EFFECTS OF ISOFLAVONES ON THE SPERMATOGENESIS OF PREPUBERAL BOVINE BULLS

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EFFECTS OF ISOFLAVONES ON THE SPERMATOGENESIS

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ABSTRACT

Several studies have been conducted over the effects of isoflavones on health and reproductive parameters of small rodent and humans due to the wide range of proposed negative and positive effects that isoflavones can have. This study was conducted on 39 prepuberal bovine Angus bulls, stratified by weaning weight, sire, and age of dam (AOD) to one of two study diets (DIET) which consisted of Soybean meal treatment group (SBMTRT) or Cottonseed meal control group (CSMCON). The purpose of this study was to investigate average daily gain (ADG), scrotal circumference (SC) and sperm production and morphology. There were profound effects observed with DIET × AOD on ADG in the 3 year and 2 year AOD groups, and semen concentration and volume differences were observed within the 3 yr AOD. All AOD in the CSM groups were significantly lower in all aspects of semen quality and ADG.

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INTRODUCTION

Over the span of several years there has been a significant improvement in efficiency of livestock reproduction. From nutritional perspectives, the upkeep of livestock is a major financial responsibility. Nutrition plays a vital role in maintaining reproductive efficiency. Producers should be interested in using methods of feeding to maintain body condition while also decreasing the financial burden of providing these vital nutrients.

Cotton seed meal (CSM) is a good source of protein that is used in livestock feed, although slightly lower in protein content compared to Soybean meal (SBM). Proteins are an important factor in livestock feed with soy being one of the most important sources used to feed animals. Soybean meal is the material that remains after the soy bean oil is removed from whole soy beans. Soybean meal has a total digestive nutrients (TDN) level of around 80%, while crude protein (CP) is around 45-50%. Soybean meal is palatable and readily consumed by animals. There are many different protein ingredients that can be added to livestock feedstuff which are high in protein. These include; canola meal, CSM, corn gluten meal, distiller's grain (dehydrated or wet), peanut meal, and soybean derived proteins (NRC 2000). Although these are not all of the protein sources available, it allows producers different choices of protein to meet the different needs of livestock. Both SMB and CSM are excellent sources of protein, but there has been concern raised in the consumption of SBM because of the isoflavones content. Soybean meal is derived from a bean which is a type of legume which contains isoflavones.

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There are few studies that have investigated the implementation of soy based isoflavones in the diets of growing ruminant livestock, and none to date that investigate the effects of isoflavones on yearling bulls and the effects on reproductive measures. Isoflavones are polyphenolic compounds that are capable of exerting estrogen like effects on the body. They are classified as phytoestrogens which are found in legumes (Cardoso and Bao, 2007). The objective of the current study is to determine the adverse effects of isoflavones in feedstuffs and the effect on reproductive anatomy, bull growth, and sperm production in prepuberal bulls.

LITERATURE REVIEW

Feed stuff availability, cost, and effective utilization can be a major concern for cattle raisers in the west Texas region. One major expense that affects livestock is feed (Holchek and Herbel, 1986). Numerous publications report that feed cost for maintenance is estimated to represent at least 65% of the total cost requirements for cowherds, these cost vary considerably between individual animals due to a number of factors, including differences in frame, body condition, desired rate of gain and the environment (Montano-Ber-mundez et al., 1990; Parnell et al., 1994; Arthur et al., 2001). It is common that commercially available rations contain corn or other cereal grains as a source of energy in a diet and cottonseed or SBM as a protein source. Because several high protein feed ingredients are available, producers can potentially decrease cost based on the availability of these protein sources and price per unit of nutrient evaluation. Eighty to 90% of the protein contained in feedstuffs with high quality protein, can be broken down in the rumen, which can result in limited protein availability for weight gain in growing ruminants. This is due to the other ten to 20% that is considered escape protein, which is released into the small intestine as intact amino acids (Grigsby et at., 1989; Beever, 1984; Rocha et al., 1995).

ISOFLAVONES

One concern associated with feeding soy and soy-derived products is that soy-derived protein sources contain isoflavones which mimic the actions of natural estrogens and may exert adverse effects on male fertility (Cederroth et al., 2010). Cederroth et al. (2010) observed an increased incidence of human male reproductive disorders due to the intake of high amounts of isoflavones through consumption of a number of foods, but mainly soy infant formulas. Cederroth et al. (2010), state that higher levels of exposure to phytoestrogens due to exposure over a lifetime or during critical periods of development, such as the period of time preceding gonadal maturity, or puberty, could have detrimental effects on fertility and reproductive functions. Isoflavones are polyphenolic compounds that are capable of exerting estrogen like effects on the body. Because isoflavones are plant derived compounds, they are classified as phytoestrogens. There are three main classes of phytoestrogens (PE); isoflavones (soybeans and clover), coumestans (alfalfa), and lignans (flaxseed) (Cardoso and Bao, 2007). Within these classes there are two specific isoflavones; genistein and diadzein which are thought to exert some of the most potent estrogenic hormone activities (Lephart et at., 2004). Estrogen is not sex specific as previously thought, as both testosterone and estrogen are important in both sexes. Estrogen is essential for normal fertility, and has important functions in the adult male reproductive tract. The primary function of estrogen in the male is regulation of fluid reabsorption in the efferent ductules. There are several estrogen receptors throughout the male reproductive tract which include the hypothalamus and pituitary glands that provide feedback and the leydig and sertoli cells. Disruption of estrogen receptors can cause interference of sperm morphology which can lead to decreased fertility (Hess, 2003). In further research by (Hess and Carnes, 2004) it was observed that estrogen has a significant impact in establishing sertoili cell function in the developing testis. Although estrogen may not be essential for spermatogenesis, there is evidence that estrogen does influence spermatogenesis indirectly.

BENEFITS OF ISOFLAVONES

It was reported by the Food and Drug Administration (FDA) that consuming soy on a daily basis could help reduce the risk of heart disease in humans. (Food and Drug Administration, 1999). Other research that has been conducted shows health benefits of consuming phytoestrogens in that it can help in easing premenopausal symptoms (Patisaul and Jefferson, 2010). Yamori et al. (2002) show that women who consume higher levels of soy foods had better spine and pelvic density compared to women who eat little to no soy. High intake of dietary phytoestrogens can be associated with lower incidence with breast and prostate cancer in populations where diets contain more soy products in countries such as Japan and China, compared to western diets of North America (Adlercreutz et al., 1991). In research conducted by Shu et al. (2009) it was shown that breast cancer survivors that consumed soy products daily showed a decreased risk of death or cancer recurrence.

BULL DEVELOPMENT

As yearling bulls mature their nutritional needs change. Younger bulls need extra nutrients to support rapid growth that occurs whereas mature bulls utilize nutrients to support body maintenance. Due to the rapid growth of lean muscle that occurs in young bulls, the protein percentages needed are higher. 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, then growing bulls may exhibit reduced muscle growth as well as permanently impaired sperm production (Hafs et al., 1959; VanDemark, 1964). Barth et al. (2008) also states that weight of the testes, epididymis, and seminal glands were significantly reduced in bulls fed protein-deficient rations, while 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. 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.

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 prepuberal period is when there is a transient increase in gonadotropin secretion and usually occurs from 8-20 weeks of age, it is often called the “early gonadotropin rise”. This causes the concentration of testosterone to rise and correlates to the period in which there is acceleration in reproductive development (Barth et al., 2008).

BULL GROWTH AND MANAGEMENT

Bull growth and development not only depends on what producers are feeding, but also what the producer’s goals are. Herd et al. (2003) states that genetic improvement for beef cattle emphasize on improved outputs such as body weight (BW) and reproduction aspects. Herd et al. (2003) continues in stating that efforts, focusing on avenues to reduce inputs, are needed in order to improve efficiency and production while increasing profitability potential.

AGE OF DAM FACTORS

Kastelic (2014) states that more indirect evidence exists regarding the effect of early life nutrition because testicular size of bulls from 2 year old dams was smaller than the testicular sizer of offspring from older dams. It is speculated that that this is due to higher yields of milk production of the older females, and a balanced distribution of nutrients within the dam herself. In further research dealing with gestation length, birth weight and growth, Bourdon and Brinks (1985) found that AOD affected all traits in beef bulls excluding postweaning growth and age at first calving. Bourdon and Brinks (1985) continue to state that with AOD being between 5 and 10 years of age, there was an association with longer gestations, heavier birthweight, and larger weights and gains.

SOY PROTEIN AND MALE REPRODUCTION ASPECTS

Much of the current published data has focused on both male and female human aspects of reproduction, and the functionality of the reproductive tract of small monogastric animal models such as rats and mini-pig boars/sows. Yuan et al. (2012) observed that exposure to soy isoflavones increased testosterone levels in blood serum and testis, delayed the growth and development of the testis, and caused structural changes in the tissues of mini-pig boars.

Testicular size, which is closely related to sperm-producing potential, is most commonly assessed using measurement of SC, which has an effect on semen it varies among individual bulls and breeds and is highly heritable. In general, more sperm, a higher percentage of morphologically normal sperm, and better reproductive performance in closely related females (Kastelic, 2014). The most commonly used definition of puberty in bulls is when ejaculates collected via electroejaculation contain a minimum of 50×10^6 total sperm with at least 10% progressive motility (Wolf et al., 1965).

Estrogen is important in the regulation of the male reproductive tract and has direct effects on leyding 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 and concludes that estrogen is important in the regulation of fluid reabsorption in the efferent ductule epithelium. Therefore, a disruption of the estrogen receptors can have adverse effects on sperm production and morphology. Lunstra et al. (1998) observed that lutenizing hormone (LH) and testosterone (T) increased gradually as bulls approached puberty. It has been reported that induced LH surges do not stimulate increased T secretion until bulls are 6 months of age or older, indicating that the testes of prepubertal bulls become more responsive to LH stimulation as puberty approaches (Mongkonpuna et al., 1975). Topparia et al. (1996) suggests that estrogen suppresses gonadotropin production in rabbits at all ages preceding puberty, causing decreased gonadotropin stimulation during critical development and may result in inadequate sertoli cell proliferation and small testis.

To date there have been several publications that report the effects of soy protein isoflavones in humans. Unfortunately, little published data is available that investigates the effects of estrogen like compounds in ruminants. Therefore, the primary objectives of this project are to determine the effects of dietary isoflavones on bull sperm production measures in young Angus bulls by detecting differences in sperm motility, sperm color, sperm concentration, and ejaculate volume of bulls exposed to isoflavones. The secondary objectives are to observe differences in traditional measures of growth performance by detecting changes in rate of gain and overall growth performance of bulls exposed to isoflavones as compared to bulls naïve of isoflavone containing feed ingredients.

MATERIALS AND METHODS

This study was conducted at the Angelo State University Management, Instruction, and Research Center in San Angelo, Texas. All animal procedures have been approved by the Angelo State University Institutional Animal Care and Use Committee (AUP # 14-05). Spring born Angus bull calves (n=39) were stratified by weaning weight, sire, and age of dam into a cottonseed meal-control (CSMCON) group and a soybean meal treatment group (SBMTRT). There were bulls that were rejected from the study due to sickness or lack of viable samples of sperm. Both diets were textured mixed rations that were formulated to meet NRC requirements (NRC, 2000) of young/yearling growing bulls. The ingredients and percentages contained in each diet formulation are presented in Table 1.

Table 1. Ingredients and Percentages of CSMCON[a] Ration and SBMTRT[b] Ration

Feed Ingredient	Percentage of the CSMCON Ration	Percentage of the SBMTRT Ration
Cracked Corn	30.00%	30.00%
Cottonseed meal, dry	10.00%	**0.00%**
Soy Bean meal, dry	**0.00%**	10.00%
Corn Glutten Feed, pellets	15.00%	15.00%
Cottonseed Hulls, Dry	18.00%	22.50%
Alfalfa Pellets	20.50%	16.00%
Molasses	4.00%	4.00%
Mineral Premix	2.50%	2.50%

[a] Cottonseed meal – Control ration
[b]Soybean meal – Treatment ration

Each ration was formulated to be isocaloric and isonitrogenous with similar nutrient composition. The calculated nutrient composition is presented in Table 2.

Table 2. Calculated diet composition of CSMCON[a] and SMBTRT[b] Diets.

Nutrient, DM	CSMCON Diet	SBMTRT Diet
NEm Mcal/CWT	65.18	64.77
NEg Mcal/CWT	40.51	40.58
TDN %	64.89	64.89
Crude Fat	3.82	3.78
Crude Fiber	17.32	17.31
ADF	24.2	24.19
NDF	35.64	35.26
Crude Protein	17.62	17.92
Calcium	0.96	0.92
Phosphorus	0.47	0.43

[a] Cottonseed meal – Control ration
[b]Soybean meal – Treatment ration

Treatment diet samples were collected at random across multiple days and multiple mixed feed batches and analyzed at SDK labs (Hutchinson, KS) to confirm ration estimates for ration nutrient availability. All bulls had continuous access to adequate shade, and *ad libitum* access to fresh water and treatment diets throughout observation days.

At day -42 all bulls were weaned and comingled into their respective diet groups. All bulls were fed CSMCON diet *ad libitum* during the concentrate diet adaptation period. At day 0, basal scrotal circumference measurements and weights were collected and then bulls in the SBMTRT group were gradually transitioned over to the SBMTRT ration. Weights and scrotal measurements were taken again at day 21, 54, and 86. At day 86 individual semen samples were harvested via electro-ejaculator and semen motility scores, and semen color scores were assessed utilizing trained personnel using a 5 point scale (Hossain et al., 2012). Ejaculate volume was observed in mL and semen concentrations were counted via Dupree model 591B densimeter which counts sperm concentration (number of spermatozoa per milliliter) at the time of ejaculation.

Mixed model procedures of SAS (SAS Inst. Inc., Cary, NC) were used to analyze scrotal circumference and body weight with a model that includes the fixed effects of day, age of dam (AOD), diet treatment group (Diet), day -42 weight as a covariate, and two-factor interactions. These models were analyzed as repeated measures with a first order autoregressive covariance structure. Average daily gain, changes in scrotal circumference, semen color, semen motility, and semen concentration (transformed to a log base of 10 for analysis) were analyzed using a similar model but excluding the repeated measures statements. In all analysis, main effects with ($P > .15$) and two-way interactions with ($P > .25$) were removed for final analysis.

RESULTS

Table 3 below displays ADG throughout the four different periods of the bulls in the study, day -42, through day 86. It also shows scrotal circumference growth in cm, as well as, bull growth, scrotal growth, semen volume, concentration, motility and color scores within the same time period. Although there was considerable individual variation for all variables analyzed, not all of the measures yield significant results.

Table 3. Summary Statistics

Variable	N	Minimum	Maximum	Mean	Std. Dev.	Coeff. of Var.
Period 1 ADG (day -42 through day 0) , in kgs	34	0.40667	3.19082	1.97356	0.66552	0.3372
Period 2 ADG (day 0 through day 21), in kgs	34	0.88051	3.45534	2.14988	0.48525	0.2257
Period 3 ADG (day 21 through day 54), in kgs	34	1.23708	3.02398	2.08647	0.45366	0.2174
Period 4 ADG (day 54 through day 86), in kgs	34	0.567	2.69323	1.76061	0.5082	0.2886
Post Weaning ADG, in kgs	34	1.43521	2.49124	1.99626	0.23836	0.1194
Day - 42 Weight, in kgs	34	290.3021	425.47401	357.30104	37.84017	0.1059
Day 0 Weight, in kgs	34	338.38338	474.00889	414.53432	43.07482	0.1039
Day 21 Weight, in kgs	34	381.0215	560.19233	487.63014	46.01698	0.0944
Day 54 Weight, in kgs	34	436.36034	644.10777	556.4835	49.93196	0.0897
Day 86 Weight, in kgs	34	474.00889	705.34337	612.82292	53.49223	0.0873
Day 0 Scrotal Circumference, in cm	34	27.5	38	33.38235	2.35185	0.0705
Day 21 Scrotal Circumference, in cm	34	31	40	35.89706	2.47955	0.0691
Day 54 Scrotal Circumference, in cm	34	33	43	38.98529	2.60095	0.0667
Day 86 Scrotal Circumference, in cm	34	33	46	40.55882	2.63934	0.0651
riod 2 Scrotal Growth (day 0 through day 21, in cm	34	0	6.5	2.55882	1.56556	0.6118
iod 3 Scrotal Growth (day 21 through day 54, in cm	34	0.5	6	3.08824	1.13131	0.3663
iod 4 Scrotal Growth (day 54 through day 86, in cm	34	0	5	1.57353	1.37693	0.8751
Semen Volume, in mL	33	0.5	5	2.28182	1.13598	0.4978
Semen Concentration (transformed: log base 2)	33	7.6721	9.24304	8.42318	0.37055	0.0440
Semen Motility Scores	33	1	5	3.0303	0.95147	0.3140
Semen Color Scores	33	1	5	2.63636	1.11294	0.4222

WEIGHT

The sources of variation for weight measures are presented in figure 1. No differences between SBMTRT and CSMCON were observed at day -42 and day 0, but weight due to Diet becomes different at day 21, 54, and 86 as the SBMTRT bulls were 28.37, 36.05, and 33.93 kgs more respectively ($P < 0.05$), than their CSMCON cohorts at the same weigh periods. These results are similar to observations of Yuan et al. (2012), where it was observed that feeding lower levels of soy isoflavones in a diet can increase growth hormone as well as liver growth hormone receptors which ultimately contribute to growth in the sows and mini-pig boars.

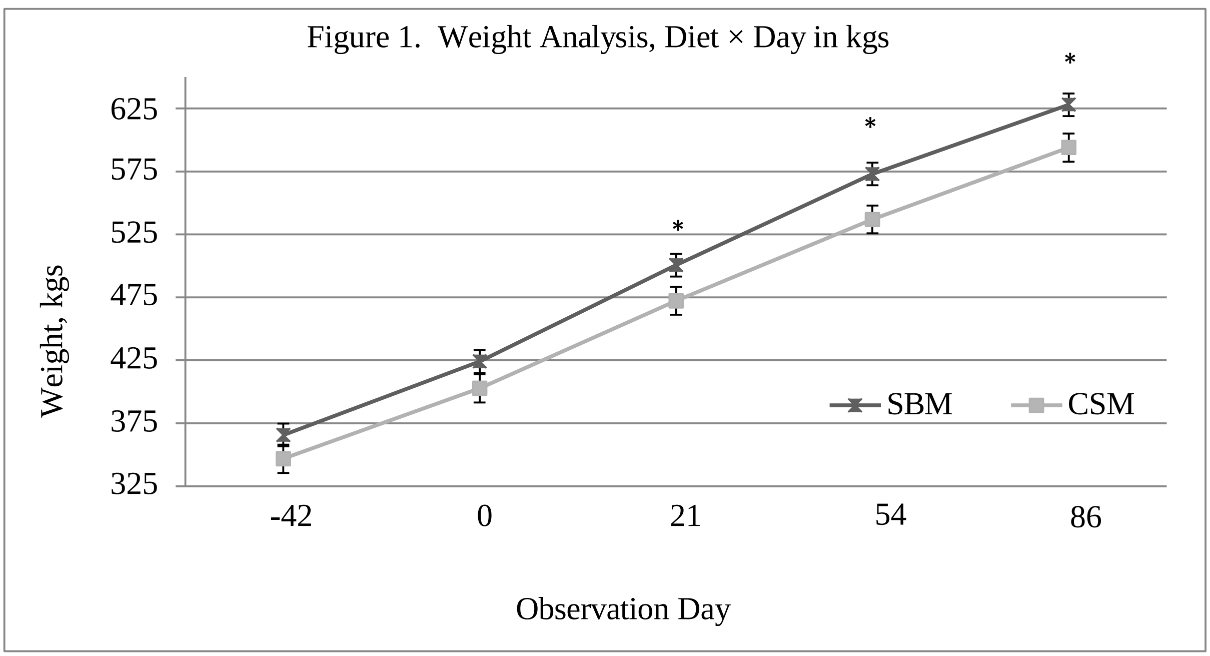

Figure 1. Least squares means of the diet × collection day interaction on weight gain in kgs. * denotes differences in weight observations within a day ($P \leq 0.05$).

ADG

Average daily gain was calculated for 5 time periods. Period 1 was calculated from day -42 to day 0, Period 2 was calculated from day 0 to day 21, Period 3 was calculated from day 21 to day 54, Period 4 was calculated from day 54 to day 86, and an overall post weaning ADG from day -42 to day 86. Sources of variation for average daily gain were the main effects of Diet and the Diet × AOD interaction.

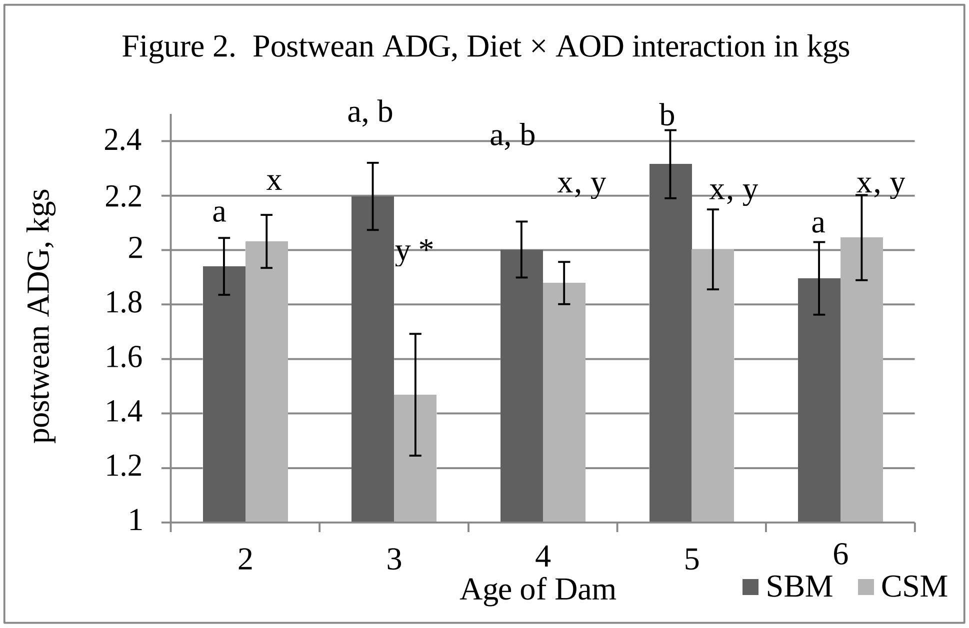

Figure 2. Least squares means of the diet × Age of dam interaction on postweaning ADG in kgs. [ab] superscripts differ across SBMTRT ($P \leq 0.05$), [xyz] superscripts differ across CSMCON ($P \leq 0.05$), and
[*] denotes SBMTRT and CSMCON differ within a specific Age of dam ($P \leq 0.05$).

It has been well understood that an important point to consider is the AOD variable when discussing observations of weight and rate of weight gain (Koch and Clark, 1955). In figure 2 it is observed that bulls born to 3 year old dams and consuming the CSMCON diet were significantly lower for postwean ADG as compared to CSMCON bulls out of 2 year old dams ($P < 0.05$) and had a strong tendency to be lower than bulls from 4, 5, and 6 year old dams, ($P = 0.09$), ($P = 0.06$), ($P = 0.06$), respectively. The reason for this is unclear since a similar pattern was not observed in the SBMTRT consuming bulls. This drastic reduction in growth rate could be due to nutritionally compromised dams as a result of physiological process of developing heifers and young females into mature cows. It should be of particular interest that bulls born to 3 year old dams that were fed SBMTRT diet gained 1.47 kg/day more than their CSMCON consuming contemporaries, and no other differences within AOD were observed, and this may warrant additional research investigating the AOD when considering sources of protein in developing bulls to maximize gain in performance testing settings.

There appears to be a large difference in postweaning ADG for the main effect of Diet as observed in figure3.

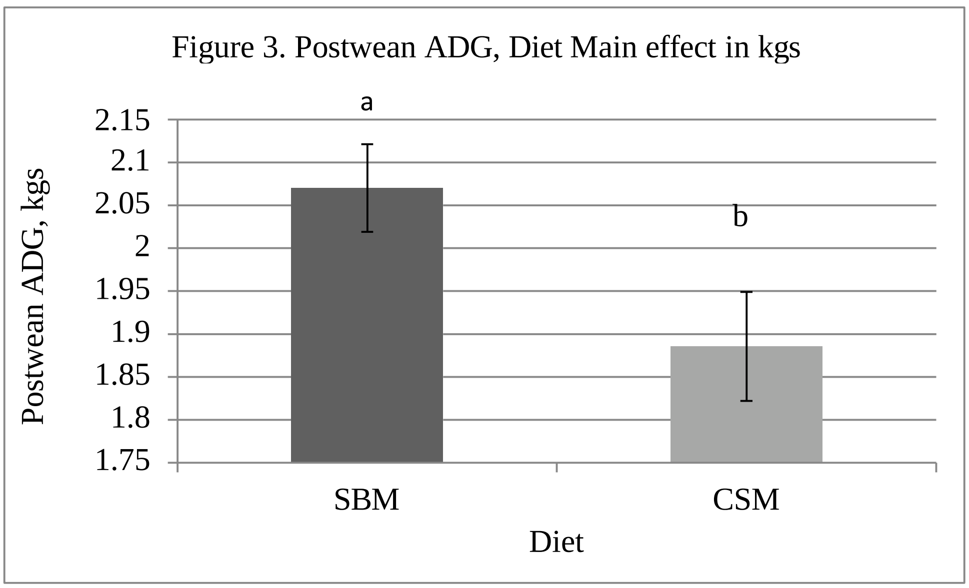

Figure 3. Least squares means of the main effect of diet on Postwean ADG in Kg.
[ab] superscripts differ ($P \leq 0.05$)

Bulls in the SBMTMT group had a 0.185 kg/day increase in postwean ADG as compared to the CSMCON group ($P < 0.05$).

No differences in ADG were observed in periods 1, 3, or 4 for any of the main effects or two-way interactions, but a Diet × AOD interaction difference was detected as a significant source of variation for ADG in period 2 (Figure 4). Bulls from three year old dams in the SBMTRT group were significantly higher for ADG as compared to bulls from two year old and six year old dams also in the SBMTRT (P = 0.05). No other differences were observed due to treatment by age of dam interaction within the SBMTRT group average daily gain. It was also observed that ADG of bulls in the CSMCON group from three year old dams and four year old dams was suppressed as compared to bulls from five year old dams ($P \leq 0.05$), but were not different when compared to bulls from dams of any other age group within the CSMCON diet treatment. Differences between CSMCON and SBMTRT within a single age of dam was only observed for bulls from 3 year old dams where SBMTRT bulls gained 2.84 kg/day as compared to CSMCON bulls from 3 year old dams which gained 1.37 kg/day ($P > 0.05$).

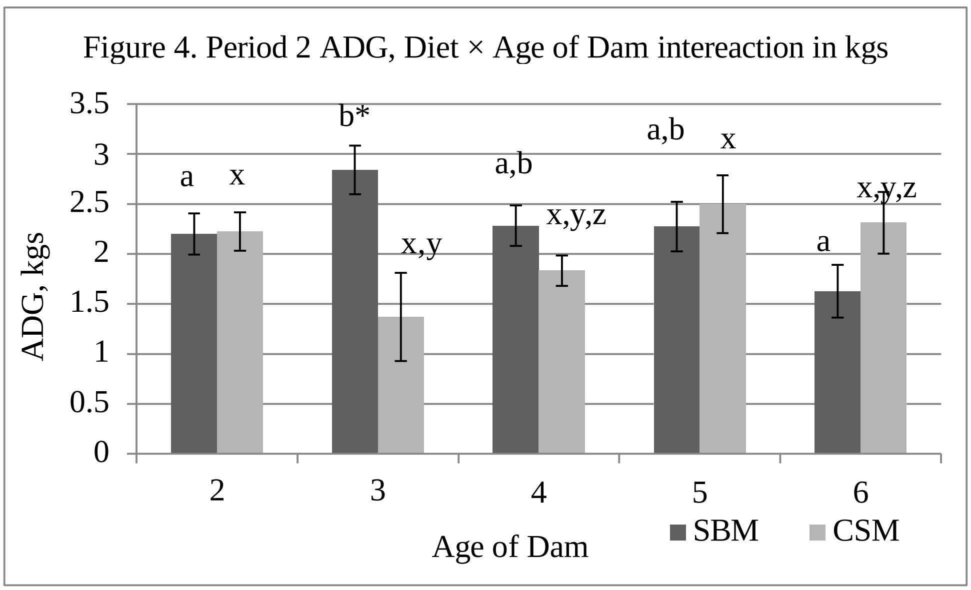

Figure 4. Least squares means of the Diet × Age of Dam interaction for Period 2 ADG. [ab] superscripts differ across SBMTRT ($P \leq 0.05$),
[xyz] superscripts differ across CSMCON ($P \leq 0.05$), and
[*] denotes SBMTRT and CSMCON differ within a specific Age of dam ($P \leq 0.05$).

Rumph and VanVleek, (2004) state that first calf-heifers are not physically or biologically mature. Most of the nutrients that they ingest are partitioned for many things such as lactation, gestation, and maintenance as well as their own growth. This causes calves from younger cows to be smaller at both, birth and weaning compared to older more mature contemporaries. However, bulls fed SBMTRT from 3 year old cows recorded a higher ADG in period 2, compared to bulls fed SBMTRT from 2 and 6 year cows. Within 2 year old cows there is a potential for inadequate energy reserve due to the continued growth and development of the cow in combination with stress and first lactation Spitzer et al. (1995).

SCROTAL

Kastelic (2014) states that testicular weight cannot be measured in a noninvasive way in cattle, and that use of scrotal tape is necessary to measure sperm producing potential. Scrotal circumference (SC) is defined as the largest diameter of the testes when both testes were pulled together down into the scrotum with thumbs and fingers without separating the two testes (Yilmaz et al., 2004). In a study of 205 beef herds, the percentage of morphologically normal sperm was lower ($P < 0.006$) in bulls with an SC $\leq$ 34 cm versus > 34 cm. In addition, cows that were exposed to bulls with a smaller SC had a smaller chance of becoming pregnant and had a longer interval from bull exposure to calving as compared to bulls with larger SC (Waldner et al., 2010). Lunstra et al. (1988) stated that measurement of SC can be a simple method in assessing puberty in beef bulls regardless in age difference, weight, or breed, and is helpful in selecting early maturing bulls with little effort. Kriese et al. (1991) found that SC was positively correlated with growth and reproductive traits and is often used in selection programs. Kastelic (2014) states that SC is highly heritable trait, and bulls exhibiting larger SC have higher counts of normal sperm. Kastelic and Thundathil. (2008) describes the use of SC as part of the breeding soundness evaluation, and the propensity of the SC measure to detect bulls that have abnormal semen characteristics.

Increasing SC is associated with a higher frequency of cows diagnosed pregnant and so it is recommend by the American Society of Theriogenology that all yearling bulls intended for breeding should have a minimum SC of 30 cm (Ajitkumar et al., 2010), but other reports by Kasari (1996) suggests the utility of higher minimums of 32 to 33 cm due to better semen quality. In general, a large SC is associated with early puberty, higher sperm concentration, and a higher percentage of morphologically normal sperm.

Least squares means of scrotal measures for SBMTRT and CSMCON by months (Top) and increase in scrotal measure for diet within each month (Bottom) is presented in figure 5.

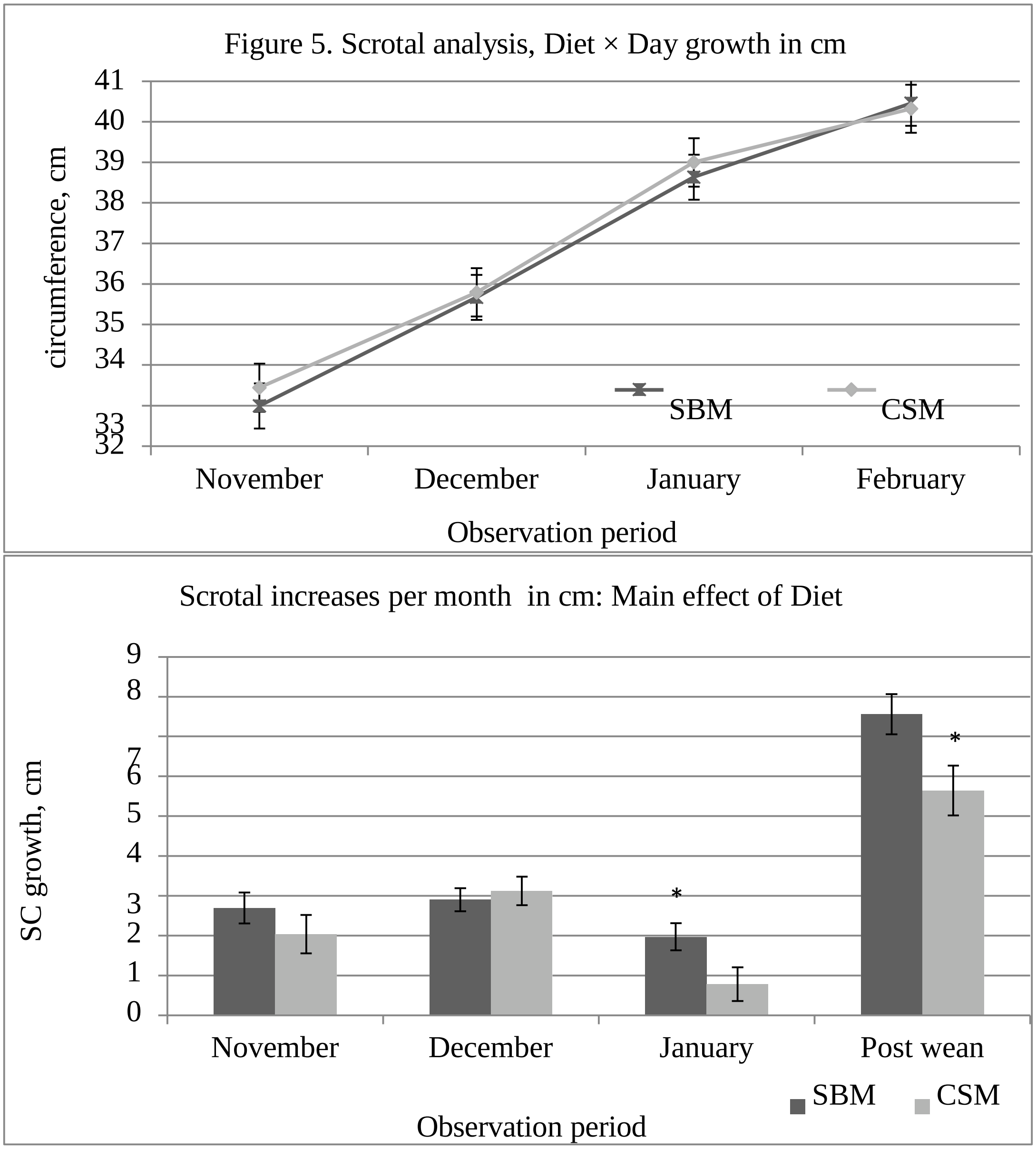

Figure 5. Least squares means of the diet × day observations of scrotal growth in cm (Top) and changes in scrotal growth within each month (Bottom). * Superscript SBMTRT and CSMCON differ within month or postweaning period ($P \leq 0.05$)

A similar increasing pattern was observed for both SBMTRT and CSMCON with no significant differences in SC within each month Figure 5 (Top). But when the increase of SC during each month was calculated and analyzed, significant differences were detected due to diet Figure 5 (Bottom). The SC of SBMTRT was greater than their CSMCON contemporaries in the month of January and for the entirety of postweaning measure (P <

0.05). This data would reflect similar results presented by Yuan et al. (2012), in which the level show that testis index in pigs fed 500ppm of isoflavone supplement were 40% lower than the control group and even lower, (58%), than the boars that were fed 250 ppm isoflavones, but the testis of the boars fed the 250 ppm were larger than the control boars consuming 500ppm isoflavone. It is not unreasonable to extrapolate that the level of isoflavone exposure in this data is likely not high enough to cause detrimental effects to SC measures. This current study was centered on investigating the potential difference in SC and weight and therefore future reports may require deeper inquiry into varying levels of isoflavones exposure to growing bulls. Coulter (1982) concluded that 13% of the bulls with a scrotal circumference of 32 cm or less produced satisfactory seminal quality, while 88 percent of the bulls in the 32 - 38 cm scrotal range were classified as satisfactory. In these data, no observations of SC below 32 cm was observed at day 54 or day 86, regardless of diet group (Table 3), implying that isoflavone consumption from SBM is not prohibitive to scrotal measures at a 10% inclusion rate as a feed ingredient in developing bull rations.

CONCENTRATION

Sperm concentration in ejaculate is one of the important criteria of semen characteristics to qualify fertile males for breeding purposes (Graffer et al., 1988) Semen concentration is imperative for successful conception rates in breeding herds. Results of the significant sources of variation for semen concentration in this study are presented in figure 6.

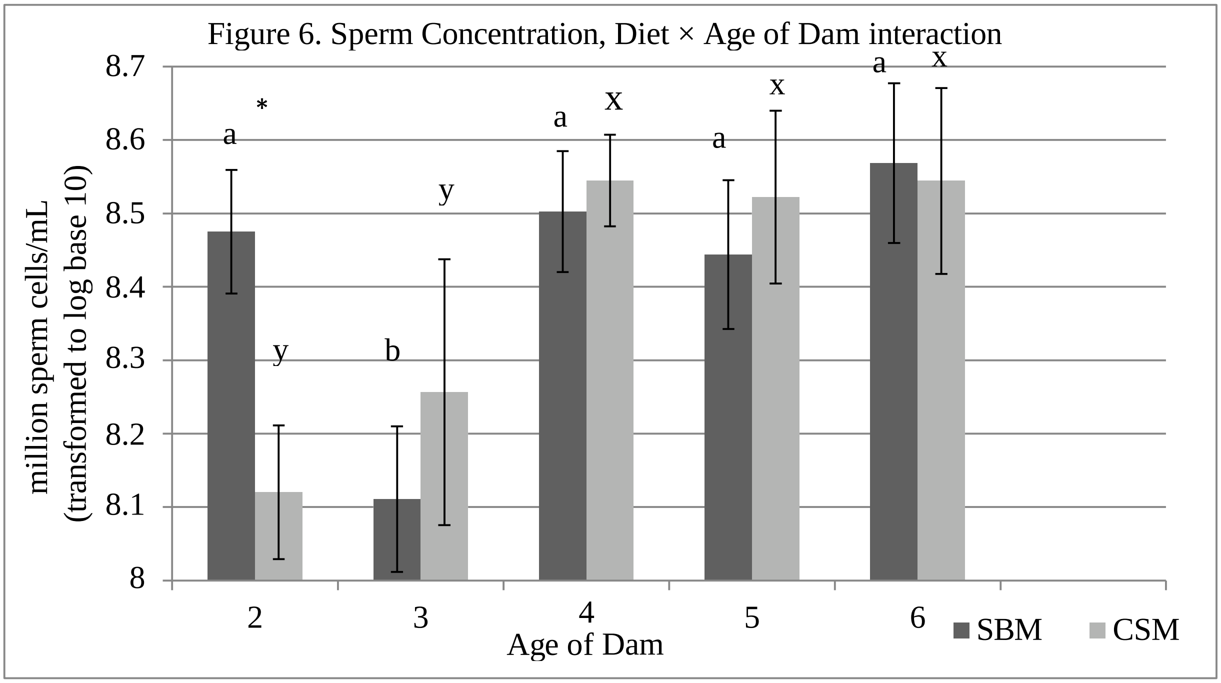

Figure 6. Least squares means of the Diet × Age of Dam interaction on semen concentration, million sperm cells/ml of ejaculate (transformed to log base of 10 for analysis). [ab]superscripts differ across SBMTRT ($P \leq 0.05$), [xyz] superscripts differ across CSMCON ($P \leq 0.05$), and [*] denotes SBMTRT and CSMCON differ within a specific Age of Dam ($P \leq 0.05$)

Of primary interest is the limited sperm cell count of the SBMTRT consuming bulls born to 3 year AOD, which was significantly lower than all other SBMTRT bulls in the remaining AOD. The CSM consuming bulls born to 2 year AOD and 3 year AOD was also significantly reduced as compared to the remaining CSM bulls from 4, 5, and 6 year AOD ($P < 0.05$). The only significant difference within AOD was observed in the 2 year AOD as the SBMTRT bulls produced a greater concentration of sperm cells/mL of semen samples than the CSM bulls. In spite of the inconsistent pattern observed in the 2 and 3 year AOD, it is plausible to conclude that neither diet offered decisive advantages in terms of sperm proliferation as measured by sperm concentration in this data.

VOLUME

One of the sources of variance in this report for volume was observed due to the Diet × Age of Dam interaction and is presented in figure 7.

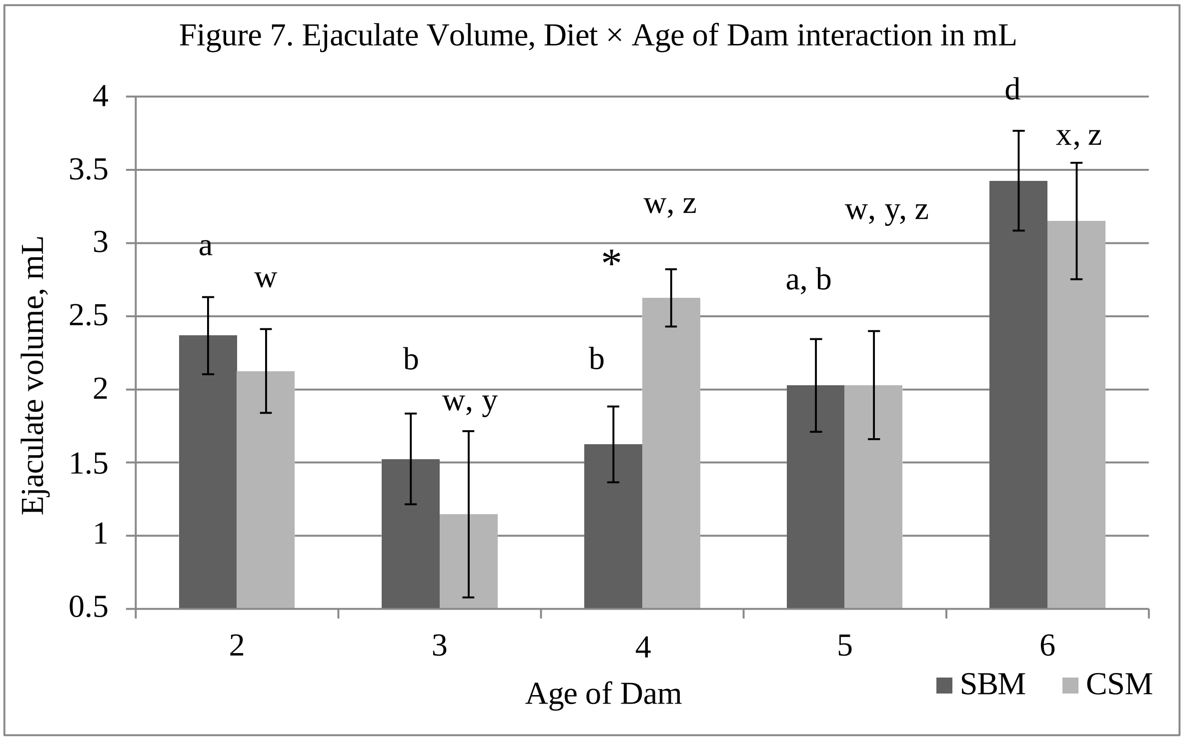

Figure 7. Least squares means of the Diet × Age of dam interaction on ejaculate volume in mL. [ab] superscripts differ across SBMTRT ($P \leq 0.05$), [xyz] superscripts differ across CSMCON ($P \leq 0.05$), and [*] denotes SBMTRT and CSMCON differ within a specific Age of dam ($P \leq 0.05$)

It was observed that bulls consuming SBMTRT from 6 year AOD produced a significantly higher volume of semen as they were 1.06, 1.90, 1.80, and 1.40 mL higher as compared to SBMTRT consuming bulls from 2, 3, 4, and 5 year AOD, respectively ($P < 0.05$). A similar pattern was also observed in bulls consuming the CSMCON from 6 year AOD as they produced 1.03 and 2.00 mL more ejaculate volume as compared to the CSMCON consuming bulls from the 2 and 3 year AOD groups ($P = 0.05$). Considering the differences that exist due to DIET within a specific AOD, the single difference in these data was observed in the 4 year AOD as the bulls consuming CSMCON produced 1.00 mL greater volume than there SBMTRT consuming contemporaries ($P < 0.05$). Hossain et al. (2012) reported that bulls that produce greater semen volume may be a good indicator of fertility. These results suggest that regardless of DIET, a selection criteria in which bulls from 3 year AOD are selected against and bulls from 6 year AOD are selected for can potentially enhance fertility and therefore reproductive efficiency.

Age of dam was a significant source of variation on the semen measures of sperm cell concentration and ejaculate volume in these data ($P \leq 0.05$). Characteristics of these observations are found in figure 8. While there is limited published data and current literature discussing the age of dam effects on semen measures, observations by Lunstra. et al. (1998) highlight the impact of age of dam on scrotal circumference and outlines standard adjustments factors due to differences in age of dam that are often utilized in standard breed association information reporting purposes.

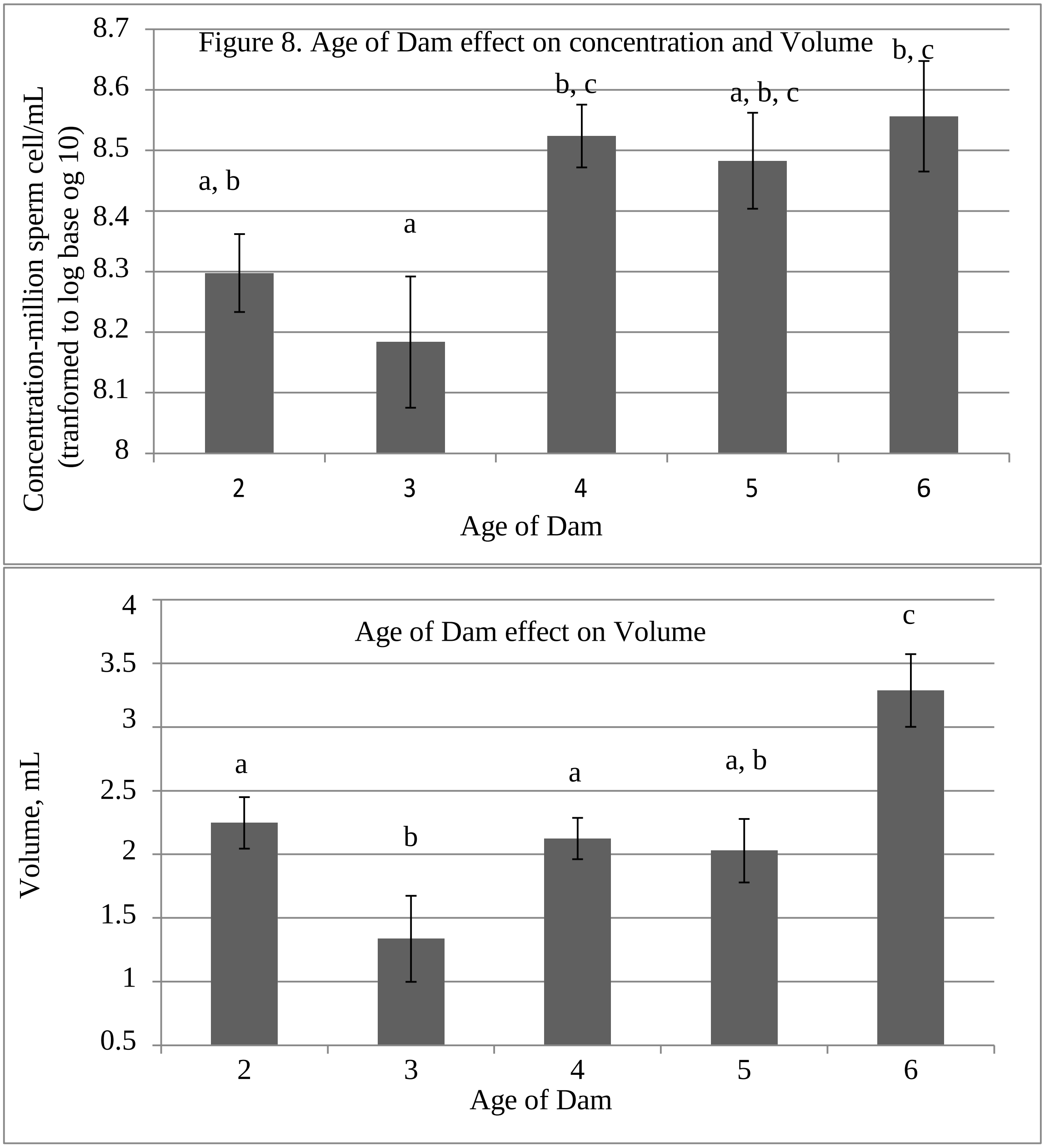

Figure 8. Least squares means of Age of dam main effects on Semen concentration (Top) million sperm cells/ml of ejaculate (transformed to log base of 10 for analysis.) and ejaculate volume, in ml (Bottom). [abc] superscripts differ across age of dams ($P \leq 0.05$).

Kastelic (2014) state that early life nutrition due to maternal milk production; can affect testicular size in growing bulls. Resulting in reduced testicular size of bulls from two year old dams compared to offspring from older dams. Sperm concentration in ejaculate is one of the important criteria of semen characteristics to qualify fertile males for breeding purposes (Graffer et al., 1988) and motility is one of the most important requirements of fertile semen. Presumably, these differences are due to potentially nutritionally compromised situations due to limited maternal milk availability of younger dams. It is not unreasonable to asses that similar results are plausible for measures of testicular function and this data outlines the need for additional research that primarily investigates the impact of early calf nutrition or the interactions with nutritional metabolites through puberty. Coulter and Kozub (1988) discovered that concentration as well as volume of sperm ejaculates score increased with age, and had a notable increase between 1 year old and 2 year old bulls.

MOTILITY

Table 4 presents the scoring methodology utilized to assess semen motility scores in this report.

Table 4. Semen grading scores based on motility.

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.

In regards to semen motility scores, much variation was observed due to Diet as a main effect, and the DIET × AOD interaction. The least squares means for the AOD × Diet interaction are presented in figure 9.

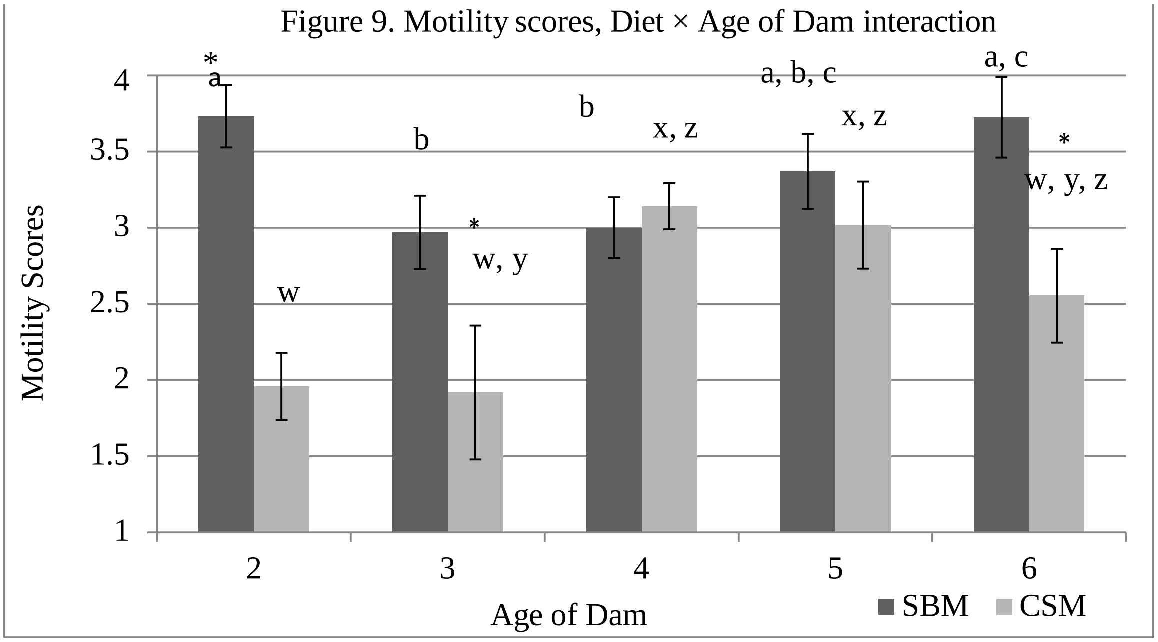

Figure 9. Least squares means of the diet × Age of dam interaction sperm motility. [abc] superscripts differ across SBMTRT ($P \leq 0.05$), [xyz] superscripts differ across CSMCON ($P \leq 0.05$), and [*] denotes SBMTRT and CSMCON differ within a specific age of dam ($P \leq 0.05$)

It was observed that bulls consuming SBMTRT within the 2, 3 and 6 year old dams scored significantly higher than the CSMCON consuming bulls within like age of dam analysis (1.77, 1.05, and 1.17) scores higher, respectively. No differences between SBMTRT and CSMCON within the 4 year old dams and 5 year old dams were observed.

The main effect of Diet on motility scores is summarized in figure 10. The least squares means for motility resulted in measures of 3.36 for the SBMTRT consuming bulls which was significantly higher than the observed 2.52 scores for the CSMCON consuming bulls ($P < 0.05$). Donham et al. (1926) found that semen below normal motility (≥ 90 %) was less than half as effective in producing maximum conception rate.

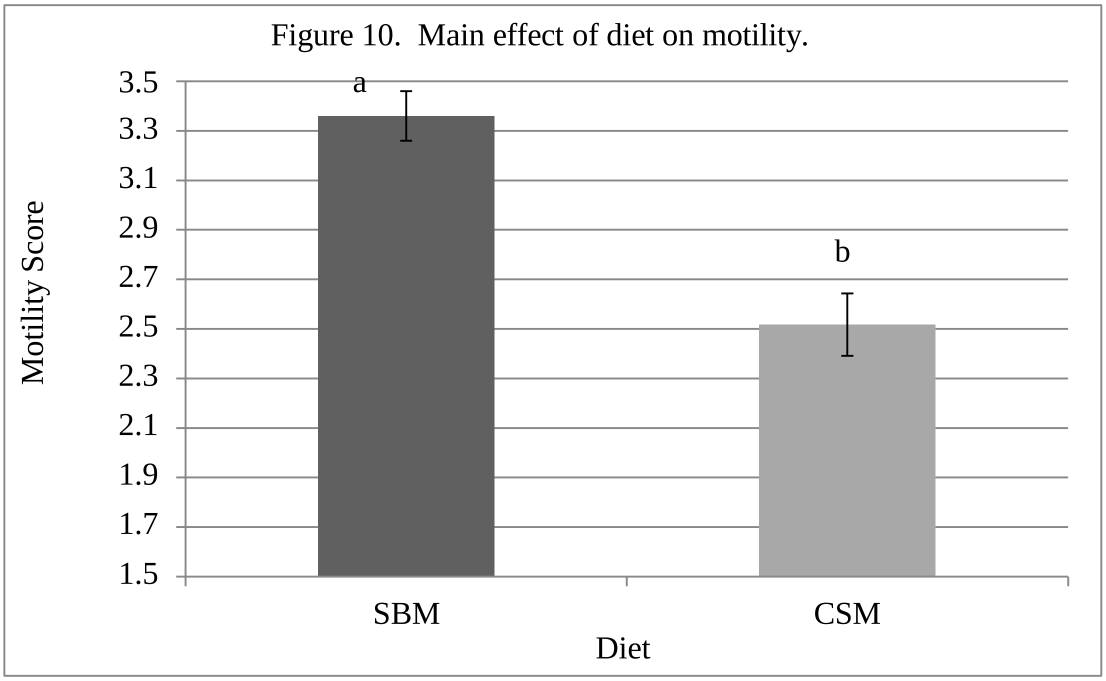

Figure 10. Least squares means for main effect of diet on motility.
[ab] superscripts differ across diet ($P \leq 0.05$).

Alexander (2008) and Chenoweth (1993) concluded that bulls must have at least a minimum of 30% progressive motility estimation to be deemed a satisfactory breeder. In these data, semen motility scores of 2 or higher represent a 30% or greater progressive motility equivalent and all bulls would therefore be deemed as acceptable in terms of semen motility as observed by oscillating activity. It is also important to indicate that as a bull advances in age, there is an improvement in semen concentration and total motile sperm output which continues to increase thereafter for a length of 20 weeks after puberty through maturity (Almquist and Cunningham, 1967). Davis (1939) reported motility of spermatozoa as one of the best single evidence of viability. These data suggest plausible advantages of inclusion of SBM as a protein source at levels of 10% of a ration or less for added benefits of viability as breeders from semen motility observations.

IMPLICATIONS

The summation of this project has shown that there are benefits to feeding SBM compared to CSM to growing beef bulls. There were noticeable gains in weight, growth, scrotal circumference as well as higher sperm values. Future research should focus on the AOD with specific attention to the 3 year old dams, the outcome of AOD effects on growth and reproduction are of primary interest due to results of this study. The SBMTRT did have profound effects on the growth and sperm production measures within the prepuberal bulls.

There was a significant drop in the post weaning ADG weight in the 3 year old dams in the CSMCON group. The scrotal growth within the study rose at a steady level for both treatment groups which is expected with the increase in age. The AOD did have affected sperm concentration and volume, as the 3 year old dams in both SBMTRT and CSMCON groups seemed to be lower measures than all other AOD interactions. Though it is necessary for further research into soybean and isoflavones as a protein source in performance tested beef bulls, these results support the inclination of positive aspects of utilizing soybean meal as a protein source in growing beef cattle without inducing detrimental effects due to isoflavone consumption

LITERATURE CITED

Adlercreutz, H., H. Honjo., A. Higashi., E. Fotsis., T. Hamalainen., T. Hasegawa., and H. Okada. 1991. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am. J. Clin. Nutr. 54:1093

Ajitkumar, G.M., herman W.B., R. Wilde, J.P. Kastelic, and J.C. Thundathil. 2010. Associations between sperm abnormalities, breed, age, and scrotal circumference in beef bulls. Can. J. Vet. Res. 75: 241-247.

Alexander, J.H. 2008. Bull breeding soundness evaluation: A practitioner's perspective. Theriogenology. 70(3): 469-472

Almquist J.O. and D.C. Cunningham. 1967. Reproductive capacity of beef bulls. I. Post puberal changes in semen production at different ejaculation frequencies. J. Anim. Sci. 25:1: 174-181.

Arthur, P.F., J.A. Archer, D.J. Johnston, R.M. Herd, E.C. Richardson, and P.F. Parnell. 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other post weaning traits in Angus Cattle. J. Anim. Sci. 79:2805-2811

Barth, A.D., L.F.C. Brito., and J.P. Kastelic. 2008. The effect of nutrition on sexual development of bulls. Theriogenology 70:485-494

Beever, D.E. 1984. Utilization of the energy and protein components of forage by ruminants- A United Kingdom perspective. In: G.W. Horn (Ed.) National Wheat Pasture Symposium. P. 65. Proc. Oklahoma Agri. Exp. Sta.

Bennetts, H.W., E.J., Underwood, and F.L. Shier. 1946. A specific breeding problem on subterranean clover pastures in Western Australia. Aust. Vet. J. 22, 2-12.

Bourdon R.M. and J.S. Brinks. 1982. Genetic, environmental and phenotypic relationships among gestation length, birthweight, growth traits and age at first calving in beef cattle. J. Anim. Sci. 55:3: 543-553.

Bourdon R.M. and J.S. Brinks. 1985. Scrotal circumference in yearling Hereford bulls: adjustment factors, heritability's and genetic, environmental and phenotypic relationships with growth traits. J. Anim. Sci. 62:958-967.

Brito L.F.C., A.D Barth., R.E Wilde., and J.P Kastelic. 2011. Effect of growth rate from 6 to 16 months of age on sexual development and reproductive function in beef bulls. Theriogenology 77:1398-1405

Brito, L.F.C., A. Barth, R.E. Wilde and J.P. Kastelic. 2012. Testicular vascular cone development and its association with scrotal temperature, semen quality, and sperm production in beef bulls. Anim. Repro. Sci. 134 (3-4):135-140

Cardoso, J.R., 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. Anim. Reprod. Sci. 97: 237-245

Cederroth, C.R., J. Auger, C., Zimmermann, F. Eustache, S. Nef. 2010. Soy, phyto-estrogens and male reproductive function: A review. Int. J. Androl. 33:304-316.

Coulter G.H. and Keller, D.G. 1982. Scrotal circumference of young beef bulls: Relationship to paired testes, weight, effect of breed and predictability. Can. J. Anim. Sci. 52:133-139

Coulter G.H.and G.C Kozub. 1988. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. J. Anim. Sci. 67:1757-1766

Davis, H.P., and N.K., Williams. 1939. Evaluating bovine semen I. Influence of the number of ejaculates upon various physical and chemical characteristics and the relationship between those factors Proc. Am. Sci. Anim. Prod 1:232-242

Donham C.R., B.T. Simms. and J.M. Shaw. 1926. Fertility studies in the bull. II. The relation of the microscopic findings in semen to its fertility. J. Am. Vet. Med. Assoc. 68: 701-715.

Dunn, T.G. and G.E. Moss. 1992. Effects of Nutrient Deficiencies and Excess on Reproductive Efficiency of Livestock. J. Anim. Sci. 70:1580-1593

Food and Drug Administration. 1999. Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final Rule. Fed Regist. 64:57700-57733

Graffer T., H. Solbu, O. Filseth. 1988. Semen production in artificial insemination bulls in Norway. Theriogenology. 30: 1011-1021.

Grigsby, K.N., F.M. Rouquette, Jr., W.C. Ellis, and D.P. Hutcheson. 1989. Self-limiting protein supplements for calves grazing Bermuda grass pastures. J. Prod. Agric. 2:222

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

Herd, R.M., J.A. Archer, and P.F. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: opportunity and challenges to application. J.Anim.Sci. 81(E. Suppl. 1):E9-E17.

Hess, R.A. 2003. Estrogen in the adult male reproductive tract: A review. Reprod. Biol. Endocrinol.. 52: 1-14.

Hess, R.A. and K .Carnes, December 2004. The role of estrogen in testis and the male reproductive tract: A review. Anim. Reprod. 1:5-30

Holechek J.L. and C.H. Herbel. 1986. Supplementing Range Livestock. Rangelands 8(1) 29-33

Hopkins F.M., J.C. Spitzer. 1997. The New Society for Theriogenology breeding soundness evaluation system. Vet Clin. North Am Food Anim. Prac13:283-293

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.

Kasari T.R., S.E. Wiske, R. Jones. 1996. Use of yearling bulls in beef cattle operations. Part 1. Economic analysis and fertility assessment. Comp Cont. Educ. Prac. Vet 18:1244-1253

Kastelic J.P., 2014. Understanding and evaluating bovine testes. Theriogenology 81, 18-23

Kastelic J.P., J.C. Thundathil. 2008. Breeding soundness evaluation and semen analysis for predicting bull fertility. Reprod. Dom. Anim.; 43(Suppl.2):368 –73.

Kotch, 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.

Kriese, L.A., J.K. Bertrand and L.L. Benyshek. 1991. Age adjustment factors, heritabilities, and genetic correlations for scrotal circumference and related growth traits in Hereford and Brangus bulls. J. Anim. Sci. 69:478.

Lephard, E.D., K.D.R. Setchell, R.L. Handa, and T.D. Lund. 2004. Behavioral Effects of Endocrine-disrupting Substances: Phytoestrogens. ILAR J. 45(4):443-454

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.

Mongkonpunya, K., H.D. Hafs, E.M.Convey, H.A. Tucker and W.D. Oxender. 1975. Serum lutenizing hormone, testosterone and androstenedione in puberal and prepuberal bulls after gonadotropin releasing hormone. J. Anim. Sci. 40:682.

Montano-Bermudez, M., M.K. Nielsen, and G.H. Deutscher. 1990. Energy Requirements for maintenance of crossbred beef cattle with different genetic potential for milk. J. Anim. Sci. 68:2279-2288.

Nolan, C.J., D.A. Neuendorff, R.W. Godfrey, P.G. Harms, T.H. Welsh, N.H. McArthur and R.D. Randel. Influence of dietary energy intake on prepubertal development of Brahman bulls. 1990. J Anim. Sci. 68(4):1087-1096.

NRC, 2000. Nutrient requirements of Beef cattle. (6th edition) 2000 update. National Academy press, Washington, DC.

Parnell, P.F., R.M. Herd, D. Perry, and B. Bootle. 1994. The Triangle experiment-Response in growth rate, size, maternal ability, reproductive performance, carcass composition, feed requirements and herd profitability. Proc. Aust. Soc. Anim. Prod. 20:17-26.

Patisaul, H.B., W. Jefferson. 2010. The pros and cons of phytoestrogens. Front Neuroendocrinol. 31 (4): 400-419.

Rocha, A., M. Carpena, B. Triplett, D.W. Forrest, and R. D. Randel. 1995. Effect of Ruminal Undegradable Protein from Fish Meal on Growth and Reproduction of Peripuberal Brahman Bulls. J. Anim. Sci. 73:947-953.

Rumph J.M., L.D. VanVeleck. 2004. Age of dam adjustment factors for birth and weaning weight records of beef cattle: a review. Genet. Mol. Res. 3 (1): 1-17Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W. 2009. Soy food intake and breast cancer survival. J. Am. Med. Assn. 302:2437-2443

Spitzer J.C. D.G Morrison, R.P. Wettemann, and L.C. Faulkner. 1995. Reproductive responses and calf birth and weaning weights as affected by body weight condition at parturition and postpartum weight gain in primiparous beef cows. J. Anim. Sci. 73:1251-1257.

Toppari, J., J.C. Larsen, P. Christiansen, A. Giwercman. P. Grandjean, L.J. Guillette Jr., J. Bernard, T.K. Jensen, P. Jouannet, N. Keiding, H. Leffers, J.A. McLachlan, O. Meyer, J. Muller, E. Rajperd-De-Meyts, T. Scheike, R. Sharpe, J. Sumpter, N.E. Skakkebaek. 1996. Male reproductive health and environmental Xenoestrogens. Enviro. H. Persp. 104:4.

VanDemark, N.L. 1964. Quantitative aspects of semen production in bulls. Intern. Congr. Anim. Reprod. (Cambridge): 80-81

Waldner, C.S., R.I. Kennedy, and C.W. Palmer. 2010 A description of the findings from bull breeding soundness evaluations and their association with pregnancy outcomes in a study of western Canadian beef herds. Theriogenology 74:871-83

Wolf, F.R., J.O. Almquist and Hale, E.B. 1965. Prepuberal behavior and puberal characteristics of beef bulls on high nutrient allowance. J. Anmi. Sci. 24:761-765

Yamori Y, E.H. Moriguchi, T. Teramoto, A. Miura, Y. Fukui, K.I. Honda, M. Fukui, Y.Nara, K. Taira, Y. Moriguchi. 2002. Soybean isoflavones reduce postmenopausal bone resorption in female Japanese immigrants in Brazil: a ten-week study. J. Anim. Coll. Nutr. 21:560-563

Yilmaz, A., M.E. Davis, and R.C. Simmen. 2004. Estimation of (co)cariance for reproductive traits in Angus beef cattle divergently selected for blood serum IGF-I concentration. J. anim. Sci. 82:2285-2292

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. Biotechol. 3:31.