EFFECTS OF ISOFLAVONES ON

REPRODUCTIVE FUNCTIONS OF RAM LAMBS

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ABSTRACT

A study was conducted to determine if the isoflavones found in soybean-based proteins were a detriment to ram lamb production and/or rate to puberty. New born Rambouillet and Suffolk ram lambs were randomly assigned either to the treatment (n=20) or control group (n=19). Treatment received soybean based feed from creep to finishing, while the control received cottonseed meal based feed. At weaning, serum samples and weights were collected to evaluate estradiol and testosterone levels. At an average of seven months of age, blood samples, final weights, semen samples and scrotal circumference measurements were taken. Scrotal circumference and weaning weight were decreased ($p<0.05$) and in general no other aspect was affected ($p>0.05$). However, numerically semen counts tended to be reduced. Additionally, estradiol was found to be higher at weaning ($p>0.05$) in the treatment and lower at final collection, testosterone acted inversely.

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INTRODUCTION

In the ever-changing and ever-progressing livestock industry, higher quality and more uniform offspring are being produced and destined to both terminal end markets, as well as the seed stock sector. An abundance of new available feed stuffs and proper utilization of these feedstuffs are of critical importance to all producers. This is of special importance to the seed stock producers and those who produce their own replacements. Different nutrient sources work to maximize different areas of production. For instance, what may be more beneficial to females is not always beneficial to their contemporary male siblings.

Seed stock producers raise animals that garner a higher price due to the higher quality associated with the animals. Studies have been performed to help determine a feedstuff that will help female offspring reach puberty at an earlier age. One such study performed by Ede et al. (2011) showed that ewe lambs fed diets rich in soy exhibited higher estradiol levels, which would insinuate quicker and more responsive reproductive functions. In order to determine the effects of soy proteins on male progeny, a study was conducted testing soy affects on ram lambs starting at an early age lasting until weaning.

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OBJECTIVES

1. Determined the effects of soy isoflavones on early life development in ram lambs from birth to post weaning
2. Determined if soy isoflavones are a delay to ram lamb puberty

LITERATURE REVIEW

Soy-based feeds are known to be high in phytoestrogens, plant derived compounds exhibiting properties similar to estrogens (Ogbuewu, 2010). Soy compounds are the most abundant source of phytoestrogens, both in animal and human feedstuffs. Soy isoflavones are non-steroidal compounds which are structurally similar to estradiol-17β (Ogbuewu, 2010). As isoflavones are structurally similar to estrogens they bind to the receptor sites of estradiol-17 β and mimic its effects. While the structure is not exactly the same as estrogen, the response to isoflavones results in similar tissue changes (Schneider, 2005).

Sellable productive male offspring begin their useful stage in life shortly after puberty. Puberty in the ram lamb can be described as reaching full testicular descent, full penile separation, and spermatogenesis with body weight playing a strong role in determination of puberty (Madani et al., 1989). Lambs born in the spring are often sexually mature by fall and used for breeding purposes. Furthermore, nutrition has been found to play an important role in determining the onset of puberty in ram lambs. Mukasa-Mugerwa and Ezaz (1992) concluded that post-weaning nutrition management had a strong influence on lamb weight gain, which in turn, was related to testicular growth and puberty onset in Menz ram lambs.

Supplemented or total mix ration feeding also affects on scrotal circumference in ram lambs. Fourie et al. (2004), divided dorper ram lambs were into intensive management and extensive management conditions. The intensive management lambs were feed an *ad libitum* diet for seven weeks while the extensively managed lambs were maintained on native pasture with lick supplements. The intensive managed lambs had larger scrotal circumferences, but they exhibited lower semen characteristics because of excess fat reserves found within the scrotum.

Other inhibitory factors to male reproductive function include estrogen and estrogen like compounds at higher than normal levels. While estrogen is important for the development of the male reproductive tract, excessive amounts will lead to underdevelopment and malformation of the male reproductive tract. It has been known as early as the 1930s that developmental exposure to a high dose of estrogen causes malformation of the male reproductive tract (Hess 2003). However, Mitchell et al. (2001) reported that soy supplementation in humans had no effect on gonadotrophin or sex hormone levels or on semen quality. While the study did not take into affect long term or higher dosage considerations, it was found that in the short term soy supplementation had no detrimental effect.

Conversely, soy-based proteins have been found to have effects on the female gender. Excessive levels of soy in the females' diet have been found to cause early development of female secondary sex characteristics and potentially premature development of reproductive organs. If premature development occurs in the reproductive tract of the female, then she will experience cyclicity at an earlier age than normal (Tan et al., 2006). Daniel (2004) reports that human girls consuming high levels of soy isoflavones exhibited signs of puberty by age three, well before puberty is normally obtained in humans. Additionally Ede et al. (2011) reported that ewe lamb fed diets high in soy had a substantial difference of circulating estrogen than that of the ewes on the control diet. These diets high in isoflavones could potentially lead to early puberty in the ewe lamb.

With soy isoflavones, puberty in ram lamb, and estrogen effects on male reproduction defined, the study attempted to tie all three together. The effects found in females logically leads to the next question of what are the effects on the male. If a negative influence is found, seed stock producers will need to manage their male offspring differently than they would their female offspring. If no difference or a positive influence is found, male progeny can be run with female offspring thus decreasing the event of mis-feeding lambs and decreasing input costs associated with carrying different varieties of feed.

MATERIALS AND METHODS

New-born Rambouillet and Suffolk ram lambs were randomly assigned to one of two treatments when moved from lambing facilities at 14 d of age. Lambs were assigned randomly to a treatment by splitting ewes in an alternating fashion and placing their lambs in respective treatment groups. The two treatments for the study are 1) control: cotton seed meal protein (n=19) 2) treatment: soy bean meal protein (n=20; Table 1). Lambs and ewes were placed in wheat fields that contain sections of native range lands.

Creep feeders were placed near water in each pasture containing the diet specific to the treatment for lambs to consume free choice. Lambs remained on creep feeders with treatment specific diets until weaning. At weaning, blood samples were collected for analysis of estrogen levels. After weaning, ram lambs were placed into pens and fed 3.5% of body weight of the diet specific to the treatment (Table 1). Lambs also had *ad libitum* access to water, shade, and haygrazer hay.

Weights and blood samples were collected at weaning and at approximately seven months of mean lamb age. Blood was centrifuged at 2000 x g for 30 minutes to separate serum. Blood was collected using jugular veinapuncture methods into corvac serum separator tubes. Serum samples were frozen at -80°C then shipped to New Mexico State University's endocrinology laboratory. Serum was analyzed for estradiol and testosterone concentrations. At seven months of mean age, the rams also underwent a breeding

Table 1. Ingredient Composition of Diets Used, as fed basis

	Creep		Post Weaning	
Ingredient %	Soy	Control	Soy	Control
Milo	64.36	60.39	44.33	44.33
Soybean Meal	9.90	---	16.75	---
Cottonseed Meal	---	14.49	---	14.78
Cottonseed Hulls	---	---	29.56	21.67
Alfalfa Pellets	19.80	19.32	2.46	12.32
Molasses	3.46	3.38	4.44	4.44
Premix	2.48	2.42	2.46	2.46

soundness exam measuring scrotal circumference and sperm cell concentration. The breeding soundness exam was conducted by suspending the rams slightly off the ground, measuring the scrotum at the thickest point using a scrotal circumference tape, and semen collection via electro ejaculation. Sperm cell concentration count was determined using an Animal Reproductive Systems densimeter, model 591 B.

Weight, serum, scrotal circumference, and sperm cell concentration data was analyzed using GLM procedures of SAS (Cary, NC) with each individual animal serving as the experimental unit and breed serving as a block. Treatments were considered different when $p \leq 0.05$.

RESULTS AND DISCUSSION

Estrogen

No breed X treatment interactions were detected. Therefore, only main effect means are presented. Estrogen levels for the two groups at weaning and final collections were found to not differ significantly, as expected based on Weber et al. (2001) finding that estradiol levels were statistically indifferent in soy based diets. Weaning estrogen levels of 7.44 ng/mL for the control and 10.31 ng/mL for the treatment were found with a standard error of 5.24, while final levels of 12.33 ng/mL and 6.36 ng/mL (respectively) with a standard error of 2.59 (Table 2). This differs from the expected, as the estrogen levels are higher in the treatment at weaning but are higher at the finish in the control, potentially caused by a change in the timing of puberty. Other unexpected blood works reveals that the two highest weaning (115 and 73 ng/mL) and final (53 and 49 ng/mL) estrogen levels came from the control group.

Testosterone

Circulating testosterone levels have been found to be lower in soy based diets (Weber et al. 2001). Testosterone levels were evaluated at weaning and at seven months of age. In general, testosterone levels for the two groups were not found to be significantly different. Weaning testosterone levels of 1.71 ng/mL for the control and 1.34 ng/mL for the treatment were calculated with a standard error of 0.29 with final collections of 2.75 ng/mL and 3.33 ng/mL (respectively) with a standard error of 0.61 (Table 2). This follows Weber's 2001 experiment but was statistically indifferent. However, a breed interaction was found

Table 2. Hormone Analysis for Weaning and Final, ng/mL

Hormone	Control	Treatment	Standard Error[a]	P-value
Estrogen, Weaning	7.44	10.31	5.23	0.70
Estrogen, Final[b]	12.33	6.36	2.59	0.11
Testosterone, Weaning	1.71	1.34	0.29	0.36
Testosterone, Final[b]	2.75	3.33	0.61	0.50

[a]Most conservative standard error of the least squares mean
[b]Final measurements were at an average age of 7 months

in the final testosterone reading. The Rambouillet ram lambs were found to be lower in circulating testosterone, 1.29 ng/mL for the control and 2.08 ng/mL for the treatment, than the Suffolk lambs of either study, 4.58 ng/mL and 5.05 ng/mL, respectively (Table 3). Upon further analysis, the interaction was determined to be solely affected by breed as the Rambouillet lambs averaged 1.7 ng/mL to the Suffolks 4.81 ng/mL (Table 4).

Table 3. Hormone Analysis for Breed X Treatment Interactions Measured in ng/mL

	Control		Treatment			
Hormone	Rambouillet	Suffolk	Rambouillet	Suffolk	Standard Error[a]	P-value
Estrogen, Weaning	12.20	1.50	16.18	2.25	7.75	0.39
Estrogen, Final[b]	13.40	11.00	7.45	4.87	3.98	0.40
Testosterone, Weaning	1.80	1.60	1.21	1.51	0.44	0.76
Testosterone, Final[b]	1.29 [c]	4.58 [d]	2.08 [c]	5.05 [d]	0.74	0.0009

[a]Most conservative standard error of the least squares mean
[b]Final measurements were at an average age of 7 months
[c,d]Means in the same row with differing superscripts are different at the indicated p-value

Table 4. Hormone Analysis for the Breed Interactions Measured in ng/mL

Hormone	Rambouillet	Suffolk	Standard Error[a]	P-value
Estrogen, Weaning	14.28	1.87	5.34	0.0886
Estrogen, Final[b]	10.28	7.93	2.84	0.5376
Testosterone, Weaning	1.49	1.55	0.31	0.8823
Testosterone, Final[b]	1.7 [c]	4.81 [d]	0.56	< 0.0001

[a]Most conservative standard error of the least squares mean
[b]Final measurements were at an average age of 7 months
[c,d]Means in the same row with differing superscripts are different at the indicated p-value

Sperm Cell Concentration

No breed X treatment interactions were detected, therefore main effects are presented. Chavarro et al. (2008) found that in human males lower sperm cell concentration could be attributed to high soy diets. However, sperm Cell concentration was found to be indifferent (Table 5) while there was a numeric difference of the average of the two groups in favor of the control. They were found to be indifferent due to a large standard error within each group. Additionally, the highest sperm cell concentrations belonged to ram lambs from the soy group ($3.28x10^9$ and $3.15x10^9$). While different from Chavarro, numerically the study behaved as expected.

Scrotal Circumference

In the study by Tan et al. (2006), soy based formula, have had no adverse affects on the male reproductive tract of marmoset monkeys, and in fact led to larger testis size. This is a complete disparity from the ram lambs as scrotal circumference between the two groups was found to be different ($P<0.05$) with the control having the larger average scrotal circumference at 30.5 cm verses the treatment average scrotal circumference of 28.8 cm (Table 5). Additionally, there was as Breed X Treatment interaction, as the Suffolks from the treatment were smaller (27.5 cm) than those of the control (29.2 cm; Table 6). However, the two breeds were found to be different in terms of scrotal circumference as the Rambouillets were larger at 30.7 cm to the Suffolks 28.3 with a standard error of .57 cm and a p-value of 0.005 (Table 7).

Table 5. Sperm Cell Concentration, Scrotal Circumference and Weights

	Control	Treatment	Standard Error[a]	P-value
Sperm Cells, $x10^8$	10.1	8.2	2.17	0.54
Scrotal Circumference, cm	30.5 [c]	28.8 [d]	0.57	0.04
Weaning Weight, kg	39.2 [c]	32.2 [d]	2.12	0.03
Final Weight, kg[b]	63.4	59.9	2.22	0.27

[a]Most conservative standard error of the least squares mean
[b]Final measurements were at an average age of 7 months
[c,d]Means in the same row with differing superscripts are different at the indicated p-value

Table 6. Breed X Treatment Interaction on Sperm Cell Concentration, Scrotal Circumference and Weights

	Control		Treatment			
	Rambouillet	Suffolk	Rambouillet	Suffolk	Standard Error[a]	P-value
Sperm Cells, $x10^8$	10.9	9.2	10.4	6.3	3.3	0.58
Scrotal Circumference, cm	31.1[c]	29.2[cd]	30.2[c]	27.5[de]	0.84	0.02
Weaning Weight, kg	45.3[c]	30.9[d]	37.1[c]	26.8[d]	2.43	0.0001
Final Weight, kg[b]	69.9[c]	55.5[d]	67.1[c]	53.3[d]	2.34	0.0001

[a]Most conservative standard error of the least squares mean

[b]Final measurements were at an average age of 7 months

[c,d,e]Means in the same row with differing superscripts are different at the indicated p-value

Table 7. Breed Interaction on Sperm Cell Concentration, Scrotal Circumference and Weights

	Rambouillet	Suffolk	Standard Error[a]	P-value
Sperm Cells, $x10^8$	10.7	7.6	2.20	0.30
Scrotal Circumference, cm	30.7 [c]	28.3 [d]	0.57	0.0051
Weaning Weight, kg	41.4 [c]	28.7 [d]	1.82	<0.0001
Final Weight, kg[b]	68.6 [c]	54.3 [d]	1.56	<0.0001

[a]Most conservative standard error of the least squares mean
[b]Final measurements were at an average age of 7 months
[c,d]Means in the same row with differing superscripts are different at the indicated p-value

Growth and Performance

In general, as indicated by the final weights (Table 5), the treatment had no effect on the ram lambs potential for growth and performance, while the treatment had a negative effect on the weaning weights of the ram lambs (P<0.05). However, when breed X treatment interactions were evaluated, both weaning and final weights were altered by the treatment. Larger lambs were obtained of both breeds on the control at weaning (45.3 kg for Rambouillet and 30.9 kg for the Suffolk vs. 37.1 kg and 26.8 kg, respectively) as well as at the final weight (69.9 kg and 55.5kg vs. 67.1 kg and 53.3 kg). In both instances a P-value less than 0.0001 was obtained (Table 6). This again had breed only implications (P<0.0001) as the Rambouillet rams were larger, both at weaning and the final at 41.4 kg and 68.6 kg while the Suffolk rams were 28.7 kg and 54.3, with a standard error of 1.82 kg and 1.56 kg (Table 7). The differences at weaning come as a surprise as the previous study by Ede et al. (2011) involving soy proteins suggests that feeding soy will have no effect on weight gains and performance. Expected weight gains and performance in these ram lambs would have mimicked those in the ewe lambs; however, the difference comes as the rams were fed soy based rations prior to weaning.

IMPLICATIONS

While, in general, the treatment seems to not be a detriment to final spermatic activity or final weights, it is a may be problem for scrotal circumference and weaning weights. Additionally, the change of estrogen levels from higher to lower and lower to higher in testosterone for the treatment leads to one believe that puberty may be altered (pushed back or forward), just not at the times tested. While not statistically different due in part to a large variation in specimen collects, the lower numeric semen count is troubling for the producer who may be selling breeding male offspring. Going forward I would recommend more frequent blood testing, scrotal measurements, and semen counts. Additionally, I believe that the long term implications need to be determined, as the treatment obviously affected scrotal circumference. Another way to improve upon the project would be a tighter age group, which is more consistent in its genetic makeup and lineage.

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