as corroborated by earlier studies (Boila and Ingalls 1994a; Ojowi et al. 1997), the dried product is a better source of RUP than the wet product generated from the same feedstock.

Wheat DDGS was similar ($P>0.05$) to blend DDGS and higher ($P<0.05$) than corn DDGS in D fraction, whereas no significant variation among DDGS samples was observed in S fraction and Kd (average 4.16 % h^{-1}) (Table 4.2). However, wheat DDGS was numerically the highest in Kd (4.56 % h^{-1}), which largely contributed to the highest ($P<0.05$) EDCP (45.6 vs. 36.2 vs. 34.0 %CP). While the degradable fraction of DDGS protein in the rumen increased as the content of wheat in feedstock increased, the undegradable fraction conversely increased with increasing the corn content in feedstock; therefore, RUP (%CP) for corn DDGS was numerically higher than blend DDGS and significantly higher than wheat DDGS (66.1 vs. 63.8 vs. 54.4 %CP). When RUP was expressed in g kg^{-1} DM, no significant variation was found among DDGS types. However, due to the higher protein content in wheat and the higher undegradability of of corn protein, RUP (g kg^{-1} DM) for blend DDGS was numerically higher than that for wheat DDGS and corn DDGS (247 vs. 223 vs. 223 g kg^{-1} DM). The lower EDCP in corn DDGS and blend DDGS relative to wheat DDGS is likely due to resistance of zein, the major corn protein source, to rumen degradation (Little et al. 1968). Similarly, Boila and Ingalls (1994) reported that the effective degradability of CP was higher in DDGS prepared from 100% wheat grain than from a mixture of 75% wheat grain and 25% corn grain.

Numerous studies have shown a negative relationship between ADICP concentration and effective degradability of CP in forages (Goering et al. 1972; Yu and Thomas 1976; Van Soest and Mason 1991a; Waters et al. 1992) as well as in by-products (Nakamura et al. 1994a; McKinnon et al. 1995). In the present study, no correlation ($r = 0.075$; $P = 0.7411$) was found between EDCP and ADICP. Boila et al (1994) observed that an increase in the concentration of ADICP among different types of wheat based DDGS resulted in a decreased degradability of CP; however, ADICP levels ranged from 8.9 to 16.7 %CP while in the present experiment were below 8 %CP. The lack of accordance in the relationship between ADICP content and effective degradability of CP between these two studies could be explained by Harty et al. 1998, who

concluded that ADICP can be utilized as a quantitative predictor of ruminal CP availability in DDGS only when the value is greater than 13 %CP. Unlike conventional feeds, approximately 60 to 80 % of ADICP in distillers grains is digested (Waters et al. 1992; Van Soest 1994; Nakamura et al. 1994a), thus greater ADICP levels than the levels observed here would be required in order to detect greater differences in the ruminal protein degradability of the current DDGS samples.

In terms of plant effect (Table 4.2), significant differences between the two types of wheat DDGS were only found in the S fraction (12.2 vs. 5.3 %CP). Differences in the S fraction can be expected as a result of differences in feedstock grain, time and temperature of drying (Arieli et al. 1989; McKinnon et al. 1995) as well as in the amount of solubles blended back. The solubles contribute to RUP by providing low ruminally degradable heated yeast and soluble sugars that increase the susceptibility to Maillard reaction (Belyea et al. 2004; Martinez-Amezcua et al. 2007; Klopfenstein et al. 2008). However, Cao et al. (2009) showed that raising the proportion of solubles in corn DDGS resulted in increased ruminal protein degradability as a result of increased S fraction. In wheat DDGS, reduced degradability of protein was attributable to a lower S fraction and reduced rate of degradation as a result of a higher ADICP (Boila and Ingalls 1994a). Although higher ADICP concentration and lower S fraction was found in wheat DDGS from SK-Plant 2, no plant effect was observed in terms of Kd and EDCP. Indeed, correlations between ADICP and Kd ($r = 0.47$; $P = 0.1685$), and between ADICP and EDCP ($r = 0.08$; $P = 0.8257$) did not exist, suggesting that dissaperance of protein in the rumen is not sentive enough to the low ADICP levels of the present wheat DDGS samples.

4.3.3. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on rumen degradation characteristics of NDF

The effects of DDGS type and bio-ethanol plant origin on degradation kinetics and effective degradability of NDF (EDNDF) is presented in Table 4.2. Comparing feedstock grain with DDGS products, no significant differences were observed in terms of T_0. Wheat was similar ($P>0.05$) to wheat DDGS in S fraction, lower ($P<0.05$) in D fraction, and similar ($P>0.05$) in Kd (average 9.52 % h^{-1}) and EDNDF (average 36.5 %NDF); however, the total amount of digestible NDF was higher ($P<0.05$) for wheat DDGS than for wheat (107 vs. 50 g kg^{-1} DM). Likewise, corn was higher ($P<0.05$) than corn DDGS in S fraction, lower ($P<0.05$) in D fraction, similar

(P>0.05) in Kd (average 6.33 % h^{-1}) and EDNDF (average 39.4 %NDF) but lower (P<0.05) in the total amount of effective degradable NDF (59 vs. 117 g kg^{-1} DM). Previously, Varga and Hoover (1983) described corn with lower Kd (5.10 vs. 8.99 % h^{-1}) but similar EDNDF (42.3 vs. 41.8 %NDF). In a recent study, Winterholler et al. 2009 reported corn DDGS with higher S fraction (35.7 vs. 0.0 %NDF), lower D fraction (54.4 vs. 98.1 %NDF), lower Kd (2.39 vs. 3.67 % h^{-1}) but higher EDNDF (67.4 vs. 37.1 %NDF). The likely causes of these differences are differences in the feedstocks, co-product processing, and *in situ* processing methods.

Dried distillers grains with solubles can replace the energy component from cereal grains in the diet. NE_{L3X}, NEm, and NEg values for wheat DDGS are similar to wheat and corn, values for blend DDGS are similar to corn, and values for corn DDGS are superior to wheat and corn (Chapter 3). This can be explained by the threefold concentration of fat and the increased ruminal NDF availability in DDGS, which replaces the energy supply from starch in original grain.

Soluble fraction was zero across the three types of DDGS (Table 4.2). This is in accordance with the CB1 carbohydrate fraction obtained by CNCPS in Chapter 3. CB1 consists of starch and soluble fiber (pectins and beta glucans) (Sniffen et al. 1992) and was 5.9, 4.0 and 3.6 %DM for wheat DDGS, corn DDGS and blend DDGS, respectively. These results are close to the starch values reported in Table 3.1 (6.7, 4.4 and 4.0 %DM), thus corroborating the lack of S fraction in the NDF component of DDGS. Although corn DDGS was higher (P<0.05) than wheat DDGS and blend DDGS in T0 and D fraction, no significant variation was found among DDGS samples in terms of Kd (average 5.54 % h^{-1}) and EDNDF (average 34.8 %NDF). When expressed as g kg^{-1} DM, EDNDF for corn DDGS was similar (P>0.05) to wheat DDGS (average 112 g kg^{-1} DM) but higher (P<0.05) than blend DDGS (117 vs. 97 g kg^{-1} DM). No significant differences in NDF degradation parameters were observed between wheat DDGS and blend DDGS; however, numerical differences indicate that NDF degradation of wheat DDGS was faster than blend DDGS while the latter was degraded more extensively due to the higher D fraction. In contrast to other studies (Varga and Hoover 1983; Mustafa et al. 2000b), the present study did not show correlation between the extent of degradation of DM and NDF (r = 0.33, P = 0.1296); however, the correlation between the extent of degradation of DM and CP was positive (r = 0.56, P = 0.0067), suggesting that differences in ruminal DM degradability among DDGS samples is largely due to differences in the ruminal degradability of CP rather than NDF.

No effect on ruminal degradation NDF variables was detected between wheat DDGS originated from SK-Plant 1 and SK-Plant 2 (Table 4.2). As fat inhibits microbial growth and reduces fiber digestibility (Nagaraja et al. 1997), the numerical difference in the NDF degradability (33.5 vs. 37.0 %NDF) can be explained by the significant difference in the fat content (6.2 vs. 4.2 %DM).

4.3.4. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on hourly effective degradability ratios between N and energy

In dairy cows, the optimal ratio between the effective extent of degradability of N and energy in order to achieve maximum microbial synthesis and minimize N loss is 25 g N kg^{-1} OM truly digested in rumen (Czerkawski 1986) or 32 g N kg^{-1} CHO truly digested in rumen (Sinclair et al. 1991). Higher ratios indicate some potential loss of N or a deficiency in the energy supply in rumen, while lower ratios indicate N shortage or excessive energy supply for microbial growth. The effect of feedstock, DDGS type and bio-ethanol plant on the hourly ED ratio of N/OM at different incubation times is shown in Figure 4.1. The largest effective degradation N/OM ratios were seen at longer incubation across all the treatments. Wheat (Figure 4.1.A) exhibited higher than optimal rumen fermentation ratio at all incubation times except at 2 h, ranging from 23 to 991 g N kg^{-1} OM, while corn (Figure 4.1.B) showed sub-optimal ratios during the entire incubation, ranging from 0 to 24 g N kg^{-1} OM (See Appendix, Table 8.3). The difference between wheat and corn can be explained by the difference in the rate and extent of degradation of protein, which were about five and two times higher, respectively, in wheat. Compared with DDGS, the hourly ED ratios of N/OM for wheat were higher ($P<0.05$) than those for DDGS samples at 0, 12 and 24 h; however, the ratios for corn were lower ($P<0.05$) at all incubation times. The extremely high value observed for wheat at 24 h (991 g N kg^{-1}) is explained by the small difference (less than 0.01) between successive hours in the effective degradability of both N and OM after 12 h incubation. This results in disproportionate ratios, suggesting that the formula is not accurate at long incubation times when feeds characterized by high degradation rates are evaluated.

Comparing among the three types of DDGS (Fig. 4.1.C), wheat DDGS generally had the highest ($P<0.05$) ratios (26 - 103 g N kg^{-1} OM), while ratios for blend DDGS (29 - 89 g N kg^{-1}

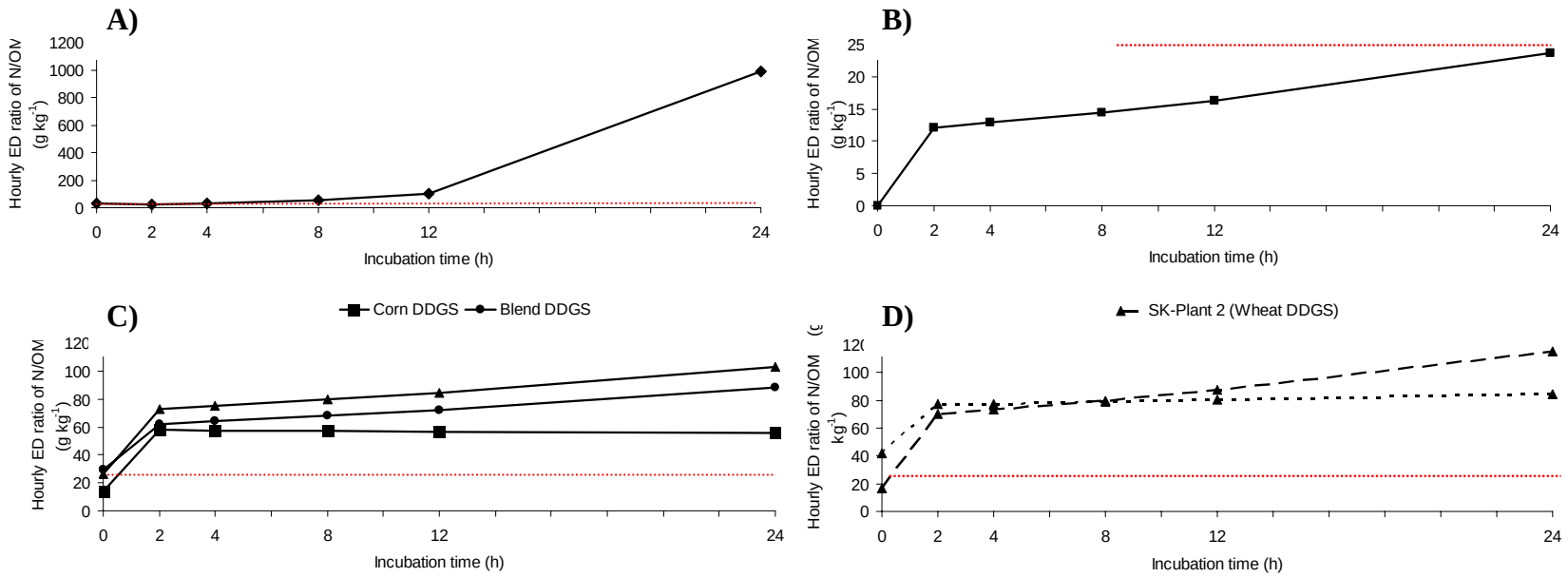

Figure 4.1. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of hourly effective degradability ratios between N and OM (Red dashed line = 25 g N kg^{-1} OM truly digested in rumen)

OM) were numerically higher (P>0.05) than those for corn DDGS (14 - 56 g N kg^{-1} OM). The hourly ED ratios of N/OM tended to rise with increasing incubation time for wheat DDGS and blend DDGS; however, they remained constant for corn DDGS after 2 h. This reflects a higher difference in the hourly effective degradation of N at later stages for blend DDGS and wheat DDGS than for corn DDGS, rather than differences in the hourly effective degradability of OM.

The bio-ethanol plant effect was significant at the beginning and end of incubations (Figure 4.1.D). The hourly ED ratios of N/OM for wheat DDGS from SK-Plant 1 was greater at 0 h (42 vs.16 g N kg^{-1} OM) but lesser at 12 h (80 vs. 88 g N kg^{-1} OM) and 24 h (85 vs. 114 g N kg^{-1} OM). In this case, rather than differences in CP degradability, the larger difference at later incubation times was mainly due to differences in the in the hourly effective degradation of OM. As shown in Table 4.2, the difference in the ED (%) between the two wheat DDGS is greater for OM than for CP.

The results shown here indicate that DDGS samples exhibited a higher than optimal rumen fermentation ratio when evaluated as a single ingredient, revealing that, in spite of being an excellent source of RUP, there is extra N in rumen that is not captured in microbial protein. The extra N will increase the ammonia concentration in the rumen, which must be absorbed into bloodstream, converted to urea in the liver, and excreted in urine. This suggests that elevated levels of DDGS in ruminant rations will have repercussions both on animal performance, in terms of extra metabolic cost associated with excreting N, and on environment, as this may lead to soil N accumulation. According to previous studies (Janicek et al. 2008; Hao et al. 2009), up to 30% corn DDGS and 20% wheat DDGS as dry matter can be included in dairy and beef rations respectively without detecting a negative effect on animal performance and N excretion.

4.3.5. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on estimated intestinal availability of rumen undegradable protein

With the development of new techniques (Hvelplund 1985; Calsamiglia and Stern 1995), several protein evaluation systems, such as NRC 2001 and DVE/OEB System, assign estimates of intestinal digestibility of RUP to each feed. The effect of DDGS type and bio-ethanol plant on the mean estimated RUP, estimated intestinal digestibility of RUP (IDP) as determined by the three step *in vitro* procedure (Calsamiglia and Stern 1995), estimated intestinally

absorbable feed protein (IADP) and total digestible feed protein (TDP) is presented in Table 4.3. Wheat was an inferior (P<0.05) source of RUP than wheat DDGS (26.4 vs. 54.4 %CP); however, no significant difference was observed between corn and corn DDGS (average 63.0 %CP). In terms of IDP, wheat was similar (P>0.05) to wheat DDGS (average 78.2 %RUP) while corn was lower (P<0.05) than corn DDGS (69.3 vs. 82.5 %RUP). As a result, IADP for wheat and corn were lower (P<0.05) than for wheat DDGS and corn DDGS, respectively. TDP, calculated as the sum of IDAP (%CP) and EDCP (%CP), showed similarity (P>0.05) between wheat and wheat DDGS and higher values (P<0.05) for corn DDGS relative to corn.

The mean values for RUP were superior (P<0.05) for corn DDGS and blend DDGS compared to wheat DDGS (66.1 vs. 63.8 vs. 54.4 %CP) (Table 4.3). IDP was the highest (P<0.05) for blend DDGS and similar (P>0.05) between wheat DDGS and corn DDGS (93.9 vs. 81.3 vs. 82.5 %RUP). As a result, IADP for blend DDGS and corn DDGS were similar (P>0.05) and superior (P<0.05) to wheat DDGS (59.9 vs. 54.5 vs. 44.0 %CP). The highest IDP for blend DDGS was largely responsible for the highest TDP (96.1 %CP). Due to the greater EDCP for wheat DDGS relative to corn DDGS, no significant difference in TDP was observed between these two types (89.5 vs. 88.4 %CP)

The results obtained here are in good agreement with Kleinschmit et al. 2007 who, using the three step *in vitro* procedure, reported RUP in corn DDGS ranging from 46.4 to 71.7 (%CP) and IDP ranging from 59.2 to 76.8 (%RUP). In that study, the lowest IDP value (59.2 %RUP) corresponded with the highest ADICP content (23.1 %CP); however, the effect of ADICP content on the digestibility of RUP was not clear when ADICP levels ranged from 7.5 to 11.9 (%CP). Cao et al. (2009) also used the three-step *in vitro* procedure and showed lower IDP (62.3 - 65.1 %RUP), IADP (36.2 - 39.6 %CP) and TDP (76.2 - 78.6 %CP) in corn DDGS; however, ADICP levels ranged from 14.1 to 17.4 %CP. According to Harty et al. (1998), ADICP can be utilized as a quantitative predictor of intestinal CP availability in distillers grains only when the value is above 13%. Similarly, Schroeder et al. (1996) reported that the digestibility of RUP of heat processed plant proteins does not decrease when ADICP levels are below 12-15 %CP. In the current study, ADICP content of DDGS samples was far below those levels but, unlike in the rumen, the relationship between ADICP content and intestinal digestibility of DDGS protein was significantly negative and moderate ($r = -0.70$; $P = 0.0003$).

In terms of plant effect (Table 4.3), significant differences were found only in IADP,

Table 4.3. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of estimated intestinal digestibility of rumen undegradable protein (IDP), estimated intestinally absorbable feed protein (IADP), and total digestible feed protein (TDP)

	Feed sources						Bio-ethanol plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK- Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
CP (%DM)	14.28 d	10.13 e	39.32 a	32.01 c	36.82 b	0.535	39.99	38.87	1.019
RUP [z] (%CP)	26.37 c	59.87 ab	54.42 b	66.05 a	63.80 a	3.209	55.20	53.90	2.893
IDP [y] (%RUP)	76.41 bc	69.30 c	81.25 b	82.54 b	93.89 a	3.263	89.35	75.85	4.177
IADP [x] (%CP)	20.22 c	41.69 b	43.96 b	54.46 a	59.85 a	3.112	49.37 a	40.36 b	1.989
TDP [w] (%CP)	96.86 ab	81.82 c	89.54 b	88.41 b	96.05 a	1.985	94.17	86.46	2.867

SEM=standard error of mean. Means with different letters in the same row are significantly different (P<0.05)
[z]Rumen undegradable protein
[y]Estimated intestinal digestibility using the three step *in vitro* procedure (Calsamiglia and Stern 1995)
[x]Estimated intestinally absorbable feed protein = RUP × IDP / 100
[w]Total digestible feed protein = EDCP + IADP

being higher ($P<0.05$) for wheat DDGS from SK-Plant 1 (49.4 vs. 40.4 %CP) mainly as a result of numerically higher IDP (89.4 vs. 75.9 %RUP). The numerical difference in IDP can be further attributed to the significant difference in ADICP content (0.7 vs. 7.6 %CP), however, the higher ADICP content for wheat DDGS from SK-Plant 2 was not reflected in reduced EDCP in the rumen. Thus, results shown here suggest that the disappearance of DDGS protein is more sensitive to low ADICP levels in the small intestine than in the rumen. In addition, since ADICP content provides an indication of the severity of the drying conditions (Goering et al. 1972; Kleinschmit et al. 2007), it can be speculated that wheat DDGS from SK-Plant 1 was exposed to more optimal heating conditions than wheat DDGS from SK-Plant 2. Optimal heating is interpreted as the heating able to increase, without damaging, RUP (Kleinschmit et al. 2007), thereby increasing the availability of feed protein in the lower tract.

4.4. Conclusions

The digestive characteristics of each feed component (DM, OM, CP and NDF), the hourly effective degradability between N and energy, and the estimated intestinal availability of feed protein differed significantly among wheat DDGS, blend DDGS and corn DDGS, and to a lesser extent between different bio-ethanol plants. The effective degradability of DM in DDGS samples increased as the content of feedstock wheat increased. DDGS are a good source of rumen undegradable protein. The protein content of DDGS derived from wheat is higher relative to that derived from corn; however, the undegradability of the protein fraction increases as the proportion of corn in the feedstock increases. In addition, DDGS provide significant amounts of rumen degradable protein, which increased as the content of wheat in the feedstock increased. This indicates a potential loss of N when high levels of DDGS are included in the diet.

ADICP levels were generally low across all DDGS samples, revealing no effect of ADICP on ruminal and intestinal disappearance of feed protein. However, consideration should be given to the numerical differences in digestibility of RUP and the relation with ADICP content. Further research with a higher number of samples and higher range in the ADICP content should be undertaken to investigate the effect of ADICP on rumen and intestinal disappearance of DDGS protein. These results indicate that it is inappropriate to assume fixed

rumen and intestinal degradation characteristics for DDGS without considering factors such as DDGS type and bio-ethanol plant origin.

The ruminal degradability of CP and OM as well as the digestibility of RUP are required inputs for modern protein evaluation systems, such as the NRC 2001 Model and the DVE/OEB System. These models can provide more detailed information regarding the effect of DDGS type and bio-ethanol plant on the potential truly absorbable protein in the small intestine.

5. USING THE DVE/OEB SYSTEM AND THE NRC 2001 MODEL TO ESTIMATE THE METABOLIC CHARACTERISTICS OF PROTEINS AND PREDICT THE NUTRIENT SUPPLY FROM CO-PRODUCTS OF BIO-ETHANOL PRODUCTION: COMPARISON AMONG WHEAT DDGS, CORN DDGS AND BLEND DDGS, AND BETWEEN DIFFERENT BIO-ETHANOL PLANTS.

5.1. Introduction

Nutrition models are fundamental for the continued success of the dairy nutritionist. They are practical tools that provide information on the animal's performance in response to changes in the ration. With the increasing understanding of events occurring in the ruminant's digestive tract, as well as the development of *in vitro* techniques and mathematical approaches that mimic these events, nutrition models are able to estimate the availability of protein in the small intestine. This is advantageous from a research standpoint, as the *in vivo* animal trial is labor-intensive, expensive, and suitable for only a few treatments, while models allow for the evaluation of a higher number of treatments.

The period from 1970 to 1995 saw the creation of various nutrition models (INRA 1978; ARC 1984; Madsen 1985; NKJ-NJF 1985; NRC 1985; Wu et al. 2000) and their corresponding updates (Verité and Geay 1987; Madsen et al. 1995). These models were developed to predict the protein value for feeds and the requirements of dairy cattle in terms of truly digested and absorbed protein in the small intestine. Some of the principles and elements of these models were used to develop the framework of two more sophisticated models; the DVE/OEB System or Non-TDN Model (Tamminga et al. 1994), currently being used in Europe, and the NRC 2001 Model or TDN Model (NRC 2001), used in North America. For each ingredient or diet, these two protein evaluation systems predict two major outputs: 1) the truly digested and absorbed protein in small intestine and 2) the degraded protein balance. The prediction of these two outputs is based on the chemical profile, the rumen degradation characteristics of different feed components, and the intestinal digestibility of dietary protein. The first output includes the truly absorbable rumen synthesized microbial protein in the small intestine, the truly absorbed bypass feed protein in the small intestine, and endogenous protein losses. The second output is a concept derived from the NKJ-NJF model and reflects the balance between available N and energy in the

rumen (Tamminga et al. 1994). This balance is critical in order to achieve efficient synthesis of microbial protein, which ultimately contributes to the postruminal pool of true protein.

In Chapter 3 and Chapter 4, it was found that DDGS are a good source of RUP, rumen degradable protein and energy. In addition, significant effects of DDGS type and bio-ethanol plant origin existed on the chemical profile, the rumen degradation characteristics, and on the intestinal availability of feed protein. However, information on how the potential protein supply to the small intestine of dairy cows from DDGS is affected by DDGS type and bio-ethanol plant origin is still lacking.

The principles of the DVE/OEB System and the NRC 2001 Model are similar; however, some of the concepts and factors used in calculations differ. For this reason, past studies observed significant differences between the two model supplies when different forages (Yu et al. 2003a; Yu et al. 2004) and concentrates (Yu et al. 2003b; Heendeniya 2008) were evaluated. Since DDGS products are being largely utilized in both North America and Europe, it is of interest to provide nutritive value based on the two models as well as to study the relationship between the two models so that the protein value of DDGS can be extrapolated from one model to another.

The objectives of the current study were 1) to investigate the effects of DDGS type and bio-ethanol plant origin on the metabolic characteristics of proteins and on the prediction of protein supply using the DVE/OEB System and the NRC 2001 model, 2) to compare the predicted protein supply of feedstock grains with their respective derived DDGS samples, and 3) to compare the two models in the prediction of protein supply from DDGS. This data will help nutritionists in Europe and North America, particularly in western Canada, to increase accuracy in the formulation of dairy rations containing wheat DDGS, corn DDGS or blend DDGS.

5.2. Materials and methods

5.2.1. Samples

Samples used in this experiment were the same seventeen feeds used in Chapter 3 and Chapter 4: wheat DDGS (5 batches), corn DDGS (3 batches), blend DDGS (3 batches), wheat (3 batches) and corn (3 bacthes). Prior to ruminal incubation, samples were processed using a Sven

Roller Mill (Apollo Machine and Products Ltd., Saskatoon, SK). The roller gap was adjusted to a size of 0.203 mm in order to increase similarity in the particle size of all samples.

5.2.2. Animals and diets

Animals and diets utilized for the rumen incubation procedure were the same as those previously described in Chapter 4.

5.2.3. Rumen incubation procedure

Rumen degradation parameters were determined using the *in situ* method described by Yu et al. (2000). Seven grams of sample were weighed and placed into numbered nylon bags (Nitex 03 - 41/31 monofilament open mesh fabric, Screentec Corp., Mississagua, ON) measuring 10 cm × 20 cm with a pore size of 41 μm. The ratio of sample size to bag surface area was calculated and equal to 17.5 mg/cm^2, which is within the range recommended by previous reports (Ørskov 1982; Nocek 1988). A polyester mesh bag (45 cm × 45 cm with a 90 cm length of rope to be anchored to the cannula) was used to hold the bags in the rumen. Sample bags were added into the polyester mesh bag according to the 'gradual addition/all out' schedule and incubated for 120, 48, 24, 12, 8, 4, 2 and 0 h. Data from Urdl et al. (2006) was used to determine the number of bags incubated from each sample, which increased in relation to incubation time. The maximum number of bags in the rumen at any one time was 30. All treatments for each incubation time were incubated in duplicates (2 runs) and randomly allocated to the three non-lactating cows. After incubation, the bags were removed from the rumen and, together with those representing 0 h, rinsed under cold tap water to remove excess ruminal contents. The bags were washed with cool water without detergent and subsequently dried at 55°C for 48 h. Dry samples were stored in a refrigerated room (4°C) until analysis.

5.2.4. Chemical analysis

Original samples and pooled residues for each treatment, incubation time, and run, were ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments (Canada) LTD, Ontario), and analyzed for DM (AOAC 930.15), ash (AOAC 942.05), CP (Leco protein/N analyzer. Model FP-528, Leco Corp., St. Joseph, MI, USA), and NDF (Ankom A200 Filter Bag Technique (pore size = 25 μm), Ankom Technology, Fairport, NY, USA). In order to prevent the high fat content of

DDGS from giving inaccurately high values for NDF, fat was extracted by 2 h incubation of samples in acetone. Sodium sulfite and heat-stable amylase were used prior to NDF extraction. All samples were analyzed in duplicate and repeated when the error was higher than 5%. The rest of the chemical components were chemically assessed as described in Chapter 3. Nutrient composition of original samples is presented in Table 3.1.

5.2.5. Rumen degradation model

The first order kinetic degradation model described by Ørskov and McDonald (1979) and modified by Robinson et al. (1986) and Dhanoa (1988) was applied to describe the rumen degradation characteristics of DM, CP and starch. This model was solved by NLIN (non linear) procedure of SAS 2005 via iterative least-squares regression (Gauess-Newton method) using the following equation:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t-T_0)}$$

where, R(t) = residue present at t h incubation (%); S = soluble fraction (%); U = undegradable fraction (%); T_0 = lag time (h); and Kd = degradation rate (% h^{-1}).

The degradation model of starch differs in that T0 and U are assumed to be zero (Tamminga et al. 1994), thus:

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

Based on the nonlinear parameters estimated in the above equations (S, U, D, Kd), rumen degraded feed CP (RDP), rumen undegraded feed CP (RUP), and rumen undegraded starch (RUSt) were predicted according to NRC 2001 as:

$$RDP\ (\%) = S + (D \times Kd) / (Kp + Kd);$$

$$RUP\ (\%) = U + (D \times Kd) / (Kp + Kd);$$

$$RUSt\ (\%) = S \times 0.1 + (D \times Kp) / (Kp + Kd);$$

where, D = 100 – S - U (%); Kp = estimated rate of outflow of digesta from rumen (%h^{-1}) and was assumed to be 6 % h^{-1} (Tamminga et al. 1994); and 0.1 is a compensation factor between *in situ* and *in vivo* results indicating that 10% of the S fraction of starch escapes rumen degradation (Nocek and Tamminga 1991; Tamminga et al. 1994; Yu et al. 2003b).

5.2.6. *In vitro* estimation of the intestinal digestibility of RUP (IDP)

The estimation of intestinal digestibility of RUP (IDP) was the same as that previously described in Chapter 4. The procedure utilized was the three step *in vitro* procedure as described by Calsamiglia and Stern (1995).

5.2.7. Estimation of fermented organic matter (FOM)

The DVE/OEB System utilizes the content of fermented organic matter (FOM) in the rumen to estimate microbial protein synthesis in the rumen, thus the term of Non-TDN Model. According to Tamminga et al. (1994), FOM was calculated as:

$$FOM\ (g\ kg^{-1}\ DM) = DOM_{120}\ (g\ kg^{-1}\ DM) - CFat\ (g\ kg^{-1}\ DM) - RUP\ (g\ kg^{-1}\ DM) - RUSt\ (g\ kg^{-1}\ DM) - FP\ (g\ kg^{-1}\ DM);$$

where, DOM_{120} = digestible organic matter after 120h rumen incubation; RUP = rumen undegraded protein; RUSt = rumen undegraded starch; and FP = fermentation products that are assumed to be zero for concentrates. Because the content of starch in the residues of DDGS samples is very limited and does not play a significant role in determining FOM, the amount of RUSt was assumed to be zero.

5.2.8. Estimation of total digestible nutrients (TDN)

The NRC 2001 Model requires TDN_{3X} value to estimate rumen microbial protein synthesis, thus it is also designated as TDN-Model. A chemical and summative approach (NRC 2001) was used to estimate the total digestible nutrient at a maintenance level (TDN_{1X}). In accordance with this approach, each sample was analyzed for DM, ash, CP, NDICP, ADICP, CFat, starch, NDF, ADF, ADL. Truly digestible non fiber carbohydrate (NFC), CP, CFat and NDF, and the consequent TDN_{1X} for each sample were estimated. Ultimately, TDN_{1X} (Table 3.3) was utilized along with a discount value to determine TDN_{3X} as described by NRC 2001.

5.2.9. The DVE/OEB System

The DVE/OEB system is outlined in detail by Tamminga et al. (1994). Below is a summarized description of the concepts and calculations that the DVE/OEB System utilizes to predict the protein supply and availability to the small intestine of dairy cows. The two major outputs of the

model are 1) the truly digested and absorbed protein in the small intestine (DVE) and 2) the degraded protein balance (DPB^{OEB}). The DVE value was calculated as follows:

DVE (g kg^{-1} DM) = $AMCP^{DVE}$ (g kg^{-1} DM) + $ARUP^{DVE}$ (g kg^{-1} DM) - ENDP (g kg^{-1} DM);

where, $AMCP^{DVE}$ = absorbable microbial protein synthesized in rumen; $ARUP^{DVE}$ = truly absorbed bypass feed protein in the small intestine; and ENDP = endogenous protein loss in the small intestine.

The DPB^{OEB} value, which shows the balance between potential microbial synthesis based on rumen degraded protein and potential protein synthesis based on energy extracted during anaerobic fermentation of OM in rumen (Non-TDN Model), was calculated as:

$$DPB^{OEB} \text{ (g kg}^{-1} \text{ DM)} = MCP_{RDP}{}^{DVE} \text{ (g kg}^{-1} \text{ DM)} - MCP_{FOM} \text{ (g kg}^{-1} \text{ DM)};$$

where, $MCP_{RDP}{}^{DVE}$ = microbial protein synthesized from rumen degraded protein; and MCP_{FOM} = microbial protein synthesized from energy available from rumen fermented organic matter. A positive DPB^{OEB} in a diet reveals a potential N loss from the rumen, while a negative value indicates a shortage of N that can impair microbial protein synthesis. Therefore, the optimal degraded protein balance in a diet is zero or slightly higher than zero (Tamminga et al. 1994).

The calculation of the components contained in these two outputs was as described below.

5.2.9.1. Estimation of microbial protein synthesis in the rumen (MCP_{FOM} and $MCP_{RDP}{}^{DVE}$) and truly absorbable rumen synthesized microbial protein in small intestine ($AMCP^{DVE}$)

Microbial protein synthesis according to DVE/OEB system is based on FOM, thus:

$$MCP_{FOM} \text{ (g kg}^{-1} \text{ DM)} = 0.15 \times \text{FOM (g kg}^{-1} \text{ DM)};$$

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

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

$$MCP_{RDP}{}^{DVE} \text{ (g kg}^{-1} \text{ DM)} = \text{CP (g kg}^{-1} \text{ DM)} \times [\, 1 - (1.11 \times \text{RUP (\%CP)} / 100)];$$

where, 1.11 represents the regression coefficient of *in vivo* data over *in situ* degradation data according to the French PDI system (Verité and Geay 1987; Tamminga et al. 1994).

The estimation of the truly absorbable microbial protein synthesized in rumen ($AMCP^{DVE}$) only contemplates $MCP_{FOM,}$ therefore:

$$AMCP^{DVE} \text{ (g kg}^{-1} \text{ DM)} = 0.75 \times 0.85 \times MCP_{FOM} \text{ (g kg}^{-1} \text{ DM)};$$

where, 0.75 and 0.85 are factors representing the assumed amount and digestibility of the true protein contained in MCP_{FOM}, respectively (Tamminga et al. 1994).

5.2.9.2. Estimation of rumen undegraded feed protein (RUP^{DVE}) and truly absorbed bypass feed protein in the small intestine ($ARUP^{DVE}$)

The content of $ARUP^{DVE}$ is based on the content and digestibility of RUP^{DVE}, which was calculated as:

$$RUP^{DVE} \text{ (g kg}^{-1} \text{ DM)} = 1.11 \times CP \text{ (g kg}^{-1} \text{ DM)} \times RUP \text{ (\% CP)};$$

$ARUP^{DVE}$ was then formulated as:

$$ARUP^{DVE} \text{ (g kg}^{-1} \text{ DM)} = IDP \text{ (\%)} \times RUP^{DVE} \text{ (g kg}^{-1} \text{ DM)}.$$

5.2.9.3. Estimation of endogenous protein losses in the small intestine (ENDP)

The estimation of DVE is corrected for ENDP in order to consider N lost as a consequence of digestive processes. ENDP is associated to the amount of undigested dry matter (UDM), which was estimated as:

$$UDM \text{ (g kg}^{-1}) = (Ash \text{ (g kg}^{-1} \text{ DM)} \times 0.35) + [OM \text{ (g kg}^{-1} \text{ DM)} - ((OM \text{ (g kg}^{-1} \text{ DM)} \times dOM \text{ (\%))})];$$

where, 0.35 is the factor utilized by CVB (1996) indicating the 35% of ash is not digested; and dOM= OM digestibility after 120 h rumen incubation (Tamminga et al. 1994).

Given that the model assumes that 75 g of absorbed protein kg^{-1} UDM is required to compensate for the endogenous losses (Tamminga et al. 1994), ENDP was formulated as:

$$ENDP \text{ (g kg}^{-1} \text{ DM)} = 0.075 \times UDM \text{ (g kg}^{-1} \text{ DM)}.$$

5.2.10. The NRC 2001 Model

The detailed concepts and formulas of the NRC 2001 Model are provided by NRC (2001). Similarly to the DVE/OEB System, the true protein that is absorbed and digested postruminally

is a very important output. Thus, the NRC 2001 Model introduced the concept of metabolizable protein, which was calculated as:

$$MP\ (g\ kg^{-1}\ DM) = AMCP^{NRC}\ (g\ kg^{-1}\ DM) + ARUP^{NRC}\ (g\ kg^{-1}\ DM) + AECP\ (g\ kg^{-1}\ DM);$$

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

Although the estimation of rumen degraded protein balance (DPB^{OEB}) is not provided by the NRC 2001 Model, it can be calculated based on predicted data and according to the principle of the DVE/OEB System. However, unlike the DVE/OEB system, DPB^{OEB} reflects the difference between the potential microbial protein synthesis based on ruminally degraded dietary protein and that based on total digestible nutrients at a production level (TDN Model). Therefore:

$$DPB^{NRC}\ (g\ kg^{-1}\ DM) = RDP^{NRC}\ (g\ kg^{-1}\ DM) - 1.18 \times MCP_{TDN}{}^{NRC}\ (g\ kg^{-1}\ DM);$$

where RDP^{NRC} = rumen degraded protein; and MCP_{TDN} = microbial protein synthesis from energy available from total digestible nutrients (discounted at three times maintenance).

The different components contained in these two outputs were calculated as described below.

5.2.10.1. Estimation of microbial protein synthesis in the rumen ($MCP_{TDN}{}^{NRC}$ and $MCP_{RDP}{}^{NRC}$) and truly absorbable rumen synthesized microbial protein in small intestine ($AMCP^{NRC}$)

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

$$MCP_{TDN}{}^{NRC}\ (g\ kg^{-1}\ DM) = 0.13 \times TDN_{3X};$$

where, 0.13 signifies that 130 g of microbial protein per kg TDN (discounted) are assumed to be synthesized (NRC 2001).

Then, RDP^{NRC} calculation was as:

$$RDP^{NRC}\ (g\ kg^{-1}\ DM) = CP\ (g\ kg^{-1}\ DM) \times (100 - RUP\ (\%CP)).$$

When, $RDP^{NRC} > 1.18 \times MCP_{TDN}{}^{NRC}$, $MCP_{TDN}{}^{NRC}$ value is used as MCP^{NRC} for the final $AMCP^{NRC}$ calculation. When $RDP^{NRC} < MCP_{TDN}{}^{NRC}$, MCP^{NRC} was calculated as:

$$MCP_{RDP}{}^{NRC}\ (g\ kg^{-1}\ DM) = 0.85 \times RDP^{NRC}\ (g\ kg^{-1}\ DM);$$

where, 0.85 indicates the assumed amount of RDP that is converted to microbial protein; and 1.18 results from 1.00 / 0.85 (NRC 2001).

Since the content of true protein and digestibility of ruminally synthesized microbial CP are assumed to be 80% (NRC 2001), $AMCP^{NRC}$ was estimated as:

$$AMCP^{NRC} \text{ (g kg}^{-1} \text{ DM)} = 0.80 \times 0.80 \times MCP^{NRC} \text{ (g kg}^{-1} \text{ DM)}.$$

5.2.10.2. Estimation of rumen undegraded feed protein (RUP^{NRC}) and truly absorbed bypass feed protein in the small intestine ($ARUP^{NRC}$)

The prediction of $ARUP^{NRC}$ is based on the content and digestibility of RUP^{NRC}, thus:

$$RUP^{NRC} \text{ (g kg}^{-1} \text{ DM)} = CP \text{ (g kg}^{-1} \text{ DM)} \times RUP \text{ (\% CP)};$$

$$ARUP^{NRC} \text{ (g kg}^{-1} \text{ DM)} = dRUP \text{ (\%)} \times RUP^{NRC} \text{ (g kg}^{-1} \text{ DM)}.$$

5.2.10.3. Estimation of truly absorbed endogenous protein in the small intestine (AECP)

The NRC 2001 Model predicts endogenous protein losses (ECP) from DM content. ECP of a feed is calculated as:

$$ECP \text{ (g kg}^{-1} \text{ DM)} = 6.25 \times 1.9 \times DM \text{ (\%)} / 100;$$

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

Out of the total rumen ECP, 50% passes to small intestine and 80% is true protein (NRC 2001). Thus, AECP was calculated as:

$$AECP \text{ (g kg}^{-1} \text{ DM)} = 0.50 \times 0.80 \times ECP \text{ (g kg}^{-1} \text{ DM)}.$$

5.2.11. Statistical analysis

Study on the effect of type of DDGS and feedstock grain on the predicted nutrient supply to dairy cows. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). 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 sources, as a fixed effect; batch and runs as replications; and e_{ij} was the random error associated with the observation ij.

Study on the effect of bio-ethanol plant on the predicted nutrient supply to dairy cows. Statistical analyses were performed using the MIXED procedure of SAS (SAS 2005). The model used for the analysis was: $Y_{ij} = \mu + P_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ij ; μ was the population mean for the variable; P_i was the effect of bio-ethanol plant, as a fixed effect; batch as replications; and e_{ij} was the random error associated with the observation ij.

Study on the comparison of the DVE/OEB System with the NRC 2001 Model in the prediction of nutrient supply to dairy cows. A Paired t test procedure of SAS (SAS 2005), correlation analysis, and regression analysis were performed in order to establish the relationship between the DVE/OEB System and the NRC 2001 Model.

For all statistical analyses, significance was declared at $P<0.05$ and trends at $P\leq0.10$. Treatment means were compared using the Fisher's Protected LSD method.

5.3. Results and discussion

5.3.1. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on the prediction of the potential nutrient supply to dairy cattle using the DVE/OEB System

Using the DVE/OEB System, the effects of DDGS type and bio-ethanol plant on the prediction of the potential nutrient supply to dairy cattle is shown in Table 5.1. Microbial protein synthesis according to the DVE/OEB system is based on FOM. Given that wheat contained greater ($P<0.05$) amount of FOM than DDGS samples, MCP_{FOM} and $AMCP^{DVE}$ were also greater ($P<0.05$) for wheat; however, no significant differences were observed between corn and DDGS products. The content and digestibility of RUP was greater in DDGS samples relative to feedstock grains (Table 4.2 and Table 4.3), consequently $ARUP^{DVE}$ values were on average five times higher ($P<0.05$) for DDGS (wheat DDGS vs. wheat: 200 vs. 36 g kg^{-1} DM; corn DDGS vs. corn: 204 vs. 56 g kg^{-1} DM). The variation in UDM and ENDP was similar, being higher ($P<0.05$) in DDGS samples than in feedstock grains. As a result of these differences, mainly in $ARUP^{DVE}$, DVE values for DDGS were on average two times greater than those for feedstock grains (wheat DDGS vs. wheat: 249 vs. 107 g kg^{-1} DM; corn DDGS vs. corn: 251 vs. 108 g kg^{-1} DM). Similarly, DPB^{OEB} values were higher ($P<0.05$) for DDGS samples than for feedstock

Table 5.1. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in the prediction of nutrient supply to dairy cows using the DVE/OEB System

	Feed sources						Bio-ethanol Plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
1. Truly absorbed rumen synthesized microbial protein in the small intestine (g kg^{-1} DM)									
FOM	792.5 a	562.6 bc	590.2 b	528.1 c	558.1 bc	15.85	563.2	608.1	19.83
MCP_{FOM}	118.8 a	84.4 bc	88.5 b	79.2 c	83.7 bc	2.38	84.5	91.2	2.98
RDP^{DVE}	126.8 c	48.5 d	185.1 a	114.4 c	163.5 b	6.43	188.8	182.7	11.31
$MCP_{RDP}{}^{DVE}$	122.1 b	40.6 d	160.6 a	89.9 c	138.8 b	6.87	163.2	158.9	12.20
$AMCP^{DVE}$	75.8 a	53.8 bc	56.4 b	50.5 c	53.4 bc	1.52	53.9	58.2	1.90
2. Truly absorbed rumen undegraded feed protein in the small intestine (g kg^{-1} DM)									
RUP^{DVE}	47.1 c	80.1 b	247.3 a	247.1 a	249.5	9.38	258.1	240.1	18.58
$ARUP^{DVE}$	35.6 c	55.8 c	199.7 b	204.0 b	234.6 a	9.42	230.8 a	178.9 b	11.86
3. Endogeneous protein losses in the digestive tract (g kg^{-1} DM)									
UDM	59.6 b	24.3 c	97.6 a	51.5 b	94.8 a	5.06	103.9	93.5	8.36
ENDP	4.5 b	1.8 c	7.3 a	3.9 b	7.1 a	0.38	7.8	7.0	0.63
4. Total truly absorbed protein in the small intestine (g kg^{-1} DM)									
DVE	107.0 c	107.8 c	248.8 b	250.6 b	280.8 a	8.45	276.8 a	230.0 b	10.67
5. Degraded protein balance (g kg^{-1} DM)									
DPB^{OEB}	3.3 c	-43.8 d	72.1 a	10.7 c	55.1 b	5.96	78.7	67.7	10.35

SEM = standard error of mean. Means with different letters in the same row are significantly different ($P<0.05$)
CP values utilized in the calculation of $MCP_{RDP}{}^{DVE}$ and RUP^{DVE} were obtained by Leco protein/N analyzer

grains (wheat DDGS vs. wheat: 72 vs. 3 g kg^{-1} DM; corn DDGS vs. corn: 11 vs. -44 g kg^{-1} DM). The negative DPB^{OEB} value for corn indicated that, when evaluated as a single ingredient, microbial protein synthesis may be compromised because of a potential shortage of N in rumen. As shown in Table 5.1 for corn, microbial protein synthesis based on energy extracted during fermentation (MCP_{FOM}) was higher ($P<0.05$) than that from rumen degradable protein ($MCP_{RDP}{}^{DVE}$) (84 vs. 41 g kg^{-1} DM), while the opposite was observed for wheat and DDGS products. These results are in accordance with the hourly ED ratios between N and OM reported in Chapter 4, in which corn was the unique feed source that remained below the optimal ratio (25 g N kg^{-1} OM) during the entire 24 h incubation.

The results showed that the different types of DDGS had an impact on the potential nutrient supply to dairy cattle (Table 5.1). As the amount of wheat in feedstock increased, FOM and consequently $AMCP^{DVE}$ increased in DDGS samples. A similar trend was observed in UDM, and subsequently in ENDP. Blend DDGS had the highest ($P<0.05$) $ARUP^{DVE}$ (235 g kg^{-1} DM) resulting in the highest ($P<0.05$) DVE (281 g kg^{-1} DM), while DVE values for wheat DDGS and corn DDGS were similar (249 vs. 251 g kg^{-1} DM). DPB^{OEB} values for DDGS samples were all positive; wheat DDGS was the highest ($P<0.05$), followed by blend DDGS, and corn DDGS (73 vs. 55 vs. 11 g kg^{-1} DM). These results are comparable with the previously reported hourly ED ratios between N and OM (Chapter 4). According to Tamminga et al (1994), the lactation requirement of a dairy cow producing 30 kg of milk with 3 % of true protein is 1414 g per day of truly digested and absorbed protein in the small intestine, implying that 3 kg of wheat DDGS or 2.5 kg of blend DDGS in the ration (on a DM basis) would cover 50% of this requirement.

Table 5.2 shows the average predicted protein supply to dairy cows from other feeds commonly included in dairy rations when evaluated by the DVE/OEB System. The protein value (DVE) of DDGS samples was on average three times higher than alfalfa (260 vs. 75 g kg^{-1} DM) and seven times higher than timothy (260 vs. 39 g kg^{-1} DM). Compared to barley, the protein value of wheat DDGS was three times greater on average (84 vs. 249 g kg^{-1} DM). Canola meal showed a lower protein value than wheat DDGS and corn DDGS (180 vs. 249 vs. 251 g kg^{-1} DM) but a greater potential for N loss in the rumen (162 vs. 72 vs. 11 g kg^{-1} DM). Even though, it has been recently reported that corn DDGS can be replaced by canola meal in dairy cattle diets without affecting animal performance (Mulrooney et al. 2009). This fact can be

Table 5.2. The prediction of the protein supply to dairy cows from different feed sources, using the DVE/OEB System and the NRC 2001 Model

Items	Alfalfa ([1])	Timothy ([2])	Barley ([3])	Oat ([4])	Canola Meal ([5])	Soy meal ([6])
Using the DVE/OEB System (g kg DM^{-1})						
$AMCP^{DVE}$	58.4	37.2	53.4	-	55.2	61.0
$ARUP^{DVE}$	32.0	23.9	47.0	-	137.6	234.8
ENDP	15.2	23.9	16.0	-	12.8	4.3
DVE	75.3	38.9	84.4	-	180.0	291.4
DPB^{OEB}	51.7	-17.1	-6.1	-	162.1	136.5
Using the NRC 2001 Model (g kg DM^{-1})						
$AMCP^{NRC}$	50.1	26.5	45.3	53.3	58.7	59.6
$ARUP^{NRC}$	28.1	18.6	47.0	18.0	124.0	211.5
AECP	4.4	4.4	4.3	4.5	4.4	4.4
MP	82.0	49.5	96.6	75.7	187.1	275.5
DPB^{NRC}	55.5	-16.5	-30.9	-15.6	156.5	145.6

Source: [1,2]Yu et al. (2003a); [3]Yu et al. (2003b); [4]Yu et al. (2008); [5,6]Heendeniya (2008)

explained by the reduced availability of lysine in corn DDGS and the more desirable biological value of canola meal protein (Mulrooney et al. 2009). The protein value of soy meal was the most similar to DDGS samples, particularly to blend DDGS (291 vs. 281 g kg^{-1} DM). Several studies showed that similar milk production is achieved when corn DDGS or soy meal are used as protein supplements (Nichols et al. 1998; Anderson et al. 2006). Differences in the amino acid availability between the two protein sources exist; while corn DDGS are first limiting in lysine, soy meal is first limiting in methionine (Chandler 1989; Kleinschmit et al. 2007).

Between wheat DDGS from SK-Plant 1 and SK-Plant 2 (Table 5.1), significant difference was detected in the DVE value (271 vs. 230 g kg^{-1} DM), mainly due to the significant difference in $ARUP^{DVE}$ (231 vs. 179 g kg^{-1} DM). The difference in $ARUP^{DVE}$ can be further attributed to a significant difference in ADICP content (0.7 vs. 7.6 %CP) between plants and the subsequent numerical but large difference in the digestibility of RUP protein (89.4 vs. 75.9 %RUP; Chapter 4). As a result, the relationship between ADICP content and DVE value for wheat DDGS was moderate and significant ($r = -0.74$; $P<0.05$). Several studies (Arieli et al. 1989; McKinnon et al. 1995) have showed a negative relationship between the ruminal and post-ruminal availability of protein and the ADICP content resulted from heating. In DDGS, the relationship ADICP and ruminal availability of protein has been found to not exist (Klopfenstein 1996), to be moderate (Nakamura et al. 1994a), or to be strong when ADICP levels were higher than 13 %CP (Harty et al. 1998). In the present wheat DDGS samples, ADICP levels were significantly different but lower than 13 %CP, which is consistent with the lack of effect on the protein disappearance in rumen. However, the difference in the ADICP content was reflected in largely numerical differences in the digestibility of RUP, which ultimately affected the intestinal availability of RUP (Chapter 4) as well as the predicted total post-ruminal protein supply and availability. This suggests a higher sensitivity of DDGS protein disappearance to low ADICP levels in the small intestine than in the rumen, as well as the important role of ADICP in the determination of truly absorbable protein in the small intestine. The difference in ADICP content and the in subsequent DVE values has also an economic impact. In this case, 0.5 kg extra of wheat DDGS from SK-Plant 2 would be required in order to meet 50 % of the truly digested and absorbed protein in the small intestine required by a lactating dairy cow producing 30 kg of milk per day with 3 % true protein (DVE for lactation = 1414 g day^{-1}). The variation in the metabolizable essential amino acids between plants was not studied; however, Kleinschmit et al.

(2007) concluded that for corn DDGS this variation was not as prominent as in the ruminal and post-ruminal availability of protein.

5.3.2. Effects of DDGS type (wheat DDGS, corn DDGS and blend DDGS) and bio-ethanol plants on the prediction of the potential nutrient supply to dairy cattle using the NRC 2001 Model

Using the NRC 2001 Model, the effects of DDGS type and bio-ethanol plant origin on the prediction of the potential nutrient supply to dairy cattle is presented in Table 5.3. Given that RDP^{NRC} was higher than 1.18 x $MCP_{TDN}{}^{NRC}$ for wheat and DDGS samples and lower for corn, MCP^{NRC} was estimated correspondingly using $MCP_{TDN}{}^{NRC}$ and $MCP_{RDP}{}^{NRC}$ formulas, respectively. The variation of MCP^{NRC} across treatments was equally detected in $AMCP^{NRC}$, which was higher ($P<0.05$) for wheat than for wheat DDGS but lower ($P<0.05$) for corn than for corn DDGS. DDGS samples had greater content and digestibility of RUP^{NRC} resulting in on average fivefold greater ($P<0.05$) $ARUP^{NRC}$ values than those for feedstock grains (wheat DDGS vs. wheat: 180 vs. 32 g kg^{-1} DM; corn DDGS vs. corn: 184 vs. 50 g kg^{-1} DM). This difference largely contributed to MP values for DDGS that were three times higher ($P<0.05$) on average than those for feedstock grains (wheat DDGS vs. wheat: 242 vs. 100 g kg^{-1} DM; corn DDGS vs. corn: 250 vs. 81 g kg^{-1} DM). For DPB^{NRC}, values were higher ($P<0.05$) for DDGS samples than for feedstock grains (wheat DDGS vs. wheat: 78 vs. 9 g kg^{-1} DM; corn DDGS vs. corn: -12 vs. -76 g kg^{-1} DM); however, it should be noted that both corn and corn DDGS had negative values. The negative balance is attributed to a greater rumen availability of energy from total digestible nutrients rather than degradable protein, indicating a shortage of N when corn and corn DDGS are evaluated as single ingredient. This result is in agreement with the hourly ED ratios between N and OM reported in Chapter 4 for corn, but not for corn DDGS, which exhibited an excess of N in rumen almost during the entire 24 h incubation. This discrepancy is explained by the fact that DPB^{NRC} calculation is based on TDN rather than fermented organic matter. As shown in Table 5.3, TDN_{3X} value for corn DDGS was as high as for corn, resulting in higher $MCP_{TDN}{}^{NRC}$ and lower DPB^{NRC} relative to other feed sources. Despite the conceptual difference between DPB^{NRC} and hourly ED ratios between N and OM, both parameters concurred that corn DDGS was the closest among DDGS samples to the optimal value (0 g kg^{-1} DM and 25 g N kg^{-1} OM),

Table 5.3. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in the prediction of nutrient supply to dairy cows using the NRC 2001 Model

	Feed sources						Bio-ethanol Plant		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-Plant 2 Wheat DDGS n = 3	SEM
1. Truly absorbed rumen synthesized microbial protein in the small intestine (g kg^{-1} DM)									
TDN_{3X} (%DM)	76.6 b	81.1 a	69.8 d	82.4 a	73.7 c	0.57	71.9 a	69.5 b	0.88
RDP^{NRC}	126.8 c	48.5 d	185.1 a	114.4 c	163.5 b	6.43	188.8	182.7	11.31
MCP^{NRC}	99.6[z] a	41.2[y] c	90.8[z] b	97.3[z] a	95.8[z] a	1.58	93.4[z] a	89.0[z] b	1.15
$AMCP^{NRC}$	63.7 a	26.4 c	58.1 b	62.2 a	61.3 a	1.01	59.8 a	57.0 b	0.74
2. Truly absorbed rumen undegraded feedl protein in the small intestine (g kg^{-1} DM)									
RUP^{NRC}	42.4 c	72.2 b	222.8 a	222.6 a	224.8 a	8.45	232.5	216.3	16.74
$ARUP^{NRC}$	32.1 c	50.2 c	179.9 b	183.8 b	211.3 a	8.49	207.9 a	161.2 b	10.68
3. Truly digested rumen endogeneous protein in the small intestine (g kg^{-1} DM)									
ECP	10.6 c	10.5 c	11.1 a	10.9 b	10.9 b	0.05	11.0 b	11.2 a	0.08
AECP	4.3 c	4.2 c	4.5 a	4.3 b	4.4 b	0.02	4.4 b	4.5 a	0.03
4. Total truly absorbed protein in the small intestine (g kg^{-1} DM)									
MP	100.1 c	80.8 c	242.4 b	250.3 b	276.9 a	8.82	272.1 a	222.6 b	10.93
5. Degraded protein balance (g kg -1 DM)									
DPB^{NRC}	9.3 c	-75.9 e	78.0 a	-12.1 d	50.5 b	6.01	78.5	77.7	10.18

SEM = standard error of mean. Means with different letters in the same row are significantly different ($P<0.05$)
CP values utilized in the calculation of RDP^{NRC} and RUP^{NRC} were obtained by Leco protein/N analyzer
[z] Predicted according to $MCP_{TDN}{}^{NRC}$
[y] Predicted according to $MCP_{RDP}{}^{NRC}$

in which the balance between microbial protein synthesis potentially possible from available rumen degradable protein and that potentially possible from energy is achieved.

Comparing the three types of DDGS (Table 5.3), MCP^{NRC} values increased as corn in feedstock increased. $AMCP^{NRC}$ for corn DDGS and blend DDGS was similar ($P>0.05$) and higher ($P<0.05$) than that for wheat DDGS. $ARUP^{NRC}$ was the greatest ($P<0.05$) for blend DDGS (211 g kg^{-1} DM) and similar ($P>0.05$) between wheat DDGS and corn DDGS (average 182 g kg^{-1} DM), while AECP was the greatest ($P<0.05$) for wheat DDGS and similar ($P>0.05$) between corn DDGS and blend DDGS. These differences resulted in the highest ($P<0.05$) MP for blend DDGS (277 g kg^{-1} DM) and in similar ($P>0.05$) values between wheat DDGS and corn DDGS (average 246 g kg^{-1} DM). DPB^{NRC} was the greatest ($P<0.05$) for wheat DDGS, followed by blend DDGS and corn DDGS (78 vs. 51 vs. -12 g kg^{-1} DM). For a dairy cow producing 30 kg of milk per day containing 3 % of true protein, NRC 2001 estimates a daily requirement of 1343 g MP, which would be met by including 3 kg of wheat DDGS or 2.5 kg of blend DDGS (on a DM basis).

Table 5.2 shows other feeds previously evaluated by the NRC 2001 Model. The protein value (MP) of DDGS samples was on average three times higher than alfalfa, five times higher than timothy, and three times higher than barley and oat (257 vs. 82 vs. 50 vs. 97 vs. 76 g kg^{-1} DM). When comparing with other protein supplements, the MP value of DDGS samples was higher than canola (257 vs. 187 g kg^{-1} DM), and it was very similar to soy meal (257 vs. 276 g kg^{-1} DM). Blend DDGS was almost identical to soy meal in both $ARUP^{NRC}$ (211 vs. 212 g kg^{-1} DM) and MP (277 vs. 276 g kg^{-1} DM). Despite these similarities, MP value does not contemplate the entire biological value of the protein. Therefore, other factors such as the availability of limiting amino acids must be considered.

The significant difference between wheat DDGS from SK-Plant 1 and SK-Plant 2 (Table 5.3) in $ARUP^{NRC}$ (208 vs. 161 g kg^{-1} DM) resulted in a significant difference in MP (272 vs. 223 g kg^{-1} DM). The relationship between ADICP content and MP value was moderate and significant ($r = -0.74$; $P<0.05$). Moreover, small but significant differences were detected in $AMCP^{NRC}$ and AECP due to numerical differences in the TDN value and DM content, respectively (Chapter 3). In accordance with Chapter 4 and the DVE/OEB System, the supply and availability of protein in the small intestine was more sensitive than the rumen to the difference in low ADICP levels of wheat DDGS samples. The difference in the ADICP content

between plants is largely the result of differing processing conditions, likely in the time and temperature of drying. The difference in the MP values between wheat DDGS from SK-Plant 1 and from SK-Plant 2 (49 g kg^{-1} DM) will result in differences in animal performance at the same inclusion level.

5.3.3. Comparison of predictions from the DVE/OEB System and the NRC 2001 Model

The average of the predicted values for wheat DDGS, corn DDGS and blend DDGS calculated according to the DVE/OEB System and the NRC 2001 Model as well as the difference and correlations between the two models are presented in Table 5.4. The results showed that the predicted values from the DVE/OEB System were 10 % lower ($P<0.05$) in the truly absorbable rumen synthesized microbial protein in the small intestine, 10% higher ($P<0.05$) in the truly absorbed bypass feed protein in the small intestine, 30 % higher ($P<0.05$) in the endogenous protein, and 2% higher ($P<0.05$) in the total truly absorbed protein in the small intestine than the predicted values from NRC 2001 Model. No significant difference was detected in terms of the degraded protein balance between the two models.

These differences are attributed to differing concepts and factors utilized in calculations by each of the models. A striking dissimilarity affecting the truly absorbable rumen synthesized microbial protein in the small intestine is the prediction of the microbial protein synthesis in the rumen; while the calculation by the DVE/OEB System is based on fermented organic matter, the calculation by NRC 2001 Model is based on total digestible nutrients. This was verified by the significant average difference (-6 g kg^{-1} DM) and a weak correlation ($r = -0.44$; $P<0.05$) between the two models in terms of the truly absorbable rumen synthesized microbial protein in the small intestine. In addition, it was clearly detected when comparing wheat DDGS with corn DDGS (Table 5.1 and Table 5.3); whereas $AMCP^{DVE}$ was higher in wheat DDGS due to the higher FOM, $AMCP^{NRC}$ was higher in corn DDGS due to the higher TDN. It is important to mention that the FOM approach does not consider the fat fraction as ruminally degradable, thus eliminating it from the calculation and contributing to the lower $AMCP^{DVE}$ compared to $AMCP^{NRC}$ for corn DDGS. Other minor differences affecting the amount of truly absorbable rumen synthesized microbial protein in the small intestine are the assumed amount of rumen synthesized microbial protein per kg of available energy (150 g MCP kg^{-1}FOM vs. 130 g MCP

Table 5.4. Comparison of the DVE/OEB System with the NRC 2001 Model in the prediction of protein supply to dairy cows from DDGS

Items ($g\ kg\ DM^{-1}$)	Mean DVE/OEB	Mean NRC 2001	Numeric difference[z]	SED[y]	P value	r[x]	P value
$AMCP^{DVE}$ vs. $AMCP^{NRC}$	54.0	60.1	-6.1	1.27	<0.0001	-0.44	0.0405
$ARUP^{DVE}$ vs. $ARUP^{NRC}$	210.4	189.5	20.9	0.66	<0.0001	1.00	<0.0001
ENDP vs. AECP	6.3	4.4	1.9	0.39	<0.0001	0.45	0.0371
DVE vs. MP	258.0	254.0	4.0	0.79	<0.0001	0.99	<0.0001
DPB^{OEB} vs. DPB^{NRC}	50.7	45.9	4.8	2.73	0.0957	0.97	<0.0001

[z]Paired t test (n = 11)
[y]Standard error of the difference
[x]Pearson correlation (n = 11)

kg^{-1} TDN) as well as the amount (0.75 vs. 0.80 %) and digestibility (0.85 vs. 0.80 %) of the true protein contained in the rumen synthesized microbial protein.

The truly absorbed bypass feed protein in the small intestine is calculated in the two models as bypass feed protein times the digestibility of feed protein in the intestine. However, the prediction of bypass feed protein differs between models; while the DVE/OEB System uses a regression coefficient (1.11) to correct the *in situ* degradation data on *in vivo* RUP results, no correction factor is used by the NRC 2001 Model. As a result, the highest significant difference on average (21 g kg^{-1} DM) but also the strongest correlation ($r = 1$; $P<0.05$) was observed between $ARUP^{DVE}$ and $ARUP^{NRC}$. Similarly, the greatest difference between the two models when evaluating other sources of RUP (canola meal and soy meal) was found in the truly absorbed bypass feed protein in the small intestine (Table 5.2).

Another notable difference is the concept and calculation of endogenous protein in the digestive process. In the DVE/OEB System, the truly digested and absorbed protein in the small intestine is corrected for endogenous protein losses. The assumed net loss of metabolic protein is 50 g kg^{-1} of undigested dry matter as the efficiency of resynthesis is 0.67, hence, the correction factor (0.075= 0.05/0.67) used in the ENDP formula. In the NRC 2001 Model, endogenous protein losses pass to small intestine contributing to the truly digested and absorbed protein. The same model assumes that the loss of metabolic protein is associated with dry matter content, 50% of the losses in rumen passes on to the small intestine, and 80% of the losses is true protein. As a result of these dissimilarities, a significant average difference (2 g kg^{-1} DM) and weak correlation ($r = 0.45$; $P<0.05$) were obtained between ENDP and AECP.

These differences between the two models in the concepts and calculations of the truly absorbable rumen synthesized microbial protein in small intestine, the truly absorbed bypass feed protein in the small intestine, and the endogenous protein losses contributed to a significant average difference (4 g kg^{-1} DM) in the prediction of the truly digested and absorbed protein in the small intestine. Despite this, the correlation was strong ($r = 0.99$; $P<0.05$) due to the fact that the range of the predicted values was similar.

In the prediction of the degraded protein balance, the DVE/OEB System assumes that 100% rumen degraded feed protein can potentially be converted to microbial protein if enough energy is supplied. The NRC 2001 Model, however, assumes that only 85% of rumen degraded feed protein can be converted by rumen microorganisms into protein, thus the factor (1.18 = 1.00

0.85) used in the American system. Moreover, the concept and calculation of the predicted microbial protein synthesis in the rumen from available energy (MCP_{FOM} vs. MCP_{TDN}^{NRC}) and the microbial protein synthesis in the rumen from available degraded protein (MCP_{RDP}^{DVE} vs. RDP^{NRC}) differed. MCP_{TDN}^{NRC} contemplates more energy in the equation than MCP_{FOM}, consequently more nitrogen is required to achieve optimal availability of nitrogen and energy in the rumen according to the NRC 2001 Model. The 1.11 correction factor is taken into consideration for the calculation of MCP_{RDP}^{DVE} but not for RDP^{NRC}. These differences generated positive DBP^{OEB} and negative DPB^{NRC} for corn DDGS, indicating N loss and N shortage respectively. Despite these factors, the average difference between the two models was not significant (5 g kg^{-1} DM), and correlation was strong ($r = 0.97$; $P<0.05$) due to the similar range of predicted values. Indeed, both models concluded that among DDGS samples studied, corn DDGS was the closest one to the optimal balance, which, at the same time, was approached by increasing the amount of corn relative to wheat in feedstock.

Linear regressions of the main average predicted nutritional values between the DVE/OEB System and the NRC 2001 Model for DDGS samples are presented in Table 5.5. All regression equations were significant. In addition, a high proportion of the variability in the truly absorbed rumen bypass feed protein in small intestine ($R^2 = 1.00$), truly digested and absorbed protein in small intestine ($R^2 = 0.99$) and degraded protein balance ($R^2 = 0.96$) predicted according to the DVE/OEB System can be accounted by the equivalent parameters predicted by the NRC 2001 Model.

Table 5.5. Regression equations for the prediction of protein supply from the DVE/OEB System based on the predicted values from the NRC 2001 Model

Items (g $kg\ DM^{-1}$)	Regression[z]	R^2 [y]	P value	RSD[x]
$AMCP^{DVE}$ vs. $AMCP^{NRC}$	$AMCP^{DVE} = 90.58 – 0.61 \times AMCP^{NRC}$	0.19	0.0405	3.72
$ARUP^{DVE}$ vs. $ARUP^{NRC}$	$ARUP^{DVE} = 1.11 \times ARUP^{NRC}$	1.00	<0.0001	0.00
ENDP vs. AECP	ENDP = -39.03 + 10.31 x AECP	0.20	0.0371	1.71
DVE vs. MP	DVE = 14.83 + 0.96 x MP	0.99	<0.0001	3.58
DPB^{OEB} vs. DPB^{NRC}	$DPB^{OEB} = 16.93 + 0.73 \times DPB^{NRC}$	0.96	<0.0001	6.50

[z]Linear regression equation (n = 11)
[y]R^2 = Coefficient of determination
[x]RSD = Residual standard deviation

5.4. Conclusions

Dried distillers grains with solubles are a good source of truly digested and absorbed protein in small intestine in dairy rations. According to the DVE/OEB System and the NRC 2001 Model, the predicted protein supply differed significantly among wheat DDGS, corn DDGS and blend DDGS and, to a lesser extent, between different bio-ethanol plants. Therefore, it is inappropriate to assume fixed protein values for DDGS without considering factors such as DDGS type and plant origin. The sensitivity between the two models to detect differences among DDGS types and between plants was similar. The two models coincided in predicting the superior protein value (DVE and MP) of blend DDGS as well as in identifying the more optimal degraded protein balance for corn DDGS. In addition, the difference in the ADICP content of wheat DDGS samples as a result of different ethanol operation decisions between plants was reflected in differing protein value, suggesting the use of ADICP is a reliable indicator for the protein value of wheat DDGS for sample comparison purposes.

It is important to remember that the current experiment evaluated DDGS as a single ingredient, thus the modeling of a total mixed ration containing different types and levels of DDGS would provide a more realistic approach of the differences in the ruminal and post-ruminal supply and availability of protein as well as of the differences in the balance between available nitrogen and energy in rumen. Also, DVE and MP values do not contemplate the entire biological value of the protein. Therefore, other factors such as the availability of limiting amino acids must be carefully considered.

Although differences between the DVE/OEB System and the NRC 2001 Model were significant for most outputs due to differences in some of the concepts and factors used in the calculations, correlations between DVE and MP values and between DPB^{OEB} and DPB^{NRC} were also significant. Moreover, the two protein evaluations systems are both supply and requirement models, involving that the requirement values also differ. The study of the nutrient balance (model requirement minus model supply) would provide a better indication of the differences between the two models.

6. GENERAL DISCUSSION

The general objective of this project was to investigate the effects of type and bio-ethanol plant origin in terms of the chemical and biological characteristics of dried distillers grains with solubles (DDGS). To achieve this, three types of DDGS currently utilized in ruminant diets in western Canada (wheat DDGS, corn DDGS, and a blend (wheat:corn = 70:30) DDGS), as well as wheat DDGS manufactured at two different bio-ethanol plants (SK-Plant 1 and SK-Plant 2), were evaluated using eight common feed evaluation methods: 1) chemical analysis, 2) Cornell Net Carbohydrate and Protein System (CNCPS), 3) energy values estimation using the NRC 2001-chemical approach, 4) energy values estimation using an in situ assay-biological approach, 5) in situ digestibility trial, 6) in vitro intestinal digestibility of rumen bypass protein (RUP) as outlined by Calsamiglia and Stern (1995), 7) prediction of the protein supply to small intestine using the NRC 2001 Model, and 8) prediction of the protein supply to small intestine using the DVE/OEB System. The parameters obtained from each of these methods were also studied in feedstock grains as compared to their derived DDGS product. In addition, the validity of the chemical profile to determine energy values and predict the potential degradability of DDGS was tested.

Due to the starch removal during processing, the chemical components of DDGS samples were generally threefold higher than in feedstock grains. Still, some unconverted starch was present in DDGS samples (on average 4.9 %DM), suggesting starch resistance or ineffectiveness of the processing conditions. Compared to feedstock grains, and in accordance with previous studies, the protein profile in DDGS samples was characterized by reduced soluble crude protein (SCP), increased neutral detergent insoluble crude protein (NDICP), and increased acid detergent insoluble crude protein (ADICP). As a result, the CNCPS System showed a reduction in rapidly degradable non-protein nitrogen (PA), rapidly degradable protein (PB1), and intermediately degradable protein (PB2), and an increase in the slowly degraded protein (PB3) and unavailable protein (PC) of DDGS, suggesting decreased overall rumen degradability relative to feedstock grain. These shifts in the protein profile between feedstock grains and the derived DDGS product may be attributed to events occurring during processing, such as partial protein degradation during fermentation and Maillard product formation as a result of heating. The CNCPS profile among the three types of DDGS varied due to differences in the parental grains as well as

differences in the processing conditions among the bio-ethanol plants. While wheat DDGS and blend DDGS were characterized by higher PB3 and rapidly fermented carbohydrates (CA) than corn DDGS, the latter was characterized by higher PB2 and slowly degraded carbohydrates (CB2). Although RUP is mainly represented by PB3 and PC, some protein in PB2 escapes to small intestine. Thus, it is necessary to look at the in situ degradation data in order to conclude more accurately what type of DDGS is providing higher RUP.

The energy values estimated according to the NRC 2001-chemical approach differed among the three types of DDGS largely due to differences in the truly digestible fatty acids (tdFA) and neutral detergent fiber (tdNDF). The energy content of corn DDGS was the highest, followed by blend DDGS and wheat DDGS. Corn DDGS values were gretaer than corn, wheat DDGS values were similar to wheat and corn, and blend DDGS values were similar to corn. Thus, wheat DDGS can be used as an alternative to wheat and corn in dairy and beef diets, while blend DDGS can be used as an alternative to corn.

Results from the in situ degradability trial showed a reduction in the effective degradability of dry matter (%EDDM), organic matter (%EDOM), and crude protein (%EDCP) of wheat DDGS relative to wheat; however, corn DDGS remained the same as corn. This can be explained by the lower effective degradability of these feed components in corn relative to wheat as well as by differences in plant processes. Rumen undegraded protein (RUP, %CP) was on average twofold higher in wheat DDGS relative to wheat, while it was similar between corn DDGS and corn. Thus, it is important to clarify that only the chemical components, and not the nutrient components (such as RUP), become threefold concentrated in the DDGS product in comparison with feedstock grain. Among DDGS types, %EDDM, %EDOM and %EDCP increased with increasing the proportion of wheat in feedstock. In addition, the protein content in wheat DDGS was higher than in corn DDGS and blend DDGS. As a result, the availability of total rumen degradable protein (g kg^{-1}) was the highest for wheat DDGS while the total supply of RUP (g kg^{-1}) was similar among the three types of DDGS. The hourly effective degradability (ED) ratios between nitrogen (N) and energy indicated some potential loss of N in rumen when the three types of DDGS were evaluated as a single ingredient. Albeit far from the optimal ratio, corn DDGS was the closest to optimal, as it provided a lower amount of ruminal effective degradable protein and higher energy than wheat DDGS and blend DDGS. This excess of N implies that elevated levels of DDGS in ruminant rations may have repercussions both on animal

performance and on environment. Although the effective degradability of NDF (%EDNDF) was similar between feedstock grains and DDGS samples, the total amount of effective degradable NDF was on average twofold greater in DDGS. This increased NDF availability, along with the threefold concentration of fat, is what provided DDGS with energy values similar to those in feedstock grains.

The high RUP in DDGS samples largely contributed to the higher protein value relative to feedstock grains. In a single feed evaluation, both DVE/OEB System and NRC 2001 Model coincided in identifying the superior protein value of blend DDGS, followed by similar values between wheat DDGS and corn DDGS. These differences among DDGS types are mainly attributed to differences in the estimated intestinal digestibility of RUP (IDP), which was significantly higher in blend DDGS. In accordance with the hourly ED ratios between N and OM, the DVE/OEB System showed that the degraded protein balance (DPB) was positive for the three types of DDGS and increased as the wheat proportion in the feedstock increased. However, the NRC 2001 Model showed a negative balance for corn DDGS, indicating a shortage of N that can impair microbial protein synthesis. This discrepancy is explained by the fact that while DPB^{DVE} and hourly ED ratios are calculated based on fermented organic matter, DPB^{NRC} is based on TDN value. As a result, the high TDN value of corn DDGS shifted the balance towards higher microbial protein synthesis based on energy supply rather than towards higher microbial protein synthesis based on rumen degradable protein, as observed for wheat DDGS and blend DDGS.

Plant to plant variation was detected in wheat DDGS samples. These differences are mainly attributed to differences in the parental wheat, in the amount of solubles blended back with wet distillers, and in the processing conditions. The ADICP levels were 0.7 and 7.6 %CP for wheat DDGS from SK-Plant 1 and SK-Plant 2, respectively. In addition to higher ADICP content, wheat DDGS from SK-Plant 2 exhibited higher lignin and lower hemicellulose, suggesting more severe heating conditions in this plant. Literature has shown that ADICP content is negatively related to protein degradability in the rumen and the small intestine; however, this relationship was studied in corn DDGS samples and was only effective when ADICP values were above 13% CP. As a result, the low but significantly different ADICP values did not caused an effect on the protein degradability of wheat DDGS samples in rumen. Differences in intestinal digestibility (IDP), however, were non-significant but numerically large,

indicating that the disappearance of DDGS protein is more sensitive to low ADICP levels in the small intestine than in the rumen. As a matter of fact, correlation analysis showed that ADICP is negatively related to protein digestibility in the small intestine but not in the rumen. For both the DVE/OEB System and the NRC 2001 Model, and mainly due to the large difference in IDP values, the truly absorbable rumen bypass protein (ARUP) was significantly different between wheat DDGS samples. This contributed to the significantly higher protein value (DVE and MP) in wheat DDGS from SK-Plant 1. In addition, both models showed the same negative and moderate correlation between ADICP and protein values (DVE and MP), implying that low ADICP levels do provide an indication of the protein value of wheat DDGS samples.

Although the predicted energy values using the NRC 2001-chemical approach and the *in situ* assay-biological approach were similar, results indicated that NRC 2001-chemical approach is not a reliable predictor of tdNDF and tdCP. The NRC 2001-chemical approach calculates tdNDF based on the acid detergent lignin content (ADL); however, only wheat DDGS samples showed a negative and strong relationship between ADL and NDF digestibility. In forages, this relationship is stronger as the ADL content increases. Thus, the accuracy of the NRC 2001-chemical approach to predict tdNDF in DDGS samples may require ADL levels similar or higher to those detected in wheat DDGS samples. Similarly, tdCP value depends on the ADICP content, which in turn is negatively correlated with ruminal protein digestibility only when levels are greater than 13%CP. Therefore, the low ADICP levels in the current experiment suggest a lack of accuracy of the NRC 2001-chemical approach to determine tdCP.

Particle size distribution has been shown to affect disgestibility and nutrient availaibility in the animal. In addition, if a large proportion of particles are smaller than the pore size of the filter paper (20 - 25 μm), ankom bag (25 μm), or nylon bag utilized (41 μm), chemical components such as fiber can be underestimated while digestibility values can be overestimated. Shurson (2004) showed that in the typical particle size distribution of corn DDGS, more than 10% of the particles are retained in a sieve opening of 37 μm. Although particle size distribution was not measured in the present experiment, data reported by Shurson (2004) would suggest that degradability values resulted from the *in situ* trial could be slightly overestimated; however, a later evaluation using wheat DDGS samples observed that the amount of insoluble particles contained in the washable fraction was minimal (N. Zhi-yuan, personal communication). In

addition, a pore size bigger than particle size does not involve the complete loss of small particles from the bag.

Therefore, results from this study clearly indicate that is not correct to assume fixed nutrient values for DDGS in ration formulation without considering factors such as DDGS type and bio-ethanol plant origin. Results have shown the role played by ADICP in the estimation of the metabolizable protein supplied to the dairy cow. This information can be used by nutritionists to increase accuracy in ration formulation, as well as by bio-ethanol companies in order to achieve better product quality consistency. For the former, it is recommended to check the chemical profile periodically, particularly ADICP levels, in order to track the quality of the DDGS product obtained from each plant and formulate rations accordingly. For the latter, it is important to control drying temperature and time in order to decrease ADICP levels in the DDGS product. Low differences in the ADICP levels of different DDGS sources will impact animal performance by achieving a more consistent supply of metabolizable protein.

For the future, much additional information is required in order to increase the accuracy of the current feed evaluation systems when evaluating the nutritive value of DDGS. As stated earlier, literature has shown the relationship between ADICP content and protein digestibility in the rumen and the small intestine in corn DDGS samples. Thus, further research could include the use of wheat DDGS samples with a higher range of ADICP levels to establish the specific relationship between the ADICP content and the potential digestibility of wheat DDGS protein. Similarly, refinement of the tdNDF formula used by the NRC 2001-chemical approach is required, not only to evaluate DDGS, but also other feeds. As suggested in the literature, not only the content of lignin but also the extent to which lignin is bonded to other components of the cell wall affects NDF digestibility. Currently, Dr. VanAmburgh' group at Cornell University is looking at the effect of lignin-carbohydrate linkages, in particular ester and ether linked phenolic acids like ferulic and p-coumaric acids, on NDF degradability. The effect of these hydroxycinnamic acids on rumen degradation characteristics of barley has been recently investigated by Dr. Yu's group, at University of Saskatchewan, and will be available soon.

Results from this study have shown nutrient variation among DDGS types and between different bio-ethanol plants; however, information on the nutrient profile of DDGS affected by different batches within the same plant has not been published yet. Similarly, the effect of bio-ethanol plant on protein molecule structure and the subsequent effect on nutrient availability are

still unknown. These two studies are being currently performed at University of Saskatchewan by Dr. Yu's group.

In the present project, and in order to investigate the rumen degradation characteristics of DDGS, samples were rolled before rumen incubation. This processing removed the presence of syrup balls; however, these agglomerates are present in the DDGS product fed to cattle, particularly in wheat DDGS batches produced by some plants. As rumen outflow rate, and consequently digestibility, is affected by particle size and density; the effect of these physical parameters on the rumen degradation characteristics of DDGS needs to be investigated for proper ration formulation.

Lysine and methionine are the first and second limiting amino acids in DDGS, respectively. Thus, nutritionists are aware of the special consideration that the lysine to methionie ratios must have when including DDGS in the rations. For concentrates, the degradative behavior of lysine in the rumen and intestine was similar to that of protein, while methionine followed the same pattern as protein in the rumen but the absorption was slightly higher (4%) in the intestine (Tamminga et al. 2007). The question is whether DDGS samples behave the same as other concentrates. Thus, further studies investigating the metabolizable lysine and methionine affected by DDGS type and bio-ethanol plant will help to increase accuracy in ration formulation by providing the proper amino acid balance.

The supplementation of corn DDGS diets with rumen protected lysine and methionine has increased milk and protein yields, while replacing corn DDGS with other protein supplements of higher biological value, such as canola meal, has tended to increase these production parameters. At the present, information on the effect of amino acid supplementation in wheat-based DDGS diets is not available. Dr. Mutsvangwa, at the University of Saskatchewan, is currently conducting a trial to investigate the effect of extruding wheat DDGS along with peas or canola meal on milk production parameters

Animal performance is the most important factor determining the acceptability of DDGS as protein supplement for dairy rations. While results obtained in the present study showed the potential loss of N when DDGS are included in the ration, literature has shown that inclusion levels up to 30% of corn DDGS can be included without detecting a negative effect on milk production and N excretion. However, the excess of N may also represent a risk for reproductive performance, which is also a determining factor of the economical viability of the dairy

operation. Thus, it could be of interest to investigate the effect of DDGS inclusion rate, in particular wheat DDGS, on the reproductive performance of commercial farms.

Several studies have recently shown an increased ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) in meat with increasing the levels of corn DDGS, suggesting that some of the fat in corn DDGS can be protected from rumen biohydrogenation. It seems that the amount of solubles blended back has a direct effect on the PUFA:SFA ratio; however, information is still scarce. Likewise, the fatty acid profile of milk as affected by the amount of solubles blended back has not been investigated yet.

In addition, the efficiency of ethanol extraction in new-generation bio-ethanol plants still can be firther optimized. The results from the present study showed residual starch in all DDGS samples. The presence of this unconverted starch can be attributed to resistance to degradability or to ineffectiveness of the processing conditions. Thus, further studies investigating the effect of granule structure and amylose:amylopectin ratio on starch degradability in both ruminants and monogastrics will help to clarify the nature of this residual starch. (Dong and Rasco 1987a; Shurson 2004)

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8. APPENDIX

Table 8.1. Total mixed ration (%DM) fed to non-lactating Holstein cows fitted with a rumen cannula	
Barley silage	56.8
Alfalfa hay	20.2
Dehydrated alfalfa pellets	4.5
Standard dairy concentrate	21.6
Fresh cow concentrate	6.8
Standard dairy concentrate[z, y] (%DM)	
Barley	56.0
Wheat	5.0
Oats	5.0
Dairy supplement pellets	33.0
Molasses	1.0
Fresh cow concentrate[x,w] (%DM)	
Barley	51.05
Oats	5.0
Canola meal	11.6
Soybean meal	10.0
Wheat distillers dried grains	9.0
Corn gluten meal	3.0
Molasses	2.5
Golden flakes[v]	2.5
Canola oil	0.5
Mineral-vitamin mix[t]	3.0
Niacin-magnesium mix[s]	0.3
Cobalt-iodized salt	0.6
Sodium bicarbonate	0.6
Ground limestone	0.3
Dynamate[r]	0.05

Cows was fed twice daily at 8:00 and 16:00 by receiving 14 kg (7 kg at each feeding time) of the total mixed ration.

[z]Grains were dry rolled and mixed with supplement pellets.

[y]Proximate composition: 18.5% crude protein, 0.7% calcium, 0.8% phosphorus (DM basis).

[x]0.48 cm (3/16") pellets

[w]Proximate composition: 22% crude protein, 0.9% calcium, 0.85% phosphorus (DM basis).

[v]Dried fat supplement (Malaysian palm oil) distributed in Western Canada by Prairie Micro-Tech Inc., Regina, Saskatchewan.

[t]Formulated to provide 45 mg manganese, 63 mg zinc, 17 mg copper, 0.5mg selenium, 11000 I.U. vitamin A, 1800 I.U. Vitamin E per kg of dairy concentrate. The mix also contributes 0.14% magnesium, 0.48% calcium, 0.26% phosphorus, 0.23% sodium and 0.38% chloride to the total dairy concentrate. Prepared by Federated Cooperatives Ltd., Saskatoon, Saskatchewan.

[s]Formulated to provide one gram of niacin and 0.3 grams of magnesium per kg of fresh cow concentrate.

[r]Contains 22% sulphur, 18% potassium, 11% magnesium (International Minerals and Chemical Corp., Mundelein, ILL).

Table 8.2. Comparison of wheat and corn in terms of *in situ* rumen characteristics of starch

	Feed sources		
Items	Wheat	Corn	SEM
T_0 (h)	0.00	0.00	
S (%)	22.76 a	2.99 b	3.384
D (%)	77.24 b	97.00 a	3.384
Kd (% h^{-1})	43.48 a	7.55 b	3.246
EDStarch (%)	87.99 a	54.99 b	2.965
EDStarch (g kg^{-1}DM)	530.89 a	350.51 b	22.218

SEM = standard error of mean. Means with the different letters in the same row are significantly different ($P<0.05$)
Starch model according to Tamminga et al. (1994)
Samples ground through a 0.5 mm screen

Table 8.3. Comparison of wheat DDGS, corn DDGS and blend DDGS (wheat: corn=70:30) and comparison of different bio-ethanol plants in terms of the hourly effective degradability ratios between N and OM (g N kg^{-1} OM)

	Feed Sources						Bio-ethanol Plant effect		
Items	Wheat Grain n = 3	Corn Grain n = 3	Wheat DDGS n = 5	Corn DDGS n = 3	Blend DDGS (W:C=70:30) n = 3	SEM	SK-Plant 1 Wheat DDGS n = 2	SK-plant 2 Wheat DDGS n = 3	SEM
0h	29.7 a	0.00c	26.4 a	14.3 bc	29.5 ab	6.327	41.6 a	16.2 b	5.139
2h	22.5 c	12.1 d	73.0 a	57.8 b	62.1 b	2.556	77.1	70.2	2.645
4h	29.9 c	12.8 d	75.1 a	57.6 b	64.0 b	2.228	77.7	73.3	2.342
8h	54.5 c	14.5 d	79.7 a	57.2 c	68.0 b	2.570	79.0	80.1	1.771
12h	104.1 a	16.3 d	84.7 ab	56.8 c	72.4 bc	8.389	80.4 b	87.5 a	1.522
24h	990.9 a	23.7 b	102.8 b	55.9 b	88.5 b	220.398	84.6 b	114.9 a	4.687

SEM=standard error of mean. Means with the different letters in the same row are significantly different ($P<0.05$).

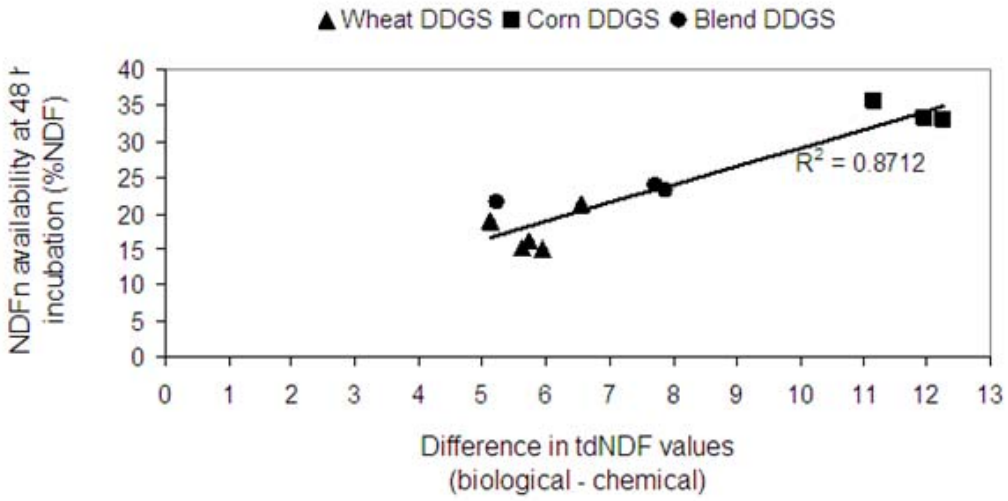

Figure 8.1. Differences between the *in situ* assay-biological approach and the NRC 2001-chemical approach in the truly digestible neutral detergent fiber (tdNDF) values of DDGS samples. NDF availability estimated as NDFn digestibility after 48 h incubation × NDFn (%DM)