REPRODUCTIVE AND NUTRITIONAL IMPACTS OF DIETARY INCLUSION OF DRIED DISTILLER'S GRAINS WITH SOLUBLES ON FEEDLOT LAMBS AND GROWING RAMS

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Byproduct supplementation in livestock rations is viable and can lead to increased returns to producers. Increasing the inclusion of dried distiller's grains with solubles (**DDGS**) was hypothesized to increase growth performance in feedlot and growing ram lambs, while negatively affecting reproductive characteristics of ram lambs. Ethanol production in the United States provides an affordable byproduct feed for livestock, in the form of DDGS. Due to its RUP and energy content, DDGS can be readily incorporated into ruminant diets, with S concentration being the main concern for livestock health. The impacts of DDGS on feedlot lamb performance were evaluated on 240 crossbred (Suffolk × Rambouillet) lambs in a completely randomized design with a 3 x 2 factorial arrangement of treatments. Lambs were placed into 24 feedlot pens (4 pens/treatment) for a 111 d finishing study. Treatments included increasing concentration of DDGS (0, 15, or 30% DM basis) and inclusion of LAS (0 or 22.05 g/metric ton LAS) resulting in treatments of: 1) 0% DDGS without LAS (**0DDGS-NL**), 2) 0% DDGS with LAS (**0DDGS-L**), 3) 15% DDGS without LAS (**15DDGS-NL**), 4) 15% DDGS with LAS (**15DDGS-L**), 5) 30% DDGS without LAS (**30DDGS-NL**), and 6) 30% DDGS with LAS (**30DDGS-L**). The inclusion of LAS increased ($P \leq 0.02$) final BW, ADG, G:F, and HCW. To evaluate the effects of DDGS on growth performance and reproductive traits in ram lambs, 112 Suffolk and Hampshire ram lambs were allocated to four treatments (n = 4 pens/treatment) in a completely random design. Basal diets were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**). Treatments were (% DM basis): 15% of the ration as DDGS substituted for corn (**15DDGS**), 30% of the ration as DDGS substituted for corn (**30DDGS**) and 45% of the ration as DDGS substituted for corn (**45DDGS**). Rams were fed to d 112 on their respective treatment (**PHASE 1**), after which rams were placed on the CON ration until d 168 (**PHASE 2**). Many growth traits

exhibited positive quadratic or cubic effects ($P \leq 0.05$), indicating a possibility of both DDGS and LAS being viable supplements for sheep in growing rations.

Key words: dried distiller's grains with solubles, feedlot, growth, lambs, reproduction, semen quality

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DEDICATION

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LIST OF ABBREVIATIONS

µL.....microliter

ADF.....Acid Detergent Fiber

ADG.....Average Daily Gain

A:P .....Acetate:Propionate Ratio

BCTRC .....Boneless Closely Trimmed Retail Cuts

BUN .....Blood Urea Nitrogen

BW .....Body Weight

˚C .....Degrees Celsius

Ca .....calcium

CK.....Creatine Phosphokinase

cm.....Centimeter

CON .....Control

CP.....Crude Protein

Cu.....Copper

CV .....Coefficient of Variation

d.....Day

DDGS.....Dried Distiller's Grains with Solubles

DIP .....Degradable Intake Protein

DM .....Dry Matter

DMI.....Dry Matter Intake

DNA.....Deoxyribonucleic acid

FSH .....Follicle Stimulating Hormone

G:F .....Kg of Gain:Kg of Feed; Feed Efficiency

G.....Gauge

g .....Gram

GnRH .....Gonadotropin Releasing Hormone

HCW .....Hot Carcass Weight

H_2S .....Hydrogen Sulfide Gas

h.....Hour

IUP .....Intestinally Undegradable Protein

kg.....Kilogram

Kg/d .....Kilograms per Day

LAS .....Lasalocid

LH .....Luteinizing Hormone

LM.....Longissimus Muscle

LEA.....*Longissimus dorsi;* Loin Eye Area

LSMEANS .....Least Squared Means

ME.....Metabolizable Energy

mg .....Milligram

min .....Minute

mL .....Milliliter

mm .....Millimeter

mo .....Month

MP .....Metabolizable Protein

mRNA .....Messenger Ribonucleic Acid

N.....Nitrogen

NAN.....Non-Ammonia Nitrogen

NDF.....Neutral Detergent Fiber

NRC .....National Research Council

OFDA.....Optical Fiber Diameter Analyzer

OM .....Organic Matter

PEM .....Polioencephalomalacia

P .....P

ppm .....Parts per Million

RUP.....Rumen Undegradable Protein

S .....S

SAS .....Statistical Analysis Software

SD .....Standard Deviation

SEM .....Standard Error of the Mean

SQ .....Subcutaneous

SSCs.....Spermatogonial Stem Cells

TDN .....Total Digestible Nutrients

UIP .....Undegradable Intake Protein

VFA .....Volatile Fatty Acids

wk.....week

CHAPTER 1: LITERATURE REVIEW

Introduction

Ethanol production in the United States continues to increase (Renewable Fuels Association, 2016). The byproduct of the ethanol industry, dried distiller's grains with solubles (**DDGS**), provides an affordable and viable feed source for livestock, especially ruminants. Dried distiller's grains can be readily incorporated into diets to provide rumen undegradable protein (**RUP**) to increase metabolizable protein, as well as increase the energy availability. However, DDGS can also be high in S and crude fat, providing animal health challenges (Olkowski et al., 1992; Ham et al., 1994; Gould et al., 1998). Newer ethanol distilleries have decreased the variability in crude fat and protein, however mineral content can still be highly variable (Spiehs et al., 2002). Many sheep producers are apprehensive about feeding DDGS to feedlot lambs above 20% of the ration for fear of S toxicity, which the NRC (2007) indicates is likely to occur when S exceeds 0.3% (DM) in a high-concentrate ration, possibly leading to polioencephalomalacia (**PEM**). Research involving DDGS inclusion in feedlot rations continues to increase, to quantify both its positive and negative traits. Dried distiller's grains with solubles have been included in both cattle (Bos Taurus) and sheep (Ovis aries) rations at rates of up to 60% with no negative effects on performance or signs of PEM (Ham et al., 1994; Peter et al., 2000; Huls et al., 2006; Schauer et al., 2008; Neville et al., 2010; Neville et al., 2011). Most of the existing data investigates the feeding of DDGS to beef cattle in the finishing phase (Ham et al., 1994; Peter et al., 2000) which resulted in improved growth performance. Increases in ADG and G:F have also been reported when including DDGS in corn-based finishing diets at inclusion rates of 40% (Ham et al., 1994). At inclusion rates of 20% of the diet, no effect on nutrient digestion or ruminal fermentation characteristics were observed (Peter et al., 2000).

The impacts of including ionophores, such as lasalocid (**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ), in rations including DDGS have not been investigated in lambs. Ionophores are typically used to improve efficiency of livestock production (Crane et al., 2014). The potential to improve growth performance and feed efficiency by including ionophores in lamb rations has been investigated (Funk et al., 1986; Crane et al., 2014), however, these studies did not include DDGS in the ration. By inhibiting hydrogen- and ammonia-producing bacteria in the rumen, LAS decreases the acetate:propionate ratio (**A:P**) and improves feed efficiency (Bartley et al., 1979). Kung et al. (2000) determined hydrogen sulfide (**H_2S**) production might increase when ruminants are fed ionophores such as LAS. There is also limited data on the metabolism of DDGS in growing and finishing (70% concentrate) diets. Therefore, with a potential increase in H_2S production in lambs fed LAS, the potential exists for an increased likelihood of S toxicity for lambs fed both DDGS and LAS.

Data is limited on the carcass effects of feeding DDGS to growing lambs, Van Emon et al. (2013) observed no effects on carcass characteristics of rams when feeding increasing levels of DDGS. Roeber et al. (2005) observed that when Holstein steers are fed up to 50% DDGS, there are no effects on tenderness or sensory traits compared to those steers fed corn-based diets. With the growing popularity of feeding DDGS by sheep producers, research needs to expand to investigate the possible impacts of DDGS on ram reproductive traits and fertility. For example, Van Emon et al. (2013) reported a linear decrease in spermatozoa concentration as DDGS increased in the diet when fed to growing ram lambs. However, this is the only trial found, that has evaluated DDGS in growing ram lamb rations, and its potential effect on male fertility.

The objective of this review is to provide a discussion on the available current literature regarding the effects and impacts of inclusion of DDGS with or without LAS on lamb growth

and performance, as well as reproductive performance. Therefore, discussions of CP supplementation, crude fat and energy supplementation, S consumption, as well as ionophore inclusion will be included. Impacts on ram fertility in relation to DDGS inclusion will also be included in the discussion.

Ethanol Production

Production of ethanol can come from many carbohydrate sources, such as corn, sorghum, wheat, barley, sugarcane, brewery byproducts, wheat straw, or corn stover (Nguessan, 2007). However, this review will focus on ethanol produced from corn, as it is the primary starch source for ethanol production in the Northern Great Plains. There are two techniques by which ethanol is produced, wet or dry milling. In the United States, most ethanol is produced through dry milling (Bothast and Schlicher, 2005). Both types of processing produce byproducts that are used in livestock feeds. However, this literature review will focus on dry milling processing which produces thin stillage, wet and dry distiller's grains, condensed distiller's solubles, and wet, and dry distiller's grains with solubles (Kalscheur et al., 2008).

When included in growing and finishing rations, corn is an adequate concentrate for growing lambs and cattle, and in fact is a standard for livestock rations. On a DM basis corn is 72% starch, 9.5% CP, and 4.5% oil (Table 1.1; McAloon et al., 2000; NRC, 2016). Dry milling, as described in Figure 1.1, begins with the whole corn kernel, indicated in the figure as corn. In processing, ethanol is made from the starch, which is converted to glucose and fermented to form ethanol. The remaining nutrients such as protein, minerals, fat, and fiber, are then concentrated into byproducts which can then be used as livestock feed (Bothast and Schlicher, 2005).

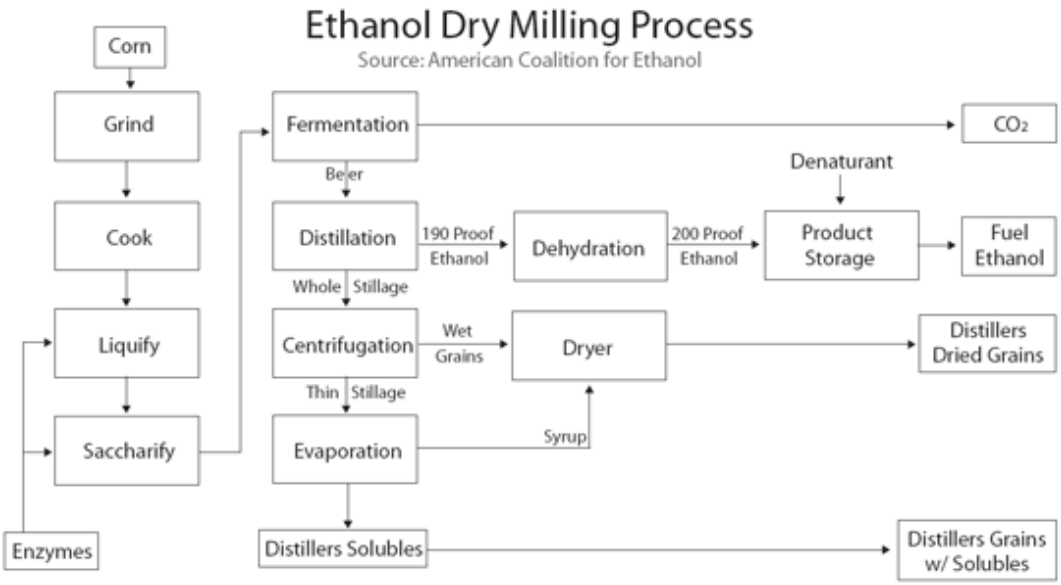

Figure 1.1. Corn dry milling process (Advanced BioEnergy, LLC).

Dried Distiller's Grains with Solubles Nutrient Content

Dried distiller's grains with solubles contains approximately 31% CP, a range of 3-12% ether extract, 68% RUP, and 34% NDF (NRC, 2000). Due to processing, the non-starch nutrients are highly concentrated in DDGS compared to corn (Bothast and Schlicher, 2005). Table 1.1 reports the nutrient composition of cracked corn in comparison to DDGS. The protein that remains in DDGS following processing is much greater in RUP than corn due to the rumen degradable protein (DIP) mostly being degraded during fermentation (Schingoethe, 2006). Undegraded intake protein values of DDGS have been reported to be as much as 2.6 times greater than that of soybean meal, making DDGS an excellent source of protein for the ruminant animal since the nutrient is available for absorption in the small intestine as RUP (Aines et al., 1987). Additionally, NDF is also more concentrated in DDGS in comparison to corn, meaning that DDGS are a good source of readily digestible, non-forage fiber (Ham et al., 1994). Due to the greater amount of digestible fiber and minimal starch content of DDGS compared to corn, incidence of ruminal acidosis may be decreased when DDGS is included in diets (Ham et al.,

1994). This decreased incidence of acidosis is most likely because of reduced starch intake, as high starch intake leads to increased production of ruminal organic acids that cause acidosis.

Table 1.1. Nutrient composition of corn and dried distiller's grains with solubles (DDGS)

Item	Corn	DDGS
DM, %	90.0	90.0
	----------DM basis----------	
TDN, %	90.0	89.0
Starch, %	72.0	5.9
NDF, %	10.5	33.7
CP, %	9.5	30.8
RUP, % CP	55.0	67.9
S, %	0.11	0.66
Fat, %	4.1	10.7
Ash, %	1.5	5.3

[1]Adapted from McAloon et al. (2000) and NRC (1996 and 2016).

One of the main concerns when feeding DDGS to livestock are the high levels of minerals, specifically S and P. Levels of P in the diet can be managed by adding other minerals to the diet, such as limestone or calcium carbonate. Phosphorus levels are a concern due to the occurrence of urinary calculi from excess S or a Ca: P ratio not being balanced to at least 1.2:1. Levels of S in the DDGS can vary depending on the ethanol plant due to the addition of Sic acid during fermentation in ethanol production (Rausch and Belyea, 2006). S in DDGS comes from two sources: 1) endogenous S contained in corn, which is concentrated by fermentation of starch to ethanol, and 2) Sic acids, added to regulate pH during fermentation, prevent undesired fermentation, and clean equipment in the distillation phase (Vannes et al., 2009). The pH of mash during fermentation must be lowered to 6.0 to activate enzymes and improve fermentation (Bothast and Schlicher, 2005).

High dietary S concentrations can cause PEM by increasing the ruminal bacterial populations that produce thiaminases (McDowell, 2000). Polioencephalomalacia is thought to be

caused by a thiamin deficiency. S might influence thiamin by cleaving it at the methylene bridge between the pyrimidine and thiazole rings, therefore mimicking the action of thiaminases (McDowell, 2000). In the rumen, there is much thiaminase activity by the microbial population, in addition to thiamin-destroying activity possibly being increase by sulfates (Olkowski et al., 1993). The increased presence of thiaminases reduces the amount of thiamin available, which could possibly result in a thiamin deficiency (McDowell, 2000). Thiamin deficiency can be treated or prevented with thiamin supplementation if caught in the early stages of deficiency. When feeding high concentrate diets to ruminants, Mathison (1986) recommends feeding 4-6 mg thiamin per kg of feed (DM) to prevent subclinical deficiency. However, supplementation of thiamin does not guarantee the prevention of PEM when feeding high S diets to ruminants. In lambs fed high S diets, Olkowski et al. (1992) reported that PEM was prevented by supplementing 243 mg thiamin/kg dietary DM, although brain lesions were not totally prevented. Schauer et al. (2008) and Huls et al. (2008) reported that feeding lambs or steers supplemental thiamin (142 mg•hd^{-1}•d^{-1} and 150 mg•hd^{-1}•d^{-1}, respectively) with up to 60% distiller's grains in the diet resulted in no incidences of PEM. In contrast, Buckner et al. (2007) ended dietary treatments fed to steers containing 50% DDGS (0.6% S) due to multiple steers dying or exhibiting visual symptoms of PEM, even while receiving 150 mg•hd^{-1}•d^{-1} of thiamin. These conflicting results not only show the need for additional research, but the importance of testing DDGS from different distilleries, feedlot water sources, and soils on individual farms.

Gould (1998) reported a link between dietary S and ruminal pH and concluded that in diets exceeding 0.3% S, the combination of S concentrations, ruminal sulfide production, and increased thiaminase production may increase the incidence of PEM. However, Alves de Oliviera et al. (1996) concluded that a decrease in ruminal pH does not decrease the microbial

production of thiamin in the rumen, although the reduction in ruminal pH does cause an increase in the population of thiaminase producing bacteria.

With the possible alterations to the rumen environment stated above, there is cause for concern when including both DDGS and an ionophore in a diet. The alterations in pH due to both the S content as well as the shift in the microbiome could potentially increase the likelihood of PEM occurrence, followed by possible exacerbation of PEM symptoms due to increases in H_2S production. However, there are many differing results across experiments and species, therefore more research is needed to be conclusive.

Dried Distiller's Grains with Solubles in Growing and Finishing Lamb Diets

Dried distiller's grains with solubles is typically fed at 15-20% of the diet (DM basis) as a replacement of corn, serving as both a CP and energy source (Klopfenstein, 2001), especially in lamb finishing rations. When compared to corn as a protein supplement, DDGS contains almost three times the concentrations of CP as corn (Table 1.1). Additionally, most of the protein is provided as RUP and is therefore more effectively utilized by the animal, rather than the ruminal microbes, unless insufficient UIP is provided. Even with the increased CP content of DDGS, the TDN values of corn and DDGS are very similar. Many researchers have considered that with the decreased starch content in DDGS, the feed is also much safer to feed than corn when considering the occurrence of acidosis. There are many factors related to the feeding of DDGS that could cause improved performance such as an increase in UIP, fat, as well as greater NDF content, reducing the potential for acidosis (Ham et al., 1994).

Intake, Performance, and Passage Rate

Dry matter intake in ruminants is a trait that is not completely understood, along with the factors that affect it. However, there is much data in cattle suggesting that the inclusion of DDGS

increases DMI (Ham et al., 1994; Mateo et al., 2004; Trenkle, 2004). Trenkle (2004) fed Holstein steers up to 40% DDGS or wet distiller's grains with solubles (WDGS) and reported steers receiving DDGS had increased DMI, and moreover, increasing levels of DDGS tended to linearly increase ADG. During the finishing phase, there were no effects on DMI, ADG, or G:F. Vander Pol et al. (2006) and Buckner et al. (2007) observed that DDGS or WDGS inclusion increased DMI in cattle. Buckner et al. (2007) also reported increases in ADG, final BW, and G:F in steers fed 15 or 30% DDGS compared to bran cake based diets. Other experiments have reported increases in lamb ADG and DMI as concentration of DDGS in the diet increased (Schauer et al., 2008), most likely due to the increased nutrient density of the diet, specifically crude fat and CP. The difference in treatment responses is likely due to the increased fiber content in the diets including DDGS since increased fiber can decrease rate of passage and therefore, decrease intake by increasing rumen fill. However, passage rate is dependent on many factors, such as feed intake, dietary fiber content, and the physical form of the diet (Faichney, 1993). Generally, as intake increases, passage rate increases (Guthrie and Wagner, 1988). Others have proposed that increased intake is accompanied by more rapid passage rate, as well as ruminal DM fill (Owens and Goetsch, 1986). Bodine et al. (2000) also reported that alterations in intake, passage rate, and digestion rate can markedly alter ruminal volume. However, limited data is available on the effects of feeding DDGS in high concentrate diets on passage rate, especially in lambs. Ham et al. (1994) reported that feeding 40% DDGS ration to steers increased passage rates compared to those fed DDGS with water included in the diet (to compare to wet distiller's grains), and both diets had faster passage rates compared to the wet and condensed distiller's grains diets. However, Firkins (1984) reported no differences in fluid dilution rates when steers were fed diets with or without DDGS.

Digestion

Mertens (1993) stated that diet, intake, ruminal fermentation, passage rate, and microbial efficiency are all factors that affect digestibility. Multiple labs have reported no differences in total tract OM digestibility when feeding DDGS to cattle (Peter et al., 2000; Mateo et al., 2004; Kalscheur et al., 2005). When observing total CP flow to the duodenum of steers, no differences were reported by Firkins et al. (1984) when feeding diets of either wet or dry corn distiller's grains, cracked corn, or corn starch grits. Firkins et al. (1985) reported similar apparent N digestibility in lambs fed either DDGS or wet distiller's grains. Chen et al. (1977) and Mateo et al. (2004) also reported no differences in CP and NDF digestion in steers fed DDGS when compared to those fed corn-based diets.

When measuring S digestibility of DDGS in lambs, Neville et al. (2010) observed that lambs excrete substantial amounts of S when consuming DDGS and that water intake and urinary output increase with increasing S intake. This could imply that although the NRC (2007) reports that feedlot type diets should not contain S in excess of 0.3% of the ration due to toxicity issues, lambs may merely excrete the excess S via their urine, theoretically preventing toxicity. Neville et al. (2010) also observed a linear increase in ruminal H_2S concentrations with increased inclusion rates of DDGS in the diet, indicating that *in vivo* S production was increased, however, not causing PE or toxicity.

Neville et al. (2010) observed no differences in ruminal pH in lambs fed diets including DDGS. It is important to consider that a reduction in ruminal pH represents an increase in hydrogen ions available to form H_2S (Morrow et al., 2013). However, Morrow et al. (2013) disproved the hypothesis that neutralizing the acid in DDGS with NaOH would improve fiber digestion, as well as feed efficiency. This is likely due to the inhibition of growth and fiber

fermenting capacity of cellulolytic bacteria at low rumen pH (Mould et al., 1983; Hoover, 1986). These findings suggest that the decrease in ruminal pH could be due to the pH of DDGS itself, rather than the altering of the rumen environment and in turn, the microbial population (Mould et al., 1983; Hoover, 1986; Morrow et al., 2013).

Felix et al. (2012) reported cattle consuming diets including DDGS exhibited linear increases in ruminal acetate, propionate, and total VFA concentrations. Kung et al. (2000) observed that excess dietary S increased ruminal sulfide production; however, it had no effect on VFA production. Smith et al. (2010) reported that increasing S concentrations in culture linearly increased H_2S production, while added S had no effect on molar proportions or total concentrations of VFA or the A:P ratio. Therefore, there are some differing results amongst DDGS and S trials and how VFA production is affected.

Ionophores and Sheep

The mechanisms by which ionophores function in the ruminant animal are clearly understood; however, when considering dietary changes and difference amongst species, details of how N metabolism are affected are not well understood. With many variables affecting the digestibility of feeds in the rumen, it is close to impossible to elucidate all of the scenarios affecting N metabolism in the rumen. One difficulty is the lack of a consolidation and comparison of results of specific subjects related to N metabolism, especially in sheep. The data needs to be reviewed in specific dietary settings to determine the possible impacts that feeding ionophores might have on N metabolism in the sheep. The objectives of this section are to outline the functions and mechanisms of ionophores affecting N metabolism in ruminants, as well as how the mechanisms can differ with changing diet types and other possible interactions with ionophores. Ionophores such as monensin (**MON**) and LAS will be the focus. The

following includes reviews of both in vitro, as well as in vivo studies, to provide a thorough analysis with some cattle data included for thoroughness.

Function and Mechanisms

Since the 1980's, ionophores have been reported to increase ruminal propionic acid yield and decrease methane production, while also decreasing proteolysis and deamination of dietary protein. The focus of this section will be on the function and mechanisms by which ionophores affect ammonia and urea production, as well as proteolysis and deamination.

Ionophores act by disrupting the membrane potential through conducting cations and anions through the lipid membrane, therefore exhibiting cytotoxic properties. Monensin for instance, creates a decrease in intracellular K concentration and intracellular pH (Russell and Strobel, 1989). This creates a large K gradient that drives an influx of H^+, and although the cells are still capable of metabolizing glucose, the activity of ATPase cannot maintain a sufficient alkaline intracellular pH (Russell and Strobel, 1989).

Ammonia and Urea

Chalupa (1980) observed that monensin can have a protein sparing effects and can cause a decrease in NH_3-N production. However, the exact cause was not known at the time**.** According to Chen and Russell (1991), MON had little effect on protein degradation, but caused a large decrease in ammonia production and an increase in non-ammonia, non-protein N. They concluded that significant quantities of peptide N could not be degraded by rumen microbes and that MON could increase peptide flow from the rumen. In the research by Chen and Russell (1991), a protonophore that inhibits both gram-positive and gram-negative bacteria, did not cause a greater decrease in ammonia than MON, an ionophore that is primarily effective against gram-

positive bacteria. Therefore, Chen and Russell (1991) suggested that the protein sparing of MON could largely be due to its inhibition of gram-positive bacteria.

Proteolysis and Deamination

The inclusion of ionophores has improved protein bypass by as much as 22 to 55% in various research trials (Poos et al., 1979; Isichei and Bergen, 1980; Chen and Russell, 1991). Monensin has been observed in many studies to decrease the flow of non-ammonia N (**NAN**) to the small intestine as well as decreasing the efficiency of microbial growth (Poos et al., 1979; Isichei and Bergen, 1980; Chen and Russell, 1991; Table 1.2). Other trials have shown that when MON was fed to steers, that NAN-non-protein nitrogen was increased in the rumen (Chen and Russell, 1991; Bergen and Bates, 1984). With these improvements in N breakdown and flow, it is quite possible that an interaction between DDGS and LAS would occur due to the increased availability of CP and RUP from DDGS.

Applied N Metabolism Trials

In a more applied setting, Smith et al. (2010) reported minimal interactions from feeding MON with DDGS to cannulated jersey steers. Increasing MON linearly increased proportions of propionate, while linearly decreasing acetate, butyrate, and isovalerate. There was also a linear decrease in the A:P ratio as MON concentrations increased. Monensin had no effect on total VFA production. Neville et al. (2010) conducted a trial feeding 0%, 20%, 40%, and 60% DDGs (DM basis) diets to finishing phase lambs. This trial included LAS in each treatment ration at the rate of 0.085% (DM basis) and observed no changes in ruminal pH or DMI as DDGS increased in the ration.

Crane et al. (2014) observed no interactions or main effects for particle size of corn and market lamb pellet mixed rations and/or LAS among diets for DMI, N intake, N balance, or

serum urea-N concentration. There is conflicting research on particle size and its effects on N digestion, N balance, and serum urea-N concentration. Although there was no particle size effect in the trial by Crane et al. (2014), previous research by Kerley et al. (1985) reported that N digestion was increased in lambs fed 6.5 mm, 5.4 mm and 0.8 mm particle size corncob diets, while the 1.4-mm diet was decreased. The 1.4-mm diet also had higher fecal N loss when compared to the other diets. Other research by Perez-Torres et al. (2010) reported no differences in DM or OM intake or digestibility in diets that differed in particle size, agreeing with results from the previously mentioned trial. Particle size results are included in this review to show how sensitive N digestibility in sheep can be and how, in some cases, much variability can be taken out of the digestibility equation when ionophores are included. The addition of LAS decreased fecal-N excretion in the trial by Crane et al. (2014), similar to findings by Ricke et al. (1984), in which LAS-fed lambs had decreased fecal-N excretion when compared to lambs fed MON or no ionophore. Varying results exist on the effect of LAS on N digestibility, with some reporting increased N digestibility (Paterson et al., 1983; Ricke et al., 1984), while others report that it and N balance remained unaffected (Funk et al., 1986). Ricke et al. (1984) also reported that LAS-fed lambs had lower fecal N loss and therefore higher N retention, which could reflect increased digestibility of the diet. Differences among reported experiments could be due to the different types of collection, ranging from N-balance trials, to in situ techniques.

Classically, as described by Thivend and Jouany (1983), ionophores substantially modify microbial activity in the rumen by targeting specific bacteria to terminate. This targeting by ionophores, in turn, directs fermentation towards a greater production of propionate and less methane. Thivend and Jouany (1983) continue by concluding this inhibition reduces the amount of microbial synthesis and limits feed protein breakdown. The effect of LAS on the microbial

population in the rumen results in decreased OM fermentation and bacterial protein synthesis in the rumen as well as increasing water intake. With these effects observed throughout many trials, Thivend and Jouany (1983) believe that this leads to accelerated turnover of the liquid fraction in the rumen, thus leading to limited development of the microbial population as well as the increased rate of passage to the small intestine and therefore reduced ruminal digestion.

Table 1.2. Effects of ionophores on N metabolism in ruminants

Effects Observed	Species and Ionophore	Reference(s)
↓ ammonia production	Lambs, Steers; Monensin	Poos et al., 1979
↓ ammonia production	Lambs, Steers; Monensin	Russell and Strobel, 1989
↓ ammonia production	Lambs, Steers; Monensin	Chen and Russell, 1991
Peptide-N degradation	Steers; Monensin	Chen and Russell, 1991
↑ NAN-NPN	Steers; Monensin	Chen and Russell, 1991
↑ NAN-NPN	Steers; Monensin	Bergen and Bates, 1984
↓ VFA production, A:P, ↑ Propionate	Steers; Monensin	Smith et al., 2010
↓ methane production	Steers; Monensin	Bergen and Bates, 1984
↓ methane production	Steers; Monensin	Russell and Strobel, 1989
No changes in ruminal pH or DMI	Lambs; Lasalocid	Neville et al., 2010
No changes in ruminal pH or DMI	Lambs; Lasalocid	Crane et al., 2014
↓ flow of NAN to SI	Steers, lambs; Monensin	Poos et al., 1979
↓ flow of NAN to SI	Steers, lambs; Monensin	Isichei and Bergen, 1980
↓ efficiency of microbial growth	Steers, lambs; Monensin	Poos et al., 1979
↓ efficiency of microbial growth	Steers, lambs; Monensin	Isichei and Bergen, 1980
↓ fecal N loss	Lambs; Lasalocid	Crane et al., 2014
↓ fecal N loss	Lambs; Lasalocid	Ricke et al., 1984
No effects: N intake, balance, BUN	Lambs; Lasalocid	Crane et al., 2014
Increased N digestibility	Lambs; Lasalocid	Paterson et al., 1983
Increased N digestibility	Lambs; Lasalocid, Monensin	Ricke et al., 1984
N balance unaffected	Lambs; Lasalocid	Funk et al., 1986
Increased passage rate	Lambs; Lasalocid	Thivend and Jouany, 1983

[1]↓=decreased and ↑=increased.

Conclusion

The previously mentioned research indicates some of the unpredictability of feeding ionophores to sheep, as well as cattle. Typically, ionophores increase growth, usually by increased N retention (Callaway et al., 2003). In some studies this is not the case, especially when ruminants are fed roughage- vs. concentrate-based diets (Fluharty et al., 1999). This raises the question as to why, in this case, would increased growth have been observed when feeding LAS, but increased N retention was not observed? Another important question is: was there any underlying interaction with DDGS, or was the linear increase in DMI and G:F as DDGS concentrations increased driven by a response to LAS and this was just not effectively tested or analyzed? With all of these questions, a thorough review of results is needed. Through this compilation of research results, there are a few likelihoods that cause these discrepancies: 1) Resistance of the microbial population to ionophores, 2) Some form of error in the trial methods, or 3) The underlying interaction of DDGS and LAS.

Many of the trials cited in this review are in the form of N balance trials, therefore, end-point error as well as other forms of error could be attributing to misrepresentation of the data. With the exception of the trials of Crane et al. (2014) and Neville et al. (2010), most of the other sheep trials mentioned here are over 30 years old. Differences could in fact be caused by genetic differences in sheep over years as well as changes in quality and processing of feedstuffs, such as DDGS. These differences could also lead to genetic alterations in the make-up of the rumen microbiota, leading to effects of resistance to ionophores by the microbes as discussed by Russell and Strobel (1989). Overall, the decrease in VFA production leads to a decrease in methane production (Bergen and Bates, 1984) and has indicated that feeding ionophores increases the ME available to the host, while sparing AA available for gluconeogenesis; all while stimulating body

protein synthesis. This shift is likely accountable for increased efficiency of the host ruminant animal, a pairing of protein sparing events and shifts in VFA production.

Ram Reproductive Performance and Dried Distiller's Grains with Solubles

Reproductive physiology is a pillar of animal production systems, especially in conjunction with genetics and nutrition. Recently, there has been a focus on understanding the female reproductive system and possible contributions it might have on future offspring. However, there has been a lack of focus on male reproductive contributions to livestock production systems. Beyond breeding soundness examinations for male livestock, many scientists and veterinarians alike fail to focus on male reproductive physiology and the possible impacts nutrition may have on production. With this in mind, there are a few labs focusing on male physiology and the possible impacts on future generations. The objective of this section is to review spermatogenesis and the importance of spermatogenic production stages, as well as outlining research techniques useful in the study of spermatogenesis, spermatozoa health, and possible impacts of DDGS.

Overview of Spermatogenesis

Endocrine Control (Preg. and Part., Senger (2003); See Figure 1.2). Males and females produce many of the same hormones, mostly originating from similar tissues. The hypothalamus releases GnRH, the anterior pituitary releases LH and FSH, while the gonads (the testes) produce testosterone, estradiol, and Inhibin. Testosterone is synthesized by the Leydig cells within the testes, while estradiol and inhibin are synthesized by the Sertoli cells. However, there is no surge center in the hypothalamus of the male, rather the tonic center discharges GnRH in a pulsatile manner to stimulate LH and FSH. Luteinizing hormone, a glycoprotein, acts on Leydig cells to stimulate production of testosterone. While some testosterone is transported across the basement

membrane into Sertoli cells, the rest goes into systemic circulation. Follicle stimulating hormone, another glycoprotein, acts on the Sertoli cells to stimulate spermatogenesis and Sertoli cell function. It is also responsible for activation of the enzyme, aromatase, in the conversion of testosterone to estradiol, taking place in the Sertoli cells. When circulating levels of FSH are reduced, impairment of Sertoli cell function and spermatogenesis occurs. Testosterone, a steroid, is bound by androgen binding proteins in Sertoli cells and then taken into the lumen of the seminiferous tubule for transport to the epididymis. Following conversion to estradiol, testosterone crosses the basement membrane into circulation. When testosterone and estradiol are present in systemic circulation, the hypothalamus responds by causing a slowdown in the release of GnRH, which then results in a reduced output of FSH and LH. Inhibin, another glycoprotein within the Sertoli cells, negatively feeds back on the anterior pituitary to selectively suppress FSH.

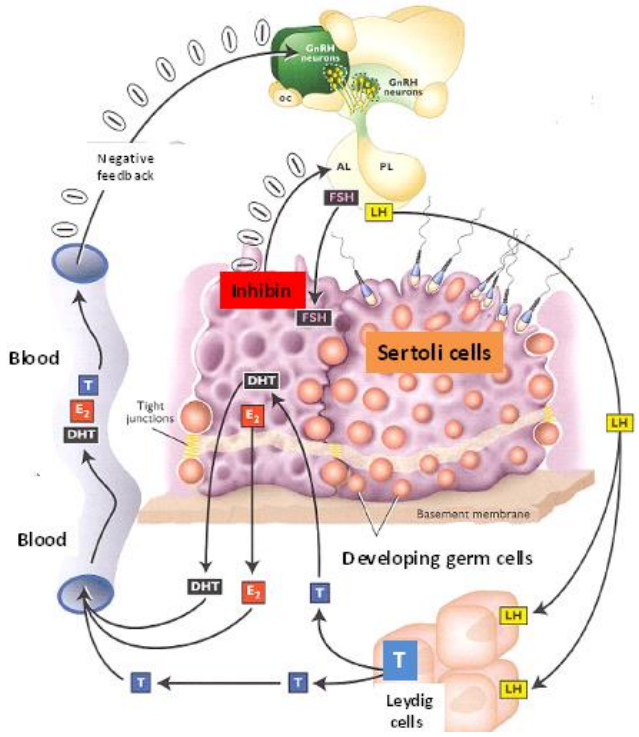

Figure 1.2. Hormonal contributions in the male and their sources. (Preg. and Part., Senger, 2003).

Spermatogenesis (Preg. and Part., Senger, 2003). Spermatogenesis occurs in the seminiferous tubules of the testes in three main phases: spermatocytogenesis, meiosis, and spermiogenesis. The spermatocytogenesis phase consists of mitotic cell division, proliferation, and maintenance of the spermatogonia and takes place in the basal compartment. Spermatogonia go through many mitotic divisions, the last of which results in primary spermatocytes (See Figure 1.3; Preg. And Part., Senger, 2003). Three types of spermatogonia are found in the basal compartment, which are spermatogonia A, spermatogonia intermediate, and spermatogonia B. Duration of spermatocytogenesis varies in different species: bulls ~21 d, rams ~ 18 d, and stallions ~ 21 d. The second stage is meiosis, taking place in the adluminal compartment of the seminiferous tubule (Figure 1.3), during which the chromosomes are reduced by half in the gamete, moving from the diploid to haploid state. Primary spermatocytes then undergo meiosis I

and become secondary spermatocytes, subsequently undergoing meiosis II resulting in the round spermatids. The lifespan of spermatocytes is short-lived (1-2 d).

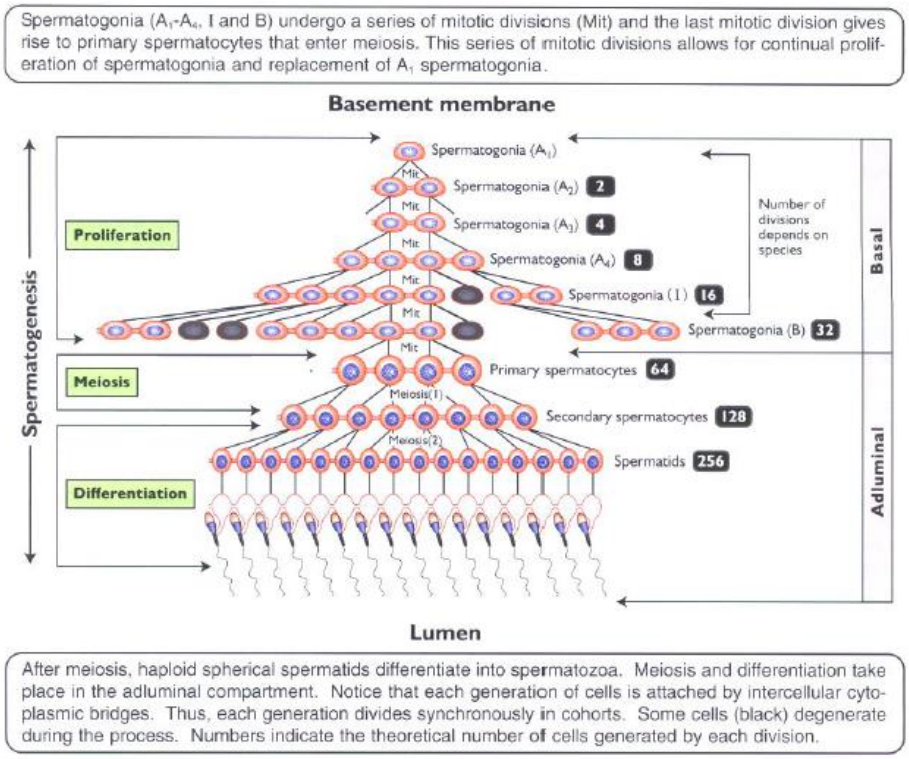

Figure 1.3. Spermatogenesis in mammals. (Preg. and Part., Senger, 2003).

The third phase of spermatogenesis is spermiogenesis, otherwise known as the differentiation phase and is composed of four sub-phases: the 1) golgi phase, 2) cap phase, 3) acrosomal phase, and 3) maturation phase. Spermiogenesis takes place in the adluminal compartment. The round spermatids mature into elongated spermatids and the DNA becomes highly condensed, followed by the formation of the acrosome during the golgi phase. In the cap phase, the flagellum starts to form, while the acrosomic vesicle spreads over the nucleus, letting the cells become potentially motile. During the acrosomal phase, the spermatid nucleus and cytoplasm elongate and the acrosome then covers the majority of the anterior nucleus. In the last phase, maturation, the mitochondria are assembled around the flagellum, forming the completed

flagellum. The elongated spermatids move closer to the lumen of the seminiferous tubule during this third phase of spermatogenesis.

Seminiferous Epithelium Cycle. In the seminiferous epithelium cycle, spermatogonia convert to spermatozoa by completing a series of cellular stages along the seminiferous tubule. It is referred to as cycle because it repeats and the time required for this progression is the duration of the cycle and is unique to each species. In rams, the seminiferous epithelium cycle is 10.4 d (Senger, 2003). Each cycle can be divided into several stages, each one consisting of 4-5 germ cell generations (See Figure 1.3). To complete the spermatogenic cycle, from spermatogonia to elongated spermatid, germ cells must go through several cycles. The ram, for instance, has a seminiferous epithelium cycle 10.4 d in length. The germ cells have to go through 4.5 cycles in order to become elongated spermatids. Therefore, the complete spermatogenic cycle of a ram is 47 d in length (10.4 d × 4.5 cycles = 47 d).

Spermatogonial Stem Cell Regulation and Research Techniques. Philips et al. (2010) suggested that spermatogonial stems cells (**SSCs**) are the foundation of spermatogenesis and male fertility. Tegelenbosch and de Rooij (1993) compare the rarity of SSCs to other stem cells types due to being outnumbered by the other differentiating spermatogonia, spermatocytes, spermatids, and sperm that they are yet to become. Research involving SSCs are extremely complex because the stem cells have no unique identifiable characteristics and they are so few. Primordial germ cells give rise to gonocytes, which lead to the SSCs stored in the testicular cords (Philips et al., 2010). There are still many debates about the stem cell pool and if it is restricted to certain spermatogonia or not (Philips et al., 2010). To date, researchers only possibility for

identifying SSCs is by observing their biological capacity to produce and maintain spermatogenesis in a transplant paradigm.

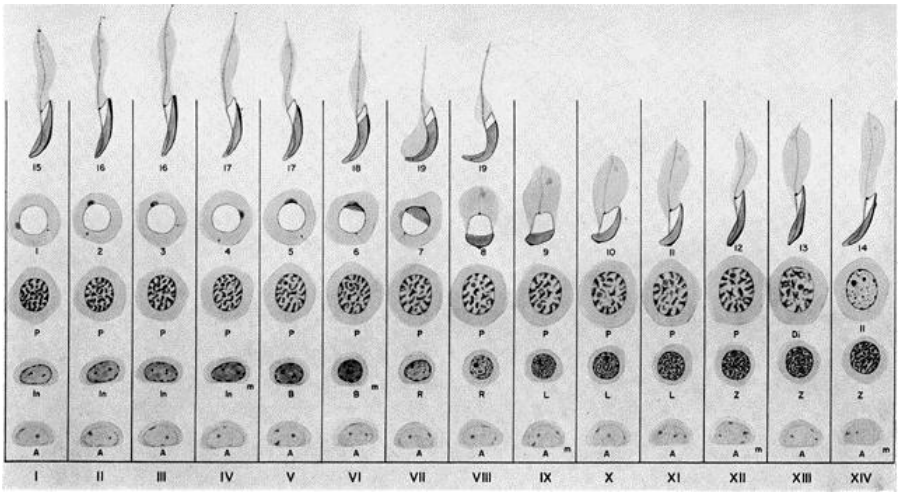

Figure 1.4. Sperm Cell Generations. Each vertical column shows the sequence of germ cell generations that would be observed in a histological section of a rat seminiferous tubule at that stage. I-XIV (the 14 stages). Spermatogonia: A (type A), In (intermediate), B (type B), R ("resting", time of final DNA replication), m (stages during which mitosis of spermatogonia occurs). Primary spermatocytes: L (leptotene), Z (zygotene), P (pachytene), Di (diakinesis). II (secondary spermatocyte). 1-19 (phases of spermatid differentiation = spermiogenesis; Adapted from Perey et al., 1961).

Transplantation of Spermatogonial Stem Cells (Philips et al., 2010). Transplant techniques for studying SSCs were first developed in the 1990s by Brinster and Avarbock (1994), as well as Brinster and Zimmermann (1994). The germ cells are first isolated from the donor testes and then transplanted into the seminiferous tubules of infertile recipients. Within the infertile recipients, normal colonies of spermatogenesis and spermatogonia are produced. In the reference studies above, all recipient mice were either infertile by genetic mutation or infertility was experimentally induced. Since only SSCs can give rise to a producing colony of spermatogenic cells, SSCs that are transplanted to be productive. Therefore, this technique is the 'gold standard' for identifying SSCs, since spermatogenesis would not take place if any other cells were mistakenly transplanted, rather than the SSCs. However, this technique is challenging

to perform, as well as having up to a two to three month of wait time before results can be observed. Yeh et al. (2007) has been refining *in vitro* techniques to culture SSCs to cut down on wait time. However, this technique is considered inferior, as it does not assess the ability of the SSCs to regenerate. Techniques to identify, culture, and further study SSCs as well as spermatogonia at different stages is important and vital for many different fields of study from preservation of species, to male infertility, to certain types of cancers.

Sperm mRNA. Messenger Ribonucleic Acids (**mRNA**) are molecules of nucleic acid which encode a 'blueprint' for a protein product. Kasimanickam et al. (2012) describes mRNA within the spermatozoa as an illustration of the life of the spermatozoa throughout spermatogenesis. Different sperm protein mRNA can serve as markers for several sperm function, such as sperm to egg interactions, fertilization, and early embryonic development (Kasimanickam et al., 2012). In the trial by Kasimanickam et al. (2012), Holstein bull sperm with higher abundances for mRNA expression of adenylate kinase 1, integrin beta 5, doppel, nerve growth factor, tissue inhibitors of metalloproteinases 2, lactate dehydrogenase C 1, small nuclear ribonucleoprotein polypeptide N, outer dense fiber 2, and phospholipase C zeta 1 also exhibited higher fertility compared with average and low fertility bulls. These genes contained within mRNA of the sperm have recently been identified and with their possible links to fertility and other traits, further study could lead to major developments in male reproductive technologies (Kasimanickam et al., 2012). The heterogeneity of RNA content of spermatozoa can be used for genomic analyses to assess semen quality (Kasimanickam et al., 2012). However, in the current study, the fertility index used limited these measures because of the small population, the age of the population, and lack of years of artificial insemination background on the bulls. This trial was performed on a single ejaculate of a small number of young bulls.

Further research in this area would be warranted on the study of more mature bulls across multiple ejaculates and amongst bulls of differing age groups. Nutritional impacts on expression abundancies would also be interesting after the basis of this field of research is established as well as other environmental components.

Ram Reproductive Characteristics and Dried Distiller's Grains with Solubles. With the growing popularity of feeding DDGS within the sheep industry, research needs to expand to investigate the possible impacts of DDGS on ram reproductive traits and fertility. Van Emon et al. (2013) reported a linear decrease in spermatozoa concentration as DDGS increased in the diet. However, this is the only trial we are aware of that has evaluated DDGS in growing ram lamb rations or in cattle, and its potential effect on male fertility.

According to Merck (1998), growing rams from 8 to 14 mo of age should have a minimum of 28 cm for scrotal circumference. Scrotal circumference is a common measurement during breeding soundness examinations used as an indicator of reproductive performance. Martin et al. (1994) and Hötzel et al. (1998) observed increases in ram scrotal circumference when they were fed high protein and energy rations for increased rates of gain. Van Emon et al. (2013) observed no differences in scrotal circumference (initial, final, or change), when feeding increasing levels of DDGS in the ration. Similar results have also been reported in bulls (Coulter and Kozub, 1984). Although Van Emon et al. (2013) did not observe increases in scrotal circumference, testosterone concentrations did increase as the trial continued, as the rams matured. These two values are normally correlated to one another. Therefore, it is unusual that Van Emon et al. (2013) observed an increase in one and not the other, as the increase in testosterone was likely due to the rams maturing throughout the progression of the trial, therefore scrotal circumferences would likely increase as well. Martin et al. (1994) and Hötzel et al. (1998)

reported that rams fed diets containing high and intermediate energy and protein had increased testosterone concentrations compared to rams fed diets containing low energy and protein.

Morphology of the spermatozoa were not measured in the trial by Van Emon et al. (2013), however, spermatozoa concentrations decreased linearly as DDGS concentrations in the diets increased. In conclusion, Van Emon et al. (2013) reported a negative effect on male reproductive traits when ram lambs are fed increasing amounts of DDGS in the ration and is the first trial of its kind to do so. Exactly what is causing the observed affects is not known. Dried distiller's grains with solubles possesses multiple factors that should be considered, such as CP, crude fat, and S.

Previous research on human sperm suggests that semen samples with low sperm concentrations, high incidence of abnormal sperm morphology, and diminished fertility had higher sperm creatine phosphokinase (**CK**) activity (Huszar and Vigue, 1993). Higher CK activity was related to increased content of CK and other proteins in the sperm resulting in those sperm heads being significantly larger and rounder, with increased morphological irregularities and increased cytoplasm believed to be due to failure of spermatogenesis (Huszar and Vigue, 1993). They concluded that higher CK activity results in cellular immaturity and a failure to complete spermatogenesis. Potentially the increased CP in the trial by Van Emon et al. (2013), as a result increasing DDGS concentration in the ration, contributes to the negative effects on sperm quality. Additional research is needed to ascertain why aspects of male reproduction are being affected.

Conclusion

The objective of this section was to review spermatogenesis and the importance of certain stages of development, as well as outlining some research techniques with potential use in the

study of spermatogenesis, spermatozoa health, and possible environmental impacts, such as feeding DDGS. Spermatogenesis is a complex cycle with many stages of proliferation, differentiation, and maturation for cells to become spermatozoa, able to fertilize oocytes. This review has outlined some newly developed techniques that can be used to study different stages of sperm development. These techniques will allow for a better understanding of spermatogenesis along with sperm health and fertility. Potential future uses of these research techniques could be in the fields of epigenetic and fetal programming effects of the male contribution. The possibilities in this field of study are unique and have great potential for studying environmental impacts on male reproductive performance.

Male reproductive physiology is not studied to a great extent, however, it is half of the genetic contribution to offspring. Conventional breeding soundness exams only provide a snapshot of a male's reproductive health. Classically, these breeding soundness exams only expose the 20% of breeding males not meeting minimal standards and provide very limited information. Future endeavors in this field of research could help identify possible genetic implication of DNA and RNA components of sperm to fertility as well as the future offspring of the male populations. Further research in these areas could shed light on seasonality and environmental impacts on sperm development like nutrition and temperature. Overall, much more research is needed to further identify possible impacts and future endeavors needed in this field of study and to elucidate existing data.

When feeding commercial lambs in the feedlot, we hypothesized that increasing levels of DDGS in that ration would increase growth, while the inclusion of LAS would increase ADG and G:F, while not affecting digestibility, VFA concentrations, and pH of the ruminal fluid. Based on the current information available, we hypothesized that ram lambs consuming

increasing concentrations of DDGS in the ration would have declining reproductive traits, with feedlot performance being unaffected.

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CHAPTER 2: EFFECTS OF DRIED DISTILLER'S GRAINS AND LASALOCID INCLUSION ON FEEDLOT LAMB GROWTH, CARCASS TRAITS, NUTRIENT DIGESTIBILITY, RUMINAL FLUID VOLATILE FATTY ACID CONCENTRATIONS, AND RUMINAL HYDROGEN SULFIDE CONCENTRATION[1]

Abstract

Our hypothesis was that increasing the inclusion level of dried distiller's grains with solubles (**DDGS**) to feedlot lambs would increase growth efficiency and the inclusion of lasalocid (**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ) would increase ADG and G:F, while not affecting digestibility, ruminal VFA concentration, and ruminal pH. Furthermore, we hypothesized that rations containing LAS and higher levels of DDGS would cause increased ruminal hydrogen sulfide gas (**H_2S**) concentrations. Two hundred forty crossbred (Suffolk × Rambouillet) lambs (31.9 ± 5.87 kg BW; approximately 90 d of age) were allocated to 6 treatments in a completely randomized design with a 3 x 2 factorial arrangement of treatments. Lambs were placed into 24 feedlot pens (4 pens/treatment; 10 lambs/pen) for a 111 d finishing study. Main effects included concentration of DDGS (0, 15, or 30% DM basis) and inclusion of LAS (0 or 22.05 g/metric ton LAS) resulting in treatments of: 1) 0% DDGS without LAS (**0DDGS-NL**), 2) 0% DDGS with LAS (**0DDGS-L**), 3) 15% DDGS without LAS (**15DDGS-NL**), 4) 15% DDGS with LAS (**15DDGS-L**), 5) 30% DDGS without LAS (**30DDGS-NL**), and 6) 30% DDGS with LAS (**30DDGS-L**). Two-day weights were taken at the beginning and end of the experiment. Two hundred eighteen lambs (64.8 ± 7.99 kg BW) were slaughtered on d 112 at

[1]The material in this chapter was co-authored by A. R. Crane, and R. R. Redden, K. C. Swanson, B. M. Howard, T. J. Frick, K. R. Maddock-Carlin, and C. S. Schauer. A. R. Crane had primary responsibility for collecting samples in the field and was the primary developer of the conclusions that are advanced here. A. R. Crane also drafted and revised all versions of this chapter. C. S. Schauer served as primary proofreader.

a commercial abattoir and carcass data collected. The inclusion of LAS increased ($P \leq 0.02$) final BW, ADG, G:F, and HCW. As DDGS in the ration increased to 30%, DMI decreased linearly ($P = 0.03$) while G:F increased linearly ($P = 0.03$). A second study was conducted utilizing the same treatments to evaluate N and S balance, ruminal VFA and H_2S concentration, and ruminal pH in 24 crossbred wethers (Suffolk × Rambouillet; 41.2 ± 12.23 kg BW). Daily urinary sulfur excretion and ruminal H_2S concentration were linearly increased ($P < 0.001$) as DDGS increased in the ration. Total ruminal VFA concentration linearly decreased ($P = 0.002$) as DDGS increased in the ration. The inclusion of LAS increased ($P = 0.02$) ruminal pH. The results confirm our hypothesis that LAS increased overall growth and increasing DDGS increased ruminal H_2S concentration and influenced growth efficiency. We reject the hypothesis that the combined effects of LAS and DDGS would have no effect on rumen pH and VFA concentrations.

Introduction

Ethanol production in the United States continues to increase (Renewable Fuels Association, 2016). Dried distiller's grains with solubles (**DDGS**) is an affordable byproduct of ethanol production and also serves as an excellent supplementary feed for livestock as it is high in crude fat and RUP. However, many producers are apprehensive about feeding DDGS to feedlot lambs above 20% of the ration for fear of S toxicity. Multiple research projects have been performed assessing the feeding of DDGS to feedlot lambs (Huls et al., 2006; Neville et al., 2010; Schauer et al., 2008), with no negative effects on performance or morbidity observed, even at inclusion levels of 60% of the diet. In fact, Crane et al. (2015) observed that feeding rations containing 45% DDGS tended to improve feedlot growth performance in growing rams. However, none of the previous DDGS research in lambs has evaluated the inclusion of lasalocid

(**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ) to potentially further improve growth performance and feed efficiency (Funk et al., 1986; Crane et al., 2014). By inhibiting hydrogen- and ammonia-producing bacteria in the rumen, LAS decreases the acetate:propionate ratio and improves feed efficiency (Bartley et al., 1979). Kung et al. (2000) determined hydrogen sulfide (**H_2S**) production may increase when ruminants are fed ionophores such as LAS. Therefore, we hypothesized that increasing the inclusion level of DDGS to feedlot lambs would increase growth, while the inclusion of LAS would increase ADG and G:F, while not affecting digestibility, VFA concentrations, and pH of the ruminal fluid. Furthermore, we hypothesized that the rations including LAS and higher levels of DDGS would cause increased ruminal H_2S concentrations. Our objectives were to evaluate the interaction of DDGS and LAS on feedlot lamb performance and ruminal fermentation and total tract digestibility.

Materials and Methods

All procedures were approved by the Animal Care and Use Committee at North Dakota State University (**NDSU**; Protocol #A15054). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Feedlot Study

Animals and Diets. At 2 wk of age, crossbred lambs (Suffolk × Rambouillet) tails were docked, male lambs were castrated, and all lambs were vaccinated against *Clostridium perfringens* types C and D as well as tetanus (**CD-T**; Bar Vac CD/T; Boehringer Ingelheim, Ridgefield, CT). Lambs were adapted to an 80% corn and 20% commercial market lamb pellet diet (DM basis; Table 2.1) from a 100% creep meal diet following weaning at approximately 60 d of age. Lambs were vaccinated with CD-T again at 60 d of age and d -1 of the study. In May 2016, two hundred forty lambs were stratified by BW (31.9 ± 5.87 kg; approximately 90 d of

age) and sex (105 wethers and 135 ewes) and randomly assigned to 1 of 24 outdoor pens (5.5m x 27m; 14.85 m^2/lamb). Pens were assigned randomly to 1 of 6 treatments, with pen serving as the experimental unit (n = 4 pens/treatment). Diets were based on an 80% corn and 20% market lamb meal (**MLM**) diet, which included LAS for respective treatments, and diets were balanced to be isonitrogenous and equal to or greater than the CP and NE requirements (NRC, 2007) for a 40 kg lamb gaining 300 g/d. Six MLM were formulated to meet these requirements (Table 2.2). Rations were formulated to have a minimum Ca:P ratio of at least 1.2:1. Rations were ground through a 1.27 cm screen (Gehl Mix-All, Model 170, Gehl, West Bend, WI), mixed, and offered for ad libitum intake via bulk feeders (48.6 cm bunk space/lamb). Lambs had continuous access to clean, fresh water and shade. Feeders were checked daily and cleaned of contaminated feed. Lambs were observed daily to monitor health and treated when necessary. No treatment related morbidity or mortality was observed. Main effects included dietary concentration of DDGS (0, 15, or 30% DM basis) and inclusion of LAS (0 or 22.05 g/metric ton LAS) resulting in treatments of: 1) 0% DDGS without LAS (**0DDGS-NL)**, 2) 0% DDGS with LAS (**0DDGS-L**, 3) 15% DDGS without LAS (**15DDGS-NL**), 4) 15% DDGS with LAS (**15DDGS-L**), 5) 30% DDGS without LAS (**30DDGS-NL**), and 6) 30% DDGS with LAS (**30DDGS-L**). Water tests indicate sulfate levels to be 141 mg/L (Stearns DHIA, Sauk Centre, MN).

Data Collection Procedures. Lambs were weighed on two consecutive d at the initiation (d -1 and 0) and end (d 110 and 111) of the trial; single day weights were taken on d 28, 54, and 84. Feed ingredient and ration grab-samples (approximately 0.2 kg) were collected from the bulk feeders at the beginning of each period and dried at 55°C for 48 h to determine DM and ration nutrient composition. Dried samples were ground to pass a 2-mm screen. Samples were analyzed for DM, ash (AOAC Int., 2010), N (AOAC Int., 2010) using a Kjeltec Auto 1030 Analyzer

(Tecato AB, Höganäs, Sweden), mineral content including S (AOAC Int., 2010), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using an Ankom 200 Fiber Analyzer without sodium sulfite, with amylase, and without ash corrections as sequentials, and ADF (Goering and Van Soest, 1970). All lambs were shorn on d 29 and 30 with final mid-side wool samples collected on d 110. The samples were clipped at skin level and washed in an 80:20 hexane to isopropyl alcohol mixture. After washing in a 220V VWR symphony ultrasonic cleaner (#97043-958, Radnor, PA) for 15 minutes, samples were allowed to dry for a minimum of 90 minutes in a controlled climate area. Following drying, the wool samples were then measured using the OFDA2000 (BSC Electronics, Ardross, Western Australia) for fiber diameter distribution (mean, SD, and CV), fiber curvature distribution (mean, SD, CV), staple length, and comfort factor. Comfort factor is defined as the percentage of fibers less than or equal to 30 micrometers. Wool data was included as there is a limited amount of wool quality data available in feedlot settings, and feedlots typically shear upon receiving lambs. On d 112 of the trial, lambs (218 hd; 64.8 ± 7.99 kg BW) were harvested at Mountain States Rosen Company (Greeley, CO). A threshold weight of 52.3 kg was required for harvest; therefore, the lambs below this threshold did not have carcass data collected and were sold at a local livestock auction. Trained personnel collected carcass data after a 24-h chill (temperature < 2°C and humidity near 100%). Carcass data collected included HCW (measured on day of slaughter), fat depth, loin eye area (**LEA**), and body wall thickness (at the 12^{th} rib), conformation score, flank streaking, lean maturity, and yield grade (Savell and Smith, 2000). The following equation, adapted from Savell and Smith (2000), was used to calculate % boneless closely trimmed retail cuts (**BCTRC**): 49.936 – (0.0848 × 2.205 × HCW) – (4.376 × 0.3937 × fat depth) – (3.53 × 0.3937 × body wall thickness) + (2.456 × 0.155 × LEA), in which HCW is measured in kilograms, fat depth and body wall

thickness are measured in centimeters, and LEA area is measured in square centimeters. Conformation score was scored on a scale of 100 to 1500 (100 = cull; 1500 = prime). All lambs were assigned bone maturity of A with varying lean maturity scored on a scale of 0 to 100 (0 = very fine; 100 = fine). Flank streaking was assigned with scores of 100 to 199 = Practically Devoid, 200 to 299 = Traces, 300 to 399 = Slight, 400 to 499 = Small, and 500 to 599 = Modest.

Digestibility Study

Animals and Diets. Twenty-four crossbred (Suffolk x Rambouillet) wethers (41.2 ± 12.23 kg BW; approximate age = 90 d) were used in completely randomized design with a 3 x 2 factorial arrangement of treatments as described in the feedlot study. Wethers were weighed on d 0 and 1, stratified by weight, and allotted randomly to treatments (n = 4 wethers/treatment). Lambs were assigned randomly to individual metabolism crates on d 1. Wethers were housed in an enclosed room with lighting from approximately 0730 to 2000 h. Lambs were adapted to diets (Table 2.1) and processed as outlined in the previous study, but lambs were also given an injection of vitamins A, D and E on d 1 of the trial. Rations were provided daily at 0830 h at 130% of the average daily intake for the previous 5 d. Feed refusals from the previous day were determined before feeding. Water troughs were cleaned and refilled daily after feeding.

Data Collection Procedures. The experimental period was 23 d. Dry matter intake was determined on d 14 to 20. Additionally, samples of DDGS, corn, and MLM were collected on d 14 to 20 and dried at 55°C for 48 h to determine DM concentration. Orts were collected on d 15 to 21 and dried at 55°C for 48 h. Wethers were fitted with fecal collection bags on d 13. Total fecal and urine output were collected on d 17 to 23. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM concentration. Urine was collected via stainless steel funnel beneath the lamb, with total urine output collected.

Sufficient 6 *N* HCL (100 mL) was added daily to urinals to maintain urine pH < 3. Total daily urine output was recorded and urine was composited daily by wether (10% of total; wet basis) and stored at 4°C. Approximately 288 g of urine were collected from each urine subsample and stored at 4°C. On d 15 to 21, 10 mL of blood were collected via jugular venipuncture 4 h after feeding using vacutainers (VWR International, Radnor, PA) and 20 gauge × 2.54 cm needles (BD Short Bevel Needles, 305178, Becton, Dickinson, and Co., Franklin Lakes, NJ) into 10 ml serum tubes (BD Vacutainer Serum, 367820, Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was cooled at 4°C for 2 h and centrifuged (3,640 × *g*, 15 degrees C, 20 min), and serum was harvested and stored (-20°C).

Dried fecal samples were ground to pass a 2-mm screen and composited by lamb. Daily samples of corn, MLM and ration were composited for the collection period, and orts were composited by lamb on an equal weight basis (20%; as-fed basis). Feed, orts, and fecal samples were analyzed for DM, ash, NDF, and ADF as described previously. Feed, orts, fecal, and urine samples were analyzed for N and S as described previously. Concentration of N and S in feed, orts, fecal, and urine samples was used to calculate daily N and S intake and excretion from feed, ort, feces, and urine weights. Nitrogen excretion (fecal N + urinary N) was subtracted from N intake (feed N – ort N) to calculate N balance (g N/kg BW basis). Sulfur excretion and balance were also determined using the same calculations. Serum samples were analyzed for urea-N using the Sigma Diagnostics Procedure 640 (Sigma Chemical Co., St. Louis, MO) and an ultraviolet-visible spectroscopy spectrophotometer (DU 800 Spectrophotometer, Beckman Coulter, Brea, CA). Serum-S concentrations were also determined by Midwest Laboratories Inc., (Omaha, NE) using Inductively Coupled Plasma Spectrometry.

Ruminal gas cap sampling was conducted on the same twenty-four lambs in the N and S balance study on d 15 and 23 utilizing procedures outlined by Gould et al. (1997) and modified by Neville et al. (2010). Gas samples and ruminal fluid were collected 4 h after feed was offered via rumenocentosis. To obtain ruminal gas cap and ruminal fluid samples, wool was shorn from a 15 cm^2 area of the left side of the animals immediately posterior to the 13th rib. Shearing was performed with surgical clippers, with care taken to remove all wool. After shearing, the area was scrubbed and disinfected with alternating isopropyl alcohol and Betadine scrubs (Purdue Products L.P., Stamford, CT). To obtain multiple samples while maintaining the integrity of the rumen gas, 2 separate portions of the sampling apparatus were developed. The first portion included a 7.6-cm 12-gauge needle, which was connected to a 20-cm (4.75-mm diam.) tubing (Tygon, S-50-HL class VI,Saint-Gobain Performance Plastics, Wayne, NJ) via a Luer-lock connection (Becton, Dickinson and Co., Franklin Lakes, NJ). The second portion of the sampling apparatus included a 140-mL catheter-tip syringe (Monoject, Sherwood Medical, Ballymoney, Northern Ireland), which was connected to an 8-cm (4.75 mm diam.) portion of tubing via a Luer-lock connection. The 2 portions were then connected or disconnected through Luer-lock connections, with ratchet tubing clamps used on both sides of the Luer-lock connectors. After the needle was introduced through the skin and into the rumen gas cap, a 120-mL sample (approximately) of ruminal gas was collected into the syringe. The first of 2 syringes was then disconnected and a second was filled in the same manner. Hydrogen sulfide gas concentrations in the rumen were determined using H_2S gas detector tubes (Gastec, Kanawaga, Japan) using techniques similar to Neville et al. (2010). A volume (100 mL) of gas was collected through the detector tube to acquire a measurement of ruminal gas cap hydrogen sulfide. On both sampling days (15 and 23), duplicate measurements were taken from each lamb for both gas and fluid

samples, and the mean of the 2 samples was used for calculations. If the detector tube failed to reach 100 mg/L of hydrogen sulfide (the least detectable concentration recommended by the manufacturer), the reading was treated as a zero. After gas and fluid sampling, the needle was removed and the sampling site was sprayed with a 10% iodine solution. Ruminal hydrogen sulfide concentrations were converted from parts per million hydrogen sulfide to grams per cubic meter through the following equation: {[hydrogen sulfide (ppm) × 139.06]/1,000,000} assuming standard temperature and pressure values. Lambs received 1 ml of penicillin per 45.36 kg of BW post collection to prevent infection from the procedure. The needle insertion site was also scrubbed with an iodine solution post collection. During collections, ruminal pH was immediately measured using an IQ Scientific pH meter (Hach Company, Loveland, CO, USA) before being allowed to cool. Once pH was recorded samples were stored frozen (−20°C) until the end of the collection period at which point they were thawed, equally composited and centrifuged at 2000 × g for 20 min. The liquid portion was filtered through a 0.45-µm filter and the supernatant separated and analyzed for NH_3 (Broderick and Kang, 1980). Ruminal VFA concentrations were determined by GLC (Hewlett Packard 5890A Series II GC, Wilmington, DE, USA) and separated on a capillary column (Nukol, Supelco, Bellefonte, PA, USA) using 2-ethyl butyric acid as the internal standard (Goetsch and Gaylean, 1983).

Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedure of SAS (v. 9.3; SAS Inst. Inc., Cary, NY), with pen serving as experimental unit in the feedlot study and lamb serving as experimental unit in the digestibility study. Serum S concentrations were only analyzed on d 18 of the digestibility trial, and were analyzed using the MIXED procedure of SAS. Repeated measures were used for the analyses of serum urea-N, individual and total VFAs,

and H_2S concentrations, as well as rumen fluid pH. Following protection with an overall F-test for treatment ($P < 0.05$), if a DDGS x LAS interaction occurred ($P < 0.05$) means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered different. Linear and quadratic contrasts were used to evaluate the effects of increasing inclusion of DDGS in the ration.

Table 2.1. Composition of diets fed to feedlot and digestibility trial lambs (DM basis)

	Dietary treatment[1]					
Item	0DDGS-NL	0DDGS-L	15DDGS-NL	15DDGS-L	30DDGS-NL	30DDGS-L
Ingredient, %						
DDGS	-	-	14.8	14.8	29.7	29.8
Corn	79.9	79.9	65.2	65.2	50.2	50.3
MLM[2]	20.1	20.1	20.0	20.0	20.1	19.9
Nutritional composition						
Ash, %	5.8	6.0	6.3	6.9	7.8	7.2
TDN, %[3]	81.0	80.8	81.3	80.8	79.8	80.5
CP, %	16.7	17.3	16.5	16.6	16.7	16.9
ADF, %	4.0	3.9	5.8	5.8	9.8	9.3
Crude Fat, %	2.5	2.6	3.9	4.0	5.0	5.1
S, %	0.2	0.2	0.3	0.3	0.4	0.4
Ca, %	1.1	1.0	1.3	1.2	1.2	1.0
P, %	0.4	0.4	0.5	0.5	0.5	0.6

[1]Diets (DM basis) were balanced to be isonitrogenous and equal or greater than CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). DDGS = dried distiller's grains with solubles; LAS = lasalocid. Treatments were: 0% DDGS without LAS (0DDGS-NL), 0% DDGS with LAS (0DDGS-L), 15% DDGS without LAS (15DDGS-NL), 15% DDGS with LAS (15DDGS-L), 30% DDGS without LAS (30DDGS-NL), and 30% DDGS with LAS (30DDGS-L).
[2]MLM = commercial market lamb meal. Nutrient composition of MLM for each treatment is reported in Table 2.2.
[3]Calculated.

Ruminal gas cap sampling was conducted on the same twenty-four lambs in the N and S balance study on d 15 and 23 utilizing procedures outlined by Gould et al. (1997) and modified by Neville et al. (2010) using H_2S gas detector tubes (Gastec, Kanawaga, Japan).Gas samples were collected 4 h after feed was offered via rumenocentosis prior to ruminal fluid samples being collected to determine H_2S concentrations in the rumen. Ruminal fluid was collected via rumenocentosis for determination of ruminal fluid VFA concentrations and pH. On both sampling days (15 and 23), duplicate measurements were taken from each lamb for both gas and fluid samples, and the mean of the 2 samples was used for calculations. Lambs received 1 ml of

penicillin per 45.36 kg of BW post collection to prevent infection from the procedure. The needle insertion site was also scrubbed with an iodine solution post collection.

Table 2.2. Nutrient composition of market lamb meal (MLM[1]) fed to feedlot and digestibility trial lambs (DM basis)

	Dietary treatment[2]					
Item	0DDGS-NL	0DDGS-L	15DDGS-NL	15DDGS-L	30DDGS-NL	30DDGS-L
Ash, %	19.9	19.8	20.9	23.0	26.3	27.4
CP, %	40.8	41.5	25.2	25.3	13.0	11.8
ADF, %	9.6	6.7	9.0	8.4	17.6	16.2
Crude Fat, %	1.5	1.6	2.2	2.0	1.2	1.2
S, %	0.6	0.6	0.6	0.6	0.6	0.6
Ca, %	5.0	4.8	5.1	5.4	5.5	6.2
P, %	0.6	0.7	0.7	0.7	0.6	0.6

[1]MLM = commercial market lamb meal contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2 mg/kg Se, 52,863 IU/kg vitamin A, 5,286 IU/kg vitamin D, and 209 IU/kg vitamin E, and LAS included into respective treatments at 22.05 g/metric ton.

[2]Diets (DM basis) were balanced to be isonitrogenous and equal or greater than CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). DDGS = dried distiller's grains with solubles; LAS = lasalocid. Treatments were: 0% DDGS without LAS (0DDGS-NL), 0% DDGS with LAS (0DDGS-L), 15% DDGS without LAS (15DDGS-NL), 15% DDGS with LAS (15DDGS-L), 30% DDGS without LAS (30DDGS-NL), and 30% DDGS with LAS (30DDGS-L).

Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedure of SAS (v. 9.3; SAS Inst. Inc., Cary, NY), with pen serving as experimental unit in the feedlot study and lamb serving as experimental unit in the digestibility study. Serum S concentrations were only analyzed on d 18 of the digestibility trial, and were analyzed using the MIXED procedure of SAS. Repeated measures were used for the analyses of serum urea-N, individual and total VFAs, and H_2S concentrations, as well as rumen fluid pH. Following protection with an overall F-test for treatment ($P < 0.05$), if a DDGS x LAS interaction occurred ($P < 0.05$) means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered different. Linear and quadratic contrasts were used to evaluate the effects of increasing inclusion of DDGS in the ration.

Results and Discussion

Feedlot Performance and Carcass Characteristics

There were no interactions ($P \geq 0.24$) of DDGS and LAS inclusion for final BW, ADG, DMI, or G:F (Table 2.3). The inclusion of LAS increased ($P \leq 0.02$) final BW (4.12%), ADG (2.30%), and G:F (0.23%). A linear decrease ($P = 0.03$) was observed for DMI as DDGS inclusion in the ration increased. Other trials have reported improvements in lamb ADG and DMI (Schauer et al., 2008; Crane et al., 2015) as concentration of DDGS in the diet increased, most likely due to the increased nutrient concentration of the diet, specifically crude fat and CP. However, Crane et al. (2014) observed that when feeding corn-based feedlot diets with or without LAS, final BW and ADG were increased with no differences in G:F. The difference in treatment responses is likely due to the increased fiber content in the diets of the current trial with the inclusion of DDGS. Associatively, G:F increased linearly ($P = 0.03$) as DDGS in the ration increased, mimicking a similar effect observed by Crane et al. (2015) in which the decreased DMI, but the increased ADG observed likely the improved G:F; however, in the current trial, we observed no improvement in ADG other than by the inclusion of LAS.

There were no interactions ($P \geq 0.21$) or main effects ($P \geq 0.18$) of DDGS and LAS inclusion on wool characteristics (fiber diameter distribution [mean, SD, and CV], fiber curvature distribution [mean, SD, and CV], staple length, and comfort factor; data not reported). Average fiber diameter, in micrometers, was 24.48, 23.95, 24.15, 24.67, 24.75, and 24.56 (± 0.42 SEM; $P = 0.44$) for 0DDGS-NL, 0DDGS-L, 15DDGS-NL, 15DDGS-L, 30DDGS-NL, and 30DDGS-L, respectively. Higher planes of nutrition have been shown to increase wool fiber diameter (Lupton et al., 1997); when the AA supply to wool growing animals is altered, specifically S-containing AA, these effects are more common. In the current trial, there were no

dietary effects observed on fiber diameter, likely due to the dietary treatments being isocaloric and isonitrogenous.

There was no interaction ($P \geq 0.23$) of DDGS and LAS inclusion for HCW, back fat, LEA, body wall thickness, flank streaking, lean maturity, yield grade, or % BCTRC; however, conformation score was affected ($P = 0.005$) by the interaction of DDGS and LAS. With a scale of 100 to 1500, the differences are minimal, however significant. This was the only interaction observed in the feedlot trial, however, this response may have been driven by increased HCW, although there was no interaction observed for HCW, only a LAS effect. The majority of carcass traits were not affected by treatment (LAS or DDGS inclusion level; $P \geq 0.08$). The inclusion of LAS increased ($P = 0.03$) HCW by 4%, similar to data reported by Crane et al. (2014) and was driven by the increase in ADG and final BW, reported previously. However, the subjective measurement, flank streaking, exhibited a tendency ($P = 0.08$) for a decreasing linear effect of DDGS. Flank streaking of the lamb carcasses all fell within the small classification (400 to 499 scale), indicating the tendency for a linear increase due to DDGS was due to very small differences between carcasses. This tendency is likely due to the decrease in DMI, which led to a decrease in overall nutrient intake and fat deposition. Maturity, another subjective measure, exhibited a quadratic effect ($P = 0.03$) for DDGS inclusion. Lean maturity decreased from 0DDGS to 15DDGS inclusion, however increased to the greatest maturity when 30DDGS was provided. This was the only quadratic effect of DDGS observed. Maturity of these lambs exhibited an A bone maturity classification, meaning there was some evidence of cartilage in the vertebrae; therefore, we only reported the lean maturity, scored from 0 to 100, with all values being relatively close, ranging from 58.6 to 64.1. It is difficult to delineate exactly why these

effects were observed and the differences among these measurements were also relatively small compared to the scale used for measuring these traits, however, still notable.

Digestibility Traits

Most observations for the N and S balance study were not affected ($P \geq 0.09$) by the inclusion of DDGS, LAS, or the interaction of the two (Table 2.4). A DDGS x LAS interaction ($P = 0.04$) was observed for serum urea-N concentration, in which 0DDGS-L and 15DDGS-L were similar. However, the similar 0DDGS-NL and 15DDGS-L differed from the also similar 15DDGS-NL, 30DDGS-L, and 30DDGS-NL treatments. Daily urinary sulfur excretion increased linearly ($P < 0.001$) with increasing inclusion rates of DDGS in the diet. This is in agreement with Neville et al. (2010), who observed that lambs excrete substantial amounts of S when consuming DDGS and that water intake and urinary output increase with increasing S intake. This could imply that although the NRC (2007) reports that feedlot type diets should not contain S in excess of 0.3% of the ration, it is possible that lambs merely excrete excess S, theoretically preventing a toxicity. Ruminal H_2S gas concentrations increased linearly ($P < 0.001$) as concentration of DDGS in the diet increased. Neville et al. (2010) also observed a linear increase in H_2S concentrations with increased inclusion rates of DDGS in the diet; however, in the current trial no differences were observed for LAS fed treatments ($P = 0.74$).

Inclusion of LAS had no effect ($P = 0.69$) on ruminal fluid pH; however, there was a tendency for a linear decrease ($P = 0.06$) in ruminal fluid pH with increased inclusion of DDGS. Neville et al. (2010) observed no differences in pH in lambs fed similar diets to ours. Although it is important to consider that a reduction in ruminal pH represents an increase in hydrogen ions available to form H_2S (Morrow et al., 2013). However, Morrow et al. (2013) disproved the hypothesis that neutralizing the acid in DDGS with NaOH would improve fiber digestion as well

as feed efficiency due to the inhibition of growth and fiber fermenting capacity of cellulolytic bacteria at low rumen pH (Mould et al., 1983; Hoover, 1986). This finding suggests that the decrease in ruminal pH could be due to the pH of DDGS itself, rather than the altering of the rumen environment and in turn, the microbial population. The observed ruminal fluid pH at or below 5 for most treatments is often an indicator of acidosis, although acidosis was not observed in the present trial. Due to the basal ration containing approximately 80% corn, and the DDGS treatments all maintaining approximately 80% concentrate consisting of corn and DDGS, it is not surprising that ruminal fluid pH was on the threshold for acidosis.

Acetate exhibited a DDGS x day interaction ($P = 0.03$; data not shown), with 15DDGS fed lambs on d 23 having the highest concentration of acetate, similar to 0DDGS on d 15, with it being a similar to all other concentrations. Acetate was expected to decrease due to LAS with propionate being increased, as observed by Smith et al. (2010); however, in the current trial, no LAS effects were observed on acetate or propionate concentration ($P \geq 0.41$; Table 2.4). It is not readily apparent as to why we did not observe an effect of LAS on acetate or propionate concentration. Additionally, no effects were observed on the effects of DDGS on acetate and propionate concentration ($P \geq 0.31$; Table 2.4). Total VFA concentration in the rumen fluid decreased linearly ($P = 0.002$; Table 2.4) with increasing levels of DDGS in the ration. This is possibly due to the increase in H_2S gas production leading to decreased microbial function, specifically of those S reducing bacteria (Gould, 1998). However, this opposes the results observed by Felix et al., (2012) in cattle that exhibited linear increases in ruminal acetate, propionate, and total VFA concentrations when the inclusion of DDGS increased. Kung et al. (2000) observed that excess dietary sulfur increased ruminal sulfide production; however, it had no effect on VFA production. The results confirm our hypothesis that LAS increased overall

growth and increasing DDGS increased ruminal H_2S concentration; however, DDGS inclusion did not increase growth. Additionally, we reject the hypothesis that the combined effects of LAS and DDGS would have no effect on rumen pH and VFA concentrations.

Implications

Dried distiller's grains with solubles decreased dry matter intake and improved feed conversion. The inclusion of lasalocid increased hot carcass weights by 4%, increasing lamb value for producers utilizing value based marketing contracts. Observations indicate when feeding increased levels of dried distiller's grains with solubles lambs may excrete excess sulfur in urine. Overall, dried distiller's grains with solubles appears to be a reliable lamb feedlot ration ingredient. Moreover, the combination of LAS and dried distiller's grains in the ration resulted in improved lamb feedlot performance, with no deleterious effects on lamb morbidity or mortality.

Table 2.3. Effects of increasing concentration of dried distiller's grains with solubles (DDGS) with or without lasalocid (LAS, 22.05 g/metric ton) on lamb performance

	Dietary treatment[1]							*P*-value[3]			
Item	0DDGS-NL	0DDGS-L	15DDGS-NL	15DDGS-L	30DDGS-NL	30DDGS-L	SEM[2]	DDGS Linear	DDGS Quadratic	LAS	DDGS* LAS
Feedlot Performance											
Initial BW, kg	40.7	40.3	40.4	40.8	40.5	41.1	1.0	0.85	0.98	0.84	0.86
Final BW, kg	63.2	63.3	62.2	65.4	61.9	66.3	1.4	0.69	0.62	0.02	0.28
ADG, kg	0.28	0.29	0.27	0.30	0.27	0.31	0.009	0.57	0.82	<0.001	0.24
DMI, kg	1.8	1.8	1.9	1.6	1.6	1.5	0.1	0.03	0.37	0.15	0.52
G:F, kg gain:kg DMI	0.16	0.16	0.14	0.19	0.17	0.21	0.005	0.03	0.67	0.003	0.28
Carcass Characteristics											
HCW, kg	30.9	31.0	31.1	32.2	29.8	32.3	0.7	0.76	0.41	0.03	0.23
Back fat, cm	0.70	0.67	0.78	0.71	0.71	0.74	0.05	0.48	0.34	0.60	0.60
Loin Eye Area, cm^2	22.3	22.3	21.6	21.6	21.6	22.3	3.9	0.33	0.14	0.21	0.79
BWT, cm^4	2.5	2.3	2.5	2.5	2.5	2.5	0.09	0.16	0.25	0.58	0.37
Conformation[4]	959.7[a,b]	975.7[a,c]	981.3[c]	956.6[b]	962.9[a]	971.8[a,c]	6.6	0.92	0.71	0.99	0.005
Flank Streaking[4]	473.6	475.0	456.1	443.1	454.6	445.6	12.9	0.08	0.16	0.51	0.85
Lean Maturity[4]	61.1	61.9	58.6	59.2	62.2	64.1	1.9	0.37	0.03	0.47	0.94
Yield Grade[4]	3.2	3.1	3.5	3.2	3.2	3.3	0.2	0.48	0.34	0.60	0.60
BCTRC, %[4]	43.3	43.5	43.2	43.0	43.5	43.1	0.2	0.39	0.20	0.21	0.28

[1]Diets (DM basis) were balanced to be isonitrogenous and ≥ CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). Treatments were: 0% DDGS without LAS (0DDGS-NL), 0% DDGS with LAS (0DDGS-L), 15% DDGS without LAS (15DDGS-NL), 15% DDGS with LAS (15DDGS-L), 30% DDGS without LAS (30DDGS-NL), and 30% DDGS with LAS (30DDGS-L).

[2]*n* = 6; SEM = standard error of the means for main effects.

[3]*P*- value for linear and quadratic effects of increasing DDGS inclusion in the diet and main effects of DDGS, LAS, and DDGS*LAS.

[4]BWT= Body wall thickness; Conformation: 100 = cull to 1500 = high prime. Flank Streaking: 100 to 199 = practically devoid; 200 to 299 = traces; 300 to 399 = slight; 400 to 499 = small; 500 to 599 = modest. Lean maturity: 0 = very fine to 100 = fine. Yield grade = 0.4 + (10 × fat depth). BCTRC = boneless closely trimmed retail cuts, % = [49.936-(0.0848 × 2.205 × HCW, kg) – (4.376 × 0.3937 × back fat, cm) – (3.53 × 0.3937 × body wall thickness, cm) + (2.456 × 0.155 × loin eye area, cm^2)].

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 2.4. Effects of increasing concentration of dried distiller's grains with solubles (DDGS) with or without lasalocid (LAS, 22.05 g/metric ton) on lamb digestibility

	Dietary treatment[1]							*P*-value[3]			
Item	0DDGS-NL	0DDGS-L	15DDGS-NL	15DDGS-L	30DDGS-NL	30DDGS-L	SEM[2]	DDGS Linear	DDGS Quadratic	LAS	DDGS*LAS
Daily Digestibility Traits, g/kg BW											
DMI	26.3	28.4	27.0	30.7	26.2	29.1	4.9	0.95	0.74	0.48	0.99
N intake	0.67	0.75	0.73	0.82	0.72	0.81	0.13	0.63	0.74	0.44	0.99
N excretion											
Fecal	0.13	0.15	0.15	0.17	0.15	0.18	0.04	0.57	0.74	0.52	0.99
Urinary	0.03	0.04	0.03	0.03	0.04	0.04	0.004	1.00	0.70	0.20	0.93
N balance	0.57	0.63	0.60	0.69	0.60	0.67	0.1	0.70	0.75	0.39	0.99
S intake	0.17	0.18	0.17	0.19	0.14	0.18	0.03	0.66	0.58	0.40	0.84
S excretion											
Fecal	0.011	0.014	0.015	0.015	0.015	0.018	0.004	0.23	0.85	0.94	0.94
Urinary	0.003	0.003	0.004	0.005	0.006	0.007	0.001	<0.001	0.93	0.73	0.73
S Balance	0.17	0.16	0.16	0.18	0.14	0.17	0.03	0.66	0.59	0.77	0.77
Serum-N, mg/dL[4]	17.1[b]	19.2[a]	14.5[c]	17.6[a,b]	13.6[c]	13.9[c]	0.6	<0.001	0.92	0.04	0.04
Serum-S, mg/dL[4]	83.2	88.2	88.4	83.78	85.3	87.2	2.1	0.86	0.96	0.09	0.09
H_2S, ppm[5]	0.005	0.012	0.082	0.056	0.190	0.195	0.02	<0.001	0.12	0.74	0.74
Rumen Fluid pH	4.96	5.08	4.9	5.08	4.85	4.92	0.06	0.06	0.52	0.69	0.69
Acetate, mmol/L	19.50	18.83	19.31	20.15	18.31	19.98	1.31	0.50	0.99	0.57	0.66
Propionate, mmol/L	60.56	58.70	51.50	55.55	50.59	56.77	3.34	0.37	0.41	0.31	0.47
Total VFA, mmol/L	3506	3403	2796	2966	1798	2745	341.9	0.002	0.95	0.29	0.23

[1]Diets (DM basis) were balanced to be isonitrogenous and ≥ CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). Treatments were: 0% DDGS without LAS (0DDGS-NL), 0% DDGS with LAS (0DDGS-L), 15% DDGS without LAS (15DDGS-NL), 15% DDGS with LAS (15DDGS-L), 30% DDGS without LAS (30DDGS-NL), and 30% DDGS with LAS (30DDGS-L).

[2]$n = 6$; SEM = standard error of the means for main effects.

[3]*P*- value for linear and quadratic effects of increasing DDGS inclusion in the diet and main effects of DDGS, LAS, and DDGS*LAS.

[4]*P*-values for serum urea-N: day ($P = 0.08$) and treatment × day ($P < 0.001$); serum-S was only analyzed on d 18 of the digestibility trial.

[5]H_2S = hydrogen sulfide gas. *P*-values for H_2S gas: day ($P = 0.30$) and treatment × day ($P < 0.001$).

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

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CHAPTER 3: INFLUENCE OF DRIED DISTILLER'S GRAINS WITH SOLUBLES ON RAM LAMB GROWTH AND REPRODUCTIVE TRAITS[2]

Abstract

The inclusion of dried distiller's grains (**DDGS**) at 15%, 30%, and 45% of the ration was hypothesized to have a decreasing linear effect on semen quality of ram lambs, while having no negative effects on growth. Following the removal of DDGS from the ration, we hypothesized that the ram lambs would recover and become reproductively sound, independent of treatment. To test this hypothesis, Suffolk and Hampshire ram lambs (n =112) were allocated to four treatments (n = 4 pens/treatment; 7 rams/pen) in a completely randomized design. Dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**), 15% of the ration as DDGS substituted for corn (% DM basis; **15DDGS**), 30% of the ration as DDGS substituted for corn (% DM basis; **30DDGS**) and 45% of the ration as DDGS substituted for corn (% DM basis; **45DDGS**). Rams were fed for 112 d on their respective treatment (**PHASE 1**), after which rams were placed on the CON ration until d 168 (**PHASE 2**). Rams were weighed on consecutive days at the beginning (d 0 and 1) and end (d 167 and 168) of the trial. Scrotal circumference was measured on all rams on d 84, 112, 140, and 168. Semen samples were collected on a subset of 64 rams (4 rams/pen) to evaluate semen quality on d 84, 112, 140, and 168. Blood samples were collected on the same subset of rams every 14 d throughout the trial. A quadratic effect on BW ($P = 0.03$) in PHASE 1, ADG in PHASE 1 and overall ADG ($P = 0.02$ and $P = 0.02$, respectively), DMI ($P = 0.007$) in PHASE 1, and a cubic effect ($P = 0.05$) for

[2]The material in this chapter was co-authored by A. R. Crane, and R. R. Redden, M. S. Crouse, J. D. Kirsch, J. E. Held, P. P. Borowicz, and C. S. Schauer. A. R. Crane had primary responsibility for collecting samples in the field and was the primary developer of the conclusions that are advanced here. A. R. Crane also drafted and revised all versions of this chapter. C. S. Schauer served as primary proofreader.

overall G:F were observed. Overall and PHASE 2 scrotal circumference had linear ($P = 0.04$) and quadratic ($P = 0.05$) effects, respectively. A treatment × day interaction ($P = 0.05$) was observed for the occurrence of distal droplets along the tails of the spermatozoa. An overall linear decrease ($P = 0.02$) was observed for the occurrence of bent heads on the sperm as DDGS increased in the diet. In PHASE 2 a linear increase in spermatozoa concentration was observed ($P = 0.03$) as DDGS increased in the ration. Overall, testosterone concentrations exhibited a linear decrease ($P = 0.004$) as DDGS increased in the ration. There were no negative effects on ram lamb feedlot or reproductive performance due to increasing DDGS in the diet.
Key words: dried distiller's grains with solubles, feedlot, growth performance, rams, reproductive traits, semen quality

Introduction

Ethanol production in the United States continues to increase exponentially (Renewable Fuels Association, 2015). The byproduct of the ethanol industry, dried distiller's grains with solubles (**DDGS**), provides an affordable and viable feed source for livestock, especially ruminants. Dried distiller's grains are readily incorporated into diets to supplement RUP as DDGS have much more available protein when compared to corn. Although, it is important to also consider that DDGS can be high in S and crude fat. New generation ethanol refineries have decreased the variability in crude fat and protein, however mineral content, such as S, can still be highly variable (Spiehs et al., 2002). Sulfur content is a concern in ruminant diets due to the possibility of inducing polioencephalomalacia (**PEM**). Due to the high energy and CP content of DDGS, its inclusion in lamb feedlot rations continues to increase. In feedlot lambs, DDGS have been included in the ration at rates of up to 60% with no negative effects on performance or symptoms of polioencephalomalacia (Schauer et al., 2008; Neville et al., 2011). With the

increased incorporation of DDGS in diets by sheep producers, research is needed that investigates the possible impacts of DDGS on ram reproductive traits and fertility. Van Emon et al. (2013) reported a linear decrease in spermatozoa concentration as DDGS increased in the diet. However, this is the only trial we are aware of that has evaluated DDGS in growing ram lamb rations, and its potential effect on male fertility. The current trial tested the hypothesis that ram lambs consuming increasing concentrations of DDGS in the ration would have declining reproductive traits, with feedlot performance, as well as wool characteristics, being unaffected. We also hypothesized that ram lambs would reproductively convalesce once being removed from diets containing DDGS and being placed on a ration that does not contain DDGS. The objectives to test these hypotheses were to determine the influence of increasing concentrations of DDGS in ram lamb rations on ram lamb growth, reproductive traits, and serum testosterone concentration.

Materials and Methods

All procedures were approved by the animal care and use committee of North Dakota State University (protocol # A14060). This study was conducted at the North Dakota State University Hettinger Research Extension Center in Hettinger, ND.

Feedlot Study

Ram lambs (Suffolk and Hampshire) were purchased from four producers in North and South Dakota, Minnesota, and Iowa. Prior to purchase, ram lambs were vaccinated for *Clostridium perfringens* types C and D and tetanus, weaned at 60 d of age, and revaccinated. At approximately 90 d of age, rams were purchased and transported to the Hettinger Research Extension Center. Ram lambs were adapted to a 60% corn, 25% oats, and 15% commercial market lamb pellet diet (**CON**; DM basis) for approximately 2 weeks. Ram lambs (n=112) were stratified by weight (48.7 ± 0.31 kg) and breed and randomly assigned to 1 of 16 outdoor pens (7

rams/pen; 18 m^2/ram). Pens were assigned randomly to 1 of 4 treatments in a completely randomized design, with pen serving as the experimental unit (n = 4). Dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**), 15% of the ration as DDGS substituted for corn (% DM basis; **15DDGS**), 30% of the ration as DDGS substituted for corn (% DM basis; **30DDGS**) and 45% of the ration as DDGS substituted for corn (% DM basis; **45DDGS**) as described in Table 3.1. Study diets were balanced to meet or exceed the CP and TDN requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007) and to maintain a Ca:P ratio of 2:1 or greater. Complete rations were ground (1.25 cm screen) and mixed in a grinder-mixer (GEHL mix-all, Model 170; West Bend, WI), and were provided to ram lambs with ad libitum access via bulk feeders (70 cm bunk space/ram). Ram lambs had continuous access to clean, fresh water. Feeders were checked daily and cleaned of contaminated feed (fecal contamination, wet feed due to precipitation, etc.). Ram lambs were weighed on two consecutive d at the beginning (d 0 and 1) and the end of the trial (d 167 and 168) and weighed once every 28 d (to assist in evaluation of lambs for morbidity). Ram lambs were fed their respective treatments until d 112 (**PHASE 1**) and on d 112 all feed was removed from self-feeders and pens were reallocated to the CON diet until d 168 (**PHASE 2**). One ram was removed from the trial due to a broken leg and six other rams died before the conclusion of the trial from complications not related to dietary treatment. Necropsies concluded that the rams had normal liver, rumen, and intestines, with chronic pneumonia being the major diagnosis for death.

Reproductive Performance Study

Scrotal circumference was measured on all ram lambs on d 84, 112, 140, and 168 of the trial. Scrotal circumference was measured with the ram standing, by retaining both testes at the base of the scrotum and measuring the circumference of the scrotal tissue and the two testes

combined (Martin et al., 1994). Sixty-four ram lambs (a subsample of the 112 ram lambs in the feedlot study described above; 4 ram lambs/pen; 16 rams/treatment; $n = 4$) were chosen for semen quality and serum testosterone concentration analysis. The four ram lambs in each pen were selected based on weight and breed to provide a representative subset. Two of the four ram lambs in each pen were selected based on weight and breed to provide a representative subset for morphology analysis.

Table 3.1. Ingredient and nutritional composition of diets fed to growing rams (DM basis)

	Dietary treatment[1]			
Item	CON	15DDGS	30DDGS	45DDGS
Ingredient, %				
DDGS[2]	-	14.93	29.7	44.33
Corn	60	44.78	29.7	14.78
Oats	25	24.88	24.75	24.63
Lamb Pellet[3]	15	14.93	14.85	14.78
$CaCO_3$	-	0.48	1.00	1.48
Nutritional composition, % DM				
Ash	4.00	4.56	5.18	5.66
TDN[4]	86.53	84.83	83.06	81.44
CP	16.42	19.89	23.32	26.74
ADF	6.56	7.88	9.16	10.59
Crude Fat	3.52	4.16	4.79	5.42
S, %	0.21	0.27	0.32	0.37
P, %	0.41	0.51	0.61	0.71
K, %	0.74	0.90	1.05	1.21
Mg, %	0.18	0.23	0.27	0.31
Ca, %	0.68	1.18	1.69	2.18
Ca:P	1.65	2.31	2.77	3.07
Na, %	0.19	0.24	0.29	0.34
Fe, mg/kg	57	69	81	92
Mn, mg/kg	109	117	125	133
Cu, mg/kg	9	13	17	21
Zn, mg/kg	32	52	72	92

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).
[2]DDGS= dried distiller's grains with solubles.
[3]Commercial market lamb pellet contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2 mg/kg Se, 52,863 IU/kg vitamin A, 5,286 IU/kg vitamin D, and 209 IU.
[4]Calculated.

Semen was collected on d 84, 112, 140, and 168 of the study via electroejaculation. Immediately post-ejaculation, volume of the ejaculate was recorded in a conical vial, followed by a 10 µL subsample of semen being placed on a glass slide and assessed via microscope (10x magnification) for spermatozoa motility scoring. The spermatozoa motility score, which is based on the rate of forward movement, was determined on a 1 to 4 scale: 1) no forward movement or all dead (0-24% live sperm cells), 2) slow forward movement (25-49% live sperm cells), 3) moderate forward movement (50-74% live sperm cells), 4) fast forward movement (≥75% live sperm cells). Spermatozoa concentration was evaluated using a 20 µL subsample of semen diluted in 3,980 µL of 3% NaCl solution and placed on a hemocytometer and assessed via microscope at 430x magnification. The hemocytometer has a counting chamber volume of 1 mm^3. Five large squares were counted for each ejaculate sample, the four corner squares and the middle square. If the spermatozoa cells were on the top or right borderline of these squares, they were counted, however, if the cells were on the bottom or left, they were not counted. The below formula was used to calculate the spermatozoa concentration:

Spermatozoa/mL = number of sperm counted (in 5 squares) × dilution factor × hemocytometer factor × conversion factor.

The dilution rate was 1:200, the hemocytometer factor was 50, and the conversion factor (converted units to spermatozoa/cm^3, or mL) was 1,000. In addition, the percent of specific morphological abnormalities of the diluted spermatozoa in a nigrosin-eosin stain was determined on a pre-warmed glass slide at 40x magnification. The morphological abnormalities observed were: distal droplets, proximal droplets, tailless heads, abaxial tail implantation, abnormal acrosomes, simple bend in tails, narrow or small heads, bent heads, pyriform heads, strongly folded tails, mid-piece defects, maldeveloped spermatozoa, or normal spermatozoa. The

remaining semen sample from each individual ram was then diluted, mixed with an extender and frozen in 200 µl pellets on a block of dry ice and stored in a liquid nitrogen tank (-196˚C). The extender recipe included 30 mL of sterile distilled water, 10 mL triladyl and 10 mL of an egg yolk from a brown chicken egg.

Every 14 d throughout the duration of the trial a 10 mL blood sample was collected via jugular venipuncture with a 20 gauge x 2.54 cm vacutainer needle into serum separator 16 x 100 mm tubes from the same subset of 4 ram lambs. Blood samples were immediately placed on ice and cooled for 4 h at 4°C and serum was harvested after centrifugation (3,640 × *g* at 15°C for 20 min). Serum was frozen at -20°C until serum testosterone analysis (IMMULITE 1000 Total Testosterone; LKTW1; Siemens Diagnostic, Los Angeles, CA). The intra- and interassay CVs were 18.7% and 7.0%, respectively. The average testosterone concentrations for the quality control samples were 123.8, 640.8, and 1241.1 ng/dL for the low, medium, and high samples, respectively. The minimal detectable concentration of testosterone was 20.0 ng/dL.

Sampling and Laboratory Analysis

Ground ration samples were collected every 28 d (approximately 2.0 kg) and dried at 55°C for 48 h (The Grieve Corporation, Round Lake, IL) to determine DM concentration. Orts were collected and weighed on d 112 and 168 of the trial and dried at 55°C for 48 h to determine DMI for PHASE 1 and 2, respectively. Dietary and ort samples were ground to pass a 2-mm screen (Wiley Mill; Arthur H. Thomas Cp., Philadelphia, PA) and shipped to a commercial lab (Midwest Laboratories, Inc., Omaha, NE) for proximate and mineral analysis (Table 3.1). Samples were analyzed for DM (method 930.15; AOAC Int., 2009), N (method 990.03; AOAC Int., 2009), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using an Ankom 200 Fiber Analyzer without sodium sulfide, with amylase, and without ash

corrections as sequentials, ADF (Goering and Van Soest, 1970), crude fat (method 945.16; AOAC Int., 2009), and minerals (inductively coupled atomic plasma and wet digest procedure).

Statistical Analysis

Ram lamb feedlot and reproductive performance, along with scrotal circumference, were analyzed as a randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen serving as the experimental unit. The fixed effect included in the model was dietary treatment with the random effect of pen nested within treatment. The random effect of day was used in the REPEATED measures analysis for scrotal circumference, testosterone concentrations, semen volume, spermatozoa motility score, morphology, and concentration. The model included the fixed effects of dietary treatment, day, and treatment × day. Random effects included pen nested within treatment, ram × pen × treatment, and day × pen × treatment. Pre-planned comparisons of linear, quadratic, and cubic contrasts were used to partition treatment effects. Significance was determined at $P \leq 0.05$. All interactions that were not clearly significant ($P \geq 0.20$) were removed from the model. To partition day effects and treatment × day interactions, least significant differences were used ($P \leq 0.05$).

Results and Discussion

Feedlot Performance

Phase One. There was a quadratic effect on PHASE 1 BW, ADG, and DMI ($P \leq 0.03$), however, there was no effect on PHASE 1 G:F ($P \geq 0.12$; Table 3.2) by dietary treatment. Van Emon et al. (2013) did not observe any effects on ram lamb final BW as DDGS increases in the diet, while Schauer et al. (2008) observed a tendency for DDGS to increase final BW compared to lambs receiving no DDGS. Van Emon et al. (2013) observed a linear increase in ADG and DMI as DDGS increased in the diets from 0 to 30%; however, a linear decrease was observed for

G:F. Previous trials have also shown an improvement in lamb ADG (Schauer et al., 2008; Van Emon et al., 2013) as concentration of DDGS in the diet increased, likely due to an increase in CP and crude fat in the diet. However, Van Emon et al. (2013) observed a linear increase in DMI as concentration of DDGS increased in the diets. Klopfenstein et al. (2008) reported cattle fed wet distiller's grains with solubles and DDGS were more efficient than corn-fed cattle. In contrast, Richter et al. (2012) observed that steers fed a high S diet (0.6% of the ration), similar to 45DDGS in the current trial, responded with decreased ADG compared to steers fed a low S diets (0.2% of the ration). In the present trial, we likely observed decreases in DMI due to a decrease in ruminal pH, driven by the increased production of H_2SO_4 (Felix and Loerch, 2011; Felix et al., 2012a). Decreases in ruminal pH have been associated with decreases in intake (Owens et al., 1998), similar to the current trial. In the present study, the quadratic effect observed for PHASE 1 ADG paired with the quadratic effect on DMI in the same time period likely drove the cubic effect ($P = 0.05$) observed for Overall G:F. Felix et al. (2012b) observed conflicting results in which lambs fed increasing levels of DDGS (0 to 60%) had a linear reduction in G:F, similar to Van Emon et al. (2013). Others have reported no effects on feedlot performance when feeding DDGS to lambs or steers at inclusion rates from 23 to 30% (Huls et al., 2006; Leupp et al., 2009). The current study indicates there are benefits of feeding DDGS to feedlot lambs at inclusion rates of up to 45DDGS.

Phase Two. PHASE 2 BW, ADG, and DMI, and G:F were not affected ($P \geq 0.06$; Table 3.2) by PHASE 1 dietary treatment. It is likely the overall G:F effect was driven by the PHASE 1 response. PHASE 2 was implemented in this trial to provide an opportunity for reproductive convalescence, however, it also allowed our lab to observe how growing rams would adjust or perform when switched to another diet. In this case, rams quickly adjusted to the CON diet,

however, when numerically comparing DMI from PHASE 1 and 2, it decreased greatly, as did ADG.

Overall. There were no treatment x PHASE interactions for feedlot performance variables ($P \geq 0.22$). There was a PHASE effect ($P \leq 0.01$) for overall BW, ADG, DMI, and G:F, all of which increased from PHASE 1 to 2 (data not shown). A quadratic effect was observed for overall BW ($P = 0.005$; Table 3.2) and overall ADG ($P = 0.02$; Table 3.2), however a cubic effect was observed for overall G:F ($P = 0.05$; Table 3.2). The overall effects for BW likely drove the similar effect seen for ADG, provided the observed effect was cubic for G:F. However, the effect on G:F is still most likely driven by the effects on BW and ADG. In general, the cubic response exhibited in all cases was likely driven by a decrease in value from the CON through the 15DDGS and 30DDGS rations, followed by an increase in the 45DDGS ration.

Reproductive Performance

Phase One. PHASE 1 scrotal circumference, seminal volume, spermatozoa motility, and concentration were not affected ($P \geq 0.16$; Table 3.3) by treatment. In PHASE 1, there were no treatment effects ($P \geq 0.07$; Table 3.4) on spermatozoa morphological abnormalities, with the exception of a linear decrease ($P = 0.04$) in the presence of strongly folded tails as DDGS increased in the diet. Most of the measured reproductive traits exhibited no effects in the first phase of this trial. This is likely due to the spermatogenic cycle of rams being 47 d long, meaning, we likely measured PHASE 1 effects in PHASE 2.

Phase Two. PHASE 2 spermatozoa volume and motility were not different ($P \geq 0.06$) due to dietary treatment. PHASE 2 scrotal circumference exhibited a quadratic response ($P = 0.05$) to increasing levels of DDGS in the diet, with circumference decreasing from 0DDGS to 15 and 30DDGS, and then increasing again to 45DDGS. It is unknown why these effects were observed.

Also, a linear increase ($P = 0.04$) in the percentage of normal spermatozoa as the concentration of DDGS in the diet increased was observed. Rams fed 0, 15, and 30DDGS treatments all had similar percentages of normal sperm; however, the 45DDGS had more normal sperm than the 0DDGS fed rams. Additionally, PHASE 2 spermatozoa concentration exhibited a linear increase ($P = 0.03$) as DDGS increased in the ration, opposed to Van Emon et al. (2013), who observed a linear decrease in spermatozoa concentrations in response to increasing levels of DDGS in the diet. For PHASE 2 spermatozoa morphological abnormalities, a quadratic effect ($P = 0.05$) was observed for the percent bent heads of spermatozoa as DDGS increased in the diet. As reported in Table 3.4, the spermatozoa of rams fed 0DDGS had a greater number of bent headed sperm when compared to the 15, 30, and 45DDGS fed rams; however, rams receiving the 15, 30, and 45DDGS diets had similar percentages.

Overall. There were no treatment x day interactions for most reproductive variables or testosterone concentration ($P \geq 0.17$). Overall spermatozoa volume and motility were not different ($P \geq 0.07$) due to dietary treatment. Overall scrotal circumference exhibited a PHASE effect ($P < 0.001$) with d 112 and 140 being similar to one another but greater than d 84 and 168. A linear decrease ($P = 0.04$; Table 3.3) in overall scrotal circumference was observed (37.30, 36.92, 36.36, 36.79 ± 0.22, respectively) as DDGS increased in the diet. According to Merck (1998), growing rams from 8 to 14 months of age should measure a minimum of 28 cm for scrotal circumference, indicating that in our trial these ram lambs fell within the acceptable guidelines for reproductive soundness. Others have observed the opposite effect on scrotal circumference when rams are fed high protein and energy rations (Martin et al., 1994; Hötzel et al., 1998), in which scrotal circumferences increased. In contrast, Van Emon et al. (2013) observed no differences in change in or final scrotal circumference for rams fed increasing

DDGS. Similar results have also been reported in bulls fed high-energy diets (Coulter and Kozub, 1984). In the current trial, diets increased in CP and crude fat with the increasing inclusion of DDGS, with slight increases in TDN as DDGS increased in the diet. Therefore, this could provide an explanation for the quadratic effect on scrotal circumference in PHASE 2. It is also possible that since PHASE 2 was not concluded until mid-November that lambs from certain treatments had more scrotal fat deposited that insulated the testes more so than other treatments. However, with a quadratic effect present, the results are difficult to explain. Overall seminal volume was affected ($P = 0.006$) by PHASE with d 84 and 140 being similar to one another, but greater than d 112 and 168, but was unaffected ($P > 0.28$) by treatment. The only treatment × PHASE interaction ($P = 0.05$) observed was for the occurrence of distal droplets along the tails of the spermatozoa. This interaction was observed on d 84 sampling (PHASE 1) in rams receiving the 15DDGS dietary treatment, with them having increased occurrence compared to other treatments and the other days. Means for this interaction were as follows: 0DDGS, 30DDGS, and 45DDGS for all four collection days were 0%, 15DDGS for d 84 collection was 16.25%, and 15DDGS for all other collection days were 0%. Distal cytoplasmic droplets are considered a minor defect, however, in natural breeding situations, have long been considered a cause of subfertility (Barth and Oko, 1989).

There was no treatment × PHASE, PHASE, or treatment effects ($P > 0.12$; Table 3.4) for abaxial tail implantation, abnormal acrosomes, and simple tail bends or coils. Proximal cytoplasmic droplets are classified as a major defect to spermatozoa, causing significant impairments to fertility. In this trial, while there was no effect of treatment on proximal cytoplasmic droplets ($P \geq 0.33$), a day effect ($P < 0.001$) was observed, decreasing from d 84 throughout the trial to d 168, with the last three collections having similarly low percentages of

the defect. This defect has been linked to poor cleavage rates of embryos (Amann et al., 2000; Thundathil et al., 2001). This is likely due to the ram lambs maturing throughout the trial, leading to the disappearance of the droplets. However, in trials observing Cu deficiencies in rams, when feeding Mo and high sulfate supplements, rams exhibited small percentages of droplets (1-4%; Van Niekerk and Van Niekerk, 1989).

Excessive S intake can be toxic and could cause decreased performance and possibly cause PEM and death (Gould, 1998). No cases of PEM were observed in the present study nor in the trial conducted by Van Emon et al. (2013). The NRC (2007) states that the maximum level of S tolerated in concentrate diets is 0.3% of DM. However, in the present study, both the 30DDGS and 45DDGS diets were above this maximum level (Table 3.1). According to the NRC (2007), high dietary levels of S can also alter selenium and copper utilization and absorption. Van Emon et al. (2013) indicated inadequate utilization of selenium and copper as a possible explanation for the observed reduction in spermatozoa concentration. The percentage of tailless spermatozoa heads also exhibited a day effect ($P < 0.001$) decreasing from PHASE 1 to PHASE 2, which each collection within the phases being similar to one another. Others have observed that this defect is usually due to testicular hypoplasia or degeneration, sexual inactivity, or more specific conditions, like decapitated sperm defect (Barth and Oko, 1989). In this trial, the presence of this defect was likely caused by sexual inactivity as the ram lambs were not exposed to females from approximately d 90 of age until trial conclusion. The presence of narrow, small, or giant heads of the sperm exhibited a day effect ($P = 0.02$) decreasing from the first collection to the last three collection days, while not exhibiting a treatment effect ($P \geq 0.54$). In the previously mentioned Cu deficiency trial, this defect was moderately observed (6-12%) in rams that were Cu deficient as a result of Mo and sulfate supplementation (Van Niekerk and Van Niekerk, 1989).

An overall linear decrease ($P = 0.02$) was observed for the occurrence of bent heads on the sperm as DDGS increased in the diet. Although the cause of this defect is not known, it can be detrimental to fertility as it can impair fertilization rate, embryonic development, and failure of cleavage (Menon et al., 2011). A PHASE effect was observed ($P = 0.02$) for the occurrence of pyriform shaped heads, with PHASE 1 collections having greater occurrence than PHASE 2, with no difference ($P \geq 0.32$) due to treatment. Similar to the other sperm head defects, pyriform shaped heads impair the spermatozoa's ability to fertilize the oocyte efficiently and effectively. A PHASE effect was also observed ($P = 0.009$) for the defect of strongly folded/coiled tails. There was also a linear decrease ($P = 0.04$) in the occurrence of this defect as the inclusion of DDGS increased in the ration. This is interesting as the Cu deficiency trial recorded this defect as one of the most prevalent (18-25%) when Cu was deficient (Van Niekerk and Van Niekerk, 1989), leading one to explicate that the S present in the DDGS in the present trial must not be leading to mineral deficiencies *in vivo*. Similar PHASE effects were observed ($P < 0.05$) for mid-piece defects as well as maldeveloped heads. Both exhibited a decrease in occurrence from PHASE 1 collections to PHASE 2 collections, with no difference due to treatment ($P \geq 0.16$). This is once again most likely due to maturation of the ram lambs, testicles, and increases in sexual activity.

A PHASE effect was observed ($P < 0.001$) for the occurrence of normal spermatozoa, with both of the PHASE 2 collections having a greater occurrence than the first collection day and the d 140 collection (3rd collection) being greater than the first collection as well. Based on the means of the collection days, ram lambs would have failed a breeding soundness exam on the first two collection days (d 84 and 112). However, there was no treatment effect ($P \geq 0.17$) on the occurrence of normal sperm. Interestingly, although there was no treatment effect, the means

for the treatments should be taken into consideration. Although the measurements of the young rams brought down the mean values, the 0DDGS, 15 DDGS, and 30DDGS treatments would have caused the rams to fail a breeding soundness exam as satisfactory percentages are listed as ≥ 75% normal spermatozoa according to Ley et al. (1990). This data suggests that supplementing growing ram lambs with a high energy and protein feed, such as DDGS, might decrease the time before young rams can effectively breed.

Overall spermatozoa concentration were not different ($P \geq 0.06$) due to dietary treatment, however there was a PHASE effect ($P = 0.02$) with d 140 and d 168 having similar increased concentrations compared to similar d 84 and 112. Previous research on human sperm revealed semen samples with low sperm concentrations, high incidence of abnormal sperm morphology, and diminished fertility had higher sperm creatine phosphokinase (**CK**) activity (Huszar and Vigue, 1993). Higher CK activity was related to increased content of CK and other proteins in the sperm resulting in those sperm heads being significantly larger and rounder, with increased morphological irregularities and increased cytoplasm believed to be due to failure of spermatogenesis (Huszar and Vigue, 1993). Their trial concluded that higher CK activity results in cellular immaturity and a failure to complete spermatogenesis (Huszar and Vigue, 1993). This experiment suggests one possibility for a potential pathway for spermatozoa to be negatively effected. It is possible that the increased CP in both our trial and that of Van Emon et al. (2013), as a result of DDGS increasing in the ration, is contributing to the negative effects on sperm quality. Additional research is needed to ascertain why these effects are occurring.

Testosterone concentrations exhibited a PHASE ($P < 0.001$) effect, increasing as the trial progressed, with the exceptions of days 126, 154, and 168. This increase is similar to the observed increasing testosterone concentrations by Van Emon et al. (2013), likely due to the

rams maturing throughout the progression of the trial. Testosterone concentrations also exhibited a linear decrease ($P < 0.005$) as DDGS increased in the ration (Table 3.4). Scrotal circumferences decreased as the concentration of DDGS in the diet increased, likely causing the decrease in testosterone concentration. These results are in contrast to those observed by Martin et al. (1994) and Hötzel et al. (1998) who observed that rams fed diets of high or intermediate energy and protein had increased testosterone concentrations compared to the rams receiving low energy and protein diets. The decrease in testosterone concentrations observed in the current study are likely due to the decrease in scrotal circumference that was most likely caused by decreasing temperatures. However, this does not explain the decreases as DDGS in the diets increased.

Implications

Dried distiller's grains with solubles improved feedlot growth performance in ram lambs when fed at up to 45% of the ration, with improved performance carrying through in body weight gains for an additional 56 days following removal of dried distiller's grains with solubles. Dried distiller's grains with solubles had no negative impacts on the morphology of spermatozoa, and for some traits, improved morphology in ram lambs when fed at up to 45% of the ration. We are only aware of two trials elucidating reproductive quality of rams when feeding dried distiller's grains with solubles in the diet. More research is needed in the area of nutritional effects on ram fertility to further elucidate what is causing the observed effects. Future research should focus on specific nutrient and mineral concentrations as well as heavy metals and their effects on both ram and ewe fertility.

Table 3.2. Effects of dried distiller's grains with solubles (DDGS) on feedlot performance traits of growing rams

	Dietary Treatment[1]					Contrasts[3]		
Item[4]	CON	15DDGS	30DDGS	45DDGS	SEM[2]	Linear	Quadratic	Cubic
Initial BW, kg	48.4	48.5	49.3	48.5	0.31	0.45	0.17	0.13
Overall BW, kg	80.76	78.91	78.95	84.89	1.28	0.04	0.005	0.49
PHASE 1 BW	92.9	90.2	90.0	100.0	2.58	0.10	0.03	0.53
PHASE 2 BW	100.9	98.1	97.5	106.2	2.84	0.25	0.06	0.60
Overall ADG, kg/d	0.33	0.31	0.30	0.35	0.01	0.25	0.02	0.39
PHASE 1 ADG	0.40	0.37	0.36	0.46	0.02	0.11	0.02	0.41
PHASE 2 ADG	0.14	0.14	0.13	0.11	0.04	0.57	0.78	0.95
Overall DMI, kg • ram^{-1} • d^{-1}	2.58	2.20	2.22	2.34	0.14	0.31	0.11	0.65
PHASE 1 DMI	2.68	2.27	2.31	2.62	0.11	0.82	0.007	0.70
PHASE 2 DMI	2.38	2.08	2.04	1.79	0.26	0.15	0.94	0.71
Overall G:F, kg of gain/kg of DMI	0.13	0.14	0.13	0.15	0.005	0.04	0.50	0.05
PHASE 1 G:F	0.15	0.16	0.16	0.18	0.009	0.12	0.80	0.36
PHASE 2 G:F	0.06	0.07	0.06	0.06	0.02	1.00	0.79	0.76

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).

[2]$n = 4$.

[3]*P*-value for linear, quadratic, and cubic effects of increasing dried distiller's grains with solubles.

[4]PHASE 1 = treatment diets fed to respective groups of ram lambs from d 1 to 112 of trial; PHASE 2 = d 112 to 168 all ram lambs were placed on the CON ration.

Table 3.3. Effects of dried distiller's grains with solubles (DDGS) on reproductive traits of growing rams

	Dietary Treatment[1]					Contrasts[3]		
Item[4]	CON	15DDGS	30DDGS	45DDGS	SEM[2]	Linear	Quadratic	Cubic
Overall scrotal circumference, cm	37.30	36.92	36.36	36.79	0.22	0.04	0.08	0.23
PHASE 1 scrotal circumference	37.67	38.14	37.60	38.23	0.50	0.62	0.87	0.34
PHASE 2 scrotal circumference	37.52	36.55	35.66	36.27	0.36	0.01	0.05	0.39
Overall spermatozoa volume, mL	1.16	1.29	1.26	1.33	0.10	0.28	0.76	0.55
PHASE 1 volume	1.02	1.17	1.22	1.07	0.17	0.79	0.38	0.88
PHASE 2 volume	0.93	0.79	1.00	1.44	0.19	0.06	0.15	0.89
Overall spermatozoa motility[5]	3.05	3.26	3.38	3.47	0.17	0.07	0.72	0.92
PHASE 1 motility	3.13	3.44	3.56	3.63	0.29	0.23	0.67	0.92
PHASE 2 motility	2.81	2.92	3.00	3.81	0.41	0.12	0.40	0.69
Overall spermatozoa concentration[6]	10.98	10.33	13.84	13.17	1.17	0.06	0.99	0.12
PHASE 1 concentration	8.11	10.73	10.03	12.22	1.71	0.16	0.90	0.43
PHASE 2 concentration	9.89	13.10	17.60	17.83	2.48	0.03	0.56	0.62
Testosterone concentration, ng/dL[7]	665.56	592.09	571.59	546.79	24.16	0.005	0.32	0.60

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).

[2]$n = 4$.

[3]*P*-value for linear, quadratic, and cubic effects of increasing dried distiller's grains with solubles.

[4]PHASE 1 = treatment diets fed to respective groups of ram lambs from d 1 to 112 of trial; PHASE 2 = d 112 to 168 all ram lambs were placed on the CON ration.

[5]Spermatozoa motility score: 1= no forward movement, 2 = slow forward movement, 3 = moderate forward movement, 4 = fast forward movement.

[6]Spermatozoa concentrations were measured as billions per milliliter. The hemocytometer has a counting chamber volume of one cubic millimeter. Five large squares were counted for each ejaculate sample, the four corner squares and the middle square. To calculate the spermatozoa concentration: Total number of sperm counted × dilution factor × hemocytometer factor × conversion factor. The dilution rate was 1:200, the hemocytometer factor was 50, and the conversion factor (converted units to spermatozoa/cubic centimeter, or mL) was 1,000.

[7]Testosterone concentration values were determined from blood collections on d 84, 112, 140, and 168 of the experiment. The values expressed are the treatment mean.

Table 3.4. Effects of dried distiller's grains with solubles (DDGS) on spermatozoa morphology of growing ram lambs

Item, %	Dietary Treatment[1]				SEM[2]	*P*-Value[3]		
	CON	15DDGS	30DDGS	45DDGS		Linear	Quadratic	Cubic
Overall distal droplets	0.19	4.25	0.44	0.31	1.28	0.55	0.11	0.05
PHASE One	0.38	8.50	0.88	0.63	2.79	0.59	0.15	0.07
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall proximal droplets	1.00	1.75	0.94	1.00	0.56	0.74	0.54	0.33
PHASE One	2.00	3.50	1.88	2.00	1.22	0.77	0.57	0.38
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall tailless heads	13.44	15.12	13.31	10.88	2.88	0.46	0.48	0.82
PHASE One	20.00	20.25	21.63	15.88	5.28	0.65	0.57	0.73
PHASE Two	6.88	10.00	5.00	5.88	2.28	0.44	0.63	0.18
Overall abaxial tail implantation	0.19	0.88	0.13	<0.01	0.42	0.48	0.33	0.27
PHASE One	0.38	1.75	0.25	<0.01	0.83	0.48	0.33	0.27
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall abnormal acrosome	<0.01	0.06	0.13	0.44	0.20	0.12	0.53	0.78
PHASE One	0.00	0.13	0.25	0.88	0.39	0.12	0.52	0.77
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall simple bend or coil tails	9.06	7.13	10.56	7.94	1.96	0.99	0.86	0.19
PHASE One	9.13	9.88	13.88	10.63	3.07	0.54	0.52	0.45
PHASE Two	9.00	4.38	7.25	5.25	2.27	0.41	0.57	0.23
Overall narrow, small, or giant heads	0.44	0.38	0.38	0.94	0.54	0.54	0.57	0.84
PHASE One	0.88	0.75	0.75	1.88	1.10	0.55	0.57	0.84
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall bent heads	3.25	1.06	1.56	1.00	0.60	0.02	0.18	0.17
PHASE One	1.63	1.13	2.75	1.63	0.70	0.61	0.66	0.13
PHASE Two	4.88	1.00	0.38	0.38	0.94	0.002	0.05	0.54
Overall pyriform heads	0.44	0.50	0.25	0.19	0.22	0.32	0.78	0.62
PHASE One	0.88	1.00	0.38	0.38	0.43	0.27	0.88	0.48
PHASE Two	0.00	0.00	0.13	<0.01	0.06	0.66	0.33	0.19
Overall strongly folded tails	1.63	0.38	0.56	0.06	0.47	0.04	0.43	0.31
PHASE One	3.25	0.75	1.13	0.13	0.97	0.04	0.45	0.34
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall mid-piece defects	0.19	0.25	0.56	0.06	0.20	0.94	0.16	0.23
PHASE One	0.38	0.50	1.13	0.13	0.38	0.94	0.15	0.22
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall maldeveloped heads	0.69	0.25	0.19	0.38	0.24	0.35	0.20	0.91
PHASE One	1.38	0.50	0.38	0.75	0.52	0.40	0.241	0.92
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall normal morphology	70.69	68.94	72.25	77.75	3.95	0.17	0.36	0.87
PHASE One	61.50	53.13	56.63	66.25	7.16	0.58	0.22	0.86
PHASE Two	79.88	84.75	87.88	89.25	3.28	0.04	0.60	1.00

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).

[2]$n = 4$.

[3]*P*-value for linear, quadratic, and cubic effects of increasing DDGS.

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CHAPTER 4: ECONOMIC ANALYSIS OF DIFFERING INCLUSION RATES OF DRIED DISTILLER'S GRAINS WITH SOLUBLES TO GROWING RAMS AND FEEDLOT LAMBS

Abstract

Our objective was to determine the profitability of feeding dried distiller's grains with solubles (**DDGS**) to ram or feedlot lambs. Our hypothesis was that for the trials described in chapters two and three, as DDGS increased in the diets, so would the economic viability of the rations due to increased efficiency of gain and intake. In the first trial, we also observed the effects of including lasalocid (**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ) in the ration. Main effects included concentration of DDGS (0, 15, or 30% DM basis) and inclusion of LAS (0 or 22.05 g/metric ton LAS) resulting in treatments of: 1) 0% DDGS without LAS (**0DDGS-NL**), 2) 0% DDGS with LAS (**0DDGS-L**), 3) 15% DDGS without LAS (**15DDGS-NL**), 4) 15% DDGS with LAS (**15DDGS-L**), 5) 30% DDGS without LAS (**30DDGS-NL**), and 6) 30% DDGS with LAS (**30DDGS-L**). These economic benefits would stem from increases in ADG, G:F, and a decrease in DMI. In the second trial feeding growing ram lambs, dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**), 15% of the ration as DDGS substituted for corn (% DM basis; **15DDGS**), 30% of the ration as DDGS substituted for corn (% DM basis; **30DDGS**) and 45% of the ration as DDGS substituted for corn (% DM basis; **45DDGS**). When feeding DDGS to growing ram lambs, we observed that the 45DDGS treatment had the least cost per kg of gain when considering the cost of the ration at $1.39 per kg of gain. In the first trial, we observed that feedlot lambs receiving the 30DDGS-L treatment exhibited the least cost per kg of gain at $1.42 and overall least cost at $35.68. Therefore, total profits per lamb from this treatment was the greatest across all treatments at $45.72. The least

profits displayed in our analysis was for the 30DDGS-NL dietary treatment at $23.76 per lamb, followed by the 15DDGS-NL treatment with a revenue per lamb at $27.60.

Key Words: dried distiller's grains with solubles, economic analysis, feedlot, lambs, rams

Introduction

Overall, there is a need for more research on the economic impacts of feeding dried distiller's grains with solubles (**DDGS**) to sheep, especially feedlot lambs. McEachern et al. (2009) reported that DDGS can replace cottonseed meal in lamb finishing diets without negatively affecting growth rate, feed conversion, wool characteristics, and can potentially reduce feed cost per kg of gain. Many trials have shown that DDGS can be a viable feed supplement in growing lambs (Schauer et al., 2008; Neville et al., 2010; Van Emon et al., 2013); however, none of these stated the economic benefits of feeding DDGS. Huls et al. (2006) observed no effects of including DDGS in lamb finishing rations on carcass characteristics. These results and those from the trials mentioned in the previous two chapters indicate a possibility of economic value in including DDGS in lamb rations, either in the feedlot or in growing purebred stock.

Considering the above information and that which is provided in the previous chapters, we hypothesized that as DDGS increased in the diets, so would the economic viability of the rations due to increased efficiency of gain and intake. Our objective for this chapter was to evaluate the cost and revenue of feeding DDGS to growing rams and feedlot lambs.

Materials and Methods

Trial One

All procedures were approved by the Animal Care and Use Committee at North Dakota State University (**NDSU**; Protocol #A15054). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Animals and Diets

At 2 wk of age, crossbred lambs (Suffolk × Rambouillet) tails were docked, male lambs were castrated, and all lambs were vaccinated against *Clostridium perfringens* types C and D as well as tetanus (**CD-T**; Bar Vac CD/T; Boehringer Ingelheim, Ridgefield, CT). Lambs were adapted to an 80% corn and 20% commercial market lamb pellet diet (DM basis; Table 2.1) from a 100% creep meal diet following weaning at approximately 60 d of age. Lambs were vaccinated with CD-T again at 60 d of age and d -1 of the study. In May 2016, two hundred forty lambs were stratified by BW (31.9 ± 5.87 kg; approximately 90 d of age) and sex (105 wethers and 135 ewes) and randomly assigned to 1 of 24 outdoor pens (10 lambs/pen). Pens were assigned randomly to 1 of 6 treatments, with pen serving as the experimental unit (n = 4 pens/treatment). Diets were based on an 80% corn and 20% market lamb meal (**MLM**) diet, which included LAS for respective treatments, and were balanced to be isonitrogenous and equal to or greater than the CP and NE requirements (NRC, 2007) for a 40 kg lamb gaining 300 g/d. Rations were formulated to have a minimum Ca:P ratio of 2:1. Rations were ground through a 1.27 cm screen (Gehl Mix-All, Model 170, Gehl, West Bend, WI), mixed, and offered for ad libitum intake via bulk feeders (48.6 cm bunk space/lamb). Lambs had continuous access to clean, fresh water and shade. Feeders were checked daily and cleaned of contaminated feed. Lambs were observed daily to monitor health and treated when necessary. No treatment related morbidity or mortality

was observed. Main effects included dietary concentration of DDGS (0, 15, or 30% DM basis) and inclusion of LAS (0 or 22.05 g/metric ton LAS) resulting in treatments of: 1) 0% DDGS without LAS (**0DDGS-NL)**, 2) 0% DDGS with LAS (**0DDGS-L**, 3) 15% DDGS without LAS (**15DDGS-NL**), 4) 15% DDGS with LAS (**15DDGS-L**), 5) 30% DDGS without LAS (**30DDGS-NL**), and 6) 30% DDGS with LAS (**30DDGS-L**). Water tests indicate sulfate levels to be 141 mg/L (Stearns DHIA, Sauk Centre, MN).

Data Collection Procedures

Lambs were weighed on two consecutive d at the initiation (d -1 and 0) and end (d 110 and 111) of the trial; single day weights were taken on d 28, 54, and 84. Feed ingredient and ration grab-samples (approximately 0.2 kg) were collected from the bulk feeders at the beginning of each period and dried at 55°C for 48 h to determine DM and ration nutrient composition. Dried samples were ground to pass a 2-mm screen. Samples were analyzed for DM, ash (AOAC Int., 2010), N (AOAC Int., 2010) using a Kjeltec Auto 1030 Analyzer (Tecato AB, Höganäs, Sweden), mineral content including S (AOAC Int., 2010), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using an Ankom 200 Fiber Analyzer without sodium sulfite, with amylase, and without ash corrections as sequentials, and ADF (Goering and Van Soest, 1970). All lambs were shorn on d 29 and 30 with final mid-side wool samples collected on d 110. The samples were clipped at skin level and washed in an 80:20 hexane to isopropyl alcohol mixture. After washing in a 220V VWR symphony ultrasonic cleaner (#97043-958, Radnor, PA) for 15 minutes, samples were allowed to dry for a minimum of 90 minutes in a controlled climate area. Following drying, the wool samples were then measured using the OFDA2000 (BSC Electronics, Ardross, Western Australia) for fiber diameter distribution (mean, SD, and CV), fiber curvature distribution (mean, SD, CV), staple length, and comfort factor.

Comfort factor is defined as the percentage of fibers less than or equal to 30 micrometers. On d 112 of the trial, lambs (218 hd; 64.8 ± 7.99 kg BW) were harvested at Mountain States Rosen Company (Greeley, CO). Trained personnel collected carcass data after a 24-h chill (temperature < 2°C and humidity near 100%). Carcass data collected included HCW (measured on day of slaughter), fat depth, loin eye area (**LEA**), and body wall thickness (at the 12th rib), conformation score, flank streaking, lean maturity, and yield grade (Savell and Smith, 2000). The following equation, adapted from Savell and Smith (2000), was used to calculate % boneless closely trimmed retail cuts (**BCTRC**): 49.936 – (0.0848 × 2.205 × HCW) – (4.376 × 0.3937 × fat depth) – (3.53 × 0.3937 × body wall thickness) + (2.456 × 0.155 × LEA), in which HCW is measured in kilograms, fat depth and body wall thickness are measured in centimeters, and LEA area is measured in square centimeters. Conformation score was scored on a scale of 100 to 1500 (100 = cull; 1500 = prime). All lambs were assigned bone maturity of A with varying lean maturity scored on a scale of 0 to 100 (0 = very fine; 100 = fine). Flank streaking was assigned with scores of 100 to 199 = Practically Devoid, 200 to 299 = Traces, 300 to 399 = Slight, 400 to 499 = Small, and 500 to 599 = Modest.

Economic Analysis

A partial budget was used to evaluate the effect of differing inclusion concentrations of DDGS in ram and feedlot lamb diets on profitability. Since the rams would theoretically be retained or sold, a revenue was not calculated; however, cost of kg of gain was used as the final determining cost in this instance (Table 4.2). Revenue was calculated for the feedlot lamb trial and was based on the cost of purchasing the lambs (USDA prices), cost of the ration, cost per kg of gain, and the HCW of the lambs after slaughter. The cost of DDGS was estimated overall at $131.40 per tonne. The HCW payment of $8.13/kg was based on the most current price for lamb

whole carcasses reported by the USDA National Weekly Lamb Market Summary (17 February 2017; Table 4.1). These lambs were not purchased, but were from the NDSU Hettinger Research Extension (HREC) flock, however a purchase price was still included for the analysis. Feed costs were calculated on an as fed basis and included whole, shelled corn, oats, dried distiller's grains with solubles, and a commercial market lamb pellet for the ram or trial one ration. The ration was the same for trial two with the exception of not including oats in the ration and the commercial market lamb pellet containing lasalocid for the respective dietary treatments. In the ram trial, as seen in Table 4.2, with the respective percentages of ration ingredients the cost of the dietary treatments per tonne were: CON ($206.16), 15DDGS ($205.39), 30DDGS ($204.62), and 45DDGS ($203.85). Whole, shelled corn was approximately $136.49 per tonne, the commercial market lamb pellet without lasalocid was $553.64, but with lasalocid was $568.63 per tonne, and DDGS cost $131.39 per tonne with delivery from the ethanol plant purchased from (Richardton, ND). The oats contained in the ration were grown at the HREC, however for the purposes of this analysis were priced at the current market price of $146.45 per tonne. The market prices for corn and oats were obtained from the USDA Agricultural Marketing Service and Grain and Feed Market News in Chicago, IL and Minneapolis, MN in December 2016. Feed costs for each year were calculated using the average daily as fed intake per lamb throughout the growing phase in the feedlot for each dietary treatment by converting the average DMI per lamb to as fed intake (Chapters 2 and 3, respectively).

Trial Two

All procedures were approved by the animal care and use committee of North Dakota State University (protocol # A14060). This study was conducted at the North Dakota State University Hettinger Research Extension Center in Hettinger, ND.

Feedlot Study

Ram lambs (Suffolk and Hampshire) were purchased from four producers in North and South Dakota, Minnesota, and Iowa. Prior to purchase, ram lambs were vaccinated for *Clostridium perfringens* types C and D and tetanus, weaned at 60 d of age, and revaccinated. At approximately 90 d of age, rams were purchased and transported to the Hettinger Research Extension Center. Ram lambs were adapted to a 60% corn, 25% oats, and 15% commercial market lamb pellet diet (CON; DM basis) for approximately 2 weeks. Ram lambs (n=112) were stratified by weight (48.7 ± 0.31 kg) and breed then randomly assigned to 1 of 16 outdoor pens (7 rams/pen; 18 m^2/ram). Pens were assigned randomly to 1 of 4 treatments in a completely random design to 1 of 4 treatments, with pen serving as the experimental until (n = 4 pens/treatment). Dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**), 15% of the ration as DDGS substituted for corn (% DM basis; **15DDGS**), 30% of the ration as DDGS substituted for corn (% DM basis; **30DDGS**) and 45% of the ration as DDGS substituted for corn (% DM basis; **45DDGS**) as described in Table 2.1. Study diets were balanced to be isocaloric and to be equal to or greater than the CP and TDN requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007) and to maintain a Ca: P ratio of 2:1 or greater. Rations were ground (1.25 cm screen) and mixed in a grinder-mixer (GEHL mix-all, Model 170; West Bend, WI) and were provided to the lambs with ad libitum access via bulk feeders (70 cm bunk space/ram). Ram lambs had continuous access to clean, fresh water. Feeders were checked daily and cleaned of contaminated feed (fecal contamination, wet feed due to precipitation, etc.). Ram lambs were weighed on two consecutive d at the beginning (d 0 and 1) and the end of the trial (d 167 and 168) and weighed once every 28 d (to assist in evaluation of lambs for morbidity). Ram lambs were fed their respective treatments until d 112 (**PHASE 1**) and on d 112 all feed was

removed from self-feeders and pens were reallocated to the CON diet until d 168 (**PHASE 2**). One ram was removed from the trial due to a broken leg and six other rams died before the conclusion of the trial from complications not related to the trial. Necropsies concluded that the rams had normal liver, rumen, and intestines, with the majority of the ram lambs succumbing to chronic pneumonia.

Sampling and Laboratory Analysis

Ground ration samples were collected every 28 d (approximately 2.0 kg) and dried at 55°C for 48 h (The Grieve Corporation, Round Lake, IL)to determine DM. Orts were collected and weighed on d 112 and 168 of the trial and dried at 55°C for 48 h to determine DMI for PHASE 1 and 2, respectively. Dietary and ort samples were ground to pass a 2-mm screen (Wiley Mill; Arthur H. Thomas Cp., Philadelphia, PA) and shipped to a commercial lab (Midwest Laboratories, Inc., Omaha, NE) for proximate and mineral analysis. Samples were analyzed for DM (method 930.15; AOAC Int., 2009), N (method 990.03; AOAC Int., 2009), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using and Ankom 200 Fiber Analyzer without sodium sulfide, with amylase, and without ash corrections as sequentials, ADF (Goering and Van Soest, 1970), crude fat (method 945.16; AOAC Int., 2009), and minerals (inductively coupled atomic plasma and wet digest procedure).

Trial One

Veterinary and medical costs were included in the total costs, however are the same across treatments since no significant sickness or disease occurred across trial treatments or were not treatment related, therefore the only medical costs were the vaccination for CD-T. This vaccination occurred three times throughout the lives of the lambs, making it a minimal cost and the same across all treatments. Yardage costs were based on commercial rates ($0.04 head-1d-1)

and accounted for fixed costs of infrastructure, the grinder-mixer wagon and tractor, and labor for feeding and daily health checks. Trucking costs were also included at $0.02/kg to transport lambs from the feedlot at the HREC to the packing plant in Greeley, CO. In this analysis (Table 4.1), the cost per kg of gain would have been the most significant cost and only differing cost across the treatments due to differences in the concentrations of ration ingredients.

In the first trial, we have the opportunity to analyze cost of the ration, cost of gain, as well as the profits from a HCW form of payment, and therefore the revenue generated from each lamb on average from each dietary treatment is presented in table 4.1.

Trial Two

In the second trial, the total cost per kg of gain (Table 4.2) is the only significant cost, and since we did not collect any reproductive data on these rams in a breeding setting, we do not know the outcome for profits from these rams.

Results and Discussion

Trial One

Table 4.1 presents the partial budget analysis for the first trial. Purely on a feed cost basis, the 30DDGS-NL diet was the cheapest; and when translated to a BW gain basis, the 30DDGS-L treatment was the most cost effective. These diets are least cost because of the concentration of DDGS replacing the higher cost corn, but most importantly, the cost of the diets diverges with the inclusion of LAS. Lasalocid increased Final BW and HCW (4% increase) in this trial, which significantly decreased the cost of gain. Since feed was the most significant cost of production in this system, as well as most other systems, the other costs included in this analysis had minimal impacts on the profitability of these dietary treatments. All of the lambs were harvested at the same time; however, since the producer is paid on a HCW basis, the

differences in these weights impacted the revenue from these lambs. The 30DDGS-L had the highest HCW and therefore, the highest revenue. This treatment was the most efficient and profitable treatment throughout the analysis as seen in table 4.1, with $3.15 more revenue per lamb compared to the next closest diet.

Trial Two

The budget for trial two is presented in table 4.2. The budget for this trial only includes the cost of the ration and the cost of each kg of BW gain per ram. The 45DDGS treatment was the most profitable ration for feeding rams. Although this diet has the highest occurrence of morphological abnormalities in the spermatozoa analysis (Chapter 2), these rams would still likely pass a veterinary breeding soundness exam. Therefore, even with the one negative quality of this diet, it remains the most profitable diet for growing ram lambs. However, no further economic analyses of these treatments was performed due to the lack of data on breeding capacity of these rams and the possible future impacts on the operation.

Implications

This analysis revealed that our original hypothesis is indeed true. Feeding 30% dried distiller's grains with solubles and lasalocid to lambs increased net return based on the current feed and lamb prices. Including dried distiller's grains with solubles in the feedlot diets decreases intake, increases gain, and the inclusion of lasalocid increased final body weights and hot carcass weights. All of these traits additively caused the profitability of the final dietary treatment. Although this analysis is not representative of market changes and does not translate to every feedlot situation, it does provide a template for producers to compare feeding dried distiller's grains with solubles as a partial replacement for corn in lamb feedlot and ram grower rations.

Table 4.1. Partial budget for the impacts of dried distiller's grains with solubles (DDGS) and lasalocid (LAS) in feedlot lambs (per hd basis)

	Dietary Treatment[1]					
Item	0DGS-NL	0DGS-L	15DGS-NL	15DGS-L	30DGS-NL	30DGS-L
Lamb Data						
Final BW, kg	63.2	63.3	62.2	65.4	61.9	66.3
HCW, kg	30.9	31.0	31.1	32.2	29.8	32.3
Dressing %	48.4	48.9	50.0	49.2	48.1	48.7
BW Gain, kg	22.5	23.0	21.8	24.6	21.4	25.2
As Fed Intake, kg/d	1.9	1.9	2.0	1.7	1.7	1.6
Costs, $						
Lamb Purchase[2]	182.67	182.67	182.67	182.67	182.67	182.67
Feed per tonne[3]	199.57	202.29	198.87	201.59	198.17	200.89
Feed per kg[4]	0.199	0.202	0.199	0.202	0.198	0.201
Per kg of gain[5]	1.87	1.85	2.02	1.55	1.75	1.42
Total cost of gain[6]	42.10	42.64	44.13	38.04	37.40	35.68
Yardage[7]	4.44	4.44	4.44	4.44	4.44	4.44
Trucking[8]	1.26	1.27	1.24	1.31	1.24	1.33
Veterinary[9]	1.01	1.01	1.01	1.01	1.01	1.01
Total Cost	231.48	232.03	233.49	227.47	226.76	225.13
Revenue, $						
Carcass Value	251.22	252.03	252.84	261.79	242.27	262.60
Pelts	8.25	8.25	8.25	8.25	8.25	8.25
Total Revenue[10]	259.47	260.28	261.09	270.04	250.52	270.85
Profit, $	27.99	28.25	27.60	42.57	23.76	45.72

[1]Diets (DM basis) were balanced to be isonitrogenous and equal or greater than CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). LAS = lasalocid. Treatments were: 0% DDGS without LAS (0DGS-NL), 0% DDGS with LAS (0DGS-L), 15% DDGS without LAS (15DGS-NL), 15% DDGS with LAS (15DGS-L), 30% DDGS without LAS (30DGS-NL), and 30% DGS with LAS (30DGS-L). MLM = commercial market lamb meal contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2 mg/kg Se, 52,863 IU/kg vitamin A, 5,286 IU/kg vitamin D, and 209 IU/kg vitamin E, and LAS included into respective treatments at 22.05 g/tonne.

[2]Lamb purchase price calculate based on USDA weekly national lamb market summary for the week of 20 March 2017.

[3]Feed costs included in the feedlot ration: whole, shelled corn ($136.53/tonne As Fed), DDGS ($206.16/tonne As Fed), and MLM (with LAS = $568.79/tonne As Fed; without LAS = $553.80/tonne As Fed), respectively for treatments.

[4]Feed costs calculated based on kg of feed (As Fed) consumed on average per lamb in that respective treatment.

[5]Feed costs calculated based on kg of BW gained per lamb, based on Final BW.

[6]Total cost of feed based on the total kg of BW gained throughout the 111 d feeding trial.

[7]Yardage rate was $0.04/lamb/d and accounted for the fixed costs of infrastructure, grinder-mixer wagon and tractor, and labor of feeding and daily health checks.

[8]Trucking costs ($0.02/kg) accounted for transporting lambs to Mountain States Lamb Cooperative in Greeley, CO for slaughter.

[9]Veterinary cost included the cost of *Clostridium Perfringens* types C and D cost at three time points according to the listed dosage of 1mL/lamb.

[10]Total revenue ($8.13/kg) was estimated from the carcass and pelt value of the USDA weekly national lamb market summary for the week of 17 February 2017.

Table 4.2. Partial budget for the impacts of dried distiller's grains with solubles (DDGS) in growing ram lambs (per hd basis)

	Dietary Treatment[1]			
Item	CON	15DDGS	30DDGS	45DDGS
Feed Cost, $/tonne[2]	206.16	205.39	204.62	203.85
BW Gain, kg	52.5	49.6	48.2	57.7
DMI, kg/d	2.58	2.20	2.22	2.34
Cost per kg of feed, $	0.206	0.205	0.205	0.204
Total cost of gain, $	89.29	75.77	76.46	80.20
Cost per kg of gain, $[3]	1.70	1.53	1.59	1.39

[1]Diets (DM basis) were balanced to be isonitrogenous and equal or greater than CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). Treatments were: 0% DDGS (CON), 15% DDGS (15DDGS), 30% DDGS (30DDGS), and 45% DDGS (45DDGS).
[2]Feed costs included in the feedlot ration: whole, shelled corn ($136.53/tonne As Fed), DDGS ($206.16/tonne As Fed), and MLM ($553.80/tonne As Fed), respectively for treatments.
[3]Feed costs calculated based on kg of feed (As Fed) consumed on average per lamb in that respective treatment.

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CHAPTER 5: GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

General Conclusions

Our results have contributed to the rather small amount of information available about feeding both growing ram lambs as well as feedlot lambs, especially when considering the supplementation of DDGS and the interaction of LAS. To our knowledge, these are the only trials that have collected digestibility data, ruminal VFA concentrations and pH, all in conjunction with feedlot performance data. Furthermore, there is only one previous trial testing the possible effects of DDGS on ram fertility. The current trial is the only trial of its kind that tested if rams were able to convalesce once exposed to DDGS in the feed.

Supplementing DDGS at up to 30% of the ration to feedlot lambs has proven to be a very profitable and efficient ration when compared to corn-based rations. When LAS is included in the ration, these results become more exaggerated. Growth of the lambs, intake, and HCW were all improved with the inclusion of DDGS and/or LAS. Supplementing DDGS to growing ram lambs produced many of the same results in the feedlot and thus far has been shown to not be reproductively detrimental and according to the data presented, possibly beneficial to the reproductive development in rams.

Future Directions

Continuing to increase growth efficiency of livestock is an ongoing research topic in the agricultural industry. Dried distiller's grains with solubles provides an excellent byproduct feed source. Lambs seem to be especially tolerant to the negative qualities of DDGS. As the need to feed a growing population continues, growing meat animals at a lower cost and more efficiently is critically important. Therefore, trials of this nature need to be pursued to continue investigating digestibility and cost.

The data extrapolated from the ram lamb project has opened doors for future research; however, there are also several ways in which it could have been improved upon. The obvious is to conduct the study with more controlled feeding parameters, and a more selective focus on the cause of effects on reproductive parameters of the ram lambs so that we might be able to better elucidate the mechanisms occurring.

Another improvement for future research would be to focus on the timing of spermatogenesis, so that we might be able to better target and elucidate when damage to the spermatozoa is occurring in the cycle. With this in mind, future projects could investigate timing of the spermatogenic cycle, possibly synchronizing spermatogenesis. By synchronizing spermatogenesis, the implications for future research could be large for studying impacts of nutritional impacts on the spermatozoa and how those effects could impact future offspring conceived from those damaged spermatozoa. Elucidating these effects could allow us to more successfully raise sheep to feed the growing population of the world once feeding strategies could be developed and implemented.