

EVALUATION OF DIETARY PHYTOESTROGEN EXPOSURE ON GROWTH, SEMEN  
PARAMETERS, AND REPRODUCTIVE ANATOMY DEVELOPMENT OF GROWING  
ANGUS BULLS

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## ABSTRACT

This study evaluated effects of phytoestrogen on scrotal growth and semen quality in bulls. Spring-born, Angus bulls were used in consecutive years (n = 39, year 1; n = 24, year 2) and stratified by weaning weight, age of dam (AOD), and sire. Dietary treatments included soybean meal (SBM) or cottonseed meal as protein source (CSM). At weaning, bulls were randomly assigned to treatment. Scrotal circumference was measured on d 0, 21, 54, and 86 and semen quality assessed on d 86. A treatment  $\times$  year interaction was detected for circumference ( $P \leq 0.05$ ). Scrotal growth d 0 to 21, 54 to 86, and 0 to 86 was greater for SBM both years ( $P \leq 0.05$ ). Semen concentration from younger cows was higher ( $P \leq 0.03$ ) with SBM both years whereas CSM in 5+ older dam concentration was greater in year 2 ( $P < 0.0001$ ). A diet  $\times$  AOD (year) interaction influenced motility ( $P < .0001$ ). Phytoestrogens from soybean meal improved scrotal growth and semen quality particularly with younger dams.

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## INTRODUCTION

Although soybean meal is a viable source for protein in livestock, it contains phytoestrogens classified as isoflavones that may impact or modify the reproductive function in males (Cederroth et al., 2009). Isoflavones are polyphenolic compounds that can exert estrogen like effects on the body and are derived principally from soybeans and clover (Cardoso and Bao, 2007). There are two specific isoflavones; genistein and diadzein which exert some of the most potent estrogenic hormone activity (Lephart et al., 2004). Estrogen is important in the regulation of the male reproductive tract and has direct effects on leydig cells and efferent ductule epithelium with potential effects on germ cells. Estrogen receptors are abundant throughout the body and reproductive tract, but are even more localized in the efferent ductule epithelium where sometimes the presence of estrogen is even more pronounced than in the female reproductive tract (Hess and Carnes, 2004). Hess (2003) observed that estrogen is found in abundance in the testis, rete testis fluid, and semen of many species such as ram, bull, stallion and boar. Therefore, a disruption of the estrogen receptors can have adverse effects on sperm production and morphology.

There is currently little published data that explains the effects of the soy based isoflavones on ruminant livestock, in particular pre-pubertal bulls. The objective of the current study is to investigate the effects of phytoestrogens in dietary soybean meal on growth, scrotal measures, and semen quality measures in growing bulls.



## LITERATURE REVIEW

### ***BULL DEVELOPMENT PROGRAMS***

Development of bulls, and effective growing bull programs, serve an important role in the US beef cattle industry. In 2017, it was estimated that there were 40.9 million beef cows in the US (USDA, NASS, 2016). It is also reported that only 7.6% of all beef cow operations in the US utilize the reproductive technology known as artificial insemination (NAHMS, 2009). Accordingly, there is still a pronounced need for the reproductive service ability of traditional bull exposure for a vast majority of the US beef cow sector. Bull development programs are imperative to afford growing bulls the opportunity to grow and develop maturity in order to reach puberty. Puberty is defined as the time at which a young bull can produce semen with  $50 \times 10^6$  spermatozoa/mL and at least 10% of the sperm are progressively motile (Arteaga et al., 2001).

The nutritional requirements of growing bulls are much higher than that of mature bulls (Geay, 1982). As young bulls grow, they first meet the nutrient requirements necessary to maintain their current body weight. Once maintenance requirements are met, remaining available nutrients contribute to the growth of the bull. In order to allow pre-pubertal bulls to reach sexual maturity more rapidly, west Texas ranchers have the option to feed higher energy concentrated rations. Pruitt et al. (1986) found that some breeds fed higher levels of energy obtain larger scrotal circumferences and maintained those advantages even after being turned out on pasture. Barth et al. (2008) states that reduced nutrient availability during early calf growth suppresses luteinizing hormone (LH) secretion during early gonadotropin development and delays puberty and development of testicular size. If the requirements are not met, growing bulls can exhibit reduced muscle growth as well as permanently impaired

sperm production (Hafts et al., 1959; VanDemark et al., 1964). Barth et al. (2008) states that weight of the testes, epididymis, and seminal glands were significantly reduced in bulls fed protein-deficient rations. As well, bulls consuming a higher quality protein diet had greater measures of body weight, scrotal circumference (SC), total sperm motility, and higher ejaculate concentrations in bulls after 12-14 months of age (Barth et al., 2008). Research conducted by Kastelic (2014), observed that bulls receiving rations on a higher plane of nutrition achieved larger testes and earlier puberty as compared to bulls that received lower planes of nutrition. Scrotal circumference is used as a standard to evaluate sexual maturity. Pruitt et al. (1986) states that utilizing scrotal circumference as a selection tool will increase reproductive potential because larger scrotal measures lead to a greater volume of sperm production and a higher quality semen sample that can be obtained at a younger age. Arteaga et al. (2001) summarized that qualitative semen traits have shown improvement with bull age, from 12-16 months in *Bos taurus* bulls and 14-18 months in *Bos indicus* influenced bulls.

While the previous discussion illustrates the potential negative effects of inadequate nutrient availability, excessive feeding of high-energy diets beyond 12 months of age can also cause deleterious effects on semen quality. Barth et al. (2008) explains the potential of excessive fat deposits around the scrotum that may interfere with temperature regulation which can, in-turn, interfere with sperm production and breeding potential. The focus of the current study however, is investigating the relationship of bull development parameters and dietary protein ingredients.

## ***PROTEIN SOURCES***

Considering protein sources to be utilized in a high energy diet can depend on price and availability. In west Texas, cottonseed meal (CSM) is a readily available and cost effective protein source due to the amount of cotton grown in the region, and consequently a large supply of cotton by-products. Certain situational circumstances, such as drought or policy changes that cause reduced cotton planting and farming, can limit the availability of CSM and thereby increases the price. During times when CSM prices are high, other protein sources can be utilized in a developing bull diet and may be acquired at more cost conservative price points.

Soybean meal (SBM) is a by-product of soybean oil extraction (Erickson, 2015). Although SBM is high in crude protein and can be used as an alternative source for protein, it contains the most abundant source of isoflavones (Setchell and Cassidy, 1999). Clarkson et al. (1995) states that there are over 300 plants that have been found to possess estrogenic activity; these compounds have been given the general name of phytoestrogens and represent several chemical classes. Isoflavones are polyphenolic compounds classified as phytoestrogens that are plant derived and are similar in chemical structure to mammalian estrogens (Setchell and Cassidy, 1999). This explains why phytoestrogens can disguise themselves to estrogen receptors, causing an increase in estrogen production in the body, and consequently may cause an adverse effect on male fertility (Cederroth et al., 2009). Isoflavones migrate with the protein fraction of the soybean during its processing, which explains why there is less of a concern with isoflavones in human Western diets, but there is still concern when considering the use of soy in livestock feeds (Setchell and Cassidy, 1999).

Historically, animal scientists believed that estrogen was female specific. Hess and Carnes (2004) explain the vital role of estrogen in normal bull fertility as estrogen regulates the fluid reabsorption in the efferent ductules. Estrogen has also been observed to have a significant impact on establishing sertoli cell function in developing testes (Hess and Carnes, 2004). Hess (2003) found that any disruption of the many estrogen receptors can cause disruption of sperm morphology and lead to decreased fertility. Cederroth et al. (2009) also states that higher levels of exposure to phytoestrogens over a lifetime, or at critical periods of development, could have detrimental effects on fertility and reproductive function. These concerns have led cattle producers to question the use of SBM as a protein source in bull diets and unfortunately, little published data is available that investigates the impact of estrogen like compounds in ruminant livestock. Therefore, the current study was designed to evaluate the impact of dietary phytoestrogens on developing bull programs by investigating the effects of SBM diet inclusion on growth and semen quality parameters in Angus bulls.

## MATERIALS AND METHODS

All animal handling procedures and data collection methods were approved by the Angelo State University Institutional Animal Care and Use Committee (AUP # 14-05). Data used in this study was collected from spring born, pre-pubertal Angus bulls (n = 63) which represent 2 consecutive, but independent, years of data collection (2014: n = 39; 2015: n = 24). In each year, bulls were stratified by weaning weight, age of dam (AOD), and sire across 1 of 2 project diet groups: a phytoestrogen consuming group with 10% soybean meal inclusion as a diet protein source (SBM), and a phytoestrogen naïve group with 10% cottonseed meal inclusion as a diet protein source (CSM). The same study rations were used in both years with weekly batch samples being composited across both years for nutrient composition analysis. Study diets were formulated, and confirmed to be isocaloric and isonitrogenous with all bulls having *ad libitum* access to fresh water supply throughout the duration of the observation days during each year. Trial ration information is presented in Table 1.

**Table 1.** Ingredient and nutrient composition of CSM ration and SBM ration

<b>Ingredient</b>	<b>CSM Diet %</b>	<b>SBM Diet %</b>
Cracked Corn	30	30
Cottonseed meal, dry	10	0
Soy Bean meal, dry	0	10
Corn Gluten Feed, pellets	15	15
Cottonseed Hulls, dry	18	22.5
Alfalfa Pellets	20.5	16
Molasses	4	4
<sup>1</sup> Premix	2.5	2.5
<b>Nutrient, DM</b>	<b>CSM Diet</b>	<b>SBM Diet</b>
DM	88.61	88.85
Crude Protein	17.62	17.92
NEm Mcal/CWT	65.18	64.77
NEg Mcal/CWT	40.51	40.58
Crude Fat	3.82	3.78
ADF	24.2	24.19
NDF	35.64	35.26
Calcium	0.96	0.92
Phosphorus	0.47	0.43

<sup>1</sup>Premix: 17.5-19% Ca, 18.1-20.6% NaCl, 1075 ppm Mn, 1780 ppm Zn, 3.95 ppm Se, 89,187.09 IU/kg Vitamin A, 29,728.03 ppm Vitamin D, 493.83 ppm Vitamin E.

At d -42 in each year, all bulls were weaned, weighed, then sorted and comingled into their respective diet groups. All bulls were initially fed the cottonseed meal diet for backgrounding and concentrate diet adaptation purposes. At d 0, basal scrotal measurements and weights were collected and the SBM group was gradually transitioned to the SBM treatment diet. Scrotal circumference measures and weights were then observed again at d 21 and 54. On d 79 an initial semen sample was collected via electro-ejaculator and discarded to verify a reproduction tract dispel of all bulls. At d 86, final weights and scrotal circumferences were documented, semen samples were collected via electro-ejaculator, and semen quality characteristics were recorded. Semen motility parameters were subjectively

assessed utilizing the same trained personnel in both observation years using a 5-point scale as described in Table 2.

**Table 2.** Semen grading scores for semen motility parameters

Scale	Grade	Characteristics
5	(+++++) Excellent	More than 80% of the sperm show vigorous motion. Swirls are formed due to the movements of the sperm. The movements are rapid and changing and hard to observe individual sperm samples in undiluted semen.
4	(++++) Very good	About 70-80% of the sperm show vigorous motion which causes waves and eddies but not as vigorous as the excellent grade.
3	(+++)	About 45-70% of the sperm are in motion. Motion is vigorous. Waves and eddies are formed slow across the sample.
2	(++) Fair	30-40% of the spermatozoa are in motion. Movements are vigorous. No waves or eddies present.
1	(+) poor	Little to no mobility found. < 20% of the spermatozoa are in motion. Not progressive and little oscillation.

Adapted from Hossain et al. (2012). This table illustrates the measure of motility of semen samples presented in this report.

Semen concentration measures were counted via Dupree model 591B densimeter and recorded as the number of spermatozoa per milliliter at the time of ejaculation. Trial procedures and day of the trial across the respective year is presented in Table 3.

**Table 3.** Dates for procedures and associated day of the trial across years

Procedure	2014 – 2015		2015 – 2016	
	Day	Date	Day	Date
Wean, collect weights, concentrate diet adaptation	-42	10/01/2014	-42	09/30/2015
Collect weights, Scrotal Measure, treatment diet begins	0	11/12/2014	0	11/11/2015
Collect weights, Scrotal Measure	21	12/03/2014	21	12/02/2015
Collect weights, Scrotal Measure	54	01/05/2015	54	01/04/2016
Collect weights, Scrotal Measure, Semen evaluation	86	02/06/2015	86	02/05/2016

### ***STATISTICAL ANALYSIS***

Data from both years was compiled and mixed model procedures of SAS (v. 9.2; SAS inst, Inc., Cary, NC) were used to analyze weights and scrotal circumferences with a model that includes the fixed effects of day(nested within year), year (2014 or 2015), diet (SBM or CSM), age of dam (2, 3, 4, 5+), sire, d -42 weight as a covariate, and two-factor interactions. These models were analyzed as repeated measures with a first order autoregressive covariance structure. Changes in scrotal circumference (SC) were calculated by time period (Period 1 scrotal growth = difference of d 0 SC and d 21 SC: Period 2 scrotal growth = difference of d 21 SC and d 54 SC: Period 3 scrotal growth = difference of d 54 SC and d 86 SC; POSTWEAN SC Growth = difference of d 0 SC and d 86 SC). Average Daily gain (ADG) was also calculated within the same time periods. Scrotal growth within time periods, ADG, semen concentration (transformed to a log base of 10 for analysis), and semen



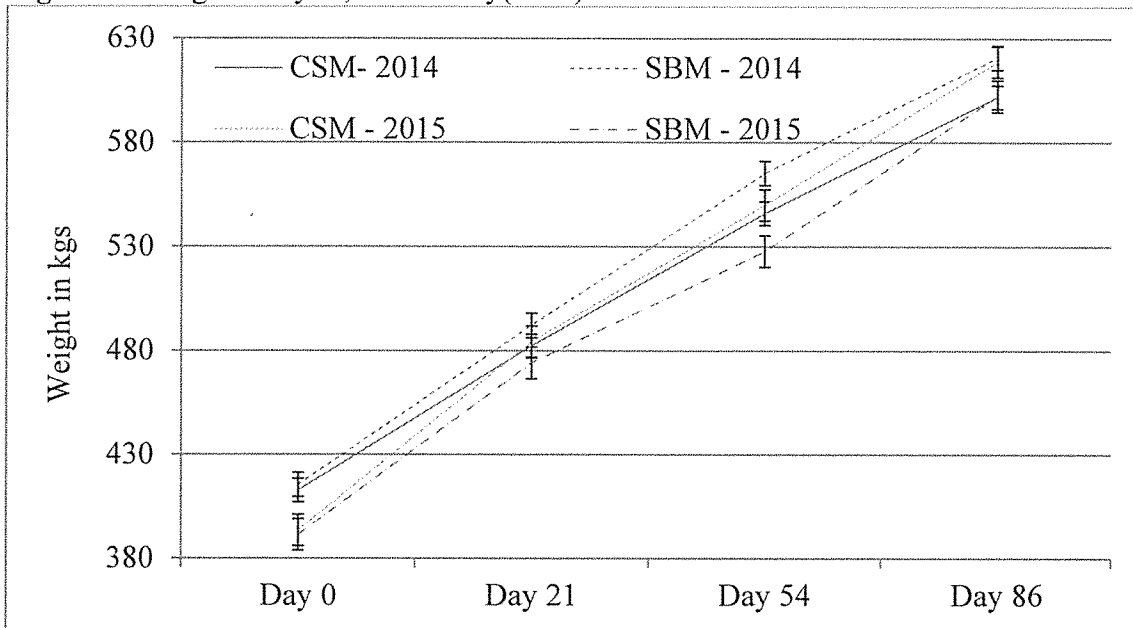
motility scores were analyzed using a similar model but excluding the repeated measures statements. In all models, main effects with ( $P \geq 0.15$ ) and interacting terms with ( $P \geq 0.25$ ) were removed from final analysis. Least squares means were separated using / PDIFF option and  $P$  – values  $\leq 0.05$  were considered different.

## RESULTS

### ***BULL GROWTH***

The Diet × Day(Year) interaction on body weight is shown in Figure 1.

**Figure 1.** Weight analysis, Diet × Day(Year)



Because the rations were isonitrogenous and isocaloric, few differences in weight were observed between diets within each day with respect to each year. This can be attributed to the weight stratification process prior to start of the study. The single difference that was observed due to diet within day was observed in the 2015 trial where the CSM bulls were heavier ( $P = 0.03$ ) than the SBM bulls at day 54 (549.96 kg and 527.79 kg, respectively).

Average daily gain is a common measure of growth rate performance across multiple species in the animal science discipline. In these data, the single term in the model that

yielded significant results was the interaction of Diet × AOD(Year) in the Postwean ADG analysis, and these results are presented in Table 4.

**Table 4.** Postweaning ADG Diet × AOD(Year)

AOD	2014				2015			
	CSM	SBM	SEM	<i>P</i> =	CSM	SBM	SEM	<i>P</i> =
2	2.04	1.95	0.06	0.1516	1.93	1.78	0.13	0.2469
3	<sup>a</sup> 1.45	<sup>b</sup> 2.20	0.10	<.0001	1.92	2.02	0.07	0.1867
4	<sup>a</sup> 1.89	<sup>b</sup> 2.02	0.06	0.0293	<sup>x</sup> 2.67	<sup>y</sup> 1.87	0.13	<.0001
5+	<sup>a</sup> 1.94	<sup>b</sup> 2.14	0.05	<.0001	<sup>x</sup> 2.01	<sup>y</sup> 1.88	0.05	0.0118

Table 4. Least squares means of ADG for the Diet × AOD(Year) effect.

<sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2014 diet effects within respective AOD.

<sup>x, y</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2015 diet effects within respective AOD.

In 2014, bulls produced from 3, and 5+ year old cows achieved a higher average daily gain when fed the SBM ( $P < 0.0001$ ), as well as the bulls produced from 4-year old cows ( $P = 0.0293$ ). However, there were no differences in bulls produced from 2-year old cows in 2014. Interestingly, in 2015, ADG was not affected across diet groups in bulls produced from 2, and 3-year old cows. The CSM bulls produced by 4 and 5+ year old cows had higher ADG ( $P < 0.0001$ ,  $P = 0.0118$  respectively).

Although differences in ADG were observed with the Diet × AOD(Year) term, a consistent pattern of superior growth rate was not clearly evident in these data due to the variation within year. Therefore, it is challenging to declare a definitive ADG advantage to the protein source of the diet.

### **SCROTAL CIRCUMFERENCE**

Differences in least squares means (LSMEANS) for scrotal circumference (SC) measures were observed at d 54 and d 86 for the Diet × Day(Year) interaction. A similar increasing pattern for all diet by year combinations across days was observed, and it is of

interest that at day 54, the SBM in 2014 recorded higher SC means as compared to SBM and CSM in 2015 ( $P = 0.05$  and  $P = 0.03$  respectively). At day 86 however, differences in SC were only detected between the SBM in 2014, which was higher than SBM in 2015 ( $P = 0.05$ ). These results are presented in Figure 2.

**Figure 2.** Scrotal Measures, in cm for Diet  $\times$  Day(Year)

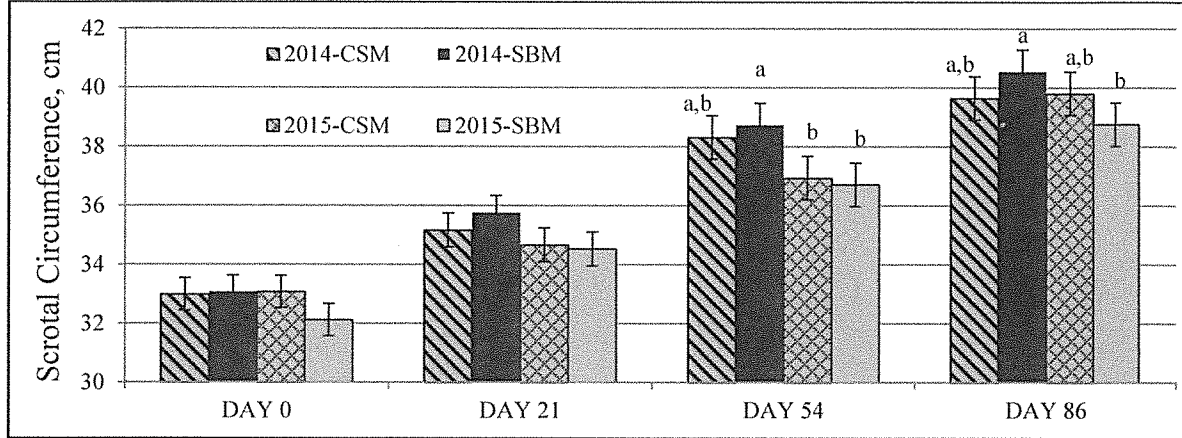


Figure 2. Least squares means for Diet  $\times$  Day(Year) interaction on scrotal measures in cm. <sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ) of diet by year within day.

While no differences in SC were observed between the diet groups in the same year and on like days, the rate of testicular growth of SBM in period 1, period 3, and in the overall postweaning period indicates enhanced teste development as compared to CSM contemporaries ( $P < 0.0001$ ,  $P = 0.01$ ,  $P < 0.0001$  respectively) (Figure 3.).

**Figure 3.** Scrotal increases per observation period in cm: Main effect of Diet

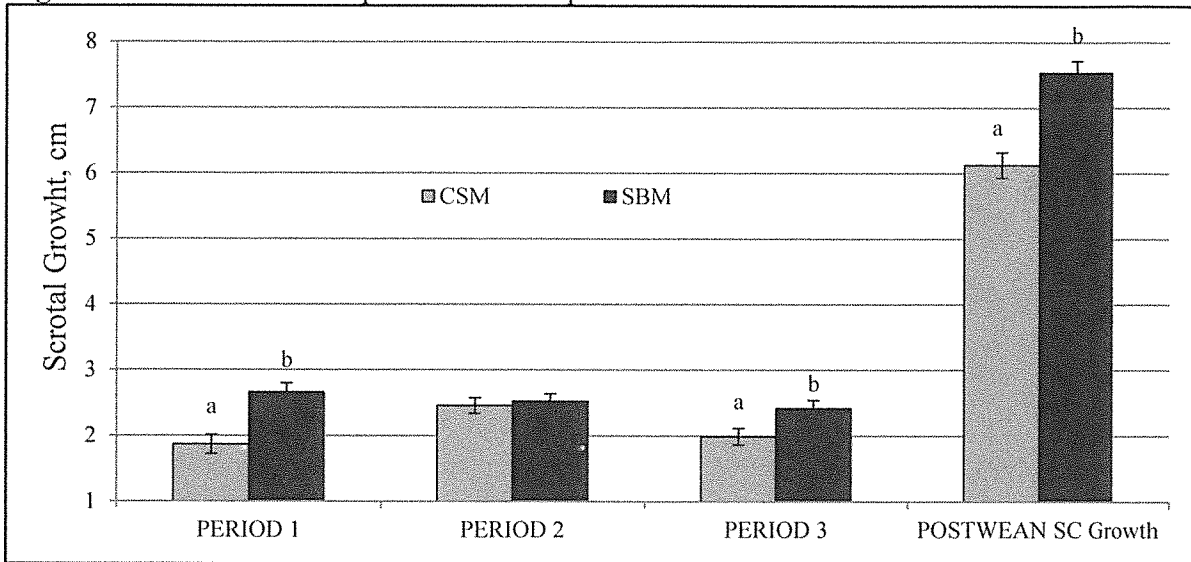


Figure 3. Least squares means of scrotal growth within time period (Period 1 scrotal growth = difference of d 0 scrotal circumference and d 21 scrotal circumference; Period 2 scrotal growth = difference of d 21 scrotal circumference and d 54 scrotal circumference; Period 3 scrotal growth = difference of d 54 scrotal circumference and d 86 scrotal circumference; POSTWEAN SC Growth = difference of d 0 scrotal circumference and d 86 scrotal circumference).

<sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ) within time period.

These data would reflect inconsistent results presented by Yuan et al. (2012), in which boar pigs consuming 500ppm isoflavone supplement were 40% lower in scrotal size index calculations as compared to a negative control group, and 58% lower in scrotal size index calculations as compared to boars consuming 250ppm isoflavone supplement. Therefore implying that the level of isoflavone exposure in these data is not high enough to cause deleterious effects to SC, but dietary soybean meal at a 10% ration inclusion rate actually augments teste SC accretion.

### ***SEMEN SAMPLE SPERM CELL CONCENTRATION***

It is well understood that age of dam (AOD) is an important point to consider when discussing bull development procedures and performance adjustment factors (Koch and

Clark, 1995; Lunstra et al., 1988). Differences and the LSMEANS of the transformed (log10) semen concentration variable due to the diet × AOD (Year) interaction are presented in Table 5.

**Table 5.** Semen Concentration (Transformed to log base of 10) of Diet × AOD(Year)

AOD	2014				2015			
	CSM	SBM	SEM	P=	CSM	SBM	SEM	P=
2	<sup>a</sup> 8.10	<sup>b</sup> 8.47	0.16	0.0266	<sup>x</sup> 7.52	<sup>y</sup> 8.67	0.33	0.0006
3	8.21	8.09	0.27	0.6621	8.02	8.03	0.19	0.9617
4	8.54	8.50	0.15	0.7792	8.74	8.29	0.33	0.1808
5+	8.46	8.53	0.13	0.5535	<sup>x</sup> 8.78	<sup>y</sup> 8.17	0.14	<.0001

Table 5. Least squares means of semen concentration (Transformed to log base of 10) of the Diet × AOD(Year).

<sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2014 diet effects within respective AOD.  
<sup>x, y</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2015 diet effects within respective AOD.

In our study, differences were observed in bulls produced by 2-year old dams where bulls in the SBM produced semen samples more heavily concentrated than the CSM born to 2 year old dams in 2014 and 2015 ( $P = 0.026$  and  $P = 0.0006$ , respectively). But in 2015, CSM from the 5+ AOD designation expelled semen ejaculate that was 7.47% more concentrated than SBM born to 5+ year old females ( $P < 0.0001$ ). Sperm cell concentration in ejaculate is an important criteria of semen charactersitics to qualify fertile males for breeding purposes (Graffer et al., 1988). This data suggests that moderate phytoestrogen exposure in bulls born to first calf females offers decisive advantages in sperm cell proliferation and semen concentration.

## SPERM MOTILITY

Semen motility scoring procedures are imperative to estimate the percent of sperm cells in an ejaculate sample. The main effect of diet on semen motility scoring analysis in this data is summarized in figure 4.

**Figure 4.** Semen motility scores, Main effect of Diet

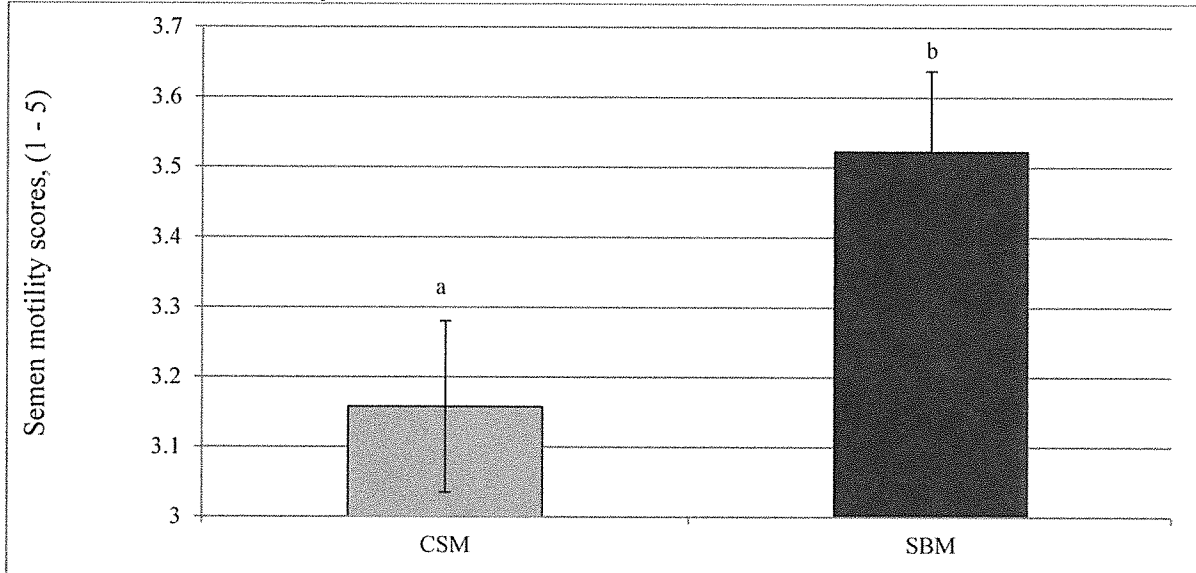


Figure 4. Least squares means of Semen motility scores (1 – 5) with scores of 1 being least motile and scores of 5 being most motile.

<sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ).

It was observed that the LSMEANS for motility of SBM was 3.52, which is higher than CSM motility scores of 3.16, and this was a significant source of variation ( $P = 0.0308$ ) in the analysis. Additionally, the Diet  $\times$  AOD(Year) interaction was also a source of model variation and LSMEANS are presented in Table 6.

**Table 6.** Semen Motility Scores of Diet × AOD(Year)

2014					2015			
AOD	CSM	SBM	SEM	P=	CSM	SBM	SEM	P=
2	<sup>a</sup> 2	<sup>b</sup> 3.75	0.35	<.0001	4	5	0.69	0.1491
3	2	3	0.56	0.0776	<sup>x</sup> 3.33	<sup>y</sup> 2.33	0.40	0.0129
4	3.14	3	0.31	0.6412	5	4	0.69	0.1491
5+	<sup>a</sup> 2.29	<sup>b</sup> 3.5	0.27	<.0001	3.5	3.6	0.30	0.7356

Table 6. Least squares means of semen motility scores for the Diet × AOD(Year) effect.

<sup>a, b</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2014 diet effects within respective AOD.

<sup>x, y</sup> superscripts designate differences ( $P \leq 0.05$ ) of 2015 diet effects within respective AOD.

In the 2014 data, the SBM produced by first parturition, 2-year old females and cows that are  $\geq 5$  years of age recorded higher semen motility scores than CSM ( $P < 0.0001$ ). But in the 2015 data, the only observable differences were in the bulls from 3-year old cows, as the CSM scored higher than the SBM ( $P = 0.0129$ ) and specific reasons for these conflicting results is not clearly interpreted. It should be noted that Alexander (2008) concludes that bulls should have a minimum of 30% progressive motility estimation to be deemed as a satisfactory breeder. In these data, semen motility scores of 2 or higher represent a 30% motility equivalent and therefore, all bulls in these study groups were deemed as acceptable potential breeders.



## IMPLICATIONS

These results support the inclination of positive aspects of exposing growing beef bulls to dietary phytoestrogen compounds by providing soybean meal as a protein source at the 10% inclusion rate of a diet of growing bulls. Additional research objectives should investigate variable rates of phytoestrogen intake, alternative timing of postnatal phytoestrogen exposure, and the potential effects of prenatal phytoestrogen exposure by modifying maternal consumption of phytoestrogens.

## LITERATURE CITED

- Alexander, J.H. 2008. Bull breeding soundness evaluation: A practitioner's perspective. *Theriogenology*. 70(3): 469-472.
- Arteaga, A., M. Baracaldo, A.D. Barth. 2001. The proportion of beef bulls in western Canada with Mature spermograms at 11 to 15 months of age. *Can Vet J*; 42:783-787.
- Barth, A.D., L.F.C. Brito, J.P. Kastelic. 2008. The effect of nutrition on sexual development of bulls. *Theriogenology*. Vol 70, Issue 3, p. 485-494.
- Cardoso, J.R., and S.N. Bao. 2007. Effects of chronic exposure to soy meal containing diet or soy derived isoflavones supplement on semen production and reproductive system of male rabbits. *Animal Reproduction Science* 97: 237-245.
- Cederroth, C.R., J. Auger, C., Zimmermann, F. Eustache, S. Nef, 2009. Soy, phyto-estrogens and male reproductive function: A review. *International Journal of Andrology*. 33:304-316.
- Clarkson, T.B., Anthony, M. S., Hughes, C. L. Jr., 1995. Estrogenic Soybean Isoflavones and Chronic Disease: Risks and Benefits. *Trends Endocrinol Metab*. 6:11-16.
- Erickson, D.R. 2015. *Practical handbook of soybean processing and utilization*. Elsevier, St. Louis, MO.
- Geay, Y., January 1982. Energy and Protein Utilization in Growing Cattle. *Journal of Animal Science*. Vol 58, No. 3, p. 766-778.
- Graffer, T., H. Solbu, O. Filseth. 1988. Semen production in artificial insemination bulls in Norway. *Theriogenology*. 30: 1011-1021.
- Hafs, H.D., R.S. Hoyt and R.W. Bratton. 1959. Libido, sperm characteristics, sperm output and fertility of mature bulls for thirty-two weeks. *J. Dairy Sci*. 42(4):626-636
- Hess, R.A., July 2003. Estrogen in the adult male reproductive tract: A review. *Reproductive Biology and Endocrinology*. 52: 1-14.
- Hess, R.A., K. Carnes, December 2004. The role of estrogen in testis and the male reproductive tract: A review. *Anim. Reprod*. 1:5-30.
- Hossain, M.E., M.M., Khatun, M.M. Islam, O.F. Miazi. 2012. Semen characteristics of breeding bulls at the central cattle breeding and dairy farm in Bangladesh. *Bang. J. Anim. Sci*. 41(1):1-5.

- Kastelic, J.P. 2014. Understanding and evaluating bovine testes. *Theriogenology*. Vol 81, Issue 1, p. 18-23.
- Koch, M.R. and R.T. Clark. 1955. Influence of sex, season of birth and age of dam on economic traits in range beef cattle. *J. Anim. Sci.* 14:386.
- Lephart, E. D., K.D.R. Setchell, R.J. Handa T.D. Lund. 2004. Behavioral effects of endocrine-disrupting substances: phytoestrogens. *J. ILAR*. 45:4: 433-454.
- Lunstra, DD, K.E, Gregory, L.V. Cundiff. 1988. Heritability estimates and adjustment factors for the effects of bull age and age of dam on yearling testicular size in breeds of bulls. *Theriogenology* 30: 127–136.
- NAHMS. 2019. References of Beef Cow-calf Management Practices in the United States. USDA:APHIS:VS: NAHMS. Ft. Collins, CO.
- NASS. 2017. Current Cattle Inventory. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1595> (Accessed 4 April 2017).
- Pruitt, R.J., L.R. Corah, J.S. Stevenson, G.H. Kiracofe. 1986. Effect of energy intake after weaning on the sexual development of beef bulls. II. Age at first mating, age at puberty, testosterone and scrotal circumference. *J. Anim. Sci.* 63:579-585
- Setchell, K.D.R., Cassidy, A. 1999. Dietary Isoflavones: Biological Effects and Relevance to Human Health. *J. Nutr.* 129: 758S-767S.
- Van Demark, N.L., R.E. Mauger. 1964. Effect of Energy Intake on Reproductive Performance of Dairy Bulls. I. Growth, Reproductive Organs, and Puberty. *Journal of Dairy Science*. Vol 47, Issue 7, p. 798-802.
- Yuan, Xiao-xue., Bin Zhang., Chao-wu Xiao. Jue-xin Fan. Mei-mei Geng, and Yu-long Yin. 2012. Effects of soybean isoflavones on reproductive parameters in Chinese mini-pig boars. *J. Anim. Sci. Biotechnol.* 3:31.



ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

*Institutional Animal Care & Use Committee*

April 24, 2017

Dr. Chase Runyan, Assistant Professor  
Agriculture  
Angelo State University  
Vincent Nursing-Physical Science Building Room 219  
San Angelo, TX 76909

Dear Dr. Runyan:

Your proposed project titled, “Evaluation of dietary phytoestrogen exposure on growth, semen parameters, and reproductive anatomy development of growing Angus bulls” was reviewed by Angelo State University’s Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective September 11, 2014, and it expires three years from this date; however, an annual review and progress report form ([www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport](http://www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport)) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 14-05. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Robert Dowler, Ph.D.  
Chair, Institutional Animal Care and Use Committee

(IACUC Use Only) IACUC APPROVAL NO.: _____ Expiration Date: _____ Category: _____
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PROTOCOL FOR THE USE OF LIVE ANIMALS  
FOR RESEARCH, TEACHING OR DEMONSTRATION  
Animal Use Form

A protocol can be reviewed only after all questions have been answered completely. Do not refer to or attach passages from grants. This is a form field document and you must provide the information in the shaded area and in the space provided.

Date Filed: 8/11/14

TITLE OF PROTOCOL: (There may be multiple titles)

Effects of isoflavones on bull growth, testicular development, and semen quality parameters

Principal Investigator: Dr. Chase A. Runyan

Department: Agriculture

Telephone Number: (325) 486-6758

E-mail: chase.runyan@angelo.edu

Proposed funding source: MIR Research

Expected starting date of project: 10/1/14

Expected completion date of project: 3/30/16

Project is:

- Biomedical
- Food/Fiber Production
- Teaching/Demonstration
- Wildlife
- Other (please describe) \_\_\_\_\_

1. Animal model(s):

A. Common name, sex, age:

Angus Bulls, 8 - 14 months of age.