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AN EXAMINATION OF THE EFFECTS OF USING GLYCEROL AND WHEAT DRY DISTILLERS' GRAINS WITH SOLUBLES IN SHEEP DIETS

Jorge Avila Stagno

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

2014

Declaration

Apart from the assistance stated in the acknowledgements, or where reference is made in the text, this thesis represents original work of the author. I certify that the work presented in this thesis has not been submitted for any other degree or qualification at any other university.

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Abstract

Five studies were conducted to evaluate the effects of using two biofuel co-products in sheep diets. The objectives of this research were to determine the effects of feeding wheat based dry distillers' grains with solubles (WDDGS) and glycerol on lamb performance, carcass characteristics and fatty acid composition of adipose tissue of lambs. The effects of glycerol on diet digestibility and methane (CH_4) emissions were evaluated *in vitro* and *in vivo*. Effects of WDDGS on rumen degradation kinetics of the diets were also assessed.

In study 1 (Chapter 3), increasing levels of glycerol were used to replace barley grain in *in vitro* batch cultures. Culture pH and total CH_4 production (as mg or as mg CH_4/g digested DM) did not differ among treatments. *In vitro* DM disappearance (IVDMD) was linearly increased with glycerol addition. Results suggested that replacing barley grain with glycerol increases IVDMD and propionate proportions of total VFA but did not reduce *in vitro* CH_4 production.

Given the propiogenic properties of glycerol in batch culture, in study 2 (Chapter 4), a semi continuous fermentation system (Rumen Simulation Technique: RUSITEC) was used to verify whether glycerol added in forage diets has the potential to reduce CH_4 production. The fermenters were fed a brome hay and corn silage diet with increasing concentrations of glycerol being substituted for corn silage. Glycerol increased total VFA production, but production of acetate was unaffected and those of propionate and butyrate were linearly increased. Glycerol linearly increased dry matter (DM) disappearance from hay and silage and neutral detergent fibre (NDF) and acid detergent fibre (ADF) disappearance

from hay were increased at glycerol concentrations of 150 g/kg. Total gas production was not affected, but CH_4 concentration was linearly increased by glycerol, resulting in an increase in mg CH_4 /g DM incubated. Our hypothesis was rejected as increasing concentrations of glycerol in a forage diet linearly increased CH_4 production despite increases in propionate concentrations. This apparent discrepancy was explained by the more reduced state of glycerol as compared to carbohydrates which implies there is no net incorporation of electrons into glycerol when it is metabolised to propionate.

In study 3 (Chapter 5), two experiments were conducted to evaluate the effects of increasing concentrations of glycerol in concentrate diets on total tract digestibility, CH_4 emissions, growth, fatty acid profiles and carcass traits of lambs. In both experiments, the control diet was based on barley grain (570 g/kg barley grain) and treatment diets contained increasing concentrations (70, 140, and 210 g/kg dietary DM) of glycerol were added to the diet as a replacement for barley grain. In the first experiment, nutrient digestibility and CH_4 emissions from 12 ram lambs were measured in a replicated 4 × 4 Latin square design. Neither diet digestibility nor CH_4 were altered by inclusion of glycerol in the diet. In the second experiment, lamb performance was evaluated in 60 weaned lambs that were blocked by weight and randomly assigned to one of the 4 dietary treatments and fed to slaughter weight. Increasing glycerol in the diet decreased dry matter intake (DMI) and tended to reduce average daily gain (ADG), resulting in a linear decrease in final body weight. Feed efficiency, carcass traits and total saturated or total monounsaturated fatty acid proportions of subcutaneous fat were not affected by inclusion of glycerol, but polyunsaturated fatty acids (PUFA) were linearly

decreased. Proportions of 16:0 (palmitic), *t*10-18:1 (*trans*-10 octadecenoic), linoleic acid (18:2 n-6) and the n-6/n-3 ratio were linearly reduced and those of 18:0 (stearic acid), *c*9-18:1 (oleic acid), linearly increased by glycerol. Results suggested that when included up to 210 g/kg of diet DM, glycerol did not affect nutrient digestibility or CH_4 emissions of lambs fed barley based finishing diets, but improved fatty acid profiles by increasing 18:0 and *c*9-18:1 and reducing *t*10-18:1 and the n-6/n-3 ratio.

Study 4 (Chapter 6) aimed to assess the effects of replacing barley grain with increasing concentrations of wheat dry distillers' grains with solubles (WDDGS) on the growth performance and fatty acid profile of adipose tissue in lambs. The barley based control diet (629 g/kg barley grain) was modified by increasing concentration (200 and 400 g/kg DM) of WDDGS as replacement of barley grain. Thirty nine weaned crossbred Dorper/Merino lambs were used in a growth experiment to determine the effect of WDDGS on growth performance, feeding behaviour, and fatty acid profile of subcutaneous tail fat. The lambs were randomly assigned to one of the three dietary treatments (Control, 200, and 400 g/kg WDDGS). Animals were fed *ad libitum* using three automatic feeders per treatment and slaughtered at a body weight > 44 kg. Increasing WDDGS in the diet did not affect eating time (min/d), but quadratically affected eating rate (g/min), total daily intake and ADG. Inclusion of WDDGS did not influence feed efficiency, but final body weights were increased in the 400 g/kg group. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA in subcutaneous fat were unaltered, but proportions of linolenic acid (C18:3 n-3) were quadratically increased. This study suggested that WDDGS fed at 400 g/kg

DM improved lamb intake and growth performance, and increased linolenic acid in backfat tissue without reducing the n-6/ n-3 ratio as compared to a barley-based diet.

Study 5 (Chapter 7) aimed to assess the effects of increasing concentrations of WDDGS as replacement of soybean meal, in iso-nitrogenous diets on *in vitro* fermentation, *in sacco* degradation, growth performance and fatty acid profile of adipose tissue in lambs. The intake of crude protein (CP) was approximately twice that required for growing lambs described in NRC (2006). In the *in vitro* incubations, CH_4 production expressed as mg/g dry matter digested (DMD) was increased in the 470 g/kg WDDGS group as compared to the control and 200 g/kg groups. In the *in sacco* study, WDDGS increased DM, CP and NDF effective degradability. In the growth trial, increasing WDDGS in the diet linearly reduced DMI, ADG and hot carcass weight. Increase in *t*-10 octadecenoic acid and decrease in oleic acid resulted in reduction of total MUFA, whereas linoleic acid was linearly increased. These findings indicate that increasing concentrations of WDDGS in iso-nitrogenous diets fed to lambs decreased animal performance and impaired the fatty acid profiles of adipose tissue.

The main findings of this thesis indicate that replacing barley grain with glycerol does not affect lamb performance at moderate concentrations in the diet (70-140 g/kg DM) and improves adipose tissue fatty composition. Glycerol fermentation in the rumen increases propionate concentrations; however, the fermentation pathways involved do not act as a hydrogen sink. Lamb intake and performance can be improved by replacing barley grain with high WDDGS concentrations. However, when the equally high concentrations of CP in the diet were met with

other sources of CP such as soybean meal, increasing concentrations of WDDGS reduced lamb performance.

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List of publications and presentations

Several publications and conference presentations have been made based on the work presented in this thesis. They are listed here for reference. A statement of my original contribution to these papers is included in Appendix 1

Peer-reviewed publications

- Avila, JS, Chaves, AV, Hernandez, LM, Beauchemin, KA, McGinn, SM, Wang, Y, Harstad, OM, McAllister, TA (2011) Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on *in vitro* fermentation and methane production. *Animal Feed Science and Technology*, 166-167**,** 265–268.

- Avila-Stagno, J, Chaves AV, Ribeiro GO, Ungerfeld EM & McAllister TA Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique (RUSITEC) system. *British Journal of Nutrition* (In Press)

- Avila-Stagno J, Chaves AV, He ML, Harstad OM, Beauchemin KA, McGinn SM, and McAllister TA. (2013) Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles and carcass traits of lambs. *Journal of Animal Science*, 91, 829–837.

- Avila-Stagno J, Chaves AV, Graham A, & McAllister TA (2013) Effects of replacing barley grain with wheat dry distillers' grains on growth performance, eating behaviour and subcutaneous fatty acid profiles of lambs. *Acta Agriculturae Scandinavica Section A*. 63, 93-100

- Avila-Stagno J, Chaves AV, He ML, McAllister TA (*in press*) Increasing concentrations of wheat dry distillers' grains with solubles in iso-nitrogenous finishing diets reduce lamb performance. *Small Ruminant Research*. 114, 10-19

Conference presentations

- Avila, JS, Chaves, AV, Hernandez, LM, Beauchemin, KA, McGinn, SM, Harstad, OM, McAllister, TA (2010). "Effects of replacing barley in feedlot diets with increasing levels of glycerol on in vitro methane production". GGAA Greenhouse Gasses and Animal Agriculture. Banff, AB, Canada, October 2010.

- Avila-Stagno J, Meale SJ, McAllister TA, He ML, Harstad OM, Beauchemin KA, McGinn SM, Chaves AV (2012). Effect of replacing barley grain with glycerol on nutrient digestibility, methane emissions, growth, carcass traits and fatty acid profiles of finishing lambs. American Society of Animal Science ADSA - ASAS Joint Annual Meeting. Phoenix, AZ, USA. *Journal of Animal Science*, 90, Suppl. 3, 601

- Avila-Stagno, J, Meale, SJ, O`Hara, AS, Horadagoda, A, Palmer D, McAllister TA, Chaves AV (2012) Effect of replacing barley grain with wheat dry distillers grains with solubles on in situ degradation kinetics, growth, and fatty acid profiles of lambs. American Society of Animal Science ADSA - ASAS Joint Annual Meeting. Phoenix, AZ, USA. *Journal of Animal Science*, 90, Suppl. 3, 620

- Avila-Stagno J, Meale, SJ, McAllister, TA, He, ML, Harstad, OM, Beauchemin, KA, McGinn, SM, Chaves AV (2012) Effect of replacing barley grain with glycerol in feedlot diets on nutrient digestibility, methane emissions, growth, fatty acid profiles and carcass traits of lambs. Nordic Association of Agricultural Scientists, NJF Seminar 453, "Agriculture and greenhouse gases" Oslo, Norway, November 2012.

- Avila-Stagno, J, Chaves, AV, Ribeiro Junior, GO, Meale, SJ, Harstad, OM, McAllister, TA (2013) Supplementing glycerol in forage diets increases *in vitro* methane production using a rumen simulation technique (RUSITEC). GGAA Greenhouse Gases and Animal Agriculture. Dublin, Ireland, June 2013.

Table of contents

List of Tables

List of Abbreviations

ADG	average daily gain
ADF	acid detergent fibre
ADICP	acid detergent insoluble crude protein
AIC	Akaike's information criterion
BW	body weight
CDDGS	corn based dry distillers' grains with solubles
CH_4	methane
CLA	conjugated linoleic acid
CNCPS	Cornell net carbohydrate and protein system
CP	crude protein
DDGS	dry distillers' grains with solubles
DE	digestible energy
DM	dry matter
DMI	dry matter intake
DMD	dry matter disappeared, dry matter digested
ED	effective degradability
eNDF	effective NDF
ERDP	effective rumen degradable protein
FA	fatty acids
FAME	fatty acid methyl esters
GC	gas chromatography
GE	gross energy
IVDMD	*in vitro* dry matter disappearance

MJ	Mega Joule
MUFA	monounsaturated fatty acids
NDF	neutral detergent fibre
NDICP	neutral detergent insoluble crude protein
NEg	net energy for gain
NFC	non fibrous carbohydrates
NH_3-N	ammonia nitrogen
OM	organic matter
PD	potential degradation
peNDF	physically effective NDF
PUFA	polyunsaturated fatty acids
QDP	quickly degradable protein
RA	rumenic acid
RDP	rumen degradable protein
RUP	rumen undegradable protein
RNB	rumen nitrogen balance
SARA	sub-acute rumen acidosis
SDP	slowly degradable protein
SFA	saturated fatty acid
SRNS	small ruminant nutrition system
TCA	trichloroacetic acid
VA	vaccenic acid
VFA	volatile fatty acids
WDDGS	wheat based dry distillers' grains with solubles
WDGS	wet distillers' grain with solubles

Chapter 1. General Introduction

The biofuel industry has grown in the last decades sustained by a series of factors that have stimulated a constant increase in the use of liquid biofuels. Historically, biofuels were developed as a geopolitical strategy to reduce dependence on fossil fuels. However, the increased demand of agricultural commodities from the biofuel industry enhanced rural economic development and increased farm income, have impelled governmental policies to increase the use of biofuels. In recent years global concern on the effects of fossil fuels on climate change have further simulated the use of biofuels as carbon neutral sources of energy (Cooper &Weber, 2012). The two major liquid biofuels, bioethanol and biodiesel generate distillers' grains and glycerine (e.g.: glycerol plus impurities) as co–products, respectively. Ethanol produced from wheat is expected to reach 8.4 billion L which account for 6% of total ethanol produced in the world whereas biodiesel production is expected to reach 40 billion L by the year 2020 (OECD-FAO, 2011). As such, these industries will generate 7 million tonnes of wheat based dry distillers' grains with solubles (WDDGS; Solomon et al., 2007) and 3 million tonnes of glycerine worldwide (Balat et al., 2008).

Glycerol has been used with relative success in beef cattle, mainly as replacement of corn grain in moderate concentrations (Parsons et al., 2009; Mach et al., 2009). However, there is a paucity of information on the effects of its inclusion in sheep diets. The inclusion of glycerol in ruminant diets has been shown to shift fermentation in favour of propionate (Rémond et al., 1993; Wang et al., 2009; Lee et al., 2011). As fermentation leading to propionate competes with methanogenesis for hydrogen, diets promoting the formation of propionate have

been suggested as a strategy to mitigate enteric methane (CH_4) emissions (Beauchemin et al., 2008). Additionally, high starch finishing diets, particularly corn or barley based-diets, have been shown to modify rumen microbial populations in such a manner that alters the biohydrogenation process in the rumen and results in an increase of *trans*-10-octadecenoic acid (*t*10-18:1) (Aldai et al., 2010). This fatty acid has been linked with cardiovascular disease in humans (Hodgson et al., 1996). Replacing part of barley grain with WDDGS in beef cattle diets has resulted in reduced proportions of this fatty acid in favour of vaccenic acid (VA; *t*11-18:1), a health promoting fatty acid and a precursor of conjugated linoleic acid (Dugan et al., 2010). Whether *trans*-10-octadecenoic acid and VA can be modified in lambs by replacing barley with WDDGS or another source of energy like glycerol has not been verified.

Wheat based DDGS have also been tested in beef cattle as replacement of barley grain showing that it can improve performance in growing beef cattle (Beliveau & McKinnon., 2008; McKinnon & Walker., 2008). However, no effects (Beliveau & McKinnon., 2008) or reduced performance were observed in finishing cattle (Gibb et al., 2008). These last authors suggested that growing cattle may benefit from increased crude protein in diets with high concentrations of WDDGS. Whether lamb performance can be improved with high concentrations of WDDGS seems plausible due to their higher protein requirements as compared to finishing cattle. The effects of increasing concentrations of WDDGS in the diet on lamb performance and fatty acid profiles have not been evaluated in growing-finishing lambs.

This thesis describes a series of studies designed to examine the effects of using glycerol and wheat based DDGS in diets for sheep on animal performance, fatty acid profiles and ruminal fermentation. Chapter 2 presents an up-to-date literature review on the effects of inclusion of glycerol and WDDGS in diets for beef cattle and lamb. Chapter 3 of this thesis describes a study that evaluated the effects of including graded concentrations of glycerol as replacement of barley grain in concentrate diets on *in vitro* batch fermentation variables and methane production. Chapter 4 presents the effects of using glycerol in forage diets on rumen fermentation as tested in a semi continuous fermentation system. This experiment assessed fermentation variables, nutrient digestibility, and methane production. Chapter 5 presents the results of a study that evaluated the effects of using glycerol in diets for finishing lambs on *in vivo* methane production and nutrient digestibility, as well as lamb performance and fatty acid profiles of adipose tissue. Chapter 6 describes the results of an experiment conducted to verify the effects of increasing concentrations of WDDGS on lamb performance and adipose tissue fatty acid profiles. This study was conducted in an automated feeding system which also allowed evaluation of eating behaviour. The improved performance of lambs fed high concentrations of WDDGS in this last study lead to the experiment described in Chapter 7, which presents the results of a last study of this thesis which was designed to assess the effects of increasing concentrations of WDDGS in iso-nitrogenous diets for lambs. Lamb performance, fatty acid profiles as well as *in sacco* degradation of nutrients and *in vitro* fermentation and CH_4 production were evaluated in this trial. A general discussion of the implications of this work is presented in Chapter 8, together with suggestions for future research.

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Chapter 2. Effects of using glycerol and wheat dry distillers' grains with solubles in beef cattle and lamb diets. A review on rumen metabolism, growth performance, and fatty acid profiles

2.1 Introduction

The rising concern over cost, secure availability, pollution and the impact of fossil fuels on greenhouse gasses emissions and climate change have led to an increasing worldwide interest in obtaining fuels from renewable carbon sources. Although the first biofuels date back to the early 1900's when ethanol was first used as fuel for cars, interest was lost due to abundance of low cost gasoline. With the oil crisis of the 1970's, biofuels gained new interest and stimulated the development of a biofuel industry that has grown in the last decade due to constant increases in oil prices (Balat, 2008).

Two biofuels, ethanol and biodiesel account for the vast majority of current global biofuel production. They are primarily made from agricultural commodities such as grain or cane, as sources of starch and sugars to produce ethanol and vegetable oils to produce biodiesel. Biofuel production has grown continuously since 2000, supported by different governmental policies providing tax exemptions or requiring that petroleum refiners blend increasing volumes of renewable fuels like ethanol and biodiesel into gasoline (Cooper & Weber, 2012). If these policies do not undergo major changes, OECD forecasts that production of ethanol should exceed 140 billion L of which 6% (8.4 billion L) will be based on wheat as feedstock, whereas biodiesel could reach 40 billion L by year 2020 (OECD-FAO, 2011). The main co-products from these industries, dry distillers' grains with solubles (DDGS) from ethanol production and glycerine from biodiesel

production are produced at a rate of 800g of DDGS (Solomon et al., 2007) and 79 g of glycerine (Balat et al., 2008), per L of ethanol and biodiesel respectively. Consequently, by 2020, wheat based DDGS (WDDGS) and glycerine production is estimated to be in excess of 7 and 3 million tonnes per year, respectively. Glycerol (major component of glycerine) and WDDGS have been used as by-product feeds for beef and dairy cattle as well as in swine and poultry. However there is a paucity of information on the use of these co-products in finishing lambs. The main objective of this review is to provide an overview of the effects of these co-products from the biofuel industry when they are included in the diets of beef cattle and sheep. Specifically, their potential effects on the rumen environment, metabolism, methane production, growth performance and adipose tissue fatty acid profiles will be discussed.

2.2 Use of glycerol in diets for beef cattle and lamb

Glycerol is the main component of crude glycerine, the main co-product of the biodiesel industry. It has many applications in the chemical, food and pharmaceutical industries in which it generally accounts for a minor proportion of complex chemical mixtures. As such, if 2020 production projections are realised, there should be an important glycerol stream in the near future, generating an impetuous for new uses including second generation biofuels, industrial chemicals and livestock feed.

2.2.1 Glycerine composition and considerations for its use as feed.

Biodiesel is produced through direct acid-catalysed esterification, or more commonly, base-catalysed esterification of plant oils or animal fats with methanol (Drouillard, 2012). The more effective and most commonly used catalysts are alkali, including sodium or potassium hydroxide (Meher et al., 2006). During the process, fatty acids are hydrolysed from the glycerol backbone of triglycerides yielding biodiesel (fatty acid methyl esters) and glycerine. These liquids differ in density and separate into a lower glycerine layer and an upper biodiesel layer (Balat, 2008).

The trans esterification process requires 3 moles of methanol for each mole of triglyceride. However, excess methanol is commonly used to ensure a high yield of methyl esters and commonly contaminates crude glycerine (Shuchardt et al., 1998). This excess methanol and the catalyst salts used for the process are usually recovered from crude glycerine to be reutilized in the trans esterification process. The purification of glycerine varies among biodiesel plants, producing variable composition of the co-product (Shröder and Südekun, 1999). Glycerine composition has been reported to vary between 650 to 950 g/kg glycerol, 2 to 200 g/kg moisture, 0 to 60 g/kg ash, and 1 to 140 g/kg methanol in Australia (Hansen et al., 2009). In Europe, large biodiesel producers purify glycerine to recover methanol and methyl esters, yielding crude glycerine with at least 800 g/kg glycerol and impurities to a maximum of 5 g/kg methanol, 50 g/kg ash, mainly sodium or potassium salts from catalysts, 12 g/kg organic non-glycerine matter, mainly fatty acids methyl esters, and water (EFSA, 2010).

The main restriction in the use of glycerine as feed is its high methanol content. Maximum concentration of methanol in glycerine for its use as non-restricted feed is 2 g/kg in Europe (EFSA, 2010) and 1 g/kg in Canada (Braid, 2012). In the United States, FDA issued a regulatory letter indicating methanol levels above 150 ppm in the final feed mix could be considered unsafe for animal consumption (Elam et al., 2008). These concentrations are extremely low for ruminants considering that methanol is rapidly metabolized by the rumen flora to methane, carbon dioxide and water (EFSA, 2010). No adverse effects were reported after low purity glycerine, containing 26.7% methanol was included at 10% of diet DM for sheep and cattle (Shröder & Südekun, 1999), likely because a large portion of the methanol evaporated from the feed. Further research, to test acceptable limits of methanol in glycerine for its use as ruminant feed should be undertaken. Nevertheless, the composition of the glycerine stock to be used for animal feeding should be assessed and constantly monitored before including in animal diets.

2.2.2 Effects of glycerol on the rumen environment

When entering the rumen, an important fraction of glycerol can be directly absorbed through the rumen wall (Rémond et al., 1993), from where it is transported to the liver and converted to glucose. Alternatively, glycerol can be fermented to volatile fatty acids (VFA), where propionate, which is in turn converted to glucose, accounts for more than 40% of total VFA (Czerkawsky & Breckenridge, 1972; Lee et al., 2011). Due to its high solubility, a small fraction of glycerol is also evacuated to the lower gut flowing with the liquid fraction of rumen contents and can be absorbed in the small intestine from where it is

redirected to the liver (Rémond et al., 1993). When glycerol is administered as a drench, more is likely to be absorbed through the rumen wall whereas when mixed with the diet a greater fraction will be fermented by rumen microbes.

2.2.2.1 Changes in microbial populations and rumen fermentation

Dietary changes in ruminants require an adaptation period to facilitate the shift in ruminal microbial populations resulting in a progressive modification of the end products of fermentation. Glycerol has been reported to be metabolized by *Megasphaera elsdenii*, *Streptococcus bovis* and *Selenomonas ruminantium* species (Stewart et al, 1997). *Selenomonas spp*. usually converts succinate to propionate but are also capable of fermenting glycerol, with the main end products being propionate, lactate, succinate and acetate. *Megasphaera elsdenii*, usually utilises lactate, an end product of *S. bovis* and *Selenomonas spp.* to form propionate and butyrate (Shin et al., 2011). Thus, feeding glycerol is usually associated with an increase in the molar proportion of propionate and to a lesser extent butyrate, at the expense of acetate. This has been consistently reported when using glycerol in *in vitro* incubations with forage diets (Rémond et al., 1993; Lee et al., 2011), wheat starch (Bergner et al., 1995), and corn (Lee et al, 2011). Glycerol has also inhibited the growth of fibre degrading *Ruminococcus flavefasciens* and *Fibrobacter succinogenes* and impeded growth and cellulolytic activity of the fibre degrading fungus *Neocallimastix frontalis* when included at 5% DM in *in vitro* culture using cellobiose as the sole energy source (Roger et al., 1992). Populations of *Butirivibrio fibrisolvens*, an important fibre degrading bacteria, as well as *S. ruminantium, Clostridium protoclasticum* and total bacterial

DNA were linearly decreased by increasing concentrations of glycerol as a replacement of corn in an alfalfa based diet at concentrations of 72 and 108 g/kg DM (Abo el Nor et al., 2010). The aforementioned changes in microbial populations and shifts towards propionate may explain reductions in fibre digestion reported in several studies using glycerol. In *in vitro* batch culture, using glycerol reduced NDF digestion (Roger et al., 1992; Abo el Nor et al., 2010). This effect was also reported in *in vitro* batch cultures using inoculum from unadapted animals fed high concentrate diets (van Cleef et al., 2011). In *in vivo* conditions, when glycerol was used in finishing beef cattle diets as replacement of corn grain at concentrations of 0, 2 and 4 % of DM, NDF digestibility tended to decrease although no effects were observed on total tract nutrient digestibility (Parsons et al, 2009). Schneider (2010) reported a linear reduction in NDF digestibility when glycerol replaced corn gluten feed. However, when using low concentrations of glycerol in forage diets (0, 1.1, 2.2, and 3.3 g/kg DM) Wang et al. (2009) reported an increase of DM, CP, and NDF digestibility of diets fed to beef cattle. Improvements in fibre digestion were found when glycerol (150 g/kg DM) was used in low starch diets but not in high starch diets (Schröder & Südekun, 1999). As such, glycerol may affect microbial populations that are important contributors to fibre digestion in ruminants fed high starch diets but is of lesser importance in animals fed high forage diets (Drouillard, 2012).

2.2.3 Effects of glycerol on beef cattle and lamb performance

2.2.3.1 Dry matter intake

Glycerol has successfully replaced grains in ruminants when fed in conjunction with diets with low to moderate concentrations of forage (Wang et al., 2009) or concentrate diets (Parsons et al., 2009). However, at concentrations above 100 g/kg DM it can have deleterious effects on feed utilisation. When replacing rolled corn with glycerine, Parsons et al. (2009) reported linear reductions in dry matter intake (DMI) at concentrations of 40 to 160 g/kg with reductions of greater magnitude at concentrations of 120 and 160 g/kg. Likewise, DMI was reduced when glycerine replaced corn grain at concentrations of 150 g/kg (Musselman et al., 2008; Gunn et al., 2010b) in lamb diets. However, no effects were reported on DMI when glycerine replaced barley grain at concentrations of up to 120 g/kg DM in diets for finishing cattle (Mach et al., 2009) or Merino ewes (Meale et al., 2013). Reductions in DMI with concentrations of glycerol above 100 g/kg may be explained by the accumulation of fermentation metabolites such as lactate and propionate (Trabue et al., 2007) in the rumen, which reduce both ruminal pH and fibre digestion, leading to a reduction in DMI. Again, this reduction seems to be of greater magnitude in ruminants fed high starch diets. When glycerine has been included at concentrations of 150 g/kg in diets containing 600 g/kg DM or less grain, DMI by steers (Schröder & Südekun, 1999) and lambs (Gunn et al., 2010a; Musselman et al., 2008) was unaffected.

2.2.3.2 Weight gain and feed efficiency

Reductions in feed intake are usually associated with reductions in average daily gain (ADG). Reduced intakes in finishing lambs fed glycerine at concentrations of 0, 150, 300 and 450 g/kg DM as replacement of rolled corn resulted in linear decrease of about 39% in ADG between control and 450 g/kg groups (Musselman et al., 2008; Gunn et al., 2010b). The gain:feed ratio in these studies was reduced in the 150 and 450 g/kg groups as compared to the control group by 8 and 24% (Gunn et al., 2010b) and 25 and 50% (Musselman et al., 2008), respectively. When glycerine replaced rolled corn in finishing heifer diets at 0, 20, 40, 80, 120, and 160 g/kg, reductions of 12.2% in DMI resulted in reductions of 23.1% in gain:feed ratio for the 160 g/kg glycerol group as compared to the control group (Parsons et al., 2009). Likewise, quadratic responses in ADG with no change in DMI resulted in quadratic reductions in feed:gain ratio of finishing beef steers (Versemann et al., 2008). However, studies replacing barley grain with glycerol found no effects on ADG or gain:feed ratio of bulls (Mach et al., 2009) or Merino ewes (Meale et al., 2013). In these two last studies high starch grain accounted for 600 g/kg or less of total DM. When glycerol was included in starch rich concentrate diets it`s energy value was reduced by 15% as compared to when it was included in a low starch concentrate diet (Schröder & Südekun, 1999). This could partially explain the reductions in feed efficiency in the aforementioned studies where glycerol replaced the grain portion of the diet.

Studies reporting effects of glycerol on carcass and meat quality are scarce. Hot carcass weight and *Longissimus* muscle were quadratically affected with an optimum at 20 to 40 g/kg DM when graded levels of glycerol replaced rolled corn

a diet fed to beef heifers (Parsons et al., 2009), whereas marbling and subcutaneous fat depth were linearly reduced by increasing glycerol in the diet. However, an optimum marbling score was demonstrated when glycerol replaced corn grain at concentrations of 100 g/kg of total DM in beef steers diets (Versemann et al., 2008). In finishing lambs, feeding glycerol as a replacement for corn grain over 150 g/kg reduced dressing percentage, 12^{th} rib fat thickness, ether extract in *longissimus* muscle and yield grade (calculated as $0.4 + (10 \times 0.393 \times$ 12th-rib fat thickness, cm; Gunn et al., 2010b). However, no effect on carcass traits or meat quality of Holstein bulls was found when glycerol replaced barley grain at concentrations of up to 120 g/kg DM (Mach et al., 2009). The few studies available in the literature with different diet ingredients and experimental conditions seem to indicate that when used in high starch diets, glycerol concentrations exceeding 100-150 g/kg may jeopardize intake and growth performance. These effects seem to be accentuated in corn based diets (Versemann et al., 2008; Parsons et al., 2009; Gunn et al., 2010b) as compared to other energy concentrates like barley (Mach et al., 2009; Meale et al., 2013) or wheat (Schröder & Südekun, 1999). The use of dry distillers' grains with solubles (DDGS) has been suggested to attenuate the negative effects of glycerol on fibre digestion (Drouillard, 2012). This could be attributed to a reduction in starch concentration in the diet when DDGS are used as an energy source. Thus, future research on combinations of high-energy and fibrous ingredients in the diet and their impact on rumen metabolism and performance is needed.

2.2.4 Methane production

Animal agriculture is facing the challenge of feeding protein to a growing human population whilst meeting global concerns on environmental consequences and greenhouse gasses emissions (Meale et al., 2013). Methane (CH_4) has a global warming potential that is 25 times greater than CO_2 (IPCC, 2007) and ruminant production has raised increasing concern about its environmental impact, as 37 % of total anthropogenic CH_4 is produced by ruminant livestock (FAO, 2006). Additionally, CH_4 production has important consequences on productive efficiency as energy lost in the form of CH_4 ranges between 2 and 12 % of total gross energy intake (Johnson et al., 1993). The variation in CH_4 emissions are caused by the rate at which carbohydrates are fermented in the rumen or escape fermentation by passing to the lower gut and by the amount of hydrogen available to reduce CO_2 to produce CH_4 (Johnson & Johnson, 1995).

As previously stated, the inclusion of glycerol in ruminant diets shifts microbial populations and favours fermentation towards the formation of propionate. The metabolic pathways leading to propionate formation in the rumen compete with methanogenesis for hydrogen and feeding systems promoting the formation of propionate have been suggested as a means to reduce enteric methane emissions from ruminants (Boadi et al., 2004; Beauchemin et al., 2008; McAllister & Newbold, 2008). However, the effects of adding glycerol in ruminant diets on methane production are less than clear. In *in vitro* conditions, including glycerol with alfalfa and corn grain substrates resulted in reduced methane production and increased lag phases (Lee et al., 2011). However, these results should be interpreted with caution as they were obtained using inoculum from animals that

were not adapted to glycerol, which has been reported to reduce total gas and methane production as compared to control diets in batch culture using inoculum from glycerol-adapted animals (van Cleef et al., 2011). This lower gas and methane production is avoided when inoculum comes from glycerol-adapted animals suggesting that microbial adaptation plays a key role in determining the ratio of fermentation gases from glycerol. As such, reductions in gas and CH_4 production with unadapted inoculum may reflect an overall reduction in fermentation as opposed to specific inhibition of the ability of methanogens to produce CH_4. In addition to propionate, acetate and butyrate are also produced during the fermentation of glycerol (Czerkawski & Breckenridge, 1972; Zhang & Yang, 2009). The fermentation pathways from glycerol to acetate and butyrate result in net release of 3 and 4 pairs of electrons per mole respectively, whereas fermentation of glycerol to propionate results in no net electron recovery given the more reduced state of glycerol as compared to glucose (Zhang & Yang, 2009).

Results from earlier research, indicate that in *in vivo* conditions, an important fraction of glycerol may avoid fermentation in the rumen by being absorbed directly through the rumen wall or by flowing with the liquid phase of rumen contents to the lower tract (Rémond et al., 1993). Whether the use of glycerol with forage diets may reduce CH_4 production as a result of absorption through the rumen wall in *in vivo* conditions warrants further research.

2.2.5 Fatty acids profiles

Ruminant meats, have generally been considered rich in saturated fatty acids (SFA) and deficient in unsaturated fatty acids (UFA). The saturated fatty acids are

reported to be associated with an increased risk of cardiovascular disease and elevated plasma cholesterol (Lourenço et al., 2010). This saturated nature of fatty acids (FA) is a reflection of the extensive transformation of the polyunsaturated fatty acids (PUFA) ingested by ruminants via biohydrogenation (Harfoot and Hazlewood, 1997; Lourenço et al., 2010). The result of this process is the transformation of PUFA, mostly linoleic (18:2 n-6) and linolenic (18:3n-3) acids to saturated stearic acid (18:0), as a strategy of the rumen bacterial populations to alleviate the toxicity of PUFA (Dugan et al., 2011). However, under some dietary regimes, a fraction of these PUFA or their intermediates may escape complete biohydrogenation and reach the tissues (Kramer et al., 2004). The major intermediates of this process under normal rumen conditions include vaccenic acid (VA; *t*11-18:1) and rumenic acid (RA; *c*9-*t*11-18:2), the most abundant of a group of 28 isomers that, due to the cis-trans configuration of their double bonds, have been described as conjugated linoleic acids (CLA). Rumenic acid has been shown to play a role in cancer prevention, decrease atherosclerosis, improve immune response and modulate fat and protein metabolism (Palmquist et al., 2005; Lourenço et al., 2010). However, the proportion of CLA in meats is frequently less than 1% of total fatty acids (Dugan et al., 2011). Griinari et al. (2000) demonstrated that the majority of RA in tissues originates from VA which is absorbed from the rumen and later desaturated in the tissues by Δ9-desaturase. As such, recent research has been oriented to manipulate rumen fermentation in a manner that stimulates the production of VA and prevent the total saturation of PUFA to stearic acid (Dugan et al., 2011).

In North America, beef and lamb are conventionally finished with diets containing high proportions of starch (barley or corn) and low fibre which lead to a rumen environment that favours the growth *Megasphaera elsdenii*, a bacterium that produces the *t*10 double bond configuration as opposed to *Butyrivibrio fibrisolvens* which produces the more desirable *t*11 double bond profile (Kramer et al., 2004). As a result, proportions of VA can be reduced in favour of *trans-10-octadecenoic acid*, which can reach concentrations above 10% of total FA in back fat (Aldai et al., 2010). This FA has been reported to be the major 18:1 *trans* fatty acid in beef cattle fed barley-based diets (Dugan et al., 2007; Aldai et al., 2008) and has also been detected in lambs fed barley based diets (McKeown et al., 2010a). The link between *trans*-10-octadecenoic acid and cardiovascular disease in humans (Hodgson et al., 1996) has stimulated efforts to reduce its concentrations in meats while increasing VA. Replacing dietary grain with forage modifies the *trans* fatty acids profiles in this favourable direction in beef (Aldai et al., 2011). Wheat DDGS are high in fibre and have been used successfully as replacement of grain in finishing diets for beef cattle (Gibb et al., 2008; Walter et al., 2010) and lambs (McKeown., 2010a). The ethanol production process extracts starch and proportionally increases the fibrous and protein fractions of the grain, thus, this dietary practice has reduced *trans*-10-octadecenoic acid and increased VA in beef cattle (Dugan et al., 2010) but not in lambs (McKeown et al., 2010a). When using glycerol as replacement of barley grain, a fraction of starch is removed from the diet. If fibre concentrations are maintained to secure rumen functionality, 18:1 fatty acid profiles may be improved. Terré et al. (2010) found no effects on fatty acid profiles of lambs when glycerol was used in a 4 week

growth trial using young lambs. However, changes in fatty acid composition of cattle have been shown to be significant after at least 6 weeks of feeding flax seed (He et al., 2012). As such, it is probable that the 4 week long study of Terre et al. (2010) may have not been long enough to detect differences in adipose tissue composition.

2.3 Use of wheat dry distillers' grains with solubles in ruminant diets.

Dry distillers' grains with solubles as a by-product from ethanol production have become a readily available source of energy and protein for ruminants and their use has been reported since 1900 (Klopfenstein et al., 2008). Corn DDGS (CDDGS) are the most common source of distillers' grains and their nutritional value for beef cattle (Klopfenstein et al., 2008) and dairy cattle (Schingoethe et al., 2009) has been well documented. However, in Western Canada and Europe, wheat is commonly used as feed stock for ethanol production due to the fact that corn is not easily grown in Northern climates (Boila & Ingalls., 1994). The quality of the co-product generated varies widely between different processing plants and even between batches within a plant.

2.3.1 Wheat DDGS composition and considerations for its use as feed

In the dry milling process, the grain is ground and subsequently starch is converted to ethanol and CO_2 by fermentation. As starch is approximately two thirds of the whole grain, the remaining nutrients are concentrated three-fold compared to the original feedstock (Erickson et al., 2012). After fermentation,

stillage is separated in distillers' grains and distillers' solubles which represent approximately 800 and 200 g/kg WDDGS on DM basis, respectively (Figure 1). Distillers' solubles are commonly added back to the distillers' grain as they are good sources of protein and fat but also include Phosphorus and Sulfur (Corrigan et al., 2007). The final wet distillers' grains with solubles (WDGS; 300-350 g/kg DM) are frequently dried to obtain dry distillers' grains with solubles (DDGS; 880-920 g/kg DM). Wet distillers grains are usually transported only short distances to nearby livestock operations whereas DDGS are the form of choice when markets are far from the ethanol plant.

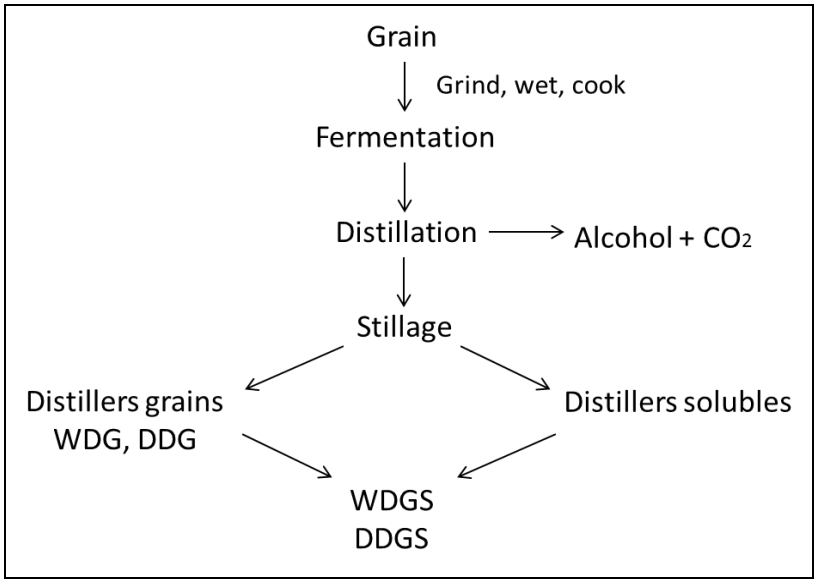

Figure 1. Dry milling ethanol process scheme. Adapted from Erickson et al. (2012). WDG, wet distillers' grains; DDG, dry distillers' grains; WDGS, wet distillers' grains with solubles; DDGS, Dry distillers grains with solubles.

2.3.1.1 Wheat DDGS as source of protein

Differences in the original feed stock, milling and drying process cause variation in the nutritional composition of WDDGS. Crude protein concentration of corn DDGS usually range between 281 and 339 g/kg DM (Klopfenstein et al., 2008). Crude protein concentrations of wheat based DDGS are greater than those of corn based DDGS, ranging widely (338-445 g/kg) with an average of 389 ± 24 g/kg; (University of Saskatchewan, 2010). The use of heat during pre-fermentation cooking of the grain and drying affects the solubility and rumen degradability of CP in the original grain (Boila & Ingalls, 1994). As such, wheat DDGS are considered to be a source of rumen degradable protein (RDP) and rumen undegradable protein (RUP). Important changes in CP fractions in WDDGS as compared with the original feed stock were reported by Nuez-Ortin & Yu, (2009). These authors found a 2.8 fold increase in total CP from 142.8 in wheat to 393.2 g/kg DM in WDDGS. However, soluble CP was reduced from 245.6 to 162.9 g/kg CP, neutral detergent insoluble CP (NDICP) was increased 4 fold from 135.1 to 560.4 g/kg CP and acid detergent insoluble CP (ADICP) was increased from 0 to 48.6 g/kg CP. The NDICP is considered to be a slowly digestible protein in the Cornell Net Carbohydrate and Protein System (CNCPS) representing heat damage and formation of Maillard products in DDGS (Li et al., 2012) whereas ADICP is considered to reflect indigestible protein (Sniffen et al., 1992). Nevertheless, increasing concentrations of WDDGS in diets for feedlot heifers (0, 200 and 400 g/kg), increased concentrations of NDICP and ADICP in the diet without affecting total tract digestibility of CP (Walter et al., 2012). These last authors reported that total tract digestibility of dietary NDCIP and ADICP were linearly

increased in WDDGS containing diets. As such, the ADICP levels in WDDGS diets are probably not always associated with heat damage nor an indicator of unavailable CP (Machacek & Kononof., 2009). This is further confirmed by the results of *in sacco* incubations of wheat and WDDGS showing that *in situ* degradability of CP was reduced in WDDGS after 24 h, but not after 48 h of incubation (Nuez-Ortin & Yu, 2009).

When WDDGS replaced barley grain and barley silage at concentrations of 250 g/kg, increased ruminal effective degradability of CP was reported (Li et al., 2011). However, these authors reported lower concentrations of NDICP (130 vs 560 g NDICP/kg CP) as compared to those reported by Nuez-Ortin & Yu (2009). The variability in results suggests that rumen undegradability of CP may change due to differences in ethanol plant drying procedures (Spiehs et al., 2002; Li et al., 2012) and that evaluation of composition of each batch of WDDGS should be determined before diet formulation.

2.3.1.2 Wheat DDGS as source of fibre

Despite being a good source of protein, wheat based DDGS are used more frequently as an energy source in feedlot diets as replacement of cereal grain (Gibb et al., 2008; Beliveau & McKinnon, 2008; McKeown et al., 2010a). This feeding pattern was justified by the rising costs of grain and growing availability of WDDGS (Gibb et al., 2008). Subsequently, the partial removal of starch by replacing grain with WDDGS, which contains 310 to 541 g/kg NDF and 112 to 233 g/kg ADF (University of Saskatchewan, 2010), suggested that the replacement could benefit the rumen environment and prevent sub-acute ruminal

acidosis (SARA; Beliveau & McKinnon, 2009). However, the effectiveness of WDDGS in SARA prevention is questionable. The magnitude of SARA is evaluated according to the time that rumen pH is below 5.8 (mild), 5.6 (moderate) and 5.2 (severe) (Nocek, 1997; Beauchemin & Yang, 2005). Previous studies have reported that replacing barley grain with wheat DDGS (Beliveau & McKinnon, 2009) or corn DDGS and wheat DDGS (Walter et al., 2012) in beef cattle diets did not modulate rumen pH. Li et al. (2011) replaced barley grain (200 g/kg) and barley silage with wheat DDGS in finishing beef cattle diets. They reported that WDDGS did not improve ruminal pH despite removing starch from the diet and that it was not a suitable substitute for the effective fibre in barley silage.

The limited effects of fibre coming from DDGS on ruminal pH in the aforementioned trials (Beliveau & McKinnon., 2009; Walter et al., 2011; Li et al., 2011) has been attributed to its low physically effective fibre (peNDF), a concept that accounts for the physical properties of NDF that stimulate chewing activity and saliva secretion (Lammers et al., 1996; Beauchemin & Yang., 2005). Physically effective fibre is calculated as the proportion of feed particles in the ration that are retained on a 19 mm and 8 mm sieve in the Penn State Particle Separator (Lammers et al., 1996) and has to be differentiated from the effective fibre (eNDF) concept proposed by Mertens (1997) which utilizes a 1.18 mm sieve and considers the need to maintain a milk fat of 3.4% in dairy cows. The particle size of WDDGS varies, but values as low as 0.67 ± 0.002 mm have been reported (Beliveau & McKinnon., 2009). As such, despite being a high fibre feed,

WDDGS does not stimulate chewing activity and has low buffering capacity and thus, is not an effective fibre source.

Additionally, the rumen degradability of NDF from WDDGS is high and contributes significantly to VFA production in the rumen. Total VFA production was unaffected in cattle fed WDDGS as replacement for up to 210 g/kg DM of barley grain (Beliveau & McKinnon, 2009) or a mixture of barley grain (200 g/kg DM) and barley silage (150 g/kg DM) (Li et al., 2011). However, when barley grain was replaced with WDDGS at concentrations of 200 and 400 g/kg DM, a linear reduction in total VFA was reported (Walter et al., 2011).

When replacing barley grain with WDDGS, molar proportions of acetate in rumen fluid remained unaltered, but propionate was reduced in a linear (Beliveau & McKinnon, 2009) or quadratic fashion (Walter et al., 2011). This has been explained by the high fermentability of the NDF fraction in WDDGS and the removal of starch from the diet which has a negative correlation to propionate concentration.

2.3.1.3 Wheat DDGS as source of energy

The use of DDGS in diets for growing or finishing ruminants has increasingly focused on their use as energy rather than as a protein source. It is a commonly accepted concept that the energy value of DDGS is derived from its digestible fibre and fat content (Klopfenstein et al., 2008). Fat concentrations in corn DDGS are higher (102-165 g/kg DM; Spiehs et al., 2002; Nuez-Ortin & Yu., 2009) than in wheat DDGS (39 – 49 g/kg DM; Mustafa et al., 2000; Nuez-Ortin & Yu., 2009). As such, the energy value of wheat DDGS is projected to be lower than

corn DDGS. For corn based DDGS commonly used in corn grain diets, concentrations used up to 150 g/kg serve to meet the protein requirements of finishing beef cattle (Erickson et al., 2012). Gibb et al. (2008) suggested that protein requirements of growing feedlot beef cattle are met with WDDGS concentrations of 50 g/kg DM in barley based diets. Consequently, when DDGS are supplemented in greater concentrations, the animal utilises DDGS as a source of both protein and energy (Erickson et al., 2012). When excess protein from WDDGS is metabolised, urea must be synthesised from ammonia to excrete the resulting excess nitrogen in the diet. Urea synthesis represents a metabolic cost to the animal (Cannas et al., 2004) which may affect the feed efficiency of cattle fed high concentrations of WDDGS or CDDGS. Feeding increasing concentrations of WDDGS (0 – 600 g/kg) as replacement of barley grain in finishing beef cattle led to linear reductions in net energy for gain (NE_g) of mixed diets (4.81 to 4.48 MJ NE_g/kg DM) and WDDGS (5.69 MJ at 200 g/kg DM vs. 5.27 MJ at 600 g/kg DM; Gibb et al., 2008). However, these NE_g and animal performance were not affected when WDDGS was used in growing cattle at concentrations of 0 to 600 g/kg DM (Gibb et al., 2008) or 0 to 400 g/kg DM (McKinnon & Walker, 2008) or at lower concentrations in the diet (0 to 230 g/kg DM) of growing and finishing cattle (Beliveau & McKinnon, 2008). The reduced impairment of high levels of WDDGS on NE_g in growing as compared to finishing cattle is likely a reflection of their higher protein requirements.

2.3.2 Effects of wheat DDGS on growth performance

The use of WDDGS as replacement of grain and its consequences on growth performance has been documented in growing and fishing cattle with variable outcomes. Inclusion of WDDGS did not affect intake when it replaced barley grain at concentrations of 0 to 230 g/kg (Beliveau & McKinnon, 2008), 0 to 400 g/kg (McKinnon & Walker, 2008) or a mixture of barley grain and barley silage (Li et al., 2011) in finishing cattle. Likewise, no effects were reported when WDDGS replaced barley grain in finishing lamb diets at concentrations of 200 g/kg (McKeown et al., 2010a). However, increases in DMI were reported when WDDGS were used at concentrations of 0 to 600 g/kg DM (Gibb et al., 2008) and 0 to 400 g/kg DM (Walter et al., 2010) in beef cattle diets. This increase in DMI was explained as a compensatory response to a reduction in DM digestibility in cattle fed WDDGS (Gibb et al., 2008).

Effects of WDDGS on ADG and feed efficiency (FE) seem to be more important in growing cattle as compared to finishing cattle. Average daily gain was increased from 1.2 to 1.28 kg/day in growing cattle when WDDGS increased from 0 to 320 g/kg DM (Beliveau & McKinnon, 2008) and the FE showed a quadratic response with lower levels of 80 and 160 g/kg DM of WDDGS. In finishing cattle, no differences in performance parameters were observed when WDDGS were included at concentrations of 0 to 400 g/kg DM (Beliveau & McKinnon., 2008; Yang et al., 2012), but negative effects were reported at concentrations of 600 g/kg DM (Gibb et al., 2008). These results suggest that growing cattle may respond better to increase degradable and undegradable CP in the diet from the

inclusion of WDDGS and that very high inclusions may reduce diet digestibility as starch from barley grain is being replaced with less digestible NDF.

2.3.3 Effects of WDDGS on adipose tissue fatty acid profiles

As explained previously, ruminants are finished in North America by using high proportions of grain in the diet in order to maximise weight gain and feed efficiency. However, this strategy has the disadvantage of promoting a shift in rumen microbial populations from those that promote the biohydrogenation of PUFA from VA to *trans*-10-octadecenoic acid thereby increasing the deposition of the unhealthy *trans*-fat isomer in adipose tissue. This response is mainly attributed to the high concentrations of starch in grain diets that result in decreased pH and conditions that favour those microbes that produce the *t*10 isomer (Bauman and Griinari, 2003). The use of WDDGS has improved the *t*10/*t*11 ratio as a result of linear reductions in proportions of *trans*-10-octadecenoic acid and linear increases in VA (Dugan et al., 2010) with WDDGS inclusions of 0 to 600g/kg DM in finishing beef cattle diets. Triticale based DDGS (200 g/kg DM) also reduced the proportions of *trans*-10-octadecenoic acid tail fat from lambs (McKeown et al., 2010b). These improvements were mainly attributed to the removal of starch and increasing NDF in the diet. These changes were also associated with an increase in total CLA due to increases in rumenic acid (*c*9, *t*11-18:2) which is derived from the desaturation of VA in mammalian tissues (Griinari et al., 2000). Additionally, increased proportions of linoleic acid (18:2n-6) were reported with increased concentrations of WDDGS (Dugan et al., 2010) and triticale DDGS in the diet (McKeown., 2010b). The reasons for this

increase are not clear but an alteration in the biohydrogenation process due to starch removal from the diet (Dugan et al., 2010) or increased passage rates due to reduced particles size of WDDGS have been proposed (He et al., 2012). The hereby described modifications in fatty acid profiles of ruminants from the use of WDDGS are promising and should be evaluated in finishing lambs.

2.4 Discussion

The growth of the biofuel industry warrants increasing stocks of glycerine and wheat DDGS in the future. Previous research with glycerine indicates that it can be used in moderate concentrations in beef cattle diets without affecting performance. Its' use in sheep, however, has been scarcely assessed and requires examination. The effects of glycerol (the main component of glycerine) on the rumen ecosystem are diverse and not conclusively understood. It is commonly accepted that glycerol increases propionic fermentation in the rumen which has been suggested to act as a hydrogen sink and to compete for hydrogen with methanogenesis. The effects of glycerol on fatty acid profiles in ruminants have not been studied. Considering that the starch rich diets used in finishing cattle and sheep diets confer undesirable fatty acid profiles to adipose tissues, the replacement of grain with other energy sources may help to improve fatty acid profiles of lambs. Wheat DDGS have also been used in growing cattle without negatively affecting performance and have been shown to improve fatty acid profiles. However, in finishing cattle, performance may be affected when it is used at high concentrations in the diet. The effects of graded concentrations of

WDDGS on lamb performance, rumen degradation kinetics and fatty acids profiles have not been tested in finishing diets for lambs.

The main hypotheses of this thesis are that glycerol and wheat DDGS can be used in finishing lamb diets to replace barley grain without affecting performance or fatty acid profiles in adipose tissue. Additionally, methane production can be reduced as a result of increased propionic fermentation from glycerol.

To verify the hypotheses, research presented here evaluates the effects of glycerol on fermentation and methane production of concentrate and forage diets in *in vitro* batch culture and semi-continuous fermentation systems as well as *in vivo* methane emissions, lamb performance and fatty acid profiles. The effects of WDDGS on animal performance, fatty acid profiles and rumen degradation kinetics of diets containing were also assessed.

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NOTE:

For ease of presentation, chapters three to seven are presented as per accepted/submitted papers according to the specific format guidelines of the stated journals.

Chapter 3. Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on *in vitro* fermentation and methane production.

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Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on *in vitro* fermentation and methane production

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Abstract

With the hypothesis that glycerol can increase propionate proportions in and thus reduce CH_4 production, this study aimed to assess impacts of increasing dietary levels of glycerol on *in vitro* ruminal fermentation and CH_4 production from a barley based feedlot diet. Glycerol was used as replacement for barley grain at inclusions of 0, 70, 140 and 210 g/kg of diet dry matter (DM) in a diet containing an equal mixture of barley grain and barley silage. Both grain and silage were dried and ground through a 1 mm screen before mixing with glycerol. The experiment was repeated (*n*=2) using ANKOM® bags in 50 ml sealed batch culture serum vials (*i.e.*, 0.5 g substrate + 25 ml media) with a 3:1 ratio of buffer:rumen liquor (*n*=5 bags/treatment/experiment). Rumen liquor was obtained from two cows fed a diet containing 710 g/kg barley silage, 250 g/kg barley grain and 40 g/kg concentrate (DM basis). Gas production was measured by water displacement at 3, 6, 12, 24, 36 and 48 h after inoculation. Volumes corrected for gas released from 15 negative controls (*i.e.*, no substrate) were used to estimate net gas production at 24 and 48 h. Gas samples collected at 24 and 48 h were analyzed for CH_4 concentration. *In vitro* DM disappearance (IVDMD) and culture pH were measured at 48 h. Cumulative gas production as ml/g DM substrate and IVDMD were similar among treatments. Culture pH did not differ among treatments. Total CH_4 production (expressed as mg or as mg CH_4/g digested DM) did not differ among treatments. Results suggest that replacing barley grain with glycerol did not reduce *in vitro* CH_4 production as a function of digested DM or substrate DM.

Keywords: methane, glycerol, *in vitro*

Abbreviations: DM, dry matter; IVDMD, *in vitro* DM disappearance; TCA, trichloroacetic acid; VFA, volatile fatty acids

3.1 Introduction

The increase of biodiesel production has led to increased stocks of glycerol with a subsequent price reduction, making glycerol a potential high energy feed source for ruminants. Until recently, glycerol was used as a minor component of the diet to prevent or treat ketosis in transition (*i.e.,* immediately before and after calving) and postpartum dairy cows (Rémond et al., 1993; Defrain et al., 2004; Chung et al., 2007). Glycerol improves glucose status in ruminants as it is readily absorbed through the rumen wall and converted to glucose in the liver (Rémond et al., 1993), or fermented to propionate, a gluconeogenic precursor that increases blood glucose levels after absorption in cattle (Chung et al., 2007) and sheep (Johns, 1953). Bergner et al. (1995) reported that replacement of wheat starch with glycerol increased production of propionate and reduced the acetate:propionate ratio *in vitro*. The same authors found no radioactivity in CH_4, acetic or lactic acid when using C^{14} labelled glycerol, confirming that most glycerol is transformed into propionate *in vitro*.

Although use of glycerol in beef cattle diets has been reported (Schröder and Südekum, 1999; Mach et al., 2008; Parsons et al., 2009), its effects on CH_4 emissions have not been assessed. Among the multitude of strategies suggested to mitigate CH_4 emissions, those that have a positive economic impact on animal production will be the ones which are most likely to be adopted (Beauchemin et al., 2008). As propionate enhancement has been suggested as a means to reduce CH_4 emissions (Boadi et al., 2004), our objective was to assess effects of

replacing barley grain with glycerol on *in vitro* CH_4 production using a mixed barley grain and barley silage diet.

3.2 Materials and method

All procedures and protocols used in this experiment were approved by the Lethbridge Research Centre Animal Care Committee (ACC1008)

3.2.1 Substrates

The substrate used for incubation was a barley grain:barley silage mixture at the ratio of (500:500 g/kg DM basis) left unmodified (Control) or supplemented with (/kg dietary dry matter [DM]) 70, 140 and 210 g of glycerol (99.5 % pure, Sigma-Aldrich, St. Louis, MO, USA) by replacing equivalent amounts of barley grain in the diet. Feed ingredients were dried at 60^{o}C for 24 h and then ground to pass a 1.0 mm screen and mixed to obtain the 4 treatments. Substrates were prepared by mixing barley silage, barley grain and glycerol in ratios of 500:500:0, 500:430:70, 500:360:140 and 500:290:210 for each treatment, respectively. For each *in vitro* incubation, 0.5 g DM of sample was weighed into an ANKOM® bag (model F57) with 5 replicates/treatment and sealed. Even at the 210 g/kg level, the glycerol was fully absorbed onto the feed leaving no free liquid. Each bag was placed into a 50 ml amber serum bottle fitted with rubber stoppers. The entire incubation procedure was repeated twice (*i.e.,* 2 incubation runs × 5 replicates per treatment, resulting in a total of 10 replicate vials per treatment).

3.2.2 Inoculum

Inoculum for the *in vitro* incubation was obtained from two ruminally cannulated cows fed a mixed diet consisting of 250 g/kg barley grain, 40 g/kg feedlot supplement and 710 g/kg barley silage. Rumen fluid was collected 2 h after feeding from 4 distinct sites in the rumen, filtered through 4 layers of cheesecloth, combined in equal portions from each animal and transported in a prewarmed Thermos® flask to the laboratory. Inoculum was prepared by mixing rumen fluid and a mineral buffer with 0.5 ml of cysteine sulphide solution (Menke et al., 1979) in a ratio of 1:3. The inoculum was then transferred (25 ml) into pre-loaded pre-warmed (39^{o}C) vials under a stream of O_2-free N gas. Vials were sealed and placed on an orbital shaker rack set at 90 oscillations/min in an incubator at 39^{o}C.

3.2.3 Determination of total gas, methane concentration and IVDMD

Net gas production of each vial was measured at 24 and 48 h of incubation with a water displacement apparatus (Fedorak and Hrudey, 1983). Headspace gas was sampled from each vial prior to gas measurement with a 20 ml syringe and immediately transferred into a 5.9 ml evacuated Exetainer (Labco Ltd., High Wycombe, Buckinghamshire, UK), which was then analyzed for CH_4 concentration by gas chromatography (Holtshausen et al., 2009). Methane was expressed as mg of CH_4/g DM disappeared, and total net gas production as ml/g of incubated DM.

After 48 h of incubation, and after gas was sampled for CH_4 and total gas production was measured, the fermentation vials were opened and the pH of the culture was measured using a pH meter (Orion Model 260A, Fisher Scientific,

Toronto, ON, Canada). The ANKOM® bags with the residues were then removed from the bottles, rinsed thoroughly with distilled water, dried at 55°C for 48 h to constant weight and weighed to estimate *in vitro* dry matter disappearance (IVDMD).

3.2.4 Determination of ammonia-N and volatile fatty acids

The liquid fraction of the fermentation at the beginning of the incubation at 0 h and after removal of the filter bag at the end of the 48 h incubation was sub-sampled for determination of ammonia and volatile fatty acids (VFA). Two subsamples (1.6 ml) of each vial were transferred to 2 ml micro-centrifuge tubes containing 150 µl of TCA (0.65; vol/vol) and centrifuged at 14,000 × *g* for 10 min at 4°C (Spectrafuse 16M, National Labnet Co., Edison, NJ, USA) to precipitate particulate matter and protein. The supernatant was transferred into 2 ml micro-centrifuge tubes (Fisher Scientific, Ottawa, ON, Canada) and frozen at -20°C until analyzed for ammonia N.

In addition, two subsamples (1.5 ml) of each vial were collected, acidified with 300 µl of metaphosphoric acid (0.25; wt/vol), and centrifuged as described for ammonia N analysis. The supernatant was frozen at -20°C until analyzed for VFA concentrations. The 0 h samples were also analyzed for ammonia N and VFA to calculate net ammonia-N and net total VFA production (Holtshausen et al., 2009). Ammonia N was determined by the salicylate-nitroprusside-hypochlorite method (Sims et al., 1995) using a flow injection analyzer. Concentrations of VFA were analyzed as described by Addah et al. (2012) using gas chromatography (Model 5890: Hewlett Packard, Wilmington, DE) with crotonic acid as an internal standard.

3.2.5 Statistical analyses

The univariate procedure of SAS was used to test for normal distribution of the data. The 5 replicate bags were averaged prior to statistical analysis and those averages, within run, were the statistical unit. Data were analysed with mixed model procedures of SAS Inc. (2011). The data were analyzed as a randomized complete block design using PROC MIXED (SAS, 2011) with treatment in the model as fixed effects and run, and the run by treatment interaction, as random effects. The run by treatment interaction was used as the error term to test the treatment effect. Planned polynomial contrasts were made to determine linear and quadratic effects of increasing levels of glycerol in the substrates. As no significant quadratic responses occurred, only linear responses are reported.

3.3 Results and Discussion

3.3.1 Gas production and DM disappearance

Cumulative gas production at 48 h as ml/g incubated DM was similar among treatments. Krueger et al. (2010) reported a linear increase in gas production when glycerol was added to alfalfa hay (at 100, 200 and 400 g/kg DM) *in vitro*, but others have found lower gas production from pure glycerol compared to alfalfa or corn silage (Ferraro et al., 2009).

In vitro DM disappearance was linearly increased (P=0.05) with higher levels of glycerol. Previous research (Rémond et al., 1993) found no difference in fermented organic matter when glycerol was added to a starch substrate, but did measure a slight increase in digestibility when the substrate was cellulose.

Krueger et al. (2010) and Schröder and Südekum (1999) reported no differences in nutrient digestibility when glycerol replaced alfalfa or wheat grain under *in vitro* or *in vivo* conditions, respectively.

3.3.2 Fermentation characteristics

Total VFA production was not affected by glycerol inclusion in the diet (Table 3.1). Effects of glycerol on fermentation profiles seem to differ according to the degradability of the diet. For example, glycerol increased total VFA production when mixed with cellulose, but not when mixed with starch (Rémond et al., 1993). Wang et al. (2009) recorded increased VFA concentration in steers by adding low amounts of glycerol (*i.e.*, 1.1, 2.2 and 3.3 g/kg DM) to high forage diets, which was mainly attributed to increased concentration of propionate and butyrate in total VFA.

Substituting increasing levels of glycerol for barley grain linearly increased propionate ($P<0.01$) and reduced acetate ($P<0.01$) concentrations resulting in a decline in the acetate to propionate ratio. This fermentation pattern is consistent with other *in vitro* (Rémond et al., 1993, Bergner et al., 1995; Trabue et al., 2007) and *in vivo* (Schröder and Südekum, 1999; DeFrain et al., 2004; Wang et al., 2009) studies, and confirms the propioneogenic properties of glycerol. Butyrate proportions of total VFA were linearly reduced ($P=0.01$) with increasing levels of glycerol. This result contrasts with others who reported increased proportions of butyrate in total VFA with inclusion of glycerol *in vitro* (Rémond et al., 1993; Trabue et al., 2007). In contrast, *in vitro* (Krueger et al., 2010) and *in vivo* (DeFrain et al., 2004; Mach et al., 2009) studies found no

effects on butyrate proportions of total VFA with increased levels of glycerol. Johns (1953) reported that almost all glycerol is fermented to propionate in *in vitro* incubations.

3.3.3 Methane production

Addition of increasing levels of glycerol did not affect CH_4 production either as a function of incubated DM (mg/g DM) or of digested DM (mg/g DMD) (Table 1). This is to our knowledge the first report on effects of glycerol on CH_4 production.

3.4 Conclusions

Replacing barley grain with glycerol in a feedlot diet increased propionate concentration in ruminal fluid and improved *in vitro* dry matter disappearance. However it did not affect *in vitro* CH_4 production as a function of digested DM or incubated DM. Results suggest that glycerol has the potential to modify fermentation characteristics towards glucogenic pathway and improve digestibility.

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Table 3.1 Effects of increasing levels of glycerol as replacement of barley grain on 48 h fermentation characteristics and in vitro methane production.

	Glycerol level (g/kg DM)					P^1
	0	70	140	210	SEM	Linear
Gas production						
Gas, ml/g DM	163.3	163.5	157.6	154.4	6.42	ns
Methane, mg/g DM	7.5	7.4	7.5	7.1	0.33	ns
Methane, mg/g DMD	12.4	12.0	12.4	11.3	0.27	ns
Fermentation characteristics						
Culture pH	5.86	5.77	5.72	6.26	0.26	ns
Total VFA, m*M*	91.3	97.4	92.8	97.1	3.40	ns
VFA, mol/100 mol						
Acetate (A)	39.4	35.3	32.6	28.3	1.87	<0.01
Propionate (P)	34.0	38.3	42.1	47.3	0.36	<0.01
Butyrate	17.7	18.2	16.6	16.2	0.91	0.01
A:P ratio	1.16	0.92	0.78	0.60	0.04	<0.01
Ammonia N, mmol	13.4	12.6	10.9	11.3	2.77	ns
IVDMD, g/kg DM	643.2	660.4	654.2	669.7	19.0	0.05

ns, *P*>0.10
IVDMD, *in vitro* dry matter disappearance;
[1] – No quadratic effect was P<0.05

Chapter 4. Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique (RUSITEC) system.

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Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique (RUSITEC) system

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Abstract

We hypothesised that inclusion of glycerol in forage diets for ruminants would increase the proportion of propionate produced and thereby decrease *in vitro* methane production. This hypothesis was examined using a semi continuous fermentation system (RUSITEC) in which each fermenter was fed a brome hay (8.5 g) and maize silage (1.5 g) diet with increasing concentrations (0, 50, 100 and 150 g/kg DM) of glycerol substituted for maize silage. Glycerol tended to increase total VFA concentration ($P = 0.065$). Concentration of acetate was unaffected ($P = 0.232$) and those of propionate and butyrate were linearly increased ($P < 0.001$). Glycerol linearly increased ($P = 0.010$) dry matter (DM) disappearance from hay and silage. Crude protein disappearance from hay was not affected (P = 0.789) but that of silage tended to increase ($P = 0.058$) with increasing glycerol. Disappearance of neutral detergent fibre ($P = 0.046$) and acid detergent fibre from hay ($P = 0.024$) were increased by glycerol. Total gas production was not affected by treatment ($P = 0.214$) but methane (CH_4) concentration in gas was linearly increased ($P = 0.002$) by glycerol, resulting in an increase ($P = 0.006$) in mg CH_4 /g DM incubated. Our hypothesis was rejected as increasing concentrations of glycerol in a forage diet linearly increased CH_4 production in semi continuous fermenters despite the increases in concentrations of propionate. We conclude that this apparent discrepancy is due to the more reduced state of glycerol as compared to carbohydrates, which implies there is no net incorporation of electrons when glycerol is metabolised to propionate.

4.1 Introduction

Glycerol has been used as a feed ingredient to replace grain in dairy[1] and finishing beef cattle[2,3] and in growing-finishing lamb diets[4]. Results suggest that glycerol at 50-100 g/kg DM does not affect weight gain and may improve feed conversion when substituted for barley or maize grain in beef cattle diets[3]. However, greater concentration (> 120 g/kg diet DM) of glycerol has resulted in reduced intakes in cattle[2,3] and sheep[4-6] fed high grain diets.

Glycerol can increase blood glucose levels in cattle and sheep by being directly absorbed through the rumen wall and converted to glucose in the liver[7] or by being fermented in the rumen mainly to propionate which can in turn be absorbed and converted to glucose in the liver [1,8]. The replacement of wheat starch[9] or barley grain[10] with glycerol linearly increased the production of propionic acid and reduced the acetic/propionic ratio *in vitro*. Shifts towards propionate fermentation have been suggested as a means to reduce methane emissions since the metabolic pathways leading to propionate have been proposed as a hydrogen sink[11-13].

Previous studies have reported increases in propionate production with only a numerical decrease of methane *in vitro*[10] (mg CH_4/g DM incubated and mg CH_4/g DM digested) and *in vivo*[4] (g CH_4/lamb/day, g CH_4/kg DMI, g CH_4/kg DM digested, % of gross and digestible energy intake lost as CH_4) when glycerol replaced barley grain in lamb finishing diets. A possible cause for this lack of effect is that the shift to propionate fermentation may be of relatively low magnitude given the propiogenic properties of high starch diets. The findings by Rémond *et al.*[7] support this concept as propiogenesis was substantially increased

when glycerol was added to high fibre as compared to high starch diets incubated *in vitro*. Methane production (mL CH_4/g DM incubated) in 24 h *in vitro* batch cultures was decreased when glycerol was added to alfalfa or maize grain-based diets[14]. Since the acetate/propionate ratio was progressively reduced over a period of 3 to 4 days after the glycerol supplementation in cattle[7,15], it is probable that enteric methane production may decrease as rumen microbial populations adapt to the inclusion of glycerol in the diet.

As feeding of forage to breeding and growing herds within the beef cattle production life cycle accounts for more than 80% of greenhouse gases emissions and 55% of CH_4 emissions[16], inclusion of glycerol in forage diets is likely to have a greater impact on reducing GHG emissions from beef production. We hypothesised that the inclusion of glycerol at up to 150 g/kg in forage-based diets may decrease CH_4 production in a semi-continuous fermentation system (RUSITEC). Thus, the objective of this study was to evaluate the effects of adding glycerol to a forage diet in a semi continuous fermentation apparatus (RUSITEC) on fermentation variables, including CH_4 production.

4.2 Experimental methods

The experiment was conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta (Canada). Donor cows used in this experiment were cared for in accordance with the guidelines of the Canadian Council on Animal Care[17].

4.2.1 Experimental design and treatments

The experiment was a complete randomized design with 4 dietary treatments replicated in each of two rumen simulation technique (RUSITEC) apparatuses[18]. The experimental period consisted of 15 d. The first 8 d were used as an adaptation period, followed by 7 d of sampling (d 9 to 15). The experimental treatments included brome hay, maize silage and glycerol in the following proportions: 1) control (CON) 8.5 g hay + 1.5 g maize silage, 2) 8.5 g hay + 1.0 g maize silage + 0.5 g glycerol, 3) 8.5 g hay + 0.5 g maize silage + 1.0 g glycerol, and 4) 8.5 g hay + 1.5 g glycerol. These amounts were selected to test inclusions of 50 to 150 g/kg based on previous *in vivo* results[4] which showed dry matter intake (DMI) reductions at glycerol concentration exceeding 140 g/kg.

Ingredients and chemical composition of the substrates are reported in Table 1. Hay and silage were ground through a 4 mm screen (Arthur Thomas Co., Philadelphia, PA). Glycerol (99.5% pure, Sigma–Aldrich, St. Louis, MO, USA) was thoroughly mixed with the hay portion of the diet for each treatment before filling the polyester bags (100 × 200 mm; pore size = 50 µm; B. & S.H. Thompson, Ville Mont-Royal, QC, Canada). Maize silage was incubated in separate bags (50 × 100 mm; pore size = 50 µm).

4.2.2 Experimental apparatuses and incubations

Each RUSITEC was equipped with eight 920 mL volume anaerobic fermenters. Each fermenter possessed an inlet for infusion of buffer and effluent output port. The fermenters were immersed in a water bath maintained at 39°C. The four dietary treatments were randomly assigned to duplicate fermenters within each

RUSITEC (4 replications per treatment). To begin the experiment, each fermentation vessel was filled with 180 mL of warmed McDougall's buffer[19] modified to contain 1.0 g/L of $(NH_4)_2SO_4$, 720 mL of strained rumen fluid, one bag containing 20 g of wet solid rumen digesta and two additional bags containing the dietary ingredients as described above. After 24 h the solid rumen digesta bag was replaced with two bags containing each feed. Thereafter bags that had been incubated for 48 h were replaced daily. Artificial saliva was continuously infused into the fermenters at a dilution rate of 2.9%/h. During nylon bag exchange, each fermentation vessel was flushed with O_2-free CO_2 to maintain anaerobic conditions. Effluent accumulation was measured daily during feed-bag exchange and collected in a 2.0-L container containing sodium azide (1 g/L) to arrest microbial growth.

Inoculum was obtained 2 h after feeding from two ruminally cannulated cows fed a forage diet containing barley silage, barley grain and a mineral vitamin supplement (71:25:4 DM basis). Rumen fluid was collected, pooled and filtered through four layers of cheesecloth into an insulated thermos and transported immediately to the laboratory. Approximately 400 g of ruminal solid digesta were also collected for the initial inoculation of the fermenters. Fermentation was initiated in the RUSITEC apparatuses on two consecutive days (two runs).

4.2.3 Sample collection

4.2.3.1 Dry matter disappearance

Dry matter disappearance at 48 h was determined daily from d 9 to 15. Feed bags were removed from each fermenter, washed in cold, running distilled water until water was clear, and dried at 55°C for 48 h. To ensure there was sufficient sample for analysis, silage and concentrate bag residues were pooled over 2 and 3 d, respectively. Samples were ground through a 1-mm screen in a Wiley mill (standard model 4; Arthur H. Thomas) prior to chemical analysis.

4.2.3.2 Fermentation metabolites

Fermentation gas was collected into reusable 2-L, vinyl urine collection bags (Bard Inc., Mississauga, ON, Canada) attached to each fermenter. Just prior to feed-bag exchange, daily total gas production from each fermenter was determined by water displacement[20]. From d 9 to 15, just prior to determination of total gas, gas samples were taken from the septum of collection bags using a 26-gauge needle (Becton Dickinson, Franklin Lakes, NJ). Samples (20 mL) were transferred to evacuated 6.8-mL exetainers (Labco Ltd., Wycombe, Bucks, UK) for immediate analysis of CH_4. Fermenter pH was recorded (Orion model 260A, Fisher Scientific, Toronto, ON, Canada) daily at the time of feed-bag exchange. To determine VFA concentration, subsamples of fermenter liquid (4.0 mL) were taken directly from the fermentation vessels[19] at the time of feed-bag exchange and placed in screw-capped vials preserved with 400 µL of 25% (w/w) metaphosphoric acid and immediately frozen at −20°C until analysis. At the same

time, 4.0-mL subsamples of fermenter fluid were also collected, placed in screw-capped vials and preserved with 400 µL of trichloroacetic acid until analysed for NH_3-N concentration. Concentrations of VFA and Ammonia-N (mM/L) were multiplied by outflow rate of fluid infused to the vessels (L/d) to obtain VFA and Ammonia-N production (mM/d).

4.2.3.3 Chemical analyses

Subsamples of each treatment were used for chemical analysis. Feed and fermentation residues were analysed for DM content (method # 930.15)[21] and ash (method # 942.05)[21]. Neutral detergent fibre (NDF) was determined and expressed inclusive of residual ash[22]. Acid detergent fibre (ADF) was determined according to the method # 973.18 (AOAC)[21]. Total N (method # 990.03)[21] was analysed using a mass spectrometer (NA 1500, Carlo Erba Instruments, Rodano, Italy)[23]. Crude fat concentration was determined by ether extraction (AOAC[21], method # 920.39) using a Goldfisch Fat Extractor (Labconco Corporation, Kansas City, MO). Concentrations of VFA and NH_3-N in the liquid effluent were analysed by gas chromatography[23]and the modified Berthelot method[24], respectively. Methane concentration in the gas samples was determined using a Varian gas chromatograph equipped with GS-CarbonPLOT 30 m × 0.32 mm × 3 µm column and thermal conductivity detector (TCD; Agilent Technologies Canada Inc., Mississauga, ON). Oven temperature was 35^{o}C (isothermal). The carrier gas was helium (27 cm/second), the injector temperature was 185°C (1:30 split, 250 µL injector volume) and the detector temperature was 150°C (TCD).

4.2.3.4 Statistical analysis

Data were analysed using the MIXED procedure of SAS (SAS Inc., 2013; SAS Online Doc 9.1.3. Cary, NC, USA). Means were compared using the least square mean linear hypothesis. The model included the fixed effects of treatment (substrate), day and treatment by day interactions with the day of sampling from each fermenter treated as a repeated measure. Therefore, the individual fermenter was used as the experimental unit for statistical analysis. The minimum values of AIC (Akaike's Information Criterion) were used to select the covariance structure among compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, Toeplitz, unstructured and banded for each parameter. Significance was declared at $P \leq 0.05$ and a trend was discussed when $0.05 < P < 0.10$. Only when effect of treatment was significant ($P \leq 0.05$), orthogonal polynomial contrasts were performed to test for linear, quadratic and cubic responses to increasing concentration of glycerol (0, 50, 100, and 150 g/kg DM) in the substrate.

4.3 Results

4.3.1 Effects of glycerol on nutrient disappearance

Increasing concentration of glycerol resulted in linear increase in DM disappearance from hay ($P = 0.001$) and maize silage ($P = 0.011$; Table 2). Crude protein (CP) disappearance from hay was not affected ($P = 0.788$) but that of silage tended to increase ($P = 0.058$). Glycerol increased disappearance of NDF

(P = 0.046) and ADF (P = 0.024) from hay, only at a concentration of 150 g/kg, but disappearance of NDF and ADF from silage was not affected (P = 0.142).

4.3.2 Effects of glycerol on fermentation

There were no interactions between treatments and sampling day for any of the fermentation variables. Inclusion of glycerol had no effect on pH (P = 0.120) but increased total VFA production (P < 0.001; Table 3). The production of acetate was not modified (P = 0.113) by increasing concentration of glycerol; whereas production of propionate was linearly increased (P < 0.001) resulting in a linear and quadratic decline (P < 0.001) in the acetate to propionate ratio. Increasing glycerol also resulted in a linear increase in production of butyrate (P = 0.003) and valerate (P < 0.001). When expressed as molar proportions, acetate was linearly decreased and propionate linearly increased by addition of glycerol whereas butyrate was not affected (results not shown). Concentration of ammonia was linearly reduced by adding glycerol (P < 0.001), although the magnitude of the effect was small.

Twenty four hour cumulative gas production was not affected by treatments (P = 0.214; Table 4) but CH_4 concentration in gas was linearly increased with increasing glycerol concentration in the substrate (P = 0.002). This resulted in a linear increase in CH_4 production when expressed as total mg CH_4 per day, mg CH_4/g total DM incubated (P < 0.004) and mg CH_4/g of hay DM disappeared (P = 0.001).

4.4 Discussion

Effects of glycerol on fibre digestion have been variable. The linear increase in DM loss from hay and silage as well as in NDF loss from hay are in agreement with Wang *et al.* [25] who reported increased DM and NDF *in sacco* effective degradability from forage and CP effective degradability from concentrate as well as an improved total tract nutrient digestibility, including NDF, when steers were fed increasing concentrations of glycerol (0, 11, 22 and 33 g/kg DM) in mixed diets (600g/kg maize stover and 400g/kg concentrate). The results in our study also seem to concur those of Schröder and Südekum [26] who reported that fibre digestion was increased in low starch diets when glycerol was included at concentration of 150 g/kg DM. In another study, increasing concentrations of glycerol (0-400 g/kg diet DM) did not affect NDF *in vitro* degradability when added to lucerne hay[27]. However, reductions in fibre digestion were reported when glycerol was added to starch-containing diets *in vivo*[26] and *in vitro*[28]. These results have been associated with an inhibition of hemicellulolitic *B. fibrisolvens*[28] and of cellulolytic bacteria and fungi[29]. Thus, discrepancies among studies on glycerol effect on fibre digestibility are difficult to explain. It is possible that some fibrolytic species may have responded differently to glycerol in the present study, but this is difficult to ascertain as microbial populations were not determined. Quantification of fibrolytic organisms and their activity would be important to resolve contradictory results of glycerol on fibre digestion.

Previous studies have consistently reported a decreased molar proportion of acetate and increases in proportion of propionate in *in vitro* conditions using glycerol in starch-rich[9,10] and forage substrates[10, 27], as well as *in vivo* in

finishing beef cattle fed concentrate diets[3,26] and in transition dairy cows[30]. This concurs with our results and confirms the propiogenic properties of glycerol. Shifts towards reduced acetate to propionate ratio derived from increased propionate concentration and increases in butyrate concentrations have also been reported *in vitro* using starch substrates[9] and *in vivo* using starch and forage based diets[26].

The linear increase in CH_4 proportion in total gas and total CH_4 production as a function of total DM disappeared contradicts our hypothesis. Fermentation of carbohydrates to propionate has been described as a hydrogen sink and feeding propiogenic substrates has been proposed as a CH_4 abatement strategy[11-13]. However, glycerol is a more reduced substrate than sugars and releases 2 electron pairs for each mole of glycerol converted to pyruvate[31], one in the oxidation of glycerol to dihydroxyacetone, which is then phosphorylated and enters glycolysis; and one in glycolysis itself, in the oxidation of 3-phosphogyceraldehyde to 3-phosphoglycerate[32]. This compensates the electron incorporation in the conversion of pyruvate or phosphoenolpyruvate into propionate. Thus, there is no net electron incorporation in the conversion of glycerol to propionate:

$$CH_2OHCHOHCH_2OH \rightarrow CH_3CH_2COOH + H_2O$$

Glycerol not only failed to decrease CH_4 production as hypothesised but increased it. There was an increase in butyrate production as glycerol replaced maize silage. Butyrate production from both carbohydrates and glycerol would result in a release of reducing equivalents and contribute to increasing CH_4 production:

$CH_2OHCHOHCH_2OH \rightarrow ½\ CH_3CH_2CH_2COOH + CO_2 + 2[2H]$

Furthermore, little glycerol seems to be fermented to acetate[33], but acetate production was unaffected by glycerol substitution for maize silage. Glycerol stimulated DM disappearance, but because glycerol replaced maize silage, total forage digested DM was actually lower because there was less maize silage to digest, and less carbohydrates were fermented. Therefore, acetate production seems to have remained unchanged because of a shift in carbohydrates fermentation towards acetate, which would also release reducing equivalents and contribute to increased CH_4, because the increase in propionate production from glycerol would not demand extra reducing equivalents. Formation of some butyrate and acetate from glycerol instead of from carbohydrates would further contribute to enhance methanogenesis, again because of glycerol being more reduced than carbohydrates this would result in a greater release of reducing equivalents per mole of acetate and butyrate produced compared to carbohydrates. An alternative explanation for the increase in CH_4 production with glycerol is based on the equimolar conversion of glycerol to formate and ethanol which has by an isolate from deer rumen identified as *Klebsiella planticola*[34]. Formate is a precursor of CH_4[35], and much ethanol is oxidised to acetate in the rumen[36,37], a process that releases reducing equivalents that can be used for CH_4 production[38]. It has been shown that pure cultures of *Ruminococcus flavefaciens*[39], *R. albus*[40] and a ruminal fungus[41] decreased formate and ethanol production when co-cultured with methanogens, as CH_4 became the main electron sink in the co-

cultures. Also, some microorganisms can convert glycerol to 1, 2-propanediol[42], and in turn there was some recovery of 1-^{14}C-1, 2-propanediol incubated in ruminal continuous cultures as $^{14}CH_4$[43].

Adaptation of donor animals to diets containing glycerol seems to have affected fermentation when glycerol was included in *in vitro* batch culture incubations. Gas and CH_4 production were increased when 150 g/kg glycerol was included in the substrates (900 g/kg concentrate based on rolled maize, maize gluten feed and soybean hulls) using inoculum from glycerol-adapted animals[44] but changes in CH_4 production were negligible when inoculum was obtained from un-adapted animals, suggesting that microbial adaptation influences digestion and fermentation end products. This explains, at least partially, the differences between previous studies reporting no effect[10] or decreased CH_4 production[14] when incubating glycerol in *in vitro* batch cultures using inoculum from non-adapted animals as opposed to the results in our study where increased propionate and total VFA production, and linear increase in DM loss was associated with increased CH_4 production (mg CH_4/g DM digested) using glycerol-adapted fermenters. When increasing glycerol was fed to adapted lambs, no effects were reported on CH_4 emissions[4]. In this case, absorption through the rumen wall or passage to the lower gut or both may have impeded fermentation of an important proportion of glycerol[7] thus reducing release of hydrogen electrons in the rumen environment as compared to *in vitro* fermenters where absorption is precluded.

4.5 Conclusions

Increasing concentration of glycerol in forage diets incubated in a rumen simulation technique (RUSITEC) improved DM disappearance from brome hay and maize silage and NDF and ADF disappearance from brome hay. Acetate to propionate ratio was linearly decreased as a result of increased production of propionate and unaltered concentration of acetate. Concentration of CH_4 in gas and total CH_4 production per unit of DM digested or incubated were increased, as fermentation of glycerol to propionate does not act as a hydrogen sink.

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Conflict of Interests

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

Authorship

The author´s contributions were as follows: J. Avila-Stagno, A.V. Chaves, and T.A. McAllister designed the study, J. Avila-Stagno and G.O. Ribeiro Jr. conducted the experimental procedures and laboratory analyses. J. Avila-Stagno and A.V. Chaves stats analysed and interpreted the data. J. Avila-Stagno wrote the first draft. A.V. Chaves, T.A. McAllister, E.M. Ungerfeld and G.O. Ribeiro Jr. critically revised the article.

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Table 4.1 Chemical composition of the substrates.

	Brome hay	Maize silage
	g/kg DM	
Dry matter	953	982
Organic matter	909	930
Neutral detergent fibre	683	459
Acid detergent fibre	485	346
Crude protein	87.5	86.3
Crude fat	24	26
Ash	91	70
Non fibrous carbohydrates*	115	359

*Calculated as 1000-[crude protein + neutral detergen fibre + crude fat + ash].

Table 4.2 Effects of increasing concentrations of glycerol on disappearance of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) of brome hay and maize silage in the rumen simulation technique (RUSITEC).

	Glycerol* g/kg					*P*-Values			
Item	0	50	100	150	SEM	Treatment	Linear	Quadratic	Cubic
DM loss, mg/g									
Hay	382[b]	377[b]	386[b]	405[a]	4.1	0.001	0.001	0.005	0.708
Silage	510[b]	523[b]	562[a]	-	13.7	0.026	0.011	0.327	-
Total DM	402[d]	424[c]	461[b]	501[a]	3.7	<0.001	<0.001	0.008	0.442
CP loss, mg/g									
Hay	622	624	642	633	15.5	0.789	-	-	-
Silage	633	674	706	-	24.5	0.058	-	-	-
NDF loss, mg/g									
Hay	213[b]	224[ab]	221[b]	245[a]	10.7	0.046	0.467	0.103	0.276
Silage	81	90	139	-	19.6	0.142	-	-	-
ADF loss, mg/g									
Hay	67.8[b]	79.3[b]	79.1[b]	113.0[a]	9.62	0.024	0.421	0.625	0.470
Silage	24.3	20.5	67.7	-	3.14	0.268	-	-	-

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre.

[a-d]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Table 4.2 (Continued)

*Experimental substrates: 0 = 8.5 g brome hay + 1.5 g maize silage; 50 = 8.5 g brome hay + 1.0 g maize silage+0.5 g glycerol; 100 = 8.5 g hay + 0.5 g maize silage + 1.0 g glycerol; 150 = 8.5 g hay + 1.5 g glycerol.

Table 4.3 Effects of increasing concentrations of glycerol on fermentation characteristics of a brome hay - maize silage diet in the rumen simulation technique (RUSITEC).

	Glycerol* g/kg					*P*-Values			
	0	50	100	150	SEM	Treatment	Linear	Quadratic	Cubic
VFA production, mM/d									
Total	18.6[b]	19.2[b]	20.8[b]	25.5[a]	1.02	<0.001	<0.001	0.045	0.665
Acetate (A)	10.2	9.2	8.8	10.6	0.56	0.113	-	-	-
Propionate (P)	4.2[c]	4.9[c]	6.3[b]	8.4[a]	0.57	0.001	<0.001	0.274	0.977
Butyrate	2.7[c]	3.1[b]	3.4[b]	4.1[a]	0.21	0.003	<0.001	0.458	0.579
Valerate	1.0[c]	1.5[b]	1.8[a]	1.9[a]	0.11	<0.001	<0.001	0.102	0.573
Caproate	0.11[b]	0.14[a]	0.15[a]	0.13[a]	0.001	0.023	0.041	0.013	0.693
A:P ratio	2.6[a]	1.9[b]	1.4[c]	1.3[c]	0.05	<0.001	<0.001	<0.001	0.376
Ammonia N, mM/d	7.0[a]	6.9[a]	6.5[b]	6.5[b]	0.12	0.001	<0.001	0.717	0.840
pH	7.11	7.10	7.09	7.07	0.011	0.120	-	-	-

VFA, volatile fatty acids.

[a-d]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

*Experimental substrates: 0 = 8.5 g brome hay + 1.5 g maize silage; 50 = 8.5 g brome hay + 1.0 g maize silage + 0.5 g glycerol; 100 = 8.5 g hay + 0.5 g maize silage + 1.0 g glycerol; 150 = 8.5 g hay + 1.5 g glycerol.

Table 4.4 Effects of increasing concentrations of glycerol on cumulative gas production and methane (CH4) production in the rumen simulation technique (RUSITEC).

	Glycerol* g/kg					*P*-Values			
	0	50	100	150	SEM	Treatment	Linear	Quadratic	Cubic
Gas volume, mL	930	1015	1058	1056	57.1	0.214	-	-	-
CH_4, %	1.13^{c}	1.59^{b}	1.78^{ab}	2.02^{a}	0.158	0.002	<0.001	0.462	0.617
CH_4, mg/day	7.51^{b}	11.54^{ab}	13.54^{a}	15.32^{a}	1.639	0.008	0.001	0.440	0.786
CH_4, mg/g hay DMD	2.62^{b}	3.64^{ab}	4.46^{a}	4.89^{a}	0.586	0.027	0.004	0.574	0.872
CH_4, mg/g substrate incubated†	0.78^{b}	1.21^{ab}	1.44^{a}	1.63^{a}	0.175	0.007	0.001	0.467	0.814
CH_4, mg/g total DMD	1.96^{b}	2.86^{a}	3.15^{a}	3.29^{a}	0.302	<0.001	<0.001	0.113	0.678

DMD, dry matter disappeared.

$^{a-c}$Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

*Experimental substrates: 0 = 8.5 g brome hay + 1.5 g maize silage; 50 = 8.5 g brome hay + 1.0 g maize silage+0.5 g glycerol; 100 = 8.5 g hay + 0.5 g maize silage + 1.0 g glycerol; 150 = 8.5 g hay + 1.5 g glycerol.

†DM basis.

Chapter 5. Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles and carcass traits of lambs

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Running head: Glycerol for finishing lambs

Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles and carcass traits of lambs

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Abstract

We hypothesised that glycerol could increase diet digestibility, reduce *in vivo* methane (CH_4) emissions, improve lamb performance and fatty acid profiles. To verify this hypothesis, two experiments were conducted to evaluate the effects of increasing concentrations of glycerol in concentrate diets on total tract digestibility, methane (CH_4) emissions, growth, fatty acid profiles and carcass traits of lambs. In both experiments, the control diet contained 57% barley grain, 14.5% wheat dried distillers grain with solubles (WDDGS), 13% sunflower hulls, 6.5% beet pulp, 6.3% alfalfa and 3% mineral-vitamin mix. Increasing concentrations (7, 14, and 21% dietary DM) of glycerol in the dietary DM were replaced for barley grain. As glycerol was added, alfalfa meal and WDDGS were increased to maintain similar concentrations of CP and NDF among diets. In Exp.1, nutrient digestibility and CH_4 emissions from 12 ram lambs were measured in a replicated 4 × 4 Latin square experiment. In Exp. 2, lamb performance was evaluated in 60 weaned lambs that were blocked by weight and randomly assigned to one of the 4 dietary treatments and fed to slaughter weight. In Exp 1, nutrient digestibility and CH_4 emissions were not altered ($P = 0.15$) by inclusion of glycerol in the diets. In Exp.2, increasing glycerol in the diet linearly decreased DMI ($P < 0.01$) and tended ($P = 0.06$) to reduce ADG, resulting in a linearly decreased final bodyweight. Feed efficiency was not affected by glycerol inclusion in the diets. Carcass traits and total saturated or total monounsaturated fatty acid proportions of subcutaneous fat were not affected ($P = 0.77$) by inclusion of glycerol, but polyunsaturated fatty acids were linearly decreased ($P < 0.01$). Proportions of 16:0, 10*t*-18:1, linoleic acid (18:2 n-6) and the n-6/n-3 ratio

were linearly reduced ($P < 0.01$) and those of 18:0 (stearic acid), 9*c*-18:1 (oleic acid), linearly increased ($P < 0.01$) by glycerol. When included up to 21% of diet DM, glycerol did not affect nutrient digestibility or CH_4 emissions of lambs fed barley based finishing diets. Lamb growth performance was optimized at 7% glycerol inclusion in the diet. Glycerol may improve backfat fatty acid profiles by increasing 18:0 and 9*c*-18:1 and reducing 10*t*-18:1 and the n-6/n-3 ratio.

Key words: biodiesel by-products, digestibility, finishing lambs, methane, trans fatty acids

5.1 INTRODUCTION

Biodiesel production has increased in recent years leading to increased stocks of glycerine, a once valuable co-product that now many consider a waste stream with disposal costs (Yazdani and Gonzalez, 2007). The inclusion of glycerol (the main component of crude glycerine) as a major component of the diet has been reported in beef cattle (Mach et al., 2009; Parsons et al., 2009), and inclusions of 10-20% in diet DM have been used without negatively affecting lamb performance (Gunn et al., 2010a). Three different fates have been reported for glycerol when entering the rumen: i) passage to the lower gut, ii) absorption through the rumen wall and conversion to glucose in the liver or, iii) fermentation to propionate resulting in increases in blood glucose in cattle (Krehbiel, 2008). Pathways to propionate production are known to act as a hydrogen sink and would therefore reduce CH_4 emissions (Boadi et al., 2004). Lee et al. (2011) reported reduced CH_4 emissions by including glycerol in *in vitro* incubations of corn grain and alfalfa hay; however, the effects of glycerol on CH_4 emissions *in vivo* have not been reported

and should be studied. High barley diets are known to produce greater proportions of *trans-10-octadecenoic acid*, a cardiovascular health risk associated fatty acid in beef fat (Dugan et al., 2011). Effects of glycerol feeding on fatty acid profiles are still to be tested. We hypothesize that the inclusion of glycerol in concentrate diets fed to lambs would reduce CH_4 emissions without affecting lamb performance and that it would modify fatty acid profiles in adipose tissue. The objectives of this study are thus to evaluate the effects of increasing concentrations of glycerol fed to lambs on nutrient digestibility, CH_4 emissions, growth performance, carcass traits and fatty acid profiles.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Procedures

All experiments were conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta between December 2010 and March 2011 with all lambs being cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

5.2.1.1 Dietary treatments

Composition of the diets are reported in Table 1 with treatment diets being formulated by substituting glycerol (99.5% pure, Sigma–Aldrich, St. Louis, MO, USA) for barley grain and beet pulp to achieve concentrations of 7, 14, and 21% glycerol (DM basis). Dietary concentrations of wheat dried distillers grains with solubles (WDDGS) and alfalfa meal were increased with increasing glycerol to

maintain similar concentrations of CP and NDF in the DM. These concentrations were selected according to the results of Musselman et al. (2008) and Gunn et al. (2010b) who reported negative impacts on lambs DMI when replacing crude glycerin for corn at concentrations above 15% dietary DM. All diets were ground and pelleted to 6.35 mm.

5.2.1.2 Digestibility and CH4 emissions study (Exp. 1)

Animals and experimental design

A replicated 4 × 4 Latin square experiment using 12 Canadian Arcott ram lambs (initial BW 34.5 ± 3.4 kg) was used to assess the impact of the 4 dietary treatments (3 lambs per treatment) on nutrient digestibility and CH_4 emissions over four 21-d periods. Lambs were grouped by weight and randomly assigned to a diet. The first 16 d of each period were used to adapt the lambs to the diet by providing them free access to feed and water in individual indoor pens (0.97 × 2.82 m) bedded with straw. Feed was delivered at 1000 h daily during the adaptation period. For determination of daily DMI, refusals were collected and weighed daily prior to feeding. On d 17, the lambs were moved to a controlled environment building and located in individual metabolism crates (0.112 × 0.92 m) located in 4 identical chambers (volume = 63.5 m^3; Model C1330; Conviron, Winnipeg, MB, Canada) for measurements total tract digestibility (McKeown et al., 2010) and CH_4 emissions. Each block of three lambs was housed in the same chamber during the trial. All lambs within a chamber received the same diet in each period. Immediately before feeding on d 17, the lambs were fitted with strap-on canvas feces collection bags. Feed offered was restricted to 90% of the intake

determined over the previous week and water was freely available. Feces samples and CH_4 measurement data were collected for 4 consecutive 24-h days starting at 1200 on d 17 and finishing at 1200 h on d 21. On the last day of each period, the lambs were removed from the chambers and transported to their individual pens in the barn for adaptation to the next diet. Interruptions to the chamber flux measurements occurred daily from 1006 to 1036 h to feed and water the lambs and collect feces samples.

Methane concentration measurements and sampling in the chambers

Chamber operation and measurements have been described in detail by McGinn et al. (2004; 2006) and Beauchemin and McGinn (2005). Briefly, the chambers were vented using individual fresh air intake and exhaust ducts (diameter 30.5 cm) with dedicated fans on each duct. Before calibration, flows were adjusted to generate a positive pressure (2 Pa or less) in each chamber. Fresh air intake flow (approximately 0.28 m^3/s) was fed into a recycling fan unit and air entered the chamber through 3 full width floor vents. The loop of recycled air passed through filters and a temperature controller to maintain air in the chamber at 15°C. Circulation within the chamber ensured that air leaving through the exhaust duct (approximately 0.22 m^3/s) was representative of the entire air volume of the chamber which was exchanged every 5 min. Air was pumped sequentially from each duct of each chamber at 1 L/min (TD3LS7; Brailsford and Company, Rye, NY) and passed through an infrared gas analyzer (Model Ultramat 5E; Siemens, Karlsruhe, Germany) via solenoids that were controlled by a datalogger (CR23X; Campbell scientific, Logan, UT). Air was dried using a Nafion® dryer (MD-110; Perma Pure, Tom Rivers, NJ) and magnesium perchlorate to a dew point of below

-50°C. To compensate for the volume in the sampling line, data were ignored for the first 3 min after switching the air stream. Afterwards, CH_4 concentration was recorded each min on the data logger. Each chamber was sampled for 7 to8 min with a cycle among the 4 chambers lasting 30 min. Each morning the analyzer was calibrated using reference gases, N_2 gas for the zero and primary standard CH_4 for the span. The difference between the intake and exhaust flow of CH_4 was used to calculate the amount of CH_4 produced by the lambs in each chamber according to the method described by Beauchemin and McGinn (2005). The emissions from the chambers were calibrated by releasing a known amount of CH_4 in each chamber and calculating the mass balance of incoming and outgoing amounts of CH_4 (McGinn et al., 2006). Cumulative daily CH_4 emissions were calculated for 4 days each period. The daily CH_4 flux was expressed per unit of DMI and digested DM for the three lambs in each chamber (i.e., chamber was the experimental unit). The daily CH_4 flux (13.3 Mcal/kg) determined for each chamber was also expressed as a proportion of GE intake and DE intake of the three lambs in each chamber.

Sample collection

Total tract digestibility of nutrients was determined during the 4 day collection phase in each period. The canvas fecal collection bags were lined with a pre-weighed plastic bag, which was changed daily between 1006 and 1036. Feces collected each day were weighed, mixed thoroughly by hand and duplicate subsamples representing 10% of daily fecal production from each lamb were retained. Samples from each lamb were combined within each period and stored at -20°C until analyzed for DM, CP, NDF, ADF and GE.

5.2.1.3 Growth study (Exp. 2)

Animals and experimental design

Sixty Canadian Arcott weaned lambs (23.03 ± 3.6 kg) were stratified by BW and randomly assigned to 1 of the 4 experimental diets. Lambs were adapted to the experimental diets for 2 weeks before the beginning of data collection. Lambs were housed in individual pens (0.97 × 2.82 m) bedded with straw, fed at 0900 daily, and weighed weekly. All lambs had *ad libitum* access to feed and water throughout the study. Feed deliveries were recorded daily. Refusals were collected daily and weighed weekly for determination of weekly DMI. Daily DMI by each lamb was estimated by summing the weekly intake and dividing by the number of days of the week. The ADG was determined by dividing weight gain (initial full BW − final full BW) by the number of days in the study. Feed conversion (g of BW gain/g of DMI) was calculated as the ratio between ADG and DMI.

Slaughter and sample collection

Lambs were slaughtered at a live weight of ≥ 45 kg, in two lots at a commercial abattoir (Sunterra Meats Ltd., Innisfail, AB, Canada). Data from 2 lambs were removed from the study due to health complications. Within 5 min of exsanguination, a fat sample (2 to 3 g) from the base of the tail was collected from each lamb. The samples were kept on ice and transported to the laboratory where

they were snap-frozen in liquid nitrogen and stored at -80°C until analyzed for fatty acids profiles. Hot carcass weights were recorded and grade rule (GR; body wall thickness) was determined from the total tissue depth of the carcass between the 12^{th} and 13^{th} rib at 11 cm from the carcass midline.

Chemical analyses

The DM concentration of the composited feed samples was determined at 135°C for 2 h (method 930.15; AOAC, 1995) followed by hot weighing and OM was determined by ashing the samples at 550°C for 5 h (method 942.05; AOAC, 1995). To determine CP (N × 6.25), feed samples were ground to a fine powder using a ball grinder (Mixer Mill MM200, Retsch Inc., Newtown, PA). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Nitrogen Analyzer 1500 series, Carlo Erba Instruments, Milan, Italy). Neutral detergent fiber was determined according to Van Soest et al. (1991) using heat stable α–amylase and sodium sulfite. Acid detergent fiber was determined according to the method 973.18 (AOAC, 1995). Gross energy was determined using an adiabatic calorimeter (model 1241, Parr, Moline, IL). Crude fat content was determined by ether extraction (AOAC 1995, method 920.39) using a Goldfisch Fat Extractor (Labconco Corporation, Kansas City, MO). Non-fibrous carbohydrate (NFC, % in the DM) was calculated as: 100-[CP + NDF + crude fat + ash] (Mertens 2002).

Lipid extraction and determination

Lipids were extracted from adipose tissue based on the method of Hara and Radin (1978). Unless otherwise stated, chemicals were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada). Briefly, samples (1 g of adipose tissue) were homogenized in 15 mL of GC grade 2-propanol using a tissue homogenizer set at 10,000 rpm (PRO 250, PRO Scientific Inc. Oxford, CT, USA). GC-grade hexane (10 mL) was added to the mixture before a second homogenization for 90 s. Samples were allowed to settle and lipids were collected from the upper hexane phase. Hexane extracts were evaporated under N_2 and lipids were stored at -80°C before methylation. Fatty acids were methylated and determined as described by He et al. (2012).

5.2.4 Statistical analyses

5.2.4.1 Digestibility and CH_4 emissions study (Exp. 1)

Data on nutrient intakes and digestibility were analyzed using the mixed procedure of SAS (SAS Inc., 2012; SAS Online Doc 9.1.3. Cary, NC, USA). Means were compared using the least squares mean linear hypothesis. The model included the fixed effects of treatment (diet), day and treatment by day interactions and the random effects of period (n = 4), chamber (group) and lamb nested within treatment as random effects with day of sampling within each period treated as repeated measure. The individual animal (n = 12) was the experimental unit for intake and nutrient digestibility because these data were obtained from individual lambs with separate access to feed. For CH_4 emissions, the model did not include the random effect of lamb as the chamber, (n = 4) representing data for three lambs was the experimental unit. The minimum values

of AIC (Akaike's Information Criterion) were used to select the covariance structure among compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, unstructured and banded for each parameter.

5.2.4.2 Growth study (Exp. 2)

For the DMI, ADG and feed efficiency initial and final LW, hot carcass weight, grading rule (GR) and fatty acid composition means (n = 15 per treatment) were compared using the least squares mean linear hypothesis test with treatment included as a fixed term (Chaves et al., 2008).

For both Exp. 1 and 2, significance was declared at a $P < 0.05$. Only when effect of treatment (diet) was significant ($P < 0.05$), orthogonal polynomial contrasts were used to determine linear and quadratic responses to the concentration of glycerol incorporation (0, 7, 14, and 21% of dietary DM) and 0% glycerol vs. glycerol addition for all variables. Otherwise, contrasts were not reported. None of the quadratic contrasts were significant and are thus not reported.

5.3 RESULTS

5.3.1 Digestion and CH_4 emissions study

Dry matter, ADF and GE intakes were not affected *(P* = 0.12) by glycerol addition in the digestibility study (Table 2). Intakes of NDF and CP tended to be lower in the 21% glycerol treated lambs (*P* = 0.10 and 0.06 respectively), likely because of numerically reduced intakes. Nutrient (CP, NDF, ADF and DE) digestibility was not affected (*P* = 0.16) by inclusion of glycerol in the diets.

Methane emissions corrected for DMI or digested DM were not affected (*P* = 0.23) by the inclusion of glycerol in the diets (Table 3). When expressed as percentage of GE or DE intake, CH_4 emissions were numerically lower in the higher glycerol treated lambs (14 and 21%) than the control and 7% treated lambs.

5.3.2 Growth study

The inclusion of glycerol in the diets linearly reduced DMI (*P* = 0.01) and tended to reduce ADG (*P* = 0.06) and final weights (*P* = 0.07). Feed efficiency was not affected by the treatments (*P* = 0.76). Hot carcass weight, dressing and GR were not affected by glycerol inclusion in the diets (Table 4).

Glycerol did not modify (*P* = 0.85) the proportion of total saturated fatty acids (SFA) in subcutaneous tail fat (Table 5), but there was a linear reduction (*P* < 0.01) in the concentrations of palmitic acid (16:0) and a linear increase (*P* < 0.01) in the concentrations of stearic acid (18:0). Glycerol inclusion in the diets linearly increased (*P* < 0.01) the proportion of oleic acid (9*c*-C18:1) and reduced (*P* < 0.01) the proportion of 10*t*-18:1 but did not affect (*P* = 0.77) the proportion of

total monounsaturated fatty acid (MUFA). Linoleic acid (18:2 n-6) and the total proportion of polyunsaturated fatty acids (PUFA) declined linearly ($P < 0.01$) with increasing concentrations of glycerol in the diet. Conjugated linoleic acids (9*c*,11*t*-CLA and 10*t*,12*t*CLA) were not affected ($P = 0.41$) by the diets. Total *trans* FA proportions were linearly decreased ($P < 0.01$). The ratio of PUFA/SFA tended to decrease ($P = 0.08$) and n-6/n-3 ratio linearly declined ($P < 0.01$) by increasing glycerol inclusion in the diets.

5.4 DISCUSSION

5.4.1 Digestibility and CH_4 emissions

Lack of effect of glycerol inclusion in the diet of lambs on nutrient intakes and digestibility is consistent with previous literature. Rémond et al. (1993) found no difference in fermented organic matter when glycerol was added to a starch substrate in continuous culture conditions, but demonstrated a slight increase in digestibility when the substrate was cellulose. No differences in nutrient digestibility were reported when crude glycerin replaced alfalfa in cattle diets (Schröder and Südekum, 1999) or wheat grain under *in vitro* conditions (Krueger et al., 2010). In contrast, Wang et al. (2009) reported increased DM digestibility with glycerol inclusion in cattle forage diets at concentrations of 0 to 3.3% of DM and Avila et al. (2011) reported linear increases in IVDMD when glycerol was included at concentrations of 0 to 21% DM as replacement of barley grain in 50% barley grain-50% barley silage based feedlot cattle diets. Based on this evidence, it is most likely that glycerol may enhance digestibility in forage diets, but may have marginal impact on digestibility of highly fermentable feedlot diets.

The lack of effect of glycerol inclusion in the diet on CH_4 emissions was unexpected. The emissions measured in our study are low in all treatments if compared with previous reports from sheep. Methane emissions of 11.5 to 25.7 g/kg DMI were reported for forage diets fed to sheep (Waghorn et al., 2002) and emissions of 14.9-15.3 g/kg DMI were reported from grazing lambs (Ulyatt et al., 1997). When expressed as % of GE loss, values reported here are lower than those suggested by IPCC (2006) for growing lambs on pasture (4.5% of GE) and more comparable to those of finishing feedlot cattle (3% ± 1%). Beauchemin and McGinn (2005) reported 2.81 and 4.03% of GE losses and emissions of 9.2 and 13.1 g CH_4/kg DMI from corn and barley based finishing cattle diets. Sheep have been reported to lose 4.6 and 3.6% of GE as CH_4 when grazing pastures with 75% and 81% DM digestibility respectively (Lassey et al., 1997; Judd et al., 1999). The low CH_4 emission values in this study may be attributable in part to the low NDF content of the diets and the use of pelleted diets. Greater NDF concentrations in feed resulted in greater CH_4 emissions and pelleted alfalfa produced 24% less CH_4 compared to fresh cut alfalfa in rams (Waghorn et al., 2002). Additionally, the ground pelleted diets might have contributed to a high fractional outflow rate which has been reported to have an important negative correlation with GE loss in the form of CH_4 (Pinares-Patiño et al., 2003).

We initially hypothesized that the propiogenic properties of glycerol would act as a hydrogen sink in the rumen and thus reduce CH_4 emissions. Glycerol fermentation to propionate does not constitute a hydrogen sink in itself, as glycerol has to donate electrons before entering glycolysis (Ungerfeld and Foster, 2011; Zang and Yang, 2009). However, we did expect it to alter the rumen environment in a manner that would favour the fermentation of carbohydrates to

propionate rather than acetate, thereby reducing CH_4 production. The lack of effect of glycerol on CH_4 in this study, together with results from previous reports, suggest that the shift towards propionate fermentation may be more likely to occur in high forage (Wang et al., 2009; Avila et al., 2011; Lee et al., 2011) than in high grain diets (Mach et al., 2009).

5.4.2 Growth performance

The linear decrease in DMI with increasing concentrations of glycerol in the diets is in accordance with previous studies (Musselman et al., 2008; Gunn et al., 2010b) in which replacing corn with increasing concentrations of crude glycerin (0-45% of DM) linearly decreased DMI when concentrations exceeded 15% of the dietary DM. Parsons et al. (2009) also reported linear decreases of intake when crude glycerin was included at more than 2% of the diet fed to finishing beef heifers. However, a previous study with steers found no effects on DMI when including crude glycerin (0-12% of DM) as a replacement of barley grain in a barley-corn grain based diet (Mach et al., 2009). Likewise, Gunn et al. (2010a) reported no changes in DMI when increasing concentrations of crude glycerin (0-20% of DM) were used to replace dry rolled corn in lamb diets.

The trend (P = 0.06) to reduced ADG with increasing concentrations of glycerol in the diets (ADG (g/d) = -2.06 × (% glycerol in diet DM) + 341.6; r^2 = 0.49) is likely due to reductions in DMI. The 12.4% reduction in DMI between the 7% and the 21% glycerol diets resulted in a 15.8% reduction in ADG. Decreases in ADG were reported when glycerol replaced 15% or more of rolled corn grain DM in diet for lambs (Musselmann et al., 2008; Gunn et al., 2010b). These authors report reductions of about 39% in ADG as a result of adding glycerin at levels up

to 45% of diet DM. A quadratic effect was reported when including crude glycerin (0, 2, 4, 8, 12, and 16% of DM) in finishing heifer diets as a replacement for steam rolled corn (Parsons et al., 2009). These authors report a 23.1% reduction in ADG associated with a 12.2% reduction in DMI between diets that contained 2% or 16% glycerol. Results presented here seem to concur with those of Parsons et al. (2009) who reported increased ADG in finishing cattle fed crude glycerin at up to 8% of dietary DM, but ADG decreased when glycerin accounted for 12 or 16% diet DM. Gunn et al. (2010a) suggested that reduced weight gain at concentrations above 15% could be due to an altered ruminal environment stemming from reduced pH and lower cellulolytic activity and thus, reduced DMI. However, previous studies in cattle using crude glycerin as a replacement of barley (Mach et al., 2009), and *in vitro* studies with glycerol incubated with corn and alfalfa (Lee et al., 2011) have reported nil or only slight reductions in pH. No effects of crude glycerin addition on ADG were reported in the aforementioned studies of Mach et al. (2009) in cattle and Gunn et al. (2010a) in lamb diets.

The lack of effect of treatments on feed efficiency is most probably explained by lower ADG in treatments with lower DMI. Similarly, feeding crude glycerin up to 12% DM did not affect feed efficiency in cattle (Mach et al., 2009). Similar results were also obtained by Gunn et al. (2010a) using up to 20% crude glycerin in feedlot diets for lambs. Other studies report linear decreases in feed efficiency when crude glycerin was included in concentrations over 15% DM (Musselman et al., 2008; Gunn et al., 2010b).

The lack of effects of glycerol inclusion on hot carcass weight, dressing percentage and grading rule are in accordance with previous reports in lambs (Gunn et al., 2010a) and beef cattle (Mach et al., 2009) who found no effects on

carcass traits when replacing corn and barley grain with crude glycerin in concentrations of up to 20 and 16% of DM, respectively.

5.4.3 Fatty acid composition

The shift in total SFA towards a reduced palmitic acid (16:0) and increased stearic acid (18:0) is interesting since palmitic acid has been considered detrimental to serum cholesterol levels and stearic acid has been shown to have a net neutral impact on serum cholesterol in humans (Yu et al., 1995). This pattern of saturated fatty acids has been described as more likely to be present in grass fed than grain fed cattle (Daley et al., 2010), because grass diets are richer in stearic and linoleic acid and cereal grain diets are richer in palmitic acid. Since the diets in this study were formulated to be isonitrogenous and isofibrous, with increasing concentrations of alfalfa (6.3% DM in the control treatment to 16.8% DM in the 21% glycerol treatment), it is yet to be clarified if this change is attributable to the changes in rumen fermentation pattern due to different concentrations of glycerol, the reduction in barley grain or changes in other ingredients of the diet.

Because 10*t*-18:1 was the major 18:1 *trans* fatty acid in our study, the linear reduction in proportions of 10*t*-18:1 implied linear reductions in the total *trans* and *trans*18:1 fatty acids. Previous studies (Dugan et al., 2007; Aldai et al., 2008) have reported that 10*t*-18:1 is the major 18:1 *trans* fatty acid in beef from cattle fed diets high in barley (i.e., low fiber) and this FA has been associated with increased risk of coronary heart disease in humans (Hodgson et al., 1996) and negative effects on plasma lipid profiles in rabbits (Roy et al., 2007). Previous reports (Bauman and Grinarii, 2003; Kramer et al., 2004; Mohammed et al., 2010) attributed this increase in 10*t*-18:1 to lower ruminal pH with a consequent

alteration of rumen bacterial flora that shifts lipid biohydrogenation pathways mainly from *Butyrivibrio fibrisolvens,* which forms the *t*11 double bond in vaccenic acid to *Megasphaera elsdenii*, which forms the *t*10 double bond, resulting in production of 10*t*-18:1 instead of vaccenic acid (11*t*-18:1). A fraction of the 10*t*-18:1 produced under these conditions will reach the tissues were it cannot be desaturated to the *t*10,*c*12-18:2 CLA (Kramer et al., 2004). Although in our study 11*t*-18:1 was not modified by diet, these findings support a previous report (Krehbiel, 2008) stating that an important fraction (> 50%) of glycerol is evacuated from the rumen through passage or absorption through the rumen wall (Rémond et al, 1993) and is thus not fermented to propionate in the rumen environment. Studies with dried distillers grains with solubles (DDGS) have reported reductions of 10*t*-18:1 when wheat DDGS (Dugan et al., 2010) or triticale DDGS (McKeown et al., 2010) were included as replacement of barley grain. Future research testing the effects of different sources and concentrations of DDGS in combination with glycerol on overall *trans* fatty acids in ruminants will be necessary to determine optimal inclusions of these ingredients.

Oleic acid has been reported to be the most abundant fatty acid in beef (Turk and Smith, 2009) and lamb (Diaz et al., 2005). The increase of this fatty acid has been associated with greater beneficial HDL cholesterol (HDL-C) plasma concentrations in humans (Gilmore et al., 2011). Glycerol addition to the diet linearly reduced the proportions of linoleic acid (18:2 n-6) and thus led to linear reductions in the total PUFA proportions and tended to reduce the PUFA/SFA ratio (P = 0.08). These results are in accordance with Diaz et al., (2005) who found a negative correlation between linoleic and oleic acid. This is attributable to the inhibiting effect of linoleic acid on of Δ9-desaturase, the enzyme responsible

for oleic acid synthesis (Jeffcoat and James, 1984). In our study, the linoleic acid content of the diets increased with greater glycerol concentrations. High concentrate diets are associated with lower ruminal pH, which decreases hydrogenase activity, producing less conversion of linoleic to stearic acid (Tove and Matrone, 1962). These changes in fatty acid profiles further suggest that animals fed glycerol added diets had better ruminal pH probably due in part to partial escape of glycerol from rumen fermentation. The n-6/n-3 ratio was linearly reduced from 7.57 to 5.93, but was still above the recommended ratio of 5:1 or less (World Health Organization, 2003).

The only previous study assessing crude glycerin effects on fatty acid composition of meat (Terré et al., 2011) found no differences in fatty acid profiles of light lambs fed 0, 5 or 10% glycerol. These authors used a 4 week feeding period and finished the lambs at a very young age (4 weeks after weaning) and low live weights (24.5 ± 0.4 kg), which might not be long enough to find differences in fatty acid profiles.

The lack of effects on diet digestibility and methane emissions contrasts with our initial hypothesis. However, results from the lamb performance and fatty acid profiles experiment support the hypothesis that glycerol does not affect lamb performance or carcass traits and has the potential to beneficially affect fatty acid profiles from subcutaneous adipose tissue in lambs.

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Table 5.1 Ingredients and chemical composition of the diets containing increasing amounts of glycerol

	Glycerol, % of dietary DM			
Ingredient	0	7	14	21
Dry rolled barley	57.0	45.0	32.5	20.4
Glycerol	0	7	14	21
Wheat DDGS[1]	14.5	16.5	18.7	21.0
Sunflower hulls	13	13	13	13
Beet pulp dehydrated	6.5	5.5	5.5	4.8
Alfalfa meal	6.3	10.1	13.3	16.8
Canola oil	0	0.1	0.2	0.3
Calcium carbonate	1.1	1.1	1.1	1.1
Mineral premix[2]	1.2	1.2	1.2	1.2
Ammonium chloride	0.4	0.4	0.4	0.4
ADE vitamin mix [3]	0.02	0.02	0.02	0.02
Chemical composition,% DM				
Organic matter	92.8	92.6	92.3	92.3
NDF	25.5	25.7	24.4	25.4
ADF	13.9	15.2	15.2	15.5
CP	16.2	16.3	15.9	16.0
Ether extract	3.3	3.9	3.6	4.0
Non fibrous carbohydrates	47.7	46.7	48.4	46.8
GE, kcal/g	4.33	4.53	4.47	4.55
Fatty acids, % of total FAME				
C16:0	28.6	17.6	15.6	15.2
C18:0	6.9	4.6	4.5	4.5
c9-C18:1	31.3	25.9	25.2	25.5
c11-C18:1	1.2	0.9	0.8	1.1
c9,c12-C18:2	26.5	44.1	45.9	45.5
C18:3	1.9	4.2	5.3	5.8
Saturated	36.3	22.5	20.5	20.0
Unsaturated	63.7	77.5	79.5	80.0
Monounsaturated	33.7	28.0	27.0	27.5
Polyunsaturated	30.0	49.5	52.5	52.5

[1]DDGS, Dry distillers grains with solubles

[2]Containing 92.6% NaCl, 4.97% Dynamate®; 0.9% ZnSO4; 0.83% MnSO4; 0.13% CuSO4; 0.1% ethylenediamine dihydroiodide, 80% preparation; 0.005% CoSO4; 0.4% canola oil (as carrier of $CoSO_4$); and 0.0014 % Na_2SeO_3. No ionophores were included in the diet.

[3]Containing vitamin A (10 000 000 IU/kg); vitamin D (1 000 000 IU/kg); and vitamin E (10 000 IU/kg).

Table 5.2 Effects of increasing concentrations of glycerol in the diet on dry matter intake and total tract nutrient digestibility of lambs housed in chambers (Exp. 1; n = 12)[1]

	Glycerol, % dietary DM					*P*-values
Item	0	7	14	21	SEM	Diet
Intake, g/d						
DM	1,324	1,362	1,354	1,213	0.12	0.12
CP	215	222	212	195	0.01	0.10
NDF	351	354	327	316	0.13	0.06
ADF	191	209	205	194	0.20	0.20
GE, Mcal/d	5.8	6.2	6.1	5.5	0.38	0.16
Digestibility, %						
DM	70.3	71.3	72.1	73.1	1.69	0.55
CP	68.0	68.8	67.2	69.8	1.79	0.62
NDF	39.2	38.9	35.5	41.6	2.48	0.22
ADF	26.1	34.1	29.6	32.3	2.58	0.16
DE	63.3	72.3	72.3	74.3	4.40	0.23

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; GE, gross energy; DE, digestible energy.

[1]Values determined for 4 d during which the animals were in the chambers.

Table 5.3 Effects of increasing concentrations of glycerol in the diet on CH_4 emissions of lambs housed in chambers (Exp. 1; n = 4)[1]

	Glycerol, % of dietary DM					*P*-values
CH_4	0	7	14	21	SEM	Diet
g/lamb.d	11.0	12.7	9.4	8.5	1.55	0.28
g/kg of DMI	8.1	8.8	6.9	7.2	0.82	0.23
g/kg of DDM[2]	11.1	11.3	9.3	10.5	0.92	0.45
% of GE intake[3]	2.6	3.0	2.2	2.0	0.34	0.15
% of DE intake	3.5	4.1	3.0	2.8	0.47	0.18

DMI, dry matter intake; DE, digestible energy.

[1]values determined for 4 d during which the animals were in the chambers.

[2]g CH_4/kg digested dry matter.

[3]percentage of gross energy intake lost as CH_4.

Table 5.4 Effects of increasing concentrations of glycerol in the diet on growth performance of lambs (Exp. 2; n = 15)

	Glycerol, % Dietary DM					*P*-values[1]	
Component	0	7	14	21	SEM	Diet	Linear[2]
DM intake, g/d	1,292	1,352	1,225	1,185	38.9	0.02	0.01
Average daily gain, g	328	354	300	298	16.7	0.06	
Gain:feed, g/g	0.26	0.27	0.24	0.25	0.013	0.60	-
Final weight, kg	52.0	53.0	49.7	50.0	1.01	0.07	-
Hot carcass weight, kg	26.1	26.9	25.8	25.8	0.49	0.29	-
Dressing, kg/kg	0.50	0.51	0.52	0.52	0.050	0.12	-
Grading rule, mm[3]	20.9	22.3	20.9	20.1	0.77	0.24	-

DM, dry matter

[1] When fixed effects of diet are not significant ($P > 0.05$), P values for contrasts are not reported.

[2]Linear effects of increasing concentrations of glycerol.

[3]Grading rule: body wall thickness between 12th and 13th rib, 11 cm from the carcass midline.

Table 5.5 Effects of increasing concentrations of glycerol in the diet on fatty acid (FA) profiles (% of total FA) in subcutaneous tail fat of lambs (Exp. 2; n = 15)

	Glycerol, % Dietary DM					*P*-values[1]		
Component	0	7	14	21	SEM	Diet	Linear[2]	0 vs.Glycerol
SFA								
10:0	0.35	0.31	0.30	0.24	0.034	0.09	-	-
12:0	0.15	0.15	0.17	0.14	0.026	0.80	-	-
14:0	2.61	2.38	1.72	1.68	0.295	0.06	-	-
15:0	1.13	1.26	1.33	1.31	0.101	0.52	-	-
16:0	20.9	20.1	19.3	18.1	0.589	0.03	<0.01	0.02
17:0	2.69	3.21	3.92	3.86	0.266	<0.01	<0.01	<0.01
18:0	11.4	11.4	13.1	13.7	0.684	0.03	<0.01	0.07
Total SFA	39.2	38.8	39.9	39.4	0.846	0.85	-	-
MUFA								
*c*9-14:1	0.11	0.09	0.10	0.88	0.011	0.46	-	-
*c*9-16:1	1.46	1.35	1.33	0.28	0.086	0.50	-	-
*t*6-*t*8-18:1	0.61	0.89	0.62	0.59	0.140	0.45	-	-
*t*9-18:1	0.58	0.50	0.49	0.49	0.036	0.11	-	-
*t*10-18:1	9.80	9.33	7.77	7.60	0.655	0.05	<0.01	0.05
*t*11-18:1	1.48	1.51	1.30	1.47	0.153	0.76	-	-
*c*9- 18:1	35.5	37.0	38.4	38.8	0.813	0.02	<0.01	<0.01
*c*11-18:1	1.12	1.04	1.05	1.07	0.036	0.35	-	-
*c*9-20:1	0.24	0.29	0.30	0.34	0.016	<0.01	<0.01	<0.01
Total MUFA	50.9	52.0	51.4	51.6	0.804	0.77	-	-
PUFA								

18:2 n-6	7.85	7.06	6.07	6.39	0.380	<0.01	<0.01	<0.01
18:3 n-3	0.90	0.89	0.89	0.93	0.046	0.88	-	-
CLA *c*9,*t*11-18:2	0.85	0.88	0.80	0.82	0.056	0.72	-	-
CLA *t*10,*t*12-18:2	0.07	0.06	0.07	0.04	0.013	0.41	-	-
20:4 n-6	0.18	0.18	0.17	0.18	0.014	0.98	-	-
20:5 n-3 EPA	0.03	0.05	0.05	0.03	0.014	0.47	-	-
22:5 n-3 DPA	0.14	0.12	0.12	0.13	0.015	0.85	-	-
22:6 n-3 DHA	0.02	0.03	0.02	0.03	0.006	0.72	-	-
Total PUFA	10.0	9.3	8.2	8.6	0.429	0.02	<0.01	<0.01
Total UFA[3]	60.9	61.2	59.6	60.3	0.955	0.63	-	-
PUFA/SFA	0.26	0.24	0.21	0.22	0.014	0.08	-	-
Trans FA[4]	13.4	13.1	11.1	10.0	0.762	0.05	<0.01	0.07
CLA+VA	2.40	2.44	2.17	2.34	0.198	0.77	-	-
n-3 FA[5]	1.08	1.08	1.08	1.11	0.055	0.96	-	-
Trans - (CLA+VA)	11.0	10.7	8.90	8.70	0.732	0.05	<0.01	0.07
C18:1 *trans*[6]	12.4	12.2	10.2	10.2	0.724	0.05	0.01	0.07
n-6/n-3	7.57	6.80	5.88	5.93	0.268	<0.01	<0.01	<0.01

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; UFA, unsaturated fatty acid; CLA, conjugated linoleic acid, VA, vaccenic acid, C18:1 *t*11.

[1] When fixed effects of diet are not significant ($P > 0.05$), values for contrasts are not reported.

[2]Linear effects of increasing concentrations of glycerol.

[3]UFA: unsaturated fatty acids = MUFA + PUFA.

[4]*Trans* FA = C18:1 *t*6-8 + C18:1 *t*9 + C18:1 *t*10 + C18:1 *t*11 (VA) + 18:2 c9-*t*11 (CLA) + 18 :2 *t*10-*t*12 (CLA).

[5]n-3 FA = C18 :3 + EPA + DHA + DHA.

[6]C18:1 *trans* = C18:1 *t*6-8 + C18:1 *t*9 + C18:1 *t*10 + C18:1 *t*11 (VA).

Chapter 6. Effects of replacing of replacing barley grain with wheat dry distillers' grains on growth performance, eating behaviour and subcutaneous fatty acid profiles of lambs.

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Effects of replacing barley grain with wheat dry distillers' grains on growth performance, eating behaviour and subcutaneous fatty acid profiles of lambs.

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Effects of replacing of replacing barley grain with wheat dry distillers' grains on growth performance, eating behaviour and subcutaneous fatty acid profiles of lambs.

Abstract

The hypothesis of this study was that wheat dry distillers' grains with solubles (WDDGS) could improve performance and fatty acid profiles of lambs. As such, this study aimed to assess the effects of replacing barley grain with increasing concentrations of WDDGS on the growth performance and fatty acid profile of adipose tissue in lambs. Increasing concentration of WDDGS was achieved by replacing barley grain with WDDGS. Thirty nine weaned crossbred lambs were completely randomized by weight and assigned to three dietary treatments (Control, 200 g/kg WDDGS and 400 g/kg WDDGS). Increasing WDDGS in the diet affected eating rate, total daily intake and average daily gain. Total saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in subcutaneous fat were unaltered but proportions of linolenic acid were increased. This study suggests that a concentrate diet containing 400 g/kg WDDGS fed to lambs as replacement of barley grain, improved lamb intake and growth performance as compared to a barley-based diet.

Keywords: ethanol by-products, linolenic acid, sheep, wheat DDGS.

6.1 Introduction

The use of dry distillers' grains with solubles as a feed ingredient for ruminants has increased with the expansion of the bioethanol industry. In areas where corn cannot be grown due to climate or water limitations, wheat is utilized as a primary source of starch for ethanol production. The resulting by-product, wheat dry distillers' grain with solubles (WDDGS), has been evaluated in beef cattle diets with variable results. The impact of replacing increasing amounts of WDDGS for barley grain has resulted in improved feed efficiency when used in beef growing diets (McKinnon and Walker, 2008; Beliveau and McKinnon, 2008), but no effect (Walter et al., 2010; Beliveau and McKinnon, 2008) or a reduction in this parameter (Gibb et al., 2008) have been reported for finishing diets suggesting that interactions of WDDGS with other dietary ingredients or fibrous components of the diet may play an important role in the overall animal performance results.

The use of increasing concentrations of WDDGS as replacement of barley grain has resulted in an improvement of *trans* fatty acid profiles in beef mainly by increasing vaccenic and conjugated linoleic acids and decreasing *trans*-10-octadecenoic acid (Dugan et al., 2010). Increases in time spent eating and eating rates have been reported in beef cattle when WDDGS replace all or a portion of the barley silage in a finishing diet (Yang et al., 2012), but no effects were observed when it only replaced barley grain (Beliveau and McKinnon, 2009).

The effect of WDDGS on growth performance in lambs has only been examined in a few studies. McKeown et al. (2010a) reported that the addition of 200 g/kg of a WDDGS to a barley-based lamb finishing diet decreased feed efficiency

while causing only minor changes in the fatty acid profiles of adipose tissue. Finishing lambs also have higher protein requirements than beef and may benefit from the higher protein content in WDDGS as compared to barley.

We hypothesized that replacement of barley grain with WDDGS in lamb finishing diets can improve subcutaneous fatty acid composition without affecting growth performance. The present study aimed to investigate the effect of pelleted total mixed rations where 0, 200 or 400 g/kg (DM basis) WDDGS was substituted for rolled barley grain on eating behaviour, feed intake, growth performance, carcass traits and adipose tissue fatty acid profiles of crossbred lambs.

6.2 Materials and methods

All experiments were conducted at Mayfarm (S34^{o}3', E150^{o}38') on the Camden Campus of the University of Sydney, with all lambs being cared for in accordance with the guidelines of the University of Sydney Research Integrity Animal Ethics Committee approval N00/5-2010/3/5318.

6.2.1 Dietary treatments

The diets were formulated using the Small Ruminant Nutrition System (Cannas et al., 2004) to meet or exceed the requirements of growing lambs of NRC (2007). The control diet contained 629 g/kg barley grain, 300 g/kg alfalfa meal, 25 g/kg molasses, and 37 g/kg mineral and vitamin additives (Table 1). Treatment diets were formulated by substituting WDDGS for barley grain to achieve concentrations of 200 WDDGS and 400 WDDGS g/kg (DM basis). Wheat DDGS

was obtained from the Manildra Group ethanol distillery (Nowra, NSW, Australia). Fatty acids and amino acids profile of WDDGS are presented in Tables 1 and 2, respectively. Ingredients and chemical composition of the diets are reported in Table 3. All ingredients were ground through a 1 mm screen, mixed and pelleted.

6.2.2 Animals and experimental design

Thirty nine Dorper/Merino weaned lambs (29.3 ± 1.3 kg) were stratified by body weight (BW) and randomly assigned to 1 of 3 experimental diets (Control, 200 and 400 g WDDGS/kg DM). Lambs were separated into 1 of 3 pens (60 m^2 each) bedded with wood chips and adapted to the experimental diets for 10 days before the beginning of data collection. All animals had *ad libitum* access to feed and water at all times throughout the study.

Diets were delivered to the lambs by three automatic feeders (ARF, Agricultural Requirements, Gatton, QLD, AU) within each pen for each treatment group. The animals were tagged with electronic transponders (Allflex, Dallas, TX, USA) that enabled recording of feeding behaviour data. All automatic feeders recorded date, time, feed consumed and time spent eating for each visit of each individual lamb throughout the day. This allowed recording and calculations of frequency of daily visits to the feeder (visits/day), daily duration of feeder attendance (min/d), individual feed intake, eating rate (g /min) and average duration of each visit (sec/visit). Methodologies of automatic feeders are described in detail by Meale et al. (2013).

6.2.3 Growth performance, slaughter and sample collection

Diets were sampled weekly and analyzed for DM at 55°C for 48 h. Samples were pooled biweekly and stored at room temperature for chemical analyses. Dry matter intakes (DMI) were calculated from the total amount of feed released by the automatic feeders in all feeding sessions of each sheep and then divided by the DM content of the ration. Eating rate was calculated by dividing feed intake by the duration of each visit to the feeder. The lambs were weighed weekly (PLUS 2000, Blue Heeler, Tamworth, NSW, Australia) until the end of the experiment. Average daily gain (ADG) was calculated by dividing the weight gain during the trial by the total number of days of the experiment. Feed conversion was calculated as the ratio between ADG and DMI (g of BW gain/g of DMI). All lambs were slaughtered at a commercial abattoir (Wollondilly Abattoir Co-Op Ltd, Tahmoor, NSW, Australia) after 54 d at a final BW of 45 ± 7.5 kg. Hot carcass weight was recorded for each lamb. Within 5 min of exsanguination, a fat sample (2-3 g) was collected from the base of the tail of each lamb. These samples were kept on ice and transported back to the laboratory where they were frozen in liquid nitrogen and stored at -80°C until analyzed for fatty acids.

6.2.4 Chemical analyses

Duplicate samples of each diet were analysed for neutral detergent fibre (NDF) using Van Soest et al. (1991) procedure modified for an Ankom 200/220 Fibre Analyser (Ankom Technol. Corp., Fairport, NY, USA), with heat-stable α-amylase and sodium sulphide included. Acid detergent fibre (ADF) was determined using the method no. 973.18 of AOAC (2006) procedure modified for

an Ankom 200/220 Fibre Analyser (Ankom Technol. Corp., Fairport, NY, USA). Concentrations of NDF and ADF were expressed inclusive of residual ash. Ash content was determined after oxidation at 600°C for 2 h in a muffle furnace using method 942.05 (AOAC, 2006). Samples were re-ground using a ball grinder (Mixer Mill MM2000, Retsch, Haan, Germany) for determination of nitrogen (N) by combustion analysis using method 990.03 (AOAC, 2006) and a Leco Nitrogen Determinator (FP-428, Leco® Corporation, St. Joseph, MI, USA). Crude protein content was estimated as 6.25 × N content of the sample. Ether extract (EE) content was determined by extraction with diethyl ether [method 920.39; (AOAC, 2006)] as modified for an Ankom XT10 Extraction System (Ankom® Technol. Corp., Fairport, NY, USA). Non-fibrous carbohydrate (NFC) was calculated as: NFC (g/kg in the DM) = 1000 - [CP+NDF+EE+ash] (Mertens, 2002).

6.2.5 Lipid extraction

Lipids were extracted from adipose tissue as well as WDDGS based on the method of Hara and Radin (1978). Unless otherwise stated, chemicals were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada). Briefly, samples (1 g of adipose tissue) were homogenized in 15 mL of 2-propanol using a tissue homogenizer set at 10,000 rpm (PRO 250, PRO Scientific Inc. Oxford, CT, USA). Hexane (10 mL) was added to the mixture prior to a second homogenation for 90 s. Samples were allowed to settle and lipids were collected from the upper hexane phase. Hexane extracts were evaporated under N_2 and lipids were stored at -80°C until methylated.

6.2.6 Methylation and determination of fatty acids

Nonadecanoic acid (C19:0) methyl ester (15 µL, 29.8 mg/mL hexane, Nu-Check Prep Inc., MN, USA) was added to the lipid samples as an internal standard. Fatty acids were methylated using a combined acid-base procedure (Lock and Garnsworthy, 2002). Sodium methoxide (2 mL, 0.5 mmol/L in methanol) was added to 15 mg of the previously extracted lipids in a 15 ml tube and mixed. Tubes were placed in a water bath at 50°C for 10 min; thereafter, 1 mL of 14% boron trifluoride in methanol was added and tubes were incubated for an additional 10 min. After cooling, ultra-pure water (5 mL) and hexane (5 mL) were mixed with the solution and samples were allowed to stand for 10 min prior to extraction of the upper layer for fatty acid determination. Fatty acid methyl esters (FAME) were quantified using a gas chromatograph (Hewlett Packard GC System 6890, Mississauga, ON, Canada) equipped with a flame ionization detector and SP-2560 fused silica capillary column (75 m × 0.18 mm × 0.14 µm; Supelco Inc., Oakville, ON, Canada). To obtain fatty acid profiles, hexane extracts (1 µL) were injected using a 20:1 split. The initial oven temperature of 50°C was held for 5 min, increased by 15°C/min to 155°C, held constant for 56 min and then increased by 10°C/min to 240°C, and finally held for an additional 15 min (He et al., 2012). Hydrogen was used as the carrier gas (head pressure 112 kPa and flow rate of 0.3 mL/min) and He was used as the make-up gas (10 mL/min). Peaks in chromatograms were identified and quantified using pure methyl ester standards (Sigma-Aldrich Inc.). The identification of *trans* C18:1 isomers and CLA (combined C18:2 *cis*-9,*trans*-11 and *trans*-7,*cis*-9) were based on relative retention times of previous reports (Cruz-Hernandez et al., 2004; He et al., 2012; Avila-Stagno et al., 2013).

6.2.7 Statistical analyses

Data were analyzed using the Mixed procedure of SAS (2013). For eating behavior (total DMI, eating time, eating rate, visits/day, time per visit and intake per visit), means were compared using the least squares mean linear hypothesis test with treatment, week, and treatment per week interactions included as fixed terms and lambs within treatment as a random effect, considering the individual lamb as the experimental unit. Repeated measures analysis with the minimum values of AIC (Akaike's information Criterion) were used for selecting covariance structure (Chaves et al., 2008). For total DMI, ADG, feed efficiency, initial and final LW, hot carcass weight, and fatty acid composition, means were compared using the least squares mean linear hypothesis test using the same model as DMI but not considering repeated measures.

Significance was declared when $P \leq 0.05$ and a trend reported when $0.05 < P < 0.10$. One lamb from the 200 g/kg WWDGS group went off feed for 1 week and was excluded from the experiment.

6.3 Results

Major fatty acids in WDDGS included C18:2 n-6 (linoleic acid; 48.08 g/100 g), C16:0 (palmitic; 22.04 g/100 g) and the C18:1 *cis* +*trans* group (21.30 g/100 g) accounting for 91.4 g/100 g of all fatty acids (Table 6.1). The amino acid composition of WDDGS indicated that glutamic acid, alanine and leucine (18.6, 10.0 and 9.6 moles/100) were the most abundant amino acids in WDDGS (Table 6.2).

6.3.1 Growth performance and eating behaviour

Total dry matter intake (DMI) and average daily gain (ADG) were increased by WDDGS (P =0.02) and final weights were increased (P = 0.02) in the 400g/kg WDDGS group as compared to the control and 200g/kg WDDGS group, but feed efficiency was not affected among groups (P = 0.62; Table 6.4). Hot carcass weight tended to be higher (P = 0.06) in the 400 g/kg WDDGS group as compared to the control and 200 g/kg WDDGS groups. Carcass dressing percentage was not affected by the diets (0.48 ± 0.01; P = 0.89).

Daily eating time was similar among treatments (P = 0.26; Table 6.5) but eating rate was reduced in the 200g/kg group and increased in the 400g/kg as compared to control (P < 0.01). The number of daily visits to the feeders was increased (P = 0.01) in the 400g/kg WDDGS group, but the average time spent per visit was reduced from 57.7 to 30.7 ± 5.61 seconds in the control and 400 g/kg WDDGS groups respectively (P < 0.01). The feed intake per visit to the feeder was reduced (P < 0.01) in both WDDGS supplemented groups (16.6, 12.8 and 11.2 ± 0.85 g for control, 200 and 400 g/kg WDDGS groups respectively; Table 6.5) .

6.3.2 Fatty acids profiles

Wheat DDGS did not modify (P = 0.30) the proportions of total saturated fatty acids (SFA) in lamb tail backfat (52.04 ± 0.794 g/100 FAME; Table 6.6), but proportions of palmitic acid (16:0) were reduced (P = 0.02). Total proportions of monounsaturated fatty acids (MUFA) were not affected by the diets but there was a trend (P = 0.07) for vaccenic acid (VA, *t*11-18:1) to be increased. Proportions of conjugated linoleic acids (CLA) were not modified by WDDGS, but CLA+VA tended to increase (P = 0.07) with increasing concentrations of WDDGS in the

diet. Proportions of linolenic acid (18:3 n-3) were quadratically increased by WDDGS, but this did not modify the n-6/n-3 ratio (5.32 ± 0.389; $P = 0.19$), levels of total polyunsaturated fatty acids (PUFA) nor the PUFA/SFA ratio (0.09 ± 0.015; $P = 0.11$).

6.4 Discussion

6.4.1 Growth performance

The quadratic effect of WDDGS on DMI in the 400 g/kg WDDGS concurs with previous reports. A quadratic increase in DMI was reported by Walter et al. (2010) when feeding finishing cattle diets containing 200g/kg DM WDDGS as compared to control and 400g/kg diets. Beliveau and McKinnon (2008) also reported a quadratic response when including WDDGS in growing cattle diets up to 320 g/kg with lower DMI at inclusion levels of 80 and 160 g/kg. However, other studies reported no effect of WDDGS on DMI when it was included in finishing beef cattle (Beliveau and McKinnon, 2008; McKinnon and Walker, 2008; Li et al, 2011; Walter et al, 2010) or lamb diets (McKeown et al, 2010a). Linear increases in DMI have been reported when WDDGS have been included in beef finishing diets at 200, 400 and 600 g/kg DM (Gibb et al., 2008). These authors suggest that increases in DMI may be a result of moderated concentrations of propionate and higher ruminal pH increases due to higher NDF and lower starch content when WDDGS replaces barley grain. Walter et al. (2012) reported that steers fed 200g/kg WDDGS were more prone to acidosis than those fed the control or 400g/kg WDDGS diets, questioning the effectiveness of the fiber in WDDGS. Higher DMI have also been attributed to

compensation for reduced DM digestibility in diets containing WDDGS (Gibb et al., 2008) but recent studies report no variations in DMI with WDDGS diets despite a reduction in total tract DM digestibility (Walter et al., 2012; Li et al., 2011). In our study, the chemical composition of the 200 g/kg diet was similar to the control diet. This may have resulted from removal of a fraction of alfalfa when WDDGS was added and may partially explain the similarities in the growth results in lambs fed those diets.

The increases in ADG and final weights with WDDGS inclusion in the diets can probably be attributed to the higher intakes found particularly in the 400g/kg WDDGS group. Increases in ADG when replacing barley with WDDGS have been reported in growing cattle (McKinnon and Walker, 2008; Beliveau and McKinnon, 2008). However, no effects were reported when WDDGS replaced barley grain at concentrations of 80 to 320 g/kg DM (Beliveau and McKinnon, 2008) and 200 to 400 g/kg DM (Walter et al., 2010) in diets for finishing cattle.

The effects of WDDGS on DMI and ADG explain the lack of effect on feed efficiency (G:F). Unaltered feed efficiency is in agreement with previous studies which reported no effects of substitution of WDDGS for barley grain (Beliveau and McKinnon, 2008; Walter et al, 2010) or barley silage (Yang et al., 2012) on feed efficiency in cattle. In contrast, others have found that replacement of barley grain with WDDGS reduced G:F in finishing lambs (McKeown et al., 2010a), and finishing cattle (Gibb et al., 2008). However, in growing cattle, improvements in feed efficiency were reported when WDDGS replaced barley at 320 g/kg, but not at 80 and 160 g/kg (Beliveau and McKinnon, 2008) and at 250 and 500 g/kg (Mc Kinnon and Walker, 2008).

6.4.2 Eating behaviour

The lack of effect on overall eating time is in agreement with the results of previous studies where WDDGS replaced barley grain in finishing cattle diets (Beliveau and McKinnon, 2008). In contrast, Yang et al. (2012) reported the longest eating times in cattle fed a finishing diet where 250 g/kg DM WDDGS replaced 200 g/kg DM barley grain and 50 g/kg DM barley silage with a linear decrease in eating time when more barley silage was replaced by WDDGS. Similar eating times among treatments in our study seem to be the result of a compensatory strategy reflected in the increase in eating rate and in the number of daily visits to the feeder with reduced time per visit to the feeder and amount eaten per visit when the level of WDDGS was increased in the diet. Eating faster, smaller meals per visit and increasing the number of meals per day resulted in increased DMI in the 400g/kg group as compared to the control and 200g/kg groups which had fewer but longer daily visits where they ate at a slower rate. In the study of Yang et al (2012), the eating rate was lowest for the 250 g/kg group where WDDGS replaced 200 g/kg of barley grain and 50 g/kg barley silage, but was compensated for by increased eating time whereas when replacing WDDGS for barley silage eating rate was increased for a reduced meal duration. These authors attributed this finding to a decreased particle size in the groups fed higher concentrations of WDDGS and to reduced pH as roughage was partially removed from the diet. The particle size is probably not a factor in this study as all diets were ground and pelleted.

Preference for WDDGS supplemented diets has been previously reported in lambs (Charles et al., 2012).Taste, diet novelty and CP contents were proposed by these authors as possible causes for preference. We believe that the intake response in our study is more related to effects of WDDGS on rumen

fermentation. Previous studies have reported quadratic increases in the period of time rumen pH is below 5.2 (i.e., indicative of acidosis) with increasing levels of WDDGS in the diets showing that at moderate (140-200 g/kg) replacement levels for barley grain, WDDGS can increase the severity of acidosis (Beliveau and McKinnon, 2008; Walter et al, 2010). The reduction of alfalfa in both WDDGS added groups of this study possibly contributed to similar pH conditions in the 200 g/kg WDDGS group than those obtained by the aforementioned authors. These conditions might have been compensated in the 400 g/kg WDDGS group by reductions in amount of rapidly fermentable barley starch, increases in NDF and rumen degradable protein, leading to more stable and higher intakes.

6.4.3 Fatty acid profiles

The fatty acid (FA) profiles of tail backfat tissue were subject of overall minor variations among diets. The slight reduction in palmitic acid (C16:0) may be attributed to a decrease in this fatty acid in high DDGS diets (McKeown et al., 2010a). As palmitic acid is the main SFA in ruminant adipose tissue, and has a negative influence on plasma cholesterol in humans (Williamson et al., 2005), any reduction in its proportions in favor of stearic acid (C18:0) which has neutral effects on plasma cholesterol (Yu et al., 1995), is positive.

The trend to increased proportions of vaccenic acid (VA; *t*11-18:1) is in agreement with a previous study reporting linear increases in this FA with increased concentrations of WDDGS in the diets of finishing cattle (Dugan et al., 2010). In contrast, no variations in this FA were reported when using WDDGS (McKeown et al., 2010a) or triticale based DDGS (McKeown et al., 2010b) in finishing lambs tail backfat. This FA is of major importance as it is the main

source of rumenic acid (RA; *c*9, *t*11-18:2) via desaturation in ruminant tissues (Griinari et al., 2000) and can also undergo desaturation in humans (Scollan et al., 2006). Dugan et al. (2010) reported linear reductions in proportions of 10*t*-18:1, a FA linked to coronary heart disease (Hodgson et al, 1996), when increasing concentrations of WDDGS were substituted for barley grain in finishing diets. These reductions were only numerical in our study. This fatty acid has been reported to be increased in high grain diets due to a shift in microbial fermentation towards a lower pH within the ruminal environment (Kramer et al., 2004). The proportions of VA in our study were higher and those of 10*t*-18:1 lower than those found in lamb tail backfat by McKeown et al. (2010a). The relatively high concentrations of alfalfa in the diets (275-300 g/kg) may have contributed to preclude more notorious changes to the more favorable proportions of VA and 10*t*-18:1.

The increases in 18:3 n-3 (linolenic acid) contrast with previous studies. Inclusions of WDDGS in the diet did not result in higher concentrations of 18:3 n-3 in brisket or diaphragm of beef cattle (Dugan et al., 2010) or lambs (McKeown et al., 2010a). Using triticale based DDGS, McKeown et al. (2010b) reported a trend for increased 18:3 n-3 in tail backfat of lambs. Despite this increase, the numerically higher proportions of 18:2 n-6 in WDDGS added groups impeded an improvement of n-6/n-3 ratio which was lower than those previously reported in beef cattle by Dugan et al. (2010) and lambs by McKeown et al. (2010a) but slightly above the recommended maximum of 5:1 (World Health Organization, 2003). Again, the inclusion of alfalfa in the diet may have contributed to these relatively low values in the n-6/n-3 ratio.

6.5 Conclusions

This study indicated that replacing barley grain with wheat DDGS in the diet of growing lambs resulted in minor improvements of the fatty acid composition of adipose tissue and at concentrations of 400g/kg, it increased eating rate and number of visits to the feeder, improved average daily gain and final live weight.

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Table 6.1 Fatty acid (FA) composition of wheat dry distillers grains with solubles expressed as g/100g fatty acid methyl esters (FAME) and mg/100g DM.

Component	% FAME	mg/100g DM
Saturated FA (SFA)		
C10:0	0.15	8.2
C12:0	0.04	2.3
C14:0	0.14	7.4
C15:0	0.18	10
C16:0	22.04	1203.1
C17:0	0.41	22.3
C18:0	2.52	137.3
C20:0	0.27	14.7
C22:0	0.25	13.5
Total SFA	26.00	1418.8
Monounsaturated FA (MUFA)		
C16:1n7	0.31	16.7
C18:1cis+trans	21.30	1162.9
C20:1	0.56	30.8
Total MUFA	22.17	1210.4
Polyunsaturated FA (PUFA)		
C18:2 n-6	48.08	2624.8
C18:3 n-6	0.12	6.6
C18:3 n-3	2.93	159.8
C20:2	0.12	6.4
C20:5 n-3 EPA	0.16	8.7
C22:5 n-3 DPA	0.35	19.0
Total PUFA	51.76	2825.3

EPA = Eicosapentaenoic acid; DPA = Docosapentaenoic acid.

Table 6.2 Amino acid composition of wheat dry distillers grains with solubles.

Name	Amino acid (-H_2O)[a] (mg/g)	Amino acid[b] (mg/g)	Mole %
Hydroxyproline	0.2	0.3	0.1
Histidine	4.1	4.6	2.0
Serine	8.3	10.0	6.5
Arginine	9.1	10.2	3.9
Glycine	7.5	9.8	8.8
Aspartic acid	13.2	15.2	7.7
Glutamic acid	35.5	40.5	18.6
Threonine	6.8	8.0	4.5
Alanine	10.5	13.2	10.0
Proline	12.0	14.2	8.4
Lysine	3.6	4.1	1.9
Tyrosine	5.0	5.5	2.1
Methionine	2.5	2.9	1.3
Valine	9.4	11.1	6.4
Isoleucine	7.1	8.2	4.3
Leucine	16.0	18.6	9.6
Phenylalanine	8.4	9.4	3.8
Total	159.2	185.9	100.0

[a] Calculation based on amino acid residue mass in protein (molecular weight minus H_2O). [b] Calculation based on free amino acid molecular weight.

Table 6.3 Ingredients and chemical composition of the experimental diets containing increasing amounts of wheat dry distillers' grains with solubles (WDDGS).

	WDDGS, g/kg		
Ingredient	0	200	400
Barley grain	629	450	250
Wheat DDGS	0	200	400
Alfalfa meal	300	275	275
Molasses	25	25	25
Calcium Carbonate	18	18	18
Canola Oil	10	10	10
Sheep mineral mix[a]	18	18	18
ADE Vitamin[b]	1	1	1
Chemical composition, g/kg			
Dry matter	924	931	921
Organic matter	945	941	933
Crude protein	165	171	219
Neutral detergent fiber	192	193	250
Acid detergent fiber	89	92	113
Ether extract	22	25	32
Ash	55	59	67
Non fibrous carbohydrates	490	483	353

[a]Containing 92.6% NaCl, 4.97% Dynamate®; 0.9% $ZnSO_4$; 0.83% $MnSO_4$; 0.13% $CuSO_4$; 0.1% ethylenediamine dihydroiodide, 80% preparation; 0.005% $CoSO_4$; 0.4% canola oil (as carrier of $CoSO_4$); and 0.0014 % Na_2SeO_3.

[b]Containing vitamin A (10 000 000 IU/kg); vitamin D (1 000 000 IU/kg); and vitamin E (10 000 IU/kg).

Table 6.4 Effect of increasing concentration of WDDGS as replacement of barley grain in feedlot diets on growth performance of lambs.

	WDDGS, g/kg				
Component	0	200	400	SEM	*P*-Value
Initial weight (kg)	29.3	29.3	29.3	1.31	0.99
Final weight (kg)	45.3[b]	44.5[b]	50.5[a]	1.63	0.02
Daily DM intake (g/d)	1266[ab]	1088[b]	1493[a]	96.7	0.02
Average daily gain (g/d)	271[b]	261[b]	364[a]	21.8	<0.01
Feed efficiency (gain:feed)	0.21	0.24	0.24	0.02	0.62
Hot carcass weight (kg)	21.3	20.3	24.1	1.04	0.06
Dressing (kg/kg)	0.47	0.48	0.48	0.01	0.89

[a-b]Within a row, means without a common letter differ ($P<0.05$).

Table 6.5 Effect of increasing concentration of wheat dried distillers' grains with solubles (WDDGS) as replacement of barley grain in feedlot diets on feeding behaviour of lambs.

	WDDGS, g/kg				
Component	0	200	400	SEM	*P*-Value
Eating time, min/d	69.7	72.5	61.4	4.62	0.26
Eating rate, g/min	19.4[b]	13.2[c]	23.9[a]	0.95	<0.01
Visits/day, *n*	100.3[b]	91.2[b]	132.7[a]	9.39	0.01
Time per visit, seconds	57.7[a]	61.7[a]	30.7[b]	5.61	<0.01
Intake per visit, g	16.6[a]	12.8[b]	11.2[b]	0.85	<0.01

[a-b]Within a row, means without a common letter differ ($P<0.05$).

Table 6.6 Effects of replacing barley grain with increasing concentrations of wheat dried distillers grains with solubles (WDDGS in the diet on fatty acid (FA) profiles (% of total FAME) in subcutaneous tail fat of lambs.

	DDGS, g/kg DM				
Component	0	200	400	SEM	*P*-Value
Saturated FA (SFA)					
C10:0	0.28[ab]	0.33[a]	0.24[b]	0.021	0.02
C12:0	0.19	0.25	0.19	0.034	0.39
C14:0	3.14	3.3	3.07	0.231	0.76
C15:0	0.97	1.37	0.86	0.305	0.47
C16:0	23.51[a]	23.27[ab]	22.08[b]	0.423	0.05
C17:0	3.54	3.04	3.32	0.356	0.62
C18:0	20.61	21.29	21.33	0.876	0.81
Total SFA	52.25	52.86	51.01	0.798	0.30
Monounsaturated FA (MUFA)					
*c*9-14:1	0.068	0.068	0.063	0.008	0.88
*c*9-16:1	1.07	1.03	0.95	0.058	0.32
*t*6-*t*8-18:1	0.31	0.28	0.31	0.023	0.51
*t*9-18:1	0.31	0.26	0.31	0.019	0.13
*t*10-18:1	2.31	2.01	1.69	0.308	0.39
*t*11-18:1	1.55	1.72	2.32	0.243	0.07
*c*9- 18:1	36.96	36.04	37.21	0.809	0.56
*c*1-18:1	0.99	0.89	0.9	0.063	0.43
*c*9-20:1	0.16	0.15	0.16	0.008	0.46
Total MUFA	43.74	42.44	43.94	0.753	0.32
Polyunsaturated FA (PUFA)					
18:2 n-6	2.72	3.33	3.49	0.274	0.13
18:3 n-3	0.43[b]	0.52[a]	0.48[ab]	0.023	0.05
CLA *c*9,*t*11-18:2	0.55	0.60	0.74	0.066	0.12
CLA *t*10,*t*12-18:2	0.02	0.03	0.02	0.005	0.35
20:4 n-6	0.08	0.09	0.1	0.007	0.47
20:5 n-3 EPA	0.02	0.02	0.02	0.004	0.81
22:5 n-3 DPA	0.17	0.09	0.09	0.050	0.47
22:6 n-3 DHA	0.02	0.02	0.02	0.002	0.42
Total PUFA	4.02	4.70	4.96	0.321	0.12
Total UFA	47.75	47.14	48.90	0.798	0.30
PUFA/SFA	0.07	0.09	0.10	0.015	0.11

trans FA	5.04	4.89	5.40	0.369	0.60
CLA+VA	2.11	2.35	3.08	0.298	0.07
n-3 FA[e]	0.64	0.65	0.62	0.060	0.92
trans - (CLA+VA)	2.93	2.54	2.32	0.323	0.42
C18:1 *trans*[f]	4.48	4.27	4.65	0.340	0.74
n-6/n-3	4.79	5.35	5.81	0.389	0.19

UFA: unsaturated fatty acids = MUFA + PUFA; *trans* FA = *t*6- 8-18:1 + *t*9-18:1 + *t*10-18 :1 + *t*11-18 :1(VA) + *c*9, *t*11-18 :2 (CLA) + *t*10-*t*12-18 :2 (CLA); CLA = conjugated linoleic acid; *c*9-*t*11-18 :2 CLA includes *t*7, c9-18 :2CLA; VA = Vaccenic Acid, C18:1 *t*11; n-3 FA = C18 :3 + EPA + DHA + DHA; C18:1 *trans* = C18:1 *t*6-8 + C18:1 *t*9 + C18:1 *t*10 + C18:1 *t*11 (VA).

[a-b]Within a row, means without a common letter differ ($P<0.05$).

Chapter 7. Increasing concentrations of wheat dry distillers' grains with solubles in iso-nitrogenous finishing diets reduce lamb performance

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Increasing concentrations of wheat dry distillers' grains with solubles in iso-nitrogenous finishing diets reduce lamb performance

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Abstract

It was hypothesized that the inclusion of wheat dry distillers' grains with solubles (WDDGS) up to 470 g/kg dry matter (DM), fed to lambs in iso-nitrogenous diets, would neither affect growth performance, nor subcutaneous adipose tissue fatty acid composition. Thus, this study aimed to assess the effects of increasing concentrations of WDDGS in iso-nitrogenous diets on *in vitro* fermentation, *in sacco* degradation, growth performance and fatty acid profiles of adipose tissue in lambs. Increasing concentrations of WDDGS (100, 300 and 470 g/kg dry matter; DM) of WDDGS was achieved by replacing soybean meal, alfalfa hay and soybean hulls. *In vitro* fermentation of the diets was conducted to measure fermentation variables and CH_4 production. *In situ* digestion kinetics of DM, crude protein (CP), and neutral detergent fiber (NDF) of the treatment diets were evaluated using three ruminally cannulated cows. Additionally, 60 lambs were used to determine the effect of WDDGS on growth performance and fatty acid profiles of subcutaneous tail fat. In the *in sacco* study, WDDGS increased (P = 0.03) DM, CP and NDF effective degradability and reduced (P < 0.01) rumen undegradable protein. In the *in vitro* incubations, CH_4 production (mg/g DMD) increased (P = 0.05) in the 470 g/kg WDDGS group. In the growth trial, increasing WDDGS in the diet linearly reduced DMI (P = 0.02), ADG (P < 0.01) and hot carcass weight (P < 0.01). Linear increases in *trans*-10 octadecenoic acid (P = 0.02) and linear decrease in oleic acid (P < 0.01) resulted in a linear reduction of total monounsaturated fatty acids (MUFA; P = 0.02) yet, linoleic acid was linearly increased (P < 0.01). In summary, increasing

concentrations of WDDGS in iso-nitrogenous diets fed to lambs decreased animal performance and negatively affected the fatty acid profile of adipose tissue.

Keywords: ethanol by-products, *in vitro*, methane, rumen digestion kinetics, sheep

7.1 Introduction

Dry distillers' grains with solubles (DDGS) produced as a by-product from ethanol production, have become a readily available source of energy and protein for ruminants. Replacing barley grain with wheat based DDGS (WDDGS) has improved body weight (BW) gain in growing beef cattle (McKinnon and Walker, 2008; Beliveau and McKinnon, 2008). However, similar improvements have not been observed in cattle fed high grain, finishing diets (Beliveau and McKinnon, 2008; Gibb et al., 2008; Walter et al., 2010). In finishing lamb diets, feed intake and BW gain was not altered when WDDGS replaced barley grain at 200 g/kg DM (McKeown et al, 2010), but increased with inclusion rates of 400 g/kg DM (Avila et al., 2012). This suggests that lambs may respond better to the increased crude protein (CP) supplied by high WDDGS concentrations in the diet, given their relatively greater CP requirements, compared to finishing beef cattle. Wheat DDGS is a complex source of nutrients, originally considered to be a significant source of digestible rumen undegradable protein (RUP; Boila and Ingalls, 1994). However, this has been challenged by recent studies which demonstrated the potential degradability of CP in WDDGS is high at slower passage rates, making it a source of rumen degradable protein (RDP) as well as RUP (Nuez and Yu, 2009; Avila et al., 2012). Wheat DDGS are also a good source of highly digestible NDF (McKinnon and

Walker, 2008; Nuez and Yu, 2009) which could modify rumen pH. Whether the substitution of high concentrations of WDDGS for barley grain, improved the performance of lambs in our previous study (Avila et al., 2012) as a result of increased CP, is unknown.

When fed as a replacement for barley grain to finishing cattle, WDGGS improved fatty acid profiles by increasing vaccenic acid (VA; *t*11-18:1) and rumenic acid (CLA; *c*9, *t*11-18:2) and decreasing *trans* -10 octadecenoic acid (*t*10-18:1) (Dugan et al., 2010). Increases in linoleic acid (*c*9, *c*12-18:2) and α-linolenic acid (*c*9, *c*12, *c*15-18:3) have also been reported when WDDGS replaced barley grain and barley silage in beef cattle (He et al., 2012b). However, the results have not been as conclusive for lambs, as only minor changes in fatty acid profiles from tail adipose tissue were reported when finishing diets contained 200 g/kg DM WDDGS (McKeown et al, 2010) and a quadratic response in linoleic acid proportions were observed when WDDGS was increased to 400 g/kg DM (Avila et al., 2012).

We hypothesized that the inclusion of WDDGS up to 470 g/kg DM fed to lambs in iso-nitrogenous diets, would neither affect growth performance, nor fatty acid composition. Thus, the objectives of this study were to examine the effects of WDDGS on growth performance, carcass traits and adipose tissue fatty acid profiles of Canadian Arcott ram lambs. *In sacco* and *in vitro* studies were also conducted to assess the effects of WDDGS on rumen digestion kinetics of the formulated diets and on methane (CH_4) production.

7.2 Materials and methods

All experiments were conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta, Canada, with all lambs being cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

7.2.1 Dietary treatments

Treatment diets were formulated by substituting WDDGS for soybean meal, alfalfa and soybean hulls to achieve concentrations of 0, 100, 300, and 470 g/kg WDDGS (DM basis; Table 1). The concentrations of WDDGS were selected according to the results of Avila et al. (2012) and Gibb et al. (2008) who reported optimum performances in cattle at concentrations of 400 - 600 g/kg WDDGS in dietary DM. All ingredients were ground through a 1-mm screen, mixed and pelleted (Chaves et al. 2008).

7.2.2 *In sacco* incubations

The pelleted experimental diets were ground through a 2-mm screen and approximately 5 g were weighed into 50 × 10-mm Dacron bags (pore size = 50 µm). The bags were sealed and stored in airtight containers until incubation. The experiment considered triplicate bags for each diet (n = 4) and each incubation time (n = 6).

Three ruminally cannulated Holstein Friesian cows fed a mixed diet consisting of 250, 400 and 710 g/kg DM barley grain, feed lot supplement, and barley silage respectively, were used to conduct the incubations. The Dacron bags with the

experimental diets were sorted according to treatment, incubation time point, and cow and placed in large net bags attached to a weight (~1 kg) to ensure anchorage at the bottom of the rumen (Chaves et al, 2006).

The bags were incubated in the rumen for 3, 6, 12, 24, and 48 h. Once removed from the rumen, the bags were immediately rinsed in cold water to remove external rumen contents. Zero hour bags were not placed in the rumen, but along with the treatment bags, they were placed in water at 25 ^{o}C for 10 min and refrigerated until all replicates, at all time points, were recovered from the cows. All bags were placed together in a washing machine on a cold water cycle, without the spin cycle. After washing, the bags were oven dried at 55 °C for 24 h and residues were hot-weighed.

7.2.2.1 Calculations of *in sacco* digestion kinetics

Disappearance of DM, crude protein (CP), and neutral detergent fibre (NDF) were analyzed using a non-linear model to determine fractional disappearance rate (k, % h^{-1}) and potential degradation (PD) according to the equation of Orskov and Mc Donald (1979):

$$PD = A + B\,(1 - e^{-k \times t})$$

where A = soluble fraction (g/kg of DM, CP or NDF at t=0), B = degradable insoluble fraction and t is time in h.

The effective degradability (ED) was calculated from the kinetic parameters obtained from exponential adjustments assuming fractional passage rates (k_p) of 0.02, 0.05, and 0.08 h^{-1} according to:

$$ED = A + B \times [k/(k+k_p)]$$

The metabolizable protein system (AFRC, 1992) for defining ruminal degradation was used to calculate CP degradability parameters:

Quickly degradable protein (QDP, g/kg DM) = A × [CP] where [CP] is CP concentration (g CP/kg DM)

Slowly degradable protein (SDP, g/kg DM) = [(B × k) / (k + *kp*)] × [CP] (g/kgDM)

Effective rumen degradability of crude protein (ERDP, g/kg DM) = [(0.8 × QDP) + SDP]

Rumen degradable protein (RDP, g/kg DM) = QDP + SDP

Rumen undegradable protein (RUP, g/kg DM) = [CP] – RDP.

Additionally, the values obtained for diet composition and disappearance rates of DM, CP and NDF were utilized in the Small Ruminant Nutrition System (SRNS version 1.8.7; Cannas et al., 2004) to compare the models predictions for DMI, metabolizable energy (ME) intake, rumen nitrogen balance (RNB), metabolizable protein (MP) intake, pH and live weight gain, with our results. The data input used the values of the whole total mixed ration for DM, NDF, CP, non-fibrous carbohydrates (NFC), fat, ash, and *in sacco* digestion parameters. The data for lignin, neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were proportionally calculated from values presented by the model for each feed ingredient of the diets. Alfalfa hay and soy bean hulls were considered as forage. After several simulations testing different full BW and effective NDF (eNDF), a critical requirement for the model as it affects rumen pH, passage rates, nutrient digestibility, ME and MP of the diets (Cannas et al., 2004), the values for

eNDF were set at 30 % of NDF as described for finely ground, pelleted concentrates (Mertens, 1997). The lambs' full BW was fixed at 54 kg for all treatments because the model predicted DMI more accurately at that full BW.

7.2.3 In vitro incubations

The substrates used for the incubation were the same previously described experimental diets. Pellets were dried at 60 ^{o}C for 24 h and then ground to pass 1 mm and 0.5 g DM of sample was weighed into an ANKOM® bag (model F57), with five replicates per treatment and sealed. Each bag was placed into a 50-mL amber serum bottle fitted with rubber stoppers. The entire incubation procedure was repeated twice (i.e., two incubation runs).

7.2.3.1 Inoculum

Inoculum was obtained from two ruminally cannulated Holstein Friesian cows fed a mixed diet consisting of 250, 40, and 710 g/kg DM barley grain, feedlot supplement, and barley silage respectively. Rumen fluid was collected 2 h after feeding from four distinct sites in the rumen, filtered through four layers of cheesecloth, combined in equal portions from each cow, stored and transported in a pre-warmed Thermos® flask to the lab. The inoculum was prepared by mixing rumen fluid and a mineral buffer with 0.5 mL of cysteine sulfide solution as a reducing agent in the ratio of 1:3 (Wang et al., 2010). The inoculum was then transferred (25 mL) into pre-loaded and pre-warmed (39 ^{o} C) vials under a stream of CO_2 gas. The vials were sealed and

placed on an orbital shaker rack setting at 90 oscillations/min in an incubator at (39 ^{o}C).

7.2.3.2 Determination of total gas, methane concentration and *in vitro* dry matter disappearance (IVDMD)

Net gas production of each vial was measured at 24 and 48 h of incubation with a water displacement apparatus (Fedorak and Hrudey, 1983). Headspace gas was sampled from each vial prior to gas measurement with a 20-mL syringe and immediately transferred into a 5.9-mL evacuated exetainer (Labco Ltd., Buckinghamshire, UK), which was then analyzed for CH_4 concentration by gas chromatography (Holtshausen et al., 2009). Methane was expressed as mg of CH_4 per gram of DM incubated and disappeared, and total net gas production as mL per gram of incubated DM.

At the end of 48 h of incubation and after gas was sampled for CH_4 and total gas production was measured, vials were opened and the pH of the whole culture was measured using a pH meter (Orion Model 260A, Fisher Scientific, Toronto, Ontario, Canada). The ANKOM® bags with the residues were then removed from the bottles, rinsed thoroughly with distilled water, dried at 100°C for 48 h (constant weight) and weighed to estimate IVDMD.

7.2.4 Growth study

7.2.4.1 Animals and experimental design

Sixty Canadian Arcott weaned ram lambs (57 ± 3.8 d of age; 21.1 ± 4.01 kg) were stratified by BW and randomly assigned to 1 of 4 experimental diets. Lambs were adapted to the experimental diets for 2 weeks before the beginning of data collection. Lambs were housed in individual pens (0.97 × 2.82 m) bedded with straw, fed at 0900 daily, and weighed weekly. All lambs had *ad libitum* access to feed and water throughout the study. Feed deliveries were recorded daily. Refusals were collected daily and weighed weekly for determination of weekly dry matter intake (DMI). Daily DMI by each lamb was estimated by summing the weekly intake and dividing by seven. The ADG was determined by dividing BW gain (initial full BW − final full BW) by the number of days (72 d) in the study. Feed conversion ratio was calculated as the ratio between DMI and ADG (g feed eaten/g of BW gain). Hot carcass weight was recorded for each lamb.

7.2.4.2 Slaughter and sample collection

Lambs were slaughtered at a BW of ≥ 45 kg, in two lots at a commercial abattoir (Sunterra Meats Ltd., Innisfail, AB, Canada). Within 5 min of exsanguination, a fat sample (2 to 3 g) from the base of the tail was collected from each lamb. The samples were kept on ice and transported to the laboratory where they were snap-frozen in liquid nitrogen and stored at -80°C until analyzed for fatty acids profiles. Hot carcass weights were recorded and grading rule (GR; body wall thickness) was determined from the total tissue depth of the carcass between the 12^{th} and 13^{th} rib at

11 cm from the carcass midline (McKeown et al., 2010). Rumen fluid was collected from each lamb into 50-mL plastic tubes and pH was measured immediately (Orion model 260A, Allometrics, Inc., Baton Rouge, LA, USA). Tubes were capped and placed in an ice bucket and immediately transferred to the laboratory. Ruminal fluid samples were centrifuged at 1000 × *g*, for 10 min, at 4 °C (Spectrafuse 16M, National Labnet Co., Edison, NJ, USA) and supernatants were collected for later analysis of ammonia (NH_3) and volatile fatty acid (VFA) concentrations.

7.2.4.3 Determination of ammonia-N and volatile fatty acids

The liquid fractions of the lamb's rumen fluid samples were sub-sampled for determination of NH3 and VFA concentrations. Two subsamples (1.6 mL) of each 50-mL tube were transferred to 2-mL micro-centrifuge tubes containing 150 μL of TCA (0.65; vol/vol) and centrifuged at 14,000 × *g* for 10 min to precipitate particulate matter and protein. The supernatant was transferred into 2-mL micro-centrifuge tubes (Fisher Scientific, Ottawa, Ontario, Canada) and frozen at -20°C until analyzed for ammonia-N.

In addition, two subsamples (1.5 mL) of each 50-mL tube were taken, acidified with 300 μL of metaphosphoric acid (0.25; wt/vol), and centrifuged as described for ammonia-N analysis. The supernatant was frozen at -20°C until analyzed for VFA concentrations.

7.2.5 Chemical analyses

The analytical DM concentration of the composited feed samples was determined after 135°C for 2 h (method 930.15; AOAC, 2006) followed by hot weighing. Organic matter was determined by ashing the samples at 550°C for 5 h (method 942.05; AOAC, 2006). To determine CP (N × 6.25), feed samples were ground to a fine powder using a ball grinder (Mixer Mill MM200, Retsch Inc., Newtown, PA). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Nitrogen Analyzer 1500 series, Carlo Erba Instruments, Milan, Italy). Neutral detergent fiber was determined according to Van Soest et al. (1991) using heat stable α-amylase and sodium sulfite and expressed as inclusive of residual ash. Acid detergent fiber (ADF) was determined according to the method 973.18 of AOAC (2006). Gross energy (GE) was determined using an adiabatic calorimeter (model 1241, Parr, Moline, IL). Crude fat concentration was determined by ether extraction (AOAC 2006, method 920.39) using a Goldfisch Fat Extractor (Labconco Corporation, Kansas City, MO). Non-fibrous carbohydrate was calculated as: 100 - [CP + NDF + crude fat + ash] (Mertens 2002).

7.2.6 Lipid extraction

Lipids were extracted from adipose tissue and experimental diets based on the method of Hara and Radin (1978). Unless otherwise stated, chemicals were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada). Briefly, samples (1 g of adipose tissue) were homogenized in 15 mL of 2-propanol using a tissue homogenizer set at 10,000 rpm (PRO 250, PRO Scientific Inc. Oxford, CT, USA).

Hexane (10 mL) was added to the mixture prior to a second homogenation for 90 s. Samples were allowed to settle and lipids were collected from the upper hexane phase. Hexane extracts were evaporated under N_2 and lipids were stored at -80°C until methylated. Fatty acids were methylated and determined as described by He et al. (2012a).

7.2.7 Statistical analyses

7.2.7.1 *In sacco* data

The univariate procedure of SAS was used to test for normal distribution of the data. Data from *in sacco* incubations were used in the nonlinear (PROC NLIN) least squares procedure of SAS (SAS Inc., 2013; SAS Online Doc 9.1.3. Cary, NC, USA) to provide estimates for *A*, *B* and *k* calculated from DM, NDF and CP disappearance data. The data were analyzed as a randomized complete design using PROC MIXED of SAS with treatment in the model as fixed effect and cow and cow × treatment interaction as random effects.

7.2.7.2 *In vitro* data

Data were analysed with the mixed model procedure of SAS. The model included the fixed effects of treatment with the average of the 5 replicates (individual vials) from each incubation (run) as the experimental unit. Treatment means were compared using the least squares mean linear hypothesis test with treatments included as fixed terms.

7.2.7.3 Animal data

For DMI, means were compared using the least squares mean linear hypothesis test with treatment, week, and treatment per week interactions included as fixed terms, lambs as a random effect, and week as a repeated measure. Repeated measures analysis with the minimum values of AIC (Akaike's Information Criterion) were used for selecting covariance structure (Chaves et al., 2008). Average daily gain, feed conversion ratio, initial and final BW, hot carcass weight, and fatty acid composition, means were compared using the least squares mean linear hypothesis test using the same model as DMI but not considering repeated measures.

For all data, significance was declared when $P \leq 0.05$ and a trend reported when $0.05 < P < 0.10$. When fixed effects of treatment (diet) were significant, orthogonal polynomial contrasts were used to determine linear and quadratic responses to the concentration of WDDGS. Otherwise, contrasts were not reported. When fixed effects of treatment were significant and contrasts were not, least square linear hypothesis test results were presented to separate differing means. Data from two lambs from the control group which died accidentally were excluded from the experiment.

7.3. Results and discussion

The experimental diets had similar CP concentrations (268.3 ± 2.7 g/kg DM; Table 1). The main fatty acids present in the diets included linoleic acid, which increased at greater concentrations of WDDGS (26.5 - 45.5 g/100 g fatty acid methyl esters (FAME)), palmitic acid (16:0) which decreased at greater concentrations of WDDGS

(28.7 - 15.2 g/100 g FAME) and oleic acid (27.0 ± 4.3 g/100 g FAME). These 3 fatty acids (FA) accounted for 81.08 ± 0.25 g/100 g of all FA in the 4 experimental diets (Table 1) resulting in greater proportions of polyunsaturated (PUFA) and lower proportions of monounsaturated (MUFA) and saturated FA (SFA) in diets with greater WDDGS concentrations.

7.3.1 In sacco degradation kinetics

The DM in the "*A"* fraction was linearly increased and DM in the "*B"* fraction was linearly reduced by increasing concentrations of WDDGS in the diet (Table 2). The degradation rate ("*k*") of DM tended to increase (P = 0.07) in the 470 g/kg DM WDDGS diet. Dry matter effective degradability was not affected by increasing dietary concentrations of WDDGS at *kp* = 0.02, but was linearly increased at *kp* = 0.05 and *kp* = 0.08. Conversely, previous studies report a reduction in total tract digestibility when increasing concentrations of WDDGS were included as a replacement of barley grain in the diets of beef cattle (Gibb et al., 2008; Walter et al. 2012). Diets containing WDDGS are richer in NDF, but the effectiveness of this fiber is questionable as it exhibits greater *in sacco* DM degradability than barley (Avila et al., 2012) or wheat grains (Nuez and Yu, 2009). The greater NDF degradability of distillers' grains is considered to be the result of exposure to processing and fermentation during ethanol production (Ham et al., 1994; Walter et al., 2012).

Linear increases in the "*A"* fraction and reductions in the "*B"* fraction of CP were observed as WDDGS increased in the diet. Degradation rate (k, h^{-1}) of CP tended to increase in the 470 g/kg DM WDDGS diet, and effective degradability of CP was

linearly increased with increasing WDDGS. Consequently, quickly degradable protein, effective rumen degradable protein and rumen degradable protein increased, whereas, slowly degradable protein and rumen undegradable protein was linearly reduced. Solubility of CP is negatively affected by temperature and time of drying, however, these conditions vary widely between ethanol producing plants (Nuez and Yu, 2010). Despite this variability, effective rumen degradability of CP from WDDGS has been reported to be greater than that of protein from corn based DDGS (Nuez and Yu, 2010). Our results contrast with those of Hedqvist and Udén (2006) who reported similar *in vitro* degradability of protein from WDDGS, compared to protein from solvent extracted soybean meal. Changes in degradability of CP from DDGS between different studies may be due to different experimental conditions but have also been attributed to differences in the drying process at the ethanol plant which can increase acid detergent insoluble CP due to heat damage (Spiehs et al., 2002; Li et al., 2012).

The soluble fraction (*A*) in the neutral detergent fiber (NDF) in the WDDGS added diets showed a slight but significant linear increase ($P = 0.01$). The "*B"* fraction in NDF was linearly increased by WDDGS inclusion in the diets ($P<0.01$) but degradation rate was not affected ($P = 0.15$). Effective degradability of NDF (ED_{NDF}) was not affected ($P = 0.18$) at kp = 0.02, but was linearly increased with increasing concentrations of WDDGS in the diet at kp = 0.05 ($P = 0.01$) and kp = 0.08 ($P = 0.02$). Increases in NDF effective degradability (ED_{NDF}) at higher WDDGS inclusions are in agreement with Walter et al. (2011) who demonstrated increases in NDF and ADF total tract digestibility as WDDGS replaced increasing proportions of barley grain. In our study this result may be partially attributed to decreased alfalfa

and soybean hulls in WWDGS diets to compensate for the increase in NDF from WDDDGS.

7.3.2 *In vitro* fermentation

The inclusion of WDDGS in the diet did not affect gas production when expressed as a function of incubated DM (Table 3). However, it linearly increased total gas production when expressed as a function of digested DM. Similarly, CH_4 production (mg CH_4/g DM digested) at 48 h increased with the substitution of 470 g/kg DM WDDGS. Increasing WDDGS resulted in a linear reduction in IVDMD. The increase in CH_4 production, as a function of digested DM, may be partially explained by the increase in total gas production and the reduction in IVDMD when comparing 470 g/kg DM WDDGS, with the control diet. Li et al. (2012) demonstrated no effect of WDDGS when included in the diet at 300 g/kg DM on CH_4 production, when replacing barley grain, despite an increase in DM disappearance in substrates containing WDDGS in an artificial rumen. Additionally, WDDGS had no effect on CH_4 production when used in growing and finishing cattle diets, when compared to barley grain (Hünerberg et al., 2012). However, a reduction in gas production and IVDMD was reported in batch cultures when 200 g/kg DM WDDGS replaced barley grain (McKeown, 2010), or when it replaced a combination of canola meal and barley grain (Au et al., 2010). Increases in NDF and degradable NDF concentrations in the diet have been associated with increased CH_4 production (Holtshausen et al., 2010; Johnson and Johnson, 1995) indicating that the increase in NDF in diets containing WDDGS in our study, may have contributed to the increase in CH_4 production.

7.3.3 Rumen fermentation

Increasing WDDGS in the diet linearly reduced the production of total VFA in rumen fluid (Table 4), which may be explained in part, by the reduced DMI for lambs fed the 470 g/kg DM WDDGS diet. Propionate, as a proportion of total VFA, increased in a quadratic manner in lambs fed increasing concentrations of WDDGS, resulting in a quadratic reduction in the acetate: propionate ratio. Conversely, previous studies have shown that when WDDGS was used as replacement of barley grain in cattle diets, ruminal propionate production was reduced in a linear (Beliveau and McKinnon, 2009) or quadratic (Walter et al., 2012) fashion. This is explained by the reduction of starch and a corresponding increase in dietary NDF. However, NDF from WDDGS has been questioned as a source of effective fiber (Walter et al., 2012), and its effect on rumen fermentation is not always associated with a modulation of rumen pH or reduced propionate concentration. In our study, NDF concentrations increased and non-fibrous carbohydrate (NFC) concentrations decreased with greater inclusions of WDDGS in the diet DM.

7.3.4 Growth performance

Total DMI, average daily gain (ADG) and hot carcass weights were linearly reduced (Table 5) with increasing concentrations of WDDGS in diet DM. Final BW tended to decrease (P = 0.07) with WDDGS inclusion. Feed conversion ratio, dressing percentage and grading rule (body wall thickness between 12^{th} and 13^{th} rib) were similar across dietary treatments. These results contrast with previous studies, which reported no effect of WDDGS on DMI when it was included as a replacement of

barley grain in finishing diets for beef cattle (Beliveau and McKinnon, 2008; McKinnon and Walker, 2008; Li et al, 2011) or lambs (McKeown et al, 2010). Furthermore, the use of WDDGS has resulted in linear (Gibb et al., 2008) and quadratic (Walter et al., 2010) increases in DMI of finishing beef cattle and quadratic increases in finishing lambs (Avila et al., 2012). Increased DMI have been attributed to a number of factors, including improved pH conditions in WDDGS fed animals, as a result of reducing starch intake, or to a reduction in DM digestibility with increasing WDDGS in the diet (Gibb et al., 2008). The *in sacco* effective degradability of DM was not affected at low passage rates (*kp* = 0.02), but was increased at greater passage rates (*kp* = 0.05–0.08; Table 3). Rumen pH was similar for all diets (Table 4). The slight increase in the proportion of propionate and reductions in the acetate: propionate ratio, suggest that eNDF from WDDGS may be low.

Increased ADG was reported when using WDDGS as replacement of barley grain in growing cattle (McKinnon and Walker, 2008; Beliveau and McKinnon, 2008), but negative (Gibb et al., 2008) or neutral (Beliveau and McKinnon, 2009) effects were reported in finishing cattle. In a recent study, Avila et al. (2012) found increased DMI and ADG with unmodified feed conversion ratio, when replacing WDDGS for barley grain at concentrations of 400 g/kg DM in lamb finishing diets, attributing these results to increased DMI and increased CP in the diet. In this study, the diets were iso-nitrogenous, suggesting the reduced DMI and lower digestibility (IVDMD) observed when increasing WDDGS in the diet, may have affected ADG.

7.3.5 Small ruminant nutrition system simulation

The output from the SRNS resulted in a slight underestimation of DMI (predicted for lambs weighing 54 kg) by 0.042 ± 0.018 kg/d when compared with the actual values (Table 5). Metabolizable energy and CP intake were reduced in a manner proportional to the reduction in DMI when WDDGS increased in the diet. This led to a reduction in the availability of ME for growth. As energy, and not protein, was the first limiting nutrient for BW gain, this reduction resulted in declining predicted BW gains with increasing WDDGS in the diet.

Predicted metabolizable protein (MP) and nitrogen balance were reduced with increasing WDDGS in the diet and consequently, led to lower predicted urea costs (energy cost of metabolizing excess N in the rumen). These reductions are also a consequence of reduced DMI, which reduced MP from feed.

Although the predicted performance (BW gain) estimates showed the same decline with increasing WDDGS in the diet, the model underestimated performance by 91 ± 18 g/d in comparison to the observed results (Table 5). The excessive CP in the diets of this study are not common in sheep, as such, the SNRS model does not account for the increased energy availability from excess dietary protein, which could partially explain the differences between predicted and actual performance parameters.

7.3.6 Fatty acid profiles

Wheat DDGS did not modify the proportions of total SFA in lamb tail adipose tissue (Table 6). This concurs with studies using WDDGS as a replacement for barley in

lamb (McKeown et al., 2010) and beef cattle diets (Dugan et al., 2010; He et al., 2012b), but contrasts with the results of Avila et al. (2012) who reported slight reductions of palmitic acid (C16:0) in lambs.

Total proportions of oleic acid were linearly reduced and those of *trans*-10 octadecenoic acid (*t*10-18:1) were linearly increased with increasing concentrations of WDDGS in the diet. This resulted in a linear reduction of total MUFA from 48.1 to 45.6 ± 1.06 g/100 g FAME. The linear increase in *trans*-10-octadecenoic acid contrasts with previous results. McKeown et al. (2010) found no variations in this FA when including 200 g/kg DM of WDDGS as a replacement of barley and Avila et al. (2012) reported only numerical reductions with increasing concentrations of WDDGS (up to 400 g/kg DM). Moreover, linear decreases were found in brisket and diaphragm fat in beef cattle fed increasing concentrations of WDDGS (Dugan et al., 2010). This *t*10-18:1 FA was reported as the major 18:1 *trans* FA in meat when high barley grain diets were fed to cattle (Dugan et al., 2007; Aldai et al., 2008) or lambs (McKeown et al., 2010; Avila-Stagno et al., 2012). Additionally, it has been related to increased risk of coronary heart disease in humans (Hodgson et al., 1996) and negative effects on plasma lipid profiles in rabbits (Roy et al., 2007).

Increases in *t*10-18:1 are associated with high grain diets, particularly barley, due to a reduction in rumen pH shifting the rumen bacterial population. This shift alters ruminal biohydrogenation of linoleic and α-linolenic acids, from the *t*11 double bond producing *Butyrivibrio fibrisolvens,* to the *t*10 double bond producing *Megasphaera elsdenii,* reducing the concentrations of vaccenic acid (*t*11-18:1) in favour of *t*10-18:1 (Baumann and Grinarii, 2003; Kramer et al., 2004). During the biohydrogenation process in the rumen, fractions of vaccenic acid and *t*10-18:1 are

not completely biohydrogenated and subsequently, reach the tissues where only vaccenic acid can be desaturated to produce rumenic acid (Kramer et al., 2004).

Recently, Avila et al. (2012) demonstrated no effects of increasing WDDGS up to 400 g/kg DM on lamb back fat proportions of *t*10-18:1, but the proportions of *t*10-18:1 were notably lower than those reported in the current study or other studies in western Canada (McKeown et al., 2010; Avila-Stagno et al., 2012). Additionally, in the experiment of Avila et al. (2012) there was a trend to increase vaccenic acid which could be at least partially explained by the greater proportions of alfalfa in the diets (300 g/kg DM) as compared to the diets of the current study (100-160 g/kg DM). Greater forage inclusion in the diet reduced the proportions of *t*10-18:1 in FA profiles in a study by Dugan et al. (2011) and may explain the differences observed in our study.

Proportions of conjugated linoleic acids (CLA) or *n*-3 FA were not modified by WDDGS. However, linoleic acid (LA) was linearly increased by increasing WDDGS in the diet, without affecting the *n*-6/*n*-3 ratio. This is consistent with studies in beef cattle where WDDGS replaced barley grain (Dugan et al., 2010) or barley grain and barley silage (He et al., 2012b). The authors suggested this may be an effect of reduced starch in the diet as increasing levels of WDDGS were included, causing a consequent alteration of the biohydrogenation process. Additionally, it was noted that the smaller particle size of WDDGS, compared to silage, would result in an increased passage rate of digesta to the lower tract. In our experiment, the concentration of barley grain in the diet was identical in all treatments, the reductions in NFC were of minor magnitude and all diets were ground and pelleted, thus, these factors are likely, not responsible for the increase in LA. However, the reduced

acetate: propionate ratio of rumen fluid in lambs fed WDDGS and the increased *in sacco* effective degradability of DM, CP and NDF, suggest that WDDGS is a limited source of fiber and that biohydrogenation may have been affected. This is supported by the observed increase in *t*10-18:1, an intermediate of the biohydrogenation process.

7.4 Conclusions

This study indicated that increasing concentrations of WDDGS in iso-nitrogenous diets of growing lambs increases *in vitro* CH_4 production per unit of digested DM. Increasing WDDGS in the diet reduced intake, ADG and hot carcass weights. *In sacco* digestion kinetics demonstrated that increasing concentrations of WDDGS in diet DM, decreased both slow degradable protein and RUP, without affecting rates of protein degradation. Fatty acid profiles of subcutaneous tail adipose tissue were not improved as linoleic acid and *trans*-10 octadecenoic acid (*t*10-18:1) increased. The main findings reject our initial hypothesis as increasing concentrations of WDDGS in iso-nitrogenous diets fed to lambs decreased animal performance and negatively affected the fatty acid profile of adipose tissue.

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Table 7.1 Ingredients and chemical composition of the iso-nitrogenous diets containing increasing amounts of wheat dry distillers' grains with solubles (WDDGS).

	WDDGS, g/kg DM			
	0	100	300	470
Ingredient				
Soybean meal	305	241	112	0
WDDGS	0	100	300	470
Dry rolled barley grain	200	200	200	200
Soybean hulls	194	192	152	129
Alfalfa hay	152	122	102	75
Beet pulp	60	60	60	60
Beet molasses	25	25	25	25
Calcium carbonate	18	18	18	18
Mineral premix[a]	18	18	18	18
Urea	10	8	3	0
Canola oil	13	11	5	0
Ammonium chloride	5	5	5	5
ADE Vitamin mix[b]	0.1	0.1	0.1	0.1
Chemical composition, g/kg DM				
Organic matter	907	912	911	911
Neutral detergent fiber	249	257	274	292
Acid detergent fiber	147	152	153	155
Crude protein	271	271	267	266
Ether extract	62	62	61	59
Non fibrous carbohydrates	418	410	399	384

Ash	93	88	89	89
Fatty acids, g/100 g of total FAME[c]				
16:0	10.0	10.6	13.1	15.6
18:0	2.6	2.3	2.0	1.7
*c*9-C18:1	33.2	29.6	21.1	14.3
*c*9,*c*12-C18:2	37.5	40.2	46.5	52.6
*c*9,*c*12, *c*15-18:3	9.7	8.7	7.4	6.3
Saturated fatty acids	14.0	14.7	17.3	19.7
Unsaturated fatty acids	86.0	85.3	82.7	80.3
Monounsaturated fatty acids	35.7	31.9	22.7	15.4
Polyunsaturated fatty acids	50.2	53.5	60.0	64.9

[a] Containing 92.6% NaCl, 4.97% Dynamate®; 0.9% ZnSO4; 0.83% MnSO4; 0.13% CuSO4; 0.1% ethylenediamine dihydroiodide, 80% preparation; 0.005% CoSO4; 0.4% canola oil (as carrier of CoSO4); and 0.0014 % Na2SeO3. No ionophores were included in the diet.

[b] Containing vitamin A (10 000 000 IU/kg); vitamin D (1 000 000 IU/kg); and vitamin E (10 000 IU/kg).

Table 7.2 Dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) degradation characteristics of treatment diets defined as soluble (*A*, %), degradable insoluble (*B*, %), and fractional degradation rate (*k*, h^{-1}), effective degradability (*ED*, %) and effective rumen degradable protein (*ERDP*, g/kg DM).

	WDDGS, g/kg DM					*P*-values		
	0	100	300	470	SEM	Diet	L	Q
Dry matter								
A, % of total DM	51.7	52.5	55.8	56.5	0.21	<0.01	<0.01	<0.01
B, % of total DM	36.5	36.5	32.5	29.8	0.76	<0.01	<0.01	0.03
Degradation rate, *k* h^{-1}	0.058	0.050	0.058	0.071	0.006	0.07	-	-
ED_{DM} [*kp*=0.02], %	79.0	79.7	80.4	79.8	0.39	0.21	-	-
ED_{DM} [*kp*=0.05], %	71.2	71.6	73.5	74.1	0.37	<0.01	<0.01	0.12
ED_{DM} [*kp*=0.08], %	66.9	67.2	69.7	70.6	0.37	<0.01	<0.01	0.04
Crude protein								
A, % of total CP	44.6	48.8	60.1	62.9	0.74	<0.01	<0.01	<0.01
B, % of total CP	56.6	55.3	39.2	34.8	0.48	<0.01	<0.01	<0.01
Degradation rate, *k* h^{-1}	0.055	0.042	0.056	0.064	0.002	0.31	-	-
ED_{CP} [*kp*=0.08], %	67.7	68.6	76.3	78.2	0.368	<0.01	<0.01	0.02
QDP[a], g/kg DM	122.4	133.7	159.3	166.9	0.33	<0.01	<0.01	<0.01
SDP[b], g/kg DM	61.5	52.7	43.6	40.4	0.68	<0.01	<0.01	0.89
ERDP[c], g/kg DM	159.4	153.6	171.0	174.0	0.46	<0.01	<0.01	<0.01
RDP[d], g/kg DM	183.9	186.4	202.9	207.4	0.42	<0.01	<0.01	<0.01
RUP[e], g/kg DM	87.7	85.2	62.9	57.7	0.41	<0.01	<0.01	<0.01
Neutral detergent fiber								
A, % of total NDF	23.9	24.6	25.4	23.4	0.27	0.02	0.01	0.85
B, % of total NDF	40.0	43.7	49.1	49.3	1.44	<0.01	<0.01	0.48
Degradation rate, *k* h^{-1}	0.032	0.022	0.022	0.038	0.0051	0.15	-	-

ED_{NDF} [*kp*=0.02], %	49.2	55.4	56.5	54.5	2.15	0.18	-	-
ED_{NDF} [*kp*=0.05], %	40.1	41.6	43.2	43.6	0.58	0.02	0.01	0.95
ED_{NDF} [*kp*=0.08], %	35.8	36.4	38.0	38.3	0.50	0.03	0.02	0.46

[a] QDP = *A* ×CP.

[b] SDP =((*B*×*k*)/(*k*+0.079))×CP.

[c] *ERDP* = (0.8×QDP +SDP).

[d] RDP = QDP + SDP.

[e] RUP = (CP - RDP).

Table 7.3 Effects of increasing levels of wheat dry distillers' grains with solubles (WDDGS) on 48 h *in vitro* fermentation characteristics and methane production.

	WDDGS, g/kg DM					*P*-values		
	0	100	300	470	SEM	Diet	L	Q
Gas production								
Gas, mL/g DM	153.5	154.3	153.8	159.7	2.64	0.32	-	-
Gas, mL/g DMD[a]	233.7	236.8	241.0	252.6	3.95	<0.01	<0.01	0.39
Methane, mg/g DM	13.7	13.8	12.9	15.1	0.66	0.12	-	-
Methane, mg/g DMD	20.8[b]	21.2[ab]	20.2[b]	23.9[a]	1.00	0.05	0.07	0.08
Fermentation characteristics								
Culture pH	6.24	6.19	6.09	6.17	0.039	0.08	-	-
IVDMD[b], g/kg DM	657	652	638	633	6.05	0.03	<0.01	0.70

[a] Dry matter disappeared.

[b] *In vitro* dry matter disappearance.

Means without common superscripts ([a, b]) differ by least square linear hypothesis test ($P < 0.05$).

Table 7.4 Fermentation characteristics of rumen fluid from lambs (*n* = 15 per treatment) fed isonitrogenous diets with increasing concentrations of wheat dry distillers'grains with solubles (WDDGS).

	WDDGS, g/kg DM					*P*-values		
	0	100	300	470	SEM	Diet	L	Q
Fermentation characteristics								
Rumen pH	5.98	6.08	6.20	6.24	0.091	0.21	-	-
Total VFA, m*M*	170.3	146.4	110.7	98.0	14.41	<0.01	<0.01	0.44
VFA, mol/100 mol								
Acetate (A)	54.9	53.7	52.2	54.6	1.03	0.28	-	-
Propionate (P)	25.3	27.4	30.1	27.3	1.09	0.03	0.10	0.01
Butyrate	12.4	11.1	11.0	10.6	0.67	0.33	-	-
A:P ratio	2.17	1.96	1.73	2.00	0.105	0.03	0.10	0.01
Ammonia-N, mmol	41.1	40.8	33.4	40.6	6.01	0.77	-	-

VFA, volatile fatty acids.

Table 7.5 Effects of increasing concentrations of wheat dry distillers' grains with solubles (WDDGS) in the diet on lamb (*n* = 15 per treatment) performance.

	WDDGS, g/kg DM					*P*-values[a]		
	0	100	300	470	SEM	Diet	L	Q
Initial BW, kg	27.4	29.0	26.1	27.1	1.32	0.45	-	-
Final BW, kg	55.5	54.7	53.4	51.6	1.05	0.07	-	-
Dry matter intake (DMI), g/d	1,911	1,855	1,820	1,700	45.3	0.01	0.02	0.74
Average daily gain, g/d	441	444	400	397	14.3	0.03	<0.01	0.76
Feed conversion ratio, g/g[b]	4.41	4.18	4.55	4.28	0.142	0.51	-	-
Hot carcass weight, kg	27.1	26.1	25.4	24.5	0.62	0.04	<0.01	0.81
Dressing	0.49	0.48	0.47	0.47	0.005	0.26	-	-
Grading rule, mm[c]	19.84	20.40	20.33	19.13	0.93	0.76	-	-
SRNS[d] predicted values								
DMI, g/d	1,850	1,820	1,800	1,750				
ME[e] intake, MJ/d	21.7	21.1	20.5	18.9				
Crude protein intake, g/d	518	503	486	452				
MP[f] intake, g/d	243	234	185	167				
Rumen N balance, g/d	56.8	53.3	52.6	47.7				
Urea cost, MJ/d	1.465	1.603	1.411	0.954				
BW gain, g/d	371	350	337	304				
Rumen pH	5.95	5.97	6.00	6.04				

[a] When fixed effects of diet are not significant ($P > 0.05$), *P* values for contrasts are not reported.

[b] Feed conversion conversion: grams of feed eaten/ grams of BW gain.

[c] Grading rule: body wall thickness between 12th and 13th rib, 11 cm from the carcass midline.

[d] Small ruminant nutrition system (Cannas et al., 2004). Values predicted for 54 kg BW

[e] Metabolizable energy.

[f] Metabolizable protein.

Table 7.6 Effects of increasing concentrations of wheat dry distillers' grains with solubles (WDDGS) in the diet on fatty acid (FA) profiles (% of total FA) in subcutaneous tail fat of lambs (n = 15 per treatment).

	WDDGS, g/kg DM					*P*-values[a]		
	0	100	300	470	SEM	Diet	L	Q
Saturated FA (SFA)								
10:0	0.26	0.26	0.26	0.25	0.015	0.52	-	-
12:0	0.12	0.12	0.12	0.12	0.009	0.95	-	-
14:0	2.11	1.64	2.23	2.14	0.272	0.47	-	-
15:0	1.33	1.43	1.25	1.18	0.108	0.40	-	-
16:0	19.2	17.9	19.7	20.4	0.921	0.20	-	-
17:0	3.10	3.10	2.85	2.90	0.188	0.27	-	-
18:0	15.8	14.3	15.2	14.4	0.795	0.44	-	-
Total SFA	41.9	38.7	41.6	41.4	1.538	0.46	-	-
Monounsaturated FA (MUFA)								
*c*9-14:1	0.08	0.08	0.07	0.08	0.006	0.72	-	-
*c*9-16:1	0.84	0.85	0.81	1.04	0.090	0.26	-	-
*t*6-*t*8-18:1	0.85	0.94	0.92	0.89	0.056	0.75	-	-
*t*9-18:1	0.76	0.82	0.81	0.82	0.047	0.79	-	-
*t*10-18:1	7.2	9.7	9.9	10.2	0.789	0.05	0.02	0.19
*t*11-18:1 (VA)	2.50	2.22	2.10	1.80	0.238	0.24	-	-
*c*9-18:1	34.1	33.4	29.9	29.2	0.764	<0.01	<0.01	0.49
*c*11-18:1	1.10	1.13	1.04	1.04	0.041	0.31	-	-
*c*9-20:1	0.70	0.73	0.49	0.58	0.126	0.58	-	-
Total MUFA	48.1	49.7	46.0	45.6	1.061	0.03	0.02	0.75
Polyunsaturated FA (PUFA)								

*c*9,*c*12-18:2, *n*-6	6.8	7.9	9.0	9.8	0.588	<0.01	<0.01	0.62
*c*9,*c*12, *c*15-18:3, *n*-3	0.93	1.03	0.95	0.88	0.057	0.27	-	-
*c*9,*t*11-18:2, CLA	1.14	1.04	0.90	0.86	0.131	0.43	-	-
*t*10,*t*12-18:2, CLA	0.17	0.25	0.16	0.22	0.055	0.66	-	-
20:4, *n*-6	0.13	0.13	0.13	0.10	0.015	0.72	-	-
20:5, *n*-3, EPA	0.02	0.02	0.02	0.01	0.015	0.69	-	-
22:5, *n*-3, DPA	0.08	0.09	0.09	0.09	0.008	0.71	-	-
22:6, *n*-3, DHA	0.61	0.88	0.92	1.03	0.301	0.81	-	-
Total PUFA	9.9	11.3	12.1	13.0	0.770	0.08	-	-
Total UFA[b]	58.0	61.1	58.1	58.5	1.494	0.45	-	-
PUFA/SFA	0.24	0.29	0.29	0.31	0.029	0.17	-	-
trans FA[c]	12.9	15.1	14.6	15.1	1.041	0.43	-	-
CLA+VA[d]	3.82	3.41	3.04	2.89	0.340	0.25	-	-
n-3 FA[e]	1.65	2.02	1.97	2.02	0.305	0.82	-	-
trans - (CLA+VA)	9.04	11.69	11.59	12.18	0.886	0.09	-	-
18:1 *trans*[f]	11.3	13.6	13.6	13.7	0.944	0.27	-	-
n-6/*n*-3	4.93	4.48	5.04	5.34	0.569	0.63	-	-

[a] When fixed effects of diet are not significant ($P > 0.05$), values for contrasts are not reported.

[b] UFA: unsaturated fatty acids = MUFA + PUFA.

[c] *Trans* FA = C18:1 *t*6-8 + C18:1 *t*9 + C18:1 *t*10 + C18:1 *t*11 (VA) + 18:2 c9-*t*11 (CLA) + 18 :2 *t*10-*t*12 (CLA).

[d] VA = Vaccenic Acid, *t*11-18:1; CLA = conjugated linoleic acid.

[e] *n*-3 FA = C18 :3 + EPA + DHA + DHA.

[f] 18:1 *trans* = *t*6-8 -18:1 + *t*9-18:1 + *t*10-18:1 + C18:1 *t*11 (VA).

Chapter 8. General discussion

This thesis has examined the potential of two important biofuel co-products; glycerol and wheat dry distillers' grains with solubles (WDDGS) as dietary ingredients in sheep diets. The approach has involved a review of the state-of-the-art of the use of glycerol and WDDGS in diets for beef cattle and sheep, and the completion of 3 studies assessing the effects of glycerol on ruminal fermentation, methane production, animal performance and the fatty acid profiles of lambs. Likewise, the effects of using WDDGS as replacement of barley grain were assessed in two studies that evaluated lamb performance, *in sacco* rumen degradation kinetics and *in vitro* fermentation of this substrate.

The primary objectives of the *in vitro* studies with glycerol (Chapters 3 and 4) were to assess the effects of glycerol on ruminal fermentation and methane production. Total gas production was increased in the semi continuous culture of forage diets (Chapter 4) but not in batch culture incubations of barley grain-based diets (Chapter 3). The 8 d adaptation period in the semi continuous culture may partially explain these differences as fermentation of glycerol in batch cultures using rumen fluid from glycerol adapted steers has been shown to produce more gas and methane than those conducted with inoculum obtained from nonadapted herd counterparts (van Cleef et al., 2011). Concurring with the higher gas production and DM disappearance, NDF and ADF disappearance from hay were also increased in the semi continuous culture. Even though we did not study changes in the microbial cellulolytic populations, the results suggest that the activity of this sub-population may have been enhanced with glycerol addition. Consequently, research should be undertaken to characterise the effects of

glycerol on rumen microbes under different dietary managements such as concentrate or forage based diets. Studies reporting effects of glycerol on microbial populations have been conducted under *in vitro* conditions. However, in *in vivo* conditions, direct absorption of glycerol through the rumen wall and passage to the lower gut can result in different consequences as compared to *in vitro* conditions. Studies characterizing effects of glycerol on microbial populations under *in vivo* conditions are scarce and should be undertaken.

In agreement with previous reports (Rémond et al., 1993; Lee et al., 2011), glycerol increased propionate production at the expense of acetate in *in vitro* conditions (Chapters 3 and 4). This shift in ruminal fermentation has been linked to reduced methane (CH_4) production as fermentation pathways that produce propionate compete for hydrogen with methanogenesis. However, results from the aforementioned studies indicate that when glycerol was included as a substrate in concentrate diets, CH_4 production (mg/g DMD) was not affected (Chapter 3) and was actually increased when included in forage diets (Chapter 4). These apparently contradictory results are explained by the chemical structure of glycerol which is more reduced than carbohydrates and has to release 2 electron pairs before entering the propionate fermentation pathway resulting in a neutral electron balance (Chapter 4). Additionally, glycerol is not exclusively fermented to propionate. Acetate and butyrate are also produced in the rumen as a result of glycerol fermentation, resulting in a net electron release that increases the amount of hydrogen available for methanogenesis. However, fermentation to VFA is not the only fate of glycerol in the rumen in *in vivo* conditions. The passage of glycerol to the lower gut and direct absorption through the rumen wall has also

been shown to occur. Whether these alternative fates of glycerol still result in it contributing to a decrease in CH_4 production in the rumen had not been assessed *in vivo,* thus justifying the *in vivo* study that I undertook in Chapter 5. Results from this study indicated that CH_4 emissions were not affected by the inclusion of glycerol in the diet. It should be noted that CH_4 emissions were low in all diets in this study and this situation may have impeded further reductions in emissions. Conditions in this study that contributed to these low emissions included: i) the replacement of barley grain, a source of fast fermenting starch which shifts fermentation to propionate with glycerol; ii) the low NDF concentrations in the diets; and iii) the use of ground and pelleted diets which may have contributed to an increased outflow rate from the rumen and thus, a reduction in ruminal fermentation. All these conditions have been associated with reduced methane production in the rumen. Enteric methane emissions from lambs were not reduced when tested *in vivo,* however emissions from manure could have been reduced as a result of changes in carbohydrate and or N fractions in faeces. A life cycle analysis examining CH_4, N_2O and CO_2 emissions from sheep operations should be conducted to evaluate these potential effects.

The *in vivo* experiment showed that dry matter intake (DMI) was reduced when glycerol replaced barley at concentrations of 140 and 210 g/kg DM (Chapter 5). Average daily gain (ADG) and final live weights tended to decrease at glycerol concentrations of 210 g/kg without affecting feed conversion or hot carcass weights. These results concur with previous reports examining the use of glycerol in beef cattle diets as replacement of corn grain (Parsons et al., 2009) or barley grain (Mach et al., 2009) and in lamb diets as replacement of corn grain (Gunn et

al., 2010a). These findings suggest that glycerol can replace barley grain without affecting lamb performance at concentrations up to 100 g/kg in concentrate diets. However, results from the Rusitec experiment suggest that when utilised in forage diets, glycerol concentrations can be increased without adverse effects on rumen fermentation.

Replacing barley grain with glycerol modified adipose tissue fatty acid profiles in a manner that produced a healthier fatty acid profile in lamb from a human nutrition perspective (Chapter 5). Stearic acid (18:0) was increased at the expense of palmitic acid (16:0) without modifying the proportion of total saturated fatty acids whereas the *trans*-10-octadecenoic acid (*t*10-18:1), a cardiovascular disease linked fatty acid, was linearly reduced in favour of oleic (*c*9-18:1). Likewise, the proportion of linoleic acid was reduced with increasing glycerol in the diet, thus reducing the n-6/n-3 ratio. These changes are probably due to multiple factors associated with increasing glycerol in the diet. Substitution of glycerol for barley grain resulted in a direct reduction in concentrations of palmitic acid in the diet, an outcome that was reflected in the fatty acid profile of lamb adipose tissue. Additionally, the substitution of glycerol for barley reduced the concentration of starch in the diet. This reduction in barley starch may have reduced those members of the bacterial populations responsible for producing *trans* fatty acids such as *Megasphaera elsdenii.* In order to balance for the reduction in fibrous carbohydrates and protein as a result of glycerol addition the concentrations of alfalfa and WDDGS in the diet were increased. These changes in dietary ingredients may have also contributed to the improvement in the fatty acid profile of lambs.

Increasing concentrations of WDDGS resulted in greater eating rates and number of daily visits to the feeders and consequently, greater DMI and ADG (Chapter 6). These responses could arise from the reduced concentration of starch and increased concentrations of NDF and protein in the diet. However, the increases in DMI (16%) were not proportional to the increases in ADG (26%) when comparing control and 400g/kg WDDGS fed lambs. These results concur with previous reports that included WDDGS in the diets of growing beef cattle (Beliveau and McKinnon, 2008) and suggest that, as opposed to with finishing beef cattle, growing beef cattle and lambs respond better to the increased protein concentration that arises from inclusion of WDDGS in the diet. It is possible that the reduced DMI in lambs from the control and 200 g/kg WDDGS groups resulted in borderline or marginally deficient metabolisable protein intakes despite the adequate CP concentration of these diets. Increase in both DMI and CP concentration in the 400 g/kg WDDGS may have compensated for this possible deficiency resulting in an enhanced growth rate. As such, direct replacement of barley grain with WDDGS at concentrations of 400 g/kg seems recommendable from a productive perspective. However, when WDDGS are fed at concentrations above 200 g/kg in diets for beef cattle, nitrogen (N) and phosphorus (P) excretion into the manure are significantly increased (Hao et al., 2009). Even though increased N in manure can be beneficial for crop production, nitrous oxide (N_2O) is produced and emitted at significantly higher levels resulting in increased greenhouse gases emissions. Likewise, given the high solubility of P, excretion may negatively affect water quality and aquatic life. Efforts are currently undertaken to develop mitigation strategies to reduce negative impacts of higher

N excretion from DDGS feeding by binding N in the manure using compounds such as condensed tannins (Hao et al., 2011).

In the previous chapter of this thesis (Chapter 7), the concentrations of WDDGS were increased as replacement of soybean meal and alfalfa to maintain similar concentrations of protein in the diet. However, *in sacco* rumen undegradable protein was reduced and, effective degradability of DM, CP and NDF were increased. In these conditions, and contrasting with the results presented in Chapter 6, DMI and ADG were linearly reduced by increasing concentrations of WDDGS. These results suggest that reduction of starch and increase of NDF by replacing barley grain with WDDGS may increase the acetate to propionate ratio, allowing for a faster recovery of postprandial pH (Beliveau &McKinnon, 2009). These improved rumen conditions also likely contributed to improved DMI and ADG. However, when WDDGS was used to replace barley grain, soybean meal and forage fractions of the diet, physically effective NDF (peNDF) of the diet was decreased, implying reductions in DMI and overall performance. Increased *in vitro* gas and CH_4 production per unit of DM digested at high WDDGS concentrations (470g/kg DM), may have resulted from a combination of increases in NDF and NDF degradability which have been associated with higher CH_4 production (Holthausen et al., 2009).

Some minor improvements in fatty acid profiles of adipose tissue were found when WDDGS replaced barley grain (Chapter 6). These were reflected in increased proportions of 18:3 n-3 (linolenic acid) and a trend for increased *t*11-18:1 (vaccenic acid) with unaffected proportions of *t*10-18:1 (*trans*-10-octadecenoic acid). However, the proportions of *t*10-18:1 (*trans*-10-octadecenoic

acid) were notably lower (2 ± 0.31 *vs.* 8.7 ± 1.5 % of total fatty acid) than the ones reported in the subsequent study (Chapter 7) in which increasing concentrations of WDDGS were used to replace soybean meal and alfalfa. In this last study, the proportions of *t*10-18:1 (*trans*-10-octadecenoic acid) and 18:2 n-2 (linoleic acid) were linearly increased. This suggests that WDDGS modulates the biohydrogenation process in a different manner when it is used to replace cereal grain as opposed to the forage portion of the diet. Future research should be conducted to evaluate the effects of WDDGS on other biohydrogenation intermediates and clarify the consequences of its use in combination with different forage and grain sources on rumen microbial populations.

In this thesis, 99.5% pure glycerol was used in the *in vitro* and *in vivo* experiments. This suppressed the issue of crude glycerin composition in which it is often contaminated with methanol, water, ashes and fatty acid methyl esters at variable concentrations. Usually, glycerine composition ranges widely and concentrations of up to 500g of methanol per kg have been reported. Such high concentrations will require additional purification of glycerine before it can be used as animal feed. Variation in glycerol composition is particularly important in small scale biodiesel plants and nutritionists should always consider local regulations for methanol concentrations in animal feed before formulating diets.

The global production of biofuels is projected to grow and generate abundant stocks of glycerine and DDGS. This growth is sustained on governmental mandates to blend liquid fossil fuels with biodiesel or ethanol. New mandates in the US and Europe are expected to substantially increase the share of agricultural

products (e.g., corn in the US, oilseeds in the EU, and sugar in Brazil) utilised by the biofuels sector. However, increasing concern is being raised on the consequences of these mandates on food costs and land use change; especially in tropical countries where biofuel oriented crop cover are increasing sharply, mostly at the expense of pastureland. As such, the biofuel industry is developing advanced technologies that allow biofuel production from non-food feedstock such as algae to produce biodiesel and lignocellulose biomass to produce ethanol. However, the impact of these technologies is not expected to be realised in the near future and availability of large streams of biofuel co-products will continue for at least the next decade (Hentel et al., 2010). The increasing availability of WDDGS and glycerol should be considered positive as both co-products provide an economically viable source of energy which will be available worldwide. Grain feeding has been associated with negative changes in fatty acid profiles in ruminant meat and the use of these alternative sources of energy to replace cereal grains in the diet can be used as part of a strategy to mitigate these effects.

From the studies carried out in this thesis, it can be concluded that:

1. Glycerol can be used to replace cereal grains in sheep diets at moderate (10% DM) concentrations.
2. Rumen fermentation is affected by dietary glycerol resulting in increased propionate and reduced acetate to propionate ratios. However, these effects are not linked to reduced methanogenesis as fermentation of glycerol to propionate does not act as a net hydrogen sink in the rumen.

3. Glycerol has the potential to modify rumen metabolism of unsaturated fatty acids by altering concentrations of biohydrogenation intermediates such as *trans* fatty acids and may be used to improve fatty acid profiles of meats from grain fed ruminants.
4. Glycerol has the potential to improve fibre digestion when used in forage diets.
5. Wheat based DDGS can improve lamb performance when used at high concentrations in replacement of barley grain by improving feed intake.
6. However, when WDDGS replaced soy bean meal and alfalfa hay it increased nutrient ruminal degradability and reduced rumen undegradable protein, feed intake and lamb overall performance.
7. Using WDDGS in lamb diets can result in minor improvements in adipose tissue fatty acid profiles when used as replacement of barley grain. However when used as partial replacement of dietary forage these improvements can be reverted.

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Appendix 1.

To whom it may concern:

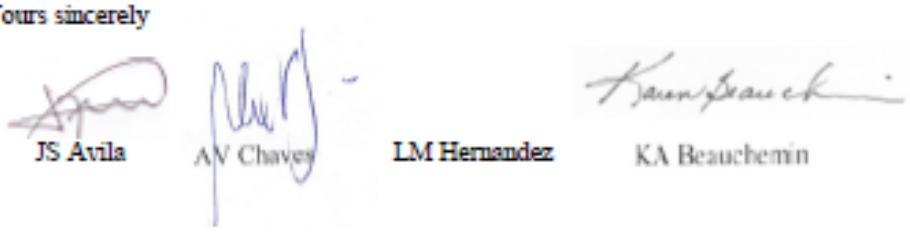
This is a statement describing our contribution to the published journal article presented in chapter 3 of this thesis:

Avila, JS, Chaves, AV, Hernandez-Calva, LM, Beauchemin, KA, McGinn, SM, Wang, Y, Harstad, OM and McAllister, TA (2011) Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on in vitro fermentation and methane production. *Animal Feed Science and Technology*. 166–167, 265–268.

The authors' contributions were as follows:

- Avila JS, Chaves AV and McAllister TA designed the research.
- Avila JS and Hernandez LM conducted the experimental procedures and laboratory analysis.
- Avila JS and Chaves AV analysed and interpreted the data.
- Avila JS drafted the manuscript.
- Chaves AV, McAllister TA, Wang Y, Harstad OM, Beauchemin KA and McGinn SM critically revised the article.

Yours sincerely



JS Avila AV Chaves LM Hernandez KA Beauchemin

SM McGinn Y Wang OM Harstad TA McAllister

To whom it may concern:

This is a statement describing our contribution to the published journal article presented in chapter 4 of this thesis:

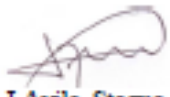
Avila-Stagno, J, Chaves, AV, Ribeiro Jr., GO, Ungerfeld, EM and McAllister, TA. Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique (RUSITEC) system.

British Journal of Nutrition, Published on line 07 October 2013 doi:10.1017/S0007114513003206.

The authors' contributions were as follows:

- Avila-Stagno J, Chaves AV and McAllister TA designed the research.
- Avila-Stagno J and Ribeiro Jr. GO conducted the experimental procedures and laboratory analysis.
- Avila-Stagno J and Chaves AV analysed and interpreted the data.
- Avila-Stagno J drafted the manuscript.
- Chaves AV, McAllister TA, Ungerfeld EM and Ribeiro Jr. GO critically revised the article.

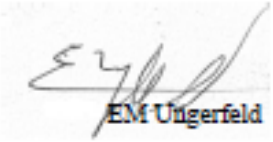
Yours sincerely



J Avila-Stagno

AV Chaves

GO Ribeiro Jr.



EM Ungerfeld

TA McAllister

To whom it may concern:

This is a statement describing our contribution to the published journal article presented in chapter 5 of this thesis:
Avila-Stagno, J, Chaves, AV, He, ML, Harstad, OM, Beauchemin, KA, McGinn, SM, and McAllister, TA (2013) Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles and carcass traits of lambs. *Journal of Animal Science*, 91, 829-837

- Avila-Stagno J, Chaves AV, KA Beauchemin, SM McGinn and McAllister TA designed the research.
- Avila-Stagno J, He ML, conducted the experimental procedures and laboratory analysis.
- KA Beauchemin and SM McGinn supervised the measurements in the chambers
- Avila-Stagno J, SM McGinn and Chaves AV analysed and interpreted the data.
- Avila-Stagno J Drafted the manuscript.
- Chaves AV, He ML, Harstad OM, Beauchemin KA, SM McGinn and TA McAllister critically revised the article

Yours sincerely

J Avila-Stagno AV Chaves ML He OM Harstad

KA Beauchemin SM McGinn TA McAllister

To whom it may concern:

This is a statement describing our contribution to the published journal article presented in chapter 6 of this thesis:

Avila-Stagno, J, Graham, A, McAllister, TA and Chaves, AV (2013) Effects of replacing barley grain with wheat dry distillers' grains on growth performance, eating behaviour and subcutaneous fatty acid profiles of lambs.
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Published online 24 May 2013 doi.org/10.1080/09064702.2013.800906

- Avila-Stagno J, Chaves AV, and McAllister TA designed the research.
- Avila-Stagno J, Graham A and Chaves AV, conducted the experimental procedures and laboratory analysis.
- Avila-Stagno J and Chaves AV analysed and interpreted the data.
- Avila-Stagno J Drafted the manuscript.
- Chaves AV, Graham A and TA McAllister critically revised the article

Yours sincerely

J Avila-Stagno

A Graham

TA McAllister

AV Chaves

This is a statement describing our contribution to the published journal article presented in chapter 7 of this thesis:

Avila-Stagno, J, Chaves, AV, He, ML and McAllister, TA (2013) Increasing concentrations of wheat dry distillers' grains with solubles in iso-nitrogenous finishing diets reduce lamb performance
Small Ruminant Research.
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http://dx.doi.org/10.1016/j.smallrumres.2013.05.003

- Avila-Stagno J, Chaves AV, and McAllister TA designed the research.
- Avila-Stagno J, He ML, conducted the experimental procedures and laboratory analysis.
- Avila-Stagno J, and Chaves AV analysed and interpreted the data.
- Avila-Stagno J Drafted the manuscript.
- Chaves AV, He ML, and TA McAllister critically revised the article

Yours sincerely

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AV Chaves

ML He

TA McAllister