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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