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Effect of dried distillers' grains with solubles on greenhouse gas emissions from beef cattle

by

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ABSTRACT

Four experiments were conducted to determine the impact of dried distillers' grains with solubles (**DDGS**) on greenhouse gas (**GHG**) emissions from beef cattle. The first compared *in vitro* methane (**CH_4**) production from corn DDGS (CDDGS, ~30% crude protein [**CP**]) and wheat DDGS (**WDDGS**, ~40% CP dry matter [**DM**]). Wheat DDGS or CDDGS replaced barley silage at 20, 40, 60, 80 and 100% DM. Methane (mg CH_4/g DM) was lower for CDDGS than WDDGS at up to 80% DM. In a second experiment, heifers fed a growing (high silage) diet showed a reduction in CH_4 (g CH_4/kg DM intake [**DMI**]) when 35% barley grain and 5% canola meal DM were replaced with CDDGS (10.0% fat DM). Inclusion of 40% WDDGS (4.1% fat DM) had no effect on enteric CH_4 emissions. In contrast, feeding 40% DM WDDGS with added corn oil (9.5% fat DM) reduced CH_4 to the same extent as CDDGS. In a third experiment, replacing 40% DM barley grain with CDDGS (9.7% fat DM) in a finishing (high grain) diet reduced CH_4 (g/kg DMI). Whereas feeding 40% DM WDDGS along with corn oil (9.9% fat DM) resulted in similar CH_4 losses as CDDGS. Results from both *in vitro* and *in vivo* experiments indicate that the higher fat content of CDDGS vs. WDDGS was responsible for CH_4 reductions. The benefit of replacing 40% DM barley grain with CDDGS or WDDGS on GHG emissions from beef production was further evaluated using life cycle assessment. Replacing barley grain with CDDGS or WDDGS increased N intake and subsequently N excretion. Increased N excretion was predicted to outweigh reductions in CH_4 through increased formation of nitrous oxide (**N_2O**). Therefore, feeding CDDGS and WDDGS resulted in 6.3 and 9.3% higher GHG

intensity (kg CO_2 equivalent [**CO_2e**]/kg beef) compared to the control. To reduce the environmental impact, DDGS should not be fed at inclusion levels that exceed N requirements of feedlot cattle.

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A:P	Acetate: propionate ratio
AA	Amino acid
ADF	Acid detergent fiber
ADFD	Apparent total tract digestibility of acid detergent fiber
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
AIC	Akaike information criterion
ARA	Acute ruminal acidosis
AUC	Area under the curve
BUN	Blood urea nitrogen
BW	Live body weight
CDDGS	Corn-based dried distillers' grains with solubles
CDS	Condensed distillers' solubles
CH_4	Methane
CO_2	Carbon dioxide
CO_2e	CO_2 equivalent
CP	Crude protein
CPD	Apparent total tract digestibility of crude protein
DDG	Dried distillers' grains
DDGS	Dried distillers' grains with solubles
DE	Digestible energy
DM	Dry matter

DMD	Apparent total tract digestibility of dry matter
DMD	Digested dry matter
DMI	Dry matter intake
G:F	Gain to feed ratio
GE	Gross energy
GEI	Gross energy intake
GHG	Greenhouse gas
G_P	Gas pressure
GWP	Global warming potential
IPCC	Intergovernmental Panel on Climate Change
IVDMD	In vitro dry matter disappearance
LCA	Life-cycle assessment
LCFA	Long-chain fatty acids
LRC	Lethbridge research center
LRCpH	LRC Ruminal pH Measurement System
MCFA	Medium-chain fatty acids
ME	Metabolizable energy
MFS	Methyl green-formalin-saline solution
MP	Microbial protein
N	Nitrogen
N_2O	Nitrous oxide
NDF	Neutral detergent fiber
NDFD	Apparent total tract digestibility of neutral detergent fiber

NEg	Net energy for gain
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
OM	Organic matter
OMD	Apparent total tract digestibility of organic matter
P	Phosphorous
PEM	Polioencephalomalacia
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
S	Sulfur
SARA	Sub-acute ruminal acidosis
SD	Standard deviation from the mean
SE	Standard error of the mean
VFA	Volatile fatty acid
WDDGS	Wheat-based dried distillers' grains with solubles
WDDGS+oil	WDDGS supplemented with corn oil
WDG	Wet distillers' grains
WDGS	Wet distillers' grain with solubles
wt/vol	Weight per unit of volume concentration
Ym	Methane production as a proportion of gross energy intake

CHAPTER 1 – Literature review

1.1 Introduction

Government initiatives that support the production of renewable fuels, along with a growing demand for transportation fuel has led to an exponential increase in ethanol production worldwide. In 2011, global ethanol production was 85 billion L, with the United States (52.6 billion L) accounting for > 60% of global production. Canada (1.7 billion L) is the fifth biggest ethanol producer worldwide after Brazil (21.1 billion L), the European Union (4.5 billion L) and China (2.1 billion L; RFA, 2012). While ethanol in Brazil is mainly produced from sugar cane, ethanol in United States is produced primarily from corn which accounted for 88% of the estimated 142.5 million tonnes of grain used for ethanol production worldwide (FAO, 2012). Similarly to the United States, ethanol production in Canada is based on cereal grains, with wheat and corn being the primary feedstock (CRFA, 2010; RFA, 2012).

Dried distillers' grains plus solubles (**DDGS**) is the most common co-product of the ethanol industry in Canada and the United States. As most of the starch is removed from the grain during fermentation to ethanol, the remaining nutrients in DDGS (fiber, crude protein [**CP**], fat and minerals) are concentrated about three fold (Spiehs *et al.*, 2002). Due to its high CP and fiber content, DDGS is used as protein as well as energy source for livestock with ruminants consuming the majority of this co-product (Klopfenstein *et al.*, 2008; FAO, 2012). As all feed co-products, the nutrient composition of DDGS is variable and largely depends on the processing conditions, and the type and composition

of the original grain (Spiehs *et al.*, 2002). Due to the inherent differences in CP and fat content between corn (9% CP and 4.1% fat dry matter [**DM**] basis; NRC, 2000) and wheat (14% CP and 2.3% fat DM basis; NRC, 2000), corn DDGS (**CDDGS**; ~30% CP and ~10% fat DM basis; Spiehs *et al.*, 2002; Klopfenstein *et al.*, 2008) is lower in CP but higher in fat compared to wheat DDGS (**WDDGS**; ~40% CP and <5% fat DM basis; Gibb *et al.*, 2008). However, the effects of differences in CP and fat in DDGS on rumen metabolism including nitrogen (**N**) metabolism and methane (**CH_4**) production have not been extensively studied.

Methane is a potent greenhouse gas (**GHG**) with 25 times the global warming potential (**GWP**) of carbon dioxide ([**CO_2**]; IPCC, 2007a). Losses of enteric CH_4 from domestic ruminants account for nearly one third of total anthropogenic CH_4 emissions (Beauchemin *et al.*, 2008; Lassey, 2008). Supplementation of diets with lipids that are unprotected from ruminal digestion is recognized as a nutritional strategy with high probability of reducing enteric CH_4 production (Beauchemin *et al.*, 2008). Replacing barley grain (35% DM basis) with CDDGS (12.7% fat DM) in a growing (high forage) diet decreased enteric CH_4 of growing beef cattle from 23.8 to 19.9 g CH_4/kg DM intake (**DMI**); a response that was thought to be due to the high fat level in CDDGS (McGinn *et al.*, 2009). However, one limitation of using DDGS as an energy source in ruminant diets is that it increases dietary CP leading to greater N excretion if the diet is not balanced for protein. Nitrogen released into the

environment can lead to the formation of nitrous oxide (**N_2O**) a powerful GHG with 298 times the GWP of CO_2 (IPCC, 2007a).

Thus five objectives were set to test the overall goal understanding the impact of feeding CDDGS and WDDGS on GHG emissions from growing and finishing beef cattle from a life cycle perspective. The objectives were:

1) Compare *in vitro* CH_4 production from CDDGS and WDDGS as substitution for whole crop barley silage and to describe the responses of CH_4 and other fermentation parameters to increasing levels of both DDGS types as fermentation substrate.

2.) Determine if the inclusion of CDDGS or WDDGS in growing (high forage) diets reduces enteric CH_4 emissions from beef cattle and if the oil in corn was responsible for any response observed.

3.) Determine if the inclusion of CDDGS or WDDGS in finishing (high concentrate) diets reduces enteric CH_4 emissions from beef cattle and if the oil in corn was responsible for any response observed.

4.) Evaluate the impact of CDDGS and WDDGS inclusion on GHG emission of the beef feedlot life-cycle using a life-cycle assessment (**LCA**) approach.

The following section provides a comprehensive overview on the production, nutrient composition and nutritional properties of CDDGS and WDDGS. Furthermore, this section describes the mechanisms whereby ruminant livestock contribute to GHG emissions and the approaches aimed to mitigate enteric CH_4 and N_2O losses from ruminant production systems.

1.2 Dried Distillers' Grains plus Solubles (DDGS)

1.2.1 Production and Utilization of DDGS

Ethanol can be produced from either dry or wet milled grains. The majority of ethanol plants in North America use a dry milling process as it is relatively simple and cost efficient (U.S. Grain Council, 2012). In contrast, wet milling is a more expensive and does not yield distillers' grains as an end product (Erikson *et al.*, 2006), but rather dry or wet corn gluten meal. Therefore the wet milling process, and its co-products, will not be discussed further. The dry milling process begins with cleaning and milling of the grain using hammer or roller mills to break the kernel and expose starch (Figure 1-1).

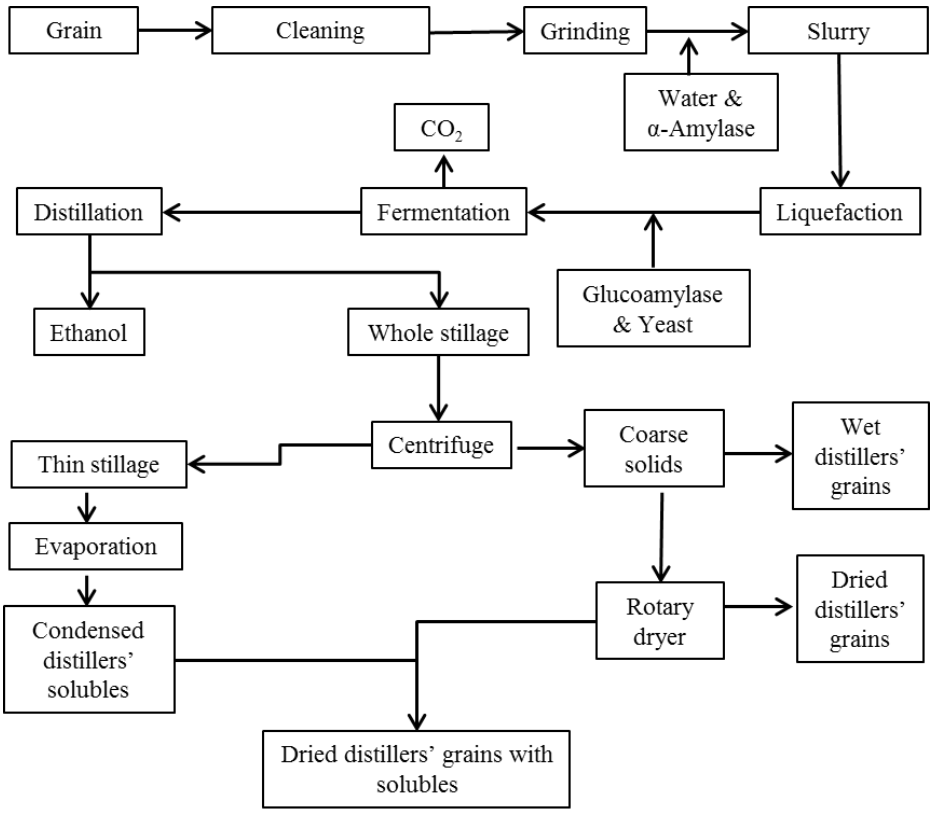

Figure 1-1 Dry milling ethanol production (Adapted from U.S. Grains Council, 2012).

The kernel particles are then mixed with water and heat stable α-amylase during liquefaction. In this process, heat (tp >100°C) is applied to create a slurry or mash and solubilize the starch granules. The added α-amylase hydrolyzes starch by cleaving α-1,4-glycosidic bonds at random points of the starch molecule. End products of liquefaction are dextrin (short glucose chains) and small amount of glucose and maltose (Borglum, 1980). The slurry is then cooled (tp ~60°C) and glucoamylase and yeast (*Saccharomyces cerevisiae*), are added in the subsequent two processes known as saccharification and fermentation, respectively (Bothast and Schlicher, 2005). As natural starch consists of amylose (linear α-1,4 linkages) and amylopectin (branched α-1,6 linkages) but α-amylase cannot hydrolyze α-1,6 bonds, glucoamylase, a debranching enzyme that can hydrolyze both α-1,4 and α-1,6 bonds, is added to the cooled slurry (Borglum, 1980).

Glucoamylase converts the liquefied starch into glucose and yeast convert the glucose monomers into ethanol and CO_2. Sulfuric acid is commonly added to the slurry to lower the pH to an optimal level for glucoamylase activity (~pH 4.5) and prevent microbial growth (Bothast and Schlicher, 2005). Furthermore antibiotics, most commonly penicillin and virginiamycin, are added to control overgrowth by bacteria or wild yeast infections during fermentation (U.S. Grain Council, 2012). As yeast cells are incapable of degrading grain protein, urea is added to provide a rapidly available non-protein N source (Belyea *et al.*, 2004). Some ethanol plants add proteases that break down grain protein to free amino acids, which serve as an additional source of N for yeast

(Bothast and Schlicher, 2005). The fermentation process requires 2–3 days to reach a final ethanol concentration of 10 to 12% (Bothast and Schlicher, 2005). The fermented slurry is then distilled to remove the ethanol. The remaining fermented slurry is called whole stillage and is centrifuged or screened to remove coarse particles from the liquid fraction (Erikson *et al.*, 2006). The extracted coarse grain particles are termed wet distillers' grains (**WDG**). Due to the high cost of shipping and its fast rates of spoilage, **WDG** (~35% DM; Erikson *et al.*, 2006) are typically fed to livestock within the vicinity of the ethanol plant. To prevent spoilage and to reduce shipping costs, WDG can be dried to form dried distillers' grains ([**DDG**] ~ 90% DM; Erikson *et al.*, 2006). The remaining liquid fraction, known as thin stillage, is usually evaporated to produce condensed distillers' solubles ([**CDS**] ~30% DM; U.S. Grains Council, 2012). Commonly, CDS are added back to the WDG to create wet distillers' grains with solubles (**WDGS**) or dried together to create DDGS. Thus, the end products of the dry milling ethanol production are ethanol, CO_2 and either WDGS or DDGS including spent yeast cells. Depending on the grain source and its starch content, 1 t of grain yield about 350 to 400 L of ethanol and 300 to 370 kg of DDGS (Bothast and Schlicher, 2005; FOBI, 2011). The CO_2 produced is either released into the atmosphere or captured and sold to the beverage industry (Bothast and Schlicher, 2005).

As corn grain has a high oil content (4.1% fat DM basis; NRC, 2000) and corn oil can be marketed for other applications such as biodiesel production most ethanol plants are installing enhanced oil extraction technologies to improve the

recovery of corn oil (U.S. Grains Council, 2012). Corn oil can be extracted by separating the endosperm, germ, and bran fractions of the kernel prior to fermentation and extracting the oil directly from the germ (front-end fractionation); or by extracting the oil from thin stillage after fermentation and distillation (back-end fractionation; Shurson and. Alghamdi, 2008). As front-end fractionation is more costly, the majority of dry milling ethanol plants are using the back-end oil extraction procedure (U.S. Grains Council, 2012).

Due to its high fiber content DDGS is most efficiently utilized as feed for ruminants, replacing concentrates (Klopfenstein *et al.*, 2008), or to a lesser extent forages (Li *et al.*, 2011). As DDGS has been included in the beef cattle diets at levels up to 60% of DDGS (DM basis; Gibb *et al.*, 2008; Felix et. al 2012), most DDGS in the United States is consumed by beef cattle (48%) followed by dairy cattle (32%), swine (11%) and poultry (8%), respectively (RFA, 2012). Other minor uses of DDGS include as a feed source in aquaculture, crop fertilizer or as a substrate for combustion (FOBI, 2011).

1.2.2 Chemical Composition of DDGS

Corn is the main feedstock used for ethanol production in the United States and eastern Canada. Consequently, CDDGS is the most abundant source of DDGS throughout North America (Erickson et al., 2006). In general, climatic conditions in western Canada are not suitable for the production of corn grain, therefore spring wheat is the primary feedstock utilized for ethanol production in western Canada (Beliveau and McKinnon, 2008). Although of lesser importance,

research has also studied DDGS from other sources such as sorghum, triticale, barley and rye.

As for all co-products, the chemical composition of DDGS is variable and mainly affected by the nutrient composition of the grain and the procedures employed during the fermentation process. Large variation in nutrient composition can have a negative impact on the market value of DDGS as it makes diet formulation more difficult (Belyea et al., 2010). Consequently, ethanol producers try to minimize variation in DDGS composition, mainly by standardizing processing conditions (U.S. Grains Council, 2012). With the starch being almost completely fermented to produce ethanol, DDGS contains a threefold concentration of CP, fibre, fat and minerals as compared to the grain entering the ethanol plant (Spiehs et al., 2002). Spent yeast cells supply additional protein to DDGS. For example, Belyea et al. (2004) estimated that yeast protein may make up nearly half of the protein in CDDGS.

Protein

The CP content of DDGS can range from 26.9 to 45.3% DM (Kelzer *et al*., 2010; Hünerberg *et al*., 2013a) and is largely depending on the type of grain used in the ethanol production process. Corn grain has lower protein content (~9% CP) than wheat grain (~14% CP DM basis; NRC, 2000). Therefore the CP content in CDDGS (~30% DM; Klopfenstein *et al*., 2008) is typically lower than in WDDGS (~40% DM; Gibb et al., 2008). Dried distillers' grains are a good source of rumen undegradable protein (**RUP**; Boila and Ingalls, 1994). For

example, Kleinschmit *et al.* (2007) reported RUP content in CDDGS of up to 70.7% of total CP (DM basis). However, the RUP content of DDGS varies and is dependent on the type of grain used, processing conditions (e.g. drying temperature) and amount of CDS added back to the solids (Cao *et al.*, 2009; Klopfenstein et al., 2008). Wheat protein is mainly composed of gluten which is less resistant to ruminal degradation than zein in corn (Little *et al.* 1968). As a result, ruminal CP degradability of WDDGS is typically higher than CDDGS (Boila and Ingalls, 1994). Cao *et al.* (2009) reported that ruminal CP digestibility in Holstein cows was improved when increasing amounts of CDS were added to CDDGS. A high content of acid detergent insoluble N (**ADIN**) is generally accepted to be indicative of heat damage of DDGS and a reduction in protein digestibility (Sniffen *et al.*, 1992). The availability of lysine is especially negatively affected by heating or drying, as it is particularly susceptible to the formation of Maillard products owing to its free amino group (Schwab, 1995). However, the usefulness of ADIN as predictor of RUP and total tract protein digestibility of DDGS has been challenged. Nakamura *et al.* (1994) reported a weak correlation ($r^2 = 0.24$) between ADIN content and N digestibility of CDDGS in wethers. Similarly, Kleinschmit *et al.* (2007) found ADIN to be a poor predictor of ruminal and total tract protein digestibility in ruminally cannulated Holstein cows. Alternatively, a darker color of DDGS was proposed to be more indicative of protein damage in CDDGS (Powers *et al*., 1995). However, others have questioned the validity of a colour scoring system to predict ADIN especially at low ADIN concentrations (Cromwell *et al*., 1993;

Harty *et al*., 1998). Furthermore particle size, moisture content, and the amount of CDS added also influence the color of DDGS. Therefore, using color as only indicator of the protein quality of DDGS is not recommended (U.S. Grains Council, 2012).

Fat

Due to the higher level of oil associated with corn germ, the fat content of CDDGS (8.2 to 12.7% DM; Spiehs *et al.*, 2002; McGinn *et al.*, 2009) is substantially higher than WDDGS (3.4 to 4.9% DM; Hünerberg *et al.*, 2012; Hünerberg *et al.*, 2013b). As described, improved oil extraction methods are producing low-fat CDDGS with an average fat content between 6.0 to 9.0% DM (U.S. Grains Council, 2012). Distillers' grains are rich in unsaturated fatty acids (McKeown *et al*., 2010) and results from metabolism studies suggest the fat in DDGS may be partially protected from ruminal biohydrogenation, increasing the flow of unsaturated fatty acids to the duodenum (Klopfenstein *et al*., 2008).

Energy and fiber

Although starch is almost completely utilized during fermentation, DDGS is often added to the diet of feedlot cattle as a source of energy source rather than protein (Klopfenstein *et al*., 2008). When fed to cattle at inclusion levels of 15 to 40% diet DM CDDGS has an energy value equal or higher to corn grain (Ham *et al*., 1994; Klopfenstein *et al*., 2008). Similarly, the energy value of WDDGS, at inclusion levels between 20 and 50% diet DM, is at least equivalent to barley grain (Beliveau and McKinnon, 2008; Gibb *et al*., 2008).

The unexpectedly high energy value of DDGS despite its low starch content can be attributed to the highly digestible fiber in DDGS as well as an increase in fat content, especially with CDDGS (Schingoethe *et al*., 2009; Wierenga *et al*., 2010). Due to its higher fat content the gross energy (**GE**) content of diets supplemented with CDDGS is typically higher than in diets supplemented with the same amount of WDDGS (Walter *et al.,* 2012).

Neutral detergent fiber (**NDF**) content of DDGS varies from 35.4% to 49.1% DM for CDDGS (Spiehs *et al*., 2002) and 23.8 to 48.9% for WDDGS (Hünerberg *et al*., 2013a; Beliveau and McKinnon, 2008). Acid detergent fiber (**ADF**) content ranges from 13.8 to 23.0% for CDDGS (Spiehs *et al*., 2002; Hünerberg *et al*., 2013b) and 12.9 to 21.5% for WDDGS (Beliveau and McKinnon, 2008; Hünerberg *et al*., 2013b). While the extensive processing and fermentation of the grain during the ethanol production results in higher ruminal and total tract NDF and ADF digestibility of DDGS compared to most cereal grains and forages (Ham *et al*., 1994).

Minerals

With the removal of starch minerals are also concentrated in DDGS as compared to the original cereal grain (Spiehs *et al*., 2002). Of special concern for cattle producers are high level of P and S in DDGS. The high P concentration in CDDGS (0.70 to 0.99% DM; Spiehs *et al*., 2002) and WDDGS (0.96 to 1.07% DM; Gibb *et al*., 2008; own data) can result in increased fecal P excretion; particularly when DDGS is used as an energy source (>15 to 20% of diet DM;

Klopfenstein *et al*., 2008). Increasing fecal excretion of P is an environmental concern as P applied to the land can be lost due to run off into water bodies, resulting in eutrophication (Rausch and Belyea, 2006).

As sulfuric acid is typically added to the mash to control the pH and to prevent microbial overgrowth during fermentation, DDGS can be high in sulfur (**S**). The average S content of CDDGS from 10 ethanol plants in Minnesota and South Dakota ranged from 0.33-0.74% (DM basis; Spiehs *et al*., 2002). Gibb *et al*. (2008) reported that WDDGS contained 0.48% S (DM basis). In the rumen, sulfate is reduced to H_2S by sulfate-reducing bacteria. High ruminal H_2S concentrations have been associated with the neurological disorder polioencephalomalacia (**PEM**). Feeding high concentrations (>40% diet DM) of WDGS or DDGS with elevated S content can result in an increase of PEM (NRC, 2001). With high S level of DDGS being discussed as cause for PEM, ethanol plants monitor S levels in DDGS as part of their quality assurance protocols and have reduced the usage of sulfuric acid to regulate the pH during fermentation. However, sulfuric acid is still commonly added during ethanol production because of its lower cost relative to other acids (U.S. Grains Council, 2012). In some cases, high S content of the drinking water may contribute to the occurrence of PEM in cattle (NRC, 2000). Therefore, in geographic regions with high S level in the drinking water, the level of DDGS included in the diet may need to be reduced (Schingoethe *et al*., 2009).

1.2.3 Effect of DDGS on Rumen Fermentation, N Metabolism, and Digestibility

Rumen fermentation

Rumen fermentation and volatile fatty acid (**VFA**) production is diet dependent; with the form of carbohydrate present being the most important factor influencing the concentration and profiles produced (Van Soest, 1994). As described, DDGS is high in structural but very low in non-structural carbohydrates. Ruminal fermentation of structural as compared to non-structural carbohydrates typically results in lower total VFA and propionate production but higher acetate production (Van Soest, 1994). Consequently, it could be expected that feeding DDGS would produce a ruminal VFA profile more similar to forages than grains, lowering total VFA production while increasing the acetate:propionate ratio (Russell, 1998).

Walter *et al.* (2012) reported a linear decrease of propionate but no effect on acetate when WDDGS and CDDGS replaced barley grain at levels of 20 and 40% dietary DM in finishing (high concentrate) diets. Total VFA concentration linearly decreased with increasing WDDGS in the diet, but was not affected by CDDGS (Walter *et al.*, 2012). Similarly, Beliveau and McKinnon (2009) reported a decrease in propionate, but acetate concentration was not affected as WDDGS replaced barley grain at levels of 7, 14 and 21% DM in finishing diets. With respect to corn based diets, Ham *et al.* (1994) found no difference in total or individual VFA production in steers fed finishing diets containing 40% dietary DM corn, CDDGS or corn based WDGS. In contrast, replacing dry rolled corn with CDDGS at levels of 15, 30, 45 and 60% DM resulted in a linear

reduction of total VFA and acetate concentration whereas propionate concentration linearly increased (Leupp *et al.*, 2009). The type of grain fed impacts VFA production, with barley diets increasing propionate and total VFA production in the rumen relative to corn diets (Huntington, 1997). Furthermore, DDGS is extensively processed and as a result its ruminal NDF and ADF digestibility are higher than high-fiber feeds that possess a larger particle size (Ham *et al.*, 1994). Consequently, even though DDGS is high in fiber, VFA profiles and concentrations in cattle fed DDGS do not coincide with those offered typical high-fiber diets.

Replacing grain starch with a combination of highly digestible fiber, fat and CP could reduce the amount of total VFA produced in the rumen, possibly reducing sub-acute ruminal acidosis ([**SARA**]; Klopfenstein *et al.*, 2008). The occurrence of SARA, commonly defined as a prolonged time of rumen pH below 5.5 (Penner *et al.*, 2007), is a major problem in feedlot cattle fed high concentrate diets (Owens *et al.*, 1998). Substituting dry-rolled barley with 20% DM of triticale DDGS in a finishing diet decreased the prevalence of SARA (Wierenga *et al.*, 2010). In contrast, replacing 20 or 40% of barley grain DM with CDDGS or WDDGS in barley-based finishing diets had no effect on daily mean pH or time spend below pH of 5.5 (Walter *et al.*, 2012). Correspondingly, replacement of barley grain with 7, 14 and 21% DM WDDGS in a finishing diet did not increase mean rumen pH or reduce SARA (Beliveau and McKinnon, 2009). The failure of DDGS to modulate rumen pH when added to barley (Beliveau and McKinnon, 2009; Walter *et al.*, 2012) as well as corn grain diets

(Ham *et al.*, 1994), likely reflects high ruminal fermentability of DDGS fiber. Furthermore, the particle size of DDGS is small (e.g., 0.679 ± 0.002 mm, mean ± standard deviation [**SD**]; Beliveau and McKinnon, 2009) and ineffective in stimulating saliva production to buffer pH in the rumen. The low effectiveness of DDGS fiber is furthermore supported by studies that report a decrease in ruminal pH with partial replacement of barley silage by WDDGS (Li *et al.*, 2011) or triticale DDGS (Wierenga *et al.*, 2010).

Nitrogen metabolism

Feed N entering the rumen can be in the form of non-protein N or true protein N, with the latter being divided into RUP or bypass protein and rumen degradable protein (**RDP**) (Bach *et al.*, 2005). Rumen degradable protein is rapidly hydrolyzed and broken down to peptides and amino acids (**AA**) which can be deaminated to form ammonia (**NH_3**) and carbon skeletons. Ammonia N, amino acids and peptides can be incorporated into microbial protein ([**MP**]; Van Soest, 1994). Thus the nitrogenous compounds leaving the rumen include NH_3, RUP, endogenous protein and MP. If NH_3 is not directly incorporated in MP it can be absorbed across the rumen wall and metabolized to form urea in the liver (Bach *et al.*, 2005). Urea is released into the blood stream, recycled back to the rumen and lower intestinal tract or excreted via urine. Urea that is recycled back to the gastrointestinal tract can be turned into NH_3 by microbial urease and used by some members of the microbial community to synthesize amino acids. During times of low RDP availability renal urea excretion is down regulated (Van Soest, 1994). The energetic costs of NH_3 detoxification and urea synthesis

are estimated at about four ATP per mole of urea produced; furthermore, energy expenditure of animals consuming high CP diets may increase as liver and kidneys can undergo cell hypertrophy in response to increased N metabolism (Summers *et al.*, 1988; McBride and Kelly, 1990).

Even though DDGS is rich in RUP, its CP content is three fold higher than that of grains (Spiehs *et al.*, 2002), and feeding DDGS often results in an increase in ruminal NH_3 concentration (Walter *et al.*, 2012; Beliveau and McKinnon, 2009). Lower ruminal NH_3 concentration of cattle fed CDDGS compared to WDDGS at the same inclusion level likely reflect the reduced RDP content of CDDGS (Walter *et al.*, 2012). Depending on its inclusion level, CP content and CP digestibility, feeding DDGS also cause an increase in blood urea N ([**BUN**]; Wierenga *et al.*, 2010).

Rumen MP accounts for the majority of protein that flows to the small intestine of ruminants, accounting for ~50 to 80% of total post-ruminal, absorbable protein (Storm and Ørskov, 1983; Bach *et al.*, 2005). The amount of MP flowing to the small intestine is mainly influenced by the availability of protein and carbohydrate to ruminal bacteria (Van Soest, 1994). The contribution of protozoa protein to total MP is believed to be small owing to their ability to sequester within the rumen (Van Soest, 1994). However, the presence of protozoa typically increases bacterial protein turnover in the rumen and comprehensive *in vivo* results indicate that duodenal flow of MP generally increases after partial or complete defaunation (Firkins *et al.*, 2007). This response likely arises due to the fact that rumen protozoa predate bacteria.

With respect to the impact of DDGS on MP synthesis, Chibisa *et al.* (2012) reported that substitution of WDDGS for canola meal at 10, 15, or 20% diet DM had no negative effect on MP yield of dairy cows. Similarly, replacing a mixture of cottonseed and soybean meal by 30% diet DM of CDDGS, in a diet fed to lactating dairy cows, produced a similar amount of MP (Janicek *et al.*, 2008). Replacing a mixture of dry-rolled corn, sunflower meal, and urea with 15, 30, 45, or 60% DM CDDGS in a 70% concentrate diet (DM basis) did not change ruminal MP yield in steers (Leupp *et al.*, 2009). These results indicate that CDDGS and WDDGS have no adverse effects on MP synthesis despite being high in RUP. Dried distillers' grains can supply large quantities of RUP or bypass protein and indeed, prior to the expansion of the fuel ethanol industry, DDGS was most commonly used to maximize the amount of metabolizable protein available to dairy cows (Schingoethe *et al.*, 2009). Even though beef cattle have lower protein requirements than dairy cows, and the concept of bypass protein is far less relevant to beef than to dairy nutrition, bypass protein could in part account for the high feeding value of DDGS, as the energetic efficiency of metabolizable bypass protein is higher compared to protein that is degraded in the rumen and incurs energy losses due to fermentation (Klopfenstein *et al.*, 2008).

Depending on its CP content and dietary inclusion level, feeding DDGS can lead to increased N excretion. Walter *et al.* (2012) replaced 20 and 40% of barley grain DM with CDDGS (31.8% CP) and WDDGS (39.3% CP; both DM basis) in a beef finishing diet and reported that total N excretion increased

linearly from 142 g to 207 g and 266 g/d with increasing inclusion level of CDDGS and WDDGS, respectively. Increasing the amount of WDDGS also linearly increased fecal as well as urinary N excretion, whereas CDDGS only resulted in a linear increase in urinary N. Although, urine was the major route of N excretion for both DDGS sources, the linear increase in fecal N excretion for WDDGS likely reflects its higher ADIN content (23.6% ADIN) as compared to CDDGS (11.6% ADIN; both as % of N DM basis). Salim *et al.* (2012) replaced 16.7, 33.3, and 50% of corn grain DM with CDDGS in a finishing diet and reported linear increases in total N (109.3 to 145.9 g/d), urinary N (46.3 to 69.8 g/d) and fecal N excretion (63.0 to 76.2 g/d). Increases in total N, fecal N and urinary N excretion in response to increasing levels of WDGS and DDGS has been reported in numerous other studies (Cole *et al.*, 2005; Luebbe *et al.*, 2008; Spiehs and Varel, 2009).

Walter *et al.* (2010) reported that apparent N retention of beef heifers increased from 48 to 86 g/d when WDDGS replaced barley grain at an inclusion level of 40% diet DM in a finishing diet. This apparent N retention value is high as it indicates an average daily gain (**ADG**) in excess of 2 kg/d (Walter *et al.*, 2012). Nitrogen retention values of up to 72.6 g/d have been reported for a diet containing 60% DM corn WDGS (Spiehs and Varel, 2009). An even higher apparent N retention of up to 127.4 g/d was reported for steers fed a finishing diet containing 50% diet DM of CDDGS (Salim *et al.*, 2012). However, apparent N retention is often skewed, as it is calculated as N intake – N excretion, and measurement errors or sampling losses such as volatile N losses from urine, or

fecal samples can confound apparent N retention estimates (Spanghero and Kowalski, 1997). Furthermore, dermal, hair and scurf losses are unaccounted for in most N balance studies (Spanghero and Kowalski, 1997).

Overall, increasing concentrations of either CDDGS or WDDGS in beef cattle diets results in higher CP supply and a subsequent linear increase in total N (g/d) excretion. Nitrogen metabolism variables such as ruminal NH_3, BUN and MP are, amongst other factors, impacted by the availability of protein (e.g., ratio between RDP vs. RUP) in DDGS. Even though the rate of N excretion (% of intake) of cattle fed DDGS as an energy source (>15 to 20% of diet DM; Klopfenstein *et al.*, 2008) may not be different from cattle fed diets with lower CP content; N intake of cattle fed DDGS as energy source is high and result in substantially greater daily N excretion. Furthermore, metabolic costs associated with removing excess N through urea synthesis could reduce the energy efficiency of cattle fed diets supplemented with high percentages of DDGS.

Digestibility

Due to their low content of non-structural carbohydrates, CDDGS and WDDGS typically reduce apparent total tract DM and organic matter (**OM**) digestibility when they replacing cereal grains in beef cattle diets (Gibb *et al.*, 2008; Depenbusch *et al.*, 2009; Salim *et al.*, 2012). In contrast, replacement of forages with DDGS usually increases total tract DM and OM digestibility (Depenbusch *et al.*, 2009; Li *et al.*, 2011). Even though fibrolytic enzymes are usually not added during the fermentation process, digestibility of NDF and ADF in DDGS is high (Bothast and Schlicher, 2005). For example, Walter *et al.*

(2012) found that increasing levels of CDDGS or WDDGS (0, 20 and 40% DM) a beef finishing diet linearly increased apparent total tract ADF and NDF digestibility. High apparent total tract NDF digestibility in a finishing diet suggests that a significant amount of this fiber is being digested postruminally (Ham *et al.*, 1994). Leupp *et al.* (2009) who replaced dry rolled corn with CDDGS at inclusion levels of 15, 30, 45 and 60% DM, found that ruminal OM digestion linearly decreased, while post-ruminal OM digestion linearly increased. The authors attributed the linear decline in ruminal OM digestion to increasing DDGS levels and its accelerated ruminal passage to its small particle size. As discussed earlier, a shift in digestion from the rumen to the intestine could contribute enhance the energy value of DDGS provided energy losses associated with rumen fermentation were lowered (Klopfenstein *et al.*, 2008). However, studies examining the extent of ruminal versus post ruminal digestion of DDGS are rare and more research is required. Leupp *et al.* (2009) reported that total tract CP digestibility linearly increased with increasing levels of DDGS, an observation that might reflect that a substantial amount of DDGS protein is post-ruminally digested. However, replacement of 20 and 40% of barley grain DM with WDDGS did not affect apparent CP digestibility, whereas inclusion of the same levels of CDDGS resulted in a linear increase in apparent CP digestibility. As discussed in section 1.2.2, differences in CP availability between CDDGS and WDDGS could be due to differences in the nature of the protein contained in the two grains (gluten vs. zein protein) as well as the

processing procedures such as drying temperature and fate of CDS, used in the ethanol production process.

Digestion of lipids in DDGS is important from an energetic as well as a meat, and milk composition perspective (Klopfenstein *et al.*, 2008, Walter *et al.*, 2010). Distillers' grains are rich in unsaturated fatty acids that may be partially protected from ruminal biohydrogenation and alter the fatty acid composition of meat and milk (Vander Pol *et al.*, 2007; Klopfenstein *et al.*, 2008). For example, He *et al.* (2012) reported a linear increase in unsaturated fatty acid content of beef when 25, 30 and 35% diet DM WDDGS replaced barley silage and barley grain. Similarly, milk fat from cows fed diets containing 20% DM corn WDGS or CDDGS was more unsaturated and contained more cis-9, trans-11 conjugated linoleic acid compared to milk fat from cows supplemented with of soybean meal (Anderson *et al.*, 2006).

1.2.4 Effect of DDGS on Growth Performance and Carcass Traits

Several researchers have examined the impact of feeding DDGS on growth performance and carcass traits of beef cattle. Most studies have investigated this from the perspective of substituting DDGS for the grain portion of the diet. For example, replacing 10, 20, 30 and 40% of dry rolled corn DM in a finishing diet resulted in a quadratic response in final body weight (**BW**) and ADG, with the highest ADG observed at 20% inclusion (Buckner *et al.*, 2008). However, feeding up to 40% of the diet DM as DDGS had no adverse effect on gain:feed or carcass characteristics as compared to steers fed a corn grain diet. Similarly, Benson *et al.* (2005) reported that CDDGS can be included in beef

cattle finishing diets at levels up to 35% of DM without negatively affecting growth performance. Carcass characteristics including subcutaneous fat thickness and yield grade of steers linearly increased with increasing level of CDDGS. When CDDGS or WDDGS replaced barley grain at levels up to 40% of DM, Walter *et al.* (2010) reported an improved gain:feed and reduced days on feed with no detrimental effect on quality grade or carcass yield. Replacement of barley with WDDGS at 25 and 50% of diet DM in a growing diet increased ADG and improved feed efficiency (McKinnon and Walker, 2008). Substitution of up to 23% WDDGS for rolled barley in a finishing diet had no effect on ADG or gain:feed (Beliveau and McKinnon, 2008). While Gibb *et al.* (2008) observed that finishing cattle fed 20, 40 or 60% diet DM WDDGS in a barley grain diet had similar ADG but linear increases in DM intake (**DMI**) and consequently poorer feed efficiency at the higher inclusion level.

Increased frequency of abscessed livers from 16.2 to 47.2% was reported by Wierenga *et al.* (2010) who replaced 20% dietary DM of barley grain with triticale DDGS in a finishing diet. Similarly, Beliveau and McKinnon (2008) fed WDDGS and reported a greater prevalence of liver abscesses in steers fed WDDGS as compared to a barley grain-based diet containing no DDGS. As recent work identified an enzymatic link between NH_3 detoxification and lipopolysaccharide detoxification in the liver (Satoh *et al.*, 2008), excess CP in diets supplemented with high levels of DDGS could be linked to the development of liver abscesses (Wierenga *et al.*, 2010). However, Beliveau and McKinnon (2008) and Wierenga *et al.*, (2010) did not include antimicrobial feed

additives such as tylosin which are known to reduce the development of liver abscesses in cattle.

The positive impact of DDGS on growth performance of beef cattle can be explained by its relatively high feeding value. Due to its high fat content the energy value of CDDGS is typically higher (~1.87 Mcal/kg of NEg; Ham *et al.*, 1994) than corn (1.55 Mcal/kg of NEg; NRC, 1996), barley (1.40 Mcal/kg of NEg; NRC, 1996) and WDDGS (1.26 to 1.40 Mcal/kg of NEg; Beliveau and McKinnon, 2008; Gibb *et al.*, 2008), respectively. As discussed, other factors that might be responsible for the high feeding value of CDDGS and WDDGS are their highly digestible fiber and elevated bypass protein content (Klopfenstein et. al., 2008).

1.3 Ruminant Production and Climate Change

1.3.1 Ruminal Methanogenesis

Methanogenisis is carried out by members of the domain Archaea, even though they account only for 0.3 to 4.0% of rumen microbial biomass (Janssen and Kirs, 2008; Yanagita *et al.*, 2008). Methanogens play a key role in rumen microbial fermentation (McAllister *et al.*, 1996) as they utilize H_2 as an energy source to reduce CO_2 to CH_4, thereby oxidizing cofactors (e.g., NAD^+) that can be subsequently reduced by other rumen microbes (Hungate *et al.*, 1970). Ruminal carbohydrate fermentation results in the production of H_2 and if H_2 is not efficiently removed from the rumen, it can inhibit the metabolism of rumen microorganisms (Janssen, 2010). Therefore, efficient H_2 removal is essential to maintain a high rate of ruminal fermentation (McAllister and Newbold, 2008).

Formate, which is formed in the production of acetate, or methylamines can also be used by as energy source by rumen methanogens, but these substrates are much less important compared to H_2 (Hungate *et al.*, 1970; Patterson and Hespell, 1979).

Methanogens are distinct from bacteria in that they possess a unique cell wall, and key enzymes for methanogenesis (Zhou *et al.*, 2011). The cell walls of methanogens consist of pseudomurein and surface layer proteins instead of peptidoglycan as the principal component of bacterial cell walls (Balch *et al.*, 1979). In addition, membrane lipids of methanogens are joined by ether linkages while ester linkages are the norm in bacterial cell membranes as they are formed by the condensation of alcohols and fatty acids (De Rosa *et al.*, 1986). Methanogens also possess specific cofactors and enzymes (e.g. F_{420}, methanopterin and coenzyme M) involved in methanogenesis (Baker, 1999; Kletzin, 2007). The reduction of CO_2 to CH_4 is carried out by a cascade of different reactions that require cofactors and enzymes that direct electron flow through four reductive intermediates: formyl, methenyl, methylenyl and methyl, with the final product being CH_4 (McAllister *et al.*, 1996).

Methanogens also share a symbiotic relationship with rumen protozoa through interspecies H_2 transfer (Finlay *et al.*, 1994), and it has been estimated that 9 to 37% of methanogens exist in an ecto- or endosymbiotic relationship with protozoa (Newbold et. al., 1995; Machmüller *et al.*, 2003). Even though the vast majority of enteric CH_4 is produced in the rumen CH_4 production can also occur in the hind gut. However, about 90% of the CH_4 produced in the hind gut

is absorbed into the blood and released by respiration, as a result only ~1% of enteric CH_4 is typically lost through the rectum (Murray *et al.*, 1976). As CH_4 has a high energy content (13.3 Mcal/kg) anywhere from 2 to 12% of the GE intake (**GEI**) of ruminants can be lost as CH_4 (Johnson *et al.*, 1993). Feeding low-quality, high forage diets results in higher CH_4 losses (% of GEI) as compared grain diets (Johnson and Johnson, 1995). This relationship arises as a result of the stoichiometry of CH_4 production in the rumen. More H_2 is generated during fermentation of structural carbohydrates where acetate and butyrate are the primary VFA produced (Van Soest, 1994). In contrast, fermentation of starch and other non-structural carbohydrates favors propionate production, which acts as a hydrogen sink and thereby reduces the amount of H_2 available for the reduction of CO_2 to CH_4 (Janssen, 2010). Replacing structural with non-structural carbohydrates also typically increases the ruminal passage of feed, thereby reducing the amount of substrate available for CH_4 production in the rumen passage (McAllister *et al.*, 1996).

Furthermore, higher intake of non-structural carbohydrates decreases ruminal pH (Owens *et al.*, 1998). Van Kessel and Russell (1996) reported that methanogens are sensitive to low ruminal pH and *in vitro* CH_4 production has been shown to cease at pH < 6.0. However, methanogens are capable of adapting to changing the rumen environment as they have been shown to be less sensitive to low pH in cattle fed high-concentrate diets (Hook *et al.*, 2011). Mechanisms that could make high-grain adapted methanogens more tolerant to low ruminal pH may include an increase in the endosymbiotic association with protozoa,

where pH may be higher from that in the greater rumen environment (Hook *et al.*, 2011). However, studies that discuss the relationship between *in vivo* CH_4 production and ruminal pH are scarce and given that numbers of protozoa are typically lower on high-concentrate compared to high-forage diets, the mechanisms of this enhanced endosymbiotic relationship, so it exists, are unclear. Regardless, inducing sub-acute or acute acidosis as a CH_4 mitigation strategy would be infeasible as it would also have a negative impact on animal health and growth performance.

1.3.2 Nitrogen Excretion and Nitrous Oxide Formation

Ruminant production causes losses of N in feces and urine. Manure N can be transformed and lost as NH_3, N_2O and N oxides in the air, or as nitrate ($\mathbf{NO_3^-}$) in ground water and runoff (Steinfeld and Wassenaar, 2007). The N cycle (Figure 1-2) of agricultural systems is complex. Nitrogenous inputs, such as synthetic fertilizer, crop residue and manure, are transformed through the processes of N fixation; mineralization and nitrification that increase crop available N. In contrast, denitrification, volatilization, and leaching result in losses of crop available N. Formation of N_2O results from nitrification and denitrification processes (de Klein and Eckard, 2008).

Nitrification is the aerobic microbial oxidation of ammonium ($\mathbf{NH_4^+}$) to NO_3^-. Nitrate is form of N that is most available to plants, but is also highly susceptible to leaching and a major water pollutant. Denitrification is the anaerobic microbial reduction of NO_3^- to N_2 gas.

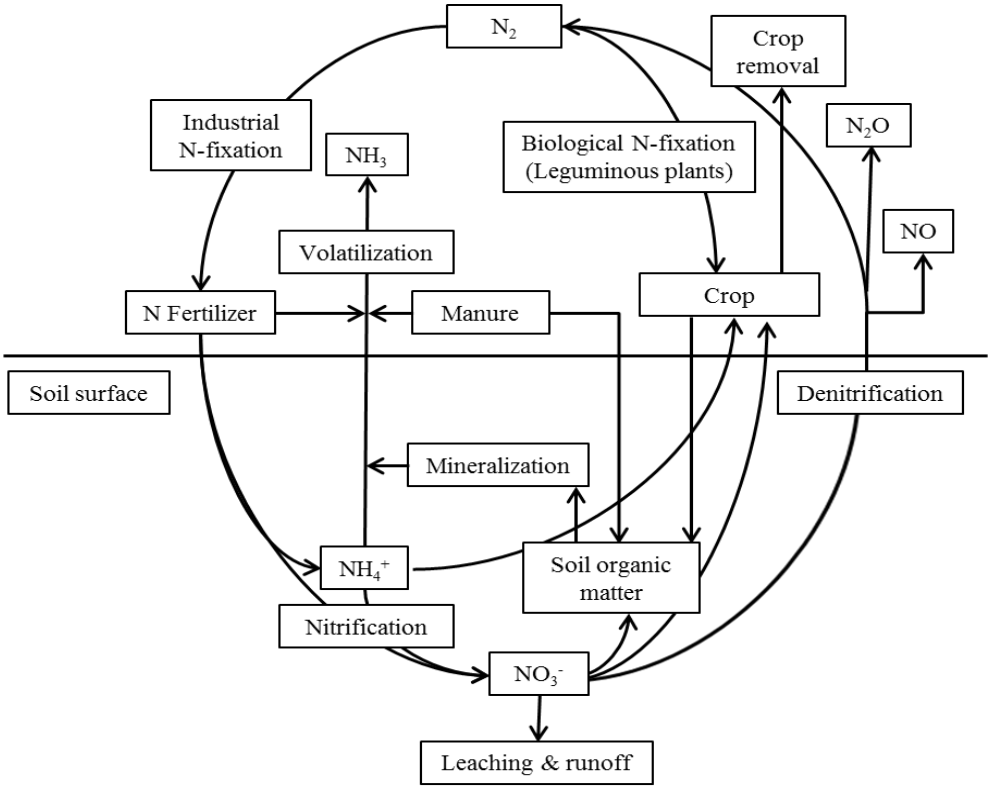

Figure 1-2 Basic nitrogen cycle of agricultural systems. Direct N_2O emissions include microbial nitrification and denitrification of fertilizer and manure N. Indirect N_2O emissions result from NH_3 and NO_3^- that is removed from agricultural soils via volatilization, leaching, and runoff (IPCC, 2000b).

Nitrous oxide is a gaseous intermediate of both processes and leaks from microbial cells into the soil atmosphere (Firestone and Davidson, 1989). High N_2O emissions rates typically coincide with soil conditions that are favorable for denitrification (wet soil, high NO_3^- concentrations); suggesting that denitrification is the main source of N_2O losses (de Klein and Eckard, 2008). However, nitrification is closely linked to denitrification as nitrification converts N from manure and urea or NH_4^+-based fertilizers into NO_3^- (Figure 1-2; de Klein and Eckard, 2008). Direct N_2O emissions include microbial nitrification and denitrification of fertilizer and manure N that are applied to agricultural

soils. Indirect pathways that can lead to the formation of N_2O involve N that is removed from agricultural soils and animal waste management systems via volatilization, leaching, runoff, or harvest of crop biomass (IPCC, 2000b).

More than half of the N excreted in the urine of cattle is in the form of urea (Hristov *et al.*, 2011). Even though urea itself is not volatile, when it comes in contact with feces or soil, it is rapidly hydrolyzed to NH_3 and CO_2 by microbial urease activity and the majority of the NH_3 is volatilized, depending to some extent of the environmental conditions at the time of urination (Bussink and Oenema, 1998). Fecal N is mainly in form of indigestible proteins or nucleic acids and is therefore non-volatile. Ammonia is a precursor to the formation of atmospheric fine particulate matter which has a negative impact on human health (U.S. EPA, 2009). Volatilized NH_3 can also be re-deposited on soil surfaces and contribute to eutrophication, soil acidity and indirect N_2O emissions (IPCC, 2006; Hristov *et al.*, 2011). In addition, NO_3^- in leachate and soil run-off can be converted into N_2O through aquatic denitrification (IPCC, 2000b).

1.3.3 Greenhouse Gas Emission from Ruminants

As assessed using an Intergovernmental Panel on Climate Change (**IPCC**) approach, and on basis of life-cycle analysis animal agriculture is estimated to be responsible for 8 to 18% of global anthropomorphic GHG emissions (IPCC, 2007b; Steinfeld and Wassenaar, 2007; O'Mara, 2011). Ruminant production releases GHG in the form of CH_4 from enteric fermentation, N_2O from use of N fertilizers, CH_4 and N_2O from manure, and CO_2 from on-farm fossil fuel and energy usage (O'Mara, 2011). Livestock

production in general and ruminant production in particular, is also closely linked to land-use changes, such as deforestation for crop and pasture production and grassland degradation due to overstocking (Steinfeld and Wassenaar, 2007). Land-use changes can lead to substantial releases of GHG and changes in soil carbon. However, under IPCC inventory methodology, emissions and removals of GHG resulting from direct human-induced land-use change are not assigned to the agriculture sector but classified separately under land-use change and forestry activities (IPCC, 2000a).

To account for the different GWP of the individual GHG gases, GHG emissions are commonly expressed in CO_2 equivalent (**CO_2e**). Emissions as CO_2e are calculated by multiplying the amount of the respective GHG gas by its GWP (IPCC, 2006). The GWP is a relative measure of how much heat a GHG traps in the atmosphere. The GWP is calculated over a specific time horizon (e.g. 20, 100 or 500 years), and expressed as a factor of CO_2, whose GWP is standardized to 1 (IPCC, 2007a). The concept of expressing GHG emission as CO_2e is especially importance in systems that emit multiple GHGs, such as ruminant production, which as discussed, produces CO_2, CH_4 and N_2O.

While CO_2 accounts for approximately 72% of global GHG emission, CH_4 and N_2O are the second and third most prevalent GHG; accounting for about 20 and 7% of emissions (IPCC, 2007a; U.S. EPA, 2012). With GWP of 25 and 298, and average atmospheric life times of 12 and 114 years respectively, CH_4 and N_2O, respectively are important targets for GHG mitigation (IPCC, 2007). Agriculture production is responsible for about 50 and 60% of all

anthropogenic CH_4 and N_2O emission, respectively (IPCC, 2007b). Ruminant livestock produces approximately 80 million tonnes of CH_4/yr, accounting for nearly one-third of anthropogenic CH_4 emissions (Beauchemin *et al.*, 2008; Lassey, 2008). Similar to CH_4, ruminant production is also a significant source of N_2O (Eckard *et al.*, 2010). The efficiency of N utilization of ruminants is low as 60 to 85% of intake N is excreted in urine and feces (Calsamiglia *et al.*, 2010). Consequently, direct and indirect N_2O emissions from manure are high and present a major environmental issue for beef cattle production (de Klein and Eckard, 2008; Eckard *et al.*, 2010).

Undoubtedly, the growing human population will increase the production of livestock and use of N fertilizers for the production of food and feed. A trend that will invariable lead to higher emissions of CH_4 and N_2O (IPCC, 2007b; Steinfeld and Wassenaar, 2007) if effective mitigation practices for agriculture are not developed. Although the use of intensive cattle production systems lowers GHG emissions per unit product, demand for livestock is expected to increase more rapidly than intensification, leading to an increase in global GHG emissions as a result of cattle production (IPCC, 2007b; Steinfeld and Wassenaar, 2007).

Beef cattle related CH_4 and N_2O emission are also a relevant issue in western Canada. In 2012, there were 12.5 million cattle in Canada, of which 9.3 million or 74% respectively were located in the western provinces (Manitoba, Saskatchewan, Alberta and British Columbia). Alberta is the province with the highest number of cattle (4.9 million), of which 3.1 million or 63% are classified

as beef cattle (Statistics Canada, 2013). Using an LCA approach Beauchemin *et al.* (2010) estimated an average GHG intensity of 21.7 kg CO_2e/kg carcass for beef produced in western Canada. Within the beef life cycle, enteric CH_4 accounted for 63% and N_2O from soil and manure for 27% of the total emissions, while CH_4 emissions from manure and CO_2 energy emissions (5% each) were only minor contributors (Beauchemin *et al.*, 2010).

1.3.4 Impact of Distillers' Grains on Methane and Nitrous Oxide emissions

McGinn *et al.* (2009) reported that replacing barley grain (35% DM basis) with CDDGS (12.7% fat DM) in a growing diet decreased enteric CH_4 emission from 23.8 to 19.9 g/DMI or 7.1 to 5.4% of GEI. This reduction in CH_4 was thought to be due to the high fat level in CDDGS. Fat that is unprotected from ruminal digestion decrease CH_4 emissions by a number of different mechanisms. Particularly medium-chain fatty acids (**MCFA**) reduce CH_4 through the toxicity they exhibit on methanogens (Machmüller *et al.*, 2003, Beauchemin *et al.*, 2008). Supplemented fat also decrease ruminal protozoal counts (Doreau and Ferlay, 1995). Methanogens and protozoa are physically and metabolically associated, therefore a reduction in protozoa numbers or activity is frequently associated with a reduction in CH_4 (Newbold *et al.*, 1995). Additionally fat supplementation has adverse effects on ruminal bacteria, with cellulolytic bacteria being more sensitive to long-chain fatty acids (**LCFA**) compared to amylolytic bacteria (Galbraith *et al.*, 1971). Depending on the type of fat and dietary inclusion level, this can lead to a depression of ruminal fiber digestion and decreased acetate:propionate ratio (Johnson and Johnson, 1995;

Elliott *et al.*, 1997). Formation of acetate results in a net release of H_2 and favours CH_4 production, while propionate and CH_4 formation are inversely related since both pathways compete for H_2 (Janssen, 2010). In some cases, dietary fat can also replace structural carbohydrates that would otherwise contribute to CH_4 production in the rumen (Johnson and Johnson, 1995), or lower CH_4 (g/d) by a reduction of DMI (Beauchemin *et al.*, 2008). Biohydrogenation of unsaturated fatty acids also serves as alternative H_2 sink to the reduction of CO_2 to CH_4 (Czerkawski, 1986). However, the total amount of H_2 utilized for ruminal biohydrogenation of unsaturated fatty acids is infinitesimal compared to the H_2 used for methanogenesis (Czerkawski, 1986; Johnson and Johnson, 1995).

Similar to McGinn et al (2009), Moate *et al.* (2011) reported a reduction in CH_4 from 25.0 to 23.7 g/kg DMI in dairy cows fed diets that contained 26% of the DM as brewers' grains (11.0% fat DM basis). Replacement of 30% of corn bran DM with CDDGS in a brome hay-based diet reduced CH_4 from 69.4 to 57.7 mL/min × kg digested DM in lambs (Behlke *et al.*, 2007). In contrast, in a second experiment, Behlke *et al.* (2007) observed that substitution of 30% corn with CDDGS increased CH_4 production (mL CH_4/min × kg digested DM) in lambs. The fat content of the CDDGS was not reported in either study. The reason that feeding CDDGS increased CH_4 emission in the second study of Behlke *et al.* (2007) could be due to the fact that it replaced corn grain. Corn starch is less intensively digested in the rumen as compared to barley or wheat

starch, therefore CH_4 emission of corn-based diets are typically lower compared to most other grains (Beauchemin and McGinn, 2005).

As discussed in section 1.2.3, usage of CDDGS and WDDGS as an energy source above an inclusion level of ~20%, results in high levels of N excretion (Walter *et al.*, 2012). Nitrogen excreted as urea via urine is readily hydrolyzed contributing to volatile N losses as NH_3, which can subsequently be converted to NO_3^- or N_2O. Consequently, McGinn *et al.* (2009) suspected that the reduction in CH_4 as result of feeding 35% diet DM CDDGS could be offset by increased manure related N losses and the production of N_2O. Unlike CH_4, N_2O is not directly emitted by cattle but arises primarily as a result of denitrification of manure N in soils (de Klein and Eckard, 2008; section 1.3.2). Therefore, direct measurement of N_2O is difficult. Consequently, Erickson and Klopfenstein (2010) used a N mass balance approach to estimate N losses from feedlot steers fed 0, 15, or 30% WDGS (DM basis). They reported that feeding corn WDGS resulted in an increase in total manure N and an increase in volatile N losses (kg/steer). Losses of N in the form of NH_3 from beef feedlots accounts for up to 50% of excreted manure N (Hristov *et al.*, 2011) and is particularly high during the summer months (Todd *et al.*, 2006). Similarly to Erikson *et al.* (2010), Hao *et al.* (2009) reported that feeding 20, 40, 60% or 60% WDDGS (DM basis) increased total N excretion as compared to a barley based control diet. In addition, water soluble NH_4^+ in feces and manure (urine and feces) were higher with 40 and 60% DDGS diets compared to the control, indicating an increased risk of NH_3 volatilization (Hao *et al.*, 2009).

1.4 Summary and Thesis Objectives

As discussed, fuel ethanol production from cereal grains has increased exponentially. Whereas ethanol in the United States is mostly produced from corn, Canadian plants use corn as well as wheat grain for the production of ethanol. Due to their high fiber content co-products of the grain-based ethanol industry, such DDGS are predominantly utilized as a protein source for ruminant livestock. However, depending on its price, DDGS is also being utilized as an energy source with the highest dietary inclusion levels in beef cattle diets (Klopfenstein *et al.*, 2008). As for all co-products, the chemical composition of DDGS is variable and dependant on the processing conditions and grain source (Spiehs *et al.*, 2002; Belyea *et al.*, 2010). Wheat grain is higher in CP, but lower in oil than corn grain, consequently WDDGS is higher in CP (~40 vs. ~30% DM) and lower in fat (~5 vs. ~10% DM) compared to CDDGS.

Enteric CH_4 emissions and losses of manure N, which can lead to formation of N_2O, are major concerns associated with beef cattle production. Methane and N_2O are not only potent GHGs with 25 and 298 times the GWP of CO_2 (IPCC, 2007a), but also represent energy and N inefficiencies in ruminant production systems (Eckard *et al.*, 2010). Thus, CH_4 and N_2O mitigation strategies that are not detrimental to growth performance would reduce the environmental impact and improve the efficiency of the western Canadian beef cattle industry. Recent research results have stimulated an interest in exploring the impact of DDGS on CH_4 emission and N excretion from beef cattle. Inclusion of 35% diet DM CDDGS in a high forage beef cattle diet reduced

enteric CH_4 (g/DM intake) by 16.4%; a response that was attributed to the high fat content of CDDGS (McGinn *et al.*, 2009). However, there is no information on the impact of WDDGS on CH_4 emission or how dietary inclusion of even higher levels of DDGS impact CH_4 emission from beef cattle. Furthermore, usage of CDDGS or WDDGS as energy source will increase N excretion and subsequently N_2O losses which could offset any reduction in CH_4. In order to fully assess the impact of CDDGS and WDDGS inclusion on net GHG emission an LCA that accounts for all GHGs from the beef life cycle is required.

Our overall null hypothesis was that, due to its higher fat content, feeding CDDGS would be more effective in reducing CH_4 emissions from beef cattle than WDDGS in both growing and finishing diets. Furthermore we hypothesized that feeding CDDGS and WDDGS would increase the excretion of N in urine and feces as compared to standard barley-based supplemented feedlot diets; and that the predicted increase in N_2O emission would offset the reduction in CH_4.

The overall goal of this thesis was to assess the impact of feeding CDDGS and WDDGS on GHG emissions from growing and finishing beef cattle from a life cycle perspective. The following objectives were formulated to achieve these set goals:

1.) Compare *in vitro* CH_4 production from CDDGS and WDDGS as substitution for whole crop barley silage, and to describe the responses of CH_4 and other fermentation parameters to increasing levels of both DDGS types as fermentation substrate (Chapter 2).

2.) Determine if the inclusion of CDDGS or WDDGS in growing (high forage) diets reduces enteric CH_4 emissions from beef cattle, and if the oil in corn was responsible for any response observed (Chapter 3).

3.) Determine if the inclusion of CDDGS or WDDGS in finishing (high concentrate) diets reduces enteric CH_4 emissions from beef cattle, and if the oil in corn was responsible for any response observed (Chapter 4).

4.) Evaluate the impact of CDDGS and WDDGS inclusion on GHG emission of the beef feedlot life-cycle using a LCA approach (Chapter 5).

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CHAPTER 2 - In vitro production of methane with increasing levels of corn- or wheat-based dried distillers' grains with solubles in a barley silage-based diet[1]

2.0 Introduction

Dried distillers' grains with solubles is a major by-product from the biofuel industry wherein cereal grains are fermented to produce ethanol. As ethanol production has increased considerably in the last decade, large amounts of DDGS are available and predominantly used as feed for ruminant livestock (Klopfenstein *et al.*, 2008). Corn based DDGS is the most abundant DDGS in the USA whereas in Canada WDDGS accounts for almost one third of total DDGS production (USDA Foreign Agricultural Service, 2010). As a result of the fermentation process, DDGS is largely starch free, but concentrated three fold in protein, fibre and fat (Spiehs *et al.*, 2002). The fat content is higher in CDDGS (~10% DM; Spiehs *et al.*, 2002) than in WDDGS (~5% DM; Gibb *et al.*, 2008) owing to the higher level of fat in corn. Supplementation of ruminant diets with fat reduces ruminal CH_4 through a number of mechanisms including reduction in ruminal DM digestibility, direct effects of fatty acids on ruminal methanogens and protozoa, and by biohydrogenation of unsaturated fatty acids (Czerkawski *et al.*, 1966; Johnson and Johnson, 1995). Additionally, dietary fats often replace fermentable carbohydrates that otherwise would contribute to an

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increase in the reducing equivalents available to reduce CO_2 to CH_4 (Beauchemin *et al.*, 2008).

Replacing a mixture of 35% barley grain and 5% canola meal (DM basis) by CDDGS (10.0% fat DM) in a high-forage diet reduced enteric CH_4 emissions of beef cattle from 25.3 to 21.5 g CH_4/kg DMI, while including 40% DM WDDGS (4.1% fat DM) had no effect on CH_4 emissions (23.9 g/kg DMI; Hünerberg *et al.*, 2013a). In a second study by Hünerberg *et al.* (2013b), replacing 40% DM of barley grain with CDDGS (9.7% fat DM) in a high-grain diet reduced CH_4 emissions from 16.6 to 13.6 g/kg DMI; while WDDGS (3.4% fat/kg DM) had no effect on enteric CH_4 production (18.4 g/kg DMI). Results from both *in vivo* studies indicate that high-fat CDDGS can effectively reduce CH_4 emissions at dietary inclusion level of 40% DM. However, it is unknown how CDDGS and WDDGS at inclusion level different from Hünerberg *et al.* (2013a; 2013b) affect CH_4 production. Measuring *in vivo* CH_4 production is expensive, labor intensive and time consuming; while *in vitro* batch culture fermentation is an effective technique to screen CH_4 production of several substrates simultaneously under standardized laboratory conditions (Soliva and Hess, 2007).

The objective of this study was to compare *in vitro* CH_4 production from CDDGS and WDDGS as these by-products over a range of substitution for whole crop barley silage, and to describe responses of CH_4 and other fermentation parameters to increasing levels of both DDGS types as a substrate.

2.1 Materials and Methods

Substrates, inoculum and incubation

The substrates used were mixtures of whole crop barley silage and CDDGS or WDDGS in the ratios of 80:20, 60:40, 40:60, 20:80 and 0:100 (% DM). It has to be acknowledged that DDGS concentrations above 40 to 60% DM are typically not fed *in vivo* because of adverse effects on feed intake and animal performance. The levels of DDGS used for this study were chosen to characterize *in vitro* CH_4 production and fermentation parameters for a theoretical range of DDGS inclusion level of up to 100% DM.

All substrate components were dried separately at 55°C for 24 h and ground through a 1 mm screen (Wiley mill standard model 3, Arthur H. Thomas, Philadelphia, PA, USA) before being combined. The incubation included 5 replications for each DDGS type at each inclusion level. The substrates 0.3 ± 0.005 g were weighed into ANKOM bags (model F57, ANKOM Technology, Macedon, NY, USA) and heat sealed. Bags were placed in 125 ml serum vials 1 day prior to incubation.

Rumen fluid was obtained from two ruminally cannulated non-lactating Holstein cows 2 h after feeding. Cows were fed a high forage diet (65% whole crop barley silage, 20% barley grain, 10% canola meal and 5% vitamin/mineral supplement; DM basis) *ad libitum*. Rumen contents were collected from three sites within the rumen (i.e., reticulum and dorsal and ventral sac), thoroughly mixed and squeezed through two layers of PeCAP® polyester 355 μm pore size screen into a preheated and insulated transport bucket. Donor cows were cared

for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Rumen fluid was immediately transferred to the laboratory and re-strained through 4 layers of cheesecloth. Filtrate was maintained at 39°C in a water bath and the headspace continuously flushed with CO_2. Strained rumen fluid (10 ml) was dispensed into pre-warmed 39ºC culture flasks, which were preloaded with a substrate filled ANKOM bag, 40 ml of buffer solution and 0.5 ml of cysteine sulfide solution as a reducing agent (Menke *et al.*, 1979). The incubation flasks were sealed with aluminum crimp-sealed rubber stoppers and placed on two rotary shaker platforms (Lab-Line Instruments Inc., Melrose Park, IL, USA) oscillating at 90 rpm in an incubator (model 1915, Sheldon Manufacturing, Cornelius, OR, USA) at 39°C. Triplicate flasks containing only rumen fluid and buffer solution were used as blank controls. All flasks were incubated for 24 h.

Gas measurement and sample collection

A pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC, Canada) attached to a 22 gauge (0.6 mm) needle was used to measure gas pressure [P_t (kPa)] inside the flasks by inserting the needle into the flasks after 3, 6, 12 and 24 h of incubation. Gas pressures were used to calculated gas production [G_P (ml)] using the equation of Mauricio *et al.* (1999) as:

$$G_P = 0.18 + (3.697 \times P_t) + (0.0824 \times P_t^2)$$

Gas production was corrected for the amount of substrate incubated and gas produced from blank controls. After each Pt measurement, a 15 ml gas sample was collected from each flask using a syringe. The gas sample was then injected into a 5.9 ml evacuated Exetainer (Labco Ltd., High Wycombe, Buckinghamshire, U. K.) and analyzed for CH_4. The remaining gas was released from the flask after the gas sample was collected. Gas production (mL/g DM) and CH_4 production per g incubated DM (mg/g DM) or digested DM (mg/g **DMD**) were summarized and reported for the duration of incubation.

After 24 h of incubation, flasks were opened and the pH of the incubation fluid measured using a pH meter (model Accumet 25, Denver Instrument Company, Arvada, CO, USA). Subsequently, flasks were placed on ice and a 1.6 ml subsample of fluid was removed from the bottle, acidified with 400 µl of metaphosphoric acid (0.25; wt/vol) and stored at -20°C for analysis of VFA. Bags containing the residual substrate were removed from the flasks, washed under cold tap water until the water became clear, dried at 55°C for 48 h and weighed to estimate *in vitro* DM disappearance (**IVDMD**).

Laboratory analyses

Methane concentrations were analyzed using a gas chromatograph (model 6890, Agilent Technologies, Wilmington, DE, USA) coupled to a thermal conductivity detector. The correlation coefficients for all standard curves exceeded 99.9%. The VFA concentrations were determined by gas chromatography as described by Holtshausen *et al.* (2009). Analytical DM was

determined by drying at 135 °C for 2 h (AOAC, 2005; method 930.15), followed by hot weighing. Organic matter was calculated as the weight lost upon ignition at 550°C for 5 h (AOAC, 2005; method 942.05). Crude fat was determined by ether extraction (AOAC, 2006; method 2003.05) using a hot extraction unit (model E-816 HE, Buchi Labortechnik AG, Flawil, Switzerland). Total N was determined by combustion analysis (model NA 1500, Carlo Erba Instruments, Milan, Italy). Neutral detergent fiber and ADF were quantified as described by Van Soest *et al.* (1991), using conventional filtration through fritted glass crucibles, and expressed inclusive of residual ash. Neutral detergent fiber was determined with inclusion of a heat stable amylase and sodium sulphite. Starch was determined as described by Rode *et al.* (1999). Chemical analyses were completed on each sample in duplicate (Table 2-1).

Table 2-1 Chemical composition (% DM) of barley silage, corn and wheat dried distillers' grains (CDDGS, WDDGS [means ± SD; n=2]).

Item	Barley silage	CDDGS	WDDGS
Dry matter, %	43.3 ± 0.4	91.7 ± 0.3	91.7 ± 0.3
Organic matter	92.1 ± 0.1	96.5 ± 0.1	93.7 ± 0.1
Crude protein	12.1 ± 0.1	31.5 ± 0.2	45.7 ± 0.2
ADF[1]	34.5 ± 0.4	14.3 ± 0.5	14.4 ± 0.3
NDF[2]	52.2 ± 1.1	47.4 ± 1.4	35.2± 0.9
Crude fat	2.5 ± 0.1	11.5 ± 0.4	4.9 ± 0.1
Starch	24.7 ± 0.8	4.3 ± 0.1	1.0 ± 0.1

[1]Acid detergent fiber expressed inclusive residual ash.
[2]Neutral detergent fiber assayed with heat stable amylase and expressed inclusive residual ash.

Statistical analysis

Data were analyzed using the mixed model procedure of SAS (SAS Institute, 2001). The incubation flask was the experimental unit for all variables. The statistical model was:

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where: y_{ij} was the dependent fermentation variable; μ the overall mean; α_i the fixed effect of type of DDGS i (CDDGS or WDDGS); β_j the fixed effect of DDGS inclusion level j (20, 40, 60, 80 or 100% DM); $(\alpha\beta)_{ij}$ the interaction of DDGS type i by inclusion level j; and ε_{ij} the residual error term. Denominator degrees of freedom were estimated using the Kenward-Roger option in the model statement. Pre-planned comparisons between CDDGS and WDDGS at the same inclusion level were completed using the contrast statement. Polynomial contrasts were used to determine linear and quadratic responses of dependent variables to increasing level of CDDGS or WDDGS. Data are presented as least squares means ± standard error of means. Differences were declared significant if $P < 0.05$.

2.3 Results and Discussion

The IVDMD (Table 2-2) decreased linearly ($P < 0.01$) with increasing levels of CDDGS or WDDGS in the diet, likely attributable to the higher concentrations of crude fat in CDDGS (11.5% fat) and WDDGS (4.9% fat) compared to barley silage (2.5% fat; all DM basis). Elevated dietary fat levels can depress *in vitro* fibre and OM digestion by exerting toxic effects on protozoa

and cellulolytic bacteria (Henderson, 1973), and by limiting microbial attachment to feed particles (McAllister *et al.*, 1994). The depression in IVDMD was higher ($P < 0.05$) for CDDGS than for WDDGS at inclusion levels above 40% DM, which corresponds with the lower ($P < 0.05$) gas production (ml/kg DM) for CDDGS compared to WDDGS at all inclusion levels.

Production of CH_4 (mg/g DM) increased ($P < 0.05$) from 5.7 to 10.0 mg CH_4/g DM as the concentration of CDDGS increased from 20 to 80% DM. However, this response is not typical of that observed *in vivo* as increased levels of concentrate in the diet are usually associated with lower CH_4 emissions per unit feed intake (Johnson and Johnson, 1995). However, it is important to consider that substitution of DDGS for barley silage also results in a substantial change in both the protein content and the nature of the fiber within the mixed substrate. Our results suggest that substitution of DDGS for barley silage results in an increase in the amount CH_4 produced/g DM fermented.

Methane production (mg) per g/DM and g/DMD from CDDGS was lower ($P < 0.05$) than from WDDGS when DDGS was included at levels of 20 to 80% DM, with the difference being more pronounced at lower DDGS inclusion levels. In contrast, CH_4 production (mg/g DM; mg/g DMD) was similar when WDDGS or CDDGS were the sole substrate incubated. Decreased CH_4 emissions (mg/g DM; mg/g DMD) from samples containing 20 to 80% DM CDDGS as compared to WDDGS likely reflect the higher fat content in CDDGS, which could have lowered OM fermentation and exerted toxic effects on methanogens and protozoa (Czerkawski *et al.*, 1966).

Table 2-2 Effect of inclusion level of corn or wheat dried distillers' grains with solubles on *in vitro* dry matter disappearance (IVDMD), gas and CH_4 production, pH and volatile fatty acids (VFA) after 24 h *in vitro* incubation.

	Dried distillers' grains with solubles,% DM											P-values[6]						
	20		40		60		80		100		Pooled				CDDGS		WDDGS	
	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	CDDGS	WDDGS	SEM	Type[1]	Level[2]	Type × Level[3]	L[4]	Q[5]	L	Q
IVDMD, % DM	49.3	50.9	48.3	49.6	44.7	49.5*	43.0	45.8*	39.2	44.6*	0.97	<0.01	<0.01	0.16	<0.01	0.23	<0.01	0.42
Gas, mL/g DM	122.8	177.9*	130.2	183.2*	143.1	180.9*	147.1	174.1*	146.0	162.0*	4.89	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.02
CH_4, mg/g DM	5.7	12.5*	7.4	12.4*	8.8	12.2*	10.0	11.5*	9.9	9.5	0.30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CH_4, mg/g DMD	10.9	23.3*	14.5	23.5*	18.7	23.4*	22.0	23.8*	21.6	20.2	0.74	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	0.02
pH	6.45*	6.41	6.42	6.41	6.43	6.43	6.44	6.45	6.45	6.45	0.005	0.03	<0.01	<0.01	0.11	<0.01	<0.01	0.55
Total VFA, mM	68.3	81.5*	72.1	79.6*	73.4	80.0*	73.5	77.0*	73.7	75.8*	0.85	<0.01	0.16	<0.01	<0.01	<0.01	<0.01	0.56
VFA, mol/100 mol																		
Acetate (A)	49.3	51.4*	50.3	51.6*	50.6	52.0*	51.2	51.9*	51.4	52.2*	0.13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.55
Propionate (P)	22.3*	19.4	21.3*	19.4	20.3*	19.5	19.7	19.5	19.4	19.7	0.14	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	0.63
Butyrate	18.1	17.9	17.8	17.7	17.8*	17.1	17.6*	17.0	17.5*	16.4	0.09	<0.01	<0.01	<0.01	<0.01	0.41	<0.01	0.52
A:P ratio	2.21	2.65*	2.36	2.66*	2.50	2.66*	2.60	2.66	2.64	2.65	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	0.95	0.62

[1]Type = CDDGS or WDDGS.
[2]Level = 20, 40, 60, 80 and 100% DM of DDGS.
[3]Type × Level = interaction of DDGS type × inclusion level.
[4]L = linear and
[5]Q = quadratic effects of different types of DDGS.
[6]Means within an inclusion level differ at (*; $P < 0.05$).

Additionally, biohydrogenation of fatty acids in CDDGS may have directed reducing equivalents away from reduction of CO_2 to CH_4 formation, as previously described *in vitro* (Jenkins, 1987; Getachew *et al.*, 2001).

Total VFA production and proportions of acetate were consistently higher ($P < 0.05$) in samples containing WDGGS compared to CDDGS. Addition of CDDGS increased ($P < 0.05$) propionate proportions at levels of 20, 40 and 60% DM compared to WDDGS. This resulted in higher ($P < 0.05$) acetate to propionate ratios for WDDGS compared to CDDGS at levels up to 60% DDGS DM and likely reflects reduced fibrolytic activity (Getachew *et al.*, 2004) with CDDGS. Higher concentrations of propionate and lower acetate to propionate ratios, in batch culture *in vitro* incubation of 20% DM CDDGS compared to WDDGS have been reported by others (Au *et al.*, 2010; McKeown *et al.*, 2010). Production of CH_4 and propionate are closely linked since both pathways utilize reducing equivalents. Therefore, increased propionate production in diets containing CDDGS compared to WDDGS may have been responsible for the lower CH_4 concentration at DDGS inclusion rates up to 60% DM. Culture pH remained above 6.4 in all incubations and was only lower ($P < 0.05$) in WDDGS versus CDDGS at an inclusion level of 20% DM.

Results of this *in vitro* study suggest that compared with WDDGS, adding CDDGS to whole crop barley silage at dietary inclusion levels of up to 80% DM could reduce CH_4 production *in vivo*. The lower CH_4 production was due to greater reduction in IVDMD/unit CDDGS compared to WDDGS,

as well as higher concentrations of propionate when up to 60% DM CDDGS was included in the diet. These predictions were subsequently confirmed *in vivo* when WDDGS and CDDGS were included in barley silage-based diets at 40% DM (Hünerberg *et al.*, 2013a; 2013b).

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CHAPTER 3 - Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from growing beef cattle[2]

3.1 Introduction

It is estimated that animal agriculture is responsible for approximately 2.9% of total anthropogenic GHG emissions in the United States (Council for Agricultural Science and Technology, 2011). Ruminant livestock have been estimated to account for 17 to 37% of global anthropogenic CH_4 emissions (Steinfeld and Wassenaar, 2007; Lassey, 2008). Recent research has shown that enteric CH_4 is the largest source of GHG emissions in the Canadian beef production cycle, accounting for 63% of total emissions (Beauchemin *et al.*, 2010).

Co-products from the ethanol industry, such as DDGS are a source of protein and energy for beef cattle diets. In Canada, both CDDGS and WDDGS are frequently used in cattle diets. Recent shortages on national grain markets have led to an increase of DDGS prices. In addition, reduction of subsidies for production and use of grain-based ethanol may impact ethanol production capacity leading to higher and more volatile DDGS availability and pricing in the future (USDA, 2013).

McGinn *et al.* (2009) reported that inclusion of 35% dietary DM as CDDGS reduced CH_4 emissions (g/kg DMI) by 16.4% in cattle fed a barley silage-based diet. This response was thought to be due to the high fat level in

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CDDGS (>12% DM basis). However, WDDGS (< 5% fat DM basis) has less than half the fat content of CDDGS and there is no information on the impact of feeding WDDGS on CH_4 emission from growing beef cattle. Furthermore, it is unknown if supplementing corn oil to WDDGS has the same effect on CH_4 emission as corn oil naturally contained in CDDGS. Despite the potential reduction in CH_4, a limitation to using DDGS as an energy source in beef cattle diets is that its high protein results in a dramatic increase in N excretion (McGinn *et al.*, 2009). Excessive N excretion contributes to higher NH_3 emissions that negatively impact air quality and contribute to emissions of N_2O, another potent GHG (Todd *et al.*, 2006). Urinary N is more susceptible to leaching and volatilization losses than fecal N (Bussink and Oenema, 1998). Further research is needed to evaluate if N excretion as a result of feeding DDGS offsets gains in reducing GHG emissions through a reduction in CH_4. Due to its higher fat content, we hypothesized that feeding CDDGS would be more effective in reducing CH_4 emissions from growing beef cattle than WDDGS.

The objective of this study was to examine the impact of CDDGS or WDDGS on CH_4 emissions and partitioning of N excretion from growing beef cattle, and determine if the oil in CDDGS was responsible for any response observed.

3.2 Materials and Methods

This study was conducted using the Metabolism Barn and the Controlled Environment Facility at Agriculture and Agri- Food Canada's

Research Centre in Lethbridge, Alberta. The experimental protocol received institutional approval and was conducted in accordance to the guidelines of Canadian Council on Animal Care (1993).

Experimental Design and Animals

Sixteen spayed crossbreed beef heifers (388.5 ± 34.9 kg of initial BW) were used in this experiment, which was designed as a replicated 4 × 4 Latin square with 2 groups of 8 animals, four 21-d periods, and 4 dietary treatments. Heifers were ruminally cannulated prior to the start of the study and vaccinated with Express 5-PHM (Boehringer Ingelheim Ltd., Burlington, ON, Canada), a modified live vaccine against bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncytial virus, *Mannheimia haemolytica*, and *Pasteurella multocida*.

Methane emissions were measured using 4 open circuit respiratory chambers with 2 heifers housed in each chamber during each measurement period. Within each group, heifers were paired such that each pair had similar BW. The 4 pairs within each group were randomly allocated to 1 of 4 treatment diets. As only 4 respiratory chambers were available at a time, the 2 groups were offset by 1 wk to facilitate CH_4 measurements.

Treatment Diets and Feed Sampling

Treatment diets were formulated as growing (high forage) diets typical of that fed during the first 80 d in western Canadian feedlots. The control diet (control) contained (DM basis) 55% whole crop barley silage, 35% barley grain, 5% canola meal, and 5% vitamin and mineral supplement

(Table 3-1). Three DDGS diets were formulated by replacing barley grain and canola meal (40% of the dietary DM) with CDDGS, WDDGS, or WDDGS plus corn oil **(WDDGS+oil)**. For the WDDGS+oil treatment, corn oil (Great Value; Wal-Mart, ON, Canada) was added to WDDGS (which contained 4.11% fat on DM basis) in a ratio of 6:94 to achieve the same fat level as in CDDGS (9.95% fat on DM basis). Total mixed rations were prepared daily (Data Ranger, American Calan Inc., Northwood, NH). Heifers were fed for ad libitum intake (5% refusal) once daily at 1100 h. Quantities of feed offered and refused were recorded daily.

Diets and ingredients were sampled once weekly and analyzed for DM by drying at 55°C for 48 h. The forage inclusion level (as fed basis) was adjusted if the DM concentration of barley silage deviated more than 3 percentage units from the average. Weekly subsamples were composited by period. Orts were sampled daily during the digestibility trial (only group 1) and CH_4 measurements (both groups) and pooled by animal at the end of each period. Samples were stored at −20°C until determination of DM and chemical composition.

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Table 3-1 Composition of experimental diets.

	Treatment[1]			
Item	Control	CDDGS[2]	WDDGS[3]	WDDGS+oil
Ingredient, % of DM				
Barley silage	55	55	55	55
Barley grain, steam-rolled	35			
Canola meal	5			
CDDGS[2]		40		
WDDGS[3]			40	37.6
Corn oil				2.4
Barley grain, ground	3.4	3.4	3.4	3.4
Calcium carbonate	1.25	1.25	1.25	1.25
Salt	0.15	0.15	0.15	0.15
Molasses, dried	0.13	0.13	0.13	0.13
Mineral and vitamin premix[4]	0.06	0.06	0.06	0.06
Vitamin E (500,000 IU/kg)	0.003	0.003	0.003	0.003
Flavouring agent[5]	0.003	0.003	0.003	0.003
Chemical composition[6]				
DM, %	51.3 ± 2.4	52.0 ± 2.0	52.7 ± 2.1	52.6 ± 1.6
OM, %	93.3 ± 0.2	92.6 ± 0.1	91.4 ± 0.3	91.3 ± 0.1
CP, %	13.0 ± 0.5	18.6 ± 0.3	23.5 ± 0.2	22.0 ± 0.6
NDF, %	32.5 ± 2.7	38.5 ± 1.1	33.9 ± 1.4	33.3 ± 2.3
ADF, %	18.0 ± 1.1	23.7 ± 0.6	23.6 ± 0.7	23.0 ± 1.6
Fat, %	3.0 ± 0.1	5.4 ± 0.1	3.7 ± 0.2	5.6 ± 0.2
Starch, %	35.8 ± 1.3	17.9 ± 0.9	16.8 ± 0.6	17.4 ± 0.7
GE, Mcal/kg of DM	4.31 ± 0.03	4.42 ± 0.06	4.38 ± 0.06	4.50 ± 0.08

[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]Corn dried distiller' grains plus solubles.
[3]Wheat dried distiller' grains plus solubles.
[4]Supplied per kilogram of dietary DM: 65 of mg Zn, 28 mg of Mn, 15 mg of Cu, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6,000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.
[5]Anise 422 powder containing ground cumin, fennel, fenugreek, silicon dioxide and wheat bran (Canadian Bio-Systems Inc., Calgary, Alberta, Canada).
[6]Determined using samples pooled by diet within each period; all values except DM are expressed on a DM basis (n = 4; mean ± SD).

Nitrogen Excretion and Digestibility

Excretion of N and apparent total tract digestibility of the diets were determined using the 8 animals in group 1 (376.4 ± 29.7 kg of initial BW). From d 1 to 17 heifers were housed in individual tie stalls in a metabolism barn. After they were adapted to the diets over the first 10 d of each period, total urinary and fecal collection were conducted between d 11 and 14. The heifers were fitted with urinary indwelling balloon catheters (Bardex® Lubricath® Foley catheter, 75 c.c. and 26 Fr.; Bard Canada Inc., Oakville, ON, Canada) to ensure separation of urine and feces. Urine was preserved by acidification (pH < 2) with 4 N H_2SO_4 to prevent volatilization of NH_3. Feces were collected using pans placed behind the heifers. Total output of urine and feces was measured every 24 h, and mixed samples were sub-sampled. Aliquots of the urine (1% of total daily output) were composited by heifer within period, diluted with distilled water at a ratio of 1:5 and stored at −20°C until analyzed. A sub-sample of the daily feces (~500 g) was oven-dried at 55°C. A representative composite sample was obtained by pooling the dried daily feces based on their respective DM content.

Ruminal Fermentation Measurements

On d 14, composite rumen samples (500 g) were obtained from three sites (reticulum, dorsal and ventral sac) within the rumen of each animal at 0, 2, 6, 12 and 24 h after feeding. Rumen contents were thoroughly mixed, squeezed through 2 layers of polyester monofilament fabric (pore size 355 μm; B. & S. H. Thompson, Ville Mont-Royal, Quebec, Canada) and filtrate

(5 mL) was mixed with 1 mL of 25 % (wt/vol) metaphosphoric acid for VFA analysis, with an additional 5 mL of filtrate being mixed with 1 mL of 1% (wt/vol) H_2SO_4 for NH_3-N analysis. Samples were stored at −20°C until analyzed. For enumeration of protozoa, filtrate (5 mL) was mixed with 5 mL of methyl green-formalin-saline solution (**MFS**). The samples were stored in the dark at room temperature until analyzed.

Ruminal pH was recorded continuously during the periods of CH_4 measurement using the LRCpH data logger system (Dascor, Escondido, CA; Penner *et al.*, 2006). Loggers were standardized in pH 4 and 7 at the start and end of each measurement period with pH being recorded every min. The pH loggers were placed in the ventral sac of the rumen 2 h before the heifers entered the chambers on d 18 and removed immediately after the heifers were returned to the metabolism barn on d 21.

Methane Emission Measurements

On d 18 of each period, heifers were moved to the Controlled Environment Facility to measure CH_4 production over 4 d using 4 large environmental chambers. The chambers measured 4.4 m wide × 3.7 m deep × 3.9 m tall (63.5 m^3 volume, C1330, Conviron Inc., Winnipeg, Manitoba, Canada) and housed 2 heifers in individual tie stalls equipped with comfort mats. Heifers were provided with free access to feed and water. The chamber doors were opened once daily for feeding and cleaning. The emission data corresponding to the door opening as well as the time for chambers to return to steady state conditions were omitted from the analysis.

Methane measurements were conducted as described by Beauchemin and McGinn (2006). Briefly, samples from the fresh-air intake and exhaust air duct of each chamber were pumped sequentially at 1 L/min (TD3LS7; Brailsford and Company, Rye, NY) and passed through an infrared gas analyzer (Ultramat 6; Siemens, Karlsruhe, Germany) via a set of solenoids controlled by a data logger (CR23X; Campbell Scientific, Logan, UT). The difference between the incoming and outgoing flow of CH_4 was used to calculate the amount generated by the 2 animals inside each chamber. The chambers were ventilated using fans in the fresh-air intakes and exhaust ducts. The air volume of each chamber was exchanged every 5 min. Temperature within the chambers was maintained at 10°C. Air velocity was continuously monitored in each intake and exhaust duct for each chamber (model 8455 Air velocity transducer, TSI Inc., Shoreview, MN, U.S.A.). Air flow rates in the ducts were adjusted to generate a slight positive pressure (approximately 2 Pa) inside each chamber. Intake and exhaust air stream CH_4 concentrations of each chamber were sampled every 30 min using the same analyzer. The gas analyzer was calibrated daily, directly after feeding time using N_2 as zero and 405 ppm of CH_4 as standard gases.

Before the start of the experiment the system was calibrated by sequentially releasing 0, 0.2, and 0.4 L/min of CH_4 separately into each empty chamber using a mass-flow meter (Omega Engineering, Stamford, CT). A three point regression was developed by plotting actual against

calculated CH_4 emission. The slopes of these best fit linear relationships were used to correct for between-chamber variability.

Blood sampling

Blood samples for the determination of BUN were collected from all 16 heifers by jugular vein puncture on d 21 of each period 22 h after feeding using 10-mL vacuum tubes containing Li-heparin solution (Vacutainer, Becton Dickinson, Mississauga, Canada). After centrifugation (3,000 × g at 4°C for 20 min) samples were stored at −20°C until analyzed.

Laboratory Analyses

Samples of composited ingredients, diets, orts and feces were oven dried at 55°C and ground through a 1 mm screen (Standard model 4 Wiley mill, Arthur H. Thomas, Philadelphia, PA). Analytical DM was determined by drying at 135 °C for 2 h (AOAC, 2005; method 930.15), followed by hot weighing. The OM content was calculated as the difference between 100 and the percentage of ash (AOAC, 2005; method 942.05). The NDF and ADF concentrations were quantified as described by Van Soest *et al.* (1991), using amylase and sodium sulfite for the NDF analysis. Fat was determined according to AOAC (2006; method 2003.05) using ether extraction (Extraction Unit E-816 HE, Büchi Labortechnik AG, Flawil, Switzerland). Gross energy in diets, orts and feces was determined using a bomb calorimeter (model E2k; CAL2k, Johannesburg, South Africa). For the measurement of CP (N × 6.25) and starch, samples were ground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany). Nitrogen was

quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). Total urinary N was analyzed in the same fashion using freeze dried urine. Starch content was determined by enzymatic hydrolysis as described by Rode *et al.* (1999).

Concentration of NH_3 in urine and rumen fluid was determined by the salicylate-nitroprusside-hypochlorite method (Sims *et al.*, 1995) using a flow injection analyzer. Concentrations of VFA in ruminal fluid were analyzed as described by Addah *et al.* (2012) using gas chromatography (model 5890; Hewlett Parkard, Wilmington, DE, USA) with crotonic acid as an internal standard. Concentration of urea in urine and blood plasma was analyzed using micro-Segmented Flow Analysis (model Astoria2; Astoria Pacific Inc., Clackamas, OR, USA). Ruminal protozoa were enumerated under a light microscope using a counting chamber (Neubauer Improved Bright- Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA, USA) as described by Ogimoto and Imai (1981).

Calculations and Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) with animal as the experimental unit for all variables, except for CH_4 production, where chamber was considered the experimental unit. Continuous ruminal pH data were summarized for daily average, minimum, maximum, SD, as duration below pH 6.0, and area under the curve (**AUC**). The AUC was calculated as the sum of the absolute value of pH deviations below pH 6.0 multiplied by the duration below pH 6.0 and

reported as pH × h. Intake corrected AUC was calculated as AUC divided by DMI. Durations and AUC below pH 6.0 were considered as critical pH threshold levels below which degradation of fiber was impaired (Weimer, 1996). Protozoa numbers were $\log_{10}$ transformed before statistical analysis. The model for DMI and ruminal fermentation variables included the fixed effect of diet and the random effects of group, heifer nested within group, and period nested within group. For ruminal fermentation variables sampling time (0, 2, 6, 12 and 24 h after feeding) was treated as a repeated measure. Data for N excretion and total tract digestibility trial were analyzed using the same model, but without the random effect of group because only group 1 heifers were used in this part of the study. Sampling days (1to 4) were treated as a repeated measure.

Daily CH_4 production (g CH_4/d) from each chamber was expressed per unit of combined DMI (g CH_4/kg DMI) and proportion of GE (%) and DE (%) intake of the 2 heifers within each chamber on that same day. The model used for CH_4 production included the fixed effect of diet and the random effects of group, period nested within group, and chamber nested within group. Day of sampling (d 1 to 4) within each period was treated as repeated measure. Denominator degrees of freedom were estimated using the Kenward-Roger option in the model statement. The PDIFF option adjusted by the Tukey method was included in the lsmeans statement to account for multiple comparisons. The best time series covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Differences

among means were tested using a protected ($P < 0.05$) LSD test. Treatment effects were declared significant at $P < 0.05$.

3.4 Results

Ruminal fermentation and pH

Even though concentration of total VFA (Table 3-2) in the rumen fluid was unaffected by treatment ($P = 0.09$), feeding CDDGS decreased ($P < 0.05$) the molar proportion of acetate as compared to all other treatments and increased ($P = 0.02$) the proportion of propionate compared to WDDGS. Furthermore, CDDGS lowered ($P < 0.05$) the acetate:propionate ratio as compared to the control and WDDGS diet. Heifers fed WDDGS and WDDGS+oil had higher ($P < 0.001$) molar proportion of valerate and concentration of NH_3 ($P < 0.001$) compared to the control and CDDGS diets. Furthermore, the proportion of valerate ($P = 0.01$) and concentration of NH_3 ($P = 0.006$) were higher for WDDGS compared to WDDGS+oil. Numbers of total protozoa were similar among diets that contained DDGS, but were lower ($P < 0.01$) relative to the control.

Table 3-2 Ruminal fermentation variables of ruminally cannulated beef heifers fed a barley silage-based high-forage diet supplemented with barley grain and canola meal, corn- or wheat dried distillers' grains plus solubles (CDDGS, WDDGS) or WDDGS and corn oil (n=16).

	Treatment[1]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Total VFA, m*M*	151	139	144	144	5.0	0.09
VFA, mol/100 mol						
Acetate	60.3^{a}	57.9^{b}	60.5^{a}	59.8^{a}	0.70	<0.001
Propionate	22.1^{ab}	23.1^{a}	20.9^{b}	22.7^{ab}	0.76	0.025
Butyrate	12.4^{b}	14.1^{a}	13.5^{ab}	12.6^{b}	0.57	0.010
Isovalerate	1.97^{a}	1.83^{a}	1.45^{b}	1.51^{b}	0.114	<0.001
Valerate	1.69^{c}	1.66^{c}	2.23^{a}	2.02^{b}	0.061	<0.001
Isobutyrate	1.06	1.01	1.02	0.96	0.049	0.18
Acetate:propionate	3.42^{a}	3.07^{b}	3.54^{a}	3.24^{ab}	0.139	0.002
NH_3, m*M*	6.1^{c}	6.3^{c}	15.8^{a}	14.0^{b}	0.68	<0.001
Protozoa						
Total, n × 10^5/mL	7.9^{a}	4.4^{b}	4.0^{b}	3.1^{b}	1.31	<0.001
Entodiniomorphs[2],%	99.3	99.8	98.6	98.8	0.37	0.11
Holotrichs[3],%	0.7	0.2	1.4	1.2	0.40	0.12

[a,-c]Within a row, means without a common superscript letter differ, P < 0.05.
[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]*Entodiniomorphs = Entodinium + Diplodinium + Polyplastron + Eudiplodinium + Epidinium + Ophryoscolex*.
[3]*Holotrichs = Isotricha + Dasytricha*.

The mean and minimum ruminal pH of heifers fed CDDGS and WDDGS+oil was lower (P < 0.05) compared to those fed WDDGS (Table 3-3). Feeding the control diet resulted in a lower minimum pH as compared to WDDGS (P < 0.001) and WDDGS+oil (P = 0.03) and a higher (P < 0.05) SD of ruminal pH as compared to all other treatments. Ruminal pH in heifers fed CDDGS and WDDGS+oil spent more time (P < 0.05) below pH 6.0 as compared to those fed WDDGS. Feeding WDDGS decreased the AUC expressed as pH × h per d at pH 6.0 (P = 0.05) as compared to the control.

The AUC < pH 6.0 per kg DMI decreased (P < 0.05) for heifers fed WDDGS as compared to those fed control, CDDGS or WDDGS+oil diets.

Table 3-3 Ruminal pH of ruminally cannulated beef heifers fed a barley silage-based high-forage diet supplemented with barley grain and canola meal, corn- or wheat dried distillers' grains plus solubles (CDDGS, WDDGS) or WDDGS and corn oil (n=16).

	Treatment[1]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Ruminal pH[2]						
Mean	6.22[ab]	6.18[b]	6.34[a]	6.18[b]	0.061	0.025
Minimum	5.41[c]	5.47[bc]	5.76[a]	5.57[b]	0.063	<0.001
Maximum	6.89	6.84	6.85	6.83	0.051	0.67
SD of mean pH	0.37[a]	0.32[b]	0.24[c]	0.29[b]	0.019	<0.001
Duration of pH, h/d						
<6.0	6.9[ab]	8.0[a]	4.0[b]	7.6[a]	1.27	0.021
AUC[3], pH x h/d						
<6.0	3.0[a]	2.9[ab]	1.0[b]	2.0[ab]	0.60	0.033
AUC/kg DMI, pH x min						
<6.0	20.7[a]	22.1[a]	7.6[b]	19.4[a]	4.63	0.026

[a,b,c]Within a row, means without a common superscript letter differ, P < 0.05.
[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]Ruminal pH determined for 4 d during which the animals were in the chambers.
[3]AUC = area under the curve.

Digestibility and Nitrogen excretion

The DMI of heifers fed CDDGS was 9.8% lower (P = 0.002) than those fed WDDGS and 12.1% lower (P = 0.014) than the control diet (Table 3-4). Consequently, feeding CDDGS resulted in lower (P < 0.05) intakes of OM and GE as compared to WDDGS and control diets, whereas OM and GE intakes were similar between CDDGS and WDDGS+oil. Intake of CP differed (P < 0.01) among all four diets. Heifers offered WDDGS ingested

the most CP followed by WDDGS+oil, CDDGS, and those offered the control diet. Heifers fed the control diet ingested less ($P < 0.01$) ADF as compared to those fed CDDGS, WDDGS or WDDGS+oil.

Table 3-4 Nutrient intakes and total tract digestibility measured in beef heifers fed a barley silage-based high-forage diet supplemented with barley grain and canola meal, corn- or wheat dried distillers' grains plus solubles (CDDGS, WDDGS) or WDDGS and corn oil (n=8).

	Treatment[1]					
Item[2]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Intake						
DM, kg/d	9.58^{a}	8.42^{b}	9.39^{a}	8.84^{ab}	0.367	0.001
OM, kg/d	8.94^{a}	7.78^{c}	8.58^{ab}	8.05^{bc}	0.340	0.001
CP, kg/d	1.24^{d}	1.58^{c}	2.18^{a}	1.94^{b}	0.079	<0.001
NDF, kg/d	3.07	3.22	3.18	2.96	0.168	0.11
ADF, kg/d	1.68^{c}	1.98^{b}	2.22^{a}	2.05^{b}	0.106	<0.001
GE, Mcal/d	41.3^{a}	37.2^{b}	41.1^{a}	39.7^{ab}	1.66	0.016
Digestibility, %						
DM	70.9^{a}	66.4^{b}	69.0^{a}	66.6^{b}	0.79	<0.001
OM	71.8^{a}	66.4^{c}	69.3^{b}	66.5^{c}	0.83	<0.001
CP	64.1^{b}	70.1^{a}	70.8^{a}	69.3^{a}	0.89	<0.001
NDF	51.3^{a}	46.3^{b}	50.4^{a}	44.1^{b}	2.24	<0.001
ADF	31.8^{c}	38.5^{ab}	43.0^{a}	37.5^{b}	2.24	<0.001
GE	69.8^{a}	65.7^{c}	68.4^{ab}	66.8^{bc}	0.96	<0.001

[a-d]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]Nutrient intakes and total tract digestibility determined for 4d.

Feeding CDDGS and WDDGS+oil reduced apparent total tract digestibility of DM (**DMD**; $P < 0.05$), OM (**OMD**; $P < 0.01$) and NDF (**NDFD**; $P < 0.05$) as compared with heifers fed WDDGS or the control diet. Digestibility of OM in WDDGS was lower ($P = 0.03$) then the control diet. In contrast, apparent total tract digestibility of CP (**CPD**; $P < 0.001$) and ADF (**ADFD**; $P = 0.02$ to < 0.001) in heifers fed CDDGS, WDDGS and WDDGS+oil were greater than for those fed the control diet. Apparent total

tract digestibility of ADF tended ($P = 0.08$) to be lower for CDDGS than WDDGS, whereas ADFD in heifers fed WDDGS+oil was reduced ($P = 0.02$) as compared to those fed WDDGS.

Total N excretion (g/d) differed ($P < 0.001$) among all four treatments (Table 3-5). Feeding WDDGS resulted in the highest total N excretion (303 g/d) followed by WDDGS+oil (259 g/d), CDDGS (206 g/d) and the control diet (170 g/d). Furthermore, feeding WDDGS, CDDGS and WDDGS+oil dramatically increased ($P < 0.001$) urinary N excretion, with diets that contained WDDGS also exhibiting increased ($P < 0.001$) fecal N excretion as compared to control. Heifers offered WDDGS, CDDGS and WDDGS+oil compared to the control excreted less N ($P < 0.001$), expressed as percentage of total N excretion, through feces but more N ($P < 0.001$) through urine.

Additionally, excretion of urea N (g/d) and NH_3 N (g/d) as well as BUN concentration of heifers fed CDDGS, WDDGS and WDDGS+oil were higher ($P < 0.001$) compared to heifers fed the control diet. Excretion of fecal N (% total N excretion) of heifers fed WDDGS was lower ($P < 0.05$) than those fed CDDGS or WDDGS+oil; whereas, urinary N excretion (% total N excretion) of heifers fed WDDGS increased ($P < 0.05$) compared with those fed CDDGS or WDDGS+oil. While feeding WDDGS+oil reduced ($P < 0.001$) daily excretion of urea N compared to WDDGS, NH_3-N output of heifers fed WDDGS+oil and WDDGS were similar.

Table 3-5 Nitrogen intake and excretion measured in beef heifers fed a barley silage-based high-forage diet supplemented with barley grain and canola meal, corn- or wheat dried distillers' grains plus solubles (CDDGS, WDDGS) or WDDGS and corn oil (n=8).

	Treatment[1]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
N intake, g/d	199[d]	252[c]	350[a]	310[b]	12.7	<0.001
N excretion[2], g/d	170[d]	206[c]	303[a]	259[b]	10.4	<0.001
Fecal excretion						
Output, kg/d	2.87	2.90	3.04	3.09	0.15	0.096
Total N, g/d	71.1[c]	73.3[c]	101.9[a]	94.3[b]	4.17	<0.001
Total N, % N excretion	42.1[a]	36.3[b]	33.8[c]	36.6[b]	1.13	<0.001
Urinary excretion						
Output, L/d	6.9[c]	6.8[c]	11.2[a]	9.9[b]	0.65	<0.001
Total N, g/d	98.5[d]	133[c]	201[a]	165[b]	7.7	<0.001
Total N, % N excretion	57.9[c]	63.7[b]	66.2[a]	63.4[b]	1.13	<0.001
Urea N, g/d	53.4[d]	89.6[c]	140[a]	118[b]	5.8	<0.001
NH_3 N, g/d	0.9[c]	1.8[b]	4.6[a]	4.5[a]	0.41	<0.001
Plasma urea N[3], mg/dL	6.6[b]	11.6[a]	12.1[a]	11.2[a]	0.63	<0.001

[a-d]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]Nitrogen intakes and excretion was measured over 4 d during the total collection period.
[3]Samples taken on d 21 (n=16 per treatment).

Methane Emissions

Once in the chamber, DMI of heifers fed WDDGS was 10.2% lower ($P = 0.015$) as compared with those fed the control (Table 3-6). Compared to the control, feeding CDDGS, WDDGS or WDDGS+oil reduced ($P < 0.01$) total CH_4 emission (g/d) by 19.5, 16.1 and 23.8%, respectively. The decrease in CH_4 emission compared to the control was maintained for CDDGS ($P < 0.001$) and WDDGS+oil ($P < 0.001$) when corrected for differences in DMI. However, feeding WDDGS had no effect ($P = 0.21$) on CH_4 emissions when corrected for differences in DMI. This suggests that the decline in total CH_4

emissions when feeding WDDGS reflects a decline in feed intake of heifers fed this diet vs. the control.

Table 3-6 Daily methane emissions from beef heifers fed a barley silage-based high-forage diet supplemented with barley grain and canola meal, corn- or wheat dried distillers' grains plus solubles (CDDGS, WDDGS) or WDDGS and corn oil (n=8).

	Treatment[1]					
Item[2]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
DMI, kg/d	9.05[a]	8.57[ab]	8.13[b]	8.42[ab]	0.291	0.024
Methane						
g/d	228[a]	184[b]	191[b]	174[b]	11.7	<0.001
g/kg of DMI	25.3[a]	21.5[b]	23.9[a]	21.1[b]	1.15	<0.001
% of GE intake	7.8[a]	6.6[b]	7.3[a]	6.3[b]	0.36	<0.001
% of DE intake	11.1[a]	10.0[bc]	10.7[ab]	9.4[c]	0.53	<0.001

[a-c]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]Treatments were: Control=35% barley grain + 5% canola meal, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.6% wheat dried distiller' grains plus solubles + 2.4% corn oil (DM basis).
[2]Methane emissions and corresponding DMI determined over 4 d during which the animals were in the chambers. Chamber (data for 2 animals) was the experimental unit.

Methane emissions as percentage of GE intake (**GEI**) decreased from 7.8% of GEI for control to 6.6% for CDDGS ($P < 0.001$) and to 6.3% for WDDGS+oil ($P < 0.001$). Emissions of CH_4 as percentage of DE intake decreased from 11.1% for the control to 10.0% for CDDGS ($P < 0.02$) and 9.4% of DE for WDDGS+oil ($P < 0.001$). Feeding WDDGS+oil reduced CH_4 emissions per kg DMI ($P = 0.004$), as a % of GEI ($P = 0.003$) and as a % of DE intake ($P = 0.006$) as compared to WDDGS alone. There were no differences in CH_4 emission between CDDGS and WDDGS+oil ($P = 0.35$ to 0.93), regardless of how emissions were expressed.

3.5 Discussion

Grain-based ethanol production throughout North America has grown considerably in the last few years due to the mandated inclusion of renewable fuel in gasoline. Whereas most of the ethanol produced in the United States is derived from corn, Canadian ethanol plants ferment wheat as well as corn. As the starch is fermented to ethanol, the remaining nutrients in DDGS (fiber, protein, fat and minerals) are concentrated about three fold (Spiehs *et al.*, 2002). Corn grain is lower in protein but higher in fat (9.8% CP and 4.1% fat DM basis; NRC, 2000) than wheat grain (14.2% CP and 2.3% fat DM basis; NRC, 2000). Consequently, CDDGS is typically lower in protein but higher in fat content than WDDGS as was the case in our study where the DM composition of CDDGS was 31.5 ± 0.5% CP, 10.0 ± 0.3% fat, 37.3 ± 1.3% NDF, 17.9 ± 1.4% ADF, and 4.4 ± 0.6% starch (DM basis) and WDDGS 45.3 ± 1.0% CP, 4.1 ± 0.1% fat, 23.8 ± 1.0% NDF, 15.3 ± 1.8% ADF, and 8.4 ± 0.9% starch. These values are similar to those previously reported for CDDGS (Spiehs *et al.*, 2002; Klopfenstein *et al.*, 2008) and WDDGS (Beliveau and McKinnon, 2008; Gibb *et al.*, 2008).

Methane Emissions

The control diet fed in our study was a typical high-forage diet fed to growing cattle in western Canadian feedlots with whole-crop barley silage and barley grain as predominant feed components. Because CH_4 emissions from feedlot cattle are greater during the growing compared to the finishing phase, effective CH_4 mitigation strategies targeting high-forage growing

diets are even more desirable to the North American beef industry (Beauchemin *et al.*, 2010). The DDGS inclusion level used in this study is within range of practical feeding strategies as inclusion of up to 40% CDDGS (Klopfenstein *et al.*, 2008) and WDDGS (Gibb *et al.*, 2008) have been shown to have no negative impact on animal performance.

Methane emissions of heifers offered the control diet are in accordance with Beauchemin and McGinn (2006) who reported heifers fed a high-forage diet containing 75% DM of barley silage lost 7.93% of their GEI as CH_4. Beauchemin and McGinn (2005) and McGinn *et al.* (2009) reported decreased CH_4 emissions of 7.3 and 7.1% of GEI for growing diets containing 70 and 60% barley silage DM. These CH_4 emissions tend to be higher than 6.5% (± 1.0%) of GEI as estimated using IPCC tier 2 methodologies for cattle fed a high forage growing diet (IPCC 2006). The accuracy of IPCC estimates for dairy and beef cattle diets have previously been challenged (Kebreab *et al.*, 2008). Information on CH_4 emissions from beef cattle diets containing CDDGS is limited. McGinn *et al.* (2009) reported that CH_4 emissions were reduced by 16.4% (g/kg DMI) or by 23.9% (% for GEI) when CDDGS (35% of DM) replaced barley grain in a growing diet containing 60% barley silage (DM basis). The response was thought to be due to the high fat level of the CDDGS (12.7% DM basis). In the present study the reduction in CH_4 for CDDGS (40% of DM CDDGS) relative to the control diet (Table 3-6) was similar to that observed by McGinn *et al.* (2009). Lower CH_4 emissions of heifers fed WDDGS+oil relative to the

control diet and WDDGS alone support the hypothesis that the high fat content of CDDGS and WDDGS+oil is responsible for the decrease in CH_4. It is unlikely that this reduction was due to other changes in feed composition since all three diets supplemented with DDGS contained more NDF, ADF and less starch compared to the barley grain control (Table 3-1). Methane emissions usually increase, rather than decrease, with increasing dietary fiber content especially when substituted for starch (Johnson and Johnson, 1995).

Moate *et al.* (2011) compared CH_4 emissions of dairy cows offered diets containing different percentages of byproducts with high residual fat content and concluded that diets supplemented with brewers' grain (11.0% fat DM basis), cold-pressed canola meal (12.0% fat DM basis) and hominy meal (16.1% fat DM basis) produced less enteric CH_4 emissions than cows fed a control cracked wheat diet. Similarly, Behlke *et al.* (2007) reported lower CH_4 emissions for lambs fed brome hay-based ration containing 30% DDGS (DM basis), although in that study DDGS replaced corn bran (30% DM basis) instead of grain. In a second experiment, Behlke *et al.* (2007) observed that a partial replacement of corn grain with DDGS (30% DM basis) increased CH_4 production in lambs fed a corn-based high grain diet (71% DM basis), but the fat content of the DDGS used in either study was not reported. The authors conclude that in order to reduce CH_4 emissions from ruminants, DDGS should replace forage rather than grains (Behlke *et al.*, 2007). That recommendation is not supported by our results; CDDGS and WDDGS+oil reduced CH_4 emission due to their high fat content even

though they replaced grain and lowered dietary starch while increasing dietary fiber content. However, CH_4 emissions per kg of DMI are generally lower for cattle fed a high concentrate diet as compared to a high forage diet (Johnson and Johnson, 1995), with the amount of concentrate used in the second experiment by Behlke *et al.* (2007) was greater than in our study. Therefore, replacement of the forage portion of the diet with high-fat DDGS could have the added benefit of lowering CH_4 emissions through increasing the concentrate portion of the diet.

Lipids that are not protected from ruminal digestion decrease CH_4 emissions by exerting toxic effects on methanogens and protozoa, which are physically and metabolically associated with methanogens (Martin *et al.*, 2009). Added fat can enhance propionic acid production, an observation consistent with CDDGS in our study, as well as replace structural carbohydrates that could otherwise contribute to CH_4 production (Johnson and Johnson, 1995). Additionally biohydrogenation of unsaturated fatty acids is thought to reduce CH_4 formation since both pathways require H_2 (Czerkawski *et al.*, 1966). Sources of medium-chain fatty acids (**MCFA**), such as coconut oil reduce CH_4 primarily by being directly toxic to methanogens while long-chain fatty acids (**LCFA**) seem to decrease CH_4 emissions more through decreased DMI and reduced fiber digestion (Machmüller and Kreuzer, 1999; Beauchemin *et al.*, 2008). Fatty acid profiles of CDDGS and WDDGS are similar and not particularly rich in fatty acids that have specific inhibitory effects on CH_4 emissions (i.e., myristic

acid). Additionally, results from metabolism studies suggest the fat in DDGS may be partially protected from ruminal hydrogenation (Klopfenstein *et al.*, 2008). Therefore, the total level of fat in DDGS as opposed to the fatty acid profile may be the factor responsible for the reduction in enteric CH_4 emissions. The reduction in CH_4 in our study was relatively high as each 1% addition of supplemental fat reduced CH_4 emissions (g/kg DMI) by 6.3% for CDDGS and 6.4% for WDDGS+oil as compared to the control. Based on 17 studies with beef cattle, dairy cows and lambs over a broad range of conditions, CH_4 (g/kg DMI) was calculated to be reduced by 5.6% with each 1% addition of supplemental fat (Beauchemin *et al.*, 2008). Similarly for cattle, Grainger and Beauchemin (2011) calculated that an increase in dietary fat from 5 to 6% (DM basis) decreased CH_4 (g/kg DMI) by 5.1%. The fact that WDDGS alone failed to reduce CH_4 emission compared to the control is attributable to the relatively low dietary fat level (3.7% DM) as feeding WDDGS+oil with a dietary fat level of 5.6% DM substantially reduced CH_4 emissions.

Sulphate (SO^{2-}_4) can also act as an alternative electron acceptor in the rumen and in fact the reduction of SO^{2-}_4 to sulfite (SO^{2-}_3) is thermodynamically more favorable than the reduction of CO_2 to CH_4 (McAllister *et al.*, 1996). Dietary S is metabolized to form SO^{2-}_4 in the rumen which in turn is reduced to SO^{2-}_3 by ruminal bacteria (Burgess, 2008). Consequently, differences in S levels among sources of DDGS could also impact ruminal CH_4 production. However, previous work using DDGS

sourced from the same plants as in the current study showed that the S content of WDDGS (1% DM) was only slightly higher than CDDGS (0.8% DM) and that serum SO^{2-}_4 levels did not differ in feedlot cattle fed WDDGS vs. CDDGS (Amat *et al.* 2012). High S levels in DDGS primarily results from the use of sulphuric acid in the cleaning of ethanol fermentation tanks, a practice that has largely ceased in the industry due to concerns that high S levels in DDGS can lead to polioencephalamolacia in cattle (Buckner *et al.*, 2008). Given the similar S levels in CDDGS and WDDGS as reported by Amat *et al.* (2012), it seems unlikely that differences in the concentration of this alterative electron acceptor played a role in the observed differences in CH_4 emissions between WDDGS and CDDGS in the current study.

Digestibility, Ruminal fermentation, and pH

Differences in chemical composition of the diets (Table 3-1) and DM intake caused different nutrient intakes (Table 3-4) among diets. Treatments containing DDGS supplied more CP and ADF than the control diet. Based on the starch and fat content of the diets it can be assumed that starch intake of heifers offered the control diet and the fat intake of heifers offered CDDGS and WDDGS+oil diet were higher than for other treatments.

The reduction in DMD and OMD in heifers fed CDDGS and WDDGS+oil is consistent with lower CH_4 emissions observed for these diets and was mainly caused by lower NDFD relative to WDDGS and the control diet. Although CDDGS failed to reduce ADFD compared to heifers fed WDDGS the reduction in ADFD in heifers fed WDDGS+oil compared to

WDDGS alone suggest that the oil reduced overall fiber digestion. High NDFD and ADFD of diets containing WDDGS or CDDGS are likely a reflection of the extensive processing of the grain prior to ethanol production and possibly the direct impact of ethanol fermentation on the structural integrity of fiber (Ham *et al.*, 1994; Walter *et al.*, 2012).

Increased apparent total tract digestibility of CP in diets containing CDDGS and WDDGS compared to barley grain has been previously described for high grain finishing diets with similar DDGS inclusion levels (Li *et al.*, 2011; Walter *et al.*, 2012). Surprisingly, in the current study ruminal NH_3 concentration in the rumen fluid of heifers fed CDDGS was similar to those fed the control diet even though CP intake of heifers fed CDDGS was higher. Unlike ruminal NH_3 concentration, BUN and urea N concentration (Table 3-5) of heifers fed CDDGS were substantially higher than those fed the control diet, suggesting that replacing barley grain in the control diet with CDDGS shifted the site of CP digestion from the rumen to the small intestine. Lower ruminal concentration of NH_3 and valerate in heifers fed CDDGS as compared to those fed WDDGS and WDDGS+oil likely reflect a reduced RDP content in CDDGS compared to WDDGS (Boila and Ingalls, 1994) as DDGS is generally high in RUP (52% of CP; NRC, 2000). Lower NH_3 concentration in the rumen fluid of heifers fed WDDGS+oil compared to WDDGS alone are likely caused by the decline in OM fermentation in response to fat.

Fat feeding has also been shown to reduce CH_4 through a reduction in protozoal numbers. Methanogens are metabolically associated with protozoa, and feeding oil can cause substantial decreases in protozoal populations (Ivan *et al.*, 2004). Lower numbers of protozoa in the rumen fluid of heifers fed DDGS as compared to those fed the control diet were likely caused by factors other than fat, because protozoa numbers were similar across the DDGS diets. Lowering the starch content of the diet by replacing barley grain with fiberous feeds has previously been shown to reduce protozoal populations (i.e., *Entodinium* spp. in particular) in the rumen (Hristov *et al.*, 2001).

The reason for lower mean and minimal ruminal pH of heifers fed CDDGS and WDDGS+oil as compared to WDDGS alone is unclear. As discussed earlier, the high fat content of CDDGS and WDDGS+oil most likely depressed ruminal digestion causing a reduction in DMD and OMD compared to WDDGS. Consequently, an increase rather than a decrease in mean and minimum ruminal pH for heifers fed CDDGS and WDDGS+oil was anticipated. As cellulolytic microbes are particularly sensitive to low pH (Weimer, 1996), the lower mean and minimum pH of heifers fed CDDGS and WDDGS+oil as compared to WDDGS might have further impaired ruminal fiber digestion, contributing to decreased DMD and OMD. However it is possible that differences in DM intake or intake behaviour (e.g., intake frequency and sorting) between these two experimental phases may have affected ruminal pH as apparent total tract digestibility was determined

between d 11 and 14 and ruminal pH between d 18 and 21. Greater SD of the ruminal pH in heifers fed the control diet compared to those fed diets containing DDGS likely reflect a less stable pH pattern due to highly fermentable starch in barley grain. The ruminal degradability of barley starch is estimated to be 80 to 85% (Huntington, 1997). It has been assumed that substitution of a nonstarch DDGS for the highly fermentable starch in barley grain, decreases VFA concentrations and consequently increases ruminal pH, reducing the incidence of sub-acute ruminal acidosis ([**SARA**]; Klopfenstein *et al.*, 2008). Results, mostly obtained in beef cattle fed high concentrate finishing diets, show that substituting DDGS for cereal grains has less impact on ruminal pH than expected and does not reduce incidence or severity of SARA (Beliveau and McKinnon, 2009; Li *et al.*, 2011; Walter *et al.*, 2012). Because the fiber in DDGS is highly fermentable and not effective at stimulating chewing activity and salvia production, adding DDGS to a diet does little to enhance rumen buffering capacity of cattle fed high concentrate diets (Beliveau and McKinnon, 2009). Our study was not designed to compare the effect of DDGS and barley grain under low pH conditions since we fed high forage diets and the ruminal pH of all heifers was above threshold level for SARA. But decreased AUC (pH × h per d) at pH 6.0 and decreased AUC < pH 6.0 per kg DM intake of heifers fed WDDGS compared to the control is reflected by higher ADFD. Ruminal cellulolytic bacteria prefer pH near neutrality for growth (Weimer, 1996). Consequently,

less time spent below threshold levels of pH 6.0 could have resulted in greater fiber digestion in heifers fed WDDGS.

Nitrogen Excretion

Due to its increased supply and competitive price, DDGS is not only used as protein but also as an energy source in beef cattle diets. The concept of using protein rich byproducts as energy sources has the potential to negatively impact the environment. Excess N, largely excreted in the form of urea via urine is rapidly hydrolyzed to NH_3 by bacterial urease. Ammonia is very volatile and disperses easily into the surrounding air (Asman *et al.*, 1998). Once in the atmosphere NH_3 is a precursor to the formation of aerosols with a potential negative impact on human health (U.S. EPA, 2009). Furthermore, NH_3 is re-deposited on the soil surface contributing to eutrophication, soil acidity and formation of N_2O (IPCC, 2006; Hristov *et al.*, 2011). Nitrous oxide is a potent GHG with a global warming potential 298 times (100 year timeframe) that of CO_2 (IPCC, 2007) whereas the global warming potential (100 year timeframe) of CH_4 is only 25 times that of CO_2 (IPCC, 2007). Therefore, the observed reduction in CH_4 for CDDGS and WDDGS+oil diets could be offset by heightened N_2O emissions that could increase the net GHG emission when feeding DDGS. Additionally, excess N can be also lost through runoff and leaching during storage and application, possibly acting as a pollutant of ground and surface water (IPCC, 2006).

Substantial differences in CP content among diets (control: 13.0%, CDDGS: 18.6%, WDDGS 23.5% and WDDGS+oil 22.0% CP; DM basis)

resulted in dramatic difference in CP intakes (Table 3-4) among treatments. The high CP content of the WDDGS and WDDGS+oil diet exceeded CP requirements of growing beef heifers by two-fold (NRC, 2000), leading to a dramatic increase in total daily N excretion (Table 3-5). A similar increase in the excretion of total N as well as fecal and urinary N was reported for heifers fed 40% CDDGS and WDDGS in place of barley grain in finishing diets (Walter *et al.*, 2012). As expected, urine was the major route of N excretion for all diets and accounting for more than 60% of daily N excretion. Urinary N is rapidly converted to NH_3, whereas fecal N is converted to NH_3 at a much slower rate. Shifting N excretion from the urine to the feces is recognized as a means of increasing the environmental stability of manure N (Varel *et al.*, 1999). Compared to the control, the increase in urinary N excretion relative to N excreted in feces observed for all three DDGS diets would likely increase N losses in the form of NH_3, as well as direct and indirect N_2O emissions and leachate. The reduction of urinary N relative to fecal N excretion in heifers fed CDDGS and WDDGS+oil compared to WDDGS was likely caused by lowered OMD in response to fat. Consequently, feeding high fat DDGS not only decreases CH_4 emission of diets containing DDGS but could also help reduce volatile N losses. Other strategies to decrease N losses from beef feedlots are reducing N intake, increasing pen cleaning frequency, manipulating the C:N ratio of manure on the pen surface and acidification of the manure (Erikson and Klopfenstein, 2010). However, CP intake of heifers fed WDDGS was

higher compared to those fed CDDGS and WDDGS+oil. Therefore, higher urinary N excretion relative to N excreted in feces of heifers fed WDDGS could also reflect increased N intake.

Concentration of BUN is an indicator of N status in ruminants and concentrations greater than 8 mg/dL are indicative of excessive N intake and N wastage (Cole *et al.*, 2003). In our study, feeding CDDGS, WDDGS and WDDGS+oil resulted in BUN concentrations greater than 11 mg/dL, clearly indicating intake of digestible N in excess of requirements. Using a meta-analysis approach Kohn *et al.* (2005) reported that BUN (mg/dL) is linearly related to urinary N excretion rate (g/d) and concluded that blood urea N concentration can be used to predict relative differences in urinary N excretion rate for animals of a similar stage of production within a study, but is less reliable across animal types or studies. Our data supports the general relationship between BUN concentration and urinary N excretion, but indicates that the accuracy of predicting urinary N excretion from BUN concentration is low. For example, in our study, BUN concentrations among heifers fed diets containing DDGS were similar despite differences in total N excretion.

In conclusion, adding CDDGS or fat supplemented WDDGS to the diet of growing beef cattle reduces enteric CH_4 production. This response is dependent on the fat content of DDGS as WDDGS (low in fat content) alone had no effect on CH_4 emissions. However, feeding DDGS, especially WDDGS, increases N excretion. Therefore, the environmental effect of

feeding DDGS to growing beef cattle needs to be measured using a life cycle assessment that accounts for both enteric CH_4 and N excretion. An appreciation for the potential environmental consequences of feeding high levels of CDDGS is critical as many ethanol plants lower the oil levels in this by-product thereby negating its ability to reduce enteric CH_4 emissions.

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CHAPTER 4- Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle[3]

4.1 Introduction

Greenhouse gas emissions in the form of enteric CH_4 as well as direct and indirect N_2O along with N losses in the form of NH_3, NO_3^- and NO_2^- are major environmental concerns arising from ruminant production (FAO, 2006). Dried distillers' grains with solubles is a co-product of grain based fuel ethanol production and is used as a source of protein as well as energy in ruminant diets. As the majority of starch in the original grain is fermented to ethanol, the remaining nutrients in DDGS (fiber, CP, fat and minerals) are concentrated about three fold over that in the original grain (Spiehs *et al.*, 2002). Depending on inclusion level, the chemical composition of diets containing DDGS can differ substantially from grain diets for finishing beef cattle, supplying less starch and more CP, fiber and fat.

Incorporating CDDGS in high-forage growing diets effectively reduces CH_4 emissions (McGinn *et al.*, 2009; Hünerberg *et al.*, 2013). Replacing barley grain (35% DM) with CDDGS **(**12.7% fat DM) in a high-forage diet (60% barley silage, DM basis) decreased enteric CH_4 in growing beef cattle from 7.1 to 5.4% of GEI (McGinn *et al.*, 2009). Similarly, we observed a reduction in CH_4 emission from 7.8 to 6.6% of GEI when 35% barley grain and 5% canola meal DM were replaced with CDDGS (10.0% fat

[3]A version of this chapter has been published. Hünerberg, M., McGinn, S. M., Beauchemin, K. A., Okine, E. K., Harstad, O. M. and McAllister, T. A. 2013b. Effect of dried distillers' grains plus solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. Can. J. Anim. Sci. 93:373–385.

DM) in a high-forage diet (55% barley silage, DM basis) for growing beef cattle (Hünerberg *et al.*, 2013). However, inclusion of 40% DM WDDGS (4.1% fat DM) had no effect on CH_4 emissions (7.3% of GEI) in this study. In contrast to WDDGS alone, inclusion of 40% DM corn oil supplemented WDDGS (9.5% fat DM) reduced CH_4 emissions (6.3% of GEI) to the same extent as CDDGS, confirming that the oil in CDDGS was likely responsible for the reduction in CH_4 (Hünerberg *et al.*, 2013). Although the inclusion of CDDGS reduced CH_4 emissions, it increased total N-excretion in heifers from 170 to 206 g/d (Hünerberg *et al.*, 2013). Methane emissions, as % of GEI or per kg of DMI respectively, are lower for cattle fed high concentrate finishing diets as compared to high forage growing diets (Johnson and Johnson, 1995). It is not known if CDDGS elicits a further reduction in CH_4 emissions in finishing cattle with comparatively low CH_4 emissions. Furthermore, N retention in finishing cattle is lower than in growing cattle, likely augmenting the negative environmental consequences associated with high levels of N excretion in cattle fed DDGS diets.

The objective of this study was to examine the effect of CDDGS and WDDGS on enteric CH_4 emissions and N excretion from finishing beef cattle. It was hypothesized that CDDGS mediated reductions in CH_4 emissions were attributable to its oil content, consequently corn oil was added to WDDGS to determine if this practice resulted in a similar reduction in CH_4 emissions.

4.2 Materials and Methods

This experiment was conducted using the Metabolism Barn Unit and the Controlled Environment Facility at Agriculture and Agri-Food Canada's Research Center in Lethbridge, AB, Canada. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Animals and Experimental Design

Sixteen crossbreed beef heifers (529.1 ± 41.1 kg of initial BW) were used in a replicated 4 × 4 Latin square with four 28-d periods, and four dietary treatments. Heifers were paired, such that each pair had similar BW and pairs of heifers were randomly allocated between squares. The four pairs within each square were randomly allocated to one of four treatment diets. Methane was measured using four open circuit respiratory chambers with each chamber housing two heifers. Periods were staggered by one week between square 1 and 2 as only four chambers were available at a time. The pairing of heifers was consistent throughout the experiment, such that heifers within a chamber received the same treatment. All heifers were ruminally cannulated and ovariectomized. Heifers were gradually transitioned over 4 weeks from a growing diet containing 55% DM barley silage to a finishing diet containing 8% DM barley silage.

At the beginning of each period, day 1–7 were used to transition the heifers from their previous diet to the new diet. Starting on d 8 all heifers received their intended experimental diet. Apparent total tract digestibility of

nutrients and excretion of N was determined from day 18 to 21 using the eight heifers in square 1 (534.9 ± 36.5 kg of initial BW). Rumen contents of all heifers were sampled on day 21. From d 1 to 24, heifers were housed in tie stalls in the metabolism unit with individual access to feed and water. Before the morning feeding on day 25, heifers were moved to the controlled environment facility to measure CH_4 over 4 d. Except for the periods during the measurement of digestibility and CH_4 emissions, heifers were given daily exercise in an open dry lot.

Diets and Feed Sampling

High-concentrate diets were formulated to have a composition that is typical of that fed to feedlot cattle in western Canada prior to slaughter. The control diet contained (DM basis) 8% whole crop barley silage, 87% steam rolled barley grain, and 3.4% ground barley as a carrier for a 1.6% vitamin and mineral supplement (Table 4-1). The three diets containing DDGS were formulated by replacing 40% of barley grain DM with CDDGS, WDDGS, or WDDGS plus corn oil (WDDGS+oil). For the WDDGS+oil diet, 6.5% of corn oil (Great Value; Wal-Mart, ON, Canada) was added per kg DM of WDDGS (3.4% fat DM) to achieve a fat level similar to CDDGS (9.7% fat DM). The inclusion level of 40% DDGS (DM basis) was chosen to reflect the usage of DDGS as an energy source and was within a range shown to have no negative impact on the growth performance of finishing cattle (Klopfenstein *et al.*, 2008; Gibb *et al.*, 2008). Heifers were fed once daily at

1100 h for ad libitum intake (5% refusal, as fed basis). The weight of feed offered and refused was recorded daily throughout the study.

Table 4-1 Ingredient composition and chemical composition of the experimental diets.

	Treatment[1]			
Item	Control	CDDGS	WDDGS	WDDGS+oil
Ingredient, % of the dietary DM				
Barley silage	8	8	8	8
Barley grain, dry-rolled	87	47	47	47
CDDGS[2]		40		
WDDGS[3]			40	37.4
Corn oil				2.6
Barley grain, ground[4]	3.4	3.4	3.4	3.4
Calcium carbonate	1.25	1.25	1.25	1.25
Salt	0.15	0.15	0.15	0.15
Molasses, dried	0.13	0.13	0.13	0.13
Mineral and vitamin premix[5]	0.06	0.06	0.06	0.06
Vitamin E (500,000 IU/kg)	0.003	0.003	0.003	0.003
Flavouring agent[6]	0.003	0.003	0.003	0.003
Chemical composition[7]				
OM, % of DM	95.4 ± 0.5	95.6 ± 0.1	94.0 ± 0.1	94.1 ± 0.4
CP, % of DM	12.2 ± 0.7	19.6 ± 0.7	23.1 ± 0.8	22.1 ± 1.1
NDF, % of DM	19.4 ± 1.0	27.9 ± 0.8	24.5 ± 1.4	24.4 ± 1.5
ADF, % of DM	7.3 ± 0.4	13.7 ± 0.7	14.2 ± 0.4	12.9 ± 0.5
Fat, % of DM	2.0 ± 0.1	5.4 ± 0.2	3.1 ± 0.4	5.1 ± 0.1
Starch, % of DM	55.0 ± 1.3	34.7 ± 2.7	31.9 ± 3.3	33.2 ± 1.8
GE, MJ/kg of DM	18.4 ± 0.1	19.0 ± 0.2	18.5 ± 0.2	18.9 ± 0.3

[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distiller' grains plus solubles, WDDGS=40% wheat dried distiller' grains plus solubles, or WDDGS+oil=37.4% wheat dried distiller' grains plus solubles + 2.6% corn oil (DM basis).
[2]Corn-based dried distillers' grains with solubles.
[3]Wheat-based dried distillers' grains with solubles.
[4]Carrier for the pelleted vitamin and mineral supplement.
[5]Supplied kg/DM: 65 mg of Zn, 28 mg of Mn, 15 mg of Cu, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6,000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.
[6]Anise 422 powder containing ground cumin, fennel, fenugreek, silicon dioxide and wheat bran (Canadian Bio-Systems Inc., Calgary, Alberta, Canada).
[7]Determined using samples pooled by diet within each period (n = 4; mean ± SD).

Diets and ingredients were sampled weekly and analyzed for DM content by drying at 55°C for 48 h. Diets were adjusted if the DM content of barley silage deviated more than 3.0% from the average. Weekly subsamples of diets and ingredients were composited by period. Orts were sampled daily during the digestibility trial and CH_4 measurements and pooled by heifer at the end of each period. Samples were stored at −20°C until further analysis.

Nutrient Digestibility and Nitrogen Excretion

To ensure complete separation of urine and feces, the eight heifers in square 1 were fitted with urinary indwelling balloon catheters (Bardex® Lubricath® Foley catheter, 75 c.c. and 26 Fr.; Bard Canada Inc., Oakville, ON, Canada). Urine was acidified (pH < 2) with 4 N H_2SO_4 to prevent volatilization of NH_3. Total output of feces was collected using rubber mats positioned behind the heifers. Total output of urine and feces was measured every 24 h over 4 d. Urine samples were pooled (1% total volume) for each animal within period and a sub-sample (20 ml) was diluted with distilled water at a ratio of 1:5 at the end of each period and stored at −20°C until analyzed. A daily sub-sample of the feces from each animal (~500 g) was oven-dried at 55°C. At the end of the digestibility experiment, a representative composite sample from each heifer for each period was obtained by pooling the dried daily fecal samples based on their original DM content.

Ruminal Fermentation Measurements, Ruminal pH and Blood Sampling

Rumen samples were collected by sampling ~500 g of rumen content from the reticulum, dorsal and ventral sac of each heifer at 0, 2, 6, 12 and 24 h after feeding. Samples from each site were mixed and squeezed through 2 layers of polyester monofilament fabric (pore size 355 μm; B. & S. H. Thompson, Ville Mont-Royal, QC, Canada) and pH of the filtrate measured (Accumet model 25; Cole-Parmer Canada Inc, Montreal, QC, Canada) immediately. Filtrate (5 mL) was mixed with 1 mL of 25% (w/v) HPO_3 for VFA and lactate analysis and with 1 mL of 1% (w/v) H_2SO_4 for NH_3 analysis. Both samples were stored at −20 ◦C until analyzed. For enumeration of ruminal protozoa, filtrate (5 mL) was mixed with 5 mL of MFS solution. The samples were stored at room temperature in the dark until examined.

Ruminal pH was recorded every min between d 25 and 28 using the LRCpH data logger system (Dascor, Escondido, CA, USA). Loggers were calibrated using buffers at pH 4 and 7 at the start and end of each measurement period. Probes were placed in the ventral sac of the rumen 2 h before the heifers entered the chambers on day 25 and removed immediately after they were returned to the metabolism unit on day 28. Ruminal pH data were summarized by day as average, minimum, maximum, and SD of mean pH, and as duration below and area under AUC for threshold values of pH 5.5 and 5.2. The AUC was calculated as the sum of the absolute value of pH deviations below pH 5.5 or 5.2 multiplied by the duration below pH 5.5 or

5.2, and reported in pH × min. Durations and AUC for pH 5.5 and 5.2 were considered indicative of SARA and acute ruminal acidosis (**ARA**), respectively (Penner *et al.*, 2007). Intake corrected AUC was calculated as AUC divided by DMI.

Blood samples for the determination of BUN were collected into 10-mL vacuum tubes containing Li-heparin solution (Vacutainer, Becton Dickinson, Mississauga, ON, Canada) by jugular vein puncture on day 28 of each period 30 min before feeding time. Blood was centrifuged (3,000 × g at 4°C for 20 min) and plasma was collected and stored at −20°C until analyzed.

Methane Emission Measurements

Four identical open circuit respiratory chambers (4.4 m wide × 3.7 m deep × 3.9 m tall, 63.5 m^3 volume, C1330, Conviron Inc., Winnipeg, MB, Canada) were used to measure CH_4 emissions from each pair of heifers over four consecutive days. Two heifers in each chamber were placed in individual tie stalls equipped with rubber mats. Both heifers within each chamber had free access to feed and water. The chambers were vented using fresh-air intakes and chamber exhaust ducts with dedicated fans for each individual duct. The air volume of each chamber was exchanged every 5 min. Air temperature within the chambers was maintained at 10°C throughout the experiment. Air from the fresh-air intake and exhaust air duct of each chamber was sampled sequentially for 3 min each, every 27 min, by pumping 1 L/min (TD3LS7; Brailsford and Company, Rye, NY, USA)

through a common infrared gas analyzer (Ultramat 6; Siemens, Karlsruhe, Germany) via a set of solenoids controlled by a data logger (CR23X; Campbell Scientific, Logan, UT, USA). Before entering the gas analyzer the air stream was dried using magnesium perchlorate. After each 24 min cycle (8 ducts × 3 min each), pure N_2 gas was introduced into the gas analyzer for 3 min. Data generated during this time period was used to account for any drift in the analyzer between measurement cycles. The difference in the concentration of CH_4 between the incoming and outgoing flow in the fresh-air intake and exhaust duct respectively, was used to calculate the amount of CH_4 produced by each pair of heifers within each chamber as described by Beauchemin and McGinn (2006). Air velocity in each intake and exhaust duct was continuously monitored (model 8455 Air Velocity Transducer, TSI Inc., Shoreview, MN, USA). Air flow rates in the ducts were adjusted to generate a slight positive pressure (approximately 2 Pa) inside each chamber (model 265 Pressure Sensor; Setra, Boxborough, MA, USA). The chambers were opened once daily at 1100 h for cleaning and to feed the heifers. Methane emission data corresponding to the door opening times (~30 min/d to clean and feed all four chambers) as well as the time needed for each chamber to reequilibrate (15 min after the door was closed) were omitted from the analysis. The gas analyzer was calibrated daily, immediately after feeding using N_2 as zero and 405 mg/kg of CH_4 as span standard gases. The system was calibrated before the beginning of the experiment by sequentially releasing 0, 0.1, 0.2 and 0.3 L/min of CH_4 separately into each empty

chamber using a mass-flow meter (Omega Engineering, Stamford, CT, USA). The slopes of the best fit four point regressions (actual against calculated CH_4 emission) were used to correct for variability between chambers. The r^2 value of this four point regression exceeded 0.99 in all four chambers.

Laboratory Analyses

Samples of composited ingredients, diets, orts and feces were oven dried at 55°C and ground through a 1 mm screen (Cutting Mill SM100; Retsch, Haan, Germany). Analytical DM was determined by drying at 135°C for 2 h (AOAC, 2005; method 930.15), followed by hot weighing. Organic matter was calculated as the difference between 100 and the percentage of ash (AOAC, 2005; method 942.05). Neutral detergent fiber and ADF, both expressed inclusive of residual ash were quantified as described by Van Soest *et al.* (1991) using amylase and sodium sulfite for the NDF analysis. Fat was determined according to AOAC (2006; method 2003.05) using ether extraction (Extraction Unit E-816 HE; Büchi Labortechnik AG, Flawil, Switzerland). Gross energy in diets, orts and feces was determined using a bomb calorimeter (model E2k; CAL2k, Johannesburg, South Africa). For the measurement of CP (N × 6.25) and starch, ground samples were reground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). Total urinary N was analyzed in the same fashion using freeze dried urine. Ball ground

ADF residues of CDDGS and WDDGS were analysed for N to determine ADIN (Table 4-2). Starch content of the diets was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Rode *et al.* (1999).

Table 4-1 Chemical analysis of major diet ingredients.

	Ingredient		
Item[3]	Barley grain	CDDGS[1]	WDDGS[2]
OM, % of DM	97.7 ± 0.1	96.9 ± 0.1	94.1 ± 0.1
CP, % of DM	12.3 ± 1.0	31.4 ± 0.5	38.7 ± 1.1
NDF[4], % of DM	20.4 ± 2.3	38.0 ± 1.2	28.0 ± 0.7
ADF[5], % of DM	5.2 ± 0.6	23.0 ± 0.5	21.5 ± 0.4
ADIN[6], % of total N	ND[7]	14.3 ± 1.9	9.3 ± 0.5
Fat, % of DM	1.8 ± 0.1	9.7 ± 0.3	3.4 ± 0.2
Starch, % of DM	56.7 ± 1.2	4.4 ± 0.3	2.5 ± 0.3

[1]Corn-based dried distillers' grains with solubles.
[2]Wheat-based dried distillers' grains with solubles.
[3]Determined using samples pooled by period (n = 4; mean ± SD).
[4]Neutral detergent fiber, assayed with a heat stable amylase and expressed inclusive residual ash.
[5]Acid detergent fiber, expressed inclusive of residual ash.
[6]Acid detergent insoluble nitrogen.
[7]Not determined.

Concentrations of VFA and lactate in ruminal fluid were quantified using gas chromatography (model 5890, Hewlett Packard, Wilmington, DE, USA) with a capillary column (30 m × 0.32 mm × 1 μm; ZB-FFAP, Phenomenex Inc., Torrance, CA, USA) and flame ionization detection. Internal standards were crotonic acid for VFA and malonic acid for lactate analysis. Lactate samples were methylated with BF_3-MeOH prior to GC analysis. Concentration of NH_3 in urine and rumen fluid was determined by the salicylate-nitroprusside-hypochlorite method (Sims *et al.*, 1995) using a flow injection analyzer (Technicon Autoanalyzer II, Technicon Instruments, Tarrytown, NY, USA). Concentration of urea in urine and blood was

analyzed using micro-Segmented Flow Analysis (model Astoria2; Astoria Pacific Inc., Clackamas, OR, USA). Ruminal protozoa were enumerated under a light microscope as described by Ogimoto and Imai (1981) using a counting chamber (Neubauer Improved Bright- Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA, USA). Duplicate preparations of each sample were counted. If values differed from the average by more than 10%, a third sample was enumerated.

Calculations and Statistical Analyses

The data were analyzed using a Mixed procedure (SAS, 2001). Heifer was the experimental unit for intake, digestibility, N excretion, and ruminal fermentation variables as these data were obtained from individual heifers. For ruminal fermentation variables, the model included the fixed effect of diet and the random effects of square, heifer nested within square, and period nested within square. For ruminal fermentation variables sampling time (0, 2, 6, 12 and 24 h after feeding) was treated as a repeated measure. Protozoa data were $\log_{10}$-transformed prior to statistical analysis. Data for N excretion and total tract digestibility were analyzed using the same model, but without the random effect of square because only square1 heifers were used and sampling day (1-4) was treated as a repeated measure. The chamber, representing data from two heifers, was the experimental unit for CH_4 measurements. Cumulative daily CH_4 emission from each chamber was calculated for each of the 4 d within each period. Methane emission was expressed per unit of DMI and as a proportion of GEI and DE intake of the

two heifers within the chamber on that same day. The GE content of CH_4 was assumed to be 55.6 MJ/kg. Methane as % of DE was calculated based on the diet specific GE digestibility values determined between day 18 and 21 shown in (Table 4-5). The model used for CH_4 production variables included the fixed effect of diet and the random effects of square, period nested within square, and chamber nested within square. Day of sampling (day 1-4) within each period was treated as a repeated measure. For all analyses, the best time series covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Denominator degrees of freedom were estimated using the Kenward-Roger option in the model statement. The PDIFF option adjusted by the Tukey method was included in the lsmeans statement to enable multiple comparisons. Treatment effects were declared significant at $P < 0.05$, and trends were discussed at $P < 0.10$.

4.3 Results

Ruminal Fermentation and pH

Feeding CDDGS, WDDGS or WDDGS+oil as compared to the control diet decreased ($P < 0.05$) the concentration of total VFA in rumen fluid (Table 4-3). In addition, feeding CDDGS decreased the concentration of acetate compared to the control; while feeding WDDGS resulted in lower concentration of propionate compared to the control. Concentrations of butyrate, isovalerate, valerate, isobutyrate as well as the acetate:propionate ratio were similar among treatments ($P > 0.10$). Feeding WDDGS or WDDGS+oil compared to the control or CDDGS resulted in a higher ($P <$

0.001) ruminal concentration of NH_3 and an increase ($P < 0.05$) in total numbers of rumen protozoa.

Table 4-3 Ruminal fermentation variables of ruminally cannulated beef heifers (n = 16) fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil.

	Treatment[1]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Total VFA, m*M*	174.7[a]	155.2[b]	156.9[b]	157.7[b]	6.96	0.008
Individual VFA, m*M*						
Acetate (A)	80.6[a]	71.7[b]	78.2[ab]	75.3[ab]	2.92	0.016
Propionate (P)	65.2[a]	57.2[ab]	48.8[b]	54.2[ab]	4.68	0.016
Butyrate	18.5	16.7	21.3	19.2	1.86	0.216
Isovalerate	2.53	2.32	1.94	1.93	0.351	0.481
Valerate	4.97	4.78	4.29	4.44	0.655	0.775
Isobutyrate	1.89	1.75	1.63	1.81	0.342	0.301
A: P ratio	1.50	1.44	1.86	1.56	0.163	0.177
Lactate	0.083	0.108	0.116	0.122	0.0239	0.636
NH_3-N	4.24[b]	5.12[b]	10.77[a]	8.90[a]	0.859	<0.001
Protozoa, 10^5 cell/mL	3.8[b]	4.1[b]	8.8[a]	7.1[a]	1.00	<0.001

[a-b]Within a row, means without a common letter differ, $P < 0.05$.
[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).

The mean ruminal pH of heifers fed WDDGS+oil was higher ($P < 0.05$) than for those offered the control diet, but was below 6.0 in all diets (Table 4-4). Feeding CDDGS, WDDGS and WDDGS+oil resulted in higher ($P < 0.05$) minimum pH as compared to the control diet, whereas heifers fed WDDGS or WDDGS+oil had lower ($P < 0.05$) SD from the daily mean pH as compared to those fed the control diet. Additionally, feeding heifers CDDGS, WDDGS and WDDGS+oil reduced the time below a pH threshold of 5.5 (SARA; $P < 0.05$) and 5.2 (ARA; $P < 0.05$) and a decreased AUC

expressed as pH × min/d at pH 5.5 ($P < 0.05$) and 5.2 ($P < 0.001$) as compared to those fed the control diet. In contrast, the AUC adjusted for DMI (kg/DMI) was lower ($P < 0.05$) for heifers offered CDDGS and WDDGS+oil as compared to heifers fed the control diet.

Table 4-4 Ruminal pH of ruminally cannulated beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (n = 16).

	Treatment[1]					
Item	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Ruminal pH[2]						
Mean	5.79[b]	5.94[ab]	5.89[ab]	5.96[a]	0.062	0.039
Minimum	5.01[b]	5.18[a]	5.20[a]	5.24[a]	0.067	0.001
Maximum	6.80	6.76	6.71	6.77	0.051	0.388
SD of mean pH	0.47[a]	0.42[ab]	0.39[b]	0.38[b]	0.026	0.003
Duration of pH, h/d						
<5.5[3]	8.6[a]	5.5[b]	5.5[b]	4.5[b]	1.19	0.009
<5.2[4]	4.6[a]	2.1[b]	2.3[b]	1.4[b]	0.73	0.004
AUC[5], pH x min/d						
<5.5	171.5[a]	84.7[b]	93.2[b]	61.2[b]	26.98	0.003
<5.2	52.2[a]	15.7[b]	25.8[b]	9.9[b]	9.19	<0.001
AUC kg/DMI, pH x min						
<5.5	19.0[a]	9.2[b]	11.0[ab]	6.9[b]	2.90	0.005
<5.2	5.7[a]	1.7[b]	2.8[ab]	1.1[b]	1.07	0.005

[a-b]Within a row, means without a common letter differ, $P < 0.05$.
[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[2]Ruminal pH determined for 4 d during which the heifers were in the chambers.
[3]Threshold level indicative of sub-acute ruminal acidosis.
[4]Threshold level indicative of acute ruminal acidosis.
[5]AUC = area under the curve.

Apparent Total Tract Digestibility

The DMI of heifers fed WDDGS was 13.7% lower ($P < 0.01$) than those fed the control diet (Table 4-5), resulting in lower ($P < 0.05$) OM and

GE intake for heifers fed WDDGS as compared to the control. Intake of DM, OM and GE among heifers fed the three diets containing DDGS was similar. Feeding CDDGS, WDDGS and WDDGS+oil as compared to the control diet resulted in higher ($P < 0.001$) intakes of CP (Table 4-5), with these levels being higher ($P < 0.05$) in diets containing WDDGS than CDDGS.

Table 4-5 Nutrient intakes and total tract digestibility measured in beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (n = 8 per treatment).

	Treatment[1]					
Item[2]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
Intake						
DM, kg/d	10.15^{a}	9.13^{ab}	8.76^{b}	9.51^{ab}	0.511	0.008
OM, kg/d	9.68^{a}	8.71^{ab}	8.22^{b}	8.95^{ab}	0.488	0.004
CP, kg/d	1.23^{c}	1.69^{b}	1.95^{a}	2.01^{a}	0.115	<0.001
NDF, kg/d	1.98^{b}	2.06^{b}	2.09^{b}	2.40^{a}	0.157	<0.001
ADF, kg/d	0.75^{b}	1.19^{a}	1.21^{a}	1.21^{a}	0.065	<0.001
GE, MJ/d	185.7^{a}	172.8^{ab}	161.5^{b}	179.3^{ab}	10.11	0.017
Digestibility, %						
DM	82.0^{a}	77.3^{b}	76.8^{b}	73.2^{c}	1.10	<0.001
OM	83.7^{a}	78.9^{b}	78.5^{b}	74.9^{c}	1.08	<0.001
CP	77.0^{ab}	78.9^{a}	78.1^{a}	74.5^{b}	1.08	0.003
NDF	54.6	50.5	52.6	49.0	2.87	0.179
ADF	36.0^{b}	56.6^{a}	52.1^{a}	41.6^{b}	2.64	<0.001
GE	82.2^{a}	78.1^{b}	77.4^{b}	73.9^{c}	1.06	<0.001

[a-c]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[2]Nutrient intakes and total tract digestibility determined over 4 d.

Feeding WDDGS+oil resulted in highest ($P < 0.05$) intake of NDF, while ADF intake of heifers offered CDDGS, WDDGS and WDDGS+oil was higher ($P < 0.05$) compared to the control diet. Feeding CDDGS, WDDGS and WDDGS+oil reduced DMD **(**$P < 0.001$), OMD **(**$P < 0.05$) and apparent

total tract GE digestibility ($P < 0.05$) as compared to the control diet. The addition of corn oil in the WDDGS+oil diet resulted in a further reduction ($P < 0.05$) of DMD, OMD and CPD as compared to CDDGS and WDDGS. Total tract digestibility of NDF was similar among all diets. In contrast, ADFD in heifers fed CDDGS and WDDGS was greater compared to those fed WDDGS+oil or the control diet ($P < 0.001$).

Nitrogen Excretion

Feeding WDDGS and WDDGS+oil resulted in greater ($P < 0.05$) total N intake and excretion (both g/d) compared to CDDGS, while feeding diets containing DDGS increased N intake and excretion ($P < 0.001$) compared to the control (Table 4-6). Consequently, feeding diets containing DDGS increased fecal ($P < 0.05$) as well as urinary N excretion (both g/d; $P < 0.001$) compared to the control diet. In addition, heifers fed WDDGS+oil excreted more fecal N ($P < 0.05$) than those fed CDDGS or WDDGS while both diets containing WDDGS exhibited higher ($P < 0.05$) urinary N excretion as compared to CDDGS. When the loss of N in feces or urine was expressed as percentage of total N excretion, heifers offered CDDGS and WDDGS excreted less ($P < 0.05$) N through feces, but more ($P < 0.05$) through urine compared to those fed the control and WDDGS+oil diet. Excretion of urea N (g/d), NH_3 N output (g/d) as well as BUN (mg/dL) of heifers fed diets containing DDGS were substantially higher ($P < 0.001$) compared to heifers fed the control diet. Additionally, feeding WDDGS alone or WDDGS+oil resulted in higher daily excretion of urea N ($P < 0.05$)

over CDDGS and NH_3-N losses from heifers offered WDDGS were higher ($P < 0.05$) compared to those fed WDDGS+oil.

Table 4-2 Nitrogen intake, excretion, and plasma urea N concentration of ruminally cannulated beef heifers (n = 8) fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil.

	Treatment[1]					
Item[2]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
N intake, g/d	197[c]	271[b]	312[a]	322[a]	18.5	<0.001
N excretion, g/d	143[c]	220 [b]	253[a]	265[a]	15.9	<0.001
Fecal excretion						
Output, kg/d	1.8[b]	2.1[b]	2.0[b]	2.5[a]	0.19	<0.001
Fecal N, g/d	44.9[c]	58.1[b]	67.6[b]	80.9[a]	5.99	<0.001
Total N, % N intake	23.0[ab]	21.1[b]	21.9[b]	25.5[a]	1.08	0.003
Total N, % N excretion	31.4[a]	25.9[b]	26.7[b]	30.6[a]	1.35	<0.001
Urinary excretion						
Output, L/d	7.1[b]	10.1[a]	11.2[a]	10.5[a]	0.99	<0.001
Urinary N, g/d	98.3[c]	162.0[b]	185.3[a]	183.7[a]	11.30	<0.001
Total N, % N intake	50.8[b]	61.4[a]	60.7[a]	57.6[a]	1.99	<.0001
Total N, % N excretion	68.6[b]	74.1[a]	73.3[a]	69.4[b]	1.35	<0.001
Urea N, g/d	52.0[c]	100.2[b]	116.4[a]	120.1[a]	8.16	<0.001
NH_3-N, g/d	2.7[c]	7.1[ab]	7.6[a]	5.9[b]	0.82	<0.001
BUN[3], mg/dL	6.0[b]	10.0[a]	10.0[a]	10.5[a]	0.50	<0.001

[a-c]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[2]Nitrogen intakes and excretion were measured over 4 d (*n* = 8 per treatment).
[3]Blood urea N; Samples taken on d 21 (*n* =16 per treatment).

Methane Emissions

In contrast to the digestibility trial, where differences in DMI between WDDGS and control diet were measured, DMI during the period of CH_4 measurement was similar among diets (Table 4-7). Feeding CDDGS or WDDGS+oil reduced ($P < 0.05$) CH_4 emission (g/d) compared to WDDGS

alone by 17.5 and 14.3%, respectively. Methane emissions (g/d) of heifers fed diets containing WDDGS did not differ from those offered the control diet.

Table 4-3 Daily methane emissions from ruminally cannulated beef heifers fed a high concentrate barley grain-based finishing diet supplemented with corn- or wheat dried distillers' grains with solubles (CDDGS, WDDGS) or WDDGS+corn oil (n = 8).

	Treatment[1]					
Item[2]	Control	CDDGS	WDDGS	WDDGS+oil	SEM	P-value
DMI, kg/d	8.51	8.82	8.10	8.74	0.672	0.202
Methane						
g/d	136.2^{ab}	119.0^{b}	144.3^{a}	123.6^{b}	10.06	0.008
g/kg of DMI	16.6^{ab}	13.6^{c}	18.4^{a}	14.5^{bc}	1.60	<0.001
% of GE[3] intake	5.0^{ab}	4.0^{c}	5.5^{a}	4.2^{bc}	0.47	<0.001
% of DE[4]intake	6.1^{ab}	5.1^{b}	7.1^{a}	5.7^{b}	0.60	<0.001

[a-c]Within a row, means without a common letter differ, P < 0.05.
[1]Treatments were: Control=87% barley grain, CDDGS=40% corn dried distillers' grains with solubles, WDDGS=40% wheat dried distillers' grains with solubles, or WDDGS+oil=37.4% wheat dried distillers' grains with solubles + 2.6% corn oil (DM basis).
[2]Methane emissions and corresponding dry matter intake (DMI) determined over 4 d during which the heifers were in the chambers. Chamber (data for 2 heifers) was the experimental unit.
[3]Gross energy intake.
[4]Methane as % of digestible energy (DE) was calculated based on GE digestibility values determined between d 18 and 21 (Table 4-5).

The reduction in CH_4 emission of heifers fed CDDGS (P = 0.001) or WDDGS+oil (P = 0.006) compared to heifers fed WDDGS was still evident when expressed as g/kg of DMI. Adjusting for numerical differences in DMI resulted in lower (P < 0.05) CH_4 emissions g/kg DMI for heifers fed CDDGS compared to those offered WDDGS alone or the control diet. Feeding CDDGS also reduced (P < 0.05) CH_4 emissions expressed as % of GEI compared to WDDGS alone and the control diet, while there was also a trend (P = 0.08) for a similar response with WDDGS+oil. When corrected for

differences in DE intake, heifers fed CDDGS produced less ($P < 0.001$) CH_4 than those fed WDDGS. Furthermore, feeding WDDGS tended to increase ($P = 0.06$) CH_4 emissions (% of DE intake) as compared to the control, while feeding CDDGS tended ($P = 0.08$) to decrease it. Heifers fed CDDGS or WDDGS+oil had similar CH_4 emissions, regardless of how emissions were expressed. Addition of corn oil to the WDDGS+oil diet reduced CH_4 emissions expressed as g/kg of DMI ($P = 0.006$), % of GEI ($P = 0.002$) and % of DE intake ($P = 0.009$) as compared to WDDGS alone.

4.4 Discussion

In the last 10- 15 years, mandatory inclusion of renewable fuel in conventional gasoline has led to exponential growth in grain-based ethanol production in North America. Whereas ethanol production in the United States is almost exclusively from corn, wheat is used to produce 31% of the ethanol in Canada, resulting in yearly production of ~1.2 million tonnes of DDGS in Canada from both grains (USDA Foreign Agricultural Service, 2010). Due to its high fiber content, DDGS is predominantly utilized as a feed for ruminants, with inclusion being the highest in beef cattle diets (Klopfenstein *et al.*, 2008). Even though DDGS is mainly used as protein source for ruminants, depending on price, DDGS can also serve as an energy source, replacing either grain, silage or both in the diet (Klopfenstein *et al.*, 2008; Li *et al.*, 2011).

The nutrient composition of DDGS is largely dependent on grain source. Wheat is higher in CP, but lower in fat (14.2% CP and 2.3% fat DM basis; NRC, 2000) than corn (9.8% CP and 4.1% fat DM basis; NRC, 2000), WDDGS is naturally higher in CP (~40 vs. ~30% DM) and lower in fat (~5 vs. ~10% DM) than CDDGS (Table 4-2; Gibb *et al.*, 2008; Klopfenstein *et al.*, 2008). An inclusion level of 40% DDGS (DM based) was chosen for this study because it is at the high end of the range that has been shown to have no negative impact on growth performance or carcass traits of finishing cattle (Gibb *et al.*, 2008; Klopfenstein *et al.*, 2008). The control diet was typical of the barley-based finishing diets routinely fed to cattle in western Canadian feedlots.

The lower DMI of heifers fed WDDGS compared to those fed the control diet, has been previously reported for finishing diets containing > 30% (DM basis) WDDGS (Li *et al.*, 2011; Walter *et al.*, 2012). Addition of oil to the WDDGS alleviated this difference in DMI, suggesting that this response may have been related to the energy density of the diet. Moving the cattle from the metabolism unit into the respiratory chambers reduced DMI of heifers fed the control, CDDGS, WDDGS and WDDGS+oil diets by 16.2, 3.4, 7.5 and 8.1% respectively. As reductions in DMI typically result in increased CH_4 emissions per unit of DMI (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995), emissions need to be interpreted with respect to the reduction in DMI caused by the change in housing conditions. However, such reductions in DMI are common in chambered cattle, a response that we

attempted to minimize by housing the heifers as pairs within chambers. However, this precaution did not completely alleviate the impact of the change in housing environment on DMI.

As expected, higher CP and NDF intake of heifers fed diets containing DDGS compared to the control reflect differences in ingredient composition. The DDGS diets had similar NDF content; therefore higher NDF intake of heifers fed WDDGS+oil as compared to CDDGS and WDDGS must be due to differences in sorting behaviour rather than diet composition. The control diet resulted in the highest starch intake as it contained 55.0% starch (DM basis) as compared to 34.7, 31.9 and 33.2% of starch (DM basis) in the CDDGS, WDDGS and WDDGS+oil diet, respectively. Starch from dry-rolled barley is highly digestible, with 80.7 ± 3.9% (mean ± SD) of it being fermented in the rumen and total tract digestibility frequently exceeding 95% (Huntington, 1997). Therefore, the lower DMD and OMD of heifers fed DDGS diets may be attributable to their lower starch content as compared to the control diet. Lower DMD and OMD, together with a depression in CPD and ADFD with WDDGS+oil as compared to WDDGS alone may reflect the negative impact of corn oil on rumen fermentation. Despite the fact that total and individual VFA concentrations in the rumen fluid did not differ between WDDGS+oil and WDDGS, we propose that the depression in DM, OM, CP and ADF digestion occurred ruminally rather than post-ruminally. The greater impact on nutrient digestibility of added oil in WDDGS+oil as compared to

CDDGS may reflect the impact of extensive processing (e.g. heating and drying) at the ethanol plant on the ruminal activity of oil in CDDGS, although both of these co-products caused a similar reduction in CH_4 emissions. The similar NDFD of the three diets containing DDGS compared to the control is somewhat surprising. The NDFD of DDGS is generally higher than the NDFD of barley grain due to extensive processing prior to ethanol production and possibly as a result of alteration in the digestibility of fiber during the fermentation process (Ham *et al.*, 1994). Consequently, others have reported higher NDFD of finishing diets with up to 40% CDDGS or WDDGS than those containing primarily barley (Li *et al.*, 2011; Walter *et al.*, 2012). The fact that ADFD of heifers fed CDDGS and WDDGS was higher than the control diet in our study, suggests that the processing of the grain feedstock for ethanol production may have a more positive impact on the digestibility of cellulose than hemicellulose.

Although cattle fed high-concentrate diets produce less methane (g/kg DMI) than those fed high-forage diets (Johnson and Johnson, 1995), the amount of GHG emitted during the growing and finishing stages within the western Canadian beef production cycle is similar (Beauchemin *et al.*, 2010). This mainly reflects the longer duration of the finishing phase of the production cycle and emphasizes the need to explore CH_4 mitigation strategies throughout the beef production cycle. Methane emissions of heifers offered the control and WDDGS diets were 25.0 and 37.5%, higher respectively, than the IPCC tier 2 estimates of 4.0% of GE for diets

containing ≥ 90% concentrate (IPCC, 2006). Similarly, we found that our emission estimates (7.8% GEI) in heifers fed a barley silage diet were slightly higher than the IPCC estimate of 6.5% GEI for cattle fed high-forage diets (IPCC, 2006). The IPCC tier 2 default emission factors for high-concentrate diets are mainly derived from corn grain diets (Johnson and Johnson, 1995), and these values may underestimate CH_4 emissions from cattle fed barley grain, owing to its higher fiber content than corn (Beauchemin and McGinn, 2005). Furthermore, corn is generally less extensively digested in the rumen than barley and a shift in the site of digestion from the rumen to the lower intestinal tract would be expected to lower CH_4 emissions (Johnson and Johnson, 1995). Consequently, Beauchemin and McGinn (2005) reported 29.8% higher CH_4 (g/kg DM intake) emissions from barley- compared to corn-based finishing diets consisting of 9.0% barley silage and 81.4% grain (DM basis).

Lower CH_4 emissions from heifers fed CDDGS and WDDGS+oil as compared to those fed WDDGS alone appear to be related to the level of fat in the diet (5.4 and 5.1 vs. 3.1% fat; DM basis). Fat that is unprotected from ruminal fermentation reduces CH_4 production primarily by lowering the quantity of organic matter fermented in the rumen (Johnson and Johnson, 1995; Beauchemin *et al.*, 2008). In the current study, a depression in OMD may account for lower CH_4 emissions from heifers fed WDDGS+oil as compared to WDDGS alone. However, total tract digestibility of nutrients between CDDGS and WDDGS did not differ suggesting that factors other

than depression of ruminal digestion, such as a direct toxic effect of fatty acids on methanogens may have contributed to this response.

Fat exerts toxic effects on methanogens as well as protozoa (Johnson and Johnson, 1995). Methanogens and protozoa exist in a synergistic relationship involving inter-species hydrogen transfer (Finlay et al,. 1994). Consequently, a reduction in protozoa numbers or activity is frequently associated with reduced CH_4 production (Martin *et al.*, 2010). Addition of corn oil to the WDDGS+oil diet did not reduce protozoa numbers, possibly because of the low level of oil added (2.6% corn oil; DM basis). Protozoa in heifers fed the control diet were almost exclusively *Entodinium*, an observation typical of high barley grain diets (Hristov *et al.*, 2001). Adding fat to the diet also enhanced the production of propionic acid and as formation of this VFA requires reducing equivalents, it decreases the amount of hydrogen available to reduce CO_2 to CH_4 (Janssen, 2010). The inverse relationship between propionate formation and CH_4 production was apparent in this study as feeding WDDGS resulted in the lowest concentration of propionate and the highest level of CH_4 production.

Previous work in our laboratory found that substitution of 40% CDDGS (5.4% fat in the diet) or 40% WDDGS+oil (5.6% fat in the diet; all DM basis) for barley grain in a growing diet containing 55% DM barley silage, reduced CH_4 (g/kg DMI) by 6.3 and 6.4% respectively, for each percentage of added fat (Hünerberg *et al.*, 2013). This magnitude of CH_4 reduction is greater than in the current finishing study, as CH_4 (g/kg DMI)

was reduced by 5.3% for CDDGS (5.4% fat in the diet) and 4.1% for WDDGS+oil (5.1% fat in the diet, both DM basis) for each percentage of additional fat. Ruminal degradation of forages results in higher CH_4 production per kg DM as compared to concentrates (Johnson and Johnson 1995); therefore it could be expected that the reduction in CH_4 in response to added fat would be potentially greater in high-forage compared to high-grain diets. However, Lovett al. (2003) reported that the magnitude of CH_4 reduction (per kg of live weight and carcass gain) from cattle supplemented with coconut oil (350 g/d) was less for high-forage compared to high-grain diets. This suggests that the CH_4 mitigation characteristics of lipids are not solely related to their negative effects on fiber digestion (Machmüller *et al.*, 2001).

The fact that CDDGS was slightly more effective than WDDGS+oil in reducing CH_4 emissions relative to the control diet might be due to the slightly lower fat level in the WDDGS+oil diet. Based on a meta-analysis using data from 27 studies over a practical range of fat feeding (< 8% fat; DM basis), the relationship between concentration of fat in the diet and CH_4 yield was not affected by form of supplemented fat, fatty acid profile, or fat source, suggesting that the level of fat in the diet may be the most important factor influencing methanogenesis (Grainger and Beauchemin, 2011). The authors calculated that an increase from 5.0 to 6.0% dietary fat (DM basis) decreased CH_4 (g/kg DMI) in cattle by 5.1% (Grainger and Beauchemin, 2011). In the present study, the calculated reduction in CH_4 from feeding

CDDGS (5.4% fat in the diet) as compared to WDDGS+oil (5.1% fat in the diet; both DM basis) was substantially higher, as a 0.3% difference was associated with a 6.6% reduction in CH_4 (g kg/DMI). The finding that CH_4 production in response to DDGS inclusion is heavily dependent on the fat content of DDGS is important as a number of ethanol plants are installing enhanced extraction technologies that lower the oil content by as much as 6%, resulting in a slight increase in the CP, NDF and ADF content of low-oil CDDGS (U.S. Grains Council, 2012). Replacing conventional CDDGS with low-oil CDDGS could reduce the lipid mediated reduction in CH_4 emissions while at the same time increasing N excretion and possibly contributing to increased N_2O emissions.

Similar CH_4 emissions in heifers fed WDDGS vs. the control diet are somewhat surprising as the WDDGS diet contained more NDF, ADF and considerably less starch. Not unlike starch, the fiber in wheat DDGS is highly fermentable in the rumen (Walter *et al.*, 2012), but in this study it still resulted in a fermentation profile that was lower in propionate than the control diet. The small particle size of WDDGS may also have increased the flow of fiber from the rumen to the lower intestinal tract, reducing CH_4 yield. However, if this response occurred it did not result in a decrease in the digestibility of fiber in heifers fed WDDGS.

Higher NH_3 concentration in the rumen fluid of heifers fed WDDGS and WDDGS+oil as compared to CDDGS and the control diet likely arise from differences in CP content and ruminal CP degradability. Corn protein is

mainly composed of zein, which is more resistant to ruminal degradation than gluten in wheat (Little *et al.*, 1968), a relationship confirmed by Boila and Ingalls (1994). Levels of ADIN in CDDGS and WDDGS were relatively low (Table 4-2), and thus unlikely to contribute to differences in ruminal N degradability. However, higher protozoa numbers in heifers fed WDDGS and WDDGS+oil as compared to the other two diets may have increased ruminal $NH_{3,}$ through deamination of amino acids that arose from the predation of bacteria (Wallace *et al.*, 1987; Koenig *et al.*, 2000).

Reducing the starch content of the diet by replacing rapidly fermentable, cereal grains with less rapidly fermentable DDGS has been proposed as an approach to modulate ruminal pH and reduce the incidence of SARA in feedlot cattle (Klopfenstein *et al.*, 2008). This is supported by our results as feeding CDDGS, WDDGS and WDDGS+oil reduced the time below a pH of 5.5, an indicator of SARA and pH 5.2, and an indicator of ARA. Higher total VFA concentrations in rumen fluid from heifers fed the control diet as compared to those fed DDGS, suggests that DDGS were not as rapidly fermented in the rumen as barley grain. An increase in the SD of ruminal pH in heifers fed the control diet as compared to those fed WDDGS and WDDGS+oil may also be indicative of a greater risk of acidosis as previously documented by Bevans *et al.* (2005). Nevertheless, differences in mean pH among diets were limited, with WDDGS+oil being the only diet that exhibited a higher daily mean pH than the control. Ruminal concentrations of lactate were low for all diets indicating that even though

heifers fed the control had longer durations of ruminal pH below 5.5 and 5.2, ARA did not occur as lactate concentration typically exceed > 50 m*M* with this condition (Nagaraja and Titgemeyer, 2007).

Walter *et al.* (2012) fed a barley-based finishing diet supplemented with 20 or 40% DM CDDGS or WDDGS and found no decrease in daily mean pH or a reduction in SARA when CDDGS or WDDGS replaced barley grain. Similarly, replacement of barley grain with increasing levels of WDDGS (7, 14, 21% DM) in a finishing diet for feedlot steers did not lead to higher mean rumen pH or reduced SARA (Beliveau and McKinnon, 2009). Both studies attributed the lack of an increase in pH with DDGS to its high ruminal fermentability and a reduction in rumination and saliva secretion owing to its small particle size. Van Kessel and Russell (1996) reported that methanogens are sensitive to low ruminal pH and that CH_4 production ceases *in vitro* at a pH < 6.0. This is clearly not applicable *in vivo* as, even when ruminal pH of both animals within a chamber dropped well below 6, CH_4 was still produced. Methanogens within cattle adapted to high-concentrate diets appear to be less sensitive to low pH than those from cattle fed diets with higher forage content (Hook *et al.*, 2011). Although the origin of this response is unclear, it may arise from these pH tolerant methanogens having a higher affinity for hydrogen or a greater endosymbiotic relationship with protozoa, where they would be less affected by the low pH within surrounding rumen fluid (Hook *et al.*, 2011).

As expected, the increased CP content of DDGS resulted in heifers fed these diets having a higher N intake than those fed the control diet. Likewise, differences in N intake between CDDGS and WDDGS reflect the higher CP content of WDDGS. All diets containing DDGS exceeded the protein requirements of finishing beef cattle by two fold (NRC, 2000), resulting in a dramatic increase in N excretion (g/d). In DDGS diets, BUN levels also exceeded 8 mg/dL, indicating that digestible N intake exceeded requirements (Cole *et al.*, 2003). Walter *et al.* (2012) found a similar response in feedlot steers fed diets containing 40% DM of CDDGS or WDDGS, reporting excretions of 207 and 266 g N/d, respectively. Protein fed in excess of requirements is an environmental concern as N is predominantly excreted as urea in urine. Upon urination, urea is rapidly hydrolyzed to NH_3 by bacterial urease and disperses into the atmosphere (Mobley *et al.*, 1995). Once in the atmosphere, NH_3 is a precursor for particulate matter and has a negative impact on air quality and respiratory health (U.S. Environmental Protection Agency, 2009). Additionally, NH_3 can be re-deposited to the soil surface and contribute to eutrophication, acidification and the formation of N_2O (IPCC, 2006). Excess N can also be lost in the form of NO_3^- through leaching and run-off and contaminate water bodies and be transformed to N_2O via aquatic denitrification (U.S. Environmental Protection Agency, 2010). As a result, N and NH_3 volatilization from manure both directly and indirectly contribute to GHG emissions (Hristov *et al.*, 2011).

As there were no differences in CPD among CDDGS, WDDGS and the control diet it can be assumed that greater percentage of urinary N excretion in heifers fed CDDGS or WDDGS was associated with the higher total N intake of these diets. In contrast, CPD in heifers offered WDDGS+oil was reduced as compared to the other diets. Heifers fed this diet also excreted less urinary N as a % of N intake, even though they had the highest N intake. It is likely that these responses arise due to a decrease in ruminal CP and OM digestibility as result of the addition of corn oil, leading to a reduction in N loss in urine and an increase in N loss in feces. This could have environmental implications as urinary N is more susceptible to leaching and volatile losses than fecal N (Bussink and Oenema, 1998).

This study completes the first full assessment of the impact of dietary CDDG and WDDG inclusion on both CH_4 emissions and N excretion from feedlot cattle. Results show that CH_4 production in response to DDGS inclusion is heavily dependent on the fat content of DDGS. Therefore, enhanced fat extraction from CDDGS could reduce its ability to mitigate enteric CH_4 emissions in ruminants. Using DDGS as an energy source exceeds CP requirements, dramatically increasing N excretion in both urine and feces. In order to reduce the environmental impact of DDGS in feedlot cattle production, it is critical that manure be applied on the basis of N requirements of the crop.

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CHAPTER 5- Farm-based life cycle assessment of greenhouse gas emissions from beef cattle fed dried distillers' grains with solubles[4]

5.1 Introduction

As estimated by FAO (2006), animal agriculture is responsible for ~18% of global GHG emissions. Greenhouse gas emissions from beef production systems are of particular interest as beef is associated with a greater GHG emission intensity (kg CO_2e per kg product or CO_2e per kg of protein) than other livestock meat (De Vries and de Boer, 2010). Beef production systems emit GHG in the form of enteric CH_4, N_2O from use of N fertilizer for crop production, CH_4 and N_2O from manure, and CO_2 from fossil fuel usage (O'Mara, 2011). The GHG emissions associated with beef arise mainly from the formation of CH_4 during enteric fermentation in the rumen, as well as the lower feed conversion efficiency and lower reproduction rates of cattle as compared to swine and poultry (De Vries and de Boer, 2010). Intake and diet composition are the two factors that have the greatest influence on enteric CH_4 emissions and N excretion in cattle (Eckard *et al.*, 2010; Johnson and Johnson, 1995).

To reduce GHG emissions and dependence on fossil fuels, governments have supported the production of fuel from renewable sources leading to an exponential growth in ethanol production. In 2011, global ethanol production was 85 billion L, with the United States (52.6 billion L) and Canada (1.7 billion L) accounting for 63.9% of production (RFA, 2012). While ethanol in the

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United States is produced primarily from corn, wheat accounts for one third of total ethanol production in Canada (USDA Foreign Agricultural Service, 2010). Distillers' grains plus solubles is the principal co-product of ethanol production. Due to its high fiber content, DDGS is most efficiently utilized as feed to replace feed grains (Klopfenstein *et al.*, 2008), or to lesser extent forages (Li *et al.*, 2011) in ruminant diets. Replacing 35% of barley grain (DM basis) with CDDGS reduced enteric CH_4 emission (% of GEI) from growing beef cattle fed a high forage, barley silage-based diet from 7.1 to 5.4% (McGinn *et al.*, 2009). This response was thought to be due to the fat (12.7%) in CDDGS.

Recent research has shown that replacing a mixture of 35% barley grain and 5% canola meal (DM basis) with CDDGS in a high forage diet reduced enteric CH_4 emissions from beef cattle from 7.8 to 6.6 % of GEI. However, inclusion of 40% WDDGS (DM basis) had no effect on CH_4 emissions (7.3% of GEI; Hünerberg *et al.*, 2013a). Similarly, replacing 40% of barley grain DM with CDDGS in a high grain diet reduced CH_4 emissions from 5.0 to 4.0% of GEI, while WDDGS (5.5% of GE intake) had no effect on enteric CH_4 emissions (Hünerberg *et al.*, 2013b). Results from both experiments indicated that the higher fat content of CDDGS (~10% fat) as compared to WDDGS (< 5% DM fat; both DM based) was responsible for the reduction in CH_4. However, replacing barley grain (~12.0% CP) with CDDGS (~30% CP) or WDDGS (~40% CP; all DM basis) dramatically increased N intake and excretion in both studies (Hünerberg *et al.*, 2013a, b). Increases in N excretion could outweigh any reduction in enteric CH_4 through increased formation of

N_2O. Furthermore, higher net GHG emissions from beef cattle operations that use CDDGS or WDDGS compared to those that use cereal grains could reduce the GHG mitigation benefits of corn and wheat based ethanol production as compared to fossil fuel. Therefore, the impact of replacing cereal grains with CDDGS or WDDGS on GHG emissions from the beef production cycle needs to be evaluated in an in-depth assessment quantifying all changes in GHG emissions at the whole farm level.

The objective of this study was to evaluate the impact of CDDGS or WDDGS inclusion in feedlot diets on GHG emission from beef cattle using a LCA. This assessment was conducted using primary data for enteric CH_4 and N excretion generated in two experiments using growing and finishing beef cattle fed CDDGS or WDDGS (Hünerberg *et al.*, 2013a, b).

5.2 Materials and Methods

In order to estimate GHG emissions from beef cattle fed CDDGS or WDDGS we simulated a representative model farm, which implemented these feeding practices under typical western Canadian management conditions. This simulation was made relative to the previous feeding practice of using barley grain as the main supplementary energy source in the diet of feedlot cattle.

Description of the beef life cycle

The North American beef production cycle typically consists of a separate cow–calf and feedlot stage. Cow-calf farming or ranching operations maintain herds of mature cows. The cows are bred and the calves are raised to weaning (Beauchemin *et al.*, 2010; Vergé *et al.*, 2008). Cow-calf operations are

usually located on pastureland that is largely unsuitable for crop production. After the calves are weaned from the cows, a proportion of the calves are retained on-farm as replacement heifers for cull cows within the breeding herd. The remaining calves destined for market (males are typically castrated) are moved into confined feedlots where they are fed until market weight. At the beginning of the feedlot phase calves are typically fed a growing or stocker (high forage) diet. To maximize energy intake and promote marbling, cattle are later transitioned to a high grain finishing diet. Finishing diets are fed until the cattle are slaughtered. The replacement heifers are typically fed a high forage diet similar to that fed during the growing phase. Replacement heifers are reintegrated into the cow herd once they reach breeding age (Beauchemin *et al.*, 2010).

Description of the model farm

Even though CDDGS and WDDGS were fed only during the feedlot phase, emissions associated with the cow calf stage were included in our LCA. This ensures the effects of this feeding practice are cycled through the entire system to the end product, or functional unit, which in this LCA was defined as 1 kg of beef (carcass weight). The model farm simulated in this LCA is similar to that of Beauchemin *et al.* (2010); therefore only a brief description is provided.

The simulated model farm was located in the county of Vulcan in southern Alberta, Canada (Ecodistrict 793; Marshal *et al.*, 1999) and consisted of a cow-calf operation on native mixed-grass pasture, a feedlot and the

cropland required to grow barley grain and silage to feed feedlot cattle and mixed hay as winter feed for the cow herd. The soil type was a dark brown Chernozem managed under reduced tillage practices. Average growing season precipitation (May–October) for this ecodistrict is 277 mm and potential evapotranspiration is 653 mm (Marshal *et al.*, 1999). The simulated beef herd consisted of 120 cows, four bulls, and their progeny, which were fed within a feedlot. The LCA consisted of growing the breeding stock from birth to maturity within the beef production cycle. This encompassed a time period of eight years, a representative breeding life span of cows in this production system (Bailey, 1991).

After entering the feedlot at an average body weight of 240 kg, weaned calves were fed a high forage diet comprised of 55% barley silage, 38% barley grain, 5% canola meal and 2% mineral and vitamin supplement (DM basis; baseline scenario). In the CDDGS and WDDGS scenarios, 35% barley grain and 5% of canola meal were replaced by 40% CDDGS or WDDGS, respectively (DM basis, Table 5-1). All three growing diets were assumed to result in a similar ADG of 1.0 kg/d as predicted by NRC (2000). Once the calves reached an average body weight of 350 kg, they were switched to a 90% barley grain, 8% barley silage and 2% mineral and vitamin supplement (DM basis; baseline scenario) finishing diet. In the finishing DDGS scenarios, 40% of barley grain was replaced with CDDGS or WDDGS (DM basis). Similar to the growing phase, NRC (2000) predicted ADG during the finishing phase (1.5 kg/d) were utilized and assumed to be constant across diets. After being offered their

respective growing and finishing diets for 110 and 170 d, respectively, cattle were marketed at 605 kg. Carcass yield was assumed to be 60% of total carcass weight (Alberta Agriculture and Rural Development, 2002). Heifers required to replace cull cows in the breeding herd were fed the growing baseline diet for five months post-weaning.

Table 5-1 Ingredient composition of the feedlot diets and primary input data.

	Growing diets[1]			Finishing diets[2]		
Item	Baseline	CDDGS	WDDGS	Baseline	CDDGS	WDDGS
Ingredient, % of dry matter (DM)						
Barley silage	55	55	55	8	8	8
Barley grain	38	3	3	90	50	50
CDDGS[3]		40			40	
WDDGS[4]			40			40
Canola meal	5					
Supplement	2	2	2	2	2	2
Primary input data						
CP[5] content, % DM	13.0	18.6	23.5	12.2	19.6	23.1
DE[6], % DM	69.8	65.7	68.4	82.2	78.1	77.4
Ym[7], % of GE intake	7.8	6.6	7.3	5.0	4.0	5.5
N excretion, % of N intake	85.2	81.7	86.6	72.8	81.3	81.1

[1]High forage diet fed at the beginning of the feedlot phase.
[2]High grain diet fed at the end of the feedlot phase.
[3]Corn dried distillers' grains with solubles.
[4]Wheat dried distillers' grains with solubles.
[5] Crude protein.
[6]Digestible energy.
[7]Yield of enteric CH_4 as % of gross energy (GE) intake

While barley grain and silage were produced on-farm, all other diet ingredients (CDDGS, WDDGS, canola meal, and mineral and vitamin supplement) were purchased and shipped to the feedlot. Greenhouse gas burdens related to the production of CDDGS, WDDGS and canola meal were estimated using the GHGenius model (http://www.ghgenius.ca). Emissions from the

production and shipping of mineral and vitamin supplement were considered negligible as it constituted only 2% of the diet (DM basis). As described by Beauchemin *et al.* (2010) feedlot cattle and the immature breeding stock were housed in pens bedded with barley straw that was grown on-farm. The manure in the pens was removed once per year and applied to the cropland used to grow barley grain and silage.

Origin and use of primary data

The diets fed over the course of the feedlot phase of this LCA (Table 5-1) are identical to experimental diets used by Hünerberg *et al.* (2013a, b). Both studies used the same 16 beef heifers in a repeated Latin square design to compare CH_4 emission from growing (Hünerberg *et al.*, 2013a) and finishing (Hünerberg *et al.*, 2013b) beef cattle fed 40% DM CDDGS,WDDGS or a DDGS-free barley based control diet. In both studies, a subset of eight heifers was used to determine the apparent total-tract digestibility of nutrients and N excretion by total collection of urine and feces. Enteric CH_4 in both studies was measured using open circuit respiratory chambers (two animals fed the same diet per chamber). Details concerning the experimental protocols are described in Hünerberg *et al.*, (2013a, b). Primary data used as model input (Table 5-1) for beef cattle during the feedlot phase of the beef life cycle were: dietary CP content (% DM), DE (% DM), enteric CH_4 production as a proportion of GEI (**Ym**) and N excretion (% of N intake). As the immature breeding stock in all scenarios received the growing baseline diet for five months post weaning,

primary data for growing cattle fed the baseline diet were also applied to immature breeding stock.

Use of HOLOS to estimate GHG emissions, boundaries and functional unit

The impact of CDDGS and WDDGS inclusion on total farm based GHG emissions was assessed using Holos (www.agr.gc.ca/holos-ghg). Holos is a whole-farm modelling software program developed by Agriculture and Agri-Food Canada that estimates GHG emission from individual farms (Little *et al.*, 2008) including those producing beef cattle (Beauchemin *et al.* 2010; 2011).

Greenhouse gas emissions include: on farm CH_4 emissions from cattle and manure; on farm N_2O emissions from manure, soils, and growing crops; off farm N_2O emissions from N leaching, runoff and volatilization; CO_2 emissions and removals due to management induced soil C change; and CO_2 from on-farm energy use (Table 5-2).

Table 5-2 Sources of greenhouse gas (GHG) emissions, equation or emission factor (EF) used, and reference source.

Gas/source	Equation/emission factor	Reference
Methane sources		
Enteric fermentation	Mature cows:	
	Based on gross energy requirements and digestible energy in feed	IPCC 2006
	Feedlot cattle:	
	Based on primary data (Table 5-1)	Hünerberg *et al.* 2013a
	Growing	Hünerberg *et al.* 2013b
	Finishing	
Pasture manure	0.01 kg CH_4 (kg CH_4)$^{-1}$	IPCC 2006
Deep bedding manure	0.17 kg CH_4 (kg CH_4)$^{-1}$	IPCC 2006
Direct nitrous oxide sources		
Pasture manure	0.02 kg N_2O-N (kg N)$^{-1}$	IPCC 2006

Deep bedding manure	0.01 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
Soil/cropping nitrogen inputs (includes land applied manure, crop residue, synthetic nitrogen fertilizer, mineralized nitrogen)	EF_{eco}[1] = $0.022 \times P^2$[2] / PE[3] – 0.0048	Rochette *et al.* 2008

Indirect nitrous oxide sources

Pasture manure	Leaching:	
	EF =	
	0.0075 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{leach}$[4] =	
	$0.3247 \times P / PE – 0.0247$	Rochette *et al.* 2008
	Volatilization:	
	EF =	
	0.01 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{volatilization}$[5] =	
	0.20 kg N (kg N)$^{-1}$	IPCC 2006
Deep bedding manure	Leaching:	
	EF =	
	0.0075 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{leach}$ = 0 kg N (kg N)$^{-1}$	IPCC 2006
	Volatilization:	
	EF =	
	0.01 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{volatilization}$ =	
	0.30 kg N (kg N)$^{-1}$	IPCC 2006
Soil/cropping nitrogen inputs (includes land applied manure, crop residue, synthetic nitrogen fertilizer, mineralized nitrogen)	Leaching:	
	EF =	
	0.0075 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{leach}$ =	
	$0.3247 \times P / PE – 0.0247$	Rochette *et al.* 2008
	Volatilization[6]:	
	EF =	
	0.01 kg N_2O-N (kg N)$^{-1}$	IPCC 2006
	$Frac_{volatilization}$ =	
	0.1 kg N (kg N)$^{-1}$	IPCC 2006

Carbon dioxide sources

Energy to produce crop	124.6 kg CO_2 ha^{-1}	Little *et al.* 2008
Energy to apply manure to land	0.1736 kg CO_2 (kg N)$^{-1}$	Little *et al.* 2008
Fertilizer production (nitrogen)[7]	3.59 kg CO_2 (kg N)$^{-1}$	Nagy 2000
Fertilizer production (phosphorus)	0.5699 kg CO_2 (kg P_2O_5)$^{-1}$	Nagy 2000
Energy to produce herbicide	1.334 kg CO_2 ha^{-1}	Little *et al.* 2008

Energy to process, store and distribute feed	5% of total energy CO_2 emissions (kg CO_2)	Expert opinion
Trucking of wheat distiller' grains plus solubles (WDDGS) and canola meal	146.9 g CO_2e/ t-km	http://www.ghgenius.ca
Rail transportation of CDDGS	16.8 g CO_2e/t-km	http://www.ghgenius.ca

[1]EFeco = Emission factor for ecodistrict.
[2]P = Growing season (May – October) precipitation.
[3]PE = Growing season (May – October) evapotranspiration.
[4]$Frac_{leach}$ = Leaching fraction.
[5]$Frac_{volatilization}$ = Volatilization fraction.
[6]Indirect emissions due to volatilization are only calculated on nitrogen inputs from land applied manure and synthetic nitrogen fertilizer.
[7]Based on the weighted average of 1/3 anhydrous and 2/3 urea N fertilizer.

Dry matter intake for cattle in each stage of production was estimated based on net energy requirements and adjusted by the DE content of the diet (NRC, 2000). The Ym value for grazing beef cows was estimated using an IPCC (2006) Tier 2 approach. Holos estimates for CH_4 emissions from manure storage were based on the amount of volatile solids produced and the type of storage system utilized (IPCC, 2006). Direct N_2O emissions from soils were calculated based on N inputs, modified by soil type and texture, climate, tillage and topography of the ecodistrict. Nitrogen inputs include synthetic N fertilizer, land applied manure, as well as above and below ground crop residue decomposition. Net N mineralization is normally estimated from net change in soil C, but given the geologically short duration of this scenario, it was assumed to be zero in these simulations (Little *et al.*, 2008). Soil derived N_2O emission was calculated from total N inputs using Canada specific algorithms for estimating national GHG inventories (Rochette *et al.*, 2008). Indirect N_2O emissions (i.e., N lost from the farm via leaching, runoff and volatilization) were

estimated from assumed fractions of N lost from manure, residues, and fertilizer using the appropriate IPCC (2006) emission factors. Holos uses a methodology derived from that developed for the Canadian National Inventory Report to estimate soil C gains and losses (McConkey *et al.*, 2007). The approach assumes that land which has been consistently managed for decades (e.g., long term native grass land) has steady state C storage so that net exchange of CO_2 is negligible. Carbon dioxide emissions arising from burning of fossil fuels from on- and off-farm sources were also considered using general coefficients (Table 5-2). On-farm, or primary, sources included use of fossil fuels and power for tillage, seeding, harvesting, irrigation, spreading manure, processing feed, and feeding cattle. Off-farm, or secondary, sources include emissions associated with manufacture of herbicides and fertilizers. Emissions associated with manufacture of machinery (i.e., capital goods) were not considered.

In this study, ISO protocols (ISO, 2006) were followed and all GHG were expressed as CO_2e to account for the GWP of the respective gases: CH_4, kg × 25 + N_2O, kg × 298 + CO_2, kg (IPCC, 2007). The boundaries of the system were at the farm gate. Greenhouse gas emissions were expressed per kg of carcass (i.e., functional unit) as well as total CO_2e emitted over the entire life cycle.

Allocation and carbon footprint of biofuel co-products

Greenhouse gas burdens for the biofuel co-products CDDGS and WDDGS, and canola meal were determined by allocation from the carbon footprints of their respective biofuels ethanol and commercial grade canola oil.

Footprints for corn- and wheat-based ethanol and canola oil, expressed as kg CO_2e/MJ, were estimated using the GHGenius model (http://www.ghgenius.ca). GHGenius is a modelling program that uses a cradle-to-grave approach to calculate carbon footprints from fossil, as well as renewable, transportation and stationary fuels. Sources of GHG emissions, equations and emission factors used by GHGenius are primarily based on IPCC methodology as well as industry information. In our case, carbon footprints for corn- and wheat-based ethanol and canola oil included: emissions associated with the production of the crop (e.g., energy used for cultivation, direct and indirect life cycle emissions from fertilizers and pesticides); emissions associated with cultivation of the crops (e.g., N_2O from application of fertilizer, changes in soil carbon); harvesting; field to plant transportation; processing of the harvested raw material (e.g., cleaning and grinding); and extraction and processing of ethanol and canola oil (e.g., emissions for process heat/steam, electricity generation, emissions from chemicals used for fuel production). Post-plant emission associated with fuel storage, distribution and dispensing were not included in the biofuel footprints used to allocate the GHG burdens for the co-product. GHGenius can perform the LCA for specific regions of Canada and the United States. Based on the most realistic scenarios, the corn to produce corn ethanol was assumed to be grown and processed in central U.S.; the wheat to produce wheat ethanol was assumed to be grown and processed in Saskatchewan, Canada. The canola to produce canola oil was assumed to be grown and processed in Alberta, Canada.

To compare different allocation methods, GHG emissions between biofuel (corn-based ethanol, wheat-based ethanol and canola oil) and co-products (CDDGS, WDDGS and canola meal) were allotted based on mass and energy content, as well as by economic value of biofuel and co-product (BSI, 2011). Carbon footprints for the co-products were expressed as kg CO_2e/kg DM (Table 5-3). Ultimately, only carbon footprints derived from economic allocation were utilized in the beef LCA. Economic allocation specifies that the proportion of emissions assigned to each co-product is equal to the proportion of revenue generated through the sale of each (BSI, 2011).

Table 5-3 Allocation factors and greenhouse gas (GHG) intensity of co-products fed.

	Allocation method[2]		
Item[1]	Economic	Energy	Mass
Corn ethanol from central U.S. (73.0 g CO_2e/MJ)			
Allocation factor to ethanol	0.79	0.65	0.53
Allocation factor to CDDGS[3]	0.21	0.35	0.47
GHG emissions, kg CO_2e/kg DM CDDGS	0.50	0.84	1.14
Wheat ethanol from Saskatchewan, Canada (66.6 g CO_2e/MJ)			
Allocation factor to ethanol	0.76	0.57	0.44
Allocation factor to WDDGS[4]	0.24	0.43	0.56
GHG emissions, kg CO_2e/kg DM WDDGS	0.37	0.67	0.87
Canola oil from Alberta, Canada (31.2 g CO_2e/MJ)			
Allocation factor to canola oil	0.74	0.60	0.43
Allocation factor to canola meal	0.26	0.40	0.57
GHG emissions, kg CO_2e/kg DM canola meal	0.25	0.37	0.54

[1]Based on GHGenius (http://www.ghgenius.ca).
[2]Estimates are plant gate emissions and do not include GHG emission for transportation to the feedlot.
[3]Corn dried distillers' grains with solubles.
[4]Wheat dried distillers' grains with solubles

Economic allocation of GHG burdens between biofuel and co-product was based on the five-year (2008-2012) average national commodity price for ethanol (USEIA, 2012), CDDGS (USDA Agricultural Marketing Service, 2012), canola oil and canola meal (Statistics Canada, 2012). As there is no national price database for WDDGS, the market price of WDDGS was assumed to be on average 15% lower than CDDGS (Expert opinion). To estimate freight emissions from CDDGS, WDDGS and canola meal from the source of production to our model feedlot, realistic production locations were specified as, Blue Flint Ethanol, Underwood, ND, U.S. for CDDGS; Terra Grain Fuels Inc., Belle Plaine, SK, Canada for WDDGS; and ADM Agri-Industries Co., Lloydminster, AB, Canada for canola meal. Transportation emission for trucking of WDDGS and canola meal (146.9 g CO_2e/t-km), as well as rail freight of CDDGS (16.8 g CO_2e/t-km), were estimated using GHGenius. Similarly to the methodology used in Holos, emissions associated with the manufacture of machinery (i.e., capital goods) were not included in transportation emission estimates.

Boundaries and allocation of manure N

Due to the high CP content of CDDGS and WDDGS diets, the manure N in both scenarios exceeded the N requirements of the crops that were grown on-farm. The emissions for the storage of this manure were allocated to the model farm. However, upon land application, the excess manure N and associated emissions (i.e., N_2O – direct and indirect, CO_2 – due to fuel use for manure spreading) were handled in three ways: 1) all emissions associated with this

excess manure N were assigned to the farm of origin; 2) excess manure was assumed to be exported off farm along with all associated emissions; 3) excess manure N was assumed to be a co-product of the system (along with beef). In this third case, the excess manure N was assigned a price equal to the 5-year average price (2008-2012) per kg N of urea fertilizer (Alberta Agriculture and Rural Development, 2013). Similarly, the price of beef was calculated based on 5-year average (2008-2012; Agriculture and Agri-Food Canada, 2013). Manure N was assumed to replace synthetic fertilizer N on a 1:1 basis. Net life cycle emissions were apportioned using economic allocation between the co-products of beef and manure N.

5.3 Results and Discussion

Baseline scenario and earlier estimates

The GHG intensity of the baseline scenario (23.5 kg CO_2e/kg carcass; Table 5-4) was 8.3% higher compared to an earlier estimate (21.7 kg CO_2e/kg carcass) for western Canadian beef from our lab (Beauchemin *et al.*, 2010).

The slightly greater GHG emission intensity in the current study arose mainly due to the use of the directly measured primary data (dietary CP content, DE value, Ym and N excretion; Table 5-1), as most other inputs used in the baseline in this study were identical to Beauchemin *et al.* (2010). Specifically, the measured Ym values of 7.8% for the growing and 5.0% for the finishing baseline diet (Table 5-1), were higher than the IPCC Tier2 estimates of 6.5% for growing and 4.0% for finishing cattle (IPCC, 2006), resulting in increased enteric CH_4 emission from feedlot cattle and greater GHG intensity. However,

we are confident that the usage of primary CH_4 emission data improved the accuracy of our baseline scenario, because IPCC Tier 2 estimates are default values that do not account for differences in diet composition. An additional factor that may have contributed to the minor difference in GHG intensity between Beauchemin *et al.* (2010) and the current study is that the Holos version used by Beauchemin *et al.* (2010) used a lower GWP for CH_4 (23 vs. 25) and N_2O (296 vs. 298).

Results from our baseline (23.50 kg CO_2e/kg carcass), CDDGS (24.97 kg CO_2e/kg carcass) and WDDGS (25.68 kg CO_2e/kg carcass) scenarios are within range of other benchmark values from similar beef production systems in the United States (Johnson *et al.* 2003, 21.7 kg CO_2e/kg carcass; Pelletier *et al.* 2010, 24.7 kg CO_2e/kg carcass). In contrast, Vergé *et al.* (2008) in a Canada wide beef LCA reported a substantial lower GHG intensity of 17.2 kg CO_2e/kg beef carcass. However, comparisons between LCA are challenging as scope, boundaries (farm vs. region or country), model inputs, and algorithms differ greatly among studies (Beauchemin *et al.*, 2010; Vergé *et al.*, 2008).

Impact of CDDGS and WDDGS on GHG emission of the beef life cycle

Feeding CDDGS or WDDGS resulted in a 6.2 and 9.3% increase in total GHG emission and GHG intensity, respectively, as compared to the baseline scenario (Table 5-4). This increase in GHG was mainly caused by greater manure related N_2O and higher CO_2 emissions from energy use in CDDGS and WDDGS production (Table 5-5).

Table 5-4 Effects of corn-and wheat distillers' grains with solubles (CDDGS, WDDGS) on total greenhouse gas (GHG) emissions (tonnes of carbon dioxide equivalent [t CO_2e]) and GHG emission intensity (kg CO_2e/kg beef) of the beef life cycle.

Scenario[1]	Cropland area ha	Manure N in excess of crop requirements[2] kg/yr	Total GHG emissions t CO_2e	Breakdown total GHG Emissions, %			GHG intensity, kg CO_2e/kg beef		Change in GHG intensity from baseline %
				Cow-calf herd	Feedlot cattle[3]	Breeding stock[4]	Live weight basis	Carcass weight basis[5]	
Baseline	289.9	-952.2	5889.6	59.8	21.4	18.7	14.10	23.50	
CDDGS	255.9	2497.0	6257.5	56.3	26.1	17.5	14.98	24.97	6.3
WDDGS	255.9	3499.3	6435.9	54.8	28.2	17.0	15.41	25.68	9.3

[1]It was assumed all scenarios resulted in the same amount of 250,626 kg of beef (carcass weight over the entire 8-yr life cycle) produced.
[2]Calculated as: (Manure N) – (N crop requirements). All emissions from excess manure N were assigned to the farm of origin.
[3]Includes 110 d growing and 170 d finishing phase.
[4]Immature replacement animals for the cow-calf herd.
[5]Based on 60% of live weight.

Table 5-5 Effects of corn-and wheat distillers' grains with solubles (CDDGS, WDDGS) on individual greenhouse gas (GHG) emissions (tonnes of carbon dioxide equivalent [t CO_2e] and percentage of total GHG emission).

	Methane				Nitrous oxide				Carbon dioxide	
	Enteric		Manure		Manure		Soil		Energy usage	
Scenario[1]	t CO_2e	%	t CO_2e	%	t CO_2e	%	t CO_2e	%	t CO_2e	%
Baseline	3887.8	66.0	290.1	4.9	1236.9	21.0	202.9	3.4	271.8	4.6
CDDGS	3809.8	60.9	339.4	5.4	1343.8	21.5	205.6	3.3	558.9	8.9
WDDGS	3960.0	61.5	332.0	5.2	1395.4	21.7	225.7	3.5	522.9	8.1

[1]All emissions from excess manure N in the CDDGS and WDDGS scenario were assigned to the farm of origin.

Furthermore, feeding CDDGS and WDDGS resulted in an increase in manure related CH_4 and soil related N_2O emission compared to the baseline scenario. In contrast, enteric CH_4 emission in the CDDGS scenario were reduced by 2.0% compared to the baseline; whereas feeding WDDGS increased losses of enteric CH_4 by 1.9 and 3.9% compared to baseline and CDDGS scenarios, respectively.

Feeding 40% DM of CDDGS and WDDGS in the feedlot increased soil and especially manure related N_2O emissions relative to the baseline and reflects the higher losses of manure N associated with the high CP content of DDGS diets (Table 5-1). Upon excretion, manure N can be rapidly transformed and lost as NH_3, a precursor of indirect N_2O. Particularly NH_3 losses from feedlot operations are high (28 to 72% of fed N) and are a significant source of indirect N_2O emission (Hristov et.al. 2011). However, owing to its frequently lower price than grain, feeding DDGS at dietary levels that exceed protein requirements is common given that beef cattle fed DDGS exhibit growth performance that is equivalent or even superior to those fed grain-based diets (Gibb *et al.*, 2008; Klopfenstein *et al.*, 2008). As a result of high dietary CP intake, the amount of manure N in the CDDGS and WDDGS scenario exceeded crop N requirements. This surplus N was caused in part by a 34 ha reduction in cropland area (Table 5-4), as CDDGS and WDDGS partially replaced the barley grain grown on-farm in the baseline scenario. However, even at equivalent cropland area (289.9 ha in baseline) inclusion of 40% DM of CDDGS and WDDGS in the diet would

still have resulted in 1544.8 (CDDGS) and 2547.1 kg (WDDGS) of excess manure N. Distillers' grains are also high in phosphorus ([P], e.g., 1.07% DM; Gibb *et al.*, 2008) contributing to greater P excretion than the baseline scenario. Soil P saturation and surface water contamination due to inclusion of high levels of DDGS in feedlot diets could also be a concern (Hao *et al.*, 2009).

The minor increase in manure related CH_4 emission in the CDDGS and WDDGS scenario can be explained by the greater amounts of manure solids due to consistently lower DE values for growing and finishing DDGS diets as compared to the baseline diet (Table 5-1). Consequently, increased emissions from manure in the CDDGS and WDDGS scenario also reflect higher CO_2 emissions as a result of greater energy use for the transportation and spreading of more manure. However, most of this increase in CO_2 emissions over the baseline scenario is associated with off-farm emissions arising from the production and transport of DDGS. The use of natural gas and electricity in ethanol plant (i.e., DDGS drying) are primary sources of these emissions. Using the Biofuel Energy Systems Simulator (BESS), Bremer *et al.* (2011) estimated GHG emissions from gas and electricity use in ethanol plants of 30.5 g CO_2e/MJ of corn ethanol, if co-products were dried to ~90% DM. In our simulation, CO_2 emissions from ethanol production included drying of DDGS and were even higher at 38.2 g CO_2e/ MJ for corn ethanol and 43.4 g CO_2e/ MJ for wheat ethanol. This represents more than 50% of plant gate emission of corn (73.0 g CO_2e/MJ; Table 5-3)

and wheat (66.6 g CO_2e/MJ) ethanol. Marketing WDGS (~35% DM), or partially dried "modified" distillers' grains with solubles (~45% DM) substantially reduces the GHG emissions associated with ethanol production (Bremer *et al.*, 2010). However, higher transport cost and faster rate of spoilage restricts these low DM co-products to being fed within the vicinity of the ethanol plant and they must be dried for wider distribution (RFA, 2012).

Inclusion of CDDGS in the diet reduced CH_4 emissions as compared to the baseline and WDDGS diets (Table 5-1). However, the impact of this reduction from a life-cycle perspective was relatively small as it only reduced CH_4 emissions from the feedlot, and had no impact on the cow-calf herd which is responsible for more than 60% of enteric CH_4 emissions (excluding breeding stock; data not shown) and between 54.8 and 59.8% of total GHG emission (Table 5-4), respectively. Consequently, effective GHG mitigation strategies that target the cow-calf herd have a greater impact on total GHG compared to those that target only the feedlot stage (Beauchemin *et al.*, 2010; Beauchemin *et al.*, 2011; Vergé *et al.*, 2008). Feeding high-fat CDDGS to the cow-calf herd (e.g., during winter feeding period) could therefore offer additional GHG reduction potential, but as in the current study, increases in manure related N_2O emission would have to be taken into account. In addition, the economic feasibility of using DDGS as supplement feed for the cow-calf herd would need to be assessed.

As the high fat content of CDDGS (>10% DM basis; Hünerberg *et al.*, 2013a, b) is responsible for the reduction in enteric CH_4, supplementing cattle with low-fat CDDGS (3.5 to 6.7% DM basis; Mjoun *et al.*, 2009; Gigax *et al.*, 2011) will likely reduce the CH_4 mitigation potential of CDDGS. Enhanced fat extraction of CDDGS is increasingly common in the industry, but apart from reducing the CH_4 mitigation potential of CDDGS, it also potentially increases N_2O emissions by further concentrating the N content of CDDGS.

Growth performance of cattle fed high-DDGS diets

Differences in animal growth or reproductive efficiency have been identified as major impact factors on GHG emission intensity of livestock production systems (Beauchemin *et al.*, 2011; De Vries and de Boer, 2010). The assumption that replacement of 40% dietary DM barley grain (baseline scenario) by CDDGS or WDDGS will have no adverse effect on growth performance of feedlot cattle is supported by the literature. Replacing 40% barley grain by WDDGS in growing (55% barley silage) and finishing diets (10% barley silage, all DM basis) resulted in similar ADG and feed conversion efficiency of beef heifers (Gibb *et al.*, 2008). Similarly, Beliveau and McKinnon (2008) found no effect on ADG, feed efficiency or DM intake when up to 32% of WDDGS replaced barley grain in growing (24% barley silage) and finishing diets (5.2% barley silage, all DM basis). Walter *et al.* (2010) included up to 40% (DM) of CDDGS and WDDGS in beef finishing diets (7.6% barley silage, all DM basis) and concluded that neither

had a negative impact on growth performance or carcass quality. Likewise, replacement of 35% barley grain by CDDGS in growing diet (60% barley silage, DM basis) did not alter the ADG of steers (McGinn *et al.*, 2009).

Carbon footprint of biofuel co-products

Allocation of GHG emissions from CDDGS on an economic basis resulted in a substantially lower estimate (0.50 kg CO_2e/kg of CDDGS; Table 5-3) as compared to that reported in an earlier study (0.91 kg CO_2e/kg CDDGS; Adom *et al.*, 2012). Differences in the GHG intensity of CDDGS likely reflect differences in the relative allocation of emissions between DDGS and ethanol. While Adom *et al.* (2012) assigned 30% of GHG emission to CDDGS; we only allocated 21% of GHG emission to CDDGS. Adom *et al.* (2012) based their GHG intensity of CDDGS on ethanol production emission from three different studies averaging 72.3 g CO_2e/MJ ethanol (Hill *et al.*, 2006; Shapouri *et al.*, 2003; Wang, 2001), whereas emissions from corn-based ethanol production was estimated at 73.0 g CO_2e/MJ in our study. However, differences in GHG emission intensity of corn-based ethanol between Adom *et al.* (2012) and our study are minor.

In contrast, GHG burdens for the production of wheat ethanol from Saskatchewan (Canada) were lower (66.6 g CO_2e/MJ ethanol) than for corn ethanol from central U.S. This is somewhat surprising because average ethanol yield per tonne of grain is higher for corn (399 L, FAO, 2008) than wheat (376 L; McLeod *et al.*, 2010), owing to the higher starch content of corn. However, production of spring wheat from western Canada is

associated with lower N_2O emissions as compared to corn in the central U.S. This is due to lower N requirements of spring wheat compared to corn and the cooler and dryer climatic conditions in western Canada which are less favorable for the formation of N_2O. Consequently, GHG emission intensity of WDDGS in the current study is lower compared to CDDGS, regardless of the allocation method (Table 5-3). Reference values for the GHG intensity of WDDGS do not exist.

Of all three biofuels, production of canola oil resulted in the lowest GHG emission intensity (31.2 g CO_2e/MJ). Advantages of canola oil over grain ethanol production are due to lower agricultural inputs and more efficient conversion of feed stocks to fuel (Hill *et al.*, 2006). As a result, emission intensity of canola meal is also substantially lower compared to WDDGS and CDDGS across all allocation methods. In addition, canola meal was only fed during the growing phase (baseline scenario; Table 5-1) at only 5% of dietary DM. Consequently, the impact of the canola meal footprint on GHG emission intensity per kg of beef was minor as compared to CDDGS or WDDGS.

Allocation of GHG emissions based on the economic value of CDDGS, WDDGS and canola meal reflects the environmental consequences of supply and demand for these biofuel co-products (BSI, 2011). Allocation of GHG emissions from biofuel co-products based on energy or mass resulted in an increase in GHG intensity (kg CO_2e/kg beef), as coefficients were lower when assigned based on economic value. Variation in allocation

factors has been previously identified as major source of uncertainty for carbon footprint analysis of biofuel co-products (Adom *et al.*, 2012).

Allocation of excess manure nitrogen

As discussed, excretion of manure N in the CDDGS and WDDGS scenarios exceeded the N requirements of the crops needed to provide feed for the beef herd. In agreement with previous Holos methodology, all emissions associated with storage, handling, and land application of this excess manure were allocated to the farm (Table 5-4), as its feeding practices were directly responsible for these emissions. However, it is unlikely that a farmer would apply manure at rates that were 2497.0 kg N/yr (CDDGS scenario) or 3499.3 kg N/yr (WDDGS scenario) in excess of crop requirements. Losses of NH_3, NO_3^- and the formation of N_2O as a result of excessive application of manure N is of environmental concern with negative effects on air and water quality as well as representing a loss of crop available nutrients (IPCC, 2006; USEPA, 2009).

Consequently beef producers would be expected to export excess manure to a farm within an economical hauling distance from the feedlot that requires N fertilize for crop production. In this scenario, it seems reasonable to allocate the GHG emission associated with the exported manure to the farm that receives it. In this situation the manure does not exceed the N requirements of the farm of origin and in fact offsets the need to use synthetic fertilizer, reducing the emission intensity of the CDDGS and WDDGS scenario by 0.9 and 1.2 % respectively (Table 5-6). However, these

reductions in GHG intensity have only a minor impact on overall emissions from the beef life cycle and do not change the fact that GHG intensities with CDDGS and WDDGS are substantially higher than the baseline scenario.

Table 5-6 Greenhouse gas emissions as affected by allocation of excess manure N.

Scenario and allocation method	Emission allocation to beef %	GHG intensity kg CO_2e/kg beef	
		Live weight basis	Carcass weight basis[4]
Emissions from excess manure N were assigned to the farm of origin			
CDDGS[1]	100	14.98	24.97
WDDGS[2]	100	15.41	25.68
Excess manure with all associated emissions was exported off farm			
CDDGS	100	14.85	24.75
WDDGS	100	15.23	25.38
Excess manure N was assumed to be a co-product of the beef cycle[3]			
CDDGS	98	14.62	24.37
WDDGS	97	14.90	24.83

[1]Corn dried distillers' grains with solubles.
[2]Wheat dried distillers' grains with solubles.
[3]Emissions between beef and manure N were apportioned using economic allocation. Beef and fertilizer N prices were assigned based on 5-yr average prices per kg of beef carcass (Agriculture and Agri-Food Canada, 2013), and kg of N from urea fertilizer (Alberta Agriculture and Rural Development, 2013).
[4]Based on 60% of live weight.

Alternatively, manure N could be considered a co-product of the beef life-cycle as its nutrients can replace synthetic fertilizer. In this case, excess manure was assigned an emission intensity based on its potential market value (e.g., per unit of N). Economic co-product allocation between beef and excess manure N, compared to assigning all emission to beef, reduced GHG

emission intensity of the CDDGS and WDDGS scenario by 2.4 and 3.3% respectively. Rising costs for synthetic fertilizer make livestock manure an attractive alternative to synthetic fertilizers, especially for soils that have been depleted in organic matter (Schröder, 2005). However, defining a market value for manure is challenging as the composition and availability of nutrients varies substantially among manure types. The price for manure in the current study was solely based on the average price per kg N from urea fertilizer ($1.33 kg/N; Alberta Agriculture and Rural Development, 2013). The use of hedonic pricing which considers the value of other nutrients (e.g., P and K) may generate a more accurate value, but in practice manure is applied primarily on the basis of its N content. In the short-term, manure N is generally less available to plants as compared to synthetic N fertilizer. However, based on Schröder (2005) we assumed that long-term application of manure N using best management practices (i.e., rapid incorporation, no application during winter or spring) was just as effective as the use of synthetic N as a fertilizer.

Even though high-fat CDDGS reduces enteric CH_4 emissions, feeding CDDGS or WDDGS at 40% of dietary DM increased both the total (t CO_2e) and intensity (kg CO_2e/kg beef) of GHG emissions as compared to the baseline scenario. This increase mainly arose due to higher emission of N_2O from manure owing to the increased excretion of N by cattle fed CDDGS (~30% CP) and WDDGS (~40% CP) diets. Supplementing feedlot cattle with 40% CDDGS or WDDGS (DM basis) also led to manure N production in

excess of the N requirements of crops grown on the model farm. Consequently, management strategies that minimize the loss of manure N and other crop nutrients such as P need to be employed. Using DDGS as source of feed energy instead of protein will not only result in higher GHG emission from the beef life-cycle but, also decrease GHG mitigation benefits of the extended grain ethanol life-cycle. Emissions associated with the feeding of the co-products that arise from ethanol production should be considered in assessing the impact of ethanol on GHG emissions relative to fossil fuels.

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CHAPTER 6-Gerneral Discussion and Conclusion

6.1. Main Findings and Integration of Results

This thesis project, designed to determine the impact of DDGS on GHG emissions from beef cattle, consisted of an *in vitro* experiment, two animal studies using growing and finishing beef cattle and a LCA modeling study.

The first experiment (Chapter 2) compared CH_4 production from increasing levels of CDDGS and WDDGS using *in vitro* batch culture technique. Wheat DDGS or CDDGS partially or completely replaced whole crop barley silage at 20, 40, 60, 80 and 100% substrate DM. The study demonstrated that cumulative CH_4 production (mg CH_4/g DM; mg CH_4/g DMD) was lower for CDDGS than WDDGS at inclusion levels of up to 80% DM. The observed reduction in CH_4 was caused by greater reduction in IVDMD/unit CDDGS compared to WDDGS, as well as higher concentrations of propionate upon incubation of up to 60% DM CDDGS. It was concluded that the higher fat content in CDDGS (11.5% fat) compared to WDDGS (4.9% fat, both DM basis) was likely responsible for the reduction in CH_4.

Lower CH_4 production (g/kg DMI) of CDDGS compared to WDDGS was also detected *in vivo*; as inclusion of 40% DM CDDGS in the growing (Chapter 3) and finishing experiment (Chapter 4) reduced CH_4 emission compared to WDDGS. Besides that, *in vitro* and *in vivo* results are not in particularly close agreement. While *in vitro* CH_4 production (mg CH_4/g DM) increased as the concentration of CDDGS in the incubated substrate increased

from 20 to 80% DM, *in vivo* CH_4 production (g/kg DMI) in the growing experiment (forage:concentrate ratio 55:45) was 31.3% higher (average over all diets) compared to the finishing study (forage:concentrate ratio 8:92). This demonstrates that the usefulness of *in vitro* techniques to predict CH_4 production *in vivo* is limited. Specifically, batch culture techniques may be suitable to screen for CH_4 mitigation agents but do not sufficiently reflect *in vivo* rumen fermentation, digestibility, animal performance, animal health, feed intake or adaptation (Flachowsky and Lebzien, 2009). Consequently, it is difficult to extrapolate from *in vitro* to *in vivo* experiments, or field conditions.

The second and third experiments (Chapter 3 and Chapter 4) determined if inclusion of 40% DM CDDGS (~10% fat) or WDDGS (~5% fat; both DM basis) in growing or finishing diets reduced enteric CH_4 emissions from beef cattle, and if the oil in corn was responsible for observed responses. In addition, both studies examined the effects of CDDGS or WDDGS on N excretion. Inclusion of 40% DM CDDGS led to a reduction in CH_4 (g/kg DMI) compared to the control and WDDGS diets in both experiments. In contrast, inclusion of 40% WDDGS with corn oil to generate a fat content similar to CDDGS, reduced CH_4 (g/kg DMI) to the same extent as CDDGS. This finding clearly confirms that the high fat content in CDDGS was responsible for the reduction in CH_4; as hypothesized earlier by McGinn *et al.* (2009). Replacing 35% barley grain by CDDGS (12.7% fat; all DM based) in a barley silage based growing diet reduced CH_4 emission from beef cattle from 23.8 to 19.9 g/kg DMI. Similarly, Moate *et al.* (2011) reported a reduction from 25.0 to 23.7 g CH_4/kg DMI in dairy cows

fed diets containing 26% of the DM as brewers' grains (11.0% fat DM). More recently, Benchaar *et al.* (2013) replaced a mixture of flaked corn and soybean meal with 0, 10, 20, or 30% CDDGS (16.3% fat; all DM basis) in a diet for lactating dairy cows and reported a linear reduction in CH_4 from 20.6 to 18.9 g/kg DM intake. Results of all three studies support our findings.

Unsurprisingly, total N excretion (g/d) in the growing and finishing experiment followed the N content of the diets, with WDDGS and WDDGS+oil, resulting in the greatest total N excretion, followed by CDDGS and the control diet, respectively. As discussed in Chapter 1, manure N can be rapidly transformed into NH_3 and contributes to eutrophication, formation of particulate matter and indirect N_2O emission (U.S. EPA, 2009). Feedlot operations are a particularly significant source of NH_3 as beef cattle utilize N less efficient than other livestock species (Calsamiglia *et al.*, 2010). In our case, heifers in the growing and finishing experiment excreted between 81.7 to 86.6%, and 72.8 to 81.3% of their daily N intake, respectively. In addition, environmental conditions in open dry feedlots, such as infrequent feces removal, manure deposition over a large surface area, and direct environmental exposure of the excreta promote high rates of N volatilization (Hristov *et al.*, 2011). Thus, increases in N excretion in response to feeding CDDGS or WDDGS could outweigh any reduction in enteric CH_4 through increased formation of N_2O.

To account for all GHG throughout the beef production cycle, the fourth study (Chapter 5) evaluated the impact of CDDGS and WDDGS inclusion on GHG emissions from beef cattle using a LCA approach. To improve the

accuracy of our simulation, primary data for diet composition, enteric CH_4 production (% of GEI), and N excretion (% of N intake) from the growing (Chapter 3) and finishing (Chapter 4) experiments were implemented in the LCA. A representative model farm which included 40% DM CDDGS or WDDGS in growing and finishing feedlot diets was simulated using the Holos GHG model. Our simulation was made relative to the standard practice of using barley grain as the main supplemental energy source in western Canadian beef cattle diets (baseline scenario). Greenhouse gas intensity of the baseline scenario (23.5 kg CO_2e/kg carcass) was 8.3% higher compared to an earlier estimate for western Canadian beef from our lab (Beauchemin *et al.*, 2010). This difference compared to Beauchemin *et al.* (2010) was due to the use of primary data for CH_4 and N excretion, as most other model inputs used in the baseline in this LCA were identical to Beauchemin *et al.* (2010). In addition, Beauchemin *et al.* (2010) used lower GWP for CH_4 (23 vs. 25) and N_2O (296 vs. 298) compared to the revised GWP (IPCC, 2007), which are used in the updated version of Holos. Higher manure related N_2O emission, together with increased off farm CO_2 emission from the production of DDGS resulted in 6.3 and 9.3% higher GHG intensity (kg CO_2e/kg beef carcass) of CDDGS and WDDGS compared to the baseline scenario. Although CDDGS reduced CH_4 emission in both *in vivo* trials, replacing 40% DM barley grain with CDDGS or WDDGS resulted in higher GHG emission on a life-cycle basis. While previous studies only assessed the effect of DDGS inclusion on enteric CH_4 production (McGinn *et al.*, 2009; Moate *et al.*, 2011) or N excretion (Walter *et al.*, 2012), this series of

experiments is the first combined assessment of the impact of dietary CDDG and WDDG inclusion on CH_4 emissions, N excretion, as well as GHG emissions from a beef life-cycle perspective. In addition, primary CH_4 emission and N excretion data was used in the LCA study. Thus, my study makes an effort to overcome one of the identified limitations of most agricultural LCAs in that normative values may not reflect variation in GHG emissions (De Vries, and de Boer, 2010).

6.2. Future Research

As discussed in Chapter 1, the number of dry-grind ethanol plants that use improved oil extraction methods and produce low-fat CDDGS with a fat content between 3.5 to 9.0% DM increased substantially over the last years (Mjoun *et al.*, 2009; U.S. Grains Council, 2012). As the fat content of DDGS was identified as main factor responsible for the observed reduction in enteric CH_4 emissions, future research should focus on the environmental consequences of this relatively new DDGS processing method. With the fat being partially extracted, the nutritional profile of low-fat CDDGS is closer to that of WDDGS, which would not only reduce its potential to mitigate CH_4 but also possibly increase N excretion.

Due to the fact that our study focused mainly on usage of CDDGS and WDDGS as source of feed energy, future research should also assess the impact of lower DDGS inclusion level of <20% DM on GHG emission from beef cattle. This should ideally include *in vivo* measurements of CH_4 as well as N excretion,

as both parameters will be affected by the lower level of fat and N in the diet. Based on two meta analyses (Beauchemin *et al.*, 2008; Grainger and Beauchemin, 2011), and results from the growing and finishing experiment, it can be expected that each 1% decrease in dietary fat content will increase CH_4 emission (g/kg DM intake) by 5.1 to 6.4%. With respect to N excretion, reducing the CDDGS or WDDGS inclusion level in a barley-based finishing diet, from 40 to 20% DM lowered total N losses (g/d) of beef heifers by 16.3 and 23.2%, respectively (Walter *et al.*, 2012). However, as discussed in Chapter 1, the chemical composition of CDDGS is variable. Consequently, the fat and N content of diets containing DDGS, and in response CH_4 emission and N excretion, will not only depend on the DDGS inclusion level but also be impacted by varying chemical composition of DDGS.

Similarly to lower DDGS inclusion levels, the long-term effect of DDGS supplementation on CH_4 emission from cattle should be assessed. Period lengths of 21 d in the growing and 28 d in the fishing study allowed us to test three different DDGS-containing diets in a relatively short period of time. However, multiple CH_4 measurement periods over the length of an entire growing (~100d) or finishing phase (~150d) are needed to confirm if CDDGS supplementation reduces CH_4 emissions throughout the feeding period, or if adaptation of the rumen environment occurs. As only study that measured CH_4 from cattle fed CDDGS over an extended period time, McGinn *et al.* (2009), who replaced 35% DM barley grain by CDDGS in a diet for growing beef cattle, reported that the reduction in CH_4 by 16.4% (g/kg DM intake) or 23.9% (% of GE intake) was

consistent over 15 wk. Adaption of rumen methanogens to various mitigation strategies has been identified as major limitation of nutritional CH_4 abatement strategies (Beauchemin *et al.*, 2008).

Besides the obvious need for more *in vivo* CH_4 and N excretion data from cattle fed DDGS, future research should also expand the GHG footprint of beef cattle fed DDGS towards a more holistic ecological or environmental footprint. This assessment would ideally capture the overall environmental consequences of feeding DDGS to beef cattle. As discussed in Chapter 5, feeding CDDGS or WDDGS does not only impact GHG emission from beef cattle but also, amongst other factors, the nutrient balance and land use of the whole model farm. Consequently, research should not only focus on GHG emission but assess for example the impact of surplus manure N and P on water quality and eutrophication. Any assessment of that nature is complex as it would have to integrate results from different environmental impact categories (e.g. GHG emission, water quality, land use efficiency) in a final index that would offer the possibility to compare different co-product feeding strategies (Van Zanten *et al.*, 2013).

6.3. Conclusion and Industry Perspective

Exponential growth in grain-based ethanol production over the last 10 to 15 years has contributed to a demand-driven increase in grain prices and price volatility (FAO, 2012). Conversely, increased availability of ethanol co-products, which are largely marketed in the form of DDGS, offers livestock

producers the opportunity to replace more expensive feed grains during high-price situations. This is particularly true for feedlot producers, as beef cattle can utilize DDGS at comparatively high dietary inclusion level (Klopfenstein *et al.*, 2008). However, the paradigm shift to use DDGS, which was initially perceived as protein supplement, as a source of feed energy impacts net GHG emission from the feedlot production cycle.

As discussed, feeding CDDGS at a dietary inclusion level of 40% DM is, due to its high level of fat, an effective measure to reduce enteric CH_4 emission from beef cattle. However, feeding CDDGS and WDDGS at 40% dietary DM also dramatically increases N excretion. Manure related losses of indirect N_2O, together with off-farm CO_2 emission from the production of DDGS, offset the reduction in CH_4 that was observed for CDDGS, and further increased GHG emission from feeding WDDGS. In consequence, feeding CDDGS or WDDGS at high dietary inclusion level is not a GHG mitigation strategy and should, based on our results, not be recognized as eligible for carbon offsets under Alberta's Agricultural Carbon Offset Trading System.

Measures to mitigate GHG emission from feedlots that include DDGS in their diets should target a reduction in CO_2 emission from the production of DDGS, as well as a reduction of N_2O emission resulting from increased N excretion. To reduce CO_2 emission from the production of distillers' grains producers should try to source WDGS (~35% DM), or partially dried "modified" distillers' grains plus solubles (~45% DM) instead of DDGS. This would spare the GHG emissions and energy associated with the drying of the co-product.

However, higher transport costs and a faster rate of spoilage restrict the use of low DM DGS to within close proximity of the ethanol plant. In addition to direct GHG savings from not drying distillers' grains, further GHG savings (g CO_2e/kg beef) could arise from a positive response in growth performance, as WDGS has typically greater feeding value than DDGS (Klopfenstein *et al.*, 2008).

In order to reduce N_2O emission producers should try to minimize all losses of N to the environment. As discussed, the CP content of the CDDGS (up to 19.6% DM CP) and particularly WDDGS diets (up to 23.5 % DM CP) in both animal experiments exceeded the N requirement of growing and fishing beef cattle. Reducing the DDGS inclusion level in the diet would therefore be the most obvious and effective dietary change to decrease N losses. However, the dietary inclusion level of DDGS is governed by feed ingredient availability and price (i.e., the relative prices of barley and corn grain). Therefore, it is unlikely that cattle producers will limit the DDGS inclusion level solely for climate protection with no financial incentive.

Practical manure management strategies to reduce immediate NH_3 losses from feedlots include improved pen drainage and increased frequency of pen cleaning (Hristov *et al.*, 2011). In addition, manure acidification and application of nitrification or urease inhibitors could help to reduce NH_3 emissions from feedlots (Varel *et al.*, 1999; Shi *et al.*, 2001). Unfortunately, the expense of these NH_3 mitigation measures limits their implementation in commercial beef production (Hristov *et al.*, 2011).

As shown by the LCA study (Chapter 5), over-feeding N by inclusion of high-levels of DDGS in beef cattle diets has implications beyond the feedlot and impacted the N balance of the cropping part of the life-cycle. Consequently, beef producers who substitute DDGS for cereal grains need to be aware of increased N concentration in the manure and develop nutrient management programs that minimize nutrient loss to the environment. First and foremost manure application need to be matched to crop N and P requirements. Besides the negative effects on GHG emission through formation of N_2O, soil accumulation of N and P from excessive manure application can lead to runoff and potentially N and P contamination of surface and ground water. To avoid N and P accumulation on agricultural land and water bodies close to the feedlot site, manure from cattle fed high-DDGS diets might have to be transported farther away and be applied to land that is P or N or deficient. Improved nutrient management will help to minimize GHG emissions from feedlots while increasing the fertilizer value of manure from cattle fed DDGS.

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