Testicular growth morphometry and semen quality of Kalahari Red and *Kalawad* goat bucks as affected by age and season in a tropical environment

Odeyemi, A. J., [1] **Shittu, O. O.,**[2] **Adekunle, E. O.,**[1] **Odeyemi, A. Y.,**[3] **Nwosu, E. U.,**[1] **Smith, O. F.,**[1]
[1]*Department of Animal Physiology Federal University of Agriculture, (FUNAAB), P.M.B 2240, Ogun State, Abeokuta*
[2]*Institute of Food Security, Environmental Resources and Agricultural Research FUNAAB, Ogun State, Abeokuta*
[3]*Department of Animal Production and Health FUNAAB, Ogun State, Abeokuta.*
Corresponding author*:* odeyemiaj@funaab.edu.ng

Abstract

For effective ruminant production, a sound breeding evaluation especially testicular growth measurement and semen quality assessment are crucial in the herd. Based on a 2 x 3 x 4 factorial arrangement, a total of 42 bucks (Kalahari Red, KR, n= 21 and Kalawad, KW, n= 21) were allotted into three age groups (>1≤ 2, >2≤ 3 and >3≤ 4 years) with average body weights of 35 – 40kg, 45 – 55kg and 60 – 80kg; and 25 – 30kg, 35 – 45kg and 45 – 55kg respectively consist seven animals each reared under different seasons (early rainy, late rainy, early dry and late dry season). The animals were managed semi-intensively with a constant adequate nutritional regime for one year. Data on testicular traits were taken twice per week using measuring tape. Semen was collected twice a month and evaluated using Computer Assisted Semen Analyser and a total of 168 ejaculates were collected from each group of the experimental animals. Data obtained on testicular growth and semen quality were analysed. The effects of age on testicular growth and semen quality parameters of goat bucks considered were significantly (P<0.05) influenced by age except pH. Significant (p<0.05) differences were reported in all testicular parameters for both breeds as age increased. The highest scrotal length of 19.31±0.36 cm and 16.07±0.13 cm were observed in early rainy and late dry seasons in KR and KW, respectively while the maximum mean values (p<0.05) for scrotal circumference (31.09±0.11 cm and 24.29±0.08 cm) and testicular width (15.54±0.05 cm and 12.15±0.04 cm) were observed during late and early dry seasons in KR and KW both at age >3≤4 years. Semen volume (1.49 mL and 1.22 mL) was at peak (p<0.05) during the late rainy season. Spermatozoa concentration was at peak in KR and KW (5.49 x 10^6/mL and 3.99 x 10^6/mL, respectively) during the late dry season. Progressive motility was highest in KR while KW had the least value during the early dry season. Acrosome and membrane integrity had maximum (p<0.05) values (85.34% and 79.94%) in KW and (81.92% and 73.74%) in KR during the early dry season, respectively. In conclusion, the reproductive capacity of KR and KW to cope under various environmental clues and seasons depend on genetic make-up and age.

Keywords: Kalahari Red, kalawad, crossbred, testicular, semen quality

Morphométrie de la croissance testiculaire et qualité du sperme des boucs de chèvres Kalahari Red et *Kalawad* influencées par l'âge et la saison dans un environnement tropical

Résumé

Pour une production efficace de ruminants, une évaluation de reproduction solide, notamment la mesure de la croissance testiculaire et l'évaluation de la qualité du sperme, est cruciale pour le troupeau. Sur la base d'un agencement factoriel 2 x 3 x 4, un total de 42 boucs (Kalahari Red, KR, n=21 et Kalawad, KW, n=21) ont été répartis en trois groupes d'âge (>1= 2, >2= 3 et >3= 4 ans) avec des poids corporels moyens de 35 à 40 kg, 45 à 55 kg et 60 à 80 kg ; et 25 à 30 kg, 35 à 45 kg et 45 à 55 kg respectivement, chaque groupe comprenant sept animaux élevés sous différentes saisons (pluie précoce, pluie tardive, sécheresse précoce et sécheresse tardive). Les animaux ont été gérés de manière semi-intensive avec un régime nutritionnel adéquat constant pendant un an. Les données relatives aux caractéristiques testiculaires ont été recueillies deux fois par semaine à l'aide d'un mètre ruban. Le sperme a été collecté deux fois par mois et évalué à l'aide d'un analyseur de sperme assisté par ordinateur, et un total de 168 éjaculats ont été collectés de chaque animal expérimental. Les données obtenues sur la croissance testiculaire et la qualité du sperme ont été analysées. Les effets de l'âge sur la croissance testiculaire et les paramètres de qualité du sperme des boucs de chèvre considérés ont été significativement (P<0,05) *influencés par l'âge, sauf pour le pH. Des différences significatives (p<0,05) ont été observées dans tous les paramètres testiculaires pour les deux races à mesure que l'âge augmentait. La plus grande longueur scrotale de 19,31±0,36 cm et 16,07±0,13 cm a été observée respectivement pendant la saison de pluie précoce et la saison de sécheresse tardive pour KR et KW, tandis que les valeurs moyennes maximales (p<0,05) de la circonférence scrotale (31,09±0,11 cm et 24,29±0,08 cm) et de la largeur testiculaire (15,54±0,05 cm et 12,15±0,04 cm) ont été observées pendant les saisons de sécheresse tardive et précoce pour KR et KW, tous deux à l'âge de >3=4 ans. Le volume du sperme (1,49 mL et 1,22 mL) était au maximum (p<0,05) pendant la saison de pluie tardive. La concentration des spermatozoïdes a atteint son maximum dans KR et KW (5,49 x 10^6/mL et 3,99 x 10^6/mL, respectivement) pendant la saison de sécheresse tardive. La motilité progressive était la plus élevée dans KR, tandis que KW avait la valeur la plus faible pendant la saison de sécheresse précoce. L'intégrité de l'acrosome et de la membrane avait des valeurs maximales (p<0,05) (85,34 % et 79,94 %) dans KW et (81,92 % et 73,74 %) dans KR pendant la saison de sécheresse précoce, respectivement. En conclusion, la capacité reproductive de KR et KW à s'adapter sous différents indices environnementaux et saisons dépend de la constitution génétique et de l'âge.*

Mots-clés: Kalahari Red, Kalawad, croisé, testiculaire, qualité du sperme

Introduction

The main goal of animal breeding is to improve traits or features that have economic value. This depends on particular neurological and endocrine, genetic morphometry of forms, and phenotypic structural qualities that support the breeds' ability to adapt to their respective production environments, support reproduction, and maintain fitness. Age, breed, and physical qualities are factors that are closely associated to these traits (Ebegbulem *et al.*, 2011; Mullin *et al.*, 2021). The Kalahari Red (KR) breed, a meat-type breed with an excellent maternal reputation (Lomandra, 2013), was introduced to Nigeria with the aim of improving the quality and output of milk and meat from native goats. The Kalahari Red are distinct from the Nigerian goat breeds in that they produce more milk and also grow more rapidly (Lomandra, 2013). *Kalawad* (*KW*), which has evolved with faster development and larger body size compared to native West African Dwarf goats, evolved by crossbreeding using the understanding of the KR goat's unique growth performance (Omotosho *et al.*, 2020). *Kalawad* possesses excellent adaptability to the tropical climate with potential to contribute to the socioeconomic system, and capacity to increase productivity, thus, the new goat breeds in Nigeria known as KR and KW stand to have a greater market value (Sanni *et al.*, 2018). In relation to sperm characteristics and animal fertility, testicular traits are one of the most significant and vital reproductive features (Tanga *et al.*, 2021). The size and make-up of the scrotum are associated with reproductive traits in the absence of an infectious condition (Yoseph, 2007). According to research by Mekasha *et al.* (2007), buck body weight is positively correlated with the number of sperm produced. Inefficient fertility and financial loss occur from the body's delayed growth in size and testicular bulk. Breed, age, nutrition, health, and other environmental cues are some of the variables that affect the rate of testicular growth and the increase in body mass (Mekasha *et al.*, 2007). Large-sized breeds are heavier and have larger testicles than small-sized breeds, but they may not have as much or as good of a capability for producing semen (Linden *et al.*, 2014). In order to assess a male's potential fecundity, physical examination, examination of external and internal genitalia (including measuring the size of scrotum), and evaluation of the quality of semen can be done in the field. These techniques are useful for eliminating infertile male(s) from the herd, despite the fact that neither one allows for a precise computation of the pregnancy rates that males actually achieve (Mohammed *et al.*, 2018). Investigating the influence of variations in the ambience on testicular morphometry and semen production of goat breeds – Kalahari Red (Exotic) and Kalawad (crossbred between male Kalahari Red and female West African dwarf goats), is necessary, because they are just been introduced into the tropical regions. Therefore, a better understanding of the growth and reproductive fluctuations in buck goats over the year can aid in breeding and husbandry decisions.

Materials and Methods

The experiment was conducted at the Kalahari Red Goat Unit of the Institute of Food Security, Environmental Resources and Agricultural Research and the Department of Animal Physiology Laboratory, Federal University of Agriculture, Abeokuta (FUNAAB). According to Google Earth 2022, it is situated 76 metres above sea level and lies between latitudes 7º 13' 49" N and 3º 26' 12" E. The South Western region of Nigeria, which is covered in forests, has a humid climate. Rainfall in Abeokuta is bimodal, with usual peaks in July and September and a hiatus of two to three weeks in August. The year's (2022) temperature ranges from 28 to 30ºC during the rainy season to 30 to 34ºC during the dry season, with the lowest nighttime temperature being during the harmattan period between December and February. In contrast to the dry season, when relative humidity ranges from 55 to 84%, it is higher during the rainy season, with values between 63% and 96% (20.FUNAAB, 2020).

Experimental Animals and Management

A total of 42 bucks were chosen at random from the herds consist of 21 each of KR and *KW* goat bucks with three different age groups according to breeding records. Each group contained seven (7) animals each. The grouping includes: bucks of seven months old to one year old (≤ 1 year), Thirteen months old to two years (>1≤ 2 years) and bucks above two years to (>2≤3) three years of age at the onset of experiment, with body weight of about (35 - 40kg, 45 - 55kg and 60 - 80kg) and (25 - 30kg, 35 - 45kg and 45 - 55kg), respectively. However, at the end of experiment, the initial grouping has changed to this order - (>1≤ 2 years, >2≤ 3 years and >3≤ 4 years) with average body weight of about (45 - 58 kg and 62 - 84kg and 85 - 88kg) and (35 - 45kg, 45 - 55kg and 55 - 60kg) respectively. The animals were managed semi-intensively in an open-ventilated pen with concrete floor under natural light and maintained under a uniform and constant nutritional regime. Animals were allowed to graze on paddocks planted with *Chloris gayana, Brachiaria ruzizensis, Stylosanthes hamata ad libitum* on rotational basis and supplemented with concentrate feed containing 16% CP, at 2% body weight dry matter; water was supplied *ad libitum.* The experiment lasted for twelve months. Regular health routine practices were also carried out particularly for endo and ecto-parasites treatments.

Experimental Design

The experimental design for this experiment was a factorial design (i.e. 2 x 3 x 4) [where 2 indicates two breeds, 3 implies three different age groups and 4 represents four different seasons - (early rainy, late rainy, early dry and late dry)] with the testicular growth and semen quality parameters as the dependent variables while age, breed and season series as independent variables.

Model

$$Y_{ijkl} = \mu + A_i + B_j + S_k + AB_{ij} + AS_{ik} + BS_{jk} + ABS_{ijk} + \sum_{ijkl}$$

Where;

Y_{ijkl} = The value of parameters of interest

μ = Overall mean

A_i = Effect of i^{th} age (i = 1, 2, 3)

B_j = Effect of j^{th} breed (j = 1, 2)

S_k = Effect of k^{th} season (k = 1, 2, 3, 4)

AB_{ij} = Effect of ij^{th} interaction between age and breed

AS_{ik} = Effect of ik^{th} interaction between age and season

BS_{jk} =Effect of jk^{th} interaction between breed and season

ABS_{ijk} = Effect of ijk^{th} interaction between age, breed and season

$\sum_{ijkl}$= Random residual error associated with each record

Testicular Growth Parameters

Testicular Measurement: This was done twice per week throughout the experimental period. The measurements taken were as follow:

Testicular Length (TL): According to descriptions by Bratte *et al.* (1999) and Akpa *et al.* (2012) , this was measured in centimeters using a flexible measuring tape to determine the distance along the caudal surface of the scrotum from its site of attachment to the tip of the scrotum.

Testicular Circumference (TC): This is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa *et al*., 2012). It was measured in centimetres (cm).

Testicular Width (TW): This was calculated as the division of Testicular Circumference by two.

Testicular Weight (TWT): This was determined using Bailey *et al*. (1996) formula as given below;

$TWT = 0.5533 \times TL \times TW^2$

Where; TWT = Testicular weight

TL = Testicular length

TW = Testicular width

Increase in Sizes (IIS): This was calculated as the final values minus initial values for all the parameters mentioned above.

Semen Collection and Evaluation

Semen was collected with the aid of artificial vagina (AV). For stability and easy collection of semen bucks were trained twice per day consistently for three months prior to commencement of the research . A teaser doe was restrained so that the donor buck became aroused and then mounted the doe. The buck was pushed away 2 to 3 times (false-mounting) to increase or heighten its libido, after which it was allowed to mount the teaser doe. Following mounting of the doe by the buck, an AV was placed to accommodate the penis. The stimulation provided by the AV's warm water bladder, lubrication, and pressure produced an ejaculate and the semen was then collected. Semen samples were collected from each animal fortnightly at the onset till the end of data collection and labelled accordingly. Semen collections were done in the morning (7am - 9am); kept properly under temperature 36.8ºC – 37ºC throughout the 12 months duration of the experiment and immediately moved to the laboratory within 20 minutes after collection. Other semen parameters of interest except volume and pH were evaluated with the aid of computer assisted semen analyser (CASA) - (SCA®) 5.0 designed with Common Astronomy Software application package (Microscopic, S.L, Barcelona, Spain).

Semen volume and pH

The semen collected using an AV was measured by recording the value on graduated semen collecting tube before being transferred into the 15 mL graduated Falcon tubes. The semen pH was determined using a pH meter (HANNA instruments®, South Africa). The electrode was rinsed with sterile water and wiped with a sterile paper towel and then dipped into the semen sample for pH evaluation. Before the next semen sample is evaluated, the electrode is dipped into the semen sample.

Sperm motility and concentration

The microscopic slide cover chamber (20 µm) of the CASA was used to assess the spermatozoa concentration and motility. Before being added to each semen sample, which had been kept at the same temperature in a water bath, the sperm washing solution Brackett and Oliphant medium (BOM) was pre-warmed to 37°C on a heated plate. 10mL of each semen sample was diluted with 500mL of BOM. For CASA determination of sperm motility, 20 frames were tracked for sperm progressive motility assessment and not less than 1000 spermatozoa were identified.

Sperm viability and morphology

Sperm morphological abnormalities (sperm with coiled tail, bent midpiece tail, curved midpiece tail, or simple bent tail) were assessed by eosin/nigrosin staining and then determined by CASA in live normal and dead spermatozoa. 5µL of raw semen and 20mL of eosin-nigrosin staining were used at a dilution rate of 1:4. Eosin-nigrosin is made up of 1.67g of eosin and 2.9g of sodium citrate in 100mL of sterile water (dissolved in nigrosin, 100g/L, and formalin, 5mL/L). Ten fields were observed for each sample.

Acrosomal integrity

The percentage of spermatozoa with intact acrosomes were determined by adding 50µL of each sperm sample to a 500µL formalin citrate solution (96ml of 2.9% sodium citrate, with 4mL of 37% formaldehyde) and it was carefully mixed. The mixture was observed with the use of CASA and spermatozoa were determined for live sperms with intact acrosome (apical piece). Ten fields were observed for each sample.

Sperm membrane integrity

Using the Hypo-osmotic Swelling Test (HOST), which involved incubating 10µL of semen in 100µL of hypo-osmotic solution (fructose and sodium citrate) at 37ºC for 30 minutes; ten fields were observed for each sample and it was possible to identify spermatozoa that contained sperm cells with intact membrane integrity.

Data analysis

Data obtained were analyzed by method of least squares three-way Analysis of Variance using SAS, 1999. Duncan Multiple Range Test was used to compare the means.

Climatic Conditions

Two seasons each were considered in both rainy and dry seasons (i.e early and late rainy and early and late dry seasons as classified by Akinseye *et al.* (2012). Early rainy season covers the month of April to June while late rainy season spans between July and September. Early dry season begins in October and ends in December while the late dry season covers the months of January to March. However, weekly meteorological data from July 1, 2021 to June 30, 2022 were taken from FUNAAB Agrometrological Station. These data include relative humidity and atmospheric temperature by recording the daily values on digital Thermo Hygrometer; recording the values of water in a rain gauge (udometer) for the determination of liquid precipitation and seasonal rainfall. Emphasis was placed on variables such as dry bulb temperature, wet bulb temperature, relative humidity, rainfall. The temperature-humidity index (THI) was calculated using the standardised formula for small ruminants – $THI = (0.8 \times T) + (RH/100) \times [(T-14.4) + 46.4]$ where T was the dry bulb air temperature (^oC) and RH was the relative air humidity (%).

Meteorological Observations of The Experimental Location

The results of analysed meteorological data showing the statistics of experimental location during the experiment is presented in Table 1. Results showed variations in environmental variables and the level of stress the animals were exposed to during the 12 months experimental period. The highest mean temperature (^oC) and dry bulb temperature were observed in Month July and August; November and December respectively; while the least values for mean temperature and dry bulb temperature were obtained in January (25.71±0.25^{o}C and 25.20±0.16^{o}C) and relative humidity (%) in November (56.80±1.91). The highest THI values were recorded in November, December and March. Besides, late rainy season had the highest mean temperature ($P<0.05$). Wet bulb and dry bulb temperatures were at peak during the late rainy and late dry seasons respectively. The least rainfall was recorded during the early rainy season while the highest was in late dry season ($P<0.05$). THI was highest (82.83±0.49) during the early dry season and followed closely during late rainy season while the least value (77.26±0.51) was recorded during early rainy season.

Table 1: Descriptive statistics of meteorological observations throughout experimental period

MONTHS	Mean Temperature (°C)	Dry bulb Temperature (°C)	Wet bulb Temperature (°C)	Relative humidity (%)	Rainfall (mm)	THI
July	29.86±0.31[a]	28.26±0.37[de]	23.89±0.63[bc]	69.58±3.11[cd]	0. 00±0.00[c]	78.65±0.89[bc]
August	30.05±0.25[a]	29.35±0.26[c]	25.23±0.22[a]	70.19±1.39[cd]	1.58±0.89[bc]	80.37±0.37[b]
September	28.62±0.22[bc]	29.08±0.17[cd]	25.45±0.12[a]	73.87±1.26[bc]	5.34±2.14[a]	80.51±0.25[b]
October	27.82±0.19[d]	27.18±0.43[ef]	24.57±0.26[ab]	80.87±1.83[a]	3.93±1.59[ab]	78.48±0.44[bc]
November	28.64±0.25[bc]	32.01±0.29[a]	25.18±0.16[a]	56.80±1.91[e]	0.43±0.19[bc]	82.01±0.30[a]
December	28.71±0.23[bc]	32.17±0.41[a]	25.25±0.16[a]	78.23±2.83[ab]	0.08±0.06[c]	89.91±0.92[a]
January	25.71±0.25[f]	25.20±0.16[g]	23.32±0.11[cd]	83.29±1.12[a]	1.08±0.78[bc]	76.39±0.17[c]
February	26.92±0.16[e]	29.99±0.61[bc]	23.89±0.16[bc]	82.79±1.77[a]	3.01±1.25[abc]	78.30±1.09[bc]
March	27.62±0.26[d]	30.53±0.47[b]	24.13±0.18[bc]	78.13±1.59[ab]	5.03±1.98[a]	83.43±0.94[a]
April	28.07±0.27[cd]	27.58±0.21[ef]	25.20±0.14[a]	78.63±1.13[ab]	1.11±0.78[bc]	80.75±0.36[b]
May	28.95±0.27[b]	27.32±0.43[ef]	23.29±0.64[cd]	68.35±2.15[cd]	0.19±0.19[c]	77.09±1.04[bc]
June	28.84±0.29[bc]	26.79±0.39[f]	22.57±0.55[d]	65.39±2.12[d]	1.16±0.72[bc]	75.93±0.88[c]
SEASONS						
Early Rainy (ER)	28.63±0.16[b]	27.23±0.21[c]	23.67±0.31[b]	70.71±1.24[b]	0.82±0.36[b]	77.26±0.51[c]
Late Rainy (LR)	29.52±0.16[a]	28.89±0.17[b]	24.85±0.24[a]	71.18±1.22[b]	2.27±0.79[ab]	79.83±0.35[b]
Early Dry (ED)	28.38±0.14[b]	30.42±0.33[a]	24.99±0.12[a]	72.07±1.71[b]	1.51±0.57[ab]	82.83±0.49[a]
Late Dry (LD)	26.74±0.15[c]	28.53±0.36[b]	23.78±0.09[b]	81.36±0.89[a]	3.04±0.84[a]	80.94±0.60[a]

[abc..] Means with different superscripts in the same column differ significantly $P<0.05$

THI – Temperature-humidity index, ER = April - June, LR = July - September, ED = October - December, LD = January - March

RESULTS

Table 2 shows the least square means on testicular growth parameters and semen quality of the two breeds of goats. There were significant differences in scrotal length (SL), scrotal circumference (SC), testicular width (TW) and testicular weight (TWT) of Kalahari Red goats (KR) and their crossbred also known as *Kalawad* (KW). The increase gained in scrotal length (IGSL) in both breeds was significantly different but KW had high mean value while KR had high (4.16±0.60 cm) increase gained in scrotal circumference (IGSC) mean value ($P<0.05$). There were no significant differences in the mean values of both breeds for increase gained in testicular width (IGTW) and increase gained in testicular weight (IGTWT). Results on semen quality of the two breeds of bucks indicated that there were differences ($P<0.05$) in all parameters except pH. KR had highest volume, concentration, progressive motility and percentage dead while KW crossbred had highest acrosome and membrane integrity, livability and for all morphological abnormality.

The interaction showing the effects of breed and age of bucks on testicular growth parameters and semen quality is presented in Table 3. The SL, SC, TW and TWT increased as age increases and their values differ ($P<0.05$) from each other. The IGSL in KW bucks were not different ($P>0.05$) in all the age groups but differ ($P<0.05$) from the values obtained in KR. The least mean value (1.52±0.49 cm) was observed in KR at age >3≤4 years. The highest mean values (5.10±1.15 cm, 2.55±0.58 cm and 13.25±7.40 g) for IGSC, IGTW and IGTWT obtained in KR at age >1≤2 years were not significantly different from mean values in age >2≤3 years as well as values obtained in KW at age >1≤2 years. Also, there were significant differences in all semen quality parameters. KR had high ($P>0.05$) volume (1.50 ml and 1.47 mL) at age >3≤4 years and >2≤3 years, respectively. Spermatozoa concentration and progressive motility were at peak (5.48 $x10^6$/ml and 90.63 %) in KR at age >3≤4 years while high acrosome integrity, membrane integrity and livability were observed in KW at age >2≤3 years. All morphological abnormalities were at peak in KW at age >3≤4 years.

The results on the interaction effects of breed and season are presented in Table 4. There were differences ($P<0.05$) in SL, SC, TW and TWT. Scrotal length for KW was not significantly different during both early and dry seasons while highest SC and TW in KR were obtained during late dry season. However, TWT was maximal at both early and late dry seasons. Also, the KW highest values (23.40±0.07 cm and 1111.18±9.90 g) for SC and TWT were obtained at early dry season, respectively. There were significant differences in all the semen parameters considered. The volume within each breed did not differ significantly ($P>0.05$) during both early and late dry season. Semen volume was at peak ($P<0.05$) during the late rainy season for both KR and KW (1.49 ml and 1.22 ml, respectively). Spermatozoa concentration was at peak ($5.49x10^6$/mL and $3.99x10^6$/mL) during the late dry season for both breeds. Progressive motility was observed to be high (91.51 %) during the early dry season in KR while the least value (85.45 %) was observed during the early rainy season in KW. The maximum ($P<0.05$) values for KW (85.34 % and 79.94 % for AI and MI, respectively) and KR (81.92 % and 73.74 % for AI and MI, respectively) during the early dry season. Percentage live (livability) had the least value (89.76 %) and percentage dead was at peak (10.24 %) during late dry season in KR. CT, BMPT, CMPT and SBT were at peak during late dry season in KW.

The effects of age on testicular growth and semen quality parameters of goat bucks is presented in Table 5. All parameters considered were significantly ($P<0.05$) influenced by age except pH.

Table 2: Least square means showing the effects of breed on testicular growth parameters and semen quality of goat bucks

BREED	Scrotal Length (cm)	Scrotal Circumference (cm)	Testicular Width (cm)	Testicular Weight (g)
KR	16.86±0.07[a]	27.81±0.08[a]	13.91±0.04[a]	1849.89±14.56[a]
KW	14.21±0.04[b]	21.91±0.05[b]	10.96±0.03[b]	965.87±6.71[b]
P value	0.0001	0.0001	0.0001	0.0001

	IGSL (cm)	IGSC (cm)	IGTW (cm)	IGTWT (g)
KR	2.15±0.22[b]	4.16±0.60[a]	1.94±0.27	6.93±2.68
KW	4.04±0.21[a]	2.65±0.27[b]	1.33±0.13	4.40±0.76
P value	0.0001	0.0303	0.0551	0.3711

BREED	Volume (ml)	PH	Conc(10^6/ml)	PM (%)	AI (%)	MI (%)	Livability (%)	% Dead	% CT	% BMPT	% CMP	% SBT
KR	1.44[a]	7.14	5.21[a]	89.57[a]	80.27[b]	72.03[b]	92.01[b]	7.99[a]	2.98[b]	2.14[b]	3.54[b]	3.16[b]
KW	1.18[b]	7.08	3.71[b]	86.74[b]	83.68[a]	78.58[a]	93.33[a]	6.68[b]	4.38[a]	2.68[a]	4.88[a]	3.74[a]
SEM	0.01	0.06	0.02	0.026	0.16	0.17	0.17	0.16	0.05	0.03	0.05	0.04

[ab] Means with different superscripts in the same column differ significantly P<0.05

KR = Kalahari Red Goat, KW = Kalawad Goat (Crossbred), IGSL = Increase gained in scrotal length, IGSC = Increase gained in scrotal circumference, IGTW = Increase gained in testicular width and IGTWT = Increase gained in testicular weight, Conc = spermatozoa concentration, PM = Progressive Motility, AI = Acrosome Integrity, MI = Membrane Integrity, CT = Percentage coiled tail, BMPT = bent mid-piece tail, CMP = Curved mid-piece and SBT = Simple bent tail

Table 3: Interaction effect of breed and age on testicular growth parameters and semen quality of goat bucks

Age (years)	Breed	Scrotal Length (cm)	Scrotal Circumference (cm)	Testicular Width (cm)	Testicular Weight (g)
>1≤ 2	KR	15.26±0.08^{c}	25.11±0.10^{c}	12.56±0.05^{c}	1351.94±13.92^{c}
	KW	13.22±0.06^{e}	20.71±0.09^{f}	10.35±0.04^{f}	801.80±9.46^{f}
>2≤ 3	KR	16.92±0.08^{b}	28.32±0.09^{b}	14.16±0.05^{b}	1896.20±17.59^{b}
	KW	14.29±0.07^{d}	22.18±0.07^{e}	11.09±0.04^{e}	988.30±9.86^{e}
>3≤ 4	KR	18.39±0.13^{a}	30.00±0.08^{a}	15.00±0.04^{a}	2301.51±21.41^{a}
	KW	15.11±0.07^{c}	22.87±0.08^{d}	11.44±0.04^{d}	1109.42±10.74^{d}
	P value	0.0001	0.0001	0.0001	0.0001
		IGSL (cm)	IGSC (cm)	IGTW (cm)	IGTWT (g)
>1≤ 2	KR	2.72±0.24^{b}	5.10±1.15^{a}	2.55±0.58^{a}	13.25±7.40^{a}
	KW	3.78±0.26^{a}	3.68±0.36^{ab}	1.84±0.18^{ab}	7.06±1.03^{ab}
>2≤ 3	KR	2.20±0.18^{bc}	3.58±0.79^{ab}	1.79±0.39^{ab}	4.81±1.86^{ab}
	KW	4.44±0.31^{a}	2.34±0.36^{b}	1.17±0.18^{b}	3.83±1.27^{ab}
>3≤ 4	KR	1.52±0.49^{c}	3.80±1.24^{ab}	1.47±0.38^{ab}	2.74±1.28^{b}
	KW	3.90±0.48^{a}	1.94±0.27^{b}	0.97±0.14^{b}	2.32±0.63^{b}
	P value	0.0001	0.1132	0.0477	0.2009

		Volume *	PH	Conc** (10^{6}/ml)	PM (%)	AI (%)	MI (%)	Livability #	% Dead	% CT	% BMPT	% CMP	% SBT
>1≤ 2	KR	1.36^{b}	7.11^{ab}	4.96^{c}	86.45^{c}	78.84^{d}	70.58^{d}	91.18^{cd}	8.83^{ab}	3.01^{d}	2.20^{d}	3.56^{d}	3.20^{de}
	KW	1.10^{d}	7.09^{ab}	3.47^{f}	83.51^{d}	82.25^{b}	77.25^{b}	93.85^{b}	6.15^{c}	4.45^{b}	2.72^{ab}	4.96^{b}	3.79^{ab}
>2≤ 3	KR	1.47^{a}	7.14^{ab}	5.19^{b}	91.63^{a}	80.68^{c}	72.38^{c}	93.00^{b}	7.00^{c}	2.13^{e}	1.89^{e}	2.78^{e}	2.96^{e}
	KW	1.21^{c}	7.09^{ab}	3.70^{e}	88.57^{b}	84.14^{a}	78.90^{a}	95.48^{a}	4.53^{d}	3.57^{c}	2.47^{bc}	4.09^{c}	3.55^{bc}
>3≤ 4	KR	1.50^{a}	7.17^{a}	5.48^{a}	90.63^{a}	81.31^{bc}	73.14^{c}	91.84^{c}	8.16^{b}	3.78^{c}	2.33^{cd}	4.26^{c}	3.33^{cd}
	KW	1.22^{c}	7.06^{b}	3.97^{d}	88.15^{b}	84.63^{a}	79.59^{a}	90.65^{d}	9.35^{a}	5.13^{a}	2.83^{a}	5.59^{a}	3.89^{a}
	SEM	0.01	0.02	0.05	0.19	0.13	0.17	0.21	0.12	0.07	0.05	0.04	0.03
	P value	0.001	0.02	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

$^{abcd..}$ Means with different superscripts in the same column differ significantly P<0.05

Conc = spermatozoa concentration, PM = Progressive Motility, AI = Acrosome Integrity, MI = Membrane Integrity, CT = Percentage coiled tail, BMPT = bent mid-piece tail, CMP = Curved mid-piece and SBT = Simple bent tail, * = mL, ** = 10^{6}/mL, # = %,

Table 4: Least square means indicating the interaction effects of breeds and seasons on testicular growth parameters and semen quality of goat bucks

BREED	SEASON	Scrotal Length (cm)	Scrotal Circumference (cm)	Testicular Width (g)	Testicular Weight (g)
KR	ER	16.98±0.16^{a}	25.84±0.15^{d}	12.92±0.08^{d}	1632.46±31.18^{c}
	LR	16.84±0.14^{b}	27.91±0.14^{c}	13.95±0.07^{c}	1863.43±30.11^{b}
	ED	17.19±0.12^{a}	28.49±0.13^{b}	14.25±0.06^{b}	1966.19±26.88^{a}
	LD	16.41±0.11^{c}	29.00±0.12^{a}	14.500±0.06^{a}	1937.47±24.58^{a}
KW	ER	12.81±0.08^{f}	19.52±0.08^{g}	9.79±0.04^{g}	690.23±10.05^{f}
	LR	14.41±0.07^{e}	22.36±0.07^{f}	11.18±0.04^{f}	1007.23±10.26^{e}
	ED	14.54±0.07^{e}	23.40±0.07^{e}	11.70±0.03^{e}	1111.18±9.90^{d}
	LD	15.07±0.09^{d}	22.37±0.06^{f}	11.19±0.03^{f}	1054.85±10.94^{de}
P value		0.0001	0.0001	0.0001	0.0001

		Volume *	PH	Conc**	PM (%)	AI (%)	MI (%)	Livability#	% Dead	% CT	% BMPT	% CMP	% SBT
KR	ER	1.33^{c}	7.23^{a}	4.78^{c}	89.71^{b}	77.22^{e}	68.78^{f}	93.42^{abc}	6.58^{cde}	2.65^{f}	1.81^{e}	3.22^{e}	2.87^{g}
	LR	1.49^{a}	7.19^{b}	5.26^{b}	89.10^{bc}	80.30^{d}	72.15^{e}	92.13^{d}	7.86^{b}	2.55^{f}	2.05^{d}	3.14^{e}	3.05^{fg}
	ED	1.47^{b}	7.11^{d}	5.31^{b}	91.51^{a}	81.92^{c}	73.74^{d}	92.71^{bcd}	7.29^{bcd}	3.05^{e}	2.17^{d}	3.62^{d}	3.19^{ef}
	LD	1.47^{b}	7.04^{f}	5.49^{a}	87.94^{cd}	81.65^{c}	73.46^{d}	89.76^{e}	10.24^{a}	3.66^{d}	2.54^{bc}	4.16^{c}	3.53^{cd}
KW	ER	1.16^{e}	7.13^{c}	3.29^{f}	85.45^{f}	80.74^{d}	75.78^{c}	93.86^{a}	6.14^{e}	4.16^{bc}	2.38^{c}	4.65^{b}	3.37^{de}
	LR	1.22^{d}	7.06^{f}	3.76^{e}	86.06^{ef}	83.46^{b}	78.41^{b}	93.27^{abc}	6.73^{cde}	3.88^{cd}	2.61^{b}	4.38^{c}	3.60^{bc}
	ED	1.17^{e}	7.09^{e}	3.80^{e}	88.33^{bcd}	85.34^{a}	79.94^{a}	93.67^{ab}	6.33^{de}	4.35^{b}	2.69^{b}	4.89^{b}	3.77^{b}
	LD	1.15^{e}	7.05^{f}	3.99^{d}	87.13^{de}	85.16^{a}	80.19^{a}	92.51^{cd}	7.49^{bc}	5.14^{a}	3.02^{a}	5.61^{a}	4.23^{a}
SEM		0.01	0.01	0.03	0.46	0.26	0.28	0.32	0.32	0.1	0.07	0.08	0.07

$^{abcde..}$ Means with different superscripts in the same column differ significantly P<0.05

Conc = spermatozoa concentration, PM = Progressive Motility, AI = Acrosome Integrity, MI = Membrane Integrity, CT = Percentage coiled tail, BMPT = bent mid-piece tail, CMP = Curved mid-piece and SBT = Simple bent tail, * = mL, ** = 10^6/mL, # = %,*ER = Early Rainy, LR = Late Rainy, ED = Early Dry, LD = Late Dry*

Table 5: Least square means showing the effects age on testicular growth and semen quality parameters of goat bucks

	AGE (Year)			
Parameter	>1≤ 2	>2≤ 3	>3≤ 4	P value
Volume (ml)	1.23±0.01^{b}	1.34±0.01^{a}	1.36±0.01^{a}	0.001
pH	7.10±0.01	7.12±0.01	7.11±0.03	0.769
Concentration	4.36±0.15^{b}	4.45±0.05^{b}	4.72±0.05^{a}	0.017
Progressive motility (%)	84.98±0.37^{b}	90.10±0.22^{a}	89.39±0.28^{a}	0.001
Acrosome integrity (%)	80.54±0.26^{b}	82.41±0.22^{a}	82.97±0.18^{a}	0.001
Membrane integrity (%)	73.91±0.326^{b}	75.64±0.28^{a}	76.37±0.26^{a}	0.001
Livability (%)	92.51±0.17^{b}	94.24±0.15^{a}	91.25±0.25^{c}	0.001
Percentage dead (%)	7.49±0.17^{b}	5.76±0.15^{c}	8.75±0.25^{a}	0.001
Coiled tail (%)	3.73±0.08^{b}	2.85±0.07^{c}	4.46±0.07^{a}	0.001
Bent midpiece tail (%)	2.46±0.05^{a}	2.19±0.04^{b}	2.58±0.05^{a}	0.001
Curved midpiece (%)	4.73±0.07^{b}	3.85±0.07^{c}	5.45±0.06^{a}	0.001
Simple bent tail (%)	3.49±0.04^{a}	3.25±0.05^{b}	3.61±0.05^{a}	0.001
Scrotal length (cm)	14.24±0.06^{c}	15.61±0.07^{b}	16.75±0.09^{a}	0.001
Scrotal circumference (cm)	22.91±0.10^{c}	25.25±0.12^{b}	26.43±0.13^{a}	0.001
Testicular width (cm)	11.45±0.05^{c}	12.63±0.06^{b}	13.21±0.06^{a}	0.001
Testicular weight (g)	1076.78±12.24^{c}	1442.02±17.80^{b}	1704.84±22.69^{a}	0.001

^{abc}means with different superscripts in the same row differ significantly P<0.05

DISCUSSION

The measurement of scrotal circumference is an indirect measurement of testicular size, volume, and onset of active spermatogenesis; therefore, it would be helpful in the evaluation of bucks for breeding soundness (Abba and Igbokwe, 2015 and Kumbhar *et al.* 2017). The study focused on the determination/evaluation of the reproductive capabilities of goats' bucks in respect to environmental and seasonal effects on age and breed in the tropics. In order to identify a suitable breeding male for both natural and artificial breeding objectives, testicular features, specifically scrotal length and circumference, could be measured in Kalahari Red and *Kalawad* bucks. Breed has a substantial impact on scrotal dimensions like length, circumference, width, and weight. In comparison to *Kalawad* bucks, the linear expansion of the scrotum was quicker in Kalahari Red with high values compared to *Kalawad* bucks. Similar results were reported by Kumbhar *et al.* (2017) who stated that Surti bucks grow their scrotums more quickly than Marwari bucks and Zaher *et al.* (2020) reported that Assaf breed (ram) testicular features were much greater than Awassi (crossbred) breed. The genetic potential for body size disparities between the breeds could be the cause for the variations recorded in this study. The scrotal circumference values of Kalahari Red and *Kalawad* bucks (27.81±0.08cm and 21.91±0.05cm) in this study are lower than those found in Zaraibi bucks (Barkawi *et al.,* 2006) and Mountain black and Hybrid bucks (Al-Omari, 2012). This is consistent with the report by Boucif *et al.* (2007) about OD rams during the winter in western Algeria, but not with the reports by Belkadi *et al.* (2017)**.** Similar observations with Damascus and Mexican bucks in the subtropical regions were reported by Delgadillo *et al.* (1999). Testicular parameters considered in this study were changed by season in both breeds.

The semen parameters of mature Kalahari Red and *Kalawad* bucks varied significantly within breeds and seasons; the Kalahari Red was found to have high semen volume, spermatozoa concentration, and progressive motility with high percentage dead spermatozoa, whereas the *Kalawad* had excellent acrosome integrity, membrane integrity, high percentage live sperm cells, and low percentage dead sperm cells. However, the quality of the semen in the tropics had been opined to be lower during the hot months (Vilakazi and Webb, 2004), impacted by thermal stress (Menon *et al.,* 2011) and has been reported to affect semen output, particularly where there is marked seasonal variations in environmental temperature. Thermal stressors result in testicular degeneration and a decrease in the flow of semen. The results of the present study compare well with previous authors (Das *et al.* 2006). In the current study, the considerable disparity in sperm concentration between the breeds suggested that the bucks were not of equivalent fitness in terms of their size and weight. But Singh *et al.* (2016), which indicated no significant buck influence on sperm concentrations, disagreed with the present findings. Additionally, the live spermatozoa percentage in the semen of *Kalawad* bucks was higher (93.33%), compared to the live spermatozoa percentage in the semen of Kalahari Red (92.01%). These values coincide with Sultana *et al.* (2013) observation that 92.95±0.74 % of live spermatozoa were present in Black Bengal goats. In this investigation, the results for progressive motility and membrane integrity were greater than those reported by Odeyemi *et al.* (2021), who reported (80.00%) and (82.5%) in Kalahari Red, respectively. The acrosome integrity value recorded in that study, 83.50%, was comparable to the *Kalawad* value obtained in this study, 83.68%. Although age and breed were different, the bucks in this study were treated to the same environmental conditions, dietary regimen, and management. Breed variations may be the cause of the variance in the percentage of viable spermatozoa. Husain (2007) and Apu *et al.* (2008) found breed variation with 83.73±0.94 to 89.27±1.40% and 84.99±0.38 to 85.62±0.57% live spermatozoa in Black Bengal semen, respectively, which is slightly lower than the findings in the current study. Variation in the percentage of live sperm reflects the genetic superiority of a breed or specific buck. The results showed that average semen volumes were slightly higher in adult bucks than in young bucks. This is also in line with observations in a number of tropical or subtropical breeds of sheep (Kishk, 2008; Mohamed and Abdelatif, 2010). Therefore, the average volume of the ejaculate would therefore increase according to body growth. Vilakazi and Webb (2004) also reported that the bulls aged 36 to 48 months were found to produce sperm of better morphology than bulls of 72 months of age and older. Several researchers have attributed similar observations to the regulatory balancing system, fat accumulation in the scrotum, and reproductive tissues that influence semen production and quality, as well as the scrotal circumference (Brito *et al.,* 2002). This is possibly because the testicles of younger animals are still maturing, and as a result, the ejaculate's semen is of poor quality. Lower semen production in older bulls may be related to degenerative changes in the seminiferous tubule, fat deposition that may occur in the scrotum and the deterioration of body tissues with age, particularly testicular tissues. Age-related fat accumulation may occur around the scrotum in bucks. As a result, the capacity of the scrotal neck to radiate heat may be reduced, which may influence the quality of the semen (Brito *et al.,* 2002). Young bucks had significantly lower sperm concentration values than older bucks in this investigation. Ghozlane *et al.* (2005) reported similar observations but Hassan *et al.* (2009) have drawn attention to the fact that this difference is the result of age differences. In this study, it was discovered that the season had an impact on the spermatozoa concentrations in the bucks of both breeds. This differs from the finding reported by Loubser and van NieKerk (1983), who discovered that the concentration of spermatozoa is not affected by the time of year. Seasonal effects may also be influenced by changes in temperature, humidity, and photoperiodism, which may result in irregular production of hormones controlling spermatogenesis. The findings of this study showed that semen parameters taken during rainy seasons, particularly late rainy season, were superior to those collected in dry seasons. Seasonal variations were seen in the semen volume of mature Kalahari Red and *Kalawad* bucks. This finding partially corroborated those of Mann (1954), who hypothesized that seminal plasma makes up about 70% of the ejaculate's volume and that variations in that volume are primarily caused by variations in the amounts of fluids secreted by the accessory glands and the epididymis. The high semen volume was recorded during late rainy season in both breeds and lower in dry seasons. These results are comparable to those of bucks from different goats' breeds (Leboeuf *et al.,* 2000; Wang *et al.,* 2015). The progressive motility, acrosome integrity, and membrane integrity of the both breeds all increased or were positively impacted by the dry seasons. However, Elsharif and Makawi, 2004; Adil and Nasir, 2015; were among the writers who previously reported results that were incongruous. Additionally, spermatozoa concentration in the semen both Kalahari Red and *Kalawad* bucks was clearly influenced by the season. During the late dry season, when the percentage of dead spermatozoa was also at its maximum, the highest concentration of sperm cells in the semen was discovered. The spermatozoa concentration findings in this study did not accord with that of Syrian Damascus bucks (Daker and Suleiman, 2004) or Nubian bucks and their crossbred bucks (Elsharif and Makawi, 2004). According to earlier studies (Elsharif and Makawi, 2004; Elsheikh *et al.,* 2013; Wang *et al.,* 2015), the high percentage of dead sperm detected in this study is consistent with those findings. The late dry season was considerably more strongly related with the percentages of aberrant spermatozoa (curved tail, bent mid-piece tail, curved mid-piece, and simple bent tail) than the early dry season. Similar findings were reported by Aguiar *et al.* (2013), who discovered that the dry season had a decline in the number of sperm with normal morphology. The strength of these seasonal impacts might not be great enough to stop bucks from being used for breeding all year round but essentially, individual examination of semen is required to choose the most viable males for breeding due to individual variances in the quantity and quality of semen among bucks within and between breeds. This result supports the findings of earlier studies (Elsheikh *et al.,* 2013; D'Andre *et al.,* 2017) and reflects the negative effects of high or marked temperatures and some other environmental factors on goats' semen quality characteristics between the seasons. The semen quality in the current study was satisfactory for both breeds, which may be the result of improved accessory gland function supported by good body condition, feed, proper grazing, and adequate exercise. The production of spermatozoa has been revealed to have a significant impact on male fertility, and high temperatures have been reported to negatively affect spermatogenesis (Menon *et al.,* 2011). Accordingly, suitable conditions must be created so that the proven males can fully express their reproductive potential by minimizing any negative environmental effects on the quality of their semen, and these crucial conditions appear to be met during the rainy seasons.

CONCLUSION

- The testicular traits in Kalahari Red bucks during the early rainy season at age >1≤2 years were similar to *Kalawad* bucks at age >2≤3 years during late dry season.
- Testicular traits measurement, especially scrotal length and circumference in Kalahari Red breed and *Kalawad* bucks should be a good indicator to be considered when selecting suitable breeding male for both natural and artificial breeding purposes.
- The bucks aged >2≤3 years in both Kalahari Red and *Kalawad* were found to produce spermatozoa of better morphology than bucks younger and older age during rainy seasons.
- Apart from other determinant factors, semen quality of Kalahari Red and *Kalawad* were highly affected by genetic make up of the animals.

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