Relationships between Semen Cation Concentrations, Semen Characteristics, Testicular Measurements and Body Conformation Traits in Red Sokoto Goat

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Abstract: The study was conducted to determine the relationship between semen cation concentrations, semen characteristics, testicular measurements and body conformation traits using 31 Red Sokoto bucks at the Teaching and Research Farm of the Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria. The body condition was scored on a scale of 1 to 5 and then used to categorize the bucks into score 3 and 4. The linear body measurements {heart girth (HG), stature (ST), chest width (CW), withers height (WH), body depth (BD), body length (BL) and rump width (RW)} were measured in centimeters (cm) using flexible tape. The testicular measurements {testicular length (TL), testicular circumference (TC), were measured using flexible tape while testicular width (TW) and testicular weight (TWT) were estimated using the appropriate formulae}. The semen characteristics {semen volume, sperm motility, semen pH, sperm concentration and live and dead ratio} and semen cation concentrations {sodium (Na), potassium (K), calcium (Ca) and phosphate ($P0_4$)} were accordingly determined. The study lasted for one year (July, 2011 – June, 2012). The results showed that, semen volume was positively and significantly correlated with K^+ and Ca^{2+} ($P<0.05$; r= 0.27 – 0.31) but not with Na^+ and $P0_4$ ($P>0.05$; r= 0.13 – 0.14). Sperm motility was positively and significantly correlated with Na^+ and K^+ ($P<0.05$; r= 0.26 – 0.33). Sperm concentration had positive and significant correlation with K^+ ($P<0.05$; r= 0.37), while live/dead ratio was positively and significantly correlated with only Na^+ ($P<0.05$; r= 0.39) among the cation. Ca^{2+} was positively and significantly correlated with testicular measurements ($P<0.05$; r=0.34 – 0.39). Negative but significant ($P<0.05$) correlation was observed between Na^+ and BL (r= -0.26). K^+ was positively and significantly correlated with body conformation traits ($P<0.05$-0.01; r=0.29 – 0.52), except BW, BCS and HG ($P>0.05$; r=-0.09 to -0.14). Ca^{2+} was positively and significantly correlation with CW, WH, BD and RW ($P<0.05$; r=0.29-0.42). The study revealed that bucks with adequate concentation of calcium would exhibit better testicular dimensions; while bucks with good body structure would show high concentration of potassium in their seminal fluid, hence they would produce higher semen volume with increased sperm concentration and motility. Therefore, mineral status of the seminal fluid is an essential index in evaluating semen quality of Red Sokoto bucks.

[Akpa G.N, Ambali A.L, Suleiman I.O. **Relationships between Semen Cation Concentrations, Semen Characteristics, Testicular Measurements and Body Conformation Traits in Red Sokoto Goat**. *Nat Sci* 2013;11(7):94-99]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 15

Keywords: Red Sokoto goat; Body conformation; Testicular measurement; semen; cation concentrations

1. Introduction

Classical methods of semen evaluation generally measure sperm concentration, progressive motility, the percentage of viable cells, and morphology. These assays may not be enough in predicting fertility outcome because only those samples with markedly poor quality semen can be identified. To solve this problem, new procedures of in vitro seminal analysis or multiple analysis of the same sample have been suggested and evaluated (Hafez, 1987).

Among the most important aspects of the study on spermatozoa metabolism is the understanding of the chemical pathways involved in energy metabolism (White, 1958) and maintenance of osmotic balance by ion present in semen (Nath, 1988) which are important determinants of sperm viability. Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract. Cations such as Na, K, Ca, and $P0_4$ in the seminal plasma establish osmotic balance, while essential trace elements are components of many important enzymes. Thus, biochemical evaluation of seminal plasma is an important criterion for assessing fertility and diagnosing male reproductive disorders (Barrier-Battut *et al.*, 2002; Massanyi *et al.*, 2004a, 2004b).

Abnormal levels of Ca, Na, K, Zn, and Cu in seminal plasma have been reported to be correlated with infertility in humans. Ca is the trigger for the acrosome reaction in mammalian spermatozoa and there is substantial evidence that Ca is differentially involved in sperm motility, depending on the stage of sperm maturation. However, Magnus *et al*. (1990) reported no association between ionized calcium concentrations and the proportion of spermatozoa displaying progressive movement. Prien *et al*. (1990) compared sperm motility, velocity and progressive

movement with total and ionized calcium. The ions present in the semen help in stimulating the motility and glycolysis. The addition of potassium to semen extenders has been shown to improve motility of stallion (Padilla and Foote, 1991) and human sperm (Karow *et al*., 1992), but Rossato *et al.* (2002) found no correlation between the ionic composition and the osmolarity of human seminal plasma.

Intracellular concentrations of potassium are higher than those of seminal plasma, and therefore potassium levels are linked to sperm concentration. In ram, increasing potassium levels are negatively correlated to progressive motility, while the reverse is true for sodium and chloride (Abdel-Rahman *et al*., 2000). In ram ejaculates, intracellular calcium and magnesium concentrations were higher than in seminal plasma as opposed to phosphate levels. Furthermore, lower values of progressive motility has been reported to be correlated to increasing levels of calcium and decreasing magnesium and phosphate concentrations levels (Abdel-Rahman *et al*., 2000).

Considerable attention has been paid to the interrelationship of seminal cations to some measured parameters that relate to semen quality in numbers of animals. However, information is lacking as regards indigenous buck in Nigeria. The objective of this study was therefore to determine the relationship between semen cation concentrations, semen characteristics, testicular measurements and body conformation traits in Red Sokoto bucks.

2. Material and Methods

Study Location

The study was conducted at the Experimental and Research Farm of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The area is situated between latitude 11^0 and 12^0N and altitude of 640m above sea level (Encarta Encyclopedia, 2009 PC version). The area falls within the Northern-Guinea Savannah Zone, having an average annual rainfall of 1100mm, which starts from late April or early May to mid-October. The peak rainy season is between June and September, followed by the harmattan period of cool and dry weather which last from October to January. This is then followed by hot-dry weather from February to April. The mean maximum temperature varies from 26^0C to 35^0C depending on the season, while the mean relative humidity during harmattan period and wet season are 21% and 27%, respectively. Detailed description of Zaria was given elsewhere by Akpa *et al.* (2002).

Experimental Animals and their Management

A total of thirty-one Red Sokoto bucks were used for the study. The animals were under the management practices of the Department of Animal Science, Ahmadu Bello University, Zaria. The bucks were reared under semi-intensive system. The animals were released daily for grazing at 8.00am and another shift by 2.00 pm. Supplemental feed (concentrates) were provided. Animals received routine inspection and dipping (ectoparasite), as well as anti-helminthic drenching (deworming) and vaccination againt endemic diseases. Drinking water was provided *ad libitum.* The experiment commenced when the bucks were 9 – 12 months of age in July 2011 and terminated when they were 21 – 24 months, in June, 2012.

Data Collection and Traits measurement

Body Weight Measurement: The body weight of the bucks was measured in kilograms by following the procedure as described by Akpa *et al.* (1998). The weight of the observer was taken first, and then the body weight of each animal was taken by carrying the animal individually and standing on a weighing scale. The difference between this weight and that of the observer gives the weight of the animal. Weighing was done at the begining of the study and subsequently on monthly basis. A total of 372 records were generated for body weight.

Body Linear Measurement: Measurement of linear conformation traits were taken on the day of measurements in centimeters (cm) using flexible tape as described by Alphonsus *et al.* (2009) and Boisot *et al*. (2002). The measurements were taken at the onset and subsequently on monthly basis. A total of 372 records were generated for each of the body linear measurements. The traits are described as follow:

Heart Girth (HG): This is the circumference of the body at a point immediately behind the fore limbs and perpendicular to the body axis.

The Stature (ST): This was measured from the top of the spine in between the hips to the ground.

Chest Width (CW): This was measured from the inside the surface between the top of the front legs.

The Wither Height (WH): This is the highest point over the scapular vertically to the ground.

Body Depth (BD): This is the distance between the top of the spine and the bottom of the barrel at the last rib.

Body Length (BL): This was measured from the point of shoulder to the ischium.

Rump Width (RW): This is the distance between the most posterior points of pin bones.

Testicular Measurement

These were done at the onset and subsequently on weekly basis before semen collection. A total of 1488 records were generated for each of the measurement. The measurement were as follows:

Testicular Length (TL)

This was measured in centimeter with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Akpa *et al*. (2012) and Bratte *et al*. (1999).

Testicular Circumference (TC)

This is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa *et al*., 2006). It was measured in centimeters (cm)

Testicular Width (TW)

This was taken as the division of Testicular Circumference by two.

Testicular Weight (TWT)

This was determined using Bailey *et al* (1996) formulae as given below;

TWT = 0.5533 x TL x TW

Where; TWT = Testicular weight

TL = Testicular length

TW = Testicular width

Body Condition Score (BCS)

The body condition score (1-5) were employed to score the bucks. The buck's backbone, loin and rump areas were palpated and examined and then scored. These areas do not have muscle tissue covering them, hence, combination of skin and fat deposit account for any cover that were felt around these areas. Amount of fat deposit was determined by the use of fingertip pressure which was exerted on the backbone, pin bone and hip bone, respectively.

Score 1 (Very Thin): Individual short ribs have a thin covering of flesh. Bones of the chine, loin and rump region are prominent. Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones. Bony structure protrude sharply and ligament prominent.

Score 2 (Thin): Individual short ribs can be felt but are not prominent. Each rib is sharp to touch but have a thicker covering of flesh. Short ribs do not have as distinct an over-hanging shelf effect. Individual bone is the chine, loin and rump regions are not visually distinct but easily distinguishable by touch. Hook and pin bones are prominent but the depression between them is less severe. Area below tail head and between pin bones is somewhat depressed but the bony structure has some covering of flesh.

Score 3 (Moderate): Short ribs can be felt by applying slight pressure. Altogether, short ribs appear smooth and the over-hanging effect is not so noticeable. The backbone appears as a rounded ridge, firm pressure is necessary to feel individual bones. Hook and pin bones are rounded and smooth. Area between pin bone and around tail head appears smooth without sign of fat deposit.

Score 4 (Fat): Individual short rib is distinguishable only by firm palpation. Short ribs appear flat or rounded, with no overhanging shelf effect. Ridge formed by backbone in chine region is rounded and smooth. Loin and rump region appear flat. Hooks are rounded and the space between them is flat. Area of tail head and pin bones is rounded with evidence of fat deposit.

Score 5 (Obese): Bony structures of backbone, short ribs and hook and pin bones are not apparent; subcutaneous fat deposit very evident. Tail head appears to be buried in fat tissue.

Semen Collection and Evaluation

Semen collection: Semen samples were collected from each animal at the onset and thereafter on weekly basis for 52 week susing an electro-ejaculator and were labeled accordingly. This was done in the morning hours throughout the duration of the experiment. The collected semen samples were evaluated immediately for colour, volume, motility and pH as describe by Zemjanis (1970). Smear of each semen sample was prepared; air dried, labeled and kept for further examination, vis determination of sperm concentration using formaldehyde and determination of live and dead ratio using eosin nigrosin. A total of 1488 records were generated for each of the observed characteristics.

Sperm Concentration: The concentration of the spermatozoa was determined using the Red Blood Cell counting chamber of a haemocytometer that were crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. The total number of smaller squares on the haemocytometer is 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot *et al*., 1997).

Prior to counting, formaldehyde was used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The haemocytometer was mounted into the microscope and an absorbable tube and O-no pette was used to pipette a drop of the solution into the haemocytometer chamber. The absorbable tube and the O-no pette were blown before pipette to avoid air bubbles in the O-no pette. After appropriate counting in the 5 large squares, the number obtained was multiplied with 100 (dilution factor), 16 (the number of smaller squares in a larger square and the volume of the semen sample collected, multiplied by 10^6). The result obtained was recorded as the sperm cell concentration for the sample.

Live and Dead Ratio: The live and dead ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosin stain immediately after collection.

A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labeled.

This was done for each sample and they were later mounted on the microscope for counting the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained. The procedure above was developed by Hancock (1951).

Sperm Mineral Concentration

Mineral analysis was performed with a Coleman 21 Flame Photometer at the Pathological Department of Ahmadu Bello University Teaching Hospital, Shika, Zaria. The Flame Photometer is calibrated with five standard stock solutions for concentration of minerals. The semen samples were centrifuged for separation of seminal plasma. Seminal plasma was then processed and different elements: Ca, Na K and $P0_4$ were estimated.

Statistical Analysis

Correlation analysis procedure of SAS (2002) was used to assess the relationship between the measured characteristics. The weekly data (1488 records) on semen cation concentrations were used for estimating thier relationships with seminal traits and testicular measurements. However, to estimate the relationship of semen cation concentrations with body weight and body conformation traits, their weekly observations were averaged for each month to get a comparative value to the monthly body measurements.

3. Results

The result of the correlation analysis between semen characteristics and semen cation concentrations is presented in Table 1. Semen volume was positively and significantly correlated with K^+ and Ca^{2+} ($P<0.05$; r= 0.27 – 0.31) but not with Na^+ and $P0_4$ ($P>0.05$; r= 0.13 – 0.14). Sperm motility was positively and significantly correlated with Na^+ and K^+ ($P<0.05$; r= 0.26 – 0.33). Sperm concentration had positive and significant correlation with K^+ ($P<0.05$; r= 0.37), while live/dead ratio was positively and significantly correlated with only Na^+ ($P<0.05$; r= 0.39) among the cation.

Table 1: Correlated Relationships between Semen Characteristics and Semen Cation Concentrations in Red Sokoto buck

	Semen Volume	Sperm Motility	Semen pH	Sperm Concentration	Live & dead ratio
Sodium (Na)	0.14	0.26*	-0.05	0.02	0.39*
Potassium (K)	0.27*	0.33*	0.01	0.37*	0.01
Calcium (Ca)	0.31*	0.02	0.15	0.00	0.06
Phosphate ($P0_4$)	0.13	-0.04	-0.13	0.05	-0.08

*= P<0.05

Table 2: Correlated Relationships between Testicular Measurements and Semen Cation Concentrations in Red Sokoto buck

	TL	TC	TW	TWT
Sodium (Na)	-0.02	-0.10	-0.10	-0.10
Potassium (K)	0.11	0.21	0.21	0.20
Calcium (Ca)	0.21	0.39*	0.39*	0.34*
Phosphate ($P0_4$)	0.16	0.07	0.07	0.15

*= P<0.05, TL: Testicular length, TC: Testicular circumference, TW: Testicular width, TWT: Testicular weight

Table 2 shows the correlated relationships between testicular measurements and semen cation concentrations. The result showed that Ca^{2+} was positively and significantly correlated with testicular measurements ($P<0.05$; r=0.34 – 0.39), while Na^+ had negative and non-significant correlation with testicular measurements ($P>0.05$; r= -0.02 to -0.10). Other correlations were close to zero or not significant.

The correlation analysis between body conformation traits and semen cation concentrations is shown in Table 3. Negative but significant ($P<0.05$) correlation was observed between Na^+ and BL (r= -0.26). K^+ was positively and significantly correlated with body conformation traits ($P<0.05$-0.01; r=0.29 – 0.52), except BW, BCS and HG ($P>0.05$; r=-0.09 to -0.14). Ca^{2+} was positively and significantly correlated with CW, WH, BD and RW ($P<0.05$; r=0.29-0.42) but was negatively and non-significantly correlated with BW, BCS and HG ($P>0.05$; r=-0.03 to -0.05). Ca^+ had positive but non-significant correlation with Stature. K^+ and Na^+ had the strogest positive correlation with BL, followed by WH.

Table 3: Correlated Relationships between Body conformation and Semen cation oncentrations in Red Sokoto bucks

	Na	K	Ca	$P0_4$
BW	0.11	-0.09	-0.03	0.16
BCS	0.19	-0.14	-0.05	0.04
Heart girth	0.09	-0.10	-0.03	0.18
Stature	0.07	0.33*	0.21	0.06
Chest width	-0.07	0.29*	0.33*	0.14
Wither height	-0.03	0.44**	0.31*	-0.03
Body depth	-0.24	0.52**	0.42*	-0.08
Body length	-0.26*	0.29*	0.17	0.05
Rump width	0.01	0.39*	0.29*	0.02

**= P<0.01, * =P<0.05, BW: Body weight, BCS: Body condition score

4. Discussions

Semen volume showed a positive and significant correlation with Ca^+ (r= 0.31) and K^+ (r= 0.27) but non-significant correlation with Na^+ and $P0_4$ indicating that the higher the volume of semen the more will be the concentration of Ca^+ and K^+ in the seminal fluid. This finding agrees with the observation of Kanwal *et al.* (2000) who reported correlation of r = 0.36 between Ca^{2+} and semen volume in bull. Abdel-Rahman *et al.* (2000) also reported a positive correlation between K^+ and semen volume. In the same vein, Na^+ and K^+ showed a positive and significant correlation with sperm motility. This signifies that the progressive active movement of spermatozoa may be improved or increased with higher concentration of Na^+ and K^+ in the seminal fluid of the bucks. This is in contrary to the report by Kaya *et al.* (2002) who observed a negative correlation between sperm motility and Na^+ and K^+ concentrations in ram. However, a positive but non-significant correlation of r = 0.17 has been reported by Kanwal *et al.* (2000) between K^+ and sperm motility in bull.

In ram, increasing potassium levels has been reported to be negatively correlated with progressive motility, while the reverse is true for sodium (Abdel-Rahman *et al.*, 2000). Hence a positive and significant correlation as observed in the present study between Na^+ and sperm motility was supported by the finding of (Abdel-Rahman *et al.*, 2000). The observed positive and significant correlation of K^+ with sperm concentration was not in accordance with the report of Massanyi *et al.* (2003) who observed K^+ to be a natural metabolic inhibitor and higher concentration in seminal plasma decreases sperm metabolism thereby, decreasing sperm motility. However positive and significant correlation of r = 0.37 between sperm concentration and K^+ as observed in the present study was in accordance with the report of (Abdel-Rahman *et al.*, 2000) who report correlation of r = 0.74 in ram.

The observed positive and significant correlation between Ca^{2+} and testicular measurements indicates that, as Ca^{2+} concentration in the seminal plasma increases, a corresponding increase in testicular dimension may be expected. However negative but non-significant correlation as recorded between Na^+ and testicular traits suggests that Na^+ is not a good indicator of testicular growth and development in the bucks.

The positive and significant correlations of $K^+$and Ca^{2+} with some conformation traits suggest that bucks with higher concentration of K^+ and Ca^+ might possess larger body size. It was observed that these conformation traits do not involve three important traits such as BW, BCS and HG. These three triats showed negative but non-significant correlation with $K^+$and Ca^{2+} respectively. Body lenght was observed to be negatively and significant correlated with Na^+ suggesting that Na^+ is not an indicator of BL in the bucks.

Conclusions

- Bucks with good body structure would show high concentration of potassium in their semen, hence they would produce higher semen volume with increased sperm concentration and motility.
- Better seminal concentrations of sodium and potassium would improve sperm motility of Red Sokoto bucks; and bucks with adequate concentrations of calcium would exhibit better testicular dimensions. Therefore, mineral status of the seminal fluid is an essential index in evaluating semen quality of bucks.

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5/6/2013