Sheep & Goat Research Journal
Volume 35, 2020 - April

Replacing Cottonseed Meal and Sorghum Grain with Corn Dried Distillers Grains with Solubles in Lamb Feedlot Diets: Growth Performance, Rumen Fluid Parameters, and Blood Serum Chemistry[1]

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[1] *This work was supported by the USDA National Institute of Food and Agriculture Hatch Project #205866.*

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Abstract

Effects of replacing cottonseed meal (CSM) and sorghum grain with dried distillers grains with solubles (DDGS) in Dorper ram lamb (n = 46) feedlot diets on growth performance, rumen fluid parameters, and blood serum chemistry were evaluated. In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL; representative of a traditional feedlot diet) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain. Lambs fed CNTL were compared to 0DDGS and linear and quadratic effects were evaluated within the four DDGS diets. A treatment × day interaction was observed (P < 0.001) for lamb BW but not for ADG, DMI, or G:F ($P \geq 0.78$). Lambs fed CNTL had greater ($P \leq 0.02$) BW on d 42 and 56 and greater ($P < 0.008$) overall ADG and G:F than lambs fed 0DDGS. On d 42 and 56, lamb BW quadratically increased ($P \leq 0.04$) as DDGS increased in the diet. Averaged across all days, ADG quadratically increased ($P < 0.001$) and DMI and G:F tended to quadratically increase ($P \leq 0.08$) as DDGS increased in the diet. On d 56, ruminal pH quadratically decreased (P < 0.001), ruminal ammonia-N quadratically increased (P < 0.001), acetate linearly increased ($P < 0.001$), and acetate:propionate tended to linearly increase ($P = 0.08$) as DDGS increased in the diet. Various blood serum profiles were affected by diet, but data suggested that diet did not negatively affect lamb health. Results indicated that lamb growth performance is enhanced when CSM is used to increase dietary CP (CNTL vs. 0DDGS) and that, within the diets that did not contain CSM, the 66DDGS diet resulted in the greatest growth performance.

Introduction

Cottonseed meal (CSM) and sorghum grain are widely used as protein and energy sources, respectively, in sheep feedlot rations, especially in Texas. However, some feedlot operators are beginning to seek alternative protein and energy sources due to seasonality and highly variable CSM and sorghum grain pricing. Nutritional characteristics of dried distillers grain with solubles (DDGS), a corn ethanol production coproduct, suggests that it can be an economical alternative to CSM and sorghum grain. Corn DDGS are an excellent source of protein and energy for ruminants (Lardy, 2003) and have become more available in recent years due to the ever-growing ethanol industry (FAPRI, 2017). Even though NRC (2007) states that CSM is greater in CP and degradable protein than DDGS, previous studies have shown that completely replacing CSM with DDGS (up to 20% of the diet; DM basis) resulted in no differences in average daily DMI, ADG, or G:F in feedlot lambs (McEachern et al., 2009). However, feeding high concentrations of DDGS in lamb feedlot rations has been of concern to the industry due to S content that originates with industry cleaning practices. This concern is based upon the evaluation of DDGS in cattle feedlot diets, which have been reported to cause polioencephalomalacia (PEM) when incorporated at more than 20% of the diet (Lardy, 2003). However, in sheep, Schauer et al. (2008) reported that diets as high as 60% DDGS can be fed to feedlot lambs with no detrimental growth or health effects. Furthermore, Rios-Rincón et al. (2014) reported that dietary energy has a greater role on G:F than protein, suggesting that lambs on lower protein diets that are adequate in energy, gain as well as lambs on greater protein diets that are normally fed in the industry. Therefore, the hypothesis was that DDGS can completely replace CSM and grain sorghum in lamb feedlot diets without negatively affecting growth performance, health, or ruminal function.

Materials And Methods

Animals and Management

Forty-six Dorper ram lambs (approximate age = 4 mo; initial BW = 25 kg ± 6 kg), previously fed 80% alfalfa hay and a 20% commercial ration (DM basis), were brought into the Texas A&M AgriLife Research feedlot in San Angelo. Lambs received an ear tag and an oral dose of albendazole (anthelmintic: Valbazen, Zoetis, Parsippany, NJ). During the first 4 d of the adaptation period, lambs were group-fed and provided ad libitum access to long-stemmed hay, which was supplemented with a 60% concentrate diet (0.22 kg•d^{-1}•lamb^{-1}; DM basis). Data from two lambs (0DDGS) were removed from the statistical analysis because one sustained an injury to the stifle and one died from an unknown infection.

Seven days before study initiation, each lamb was weighed, stratified by BW, and randomly assigned to an individual, completely covered dirt pen (2.44 × 2.97 m) with an automatic watering system and feed bunk. Each lamb was randomly assigned to one of five treatment diets (n = 9 lambs/treatment; Table 1). The positive control diet (CNTL), representative of a traditional feedlot diet, contained CSM, sorghum grain, and other concentrates but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain. Lambs fed CNTL were compared to 0DDGS and linear and quadratic effects were evaluated within the four DDGS diets. During the last 2 d of the adaptation period, lambs did not receive hay, but were fed a common 66% concentrate diet that was gradually replaced by the assigned treatment diet.

Treatment diets were formulated (NRC, 2007) to evaluate linear and quadratic trends. However, the 0DDGS diet was allowed to remain deficient in CP due to the experimental design. Since DDGS are high in P, Ca carbonate was added to maintain a Ca:P ratio between 1.5 and 2:1 as recommended by ARC (1980). Ammonium chloride was added to reduce the incidence of urinary calculi (Crookshank, 1970) and a mineral premix specifically blended for diets containing DDGS was used. Cost/metric ton of feed (DM basis) was calculated by using ingredient prices based on local markets. Average daily feed cost per kilogram of BW gain for each treatment group was calculated on a DM basis (DMI needed to gain 1 kg of BW, kg × $/kg of feed).

Sample Collection and Measurements

Lambs were fed their respective treatment diets throughout the entire

Table 1. Ingredient composition of feeds (% DM basis) of treatment diets.

	Diet[1]				
Item[2]	CNTL	0DDGS	33DDGS	66DDGS	100DDGS
Cottonseed hulls	29.1	29.1	29.1	29.1	29.1
Cottonseed meal	12.0	-	-	-	-
DDGS	-	-	22.0	43.0	64.0
Sorghum grain, rolled	53.4	65.7	43.1	21.5	-
Molasses, cane	3.0	3.0	3.0	3.0	3.0
Limestone	1.1	0.8	1.4	2.0	2.5
Ammonium Cl	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Mineral premix	0.4	0.4	0.4	0.4	0.4
Cost/t of feed	$270.99	$288.27	$278.84	$287.49	$296.18

[1] In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no corn dried distillers grains with solubles (DDGS). Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain.

[2] Mineral premix = NaCl, KCl, S, MnO, ZnO, vitamins A, D, and E, CaCO3, cottonseed meal, cane molasses, and animal fat. Cost/t of feed (DM basis; metric ton) was calculated by using ingredient prices based on local markets.

56-d trial. All mixed diets were non-pelleted and fed once daily at 0800 h with an approximate allowance of 10% refusal. Lamb BW was recorded on d 0, 14, 30, 42, and 56. Average daily gain and average daily DMI (aDMI) were determined between days that BW was recorded and G:F calculated between weigh-day by dividing ADG by average daily DMI. Ruminal fluid was collected on d 0 and 56 and blood serum collected on d 0, 14, and 56.

Subsamples of CSH, sorghum grain, DDGS, and the mixed treatment diets were individually collected three times during the trial, kept separate, and subsamples combined before being analyzed. Samples were dried at 55°C in a forced-air oven (model 630, NAPCO, Portland, OR) for 48 h, ground through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA), and stored at –20°C. Nitrogen was analyzed by a standard method (Method 990.03; AOAC Int., 2006) and CP calculated as 6.25 × N. The NDF and ADF was analyzed according to procedures of Van Soest et al. (1991), which were modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) using -amylase and Na sulfite. In addition, N was analyzed in residue remaining after the ADF procedure and multiplied by 6.25 to determine acid detergent insoluble CP (ADICP). Standard methods were used to analyze lignin (AOAC 973.18; AOAC, 2006), crude fat (Method 2003.05; AOAC Int., 2006), ash (Method 942.05; AOAC Int., 2006), and minerals; the latter by a Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer (Thermo Instrument Systems, Inc., Waltham, MA).

An Ankom model DaisyII incubator was used to determine 48-h true IVDMD (tIVDMD) by incubating each treatment diet in separate F57 bags (3 replicates; Ankom Technol. Corp., Macedon, NY) for 48 h. Each bag contained 0.35 g of material that was hammermilled to pass a 2-mm screen (Wiley mill). Bags were placed into jars containing 400 mL of sheep rumen fluid (collected orally) and 1,600 mL of McDougal's buffer solution (1.0 g of urea/L; McDougal, 1948). One blank bag per jar was included and used to adjust for potential residue on the bags. After anaerobic incubation at 39°C, bags were gently rinsed under cold water for 5 min, subjected to the NDF procedure (using -amylase and omitting Na sulfite), gently rinsed in acetone, dried at 55°C in a forced-air oven for 48 h, and weighed.

Blood Serum and Rumen fluid Collection and Analysis.

A 10-mL blood sample was collected 4 h after feeding from each lamb via jugular venipuncture using a non-heparinized vacutainer collection tube (serum separator tube, gel, and clot activator; Becton Dickenson, Franklin Lakes, NJ). Blood was allowed to clot and then centrifuged (Beckman Coulter TJ6 refrigerated centrifuge, Fullerton, CA) at 970 × g for 25 min at 4°C. Serum was decanted and frozen at –20°C until analyzed. Serum chemistry was analyzed by The Texas A&M Veterinary Diagnostic Laboratory, Amarillo, using an Olympus AU400E analyzer (Olympus America Inc., Center Valley, PA).

Rumen fluid was collected orally from each lamb 4 h after feeding, using a stomach tube. The pH of each subsample was immediately recorded and the remaining fluid was filtered through 4 layers of cheesecloth; a subsample was immediately placed on ice and stored at −80°C. Additional subsamples (1-mL) were acidified with 4 mL of 0.1 N HCl (Farmer et al., 2004) and stored at −80°C for ammonia-N analysis using a Beckman Coulter DU640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA; methods of Broderick and Kang, 1980), and VFA using an Agilent 6890N gas chromatograph (Agilent Technology, Inc., Wilmington, DE; Baumgardt, 1964; Fritz and Schenk, 1979).

Statistical Analysis

Data for growth performance, blood serum, and ruminal fluid characteristics were analyzed using the PROC MIXED procedure for normal data sets, or PROC GLIMMIX for non-normal data sets (ammonia-N, albumin, serum urea N (SUN), Ca, creatine, creatine kinase (CK), AST, GGT, GLDH, Mg, Na, K, Na:K ratio, and Cl), procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC) using a model that included treatment with lamb as the random error. Data was reported as least squares means with greatest standard errors. Treatment effects were tested using the following orthogonal contrasts: (1) CNTL vs. 0DDGS and (2) linear and (3) quadratic effects of 0DDGS, 33DDGS, 66DDGS, and 100DDGS diets. PROC IML was used to generate orthogonal coefficients for the linear and quadratic contrasts; only the highest order contrast with a P-value < 0.10 was discussed.

Results And Discussion

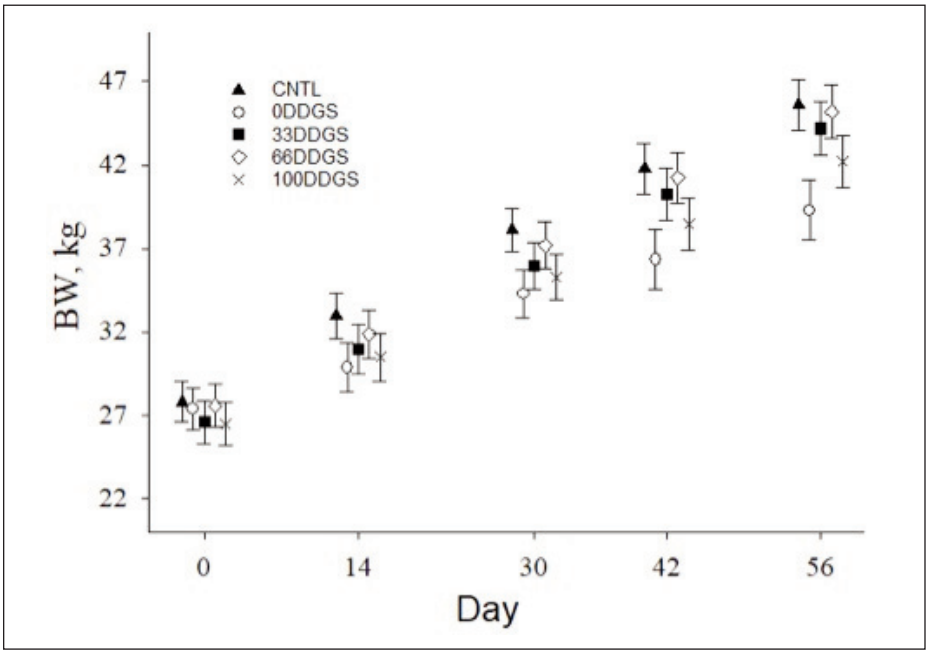
Animal Performance

A treatment × day interaction ($P < 0.001$) was observed for lamb BW (Figure 1). By design, initial lamb BW was similar ($P > 0.78$) on d 0. Lambs fed CNTL tended to have greater ($P = 0.06$) BW on d 30 and had greater ($P \leq 0.02$) BW on d 42 and 56 than lambs fed 0DDGS. Over the entire trial, ADG and G:F was greater ($P \leq 0.008$) for lambs fed CNTL vs. lambs fed 0DDGS, but DMI was similar ($P = 0.22$). Per experimental design, 0DDGS was deficient in CP (NRC, 2007), which likely resulted in decreased growth.

On d 42 and 56, lamb BW quadratically increased ($P \leq 0.04$) as DDGS increased in the diet. Positive quadratic trends were observed for ADG, DMI, and G:F ($P < 0.001$; = 0.08; = 0.06, respectively). McEachern et al. (2009) did not report any differences in final BW, ADG, or G:F when CSM was completely replaced by DDGS in lamb feedlot diets; however, urea was added to make the rations isonitrogenous, thus CP concentrations were similar. Schauer et al. (2008) reported a linear increase in lamb DMI as the concentration of DDGS increased, thus CP increased in the diet but G:F and ADG remained similar.

The observed differences in DMI are likely linked to differences in dietary CP and ADF (Table 2). Glimp et al. (1989) concluded that excessive starch in a diet can lead to decreased DMI, which could explain the reduced DMI for lambs fed 0DDGS. Fenderson and Bergen (1976) fed diets that exceeded the CP requirement of steers and reported that DMI was initially reduced but recovered after 10 d. Schauer et al. (2008) fed up to 60% DDGS and reported no decrease in DMI. In the current trial, visual inspection of the residual feed suggested that lambs tended to sort against DDGS. Thus, hammer-

Figure 1. Effects of feeding increasing levels of dried distillers grains with solubles (DDGS) on lamb BW. In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain. A treatment × day interaction was observed ($P < 0.001$) for lamb BW. Lambs fed CNTL tended to have greater ($P = 0.06$) BW on d 30 and had greater ($P \leq 0.02$) BW on d 42 and 56 than lambs fed 0DDGS. On d 42 and 56, lamb BW quadratically increased ($P \leq 0.04$) as DDGS increased in the diet. Vertical bars represent standard errors.



milling the diets may reduce sorting but rumen acidosis would need to be monitored (Welch, 1982).

The incidence of PEM is a general concern and NRC (2007) recommends that dietary S concentrations in a concentrate-based diet be kept less than 0.3% of feed (DM basis). Schauer et al. (2008) used up to 60% DDGS in lamb feedlot diets that contained dietary S concentrations of 0.55% without observing PEM; however, thiamin was included, which has been reported to reduce PEM occurrence (Edwin et al., 1979). Olkowski et al. (1992) induced PEM in two-month old feedlot lambs by feeding a diet with excess S (0.63%) and no supplemental thiamin and reported that seven of the 22 lambs on the high-S, low-thiamin diet, developed neurological signs associated with PEM.

The ethanol industry uses sulfuric acid during the cleaning process, which can result in high concentrations of S in DDGS. In the current trial, dietary S levels (from 0.36 to 0.72 %, DM basis) exceeded that of the rations fed by Schauer et al. (2008) and Olkowski et al. (1992) and the 100DDGS contained twice the amount of S recommended by NRC (2007). However, no incidence of PEM occurred, which may be due to the lambs not being at risk for acidosis. Low ruminal pH would facilitate the activity of thiaminase-producing bacteria such as *B. thiaminolyticus* (Boyd and Walton, 1977). Furthermore, S in DDGS is mainly attributed to sulfuric acid (Gray et al., 2006) that originates from the cleaning process, while Olkowski et al (1992) added S in the form of magnesium sulfate.

In a study by Huls et al. (2006), DDGS were included at 22.9% of the diet (DM basis), replacing soybean meal and a portion of corn. In the current trial, S in the 0DDGS diet was relatively low in comparison to 33DDGS, 66DDGS, and 100DDGS diets (0.36%, 0.54%, and 0.72% S, respectively). However, Huls et al. (2006) fed soy hulls, which are highly fermentable and contain less effective fiber in comparison to CSH (NRC, 2000). Cottonseed hulls are a sufficient source of dietary fiber and effective at reducing acidosis and bloat.

Corn DDGS are high in sulfuric acid and thus, acidic, as indicated by the presence of S (1.0% DM) in the DDGS used in this trial. This is supported by Whitney et al. (2014) as the DDGS when mixed in a water solution had a pH of 3.77. Acidic feedstuffs can decrease rumen pH, digestibility, and DMI (Mould et al., 1983). However, DDGS are low in starch and high in fiber, thus the risk of acidosis is reduced when feeding DDGS in mixed rations (Schingoethe, 2006). Acidic sources of dietary S can have an impact on lamb growth performance (Felix et al., 2014). Felix et al. (2014) determined that S from DDGS, primarily in the form of H2SO4, is more readily reduced to H2S in the rumen than other sources of dietary S such as Na_2SO_4. This caused reduced growth performance in lambs fed high S diets in comparison to another treatment group fed a corn-based diet supplemented with 1.4% Na_2SO_4 (Felix et al., 2014).

Rumen Fluid Profiles

Treatment × day interactions ($P \leq 0.003$) were observed for ammonia-N and acetate production (Table 4). Treatment × day interactions ($P < 0.10$) tended to be present for acetate:propriionate and ruminal pH. Lambs fed CNTL had significantly greater ruminal N ($P < 0.001$) than lambs fed 0DDGS. Lambs fed 0DDGS had a more neutral ruminal pH than lambs fed CNTL ($P < 0.001$). A positive linear increase ($P < 0.001$) was observed among treatments on d 56 for ruminal acetate concentration. On d 56, a positive quadratic change ($P < 0.001$) and a negative quadratic change ($P < 0.001$) were observed for ammonia-N and ruminal pH, respectively. No treatment × day interactions ($P > 0.21$) were observed for propionate or butyrate concentrations.

Even though DDGS are an acidic feedstuff due to the presence of sulfuric acid (Whitney et al., 2014), it is low in readily digestible starches and high in fiber (NRC, 2007; Whitney et al., 2014). This explains why ruminal pH increased and tIVDMD decreased as the proportion of DDGS increased in the diet. Fimbres et al. (2002) reported that

Table 2. Chemical composition (% DM basis) of ingredients and treatment diets.

	Diet[1]							
Item[2]	CNTL	0DDGS	33DDGS	66DDGS	100DDGS	Sorghum	DDGS	CSH
Nutrient composition								
DM, %	91.6	92.6	93.0	93.0	93.4	91.8	91.9	89.7
CP, %	13.7	10.3	15.0	19.8	24.6	11.0	33.0	6.8
ADICP, %	1.9	1.7	2.9	3.3	4.3	1.4	5.5	3.7
aNDF, %	31.8	28.4	37.0	35.3	41.2	7.6	26.3	82.1
ADF, %	23.0	21.7	25.4	26.3	25.2	5.7	16.2	66.3
Lignin, %	6.8	5.8	6.6	6.3	6.7	1.2	3.0	21.1
Crude fat, %	3.9	4.6	5.4	7.0	8.3	3.7	12.8	2.7
Ash, %	4.8	4.0	5.1	5.4	7.1	1.8	7.2	2.8
Ca, %	0.50	0.46	0.67	0.84	0.96	0.09	0.05	0.17
P, %	0.33	0.26	0.40	0.56	0.71	0.29	1.1	0.15
S, %	0.21	0.19	0.36	0.54	0.72	0.14	1.00	0.13
K, %	0.91	0.83	0.99	1.13	1.24	0.39	1.25	1.19
Mg, %	0.21	0.17	0.21	0.26	0.29	0.12	1.03	0.20
Na, %	0.30	0.32	0.34	0.38	0.35	0.10	0.13	0.02
Fe, ppm	156	86	96	124	137	47	78	45
Zn, ppm	35	42	43	50	59	23	66	13
Cu, ppm	5	4	5	5	6	3	5	5
True IVDMD	72.65	73.41	71.51	69.77	66.93	98.38	79.18	31.85
Cost of feed/kg of BW gain	$1.44	$1.71	$1.40	$1.55	$1.55			

[1] In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain.

[2] ADICP = acid detergent insoluble CP; True IVDMD = true 48-h IVDMD. Cost of feed/kg of BW gain= average daily feed cost per kilogram of BW gain for each treatment group was calculated on a DM basis as: (DMI needed to gain 1 kg of BW, kg × $/kg of feed)

Table 3. Effects of replacing cottonseed meal (CSM) and sorghum with dried distillers grains (DDGS) on lamb growth performance.

	Diet[1]						*P*-value[2]				
Item	CNTL	0DDGS	33DDGS	66DDGS	100DDGS	SEM	D	T × D	1	L	Q
ADG, kg											
d 0 to 56	0.32	0.23	0.31	0.31	0.28	0.02	0.29	0.91	<0.001	0.03	<0.001
DMI, kg/d											
d 0 to 56	1.60	1.45	1.56	1.67	1.47	0.96	<0.001	0.78	0.22	0.65	0.08
G:F, kg/kg											
d 0 to 56	0.20	0.16	0.20	0.19	0.19	0.01	<0.001	0.93	0.008	0.08	0.06

[1] In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain.

[2] Contrast 1 = CNTL vs. 0DDGS, Linear and quadratic orthogonal polynomial contrasts.

the starch content of a ration has a major influence on the rate of fermentation and pH of the rumen 4 h after feeding. Anderson et al. (2006) reported no differences in acetate production when feeding DDGS at 10% or 20% of the total diet (DM basis) fed to dairy cattle. Furthermore, no differences in butyrate, propionate, or acetate:propionate were observed according to Anderson et al. (2006). Results of Anderson et al. (2006) partially support the results of the current trial but the maximum DDGS inclusion rate was much greater in the current trial (20% compared to 65%, DM basis). The proportions of ADF and NDF in the diets fed by Anderson et al. (2006) were similar between the CNTL,

Table 4. Effects of replacing cottonseed meal (CSM) and sorghum with dried distillers grains (DDGS) on lamb rumen fluid profile.

	Diet[1]						*P*-value[2]			
Item	CNTL	0DDGS	33DDGS	66DDGS	100DDGS	SEM	D	T × D	1	L	Q
pH							<0.001	0.08			
d 0	5.94	5.81	5.95	5.85	6.25	0.17			0.52	0.10	0.42
d 56	6.34	6.87	6.31	6.45	6.57	0.09			<0.001	0.11	<0.001
Ammonia-N, mg/dL							<0.001	<0.001			
d 0	6.71	4.63	5.37	6.51	6.17	1.25			0.17	0.25	0.60
d 56	2.95	1.33	3.76	6.15	7.02	0.74			<0.001	<0.001	<0.001
Acetate, mol/100 mol							<0.001	0.003			
d 0	62.9	66.9	62.6	64.6	62.7	1.5			0.04	0.10	0.40
d 56	50.3	50.7	48.8	56.8	58.6	2.0			0.88	<0.001	0.27
Propionate, mol/100 mol							<0.001	0.21			
d 0	23.7	21.1	26.0	22.0	25.7	1.6			0.21	0.15	0.74
d 56	34.9	31.3	40.1	29.5	31.5	2.6			0.29	0.35	0.15
A:P							<0.001	0.09			
d 0	2.7	3.2	2.6	3.0	2.5	0.2			0.09	0.09	0.67
d 56	1.6	1.7	1.3	2.0	2.0	0.2			0.48	0.08	0.21
Butyrate, mol/100 mol							0.09	0.44			
d 0	11.8	11.3	10.1	11.8	10.5	1.1			0.99	0.73	0.99
d 56	10.3	10.5	9.4	12.7	8.9	1.0			0.75	0.61	0.15

[1] In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain.

[2] Contrast 1 = CNTL vs. 0DDGS, Linear and quadratic orthogonal polynomial contrasts.

10% DDGS, and 20% DDGS rations. This could also explain why no differences were observed, whereas in the current trial, there were differences in aNDF, ADICP, and crude fat. As reported in Table 2, this resulted in a linear decrease in the overall tIVDMD of the diet as the amount of DDGS increased.

Ruminal ammonia-N increased quadratically ($P < 0.001$) on d 56 as DDGS increased in the diet, which was expected due to greater dietary CP. In addition, ruminal ammonia-N was less on d 56 for lambs fed 0DDGS vs. lambs fed CNTL. This was expected because 0DDGS was deficient in CP according to NRC (2007). Even though Rios-Rincón et al. (2014) reported that dietary energy has a greater role on G:F than protein, their low protein diets contained greater than 14% CP. Thus, the decreased growth performance of the lambs fed 0DDGS can be attributed to reduced ruminal ammonia-N.. However, 66DDGS and 100DDGS diets contained excess dietary CP (approximately 7% and 13, respectively; NRC, 2007). This would likely result in dietary energy inefficiently being used to excrete excess circulating N. In contrast, as observed in the current trial with lambs fed 0DDGS, feeding diets low in N can reduce lamb performance because microbial growth and function are reduced due to ruminal N being limited (NRC, 2007; Kaya et al., 2009).

Blood Serum Profiles

Lamb blood serum chemistry profiles are presented in Table 5. Treatment × day interactions ($P < 0.05$) were observed for glucose, SUN, creatinine, albumin, GGT, Mg, and Cl. Treatment × day interactions ($P < 0.10$) tended to be observed for TSP, CK, and P. Blood SUN was greater ($P < 0.001$) for lambs fed CNTL on d 14 and 56 compared to lambs fed 0DDGS. Albumin was also greater ($P < 0.001$) on d 56 for lambs fed CNTL compared to lambs fed 0DDGS. Positive quadratic trends ($P < 0.05$) were observed for glucose, SUN, albumin, AST, P, Mg, and Cl. Positive linear changes ($P < 0.05$) were observed for TSP and GLDH. The primary purpose for analyzing blood serum chemistry was to display metabolic issues that may have occurred, which may be linked to the inclusion of high concentrations of DDGS in the diet. Although there were differences among certain enzymes (GGT and CK), minerals (Mg, Cl, and Ca), and other constituents (TSP, creatine, and glucose), values were within the normal biological range for growing lambs (Cornelius, 1989; Stämpfli and Oliver-Espinosa, 2015). This suggested that including 64% of DDGS in a mixed ration did not negatively affect lamb health. Serum GGT and alanine aminotransferase function as indicators of hepatic function disorders (Cornelius, 1989; Stämpfli and Oliver-Espinosa, 2015). Serum CK is an enzyme that

Table 5. Effects of replacing cottonseed meal (CSM) and sorghum with dried distillers grains (DDGS) on lamb blood serum profiles.

	Diet[1]						*P*-value[2]					
Item[3]	CNTL	0DDGS	33DDGS	66DDGS	100DDGS	SEM	T	D	T × D	1	L	Q
Glucose							0.007	<0.001	0.04			
d 0	79.1	77.1	82.4	77.3	76.9	2.77				0.58	0.63	0.28
d 14	87.8	79.4	87.9	88.8	77.8	3.38				0.08	0.79	0.006
d 56	85.7	78.8	78.2	79.3	66.2	3.24				0.12	0.01	0.05
SUN							<0.001	<0.001	<0.001			
d 0	9.07	8.19	10.35	8.68	8.18	0.94				0.42	0.72	0.13
d 14	8.43	4.53	9.76	14.60	19.64	2.16				<0.001	<0.001	0.04
d 56	11.09	4.28	12.82	17.33	18.60	1.45				<0.001	<0.001	<0.001
Creatinine							0.20	0.005	0.009			
d 0	0.59	0.61	0.61	0.60	0.65	0.04				0.61	0.46	0.43
d 14	0.72	0.74	0.71	0.61	0.57	0.04				0.65	<0.001	0.81
d 56	0.74	0.78	1.26	0.65	0.61	0.31				0.90	0.20	0.25
Albumin							0.15	<0.001	0.02			
d 0	2.75	2.75	2.65	2.67	2.73	0.06				0.91	0.92	0.21
d 14	2.75	2.49	2.77	2.78	2.79	0.12				0.10	0.07	0.22
d 56	3.12	2.82	3.02	3.12	3.05	0.06				0.002	0.01	0.05
Globulin, d 14 and 56	3.26	3.20	3.07	3.37	3.31	0.11	0.32	0.85	0.49	0.69	0.20	0.75
A:G ratio, d 14 and 56	0.92	0.83	0.95	0.88	0.90	0.03	0.46	0.15	0.78	0.29	0.52	0.33
TSP							0.12	<0.001	0.09			
d 0	6.15	6.08	5.64	5.82	6.01	0.14				0.68	0.98	0.02
d 14	6.07	5.68	5.89	6.14	6.06	0.18				0.12	0.09	0.41
d 56	6.32	6.15	6.02	6.51	6.40	0.14				0.38	0.05	0.95
AST, d 14 and 56	87.89	68.79	85.92	97.65	81.06	6.92	0.02	<0.001	0.77	0.02	0.06	0.006
GGT, d 14 and 56	57.63	58.02	57.40	65.10	58.32	4.30	0.63	0.15	0.04	0.94	0.64	0.46
GLDH, d 14 and 56	21.84	18.10	23.24	32.65	30.37	6.17	0.18	0.34	0.95	0.47	0.03	0.40
CK, d 14 and 56	212.6	165.9	178.8	181.0	185.6	21.4	0.55	<0.001	0.08	0.10	0.47	0.82
Ca, d 14 and 56	9.94	10.90	9.52	9.72	9.61	0.66	0.52	0.38	0.48	0.27	0.20	0.31
P						0.009	0.008	0.06				
d 0	9.84	10.44	10.42	10.36	10.24	0.50				0.36	0.75	0.92
d 14	8.69	8.91	10.03	10.01	10.60	0.42				0.70	0.01	0.53
d 56	9.83	8.82	11.41	10.72	10.95	0.47				0.12	0.009	0.01
Mg, d 14 and 56	2.60	2.30	2.74	2.52	2.46	0.09	0.01	0.53	0.008	0.01	0.45	0.006
Cl						0.29	<0.001	0.006				
d 0	113.9	112.9	113.5	113.8	111.0	1.2				0.50	0.28	0.12
d 14	107.4	112.3	108.6	111.0	110.8	1.1				0.002	0.63	0.10
d 56	107.8	107.6	108.7	109.3	104.5	1.6				0.93	0.23	0.063
Na, d 14 and 56	143.8	145.2	145.0	144.5	142.8	1.4	0.70	0.30	0.24	0.47	0.21	0.54
K, d 14 and 56	5.32	6.68	5.77	5.60	6.24	0.64	0.43	0.85	0.67	0.09	0.58	0.17
Na:K ratio, d 14 and 56	0.27	0.25	0.25	0.26	0.26	0.01	0.08	<0.001	0.76	0.01	0.14	0.93

[1] In a 56-d randomized design study, lambs were provided ad libitum 70.9% concentrate diets in individual pens. The positive control diet (CNTL) contained CSM, sorghum grain, and other concentrates, but no DDGS. Four treatment diets were similar to CNTL but did not contain CSM: corn DDGS replaced 0% (0DDGS), 33% (33DDGS), 66% (66DDGS) or 100% (100DDGS) of the sorghum grain.

[2] Contrast 1 = CNTL vs. 0DDGS, Linear and quadratic orthogonal polynomial contrasts.

[3] SUN = serum urea N; A:G = albumin:globulin; TSP = total serum protein; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; CK = creatine kinase. GLDH = glutamine dehydrogenase.

functions as an indicator of smooth muscle breakdown (Beatty and Doxey, 1983).

Differences in SUN can mainly be attributed to greater degradable protein intake (NRC, 2007). Lambs fed 66DDGS and 100DDGS received an excess of CP, which is shown by excessively high SUN concentrations on d 14 and d 56. This can increase urinary N excretion into the environment. The process of rapidly metabolizing excess ammonia into urea requires energy (Lobley et al., 2000). This, along with the reduced DM digestibility of the 100DDGS diet, potentially caused the reduced growth performance.

Conclusions

Results suggested that DDGS can entirely replace CSM and a significant portion of sorghum grain in high-concentrate finishing diets without negatively impacting ADG, DMI, G:F, or the health of the lamb. Furthermore, results support the fact that growth performance is hindered when lambs are fed diets containing less than 10.5% CP (DM basis). In addition, even though complications related to excess P in DDGS diets do not exist, feeders should remain cautious and continue to include Ca and ammonium chloride when elevated amounts of DDGS are incorporated into finishing rations.

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Sheep & Goat Research Journal
Volume 35, 2020 - June

Effects of Added Dietary Protein and Fat on Subcutaneous Adipose Tissue and Longissimus Muscle Fatty Acid Profiles of Finishing Lambs when Fed Differing Levels of Dried Distillers Grains with Solubles

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Summary

The objective of this study was to determine the effects of added dietary protein, fat, and dried distillers grains with solubles (DDGS) on longissimus muscle (LM) and subcutaneous (SQ) adipose fatty acid (FA) profiles in feedlot lambs. Sixty crossbred lambs (29.2 ± 4.6 kg) were allotted into pairs (1 ewe and 1 wether) and fed one of five dietary treatments in individual-pair pens: 1) a corn based diet with 25% DDGS included to meet CP requirements (CON), 2) 50% DDGS (50DDGS), 3) CON with added corn protein in the form of gluten meal to equal the CP in the 50DDGS diet (CON+CP), 4) CON with added vegetable oil to equal the crude fat in the 50DDGS diet (CON+VO), and 5) CON with corn protein and vegetable oil added to equal the CP and crude fat in the 50DDGS diet (CON+CPVO). Thirty wether lambs were utilized to determine differences in the fatty acid profiles of SQ adipose and LM tissues due to dietary treatment. Lambs fed increasing levels of dietary fat had greater (P = 0.04) 18:2*trans*-10, *cis*-12 concentrations in SQ adipose compared with CON lambs. Lambs fed diets with elevated CP and fat had lesser (P = 0.05) concentrations of 18:3n-6 in SQ adipose compared with CON lambs. Lambs fed diets with elevated fat had greater (P = 0.03) concentrations of 18:2*trans*-10, *cis*-12 than CON lambs. Data suggested that diets with elevated fat supply greater concentrations of CLA for biohydrogenation in SQ adipose and LM tissue of feedlot lambs.

Key Words: Dried Distillers Grains with Solubles, Fatty Acid Profiles, Feedlot Lambs

Introduction

Dried distillers grains with solubles (DDGS) have become an alternative to corn in the diets of livestock. Huls et al. (2006) reported that feeding lambs a diet containing either 23% DDGS or 10% soybean meal did not affect performance or carcass characteristics, except for a reduction in backfat thickness. However, Ham et al. (1994) reported that ADG, DMI, and G:F was greater for finishing steers fed diets containing up to 40% DDGS than steers fed a control diet containing dry-rolled corn.

Dried distillers grains with solubles contain approximately 10% fat, whereas dry corn grain contains approximately 4% fat (NRC, 2000). The increase in corn oil, which is high in monounsaturated fatty acids (FA), in the DDGS may alter the FA profile of the longissimus muscle (LM) and subcutaneous (SQ) adipose tissues of lambs. However, due to biohydrogenation of unsaturated FAs in the rumen, the FA composition of the LM tissue and SQ adipose tissue is more difficult to predict (Wood and Enser, 1997). According to Vander Pol et al. (2009), greater proportions of 18:1 *trans*, 18:1, and 18:2 FA reached the duodenum in cattle fed wet distillers grains with solubles compared with cattle consuming a corn-based control diet (Vander Pol et al., 2009). By wet distillers grains with solubles altering the flow of FA to the duodenum, this could lead to changes in the fatty acid profile of the meat.

Little information is available on how elevated levels of DDGS affect performance and carcass quality in finishing lambs. Likewise, it is also unclear as to how an increased level of dietary unsaturated fat from DDGS might affect tissue FA profile. Therefore, the objective was to determine effects of dietary protein and fat on the FA profile of lambs when feeding DDGS.

Materials And Methods

Animals and diets

The Purdue University Animal Care and Use Committee approved all animal handling procedures for this study. Sixty crossbred lambs (29.2 ± 4.6 kg; 30 ewes and 30 wethers) were stratified by BW and allotted into one of five dietary treatments (Table 1): 1) a corn-based diet with DDGS included to meet crude protein (CP) requirements of finishing lambs (25% of DM; CON), 2) CON with DDGS included at twice the amount of CON (50% of DM; 50DDGS), 3) CON with added protein to equal the CP level in the 50DDGS diet (CON+CP), 4) CON with added vegetable oil to equal the crude fat level of the 50DDGS diet (CON+VO), and 5) CON with protein and vegetable oil added to equal that of the CP and fat levels of the 50DDGS diet (CON+CPVO). Thirty wether lambs

Table 1. Dietary ingredients, chemical composition, and FA content of diets fed to feedlot lambs.

	Dietary Treatments[1]				
Item, % of DM	**CON**	**50DDGS**	**CON+CP**	**CON+VO**	**CON+CPVO**
Ingredients					
Dry-rolled corn	59.4	34.2	48.4	48.5	37.7
Dried distillers grains with solubles	25.7	51.2	25.7	25.8	25.8
Ground hay	10.7	10.4	10.6	10.7	10.7
Corn gluten meal	—	—	11.1	—	10.4
Soybean hulls	—	—	—	8.3	8.4
Vegetable oil	—	—	—	2.5	2.8
Molasses	1.2	1.2	1.2	1.2	1.2
Supplement[2]	3.0	3.0	3.0	3.0	3.0
Analyzed composition					
DM, %	89.7	90.5	90.5	90.6	90.6
CP	14.6	18.5	20.3	15.5	19.8
NDF	21.7	24.2	20.5	25.1	23.3
Crude fat	3.5	6.0	4.6	6.3	7.0
ADF	11.7	14.2	12.9	15.4	16.1
Fatty acid, mg/g of DM feed[3]					
Palmitic acid	10.11	11.89	8.36	11.68	11.15
Stearic acid	1.57	1.91	1.29	2.51	2.40
Oleic acid	16.84	20.15	13.78	19.66	18.67
Linoleic acid	40.34	47.81	34.03	50.29	48.51
α-Linolenic acid	1.39	1.76	1.26	3.79	3.80
22:2	0.19	0.24	0.19	0.21	0.22
Other	1.64	1.96	1.43	2.57	2.39
Total	72.03	85.67	60.32	90.65	87.08

[1] CON: 25% DDGS; 50DDGS: 50% DDGS; CON+CP: control diet + corn protein; CON+VO: control diet + vegetable oil; CON+CPVO: control diet + both corn protein and vegetable oil.

[2] Supplement included 150 mg·head^{-1}·d^{-1} thiamin to help prevent sulfur toxicity.

[3] Fatty acid numerical definition: palmitic acid – 16:0, stearic acid – 18:0, oleic acid – 18:1*cis*-9, linoleic acid – 18:2*cis*-9, *cis*-12, and α-Linolenic – 18:3n-3.

Table 2. Effects of differing levels of CP and dietary fat from dried distillers grains with solubles on FA intake (g/d) of feedlot lambs.

	Dietary Treatment[1]						*P*-value[2]		
Fatty acid, g of FA/d[3]	CON	50DDGS	CON+CP	CON+VO	CON+CPVO	SEM	PRO	FAT	PF
Number of lambs	6	5	6	6	6				
Palmitic acid	11.42	12.04	8.70	13.45	11.44	0.39	0.10	0.04	0.43
Stearic acid	1.77	1.93	1.34	2.89	2.46	0.07	0.10	<0.001	<0.001
Oleic acid	19.03	20.40	14.33	22.64	19.16	0.65	0.14	0.02	0.46
Linoleic acid	45.60	48.43	35.39	57.92	49.78	1.62	0.54	0.001	0.57
α-Linolenic acid	1.57	1.79	1.31	4.36	3.90	0.10	<0.001	<0.001	<0.001
SFA	13.26	14.04	10.06	16.39	13.94	0.46	0.25	0.005	0.95
MUFA	19.03	20.40	14.33	22.64	19.16	0.65	0.14	0.02	0.46
PUFA	47.38	50.45	36.90	62.52	53.90	1.73	0.87	<0.001	0.24
Other	2.06	2.23	1.68	3.20	2.67	0.08	0.15	<0.001	<0.001
Total	81.41	86.77	62.73	104.39	89.36	2.91	0.57	0.001	0.51

[1] CON: 25% DDGS; 50DDGS: 50% DDGS; CON+CP: control diet + corn protein; CON+VO: control diet + vegetable oil; CON+CPVO: control diet + both corn protein and vegetable oil.

[2] PRO: CON vs. average of elevated CP diets (50DDGS, CON+CP, and CON+CPVO); FAT: CON vs. average of elevated fat diets (50DDGS, CON+VO, and CON+CPVO); PF: CON vs. both elevated CP and fat diets (50DDGS and CON+CPVO).

[3] Fatty acid numerical definition: palmitic acid – 16:0, stearic acid – 18:0, oleic acid – 18:1*cis*-9, linoleic acid – 18:2*cis*-9, *cis*-12, and α-Linolenic – 18:3n-3.

were utilized to determine differences in the fatty acid profiles of SQ adipose and longissimus muscle (LM) tissues due to dietary treatment. As reported in a companion paper (Van Emon et al., 2012), diets were formulated to determine if differences in performance and carcass quality were associated with feeding increased concentrations of DDGS, dietary CP, dietary fat, or a synergistic effect of both CP and dietary fat. All lambs were supplemented with 150 mg/d thiamin to help prevent sulfur toxicity.

Lambs were vaccinated (Clostridium Perfringens Type C & D with Tetanus Toxoid; Boehringer Ingelheim Pharmaceuticals, Inc.; Ridgefield, CT) prior to the study at 6 wk of age and a booster was given 21 d later. Lambs were stratified by weight and blocked into pairs of one ewe and one wether (6 pens/treatment) and housed in a 1.83-m x 1.83-m pens on a mesh wire floor inside of a curtain-sided building. Three lambs (1 wether and 2 ewes) were removed from the study due to non-treatment related illness and the remaining single lambs were retained in their original pens for the remainder or the trial. Feed was offered *ad libitum* once daily at 0800 and lambs had free access to water. Feed refusals were collected twice weekly and weighed to determine DMI of the pen.

Ewe and wether lamb feedlot performance and carcass characteristics are reported in the companion paper (Van Emon et al., 2012) and the purpose of the present study is to present wether lamb fatty acid characteristics. Wether lambs were selected for harvest when they reached an approximate 12th rib fat depth of 0.5 cm. Upon harvest of wether lambs, the ewe lamb pen mate was returned to the university flock. Lambs were harvested at a common 12th rib fat depth to determine differences in carcass characteristics due to increased dietary levels of CP, crude fat, or both.

Sampling and Laboratory Analysis

Dietary samples were dried in a forced air oven for 48 h at 60°C for DM determination. Samples were then ground to pass a 1-mm screen and composited on an equal dry weight basis. Diets were analyzed for N (Leco model FP2000, Leco Instruments Inc., St. Joseph, MI) using the combustion method (AOAC, 2000; method 990.03) with EDTA as the standard. Feed NDF and ADF was analyzed by an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY), and crude fat analyzed by a standard method (AOAC, 2000; method 934.01).

Longissimus muscle and SQ adipose tissue samples were collected after a 24-h chill from the 12th to 13th rib interface and stored at -20°C for FA analysis. Subcutaneous adipose tissue was removed from the LM tissue prior to freeze-drying and stored at -20°C for FA analysis. Longissimus muscle samples were freeze-dried and ground using a coffee bean grinder.

Fatty acid analysis of the diets was accomplished using an acid catalyst (HCl) direct-*trans* esterification method of Kucuk et al. (2001). Subcutaneous adipose and LM tissue samples were analyzed using an alkaline catalyst (KOH). Preparation of the SQ adipose and LM tissues was according to procedures outlined by Murrieta et al. (2003). LM tissue consists of freeze dried LM with subcutaneous fat removed prior to freeze-drying. Each sample contained 1 mg of Methyl tridecanoic acid (T-0627, Sigma-Aldrich, St. Louis MO).

Fatty acid concentrations were determined by gas chromatography (Model 3800, Varian Inc., Palo Alto, CA; Appendix F) using a 100-m capillary column (Supelco 2560, Supelco, Bellefonte, PA). Hydrogen was the carrier gas and was maintained at a column flow of 1.5 mL/min. The oven temperature was maintained at 120°C for 2 min, ramped up to 175°C at a 6°C/min interval, and finally to 250°C at a 10°C/min interval. Injector temperature was held

at 260°C while detector temperature was held at 300°C. The split-ratio for the LM tissue was 30:1 and 100:1 for SQ adipose tissue. Purified FA standards (Sigma-Aldrich, St. Louis, MO; Nu-Check Prep, Elysian, MN, Matreya, Pleasant Gap, PA) were used to identify individual peaks. Fatty acids will presented as mg FA/g of diet DM (Table 1), g of FA/d (Table 2), mg of FA/g of SQ adipose tissue (Table 3) or mg of FA/g of LM tissue (Table 4; includes intermuscular fat extracted from lean muscle).

Statistical Analysis

Response variables included fatty acid intake and fatty acid composition of adipose tissue and longissimus muscle and were individually analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement included the fixed effect of treatment and individual lamb served as the experimental unit for FA analysis. Single-degree of freedom orthogonal contrasts were then used to test treatment effects: 1) CON diet vs. average of the diets containing elevated CP levels (50DDGS, CON+CP, and CON+CPVO; PRO), 2) CON diet vs. average of the diets containing elevated fat levels (50DDGS, CON+VO, and CON+CPVO; FAT), and 3) CON diet vs. average of the diets containing both elevated CP and fat levels (50DDGS and CON+CPVO; PF).

Results

Fatty Acid Intake

Daily FA intake is located in Table 2. Dry matter intake was reported previously in Van Emon et al. (2012). Lambs consuming the FAT diets had greater ($P \leq 0.04$) intake of all FA, which led to an increase in total FA ($P = 0.001$) compared with the lambs fed CON. Lambs consuming FAT, PRO, and PF had greater ($P < 0.001$) intakes of -linolenic acid than CON lambs. Additionally, FAT and PF had greater ($P < 0.001$) intakes of stearic acid than CON.

Subcutaneous Adipose

Total FA concentrations of SQ adipose tissue were similar ($P \geq 0.52$) between all dietary treatments (Table 3). Lambs fed FAT had greater ($P = 0.04$) concentrations of 18:2*trans*-10, *cis*-12 in SQ adipose vs. lambs fed CON. Lambs fed CON had greater ($P \leq 0.05$) concentrations of 18:3n-6 in SQ adipose than all other dietary treatments. Lambs consuming FAT tended to have increased levels of vaccenic acid ($P = 0.06$), linoelaidic acid ($P = 0.08$), and the CLA iso-

Table 3. Effects of differing levels of CP and dietary fat from dried distillers grains with solubles on subcutaneous adipose tissue FA profile in feedlot lambs.

	Dietary Treatment[1]						*P*-value[2]		
Fatty Acid, mg/g of adipose tissue[3]	CON	50DDGS	CON+CP	CON+VO	CON+CPVO	SEM	PRO	FAT	PF
Number of lambs	6	5	6	6	6				
Myristic acid	15.27	14.35	14.94	15.66	15.80	1.73	0.90	1.00	0.99
Myristoleic acid	0.38	0.39	0.73	0.53	0.43	0.19	0.50	0.72	0.26
Palmitic acid	126.99	123.55	143.47	123.48	124.34	8.11	0.69	0.71	0.48
Palmitoleic acid	10.89	10.43	11.13	11.36	10.27	0.76	0.74	0.81	0.68
Stearic acid	104.59	93.08	116.97	80.43	104.67	12.32	0.98	0.37	0.67
Vaccenic acid	74.81	95.22	54.97	110.85	103.69	13.69	0.51	0.06	0.60
Oleic acid	200.72	191.14	228.28	188.67	181.34	12.03	0.97	0.30	0.57
cis-Vaccenic acid	7.41	8.10	8.31	8.27	7.59	0.72	0.45	0.47	0.29
Linoelaidic acid	0.46	0.43	0.54	1.01	1.14	0.21	0.28	0.08	0.19
Linoleic acid	42.47	51.96	46.83	35.26	34.61	4.59	0.69	0.71	0.78
CLA – 18:2*trans*-10, *cis*-12	0.53	0.96	0.55	0.61	0.68	0.09	0.06	0.04	0.64
CLA – 18:2*cis*-9, *trans*-11	3.19	3.68	3.32	3.43	4.39	0.31	0.07	0.06	0.59
γ-Linolenic acid	0.14	0.07	0.08	0.04	0.01	0.03	0.02	0.01	0.05
α-Linolenic acid	2.37	2.51	2.07	2.76	2.62	0.22	0.90	0.28	0.86
SFA	267.41	250.01	294.59	237.88	263.33	18.01	0.92	0.38	0.95
MUFA	299.63	310.87	309.03	325.24	308.12	18.54	0.79	0.49	0.41
PUFA	49.68	60.06	54.60	43.62	43.91	4.80	0.79	0.49	0.92
Other	49.84	51.30	52.23	51.32	61.45	7.62	0.53	0.56	0.82
Total	639.58	647.19	684.42	633.68	653.04	9.00	0.52	0.88	0.59

[1] CON: 25% DDGS; 50DDGS: 50% DDGS; CON+CP: control diet + corn protein; CON+VO: control diet + vegetable oil; CON+CPVO: control diet + both corn protein and vegetable oil.

[2] PRO: CON vs. average of elevated CP diets (50DDGS, CON+CP, and CON+CPVO); FAT: CON vs. average of elevated fat diets (50DDGS, CON+VO, and CON+CPVO); PF: CON vs. both elevated CP and fat diets (50DDGS and CON+CPVO).

[3] Fatty acid numerical definition: myristic acid – 14:0, myristoleic acid – 14:1, palmitic acid – 16:0, palmitoleic acid – 16:1, stearic acid – 18:0, vaccenic acid – 18:1*trans*-11, oleic acid – 18:1*cis*-9, *cis*-vaccenic acid – 18:1*cis*-11, linoelaidic acid – 18:2*trans*-9, *trans*-12, linoleic acid – 18:2*cis*-9, *cis*-12, γ-linolenic acid – 18:3n-6, and α-Linolenic – 18:3n-3.

Table 4. Effects of differing levels of CP and dietary fat from dried distillers grains with solubles on longissimus muscle tissue FA profile of feedlot lambs.

	Dietary Treatment[1]						*P*-value[2]		
Fatty Acid, mg of fatty acid/g of LM tissue[3]	CON	50DDGS	CON+CP	CON+VO	CON+CPVO	SEM	PRO	FAT	PF
Number of lambs	6	5	6	6	6				
Lauric acid	0.12	0.07	0.06	0.13	0.08	0.02	0.03	0.22	0.30
Myristic acid	2.28	1.47	1.46	2.68	1.83	0.37	0.09	0.48	0.62
Myristoleic acid	0.02	0.02	0.00	0.07	0.02	0.02	0.76	0.44	0.47
Palmitic acid	23.52	16.46	19.20	26.19	19.68	3.50	0.19	0.47	0.83
Palmitoleic acid	1.88	1.11	1.25	2.15	1.36	0.30	0.06	0.30	0.60
Stearic acid	14.42	10.20	13.14	15.30	13.00	2.04	0.30	0.47	0.93
Vaccenic acid	6.98	6.85	3.68	12.49	9.25	2.19	0.87	0.28	0.66
Oleic acid	41.17	24.98	31.91	42.36	30.82	6.35	0.09	0.22	0.58
cis-vaccenic acid	1.84	1.30	1.34	2.05	1.43	0.23	0.06	0.32	0.58
Linoleic acid	10.80	10.64	9.93	10.81	9.31	0.73	0.29	0.49	0.60
CLA – 18:2*trans*-10, *cis*-12	0.00	0.03	0.00	0.04	0.03	0.01	0.17	0.03	0.21
CLA – 18:2*cis*-9, *trans*-11	0.46	0.35	0.35	0.58	0.54	0.08	0.59	0.75	0.97
α-Linolenic acid	0.44	0.34	0.29	0.57	0.43	0.06	0.16	0.88	0.92
SFA	42.84	29.94	35.65	46.82	36.37	6.01	0.18	0.43	0.81
MUFA	55.01	37.07	40.96	62.08	45.20	8.83	0.15	0.47	0.73
PUFA	12.26	11.87	11.03	12.57	10.82	0.82	0.25	0.56	0.62
Other	7.91	5.49	5.30	7.04	5.25	0.84	0.01	0.04	0.08
Total	111.85	79.30	87.92	122.46	93.03	15.60	0.14	0.42	0.71

[1] CON: 25% DDGS; 50DDGS: 50% DDGS; CON+CP: control + corn protein; CON+VO: control + vegetable oil; CON+CPVO: control + both corn protein and vegetable oil.

[2] PRO: CON vs. average of elevated CP diets (50DDGS, CON+CP, and CON+CPVO); FAT: CON vs. average of elevated fat diets (50DDGS, CON+VO, and CON+CPVO); PF: CON vs. both elevated CP and fat diets (50DDGS and CON+CPVO).

[3] LM tissue consists of freeze dried LM with subcutaneous fat removed prior to freeze-drying. Fatty acid numerical definition: lauric acid – 12:0, myristic acid – 14:0, myristoleic acid – 14:1, palmitic acid – 16:0, palmitoleic acid – 16:1, stearic acid – 18:0, vaccenic acid – 18:1*trans*-11, oleic acid – 18:1*cis*-9, *cis*-vaccenic acid – 18:1*cis*-11, linoleic acid – 18:2*cis*-9, *cis*-12, and α-Linolenic – 18:3n-3.

mer 18:2*cis*-9, *trans*-11 ($P = 0.06$) in SQ adipose compared with lambs consuming the CON diet. Lambs consuming PRO tended to have greater concentrations of the CLA isomers 18:2*cis*-9, *trans*-11 ($P = 0.07$) and 18:2*trans*-10, *cis*-12 ($P = 0.06$) concentrations vs. lambs fed CON. No differences were observed ($P \geq 0.19$) in the other FA in SQ adipose tissue due to dietary treatment.

Longissimus Muscle

Total FA concentrations in the LM tissue (intermuscular fat extracted from lean muscle) were similar ($P \geq 0.14$; Table 4) across dietary treatments. The lambs fed the elevated PRO had less concentrations of lauric acid ($P = 0.03$) and tended to have less concentrations of myristic acid ($P = 0.09$), palmitoleic acid ($P = 0.06$), oleic acid ($P = 0.09$), and *cis*-vaccenic acid ($P = 0.06$) in LM tissue than lambs fed CON. Lambs consuming the FAT diets had greater ($P = 0.03$) concentrations of the CLA isomer 18:2*trans*-10, *cis*-12 in LM tissue than lambs consuming CON. Concentrations of other FA in LM tissue were less for PRO ($P = 0.01$) and FAT ($P = 0.04$) and tended to be reduced for lambs consuming the PF ($P = 0.08$) diet vs. lambs fed CON. No differences were observed ($P \geq 0.15$) in the other FA in LM tissue due to dietary treatments.

Discussion

The major FA found in adipose tissue are myristic, palmitic, stearic, and oleic acids, which constitutes approximately 80% of the FAs in adipose tissue depots in ruminants (Byers and Schelling, 1988). Subcutaneous adipose FA concentrations from lambs in the current study agree with Byers and Schelling (1988). In the current study, there were relatively low concentrations of both 18:3n-3 and 18:3n-6 in the SQ adipose tissue. This may suggest that biohydrogenation was occurring in the rumen and branched chain FA are being hydrogenated to a more saturated form. Rapid biohydrogenation of FA is expected in lambs consuming diets containing vegetable oil as it is a more readily available source of FA than a protected FA source, such as DDGS, to rumen microbes (Jenkins and Bridges, 2007).

Increasing unsaturated FA composi-

tion of SQ adipose and LM tissues is difficult, primarily due to rumen microbial biohydrogenation of FA (Wood and Enser, 1997). Complete biohydrogenation of linoleic acid results in the saturated FA stearic acid (Jenkins et al., 2008). In the current study, greater concentrations of biohydrogenation intermediates, vaccenic and linoelaidic acids, in the SQ adipose tissue of lambs fed the high-fat diets (50DDGS, CON+VO, and CON+CPVO) were likely due to a reduction in complete ruminal biohydrogenation. The tendency of the elevated fat diets on the intermediates was driven by the diets containing the vegetable oil (CON+VO and CON+CPVO). Results observed from feeding beef cattle DDGS by Lancaster et al. (2007) also suggest that PUFA from the oil fraction of DDGS may be partially protected from ruminal biohydrogenation. Greater proportions of 18:1 *trans*, 18:1, and 18:2 FA reached the duodenum in cattle fed wet distillers grains with solubles compared with cattle consuming a corn-based control diet (Vander Pol et al., 2009). However, based on the current results, the deposition of linoleic acid biohydrogenation intermediates and stearic acid in the LM and SQ adipose tissue does not appear to be greatly altered by the inclusion of DDGS, CP, or fat.

Contrary to the current results, Price et al. (2007) observed that lambs fed safflower seeds, both whole and cracked, had greater duodenal flow of biohydrogenation intermediates, suggesting less complete biohydrogenation. However, Scollan et al. (2001) reported that when whole linseed was fed to steers, the seed coat provided little protection for PUFA from ruminal biohydrogenation. Duodenal flow of linoleic acid biohydrogenation intermediates were also increased in those lambs fed extracted safflower oil (Price et al., 2007). Bartoň et al. (2007) also observed greater concentrations of CLA in SQ fat in heifers that were fed a linseed-supplemented diet. The results reported here suggest that the extent of biohydrogenation was similar across all diets due to the similar concentrations of stearic acid in both the SQ adipose and LM tissues. However, Castillo-Lopez et al. (2013) suggested that intake of unsaturated FA was greater when cattle consumed DDGS compared with corn bran, which appeared to stimulate rumen biohydrogenation and increased the flow of saturated FA to the duodenum. According to Drackley (2000), the majority of the CLA produced in the rumen was most likely hydrogenated to vaccenic acid, and ultimately to stearic acid. Despite this, the current study did not observe any differences across dietary treatments in stearic acid concentrations, which would again suggest a similar extent of biohydrogenation across dietary treatments.

It has previously been concluded that increased concentrations of linoleic acid in the ration may reduce biohydrogenation in the rumen and therefore increase the flow of unsaturated FA to the small intestine (Beam et al., 2000). Although the lambs fed the added fat diets (50DDGS, CON+VO, and CON+CPVO) had increased dietary intake of linoleic acid, there was no effect on the extent of biohydrogenation in those lambs. Additionally, two different sources of fat were fed, DDGS or vegetable oil, and neither had an effect on concentrations of linoleic acid biohydrogenation intermediates.

Conclusion

Dried distillers grains with solubles has become an widely used feed for ruminants as both a protein and energy source. The increased fat and protein in DDGS does alter FA intake, but ultimately had little impact on the extent of biohydrogenation. Furthermore, diets containing both fat and/or protein had little effect on the FA concentrations of feedlot lambs. Additionally, feeding growing lambs a diet up to 50% DDGS has little effect on the FA composition of both SQ adipose and LM tissues. Fatty acid composition also had little impact on the growth and carcass characteristics measured (Van Emon et al., 2012). Therefore, more research needs to be conducted as to why feedlot cattle may be more affected by DDGS than feedlot lambs.

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Sheep & Goat Research Journal
Volume 35, 2020 - June

Impacts of Flax on Female Reproductive Traits When Supplemented Prior to Breeding in Sheep[1]

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[4] *Support for this research was provided by New Generation Supplements and AmeriFlax. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of New Generation Supplements or AmeriFlax. The authors would like to thank David Pearson, Donald Drolc, Don Stecher (Hettinger Research Extension Center, North Dakota State University), and Ethan Schlegel (New Generation Supplements) for their assistance throughout the project.*

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Summary

Fertility can be improved prior to breeding by improving nutritional management, especially in range sheep operations. Feeding fatty acids (FA), including omega-3 (ω-3) FAs, have been shown to have positive effects on pregnancy parameters. The objective of this study was to determine the effects of feeding supplemental flaxseed via a Flaxlic® Sheep Tub during the flushing period on reproductive performance of spring lambing multiparous ewes. Multiparous Rambouillet ewes (n = 240; aged 2 to 7 years) weighing an average of 70.8 ± 10.4 kg were randomly assigned to 24 pens (10 ewes/pen) and fed a flushing diet for 35 d. Ewes were assigned to receive a Flaxlic® Sheep Tub (FLX; n = 12) or not (CON; n = 12). Tubs were weighed on d 0, 3, 7, 10, 14, 17, 24, 29, and 35. Tubs weighing less than 5 kg were replaced with new tubs. Weight data for all ewes was taken on d 0, 7, 14, 21, 28, and 35, with two-day weights on d -1 and 0 and d 34 and 35. Serum samples were taken for serum progesterone concentration analysis on d 0, 7, 14, 21, 28, and 35 from 60 ewes per treatment (5 ewes/pen). On d 36, ewes and rams were comingled for breeding for 35 d. Birthweight, birth type, and sex of lambs were recorded at lambing for first cycle (149 to 166 days post ram turnout) and the overall lambing season. Initial and final weights were not different between treatment groups (P = 0.47 and 0.23, respectively). No treatment x day interactions (P = 0.82) or treatment effects (P = 0.83) were observed for serum progesterone concentration. A day effect was observed for serum progesterone concentration, which was higher on d 28 and higher still on d 35 ($P < 0.001$). No differences were observed between treatments for 1st cycle ($P \geq 0.26$) or overall ($P \geq 0.61$) pregnancy rate, prolificacy rate, or lambing rate. Our results indicate that ewes fed supplemental flax during the flushing period exhibited no economically beneficial responses in reproductive efficiency.

Key Words: Ewes, Fatty Acids, Fertility, Flax, Flushing, Sheep

Introduction

Fertility can be improved prior to breeding by improving nutritional management, especially in range sheep operations. This can be done by improving the diet through a flushing protocol. Improved management strategies prior to breeding can lead to better ewe body weight and condition score maintenance, as well as improved conception and pregnancy rates.

The addition of flaxseed to a flushing protocol has the potential to further enhance the effects of flushing (Thatcher et al., 2006; Santos et al., 2008; Silvestre et al., 2011). Flaxseed supplements two important FAs: Alpha-linolenic acid (ALA; C18:3 ω-3), an omega-3 (ω-3) FA, and linoleic acid (LA: C18:2 ω-6), an omega-6 (ω-6) FA. Of the total fats in flax oil, 57% is ALA and 16% percent is LA (Morris, 2007).

Much research has been conducted feeding supplemental fats high in polyunsaturated fatty acids (PUFA). Feeding essential FAs can also have positive effects on follicular growth, embryo quality, and pregnancy rates (Thatcher et al., 2006; Santos et al., 2008; Silvestre et al., 2011). When ω-3 FAs are supplemented, a shift in prostaglandin (PG) production can occur. Pregnancy rate may also be increased due to enhanced progesterone (P_4) production by the corpus luteum (CL) and decreased embryo mortality (Santos et al., 2008; Silvestre et al., 2011; Petit and Twagiramungu, 2006). The addition of flaxseed has been linked to increased ovulation rate in various species (Scholljegerdes et al., 2011; Abayasekara and Wathes, 1999; Trujillo and Broughton, 1995).

Research on ω-3 FA supplementation for ewe reproductive improvement is lacking. Luna et al. (2008) fed flax to ewes, however the study was focused on increasing ω-3 FAs in the milk. Alpha-linolenic acid was detectable in the milk of the flax-fed ewes, which infers ω-3 FAs can be transferred from the diet to the blood. Omega-3 FAs are available from flaxseed to be used by the body. Flaxseed supplemented in the right amount has the potential to lower PG concentrations, shift PG production to less active PGs, increase P_4 production, increase ovulation rate, decrease embryo mortality, increase follicular growth, and increase CL stability. Our hypothesis was the supplementation of flaxseed would increase ALA in the blood and therefore improve progesterone production and reproductive performance while preventing embryo death. The objective of the present study was to supplement flaxseed in an applied setting using Flaxlic® Sheep Tubs during a 35-d period prior to breeding to improve progesterone production, lambing rate, pregnancy rate, and prolificacy rate.

Materials And Methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (NDSU; protocol #A17071). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Experimental Design

Multiparous Rambouillet ewes (n = 240) aged 2 to 7 years with a mean body weight of 70.8 ± 10.4 kg were randomly assigned to 24 pens in groups of 10 ewes/pen. Twelve pens were given a Flaxlic® Sheep Tub (FLX; New Generation Feeds, Belle Fouche, SD; n = 12). The other 12 control pens (CON; n = 12) did not receive a flax tub, but instead supplemented mineral by adding to the basal ration with a commercial mineral premix described in Table 2.1. Pens were fed a diet of chopped hay for 35 days. The diet was balanced for a 70 kg ewe receiving a flushing ration prior to breeding (Table 1.; NRC, 2007). The quantity of hay offered was altered throughout the study to account for weight changes of the ewes to maintain crude protein and energy supply for flushing. Feed samples were taken at the beginning and end of the 35-d feeding trial period. Samples were sent to Midwest Laboratories (Omaha, NE) for nutrient analysis. Dry matter (calculated from moisture measurement, method 930.15; Association of Analytical Communities [AOAC] Int., 1990), acid detergent fiber (ADF; ANKOM Tech. Method; Spanghero et al., 2003), crude protein (CP; method 990.03; AOAC Int., 2006), total digestible nutrients (TDN; Weiss et al., 1992), minerals, (method 985.01 modified; AOAC Int., 2006) and ω-6 and ω-3 FA were analyzed (method 996.06; AOAC Int., 2012). The ingredients for the Flaxlic® Sheep Tub by inclusion level are beet molasses, ground flaxseed (21%), flaxseed oil (6.4%), soybeans (45%), and select vitamins and minerals (Table 1). Ewe 2-day weights and body condition

Table 1. Feedstuff Nutrient Composition of the Basal Ration for CON and FLX Treatments[1].

Nutrient, % DM[2]	Chopped Hay[3]	Flaxlic® Sheep Tub[3]	Sheep Mineral[3]
DM (% as fed)	87.45	-	99.0
ADF	37.1	2.5	0
CP	13.6	12	0
TDN	60.2	-	0
S	3.6	-	0.19
P	5.5	1.0	8.0
K	1.86	2.5	2.1
Mg	0.26	-	2.75
Ca	0.74	1.0-1.5	16.5
Na	0	-	11.0
Fe	711 ppm	-	131 ppm
Mn	67.5 ppm	0.12	52.60 ppm
Cu	5.3 ppm	0	5.50 ppm
Zn	25.0 ppm	1200 ppm	20.30 ppm
ω-3 Fatty Acids[3]	0.65 g·100g^{-1}	0.07 g·100g^{-1}	-
ω-6 Fatty Acids[3]	0.48 g·100g^{-1}	0.025 g·100g^{-1}	-

[1] CON = basal ration of chopped hay plus sheep mineral; FLX = basal ration of chopped hay plus Flaxlic® Sheep Tub.
[2] Most measurements reported on a dry matter basis; fatty acid analysis reported on an as fed basis.
[3] Chopped hay = basal ration; (-) indicates item was not measured.

score (BCS; 1-5 scale; Kenyon et al., 2014) were taken on d -1 and 0 and d 34 and 35, with ewe body weight recorded weekly to monitor ewe health.

Flaxlic® Sheep Tubs were offered ad libitum during week one. Ewe intake exceeded the recommended feeding rate due to the nature of a feedlot setting. The recommended feeding rate was 56.70-113.40 g · head^{-1} · d^{-1}. Therefore, for the remainder of the trial, ewes had access to the tubs from 8 PM to 8 AM. Flaxlic® Sheep tubs were weighed on d 0, 3, 7, 10, 14, 17, 24, 29, and 35 to monitor intake.

Blood was collected from five ewes from each pen for a total of 120 ewes to evaluate circulating P_4 to determine cyclic activity. Blood was collected via jugular venipuncture (21-gauge 3.81 cm Vacuette blood drawing needle) into 10 ml serum tubes (BD Vacutainer Serum) on d 0, 7, 14, 21, 28, and 35 before weighing. Samples were centrifuged at about 10°C for 10 min at 3300 x g. Progesterone samples were analyzed at North Dakota State University using the Immulite Immunoassay system (IMMUNULITE 1000 Progesterone; LKPW1; Siemens Diagnostic, Los Angeles, CA). The limit of detection was 0.1 ng/ml.

On d 17 following administration of tubs, ten mature rams were placed alongside the ewes for fence line contact to stimulate estrous activity. On d 35, ewes were comingled, placed on native pasture, and rams were turned in for breeding. The rams were fitted with marking harnesses with black crayons. On d 7, 14, 21, 28, and 35 post ram turnout, breeding marks were recorded and marking harnesses were checked for crayon wear and replaced, if necessary. Crayons were replaced with red crayons on d 14. After the last recording day, the breeding harnesses were removed. Ewes were moved to a new pasture in early October.

On October 10, ewes were moved to a dry lot and fed 1.81 kg/head chopped hay, 0.45 kg/head barley haylage, and 1 kg/head barley once every two days until parturition began in February. Pregnancy status was determined via ultrasound on October 10th, 53 d post ram turnout and again on November 14, 88 d post ram turn-out (ALOKA 500; convex transducer). Ewes were moved to the Hettinger Research Extension Center lambing barn at approximately d 130 of gestation.

Ewes with lambs were moved into a separate pen (0.9 m x 1.5 m lambing pen) within two hours of lambing for bonding and observation. After two hours, data were collected on lambing type (singles, twins, or triplets), birthweight, and lamb gender. Lambs were tagged and ear notched for identification at weaning. After lambs were confirmed to be healthy and suckling, the ewe and her lambs were moved into a larger pen with other ewes with lambs (7.6 m x 3.7 m lambing pen). Lamb grower pellet was available ad libitum via creep feeders for the lambs (Southwest Grain Market Lamb Supplement). Docking occurred between 7 and 14 days after birth. Males were not castrated. Ewes and lambs were moved to outside pens approximately one hour after docking. Weaning weights were taken at approximately 60 to 75 d post-lambing.

Statistical Analyses

Pen served as experimental unit (n = 12). Hay intake, ewe weights, and BCS were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). First cycle and overall pregnancy, prolificacy, and lambing rates were analyzed using the GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) in a completely random design. The models for overall and first cycle pregnancy rates were binomial designs and included pen, treatment, and age. The models of first cycle and overall prolificacy and lambing rates were a multinomial design included pen, treatment, and age. Pregnancy rate was defined as the percentage of ewes pregnant per ewe exposed in the first 16 days and overall lambing. Prolificacy rate was defined as the number of lambs born per ewe lambed in the first 16 days and overall lambing. Lambing rate was defined as the number of lambs born per ewe exposed in the first 16 days and overall lambing. Serum progesterone concentrations were analyzed using the MIXED procedure of SAS. The model for serum P_4 concentration included fixed effects of treatment, day, and treatment x day. Average P_4 concentration was a repeated measure and was analyzed using the autoregressive (1) function. Significance was determined at $P \leq 0.05$. To separate values for treatment effects, day effects, and treatment x day interactions, CONTRAST statements and LSMEANS were utilized ($P \leq 0.05$).

Results And Discussion

Ewe Weight Change and Feed Intake

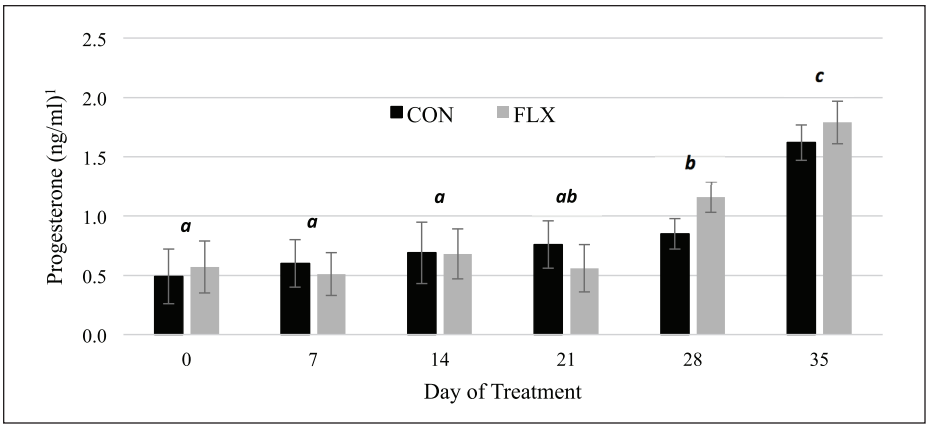
By treatment design, there was no difference of two-day ewe weights and BCS due to treatment at the start and end of the trial ($P \geq 0.23$; Table 2). Average daily hay intake on a DM basis was 1.71 ± 0.009 and 1.57 ± 0.014 kg · head^{-1} · d^{-1} for CON and FLX treatments, respectively ($P < 0.001$). Hay offered was adjusted following each weight observation. By design, the CON treatment was offered more hay to simulate weight gains similar to the FLX treatment. The goal of the trial was to evaluate supplemental flax, not changes in CP and energy supply, on ewe fertility and reproductive efficiency. Average Flaxlic® Sheep Tub consumption for the FLX treatment was 150.82 g · head^{-1} · d^{-1} for the 35-day trial. Consumption was higher than the recommended tub

Table 2. Impact of Flaxlic® Sheep Tubs on Initial and Final Weights of Ewes during a Flushing Feeding Period.

	Treatment[1]			
Item	CON	FLX	SEM	*P*-Value[3]
Initial Weight, kg	71.0	70.8	0.19	0.47
Final Weight, kg	69.5	70.1	0.37	0.23
Initial BCS[2]	3.1	3.1	0.02	0.45
Final BCS[2]	3.0	3.0	0.05	0.89

[1] FLX = Flaxlic® Sheep Tub supplemented ewes; CON =control ewes; SEM = Standard Error of the Mean.
[2] Body condition score; scale of 1-5; Kenyon et al. (2014).
[3] *P*-value across treatments (n = 12 for FLX and CON treatments).

Figure 1. Impacts of Flaxlic® Sheep Tub on Serum Progesterone Concentration by Day of Tub Exposure During the Flushing Period.



1 Serum progesterone concentration of Flaxlic® Sheep Tub fed ewes (FLX) versus control ewes (CON). The threshold for cyclicity was stated as a value over 0.4 ng/ml (Quirke et al. 1985; Wright et al., 2002; Santos et al., 2018).

a-c Groups with different letters differ ($P < 0.001$) between days.

intake of 56.70-113.40 g · head^{-1} · d^{-1}, likely due to the confined feeding situation. A pasture grazing situation, such as what the Flaxlic® Sheep Tub was intended to be used in, would require ewes to travel longer distances to reach the tubs. Tub intake would likely drop closer to the recommended rate. Total ω-3 FA intake of CON ewes was 11.11 g · head^{-1} · d^{-1}. Though intake was higher than recommended, the total ω-3 FA intake of FLX ewes was 11.27 g · head^{-1} · d^{-1}, which includes a 1.06 g · head^{-1} · d^{-1} contribution from the flax tubs. Omega-6:ω-3 ratio for FLX ewes was 1:1.36, while ω-6/ω-3 FA ratio for CON ewes was 1:1.35. The increase in omega-3 FA coming from the flax tubs was not sufficient to alter the ω-6:ω-3 FA when compared to the CON ration.

Circulating Progesterone

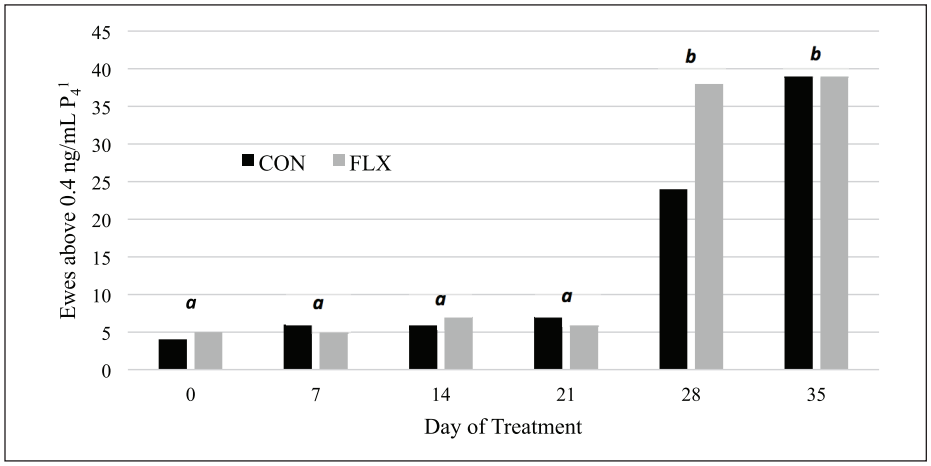
Ewe cyclicity was determined when a concentration of over 0.4 ng/ml of serum P_4 was present (Quirke et al., 1985; Wright et al., 2002; Santos et al., 2018). In the present study, particular attention was given to the trend rather than the concentration. There was no treatment x day interaction ($P = 0.82$) for serum P_4 concentration. There also was no treatment effect ($P = 0.83$). However, there was a day effect ($P < 0.001$; Figures 1 and 2). Progesterone increased as day increased, likely due to the fence line ram exposure.

Progesterone concentration on d 0 through 21 were lower than d 28 and 35 ($P < 0.001$). In addition, the rams were run along the fence line after d 17, perhaps leading to active cyclicity afterwards due to the male effect, as reported in sheep and goats (Walkden-Brown et al., 1993; Rosa and Bryant, 2002; Rivas-Muñoz et al., 2007; Delgadillo et al., 2009). These results are in agreement with Ambrose et al. (2006) and Hutchinson et al. (2012) who reported no difference in P4 concentration between control and flaxseed fed dairy cows. Dairy cattle are very different from sheep in management and production level. Ambrose fed 427.5 g of ω-3 FA in mechanically rolled flaxseed to mature dairy cows with an average weight of 650 kg. Ambrose et al. (2006) fed 4 times the amount of ω-3 FAs per kg bodyweight compared to the present study, which fed a daily ω-3 FA intake of 10.31 g · head^{-1} · d^{-1}. This may explain why a difference was not found in the present study. In agreement to this hypothesis, the results in the present ewe study are contrary to studies who reported significant increases in P_4 concentration in flax fed cows (Lessard et al., 2003; Petit and Twagiramungu, 2006). Santos et al. (2008) notes further research is required to fully understand how long chain fatty acids affect the ruminant animal, whether the effects reported are due to fatty acids in the product fed or due to the biohydrogenated forms of those fatty acids after rumen digestion. To add further evidence to this argument, Luna et al. (2008), who used whole flaxseed, found increased ALA in the blood of sheep. These studies may infer rumen protection of ALA is required to elicit an effect on the reproductive performance of the ruminant animal.

Lambing

There was no interaction between treatment and age for first cycle or over-

Figure 2. Impacts of Flaxlic® Sheep Tub on Ewe Cyclicity by Day of Tub Exposure During the Flushing Period.



1 Number of ewes above the 0.4 ng/ml progesterone (P_4) concentration on a given day; Flaxlic® Sheep Tub fed ewes (FLX) versus control ewes (CON). The threshold for cyclicity was stated as a value over 0.4 ng/ml (Quirke et al., 1985; Wright et al., 2002; Santos et al., 2018).

a-b Groups with different letters differ ($P < 0.001$) between days.

Table 3. Impact of Flaxlic® Sheep Tubs Supplementation During the Flushing Period on Ewe Lambing Data in the First Cycle and for the Overall Lambing Period.

Item[2]		Treatment[1]			P-value[3]
		CON	FLX	SEM	TRT
First Cycle	Pregnancy, %	69	72	4.3	0.76
	Prolificacy, %	148	153	6.3	0.26
	Lambing, %	103	112	7.9	0.31
Overall	Pregnancy, %	97	96	1.8	0.61
	Prolificacy, %	147	149	5.4	0.86
	Lambing, %	145	145	6.0	0.89

[1] FLX = Flaxlic® Sheep Tub supplemented ewes; CON=control ewes.
[2] Pregnancy = percentage pregnant per ewe exposed; Prolificacy = lambs per ewe lambed; Lambing rate = lambs per ewe exposed; SEM = standard error of the mean.
[3] *P*-value between treatments (TRT; *n* = 12 for FLX and CON treatments).

all pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.13$; Table 3). There were no differences between treatments for 1st cycle pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.26$). There were also no differences between FLX and CON ewes for overall pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.26$).

More focus should be placed on first cycle findings rather than overall results of the lambing season. The ewes were taken off flax supplementation upon becoming comingled with rams for breeding. Therefore, the flax supplementation effect would only be exhibited for a limited amount of time. Previous studies reported increased pregnancy rates (Ambrose et al., 2006; Silvestre et al., 2011), improved conception, and decreased embryo mortality (Ambrose et al., 2006; Petit and Twagiramungu, 2006). These improvements were not reflected in the present study's first cycle pregnancy, lambing, or prolificacy rates. As mentioned previously, Ambrose et al. (2006) fed flax at an ALA concentration that was four times as high as the present study by weight. The rolled flaxseed from Ambrose et al. (2006) would also be more vulnerable to biohydrogenation than whole flaxseed (Lashkari et al., 2015). The feeding level of flaxseed in the present study may not be at a level to sufficiently affect these pregnancy parameters. Increased prolificacy via increased ovulations was found by Trujillo and Broughton (1995), which disagrees with the present study. The ability of Flaxlic® Sheep Tub supplementation to improve pregnancy, lambing, and prolificacy rates may become more pronounced when flax is fed in larger quantities, and age is blocked by pen. Age impacted both overall and first cycle lambing and prolificacy rates.

Applications

Addition of a Flaxlic® Sheep Tub did not significantly improve pregnancy parameters or influence progesterone concentration level. An important aspect of feeding these components is to not only increase the ω-3 FAs but also to decrease the ω-6:ω-3 FA ratio in the diet and therefore in the system of the targeted animal. However, if the ω-3 FAs cannot make it through the rumen environment without being biohydrogenated, the effect may be lost altogether. Utilization of whole flaxseed or rumen protected ALA may be the answer. Studies using processed flaxseed did not find improved progesterone concentration. However, some did find

improved conception. Perhaps this improved conception is due to the products of hydrogenation of ω-3 FAs from the processed flaxseed. Studies utilizing whole flaxseed, protected by the pericarp, found increased progesterone concentrations in the blood of dairy cows and increased ALA in the milk of ewes. This may imply the use of whole flaxseed is more efficiently utilized by the ruminant and thus more beneficial to reproduction. More research is required to confirm the hypothesis that rumen protection is warranted to elicit desirable responses in reproductive performance in sheep and ruminants in general.

The desirable ratio of ω-6:ω-3 FAs is not yet known for reproductive improvement in female ruminants. More research with specific focus on controlling ω-6:ω-3 ratio is required to discover the most desirable ratio for the ruminant female. In the present study, the as-fed ω-6:ω-3 FA ratio was 1:1.36 for FLX ewes and 1:1.35 for CON ewes. These ratios were not different enough to elicit any responses, as shown by the present study.

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Sheep & Goat Research Journal
Volume 35, 2020 - July

Impacts of Flax on Male Reproductive Traits when Supplemented Prior to Breeding in Sheep[1]

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[3] *Support for this research was provided by New Generation Supplements and AmeriFlax. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of New Generation Supplements or AmeriFlax. The authors would like to thank David Pearson, Donald Drolc, Don Stecher (Hettinger Research Extension Center, North Dakota State University), and Ethan Schlegel (New Generation Supplements) for their assistance throughout the project.*

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Summary

Similar to flushing ewes prior to breeding, male fertility in sheep can be improved through nutritional management. Fatty acid supplementation has been shown to improve male reproductive characteristics, such as sperm motility, concentration, and morphology. Supplementation with flax prior to breeding is a potential strategy to increase ω-3 FAs in the diet. The objective of this study was to evaluate the effectiveness of flax supplementation on serum testosterone concentration and semen quality. One-hundred twenty Rambouillet ram lambs (42 ± 2.78 kg) were randomly assigned to 24 pens (5 rams/pen; n = 12) and fed for 112 days. Rams were assigned to either receive a Flaxlic® Sheep Tub (FLX; n = 12) or a control (CON; n = 12). Tubs were weighed on d 0, 14, 28, 48, 64, 92, 103, and 112. Tubs weighing less than 5 kg were replaced with new tubs. Weight data for all rams was taken on d 0, 28, 56, 85, and 112, with 2-d weights taken on d -1 and 0 and d 111 and 112. Serum for testosterone concentration analysis, semen for quality analysis, and scrotal circumference measurements were collected on d 83-84 and 111-112. Average daily gain (ADG) for FLX rams was not different from CON (0.73 ± 0.10 and 0.78 ± 0.11, respectively; P = 0.25). No differences were observed for testosterone concentrations between CON and FLX treatments (208.04 ng·dl^{-1}; P = 0.70). There were no significant differences in scrotal circumference, sperm motility, sperm morphology, or sperm concentration ($P \geq 0.15$). Flaxseed supplementation in a tub form did not positively or negatively affect semen quality of growing ram lambs. Our results indicate that it can be an acceptable addition to growing ram lamb rations if economically viable, but under our conditions did not improve reproductive performance.

Key Words: Fatty Acids, Fertility, Flax, Rams, Semen Quality, Sheep

Introduction

Reproduction is a vital component for any range sheep operation. The male side of reproduction is a component of overall productivity that can be overlooked. One way to improve a ram's performance during the breeding season is to improve the nutrients provided leading up to breeding. Not only will this help rams regain lost condition from the previous season, but it may also stimulate improved spermatogenesis and sperm cell function. Adding extra nutrients to the pre-breeding ration, such as essential fatty acids, has been shown to further improve a male's reproductive efficiency (Tou et al., 1999; Zanini et al., 2003; Baiomy and Mottelib, 2009; Yan et al., 2013; Esmaeili et al., 2014; Jafaroghli et al., 2014; Moallem et al., 2015; Shah et al., 2016)

Flaxseed provides two essential fatty acids (FA): Alpha-linolenic acid (ALA; C18:3 ω-3), an omega-3 (ω-3) FA and linoleic acid (LA: C18:2 ω-6), an omega-6 (ω-6) FA. Flax is approximately 45% oil. Of the total fats in flax oil, about 57% is ALA and 16% percent is LA (Morris, 2007). Flax is an excellent supplier of ω-3 FA and contains a very low ω-6 to ω-3 FA ratio (1:3.56).

Flaxseed in particular has been shown to improve sperm motility and progressive motility in bulls (Moallem et al., 2015). Flaxseed has also been shown to increase levels of the reproductive hormones such as gonadotropin-releasing hormone, follicle stimulating hormone (rats; Yan et al., 2013), luteinizing hormone (rats; Yan et al., 2013), and testosterone (rams and rats; Baiomy and Mottelib., 2009; Yan et al., 2013; Esmaeili et al., 2014). Based on the previous research, supplementing male sheep with flaxseed prior to breeding may be a way to improve semen quality and thereby improve fertility. Our hypothesis was the supplementation of flaxseed would increase testosterone in the blood and therefore improve spermatogenesis and reproductive performance while preventing sperm abnormalities. The objective of the present study was to supplement flaxseed in an applied setting using Flaxlic® Sheep Tubs during a 112-d period leading up to the breeding season.

Materials And Methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (NDSU; protocol #A18059). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Experimental Design

Rambouillet ram lambs (n = 120) were selected from the NDSU Hettinger Research Extension Center flock. At 60 days of age, lambs were weaned and vaccinated for *Clostridium perfringens* type C and D and tetanus (CD-T; Bar Vac CD/T; Boehringer Ingelheim, Ridgefield, CT). On d -1, ram lambs (approximately 4 months of age; 42 ± 2.78 kg) were randomly assigned to 24 pens (5 rams/pen; 25.2 m^2/ram), with pen serving as the experimental unit. Rams were fed a basal ration with a Flaxlic® Sheep Tub (FLX; *n* = 12) or a basal ration alone (CON; *n* = 12). The basal ration was a total mixed ration (TMR) made up of 60% soybean hulls, 10% corn, 15% soybean meal, and 15% Market Lamb Supplement (dry matter basis; Southwest Feed, Inc.). The basal ration was balanced to meet the CP and TDN requirements of a 40 kg lamb gaining 300 g/d (Table 1; NRC, 2007). The ration was mixed in a mixer-grinder (GEHL mix-all, Model 170; West Bend, WI) and provided ad libitum via bulk feeders (98 cm of bunk space per ram). Orts were taken on day 87 and 112 and tested for nutrient composition. Samples were sent to Midwest Laboratories (Omaha, NE) for nutrient analysis. Dry matter (calculated from the moisture measurement, method 930.15; Association of Analytical Communities [AOAC] Int., 1990), acid detergent fiber (ADF; ANKOM Tech. Method), crude protein (CP; method 990.03; AOAC Int., 2006), total digestible nutrients (TDN), and minerals (method 985.01 modified; AOAC Int., 2006) were measured. Omega-6 and ω-3 FA were analyzed as well (method 996.06; AOAC Int., 2012). The ingredients for the Flaxlic® Sheep Tub by inclusion level are beet molasses, ground flaxseed (21%), flaxseed oil (6.4%), soybeans (45%), and select vitamins and minerals (Table 1). Flaxlic® Sheep Tub weights were taken on d 0, 14, 28, 48, 64, 92, 103, and 112 to monitor ram tub intake. Flaxlic® Sheep Tubs that fell below 5 kg were replaced with new tubs. Rams were allowed 12-hour access to the tubs from 8 PM to 8 AM in the first two weeks. Intake during this time was below the recommendation level of 56.70-113.40 g

Table 1. Nutrient Composition of the Basal Ration and Flaxlic® Sheep Tub.

Nutrient, % DM[1,3]	TMR[2]	Flaxlic® Sheep Tub
DM (% as fed)	86.66	-
ADF	28.9	2.5
CP	22.5	12.0
TDN	72.3	-
S	0.30	-
P	0.42	1.0
K	1.54	2.5
Mg	0.36	-
Ca	1.34	1.0-1.5
Na	0.25	-
Fe	683 ppm	-
Mn	91.7 ppm	0.12 ppm
Cu	13.0 ppm	0 ppm
Zn	129 ppm	1200 ppm
ω-3 Fatty Acids	0.15 g·100g^{-1}	0.07 g·100g^{-1}
ω-6 Fatty Acids	0.71 g·100g^{-1}	0.025 g·100g^{-1}

[1] TMR = total mixed ration; Most measurements reported on a dry matter basis; fatty acid analysis reported on an as fed basis.
[2] 60% pelleted soybean hulls, 15% soybean meal, 15% Southwest Grain Market Lamb Supplement, 10% whole corn; dry matter basis.
[3] DM = dry matter; ADF = Acid Detergent Fiber; CP = crude protein.

· head^{-1} · d^{-1}. From d 14 until the trial finished on d 112, FLX rams were allowed 24-hour access to the tubs to increase intake.

The basal ration had a measured ω-6/ω-3 FA ratio of 4.7:1. The Flaxlic® Sheep Tub had an ω-6/ω-3 FA ratio of 1:2.8. In total, CON rams received an average of 13.5 g of ω-6 FAs and 2.87 g of ω-3 FAs per day based on the CON group's average intake. FLX rams received 12.86 g of ω-6 FAs and 2.75 g of ω-3 FAs per day based on the FLX group's average intake. The overall ratio is 4.73:1 and 4.69:1 for CON and FLX groups, respectively.

Two-day weights and body condition score (1-5 scale; Kenyon et al., 2014) were taken on d -1 and 0 and d 111 and 112, with ram body weight recorded every 28 d to monitor ram health. Ram scrotal circumferences were taken during a 2-day period alongside semen collection, on d 83-84, and again on d 111-112. With a standing ram, both testes were retained to the base of the scrotum, where circumference was measured of the scrotal tissue and the two testes combined (Martin et al., 1994). Four rams were removed due to non-treatment related death prior to d 84 (two FLX, two CON). One ram was removed due to non-treatment related death prior to day 112 (FLX).

Blood and semen were collected over a two-day period on d 83 and 84, then again on d 111 and 112. Blood was collected via jugular venipuncture using a 21-gauge 3.81 cm needle (Vacuette blood collection needle) into a Serum Separator Tube blood tube (SST, VWR Inc.) and placed on ice. Samples were centrifuged at 10°C for 10 min at 3300 x g for serum collection. Testosterone samples were analyzed at North Dakota State University using the Immulite Immunoassay system (IMMUNULITE 1000 Total Testosterone; LKTW1; Siemens Diagnostic, Los Angeles, CA). The intraassay and interassay coefficients of variation (CV) were 8.6% and 13.1%, respectively.

Semen was collected via electroejaculation over a two-day period on d 83 and 84, then again on d 111 and 112 for all rams. Rams were stimulated until a successful ejaculation occurred into a plastic collection sheath, no more than three times at each collection time. The first successful ejaculate from each ram was evaluated. Ejaculates were placed into a cooler held at 35°C. Contents of a sheath were transferred to a 2.5 mL conical tube for volume determination. Within 20 minutes of collection, each sample of semen was diluted with buffer (Easy Buffer B, IMV Technologies U.S.A., Maple Grove, MN) to a target cell count of 60 to 80 cells per field. Diluted semen was placed into 20 µm capillary chamber slide (Leja products B.V., Netherlands) and loaded into a computer assisted semen analysis machine (IVOS II, Hamilton Throne, Beverly, MA). Each of 10 fields were assessed. Notable measurements for abnormalities included bent tail percent of total sperm, proximal droplet percent of total sperm, and distal droplet percent of total sperm. Quantity and mobility measurements included total concentration (million cells/ml), sperm count (concentration of sperm per milliliter x total volume of the ejaculate), semen volume (ml), motile concentration (million cells/ml), motile count (concentration of motile sperm per milliliter x total volume of the ejaculate), motile sperm as a percent of total sperm, progressive concentration (million cells/ml), progressive sperm count (concentration of progressively motile sperm per milliliter x total volume of the ejaculate), progressive sperm as a percent of total sperm, and static sperm as a percent of total sperm.

Statistical Analyses

Ram body measurements, serum testosterone concentrations, and semen analysis results were analyzed in a completely random design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with pen serving as experimental unit and variance component structure. Pen nested in treatment was a random variable. Testosterone concentration and semen characteristics were repeated measures. Fixed effects were day and treatment. Models included fixed effects of treatment, day, and treatment x day. If a treatment x day interaction was not found, the model was run again without the interaction. Significance was determined at $P \leq 0.05$. To separate significant fixed and interactions, LSMEANS and CONTRAST statements were utilized (Tukey's; $P \leq 0.05$).

Results And Discussion

Ram Weight Change and Feed Intake

There were no treatment x day interactions ($P \geq 0.41$) for ram weight or BCS. Initial and final ram weight or BCS did not differ between treatments (Table 2; $P \geq 0.25$). As the rams were given free access to feed with or without the addition of the Flaxlic® Sheep Tub, this result was expected. However, there

Table 2. Impact of Flaxlic® Sheep Tubs on Weight and Body Measurements in Rambouillet Ram Lambs.

	Treatment[1]			
Item[2]	CON	FLX	SEM	*P*-Value[3]
Initial Weight, kg	41.8	42.2	2.78	0.82
Final Weight, kg	81.6	79.5	2.80	0.25
Initial BCS	3.00	2.99	0.02	0.72
Final BCS	3.62	3.58	0.05	0.65
DM Intake, kg·hd^{-1}·d^{-1}	1.91	1.81	0.05	0.23
ADG, kg/d	0.78	0.73	0.03	0.25
G:F	5.42	5.47	0.10	0.77
SC d 83-84, cm	31.8	32.1	0.29	0.56
SC d 111-112, cm	34.4	34.2	0.33	0.72

[1] FLX= Flaxlic® Sheep Tub supplemented ewes; CON=control ewes; SEM= Standard error of the mean.
[2] BCS= Body condition score; scale of 1-5; Kenyon et al. (2014); DM = Dry matter; ADG= Average daily gain; G:F= Gain to feed; SC= Scrotal circumference.
[3] *P*-value across treatments (*n*=12).

was a day effect for weight gain ($P < 0.001$), which was also expected due to the growth of a ram lamb into a mature ram. There was a treatment x day interaction ($P = 0.04$) between treatments for average daily gain (ADG). Between d 28 and 56, the CON treatment had a higher ADG than the FLX group (0.74 ± 0.32 and 0.53 ± 0.44, respectively; $P = 0.04$). The CON treatment gained more over time than the FLX group. These results are in contrast with Pesta and Drouillard (2010) who reported increased ADG and improved feed efficiency between treatments in Flaxlic® Tub fed bulls ($P < 0.05$).

There was no effect of treatment on daily dry matter intake between CON and FLX treatments (1.91 ± 0.05 and 1.81 ± 0.05 kg · hd^{-1} · d^{-1}, respectively; $P = 0.23$). FLX intake of the Flaxlic® Sheep Tub was 42.52 g · hd^{-1} · d^{-1}, 2.2% of their total feed intake per day. Gain to feed (G:F) was not different between CON and FLX treatments (5.42 ± 0.10 and 4.47 ± 0.10, respectively; $P = 0.77$). Pesta and Drouillard (2010) conversely reported the control group of bulls had higher feed intakes than Flaxlic® Tub supplemented bulls. Pesta and Drouillard (2010) also reported improved G:F ratios not found in the present study.

The present study results were opposite to the similar study in bulls, possibly indicating a difference in physiological response to flax between bulls and rams. This may be due to a species-specific response that does not occur in sheep. For example, research by Neville et al. (2012) reported that when growing lambs were fed a diet with up to 60% dried distillers grain plus solubles (DDGS), no incidences of polioencephalomalacia (PEM) were observed, which is in contrast to recommendations by Gould (1998) for preventing PEM in cattle fed DDGS. While no explanation for the disparity of results between Neville et al. (2012) and Gould (1998) has been elucidated, one possibility is that the S bonds in wool are a "S sink" that allows sheep to tolerate higher concentrations of S in the diet. While not directly applicable, it is possible that sheep and cattle have different requirements for ω-3 and ω-6 fatty acids. Another explanation may be the rams were not eating enough of the Flaxlic® Sheep Tub compared to the intake of the bulls, or that the basal diet was different. The basal diet for the Pesa and Drouillard (2010) study was a forage-based diet, vs. our diet which had primarily soybean hulls as the fiber source.

Reproductive Traits

Scrotal Circumference. There were no treatment x day interactions for scrotal circumference (SC; $P = 0.34$). There was a day effect between d 83 and 84 and d 111 and 112 (31.92 and 34.32 cm, respectively; $P < 0.001$), as expected of maturing ram lambs (Camela et al., 2018). There were no treatment effects for overall SC ($P = 0.72$) or on d 83-84 (Table 2; $P = 0.56$). The lack of change in SC is in agreement with Baiomy and Mottelib (2009), who also found no change in SC between flaxseed supplemented and control rams. Baiomy and Mottelib (2009) used flax oil, which was less protected from biohydrogenation than feed types such as whole flaxseed (Lashkari et al., 2015). The present study also used a less protected form of flax in a tub, made up of flax oil and flaxseed meal. Changes in scrotal circumference are affected by maturity and season. Rams were in the correct season to stimulate changes in SC. However, Rambouillets are known for being late maturing (Foote et al., 1970). Therefore, the lack of change in SC may simply be due to the rams' immaturity. Extending the trial for a longer period in the Rambouillet breed may reveal differences between treatments.

Testosterone. Half of the samples' testosterone concentration levels were too low for accurate measurement. Therefore, only values greater than 50 ng/dl were utilized. There were no treatment x day interactions ($P = 0.99$). Serum testosterone was not different between treatments (213.13 and 213.12 ng/dl, respectively; $P = 0.99$). A day effect was observed between d 83 and 84 and d 111 and 112 (179.2 and 247.0 ng/dl, respectively; $P = 0.02$). This increase in testosterone over time is in unison with the increase in scrotal circumference by day. Baiomy and Mottelib (2009) also measured testosterone in flax-fed vs control rams, reporting an increase in the flax-fed rams over the control after two months of treatment. The rams used were Ossimi, a breed from Egypt, which may explain a difference in testosterone response to flaxseed components due to differing climate, breed, and management systems.

Sperm Morphological Abnormalities. There were no treatment x day interactions ($P \geq 0.08$) or treatment effects ($P \geq 0.62$) for sperm abnormalities (Table 3). Percentage of bent tails and total abnormalities were affected by day ($P \leq 0.04$). Bent tail percentage decreased from 9.34 to 5.65 % from d 83-84 to d 111-112, respectively. Total abnormalities dropped from 41.24% on d 83-84 to 35.89% on d 111-112, respectively. Both bent tail percentage and total abnormalities decreased as rams aged. Therefore, this improvement is likely due to the rams' increasing maturity, as abnormalities are reported to decrease as males reach maturity (Bartlett, 1982). In rams, semen reaches optimal quality by 2 to 3 years of age (Badi et al., 2018). These results are in agreement with Pesta and Drouillard (2010), who reported no differences between control and Flaxlic® supplemented bulls for percent normal sperm. The results are in contrast with Baiomy and Mottelib (2009) who found decreased abnormal sperm in ω-3 FA supplemented rams.

The rams in the present study were fed processed flax oil and flaxseed meal in the form of a tub, in addition to the high levels of omega-6 FA found in the basal ration (Table 1). The FAs becoming biohydrogenated in both the bulls' and the rams' Flaxlic® tubs could explain why a response was not found in either study. Kronberg et al. (2012) found higher levels of ALA in the blood and tissues of lambs fed flax protected from ALA hydrogenation. Despite this, Baiomy and Mottelib (2009) found decreased abnormal sperm in rams supplemented with unprotected flax oil. The problem in the present study may be with a combination of hydrogenation of ALA and the high ω-6:ω-3 ratio. The overall ratios for the present study are 4.73:1 and 4.69:1 for CON and FLX groups, respectively. The products of biohydrogenation may be beneficial to spermatogenesis, even with the products of -6 FA biohydrogenation. Kemp et al. (1984) and Belenguer et al. (2010) reported that bacteria that specifically isomerize an desaturate long chain FA are present in rumen fluid in sheep, and may impact ω-6:ω-3 ratios. Linoleic acid is broken down into rumenic acid, further into vaccenic acid, and further still to stearic acid (18:0; Belenguer et al., 2010). Alpha-linolenic acid also can be

Table 3. Impacts of Flaxlic® Sheep Tub Supplementation on Semen Characteristics in Rambouillet Ram Lambs.

Item[3]	Treatment[1]			*P*-Value[2]
	CON	FLX	SEM	TRT
Overall bent tail, %	7.55	7.52	0.78	0.98
D 84	9.32	9.37	1.38	0.98
D112	5.71	5.58	0.58	
Overall distal droplet, %	4.89	5.03	0.34	0.86
D 84	5.40	5.10	0.56	0.87
D112	4.38	4.99	0.40	
Overall proximal droplets, %	24.71	24.64	1.23	0.97
D 84	23.94	25.14	1.87	0.62
D112	25.49	24.12	1.60	
Overall volume, ml	0.66	0.67	0.06	0.91
D 84	0.57	0.47	0.05	0.87
D112	0.75	0.88	0.10	
Overall total sperm count[4]	377.44	400.50	19.50	0.49
D 84	325.23	371.48	29.20	0.48
D112	431.55	430.05	24.46	
Overall sperm concentration, million cells/ml	994.75	1053.30	92.66	0.67
D 84	720.23	850.65	118.00	0.67
D112	1279.25	1259.66	136.85	
Overall motile concentration, million cells/ml	548.36	628.58	68.43	0.49
D 84	474.67	612.96	100.00	0.50
D112	620.68	643.34	93.30	
Overall motile sperm count[4]	192.76	216.53	16.06	0.44
D 84	189.64	236.79	26.30	0.43
D112	195.81	197.38	18.80	
Overall motile sperm, %	45.61	47.47	2.48	0.70
D 84	47.53	49.29	3.75	0.70
D112	43.73	45.68	3.27	
Overall progressive concentration, million cells/ml	457.66	465.91	48.69	0.91
D 84	434.30	501.63	83.30	0.89
D112	478.32	432.79	54.25	
Overall progressive sperm count[4]	166.29	167.58	12.28	0.96
D 84	176.24	192.61	21.50	0.94
D112	157.48	144.38	12.43	
Overall progressive sperm, %	39.84	37.64	2.20	0.55
D 84	43.74	39.73	3.42	0.52
D112	36.39	35.62	2.78	
Overall static sperm, %	56.42	52.44	2.54	0.33
D 84	55.81	50.67	3.87	0.34
D112	57.07	54.32	3.29	

[1] CON = basal ration; FLX = basal ration with Flaxlic® Sheep Tub; SEM = Standard Error of the Mean.
[2] P-values considered significant at $P < 0.05$; TRT = treatment effects.
[3] Concentrations given in million per ml of semen; percentages given as % of total sperm.
[4] Count reported as concentration of identified sperm per milliliter multiplied by the total volume of the ejaculate entered initially into the IVOS.

broken down to stearic acid, however there are more steps, including rumelenic acid (Belenguer et al., 2010). Perhaps it is the components of these pathways that are absorbed by the small intestine and used in the body. The different concentrations of ω-6 and ω-3 FA between FLX and CON groups may not end up so different after biohydrogenation.

Volume and Concentration. There were no treatment x day interactions ($P \geq 0.15$) for semen volume or concentration. There were also no effects of treatment on semen volume, total count, or concentration ($P \geq 0.48$; Table 3). The lack of change in concentration between treatments is in contrast with studies in both rams and bulls (Baiomy and Mottelib, 2009 and Shah et al., 2016, respectively). Both studies had a total samples size of 12 and an *n* of 4 per treatment. Both Baiomy and Mottelib (2009) and Shah et al. (2016) used flax oil. For these studies, it is possible the products of biohydrogenation from the rumen contribute to the improved sperm morphology, rather than ALA itself.

There was a day effect ($P \leq 0.03$) for ejaculate volume, sperm concentration, and total sperm count. Ejaculate volume

increased from 0.52 ml on d 83-84 to 0.82 ml on d 111-112 ($P < 0.001$). Total concentration increased ($P < 0.001$) from 784.86 million cells per ml on d 83-84 to 1269.45 million cells per ml on d 111-112. Total sperm count also increased ($P = 0.005$) from 348.15 sperm cells per ml on d 83-84 to 430.80 sperm cells per ml on d 111-112. An increase in total concentration by day is expected in maturing ram lambs (Badi et al., 2018).

Motility. There were no treatment x day effects ($P \geq 0.18$) for any motility measurement. There was also no effect of treatment ($P \geq 0.42$; Table 3) for any motility measurement. There was a day effect ($P = 0.05$) for progressive sperm count, decreasing from 184.85 sperm per ml on d 83-84 to 150.75 sperm per ml on d 111-112. It is unclear why progressive sperm count decreased as day progressed. However, the other measurements of progressively motile sperm, including percentage and concentration of progressively motile sperm, were not different between days ($P \geq 0.13$).

The lack of difference between treatments is in agreement with Pesta and Drouillard (2010), who found no differences between control and Flaxlic® supplemented bulls for sperm motility. The results are in contrast with multiple studies who found increased motility and mass movement in both rams and bulls (Baiomy and Mottelib, 2009; Jafaroghli et al., 2014; Shah et al., 2016) when fed supplemental ω-3 and/or ω-6 fatty acids. Jafaroghli et al. (2014) used fish oil in their study in rams, similar to flax oil used in Baiomy and Mottelib (2009). Both Baiomy and Mottelib (2009) and Jafaroghli et al. (2014) supplemented oils to increase ω-3 FAs and found improved sperm motility. Again, perhaps it is the ALA biohydrogenation intermediates that contributes positively to spermatogenesis and motility, rather than ALA itself. As stated previously, the lack of improvement in the present study could also be affected by the high ω-6/ω-3 ratio caused by the basal ration.

Applications

The addition of a Flaxlic® Sheep Tub did not improve reproductive parameters or influence testosterone concentration level. The objective of feeding the Flaxlic® Sheep Tub was to increase the availability of ALA to the testes to improve spermatogenesis. To make ω-3 FAs as ALA available, they must make it through the rumen environment to be absorbed by the small intestine. In the present study, the Flaxlic® Sheep Tub's ω-3 FAs were supplemented via flaxseed meal and flax oil. These substances are much more processed than the whole seed. The advantage of feeding whole flaxseed is the pericarp of the seed is harder for the rumen to break down than a rolled and roasted seed, which would break the pericarp. If the ω-3 FAs cannot make it through the rumen environment without being hydrogenated, their positive effects may be lost altogether. Research on protecting ALA from the rumen has been positive. However, studies reporting positive impacts to spermatogenesis use different types of oils to supplement ω-3 FAs, which is unprotected from rumen biohydrogenation. These studies could infer the present study simply did not offer enough flaxseed in these forms to impact spermatogenesis.

Research with specific focus on controlling ω-6:ω-3 ratio is required to discover the most desirable ratio for the ruminant male. Due to the similar ω-6:ω-3 ratios between CON and FLX rations, any improvement shown was not due to the ratio. The rams were fed too much soybean and too little flaxseed in the ration to achieve a ratio goal of 1:1. However, this ratio was developed in monogastrics, and may not hold true in ruminants, especially when biohydrogenation is involved. Even so, the present study's basal ration should be re-evaluated to decrease ω-6 FAs. Doing this may help preserve the beneficial effects of ω-3 FA and further explain the effect of biohydrogenation on the Flaxlic® Sheep Tub's ingredients and confirm whether or not this tub's formulation is beneficial for male sheep. The next step may be to compare the Flaxlic® Sheep Tub as is to one formulated using whole flaxseed rather than flaxseed meal, to confirm the effect of biohydrogenation and ω-6:ω-3 FA ratios on the reproductive characteristics of Rambouillet rams.

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Sheep & Goat Research Journal
Volume 35, 2020 - November

Anthelmintic Resistance in Gastrointestinal Nematodes and Associated Management Factors in Intermountain West Sheep Flocks[1-4]

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[2] *Funding for project was made possible by Western-Sustainable Agriculture, Research and Education grant #EW17-011.*

[3] *The authors wish to thank the sheep producers in Montana, Wyoming, and Utah for their participation.*

[4] *At the time of publication, W. C. Stewart and T. W. Murphy were co-editors of the Sheep & Goat Research Journal. To avoid any perceived conflict of interest, B. A. Freking refereed the entirety of the review process.*

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Summary

The objectives of this study were to provide baseline estimates of gastrointestinal nematode (GIN) prevalence and species composition on sheep operations grazing irrigated or sub-irrigated pastures, quantify anthelmintic resistance utilizing a commercially available larval development assay (LDA), and identify management risk factors from producer responses to survey data. Sampling occurred during the summers of 2017 to 2019 on 25 sheep operations in Montana (n = 15), Wyoming (n = 9), and Utah (n = 1). Operations were selected for the study based on word-of-mouth solicitation and limited to those with a history of GIN challenges. Fecal samples collected at each operation were composited into a single sample for coproculture and LDA analysis. Overall, *H. contortus* was the most commonly identified GIN across operations (68.5%) followed by *Trichostrongylus* (12.4%), *Oesphagostumum* (8.9%), *Teladorsagia* (8.4%), and *Cooperia* (1.8%). Twelve operations were evaluated for resistance to *H. contortus* using the LDA for benzimidazoles, ivermectin, and moxidectin. Results indicated that resistance to *H. contortus* was highly prevalent with benzimidazoles (91.7%), followed by ivermectin (50%) and moxidectin (8.3%). Grazing system and prior use of the corresponding anthelmintic class did not significantly impact *H. contortus* susceptibility to ivermectin. Questionnaire responses indicated that 56% of producers attributing production losses to GIN in 0 to 10% of their flock but only 25% utilized targeted treatment methods to guide anthelmintic administration. Results from the present study indicate that anthelmintic resistance to multiple drug classes is a concern in Intermountain West flocks that routinely utilize irrigated pastures as a forage base.

Keywords: Anthelmintic Resistance, Gastrointestinal Nematodes, Haemonchus Contortus, Irrigated Pasture, Risk Factors, Sheep

Introduction

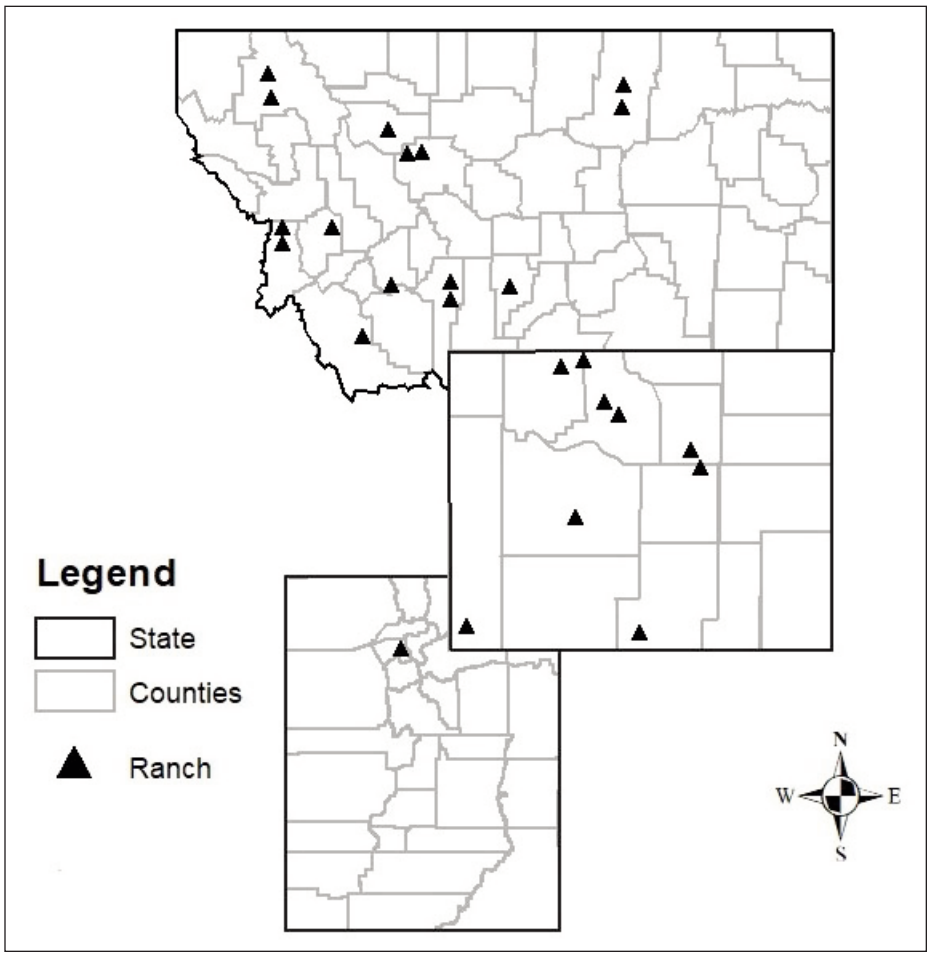
Gastrointestinal nematodes (GIN) are significant challenges to sheep production systems worldwide (Roeber et al., 2013), and exacerbated by increasing anthelmintic drug resistance (Kaplan and Vidyashankar, 2012). A broad spectrum of economically relevant GIN species challenge small ruminant production in North America, however, published reports indicate *Haemonchus contortus* and *Trichostrongylus* spp. are of the greatest concern (Howell et al., 2008). Estimates of anthelmintic resistance in common sheep GIN have been conducted in the Eastern U.S. (Howell et al., 2008; Crook et al., 2016), Eastern Canada (Falzon et al., 2013 a & b), and South Central U.S. (Tsukahara et al., 2017), indicating regionally nuanced resistance to current anthelmintic drug classes. Regional studies estimating resistance can help guide best management practices and inform adaptive strategies to combat GIN. However, differences in climate and management systems affect the prevalence of GIN and extrapolation of results can be limited. The Intermountain West region of the U.S. (MT, ID, WY, NV, UT, CO, NM, AZ) represents 33% of the total U.S. sheep inventory (USDA-NASS, 2019) with an increasing proportion of farm flock operations that may be susceptible to GIN burden due to grazing of irrigated pastures (Bullick and Anderson, 1978). Still, estimates of predominant GIN species and related resistance to anthelmintic drug classes have not previously been evaluated in the Intermountain West. The objectives of the current study were to determine the diversity and relative levels of GIN species present, estimate the prevalence of anthelmintic resistance using the DrenchRite® larval development assay (LDA), and determine related risk factors for drug resistance from questionnaire responses.

Materials And Methods

Sample collection

The locations of sampling sites are displayed in Fig. 1. A total of 25 sheep operations in Montana (n = 15), Wyoming (n = 9), and Utah (n = 1) were solicited to participate in the study via word of mouth communication and social media correspondence. Operations were only sampled during the summer months (June to August) to coincide with the grazing season and daily minimum temperatures adequate for GIN larval development. Breed composition varied across operations and included purebred and crossbred Targhee, Rambouillet, Polypay, Finn-Targhee, and Suffolk. To be eligible to participate producers needed to 1) operate on either irrigated or sub-irrigated (i.e., water table near the soil surface) acreage and 2) have a reported history of GIN burdens within their flocks. Once identified, co-authors traveled to each operation and obtained fecal samples of approximate equivalent quantity from a minimum of 10 ewes each displaying a FAMACHA© anemia score ≥ 3. Fecal samples were collected directly from the rectum using a gloved hand. Individual samples were composited within each operation and vacuum sealed to evacuate excess air from storage bags. Samples were then shipped overnight in a styrofoam cooler to the University of Georgia College of Veterinary Medicine (Athens, GA) for subsequent coproculture and LDA analysis.

Figure 1. Sampling locations (triangles) across MT, WY, and UT.



Laboratory analyses

Upon arrival in the laboratory, the sample was thoroughly homogenized, and a fecal egg count (FEC) was performed using a modified McMasters technique (M.A.F.F., 1977). An egg isolation procedure was then performed to obtain eggs for the LDA, and a coproculture was performed to determine the genus/species of GIN present in each sample. Feces were crushed, vermiculite and water were added, and the sample was incubated at ambient temperature for 10 to 14 d. Larvae were recovered using a Baermann apparatus and identified to genus level (Dinaburg, 1942; M.A.F.F., 1977). A minimum of 100 larvae were identified unless fewer were recovered, in which case all larvae recovered were identified (Howell et al.,

2008).

Larval development assays are an alternative to traditional FEC reduction tests for detecting anthelmintic resistance and were utilized in the current study due to the geographically dispersed field sampling. The maximum distance between sampled operations was ~1100 km, which represented regional diversity of sheep production, but also limited multiple visits to each location. Therefore, the LDA enabled the testing of multiple common anthelmintics at a single timepoint and reduced the logistical and financial requirements in sampling across multiple locations in the current study.

The Drenchrite LDA® was performed according to the manufacturer's recommendations and as previously described by this laboratory (Kaplan et al., 2007; Howell et al., 2008; Crook et al., 2016). Third-stage larvae (L_3) in each well were counted and identified to genus (Dinaburg, 1942; M.A.F.F., 1977). Criteria used for evaluating the resistance status of a given nematode species (*H. contortus, T. colubriformis*, or *Teladorsagia circumcinta*) on an operation was a minimum of 20% of that species in the control plate wells based on identification of L_3 larvae. Successful completion of the LDA required fecal samples with a sufficient number of *H. contortus* eggs present, ~500 eggs per gram (EPG).

For benzimidazoles and ivermectin, the plate well where approximately 50% of the eggs were inhibited from development to L_3 (critical well) was identified; this approximates the 50% effective concentration (EC_{50}). Ivermectin was also used to evaluate moxidectin efficacy (Kaplan et al., 2007) and the plate well where approximately 95% of the eggs were inhibited from development to L_3 (delineating well) was identified; this approximates the 95% effective concentration (EC_{95}). Thus, both the critical well and delineating well are used to estimate moxidectin resistance (Kaplan et al., 2007). It should be noted that levamisole was initially evaluated in the LDA, but its active ingredient appeared to have deteriorated in the testing plate and resulting data were not considered for the present study. Twelve operations had adequate *H. contortus* levels for resistance assessment (Year 1 = 6, Year 2 = 6), whereas only 3 operations had adequate *Trichostrongylus colubriformis* (Year 1 = 2, Year 3 = 1) and 1 operation had adequate *Teladorsagia circumcinta* for resistance assessment (Year 1).

Questionnaire

A written questionnaire on flock performance and husbandry was provided to the owner of each operation. Performance questions included number of ewes that lambed that year, average lamb crop near birth, and average weaning rate. Management systems were described by irrigation type (sub-irrigated, flood, sprinkler, or a combination) and grazing system (continuous, or rotational). Additional questions were designed to determine the previous 5 yr of commercial dewormer use by anthelmintic class (Valbazen® = benzimidazole/albendazole, Prohibit® = levamisole/nicotinic, Ivomec® = ivermectin/macrolide, and Cydectin® = moxidectin/macrolide) and manner of administration (use until ineffective, alternate products within a year, alternate products across years). Finally, producers were surveyed on usage of GIN management strategies in culling or selection (FAMACHA© anemia score, FEC, or visual appraisal).

Data analyses

Summary statistics of coproculture, LDA, and questionnaire results were calculated, and graphics created in R (R core team, 2018) and its *ggplot2* package (Wickham, 2016). Only questionnaire data from operations with successful LDA results (n = 12) were utilized to analyze the effect of producer response on anthelmintic resistance. These risk factor analyses were conducted using one-way analysis of variance in the GLM procedure of SAS (v. 9.4; SAS Institute Inc., Cary, NC), where $\log_{10}$ EC_{50} and EC_{95} values of each operation were the response variables and analyzed with questionnaire response as the explanatory variable.

Results And Discussion

Parasite identification

Sheep operations categorized by mean EPG measured in their composite fecal sample are displayed in Fig. 2. Forty four percent, 24%, 16%, and 16% had < 500, 500 to 1000, 1000 to 5000, and > 5000 EPG, respectively. Across operations, minimum, median, and maximum composite EPG were 100, 550, and 11,050, respectively. Percentage of genera of larvae identified from coprocultures of composited fecal samples at each operation are displayed in Fig. 3. Overall, *H. contortus* was most commonly identified from fecal samples across operations (average = 68.5%, minimum = 2%, and maximum = 100%) followed by *Trichostrongylus* (12.4%, 0%, and 98%,

Figure 2. Sheep operations categorized by eggs per gram (EPG) as measured in composite fecal samples (n = 25 total) and proportion of operations within each EPG class with > 50% prevalence of *H. contortus*.

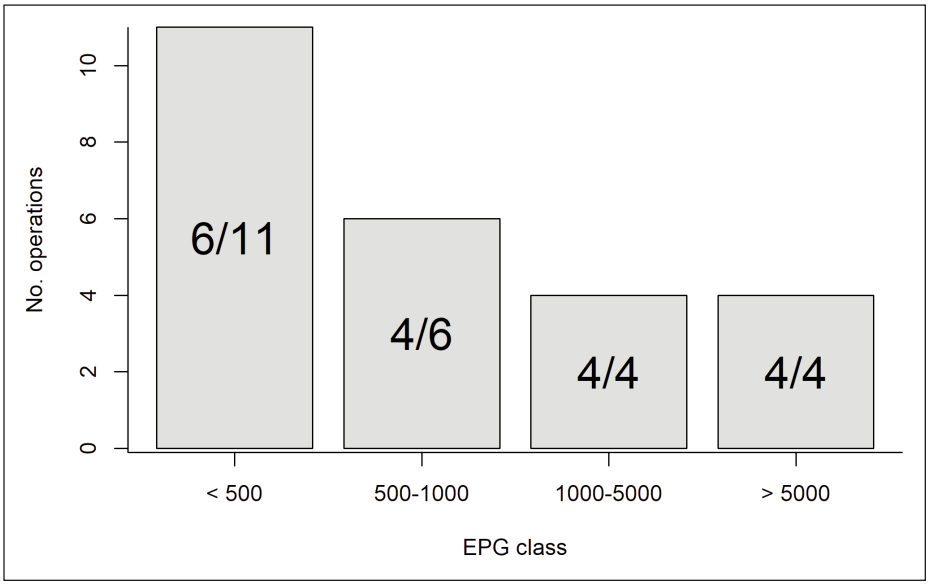
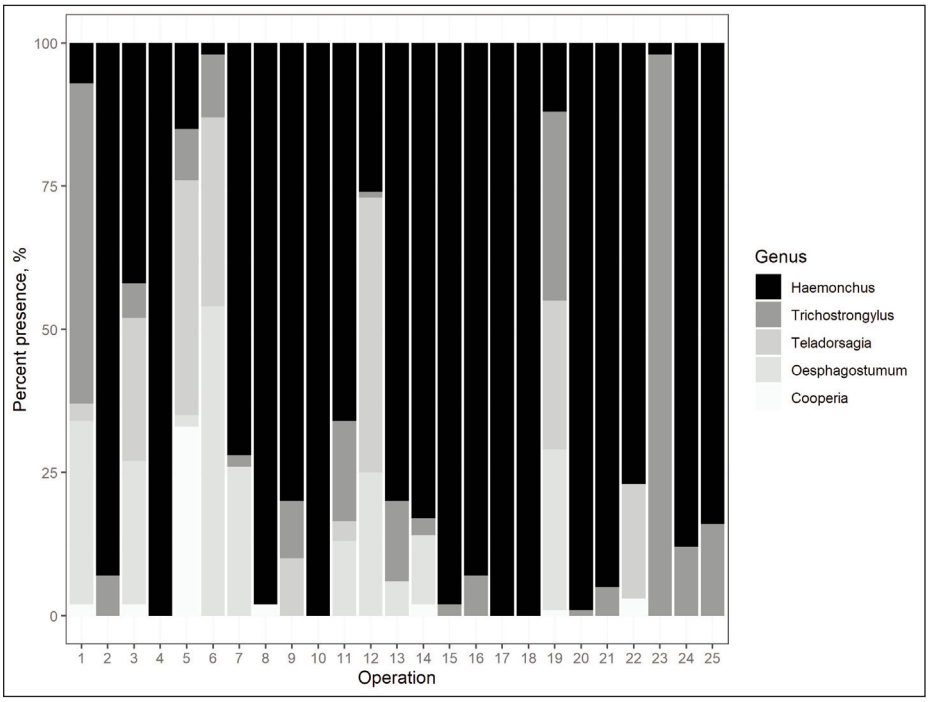


Figure 3. Percentage of the various genera identified within composited fecal samples from each sheep operation.



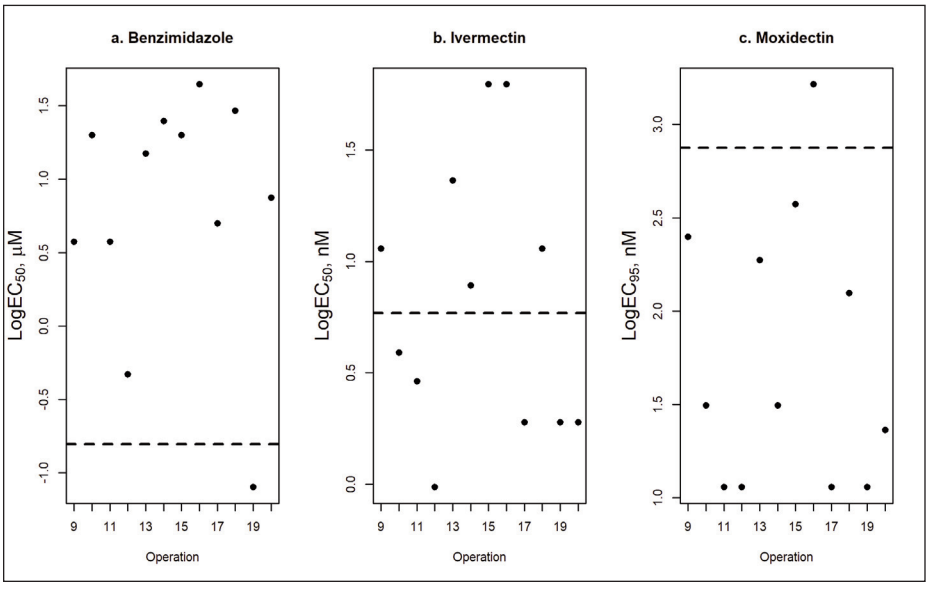
respectively), *Oesphagostumum* (8.9%, 0%, and 54%, respectively), *Teladorsagia* (8.4%, 0%, and 48%, respectively), and *Cooperia* (1.8%, 0%, and 33%, respectively).

Haemonchus contortus represented ≥ 50% of larvae identified from coprocultures on 18 of 25 (72%) sheep operations sampled, similar to reported results from previous field studies in the Mid-Atlantic (79%; Crook et al. 2016) and Ontario, Canada (>80%; Falzon et al., 2013a), but less than that reported from the Southeastern U.S. (96%; Howell et al. 2008). Additionally, the proportion of operations with ≥ 50% *H. contortus* increased with increasing EPG class (Fig. 2). Although *Trichostrongylus*, *Oesophagostumum*, and *Teladorsagia* represented the 2nd, 3rd, and 4th most predominant larvae identified, *Trichostrongylus* only represented ≥ 50% of larvae identified on 2 of 25 (8%) sheep operations, *Oesophagostumum* only represented ≥ 50% of larvae identified on 1 of 25 (4%) sheep operations whereas *Teladorsagia* did not exceed ≥ 50% of species composition in any operation sampled.

Moisture availability and temperature are key determinants for GIN development into infective larvae and subsequent migration and survival. Literature reviewed by O'Connor et al. (2006) summarized necessary temperature ranges for trichostrongylid development from unembroyonated egg to L_3 as 11 to 40°C for *H. contortus*, 6 to 39°C for *Trichostrongylus* spp., and 1 to 35°C for *Teladorsagia* spp. In the current study, historic climate data near all 25 sampled operations met temperature requirements for *H. contortus* development and survival of eggs and infective larvae approximately 135 ± 17 d in a given year, highlighting limitations for *H. contortus* persistence in semi-arid continental climates of the Intermountain West (NOAA, 2020). It is possible that the wide variation in daily temperatures and lower relative humidity of the Intermountain West can be a barrier for eggs to develop into infective larvae and for newly hatched larvae to successfully develop to L_3 stages (Smith, 1990).

Utilization of irrigation systems on sampled operations clearly favored proliferation of GIN and migration of L3 larvae onto plant material even with less than optimal temperature conditions for the survival of eggs and larvae. Studies in the Intermountain West have shown greater abundance and overall survival of *H. contortus* larvae on irrigated pastures compared to non-irrigated pastures where eggs and larvae were more easily desiccated (Bullick and Anderson, 1978). Ideal deferment "rest" periods after initial grazing to minimize reinfection from L_3 GIN, while still maximizing nutritional attributes of pasture, requires additional research in the Intermountain West region.

Figure 4. Approximate $\log_{10}$ EC_{50} and EC_{95} values for *Haemonchus contortus* from each sheep operation (n = 12) under benzimidazole (a), ivermectin (b), and moxidectin (c). The horizontal dashed line corresponds to resistance thresholds (above = resistant, below = susceptible).



Larval development assay

Twelve of 25 operations sampled in

the present study had parasite levels that yielded valid results for the *H. contortus* LDA. Resulting EC_{50} (benzimidazole and ivermectin) or EC_{95} (moxidectin) values for each anthelmintic class and their corresponding resistance thresholds are displayed in Fig. 4. Results indicate widespread *H. contortus* resistance to benzimidazole (92%) while fewer operations exceeded resistance thresholds for ivermectin (50%). Haemonchus contortus isolated from operation 15 exceeded the critical well but not delineating well threshold for moxidectin and would be considered low resistant (Kaplan et al., 2007; Crook et al., 2016), while operation 16 was the only one expected to be fully resistant to moxidectin. Discussion of results for *Trichostrongylus* LDA were limited to 4 operations (11, 12, 19, and 23) due to the minor proportion of this species across operations. *Trichostrongylus* larvae isolated from all 4 operations were susceptible to benzimidazole, while 2 of the 4 operations were resistant to ivermectin (11 and 19).

Estimates of *H. contortus* resistance in the current study, although limited in numbers, provide timely data from a region not previously represented in North American GIN resistance surveys (Howell et al., 2008; Falzon et al., 2013a; Crook et al., 2016). Agreement with these studies showing widespread resistance to benzimidazole is alarming especially considering > 84% of study participants had frequently utilized this anthelmintic class and were continuing to do so until informed by results of LDA. Resistance of *H. contortus* to ivermectin (50%) is similar to estimates from the Southeast and Mid-Atlantic (73 and 64%, respectively). A limitation of the current study is the lack of resistance data regarding imidazothiazoles (levamisoles) which were excluded due to assay quality concerns. Thus, additional resistance research that includes levamisole will help provide complete management recommendations to producers in the region. Resistance to moxidectin (8.3%) in the current study represents a new baseline for this region of the U.S.

Questionnaire

Questionnaire responses regarding GIN management strategies and related production parameters are summarized

Table 1. Summary of questionnaire responses on participating sheep operations.

Question/Response	No. of Respondents	Percentage, %
How is your pasture irrigated?		
Sub-Irrigated	5	31
Flood	6	38
Sprinkler	5	31
What is your grazing system?		
Continuous	7	44
Rotational	9	56
Estimated death loss due to internal parasites?		
0 to 5%	14	87.5
6 to 10%	2	12.5
11 to 15%	0	
Estimated proportion of flock performing poorly due to internal parasites?		
0 to 10%	9	56.3
11 to 20%	5	31.3
21 to 30%	1	6.2
31 to 40%	1	6.2
Do you utilize FAMACHA scoring?		
No	12	75
Yes	4	25
Did you treat with Valbazen® in the previous 5 yr?		
No	3	20
Yes	12	80
Did you treat with Safeguard® in the previous 5 yr?		
No	11	84
Yes	4	16
Did you treat with Ivomec® used in the previous 5 yr?		
No	6	40
Yes	9	60
Did you treat with Cydectin® in the previous 5 years?		
No	8	53
Yes	7	47
Did treat with Prohibit® in the previous 5 years?		
No	13	86.6
Yes	2	13.3
Do you treat with an anthelmintic product until it is ineffective?		
No	10	66.6
Yes	5	33.3
Do you alternate anthelmintic products yearly?		
No	12	75
Yes	4	25
Do you alternate anthelmintic products during the same season?		
No	11	68.7
Yes	5	31.3
Do you utilize combination anthelmintics (i.e., multiple products concurrently)?		
No	15	93.7
Yes	1	6.3

in Table 1. Unfortunately, of the 25 questionnaires distributed only 16 were returned with some questions left unanswered resulting in only 15 responses for certain questions. By study design, participating producers from smaller flocks were surveyed (average = 250 ewes, minimum = 32 ewes, maximum = 1400 ewes), with all operating in irrigated grazing systems predisposed to GIN burdens. Average lamb crop near birth and at weaning were 160% (minimum = 110%, maximum = 195%) and 146% (minimum = 105%, maximum = 180%), respectively. Perceived losses due to GIN infection are an important consideration as they can drive decision making in regard to frequency of treatment and what anthelmintic treatments are utilized. Approximately 88% of producers estimated death loss in the 0 to 5% range with 56% of producers attributing production losses to GIN in 0 to 10% of their flock. These perceptions combined with the fact that 75% of producers did not utilize targeted treatment methods (e.g., FAMACHA©, FEC) would indicate that non-target treatments at set production timepoints are common in the region.

The most commonly utilized anthelmintics on study operations over the past 5 yr were benzimidazoles including albendazole (Valbazen®) and fenbendazole (Safeguard®), 80 and 84% of respondents, respectively. Macrolides (Ivomec® and Cydectin®) were utilized by 60% and 47% of study operations, respectively. Nicotinic (Prohibit®) was the least utilized anthelmintic class over the previous 5 yr (13%). A common misconception with GIN management in the Intermountain West is the indiscriminate rotation of dewormers based on season or previous use rather than the recommended practice of switching products only when they become ineffective. Only 33% of respondents switched anthelmintic drug classes once determined inefficacious, 25% of respondents stated that they switch de-wormers yearly, and 31% reported rotating anthelmintic products within the same grazing season. Only 1 respondent (6.3%) reported using a combination anthelmintic (administration of 2, 3, or 4 anthelmintic drug classes; Kaplan 2017) which has proven to be an effective strategy internationally where widespread resistance to single anthelmintic drug classes is common. Administering combination anthelmintics has shown to reduce the development of resistance when incorporated with best management principles (e.g., refugia and FAMACHA; Leathwick et al., 2015).

Survey data has limited ability to quantify production practices on a fine-scale especially in instances where GIN management is given little thought beyond a routine yearly, whole-flock administration of dewormer. Though not surveyed in the current study, considerations such as accurate dosage based on body weight and calibration of drenching apparatus are not frequently accounted for by producers (Falzon et al., 2013b). Implementation of best management practices on a regional scale has the potential to reduce anthelmintic use and consequently GIN resistance (Learmount et al., 2016). Still, questionnaire data indicated that greater efforts to integrate Intermountain West extension and research efforts with current, research-based recommendations in regard to internal parasite management in small ruminants (e.g., American Consortium for Small Ruminant Parasite Control; *https://www.wormx.info*) are warranted.

Risk factors

As previously discussed, only 1 operation was susceptible to benzimidazoles and only 1 operation was resistant to moxidectin in the LDA for *H. contortus*. Therefore, risk factor analyses were limited to *H. contortus* response to ivermectin only (Table 2). As expected with reduced experimental units (e.g. operations with successful LDA), statistical power necessary to detect anthelmintic susceptibility differences among questionnaire responses was limited and none were significant ($P \geq 0.11$). Therefore, any numerical differences between response classes need to be taken with caution until a larger survey can be conducted.

Overuse of an anthelmintic class will promote GIN resistance more rapidly than targeted administration to the most susceptible animals. A comprehensive meta-analysis by Falzon et al. (2014) calculated ~4 times greater likelihood for resistance to anthelmintics in flocks with high frequency of treatment versus those with low frequency of treatment. Nevertheless, it's interesting to note that of the 6 operations that showed *H. contortus* resistance to ivermectin, 3 of them (9, 16, and 18) reported no past use of commercial ivermectin-containing products. Based on these results it is likely that many smaller flocks in the Intermountain West purchase replacement ewes and rams from flocks with anthelmintic resistance issues. Thus, best management practices when purchasing replacements should include at minimum inquiries of GIN burdens, his-

Table 2. Mean (± SE) $\log_{10}$ EC_{50} for the *H. contortus* LDA under ivermectin corresponding to producer responses to survey questions.

Question	Response (n)[1]	$\log_{10}$ EC_{50} Ivermectin, nM
Irrigation type	Sub (1)	-
	Flood (4)	0.63 ± 0.27
	Sprinkler (4)	1.28 ± 0.27
	Combination (2)	-
Grazing system	Continuous (5)	0.86 ± 0.27
	Rotational (6)	0.92 ± 0.25
Used Ivomec® in previous 5 yr.	Yes (7)	0.78 ± 0.22
	No (4)	1.09 ± 0.29
Used Cydectin® in previous 5 yr.	Yes (5)	1.20 ± 0.23
	No (6)	0.64 ± 0.21

[1] Means for classes with few responses (< 3) were not calculated.

tory of anthelmintic use, estimate of GIN burden (FEC), and quarantining animals upon arrival followed by appropriately treating with an effective anthelmintic.

Literature regarding effects of grazing management system on GIN treatment strategy are nuanced by regional-production dynamics (e.g., conventional vs. organic, forage species, climate, etc.; Burke et al., 2009; Colvin et al., 2012), but unique to the arid, and low precipitation Intermountain West is the ability to manage irrigation schedules to potentially manipulate larval desiccation. Still, other factors such as pasture infectivity, forage species composition, and soil moisture make management recommendations more complex than a time dependent irrigation process. Managing for forage quality while reducing conditions for GIN ingestion need to be jointly optimized. In more humid and tropical regions, recommendations have proposed a standard 3.5 d grazing period followed by a 35-d rest period (Barger et al., 1994). This strategy when tested in the south-central U.S. comparing rotational grazing to continuous grazing resulted in similar lamb weight gains, although continuous grazing required more anthelmintic intervention. Irrigated grazing systems can result in higher GIN burdens (Colvin et al., 2012), however, the ability to manipulate development of egg to the L_3 stage by delaying irrigation once sheep leave a paddock requires more research in the Intermountain West but could be an effective mitigation strategy. Integrated management strategies such as targeted selective treatment, condensed tannin-containing plants, nematode trapping fungi, and vaccines have proved effective in areas of the U.S. most affected by GIN (Terrill et al., 2012) and warrant more research and producer application in the Intermountain West.

Conclusion

Sheep managed on irrigated pasture in the Intermountain West are not exempt to *H. contortus* anthelmintic resistance issues common to the Southeastern and Mid-Atlantic regions. Consistent with published literature, *H. contortus* resistance to benzimidazole, and to a lesser extent, ivermectin and moxidectin were identified. Survey results indicated that breadth of knowledge regarding internal parasite management is limited in the Intermountain West, especially those operations dependent on irrigated pasture resources. Adoption rates of sustainable parasite control practices appear to be lacking and should be used as part of a long-term strategy to combat GIN resistance. Future longitudinal research that quantifies GIN burdens across calendar year and flock size demographics (farm vs range flocks), and the effects of grazing management in irrigated systems can refine future best management practices in the region. Still, data from this field study, although limited by number of observations, has potential to inform and adapt management of GIN in the Intermountain West.

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