Influence of distiller's dried grains with solubles on ram lamb growth and reproductive traits

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ABSTRACT: The hypothesis of this experiment was that increasing the inclusion level of distiller's dried grains with solubles (DDGS) in the diets would decrease semen quality but have no negative effects on growth performance. Following the removal of DDGS from the diet, it was 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 4 treatments ($n = 4$ pens per treatment; 7 rams per pen) in a completely randomized design. Dietary treatments were 60% corn, 25% oats, and 15% commercial 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). Lambs were fed for 112 d on their respective treatment, after which they were placed on the CON ration until day 168. Lambs were weighed on consecutive d at the beginning (days 0 and 1) and end (days 167 and 168) of the study. Scrotal circumference was measured on all lambs on days 84, 112, 140, and 168. Semen samples were collected on a subset of 64 rams

(4 rams per pen) to evaluate semen quality on days 84, 112, 140, and 168. Blood samples were collected on the same subset of rams every 14 d throughout the study. A quadratic effect on BW on day 112 and overall BW ($P = 0.03$ and $P = 0.005$, respectively), ADG on day 112 and overall ADG ($P = 0.02$ and $P = 0.02$, respectively), DMI ($P = 0.007$) on day 112, and a cubic effect ($P = 0.05$) for overall G:F were observed. Overall and day 168 scrotal circumference had a quadratic ($P = 0.05$) response. A linear increase in spermatozoa concentration on day 168 was observed ($P = 0.03$) as DDGS concentration increased in the treatment diets, although rams in this stage of the study were no longer receiving DDGS. Overall, testosterone concentrations exhibited a linear decrease ($P = 0.005$) as DDGS increased in the diet. The linear increase ($P = 0.04$) on day 168 in morphologically normal sperm as DDGS increased in the diets indicated that all rams, regardless of treatment, would have passed a reproductive soundness exam. In the current study, no negative effects were observed in lamb feedlot or reproductive traits due to increasing DDGS in the diet, indicating that DDGS may be included at levels up to 45% of growing lamb diets.

Key words: distiller's dried grains with solubles, feedlot, growth performance, rams, reproductive traits, semen quality

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INTRODUCTION

Ethanol production in the United States continues to increase ([Renewable Fuels Association, 2017](#)). The byproduct of the ethanol industry, distiller's dried grains with solubles (DDGS), provides an affordable and viable feed source for livestock, especially ruminants. Distiller's dried grains are readily incorporated into diets to supplement RUP as DDGS have greater CP concentration than corn. However, it is important to 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; [Gould, 1998](#)). Due to the high energy and CP content of DDGS, its inclusion in lamb feedlot diets continues to increase. In feedlot lambs, DDGS have been included in the diet at rates of up to 60% with no negative effects on performance or symptoms of PEM ([Schauer et al., 2008](#); [Neville et al., 2011](#)). [Van Emon et al. \(2013\)](#) reported a linear decrease in spermatozoa concentration as DDGS increased in the diet. Currently, this is the only study that has evaluated DDGS in lamb diets and its potential effect on male fertility. Due to this lack of research, it is not clear what nutritional component of DDGS could be affecting fertility. The current study tested the hypothesis that increasing the inclusion level of DDGS in the diet of growing lambs would decrease semen quality, without negatively affecting performance. The objectives to test these hypotheses were to determine the influence of increasing concentrations of DDGS in lamb diets on 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 No. 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 4 producers in North and South Dakota, Minnesota, and Iowa. Prior to purchase, 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, lambs were purchased and transported to the Hettinger Research Extension Center. Lambs were adapted to a 60% corn, 25% oats, and 15% commercial lamb pellet diet (CON; DM basis) for approximately 2 wk. 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 per pen; 18 m²/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% 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 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 1.5:1 or greater. Complete diets were ground (1.25-cm screen), mixed in a grinder-mixer (GEHL mix-all, Model 170; West Bend, WI), and provided to lambs with ad libitum access via bulk feeders (70 cm of bunk space per ram). Lambs had continuous access to clean, fresh water and shade. Feeders were checked daily and cleaned of contaminated feed (fecal contamination, wet feed due to precipitation, etc.). Lambs were weighed on 2 consecutive days at the beginning (days 0 and 1) and end of the study (days 167 and 168) and then weighed once every 28 d (un-shrunk weights; to assist in evaluation of lambs for morbidity). Lambs were fed their respective treatments until day 112. On day 112, all feed was removed from self-feeders and pens were reallocated to the CON diet until day 168. Seven rams were removed from the study before conclusion due to complications not related to dietary treatment.

Reproductive Traits Study

Scrotal circumference was measured on all lambs on days 84, 112, 140, and 168. 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 lambs (a subsample of the 112 lambs in the feedlot study described above; 4 lambs per pen; 16 lambs per treatment; $n = 4$) were chosen for seminal volume, spermatozoa motility, spermatozoa concentrations, and serum testosterone concentration analysis. The 4 lambs in each pen were

Table 1. Ingredient and nutritional composition of diets fed to growing rams (DM basis)

Item	Dietary treatment ^a			
	CON	15DDGS	30DDGS	45DDGS
Ingredient, %				
DDGS ^b	–	14.9	29.7	44.3
Corn	60	44.8	29.7	14.8
Oats	25	24.9	24.7	24.6
Lamb pellet ^c	15	14.9	14.9	14.8
CaCO ₃	–	0.5	1.0	1.5
Nutritional composition, % DM				
Ash	4.0	4.6	5.2	5.7
TDN ^d	86.5	84.8	83.1	81.4
CP	16.4	19.9	23.3	26.7
ADF	6.6	7.9	9.1	10.6
Crude fat	3.5	4.2	4.8	5.4
S, %	0.2	0.3	0.3	0.4
P, %	0.4	0.5	0.6	0.7
K, %	0.7	0.9	1.1	1.2
Mg, %	0.2	0.2	0.3	0.3
Ca, %	0.7	1.2	1.7	2.2
Ca:P	1.6	2.3	2.8	3.1
Na, %	0.2	0.3	0.3	0.3
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

^aDiets (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 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).

^bDDGS = distiller's dried grains with solubles.

^cCommercial lamb pellet contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75% to 4.75% Ca, 0.6% P, 3.0% to 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.

^dCalculated by Midwest Labs: (%CP × 1 × 0.85) + (%CF × 1 × 0.6) + (%NFE × 1 × 0.8) + (%EE × 2.25 × 0.8) = TDN.

selected based on weight and breed to provide a representative subset. Two of the 4 lambs in each pen were selected based on weight and breed to provide a representative subset for spermatozoa morphology analysis.

Semen was collected on days 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 (10× magnification) for spermatozoa motility scoring (Van Emon et al., 2013). The spermatozoa motility score, which is based on the rate of forward movement, was determined on a 1 to 4 scale, as previously described by Van Emon et al. (2013): 1) no forward movement or all dead (0% to 24% live sperm cells), 2) slow forward movement (25% to 49% live sperm cells), 3) moderate forward movement (50% to 74% live sperm cells), and 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 430× magnification. The hemocytometer has a counting chamber volume of 1 mm³. Five large squares were counted for each ejaculate sample: the 4-corner squares and the middle square. If the spermatozoa cells were on the top or right border-line of these squares, they were counted; however, if the cells were on the bottom or left, they were not counted (Van Emon et al., 2013). The below formula was used to calculate the spermatozoa concentration:

$$\text{Spermatozoa / mL} = \frac{\text{number of sperm counted}}{(\text{in 5 squares}) \times \text{dilution factor}} \times \text{hemocytometer factor} \times \text{conversion factor}.$$

The dilution rate was 1:200, the hemocytometer factor was 50, and the conversion factor (converted units to spermatozoa/cm³, or mL) was 1,000

(Van Emon et al., 2013). In addition, the percent of specific morphological abnormalities of the diluted spermatozoa in a nigrosin-eosin stain was determined on a prewarmed glass slide at 40 $\times$ magnification. Observed morphological abnormalities 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.

Every 14 d throughout the duration of the study, a 10-mL blood sample was collected via jugular venipuncture with a 20 gauge $\times$ 2.54-cm vacutainer needle into serum separator 16- $\times$ 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 $^{\circ}$ C and serum was harvested after centrifugation (3,640 $\times$ g at 15 $^{\circ}$ C for 20 min). Serum was frozen at -20 $^{\circ}$ 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. 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.

Although reproductive traits were measured on multiple days (days 84, 112, 140, and 168), only days 112 and 168 were utilized to determine treatment effects on scrotal circumference, seminal volume, spermatozoa motility, and spermatozoa concentration. Intermediate collection days were utilized to determine whether the rams were sexually maturing, similar to the collection of intermediate weights every 28 d, and utilized to determine day effects on reproductive traits.

Sampling and Laboratory Analysis

Ground diet samples were collected every 28 d (approximately 2.0 kg) and dried at 55 $^{\circ}$ C for 48 h (H451-A16NF; Wilder oven; NU-VU Foodservice Systems, Menominee, MI) to determine DM concentration. Orts were collected and weighed on days 112 and 168 of the study and dried at 55 $^{\circ}$ C for 48 h to determine DMI for days prior to day 112 and days 113 to 168. Dietary and ort samples were ground to pass a 2-mm screen (Wiley Mill; Arthur H. Thomas Co., Philadelphia, PA) and shipped to a commercial lab (Midwest Laboratories, Inc., Omaha, NE) for proximate and mineral analysis (Table 1). Samples were analyzed for DM (method

930.15; AOAC Int., 2009), N (method 990.03; AOAC Int., 2009), and 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

Lamb feedlot and reproductive traits, 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 fixed effect of day was used in the REPEATED measures analysis for scrotal circumference (variance components), testosterone concentrations (compound symmetry), seminal volume (variance components), spermatozoa motility score (variance components), morphology (variance components), and concentration (variance components). Random effects included pen nested within the treatment and day $\times$ pen $\times$ treatment. The model included the fixed effects of dietary treatment, day, and treatment $\times$ day. Preplanned 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 ($P \geq 0.10$) were removed from the model. To partition day effects and treatment $\times$ day interactions, least significant differences were used ($P \leq 0.05$).

RESULTS AND DISCUSSION

Feedlot Performance

There were no treatment $\times$ day interactions for feedlot performance variables ($P \geq 0.22$; Table 2). There was a day effect ($P \leq 0.01$) for overall BW, ADG, DMI, and G:F, all of which increased from days 1 to 168 (data not shown). A quadratic effect was observed for overall BW and overall ADG ($P = 0.005$ and $P = 0.02$, respectively; Table 2), and a cubic effect was observed for overall G:F ($P = 0.05$; Table 2). The overall effects for BW likely influenced 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 due to the effects

Table 2. Effects of DDGS on feedlot performance traits of growing rams

Item ^a	Dietary treatment ^b					Contrasts ^d		
	CON	15DDGS	30DDGS	45DDGS	SEM ^c	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
D 112 BW	92.9	90.2	90.0	100.0	2.58	0.10	0.03	0.53
D 168 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
D 112 ADG	0.40	0.37	0.36	0.46	0.02	0.11	0.02	0.41
D 168 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
D 112 DMI	2.68	2.27	2.31	2.62	0.11	0.82	0.007	0.70
D 168 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
D 112 G:F	0.15	0.16	0.16	0.18	0.009	0.12	0.80	0.36
D 168 G:F	0.06	0.07	0.06	0.06	0.02	1.00	0.79	0.76

^aD 112 = treatment diets fed to respective groups of ram lambs from days 1 to 112 of study; D 168 = days 113 to 168 all ram lambs were placed on the CON ration.

^bDiets (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 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).

^cGreatest standard error of the means; $n = 4$.

^d P -value for linear, quadratic, and cubic effects of increasing distiller's dried grains with solubles.

on BW and ADG. In general, the cubic response was likely influenced by a decrease in DMI from the CON through the 15DDGS and 30DDGS diets, followed by an increase in the 45DDGS diet.

The majority of the overall performance differences were influenced by performance differences during the first 112 d of the study (when DDGS were fed). There was a quadratic effect on BW, ADG, and DMI on day 112 ($P \leq 0.03$), with the 15DDGS and 30DDGS having decreased BW, ADG, and DMI compared with the CON and 45DDGS; however, there was no effect on G:F ($P \geq 0.12$; Table 2) by dietary treatment on day 112. Van Emon et al. (2013) did not observe any effects on ram lamb final BW as DDGS increased in the diet, whereas Schauer et al. (2008) observed a tendency for DDGS to increase final BW compared with 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 studies 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. In the current study, CP increased from 16% to 27% and crude fat increased from 6.6% to 10.6% in the diets.

Van Emon et al. (2013) observed a linear increase in DMI as concentration of DDGS increased in the diets. Klopfenstein et al. (2008) reported that 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) responded with decreased ADG compared with steers fed a low S diets (0.2% of the ration). In the current study, S increased from 0.2% to 0.4% of the diet as DDGS inclusion level increased. The NRC (2007) indicates potential S toxicity when greater than 0.3% of the diet in concentrate-based diets; however, there were no observed cases of S toxicity in the current study. The observed decreases in DMI were likely due to a decrease in ruminal pH, influenced 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 study. In the present study, the quadratic effect observed for ADG on day 112 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 that there are benefits of feeding DDGS to feedlot lambs at inclusion rates of up to 45DDGS, provided other dietary conditions do not

exist with a potential to create a S toxicity (i.e., high sulfate water).

Removing DDGS from the diet on days 113 to 168 did not affect ($P \geq 0.06$; Table 2) BW, ADG, DMI, and G:F. This change in dietary treatment, removal of DDGS, was implemented in this study to provide an opportunity for reproductive convalescence (not necessarily performance convalescence); however, it also allowed observation of how growing lambs would adjust or perform when switched to another diet. In this case, lambs quickly adjusted to the CON diet. Since all of the lambs were receiving the same diet for the final 56 d of the study, treatment differences in feedlot performance were not expected. No other research is available that has evaluated changing a lamb finishing diet from DDGS to a non-DDGS based ration.

Reproductive Traits

Scrotal circumference, seminal volume, and spermatozoa motility and concentration.

There were no treatment $\times$ day interactions for most reproductive traits ($P \geq 0.17$). Overall seminal volume, spermatozoa motility, and spermatozoa concentration were not different ($P \geq 0.06$) due to dietary treatment (Table 3). Overall scrotal circumference exhibited a day effect ($P < 0.001$) with days 112 and 140 being not different from one another but greater than days 84 and 168. A linear decrease ($P = 0.04$; Table 3) in overall scrotal circumference was observed as DDGS increased in the diet.

In contrast to feedlot performance results, in which the majority of the overall differences were influenced by DDGS feeding from days 0 to 112, the majority of the overall responses for reproductive traits appeared on day 168, after the rams had been consuming the non-DDGS diet for 56 d. To present the results in a similar fashion to feedlot performance, day 112 results will be presented followed by day 168 results, where the majority of the overall effects are observed (Table 3). Scrotal circumference, seminal volume, spermatozoa motility, and spermatozoa concentration were not affected ($P \geq 0.16$; Table 3) by treatment following feeding

Table 3. Effects of DDGS on reproductive traits of growing rams

Item ^a	Dietary treatment ^b				SEM	Contrasts ^c		
	CON	15DDGS	30DDGS	45DDGS		Linear	Quadratic	Cubic
Overall scrotal circumference, cm	37.30	36.92	36.36	36.79	0.22	0.04	0.08	0.23
D 112 scrotal circumference	37.67	38.14	37.60	38.23	0.50	0.62	0.87	0.34
D 168 scrotal circumference	37.52	36.55	35.66	36.27	0.36	0.01	0.05	0.39
Overall seminal volume, mL ^d	1.16	1.29	1.26	1.33	0.10	0.28	0.76	0.55
D 112 volume	1.02	1.17	1.22	1.07	0.17	0.79	0.38	0.88
D 168 volume	0.93	0.79	1.00	1.44	0.19	0.06	0.15	0.89
Overall spermatozoa motility ^{d,e}	3.05	3.26	3.38	3.47	0.17	0.07	0.72	0.92
D 112 motility	3.13	3.44	3.56	3.63	0.29	0.23	0.67	0.92
D 168 motility	2.81	2.92	3.00	3.81	0.41	0.12	0.40	0.69
Overall spermatozoa concentration ^{d,f}	10.98	10.33	13.84	13.17	1.17	0.06	0.99	0.12
D 112 concentration	8.11	10.73	10.03	12.22	1.71	0.16	0.90	0.43
D 168 concentration	9.89	13.10	17.60	17.83	2.48	0.03	0.56	0.62
Testosterone concentration, ng/dL ^{d,g}	665.56	592.09	571.59	546.79	24.16	0.005	0.32	0.60

^aD 112 = treatment diets fed to respective groups of ram lambs from days 1 to 112 of study; day 168 = days 113 to 168 all ram lambs were placed on the CON ration.

^bDiets (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 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).

^cP-value for linear, quadratic, and cubic effects of increasing distiller's dried grains with solubles.

^dGreatest standard error of the means; $n = 4$.

^eSpermatozoa motility score: 1 = no forward movement, 2 = slow forward movement, 3 = moderate forward movement, 4 = fast forward movement.

^fSpermatozoa concentrations were measured as billions per milliliter. The hemocytometer has a counting chamber volume of 1 mm³. Five large squares were counted for each ejaculate sample, the 4 corner squares and the middle square. To calculate the spermatozoa concentration: total number of sperm counted $\times$ dilution factor $\times$ hemocytometer factor $\times$ 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.

^gTestosterone concentration values were determined from blood collections on days 84, 112, 140, and 168 of the experiment. The values expressed are the treatment mean.

of DDGS for 112 d. However, scrotal circumference on day 168 exhibited a quadratic effect ($P = 0.05$) as DDGS increased in the diet, with 30 DDGS being less than CON, 15 DDGS, and 45 DDGS. Seminal volume and spermatozoa motility scores were not different ($P \geq 0.06$) due to dietary treatment on day 168, but spermatozoa concentration actually increased ($P = 0.03$) as DDGS increased in the diet. The results on day 168 are likely due to the spermatogenic cycle of rams being 47 d long, meaning, effects on semen, spermatozoa, or both made in the first 112 d of feeding DDGS were likely measured sometime from days 113 to 168.

According to Merck (1998), growing rams from 8 to 14 mo of age should measure a minimum of 28 cm for scrotal circumference, indicating that these lambs fell within the acceptable guidelines for reproductive soundness. Others have observed the opposite effect on scrotal circumference when lambs 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 or final scrotal circumference for rams fed increasing DDGS. Similar results to Van Emon et al. (2013) have also been reported in bulls fed high-energy diets (Coulter and Kozub, 1984). In the current study, 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 overall linear decrease in scrotal circumference. It is also possible that since the study was not concluded until mid-November (day 168), that lambs from certain treatments had more scrotal fat deposited that insulated the testes more effectively than other treatments. However, with a quadratic effect present, the results are difficult to explain.

The results of the current experiment for spermatozoa concentrations on day 168 were opposite of the results reported by Van Emon et al. (2013), who observed a linear decrease in spermatozoa concentrations in response to increasing levels of DDGS in the diet. Van Emon et al. (2013) indicated inadequate utilization of selenium and copper as a possible explanation for the observed reduction in spermatozoa concentration. According to the NRC (2007), high-dietary levels of S can also alter selenium and copper utilization and absorption. Excessive S intake can be toxic and could cause decreased performance and possibly cause PEM and death (Gould, 1998). 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 1), although no cases of PEM were observed in the present study nor in the study conducted by Van Emon et al. (2013).

Testosterone concentration. Testosterone concentrations exhibited a day ($P < 0.001$) effect, increasing as the study 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 lambs maturing throughout the progression of the study. Testosterone concentrations also exhibited a linear decrease ($P < 0.005$) as DDGS increased in the diet (Table 4). 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 with the rams receiving low energy and protein diets. The decrease in testosterone concentrations observed in the current study is likely due to the decrease in scrotal circumference, most likely caused by the decreasing temperatures (-6°C average temperature for November 2014; NDAWN Center, 2017). Studies in men have seen that when testosterone concentrations are inhibited, sperm concentrations decrease, as well as testicle size (Palacios et al., 1981).

Spermatozoa morphology. The only treatment $\times$ day interaction ($P = 0.05$) observed was for the occurrence of distal droplets along the tails of the spermatozoa. This interaction was observed on day 84 sampling (while DDGS was fed in the ration) in lambs receiving the 15DDGS dietary treatment, with them having increased occurrence compared with 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 day 84 collection was 16.25%, and 15DDGS for all other collection days was 0%. Distal cytoplasmic droplets are considered a minor defect; however, in natural breeding situations, they have long been considered a cause of subfertility (Barth and Oko, 1989).

A cubic response ($P = 0.05$) was observed for overall distal droplet presence, with 15 DDGS having a greater incidence of distal droplets than CON, 30DDGS, and 45DDGS (Table 4). Additionally, overall bent heads exhibited linear decreases ($P = 0.04$) 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

Table 4. Effects of DDGS on spermatozoa morphology of growing ram lambs

Item, %	Dietary treatment ^a					P-value ^c		
	CON	15DDGS	30DDGS	45DDGS	SEM ^b	Linear	Quadratic	Cubic
Overall distal droplets	0.19	4.25	0.44	0.31	1.28	0.55	0.11	0.05
D 112	0.38	8.50	0.88	0.63	2.79	0.59	0.15	0.07
D 168	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
D 112	2.00	3.50	1.88	2.00	1.22	0.77	0.57	0.38
D 168	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
D 112	20.00	20.25	21.63	15.88	5.28	0.65	0.57	0.73
D 168	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
D 112	0.38	1.75	0.25	<0.01	0.83	0.48	0.33	0.27
D 168	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
D 112	0.00	0.13	0.25	0.88	0.39	0.12	0.52	0.77
D 168	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
D 112	9.13	9.88	13.88	10.63	3.07	0.54	0.52	0.45
D 168	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
D 112	0.88	0.75	0.75	1.88	1.10	0.55	0.57	0.84
D 168	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
D 112	1.63	1.13	2.75	1.63	0.70	0.61	0.66	0.13
D 168	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
D 112	0.88	1.00	0.38	0.38	0.43	0.27	0.88	0.48
D 168	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
D 112	3.25	0.75	1.13	0.13	0.97	0.04	0.45	0.34
D 168	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
D 112	0.38	0.50	1.13	0.13	0.38	0.94	0.15	0.22
D 168	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
D 112	1.38	0.50	0.38	0.75	0.52	0.40	0.241	0.92
D 168	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
D 112	61.50	53.13	56.63	66.25	7.16	0.58	0.22	0.86
D 168	79.88	84.75	87.88	89.25	3.28	0.04	0.60	1.00

^aDiets (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 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).

^bGreatest standard error of the means; $n = 4$.

^cP-value for linear, quadratic, and cubic effects of increasing DDGS.

(Menon et al., 2011). A day effect was also observed ($P = 0.009$) for the defect of strongly folded or coiled tails as well as a linear decrease ($P = 0.04$) in the occurrence of this defect as the inclusion of DDGS increased in the diet. This is interesting as a study testing effects of Cu deficiency recorded this as one of the most prevalent defects (18% to 25%) when Cu was deficient (Van Niekerk and Van

Niekerk, 1989), leading one to conclude that the S present in the DDGS in the present study must not be leading to mineral deficiencies in vivo. Similar day effects were observed ($P < 0.05$) for mid-piece defects as well as maldeveloped heads; both exhibited a decrease in occurrence from day 112 collections to day 168 collections, with no difference due to treatment ($P \geq 0.16$). This could once again be

due to maturation of the ram lambs, testicle size, or sexual inactivity; however, the actual cause is unknown as there are many research studies studying this subject matter across species, but the results differ greatly (Levitas et al., 2005; Menon et al., 2011; Palacios et al., 2016). Current research cannot clearly distinguish the exact causes nor how much change in scrotal circumference must take place to cause some of these effects. Bent heads on day 168 also exhibited a quadratic decrease ($P = 0.05$) as DDGS increased in the diet.

Proximal cytoplasmic droplets are classified as a major defect to spermatozoa, causing significant impairments to fertility. In this study, although there was no effect of treatment on proximal cytoplasmic droplets ($P \geq 0.33$), a day effect ($P < 0.001$) was observed, decreasing from day 84 throughout the study to day 168, with the last 3 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 study, leading to the disappearance of the droplets. Although when rams with Cu deficiencies were fed Mo and high sulfate supplements, only small percentages of droplets were observed (1% to 4%; Van Niekerk and Van Niekerk, 1989). Percentage of tailless spermatozoa heads also exhibited a day effect ($P < 0.001$) decreasing from days 113 to 168, with each collection within the (days 84 and 140) being similar to one another. This defect has been observed in another study and was stated as usually being caused by testicular hypoplasia or degeneration, sexual inactivity, or more specific conditions, such as the decapitated sperm defect (Barth and Oko, 1989).

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 3 collection days, while not exhibiting a treatment effect ($P \geq 0.54$). In the previously mentioned Cu deficiency study, this defect was moderately observed (6% to 12%) in rams that were Cu deficient as a result of Mo and sulfate supplementation (Van Niekerk and Van Niekerk, 1989).

A day effect was observed ($P = 0.02$) for the occurrence of pyriform-shaped heads, with day 112 collections having greater occurrence than day 168, 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.

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). Greater 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 the failure of spermatogenesis (Huszar and Vigue, 1993). Their study 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 affected. It is possible that the increased CP in both our study 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 and why the effects were not consistent between studies.

A day effect was observed ($P < 0.001$) for the occurrence of normal spermatozoa, with days 112 and 168 collections having a greater occurrence than the first collection day and the day 140 collection (third collection) being greater than the first collection as well. Based on the means of the collection days, ram lambs would have not met the minimum standards for normal morphological spermatozoa appearance as stated by Merck (1998) on the first 2 collection days (days 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, 15DDGS, and 30DDGS treatments would have caused the rams to once again not meet the minimum standards for normal morphological spermatozoa, as satisfactory percentages are listed as $\geq 75\%$ normal spermatozoa according to Ley et al. (1990). These data suggest 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.

A linear increase ($P = 0.04$) in the percentage of normal spermatozoa as the concentration of DDGS in the diet increased was observed on day 168, but was not observed on day 112 or overall ($P \geq 0.17$), indicating that overall spermatozoa morphology improved on d 168 from d 112 measurements and as concentrations of DDGS increased in the diet. Rams fed 0DDGS, 15DDGS,

and 30DDGS treatments all had similar percentages of normal sperm, but the 45DDGS had more normal sperm than the 0DDGS-fed lambs.

DDGS improved feedlot growth performance in lambs when fed at up to 45% of the diet, with improved performance carrying through in body weight gains for an additional 56 d following removal of DDGS. DDGS had no negative impacts on the morphology of spermatozoa and for some traits, improved morphology in lambs when fed at up to 45% of the diet. Currently there are 2 studies elucidating reproductive quality of rams when feeding DDGS 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.

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