***Research paper***

# The Relationship between Phenotypic, Testicular Traits and Serum Testosterone Levels in Pre-pubertal Male Boer Goat Crosses

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## ABSTRACT

In the present study, the relationship among phenotypic traits, scrotal circumference and serum testosterone concentrations were investigated in pre-pubertal male Boer goat crosses at the Caprine Research and Education Unit, Tuskegee University, Tuskegee, AL. Phenotypic traits (chest girth -CG, height at withers-HTW, body length -BL, body condition scores -BCS, body weight –BW, shoulder width –SW), and scrotal circumference –SC were monitored at 3 week intervals for 12 weeks. Also, blood samples were collected and a calibrated IMMULITE 1000 assay system was used for the quantitative measurement of total serum testosterone. Although, results show a non-significant relationship between serum testosterone levels and many phenotypic traits, serum testosterone levels were lowly and positively correlated to BL (r = 0.19), BW (r = 0.19), CG (r = 0.13), HTW (r= 0.11) and SC (r = 0.18). Scrotal circumference was moderately correlated to BL (r = 0.30; *P*= 0.001) and SW(r = 0.33; *P*< .001) and strongly correlated with BW (0.61), CG (r = 0.53), HTW (r = 0.41) respectively. In addition, scrotal circumference and body weight increased (*P*< .01) linearly from week 6 through week 12 (23.22± 0.86cm; 31.95 ± 2.64kg) (24.53 ±1.43cm; 34.72 ± 2.98kg) and (26.05 ±1.35cm; 37.46 ± 3.44kg), respectively. Testosterone levels increased from week 0 to week 3, after which there was a declined into week 6 before peaking at week 9. It is speculated that the week 9 peak of serum testosterone levels obtained in this study represents the attainment of sexual maturity (puberty) in Boer goat male crosses. Based on the results of this study, we hypothesized that scrotal circumference measurements, when used in conjunction with serum testosterone levels and phenotypic traits can be a valuable breeding soundness evaluation tool for selecting or culling breeding Boer goat sires at an early age by limited resource producers.

***Keywords: Boer goats, Phenotypic, testicular traits, serum testosterone***

## 1. INTRODUCTION

Meat goat breeds available for production in the U.S includes: South African Boer, New Zealand Kiko, Myotonic, Savannah, Spanish [1]. The Boer goat from South Africa is a breed developed for meat production that evolved from selection pressures placed on common goats of the region by farmers [2]. To make genetic gain in the meat goat industry, there is a requirement to determine superior Boer meat goat sires within the population and increase their use. The most accurate way to test a sire's genetic worth is to perform breeding soundness evaluation (BSE) and also generate progeny from the animal [3]. BSE predicts the potential fertility of a buck. It is based on an examination that includes tests for physical soundness, testicular size, semen quality, and in some cases, serum testosterone profiles, and libido/mating ability.

The age and weight at which puberty occurs vary greatly among breeds of goats and the level of nutrition during development; however, research with various breeds [4, 5] suggests that a practical indication of imminent puberty is when scrotal circumference is between 25 and 27 cm. In bulls, there is a positive genetic correlation between a sires' scrotal circumference, the scrotal circumference of his sons, and the pregnancy rate of his daughters [6]. This indicates that bulls with a larger scrotal circumference are likely to sire sons with larger scrotal circumference, and daughters likely to reach puberty at younger ages.

Testosterone is a steroid hormone produced by cells of the testes, and is considered to be the primary circulating androgen that regulates testicular function. Testosterone levels are useful in selection of young sires, and in characterizing sexual maturity in different breeds of meat animals sire [7]. It is important to evaluate testosterone levels to determine the development of the reproductive system [8].

The relationship between body measurements and serum testosterone in 10-12 month old Dorper rams was reported by [9]. Results from [9] indicate that serum testosterone did not have a significant correlation with most of the body parameters measured. However, serum testosterone concentration in rams was positively correlated to masculinity and scrotal circumference. Scrotal circumference was positively and significantly correlated with body weight, body length, chest dept, shoulder height, shoulder width, hindquarter width, canon bone length, masculinity, wedge shape, selection index and age. Rams with a wedge shape score of between five and seven were significantly heavier than those with type scores of between two and four. Collectively, [9] concluded that there is a significant and positive relationship between scrotal circumference and serum testosterone, but no significant correlation between linear shape type score and serum testosterone levels.

Testicular development in relation to age, body weight, semen characteristics and testosterone was reported in Kivircik ram lambs [10]. All measurements of testis, body weight, and serum testosterone concentrations were positively correlated with one another. A significant positive correlation was found at seven to eight months of age between all testicular measurements, semen volume and motility. The study suggested that scrotal circumference could provide useful estimate of testicular growth, as its correlations with other testicular measurements were the highest and this information could be used as selection criteria for ram lambs at an early age.

A procedure that would link external phenotypic and testicular traits with serum testosterone or semen quality may provide a good guide to breeding soundness evaluation in meat goat sires [11]. Since limited resource producers may not be in a position to test libido and/or ejaculate qualities of males before using them for breeding, a procedure that would link external testicular measurements with serum testosterone may provide a good guide to breeding soundness evaluation, especially, where bucks are reputed to have exceptionally high libido [12, 13].

There is no evidence in literature to suggest that this approach has been used in selecting potential Boer meat goat sires for superior breeding and/or reproductive performance. Therefore, the aim of this study is to determine the relationship between certain phenotypic traits, testicular trait (scrotal circumference) and serum testosterone levels of pre-pubertal male Boer crosses. We speculate that if a positive relationship is established, that scrotal circumference measurements used in conjunction with serum testosterone levels, and body conformation traits will be an innovative method to screen potential Boer goat sires at an early age.

## 2. MATERIAL AND METHODS

### 2.1. Animal Management:

Twenty-Five pre-pubertal male Boer goat crosses were used in this study. The Tuskegee University Animal Care and Use Committee approved animal care, handling and sampling procedures. Upon arrival the animals were given an overall health check by an attending veterinarian at Tuskegee University's School of Veterinary Medicine. The animals were treated with Panacur (*fenbendezole*) to control the development and reproduction of internal parasites and quarantined for three weeks at the Tuskegee University's Caprine Research Facility. Each animal was housed for the entire experimental period in individual 1.8 x 2.1m indoor pens after the quarantine period. Throughout the experimental period animals were maintained on a daily diet that consisted of a high energy concentrate that was given at 2lbs/day. The animals were also allowed *ad libitum* access to hay, water and mineralized salt blocks.

### 2.2. Phenotypic Measurements

The phenotypic measurements recorded at 3-week intervals for 12 weeks included: Body weight, BW, kg (recorded using a standard scale), body condition score (scored subjectively on scale of 1 = emaciated to 5 = obese), shoulder width (determined with the aid of a measuring tape as the horizontal distance between the processes on the left shoulder to those of the right shoulder blade), chest girth (determined with the aid of a measuring tape as the width around the chest just behind the front legs), body length(determined with the aid of a measuring tape as the distance from the sternum to the aitch bone), hip width (determined with the aid of a measuring tape as the distance between the left and right femur bones) and height at wither (determined with the aid of a metric ruler as the length, vertically from the thoracic vertebrae to the ground).

**2.3. Testicular Measurements**

The scrotal circumference was determined for each animal by pulling the testicles firmly into the lower part of the scrotum, grasping the neck of the scrotum with one hand, squeezing and pulling down. Thereafter, the circumference was measured with the aid of a measuring tape and recorded as the largest diameter of the scrotum.

**2.4. Blood Collection**

Blood samples were collected at intervals of three weeks for12 weeks for each animal. The blood was drawn via jugular venipuncture into 10ml heparinized vacutainer tubes, and placed on ice immediately after collection. The serum was separated by centrifugation at 3000 rpm at 4ºC for 10 minutes and aliquoted into separate vials which were kept frozen at -4º C until testosterone assay.

**2.5. Serum Testosterone Assay**

A calibrated IMMULITE 1000 system (developed at Meharry Medical College, Nashville, Tennessee) was used for the quantitative measurement of total serum testosterone. IMMULITE 1000 Total Testosterone is a solid-phase, enzyme-labeled, competitive chemiluminescent immunoassay. The solid-phase, a polystyrene bead enclosed within an IMMULITE Test Unit, is coated with a polyclonal rabbit antibody specific for testosterone. Serum samples and alkaline phosphatase-labeled testosterone were simultaneously introduced into the Test Unit, and incubated for approximately 60 minutes at 37°C with intermittent agitation. During this time, testosterone in the sample competed with alkaline phosphatase labeled testosterone for antibody-binding sites on the bead. Unbound material was then removed by a centrifugal wash. Substrate is then added, and the Test Unit is incubated for another 10 minutes.

The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of these intermediate results in the sustained emission of light, thus improving precision by providing a window for multiple readings. The bound complex - and thus also the photon output, as measured by the luminometer - is inversely proportional to the concentration of testosterone in the sample.

### 2.6 Statistical Analysis

Descriptive statistics [14] was performed on the data to determined individual buck differences in selected body conformation, testicular and serum testosterone profiles. Also, data was subjected to analysis of variance using the GLM procedures of [14]; correlation coefficients (r) were established between various body, testicular parameters and serum testosterone profiles.

## 3. RESULTS AND DISCUSSION

The means and standard deviations for phenotypic traits, testicular traits and serum testosterone levels are shown in Tables 1. The average SC at week 0 was (22.87±2.42cm), with a slight decline into week 3 (22.54±1.77cm).

Table 1. The Effect of Week on Phenotypic, Testicular Traits, Serum Testosterone at week 0, 3, 6, 9, and 12)* in Pubertal Male Boer Crosses.

| Parameters<br><br>Week 12 | Week 0<br>(N= 25) | Week 3<br>(N=23) | Week 6<br>(N= 23) | Week 9<br>(N=23) | Week 12<br>(N= 23) |
|---|---|---|---|---|---|
| Body Condition Score (BCS, 1-5) | 3.40 ± 0.50$^{b}$ | 3.21± 0.51$^{ab}$ | 3.08± 0.41$^{ab}$ | 2.91± 0.59$^{a}$ | 3.13± 0.69$^{ab}$ |
| Body Length (BL, cm) | 58.62 ± 2.41$^{a}$ | 61.95± 3.21$^{b}$ | 63.72± 2.26$^{bc}$ | 65.25± 2.34$^{cd}$ | 67.13± 2.75$^{d}$ |
| Body Weight (BW, kg) | 27.66 ± 2.54$^{a}$ | 27.52± 2.20$^{a}$ | 31.95± 2.64$^{b}$ | 34.72± 2.98$^{c}$ | 37.46± 3.44$^{d}$ |
| Chest Girth (CG, cm) | 66.34± 3.22$^{ab}$ | 64.37± 1.95$^{a}$ | 67.82± 2.58$^{bc}$ | 68.91± 2.21$^{cd}$ | 70.79± 2.35$^{d}$ |
| Height at Wither (HTW, cm) | 58.01± 1.90$^{a}$ | 59.97± 2.27$^{b}$ | 61.20± 1.86$^{bc}$ | 62.29± 2.13$^{c}$ | 64.58± 2.39$^{d}$ |
| Hip Width (HW, cm) | 42.16± 3.12$^{a}$ | 41.95± 2.28$^{a}$ | 43.63± 1.65$^{ab}$ | 42.18± 1.68$^{a}$ | 45.06± 2.06$^{b}$ |
| Scrotal Circumference (SC, cm) | 22.87± 2.42$^{a}$ | 22.54± 1.77$^{a}$ | 23.22± 0.86$^{ab}$ | 24.53± 1.43$^{b}$ | 26.05± 1.35$^{c}$ |
| Shoulder Width (SW, cm) | 41.14± 2.54$^{a}$ | 40.40± 1.84$^{a}$ | 41.73± 1.52$^{a}$ | 41.29± 1.92$^{a}$ | 44.06± 2.11$^{b}$ |
| Testosterone (TT, ng/ml) | 5.625± 6.03$^{a}$<br>(N= 20) | 8.74±11.26$^{a}$<br>(N= 17 ) | 7.48±8.25$^{a}$<br>(N= 23) | 27.48±14.42$^{b}$<br>(N= 23) | 10.74±14.40$^{a}$<br>(N=23) |

*Means ± Standard Deviations

$^{a,b,c,d}$ Means in each row with the same superscript are not significantly different ($P$ > .05).

However, SC increased linearly from week 6 through week 12 (23.22±0.86cm, 24.53±1.43cm and 26.05±1.35cm), respectively. There was no significant difference for SC between weeks 0, 3, and 6; and week 6 and week 9 ($P$= .15), respectively. In addition, scrotal circumference and body weight increased correspondingly from week 6 through week 12 (23.22±0.86cm; 31.95 ±2.64kg) (24.53±1.43cm; 34.72 ±2.98kg) and (26.05±1.35cm; 37.46 ±3.44kg), respectively. The average TT level at week 0 was 5.625±6.03ng/ml, with a slight increase into week 3 (8.74±11.26ng/ml). Also, there was a decline in TT level at week 6 (7.48±8.25ng/ml), and week 9 showed an all time high (27.85±14.42ng/ml), followed by a decline at week 12 (10.74±14.40ng/ml). For weeks 0, 3, 6 and 12 there was no significant difference ($P$=.19) for TT levels. However, TT for week 9 was significantly different from week 0, 3, 6 and 12 ($P$=.05), respectively. [15] reported mean testosterone levels varying from (0.259± 0.172 ng/ml to 4.613±2.892ng/ml and 0 .521± 0.311 to 3.417± 2.021ng/ml) in Boer goats starting from 1 month to 8 months of age during dry and rainy seasons. In young Saneen and British Alpine goats, testosterone levels are marked by an initial decline followed by a peak at the time when male reach sexual maturity ([16, 17]. As previously stated testosterone levels increased from week 0 to week 3, after which there was a declined into week 6 before peaking at week 9. It is speculated that the week 9 peak of serum testosterone levels obtained in this study represents the attainment of sexual maturity (puberty) in Boer male crosses.

The correlation coefficients (r) for phenotypic traits, testicular traits and serum testosterone levels are presented in Table 2. Serum testosterone levels recorded a low to moderate correlations with most of the body conformation traits measured in this study. The serum testosterone concentration was positively correlated to BL (r = 0.19), BW (r = 0.19), CG (r = 0.13), HTW (r= 0.11) and SC (r = 0.18), respectively. However, negative relationships were observed for BCS (r = -0.19), HW (r = -0.13) and SW (r = -0.08), respectively. [9] found similar results in Dorper rams, where serum testosterone concentration was positively correlated to masculinity (r = 0.15; $P$=.05) and scrotal circumference (r = 0.23; $P$<.05), which is slightly higher than values reported in the current study. Specie difference (ram vs. bucks) could explain the discrepancy in the testosterone vs. SC correlation values.

Scrotal circumference (SC) is an important trait that is closely associated with the testicular growth and sperm production in males of all meat animals. Thus, selecting males based on their SC would result in larger testes, potentially with the capacity to produce more semen [18]. Being a highly heritable component of fertility, it is important to include SC when evaluating animals for breeding soundness [19]. In the current study Scrotal circumference was positively and significantly ($P$< .001) correlated to BL (r = 0.30), BW (0.61), CG (r = 0.53), HTW (r = 0.41) and SW(r = 0.33). Similar results were reported by [9] who found positive and significant correlations between scrotal circumference and BW (r = 0.38), body length (r = 0.34) and shoulder width (r = 0.27) in Dorper rams. [12] reported positive correlations between testosterone and body weight (r = 0.30; r = 0.43), scrotal circumference (r = 0.42; r = 0.52) and body weight and scrotal circumference (r = 0.93; r = 0.88) young Boer bucks. [20] reported a highly significant positive relationship between scrotal circumference and testis weight of West African Dwarf bucks. Testis weight is known to be highly correlated (r = 0.93) with testicular sperm reserves [13] and males with larger testes tend to produce more sperm [21]. It follows that a good measurement of scrotal circumference would be a reliable predictor of sperm producing capacity.

Overall, results from this study indicate that there are significant and positive correlations between few body confirmation traits and serum testosterone levels. However, low to moderate correlation was found between scrotal circumference and serum testosterone level in pubertal male Boer crosses. In general, correlations between serum testosterone levels and phenotypic traits in this study were either low or negative. However, testicular size was found to have a higher correlation with body weight than serum testosterone level in pubertal male Boer crosses. Based on the results of this study, it is hypothesized that monitoring scrotal circumference, serum testosterone levels and body conformation traits is a useful tool for selecting superior breeding Boer goat sires at an early age.

Table 2. Correlation Coefficients (r) for Phenotypic, Testicular traits and Serum Testosterone in Pubertal Male Boer Crosses

| | BCS | BL | BW | CG | HTW | HW | SC | SW |
|---|---|---|---|---|---|---|---|---|
| BL | -0.0819 | | | | | | | |
| BW | 0.1677 | 0.6715*** | | | | | | |
| CG | 0.1739 | 0.4941*** | 0.8280*** | | | | | |
| HTW | -0.1447 | 0.5914*** | 0.6631*** | 0.4979*** | | | | |
| HW | 0.1290 | 0.3635*** | 0.4589*** | 0.3612*** | 0.3675*** | | | |
| SC | 0.0946 | 0.3026*** | 0.6117*** | 0.5262*** | 0.4111*** | 0.1721 | | |
| SW | 0.0891 | 0.2771** | 0.4994*** | 0.4671*** | 0.3897*** | 0.4539*** | 0.3291*** | |
| TT | -0.1952* | 0.1853* | 0.1866* | 0.1279 | 0.1124 | -0.1299 | 0.1776 | -0.0851 |

BL= Body Length, BW= Body Weight, CG= Chest Girth, HTW= Height at Wither, HW= Hip Width, SC= Scrotal Circumference, SW= Shoulder Width, TT = Testosterone Level
*= Significant if $P < .05$
**= Significant if $P < .01$
***= Significant if $P < .001$

The present finding of positive correlation for SC with testosterone in the pubertal bucks may indicate the importance of monitoring SC and/or serum testosterone levels in testicular development and the onset of puberty in male Boer goat crosses. Studies in other animal species ([13, 9, 14, 15] and have increasingly shown the role of testosterone in male reproductive functions. Further studies on the activity of testosterone receptors in the caprine testis around and after puberty are needed to better understand the functional role of testosterone.

Although the present results are based on a small number of animals monitored for a short period, this is the first study of its kind in the pubertal Boer buck crosses, and it may help to shed light on the relationship between body conformation traits, testicular traits and serum testosterone levels in pubertal male goat bucks. These results represented the first detailed information characterizing changes in serum testosterone concentrations, testicular development and body conformation in pre-pubertal and pubertal male Boer goats.

## 4. CONCLUSION

1. There was an intricate relationship among testosterone concentrations, testicular volume, and various body conformation traits.
2. Overall, results from this study indicate that there is significant and positive correlations between body length; body weight and serum testosterone levels. However, low to moderate correlation was found between scrotal circumference and serum testosterone level in pubertal male Boer crosses.
3. Testicular size was found to have a higher correlation with body weight than serum testosterone level in pubertal male Boer crosses.
4. Although, the present results are based on a small number of animals monitored for a short period, the information obtained from this study, while increasing the knowledge of male meat goat reproductive biology, could also, assist the development, implementation of selection or culling criteria for breeding Boer goat sires at an early age.

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**Authors' Contributions:**

Chuck Okere designed the study, performed the statistical analysis, Latoya Keith wrote the first draft of the manuscript as partial fulfillment of her M.Sc thesis in Animal & Poultry Science entitled "The relationship between phenotypic, testicular traits, serum testosterone and insulin-like growth factor-I in pubertal male Boer goat crosses. Olga Bolden-Tiller managed the literature searches and hormone assay. All authors read and approved the final manuscript.

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