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Live Weight Related Changes in the Sperm Production Capacity of White Fulani (Bos indicus) Cattle I: Testicular Histomorphometry

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Abstract: The study investigated live weight related changes in testicular histomorphmery of White Fulani (WF) bulls of live weight range 171-320 kg under the traditional extensive system of management in Ibadan, Nigeria. Fifty-two WF bulls were randomly selected from local Fulani herds around Ibadan and placed in Giant Star grass paddocks with water available free choice for one week prior to slaughter. Morhpometric measurements were taken on the scrotum before and on the testes immediately after slaughter. Histometric measurements were taken on the testicular parenchyma tissue after histological processes. From their weight at slaughter, the bulls were grouped into four live weight groups of 171-200, 201-230, 231-260 and 261-320 kg for group I, II, III and IV, respectively. Mean values of group IV bulls were significantly ($p<0.05$) higher in all scrotal and testicular morphometric measurements than all other groups, which did not differ significantly ($p>0.05$) from one another. The gonadal index (relative weight of testes to live weight) did not differ ($p>0.05$) between groups except that group IV value was significantly ($p<0.05$) higher than all other groups. The relative proportion of paired epididymal weight to live weight did not differ between groups, while the relative proportion of tunica albuginea to the testes weight decreased insignificantly from group I to IV. The seminiferous tubule diameter did not significantly ($p>0.05$) differ between groups, while the stages in the cycle of the seminiferous epithelium also appeared stable between the different live weight groups. The volume proportion of the lumen in the semiferuos epithelium was significantly higher in group I bulls than group IV bulls while none of the testicular elements differed significantly ($p>0.05$) between groups in volume proportions. However, absolute weights of all the testicular elements were significantly higher in the group IV bulls than all the other groups, which did not differ significantly ($p>0.05$) from one another. It was concluded that the live weight range 171-320 kg corresponds to a physiologically stable state in White Fulani bulls extensively managed in Ibadan, a humid tropical environment in Nigeria.

Key words: Live weight, sperm production, white fulani, testis, histomorphometry

INTRODUCTION

Evaluation of breeding soundness and potential fertility of poultry cock have been made from the knowledge of basic morphometric characteristics of the reproductive organs (Togun *et al*., 2006). The size of the testis has been reported as a good indicator of present and future sperm production as well as breeding quality of the male (Ezekwe, 1998; Perry and Petterson, 2001; Togun and Egbunike, 2006).

Measurable reproductive criteria of tropical breeds of bulls have been reported to include growth, scrotal circumference, testicular development, sperm morphology and fertility (Osinowo *et al*., 1981; Daudu and Shoyinka, 1983). According to Kenagy and Trombulak (1986), smaller animals are expected to allocate greater proportion of body weight and energy expenditure to testicular tissue than larger animals. Togun *et al*. (2006) reported live weight to be significantly correlated with testes weight. Togun (2006) reported scrotal circumference as the most superior of all testicular parameters in estimating testis size. Brito *et al*. (2004) also reported that body weight is a good predictor of sexual maturity. Kwari and Ogwuegbu (1998) reported that scrotal, gonadal and epididymal traits were more closely related to bodyweight than the age of the bull. Quantitative aspects of spermatogenesis are particularly important to the livestock industry since there is a relationship between spermatozoa production and the number of offspring that can be produced by a sire. It becomes more important now that artificial insemination has become very prominent in animal species. The relative importance of the various cell components in the seminiferous tubules of most farm animals have been enumerated in literature (Hafez, 1987). According to Senger (1998) the time required for the duration of the cycle of the seminiferous epithelium is constant and unique for each species.

The White Fulani (WF) is a fairly large breed (Olaloku, 1972) that is very popular in Nigeria, but most of the animals are reared under the extensive system of management, which dose not allow detailed records on birth, age and growth characteristics. However, the relationship between growth, testicular development and spermatogenesis is of importance in Artificial insemination centers and progeny test stations. Such knowledge would enhance the possibility of early commencement of semen collection from bulls at a young age. The importance of WF breed to the impending national breeding programme makes it mandatory to establish the live weight related changes in the dimensions and weight as well as the histometric characteristics of its genitalia. These needs serve as the basis for this study and would allow an effective prediction of the sperm production capabilities of the breed in its natural environment, based on the live weight at any point in time. It will also satisfy the need for a greater exploitation of the spermatogenic potential at the young age, thus allowing a longer period of use of individual bull in each herd.

MATERIALS AND METHODS

Site and period: The study was carried out in Ibadan, South West of Nigeria. Ibadan is situated at an elevation of 200 m above sea level. It is in the rain forest zone, with main seasons (wet and dry) of about equal period. Vegetative growth is retarded in the dry season when temperature is highest. The study spanned a period of 2 years, between 2003 and 2005, which allowed data collection twice during the two different seasons in the two years. The data represent the means of data, spanning the experimental period.

Animals: Fifty-two White Fulani bulls of body weight range 171-320 kg involved in this study were owned by nomadic Fulani herdsmen. The animals were railed down from the Northern part of the country and placed principally on extensive system of management, where they were grazed from place to place with no attempt to feed any form of supplement. Attempts were made to supply water only as much as could be available.

Experimental design: The bulls were randomly selected from different White Fulani herds and placed in a Giant Star grass paddock for a period of one week prior to slaughter. The animals were weighed at slaughter and from their weights; they were grouped into four body weight groups viz.

- **Group I:** 171-200 (11 bulls)
- **Group II:** 201-230 (16 bulls)
- **Group III:** 231-260 (19 bulls)
- **Group IV:** 261-320 (6 bulls)

Morphometric studies

Measurements before slaughter: Scrotal circumference was measured with a tape, which was passed round the broadest point of the scrotum.

Scrotal width was measured with a veneer caliper as the distance between the two sides of the broadest part of the scrotum.

Scrotal length was taken as the length of the testis while still in the scrotum. The upper part of the testis was located with the forefinger and a steel tape was used to measure from this end to the lower part of the testis in the scrotum.

Scrotal skin fold thickness was measured with a veneer caliper taking care not to make the caliper too tight as this would underestimate the thickness of the skin.

Measurements immediately after slaughter: The testes were removed immediately after slaughter and taken to the laboratory for further processing. They were weighed individually after the epididymis has been trimmed off each of them. The length and width of each testis were taken by the use of a veneer caliper. The volume of each testis was recorded, using Archimedes principle of water displacement. The tunica albiginea were then peeled off the testes and weighed individually to know the weight of testicular parenchyma.

Histometric studies

Histology: The testes were cut mid-sagitally and tissue samples were taken from each half of each testis. The epididymides were cut into the differentiating parts of caput, corpus and cauda. A part of each, along with the testicular samples were fixed in more than 20 times the volume of each in Bouin's fixative for 24 h, dehydrated in series of ethyl alcohol, cleared in chloroform and embedded in paraffin. The completed histological procedures were according to the instructions detailed in previous studies (Togun, 1981). Histological sections, 7 μ thick were cut and left to float and flatten out on water (40°C) and then picked up carefully with clean slides, which have been smeared with Mayer's egg albumin. Successful sections, about 140th away were mounted (i.e., every 21st section) so as to ensure that the positions of the tissue examined were not of the same portion of the testis and epididymis. The slides were stored in air incubator for 30 min and later stained with Haematoxylin-Eosin (H and E). Each slide was clean-blotted and

mounted in Canada balsam under a cover slip. Four slides, made up of two slides per testis, were prepared for each bull.

Histometric measurements

Testis

Seminiferous tubule diameter: Tubular diameters of seminiferous tubules were determined by measuring twenty approximately round tubules per slide with a microscope, having its eyepiece already calibrated with a stage micrometer. Two measurements at right angles to each other were taken on each tubule and the average recorded (i.e., two slides per testis). Twenty tubules were measured in each of the four slides to give a total of eighty tubules per animal.

Volumetric proportions of cellular elements in the seminiferous epithelium were determined by the method of Chalkley (1943) as modified by Egbunike and Steinbach (1972). It essentially involved the counting of the number of hits by cellular elements in 20 fields in each of the four slides per animal with an integrating eye piece (Zeiss Oberkochen) having 25 points asymmetrically arranged in a cycle and calculating accordingly.

Stages in the cycle of seminiferous epithelium were determined by classifying twenty seminiferous tubules in each of the four slides per bull. The frequency of occurrence of each stage was calculated on percent basis.

Epididymis: Tubular diameters of the epididymal tubules were separately determined for the caput, corpus and cauda epididymides. Twenty tubules were measured (each with two measurements at right angles to each other) per slide to give a total of forty tubules per section of the epididymis.

Epithelial heights of epididymal tubules were measured from the basement membrane to the coat of the tubule for the caput, corpus and cauda epididymides.

Statistical analysis: Data were expressed as Mean±SEM. They were subjected to General Linear Model (GLM) of the Analysis of Variance (ANOVA). Means, where significant, were separated by Duncan multiple range test (SAS, 2002).

RESULTS

Scrotal and testicular morphometry: The mean scrotal circumference, width and length were significantly ($p<0.05$) higher in group IV bulls than all the other groups, which did not significantly ($p>0.05$) differ from one another, except that group III value for scrotal circumference was significantly ($p<0.05$) higher than group I value (Fig. 1). The mean paired testicular parenchyma weight and paired testicular volume were significantly ($p<0.05$) higher in group IV than all other groups, which did not differ significantly ($p<0.05$) from one another except that group III values were significantly ($p<0.05$) higher than group I value (Fig. 2). The mean group testes length and width are shown in Fig. 3 group IV values were significantly ($p<0.05$) higher than the values observed in all the other groups, which did not significantly ($p>0.05$) differ from one another.

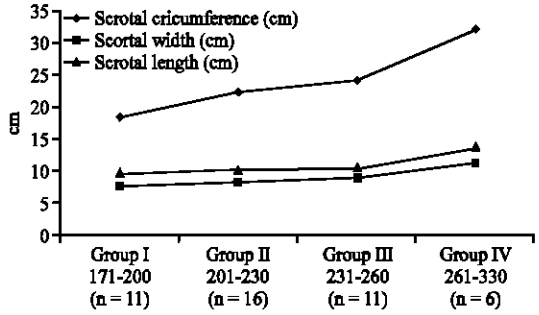

Fig. 1: Scrotal morphometry

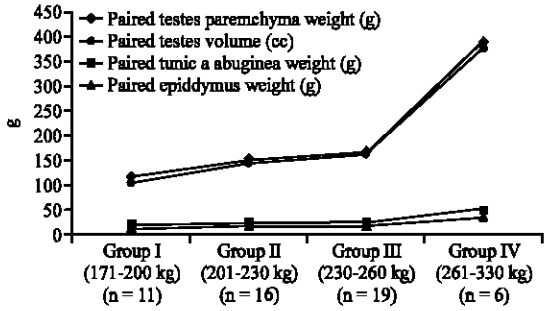

Fig. 2: Testicular morphometry

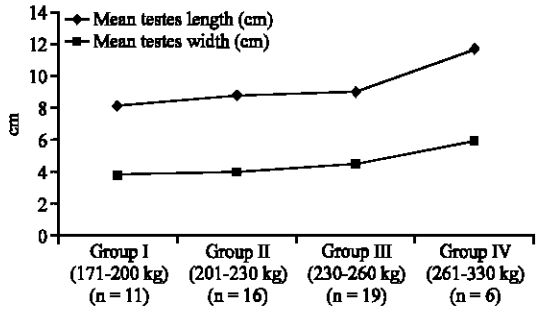

Fig. 3: Mean testicular length and width

Table 1: Derivations from testicular morphometry and seminiferous tubule diameter of white fulani bulls (Mean±SEM)

Liveweight groups	PTW/Liveweight (%)	P.ep.w/Liveweight (%)	P. Tunica/PTW (%)	Mean testis density (g/cc)	Seminiferous tubule diameter (μ)
Group I (171-200 kg) n = 11	0.06±0.01	0.01±0.00	11.23±0.70	1.07±0.17	179.94±4.64
Group II (201-230 kg) n = 16	0.07±0.01	0.01±0.00	10.08±0.62	1.04±0.01	181.31±6.01
Group III (231-260 kg) n = 19	0.07±0.00	0.01±0.00	9.79±0.48	1.03±0.00	174.84±4.72
Group IV (>260 kg) n = 6	0.15±0.02	0.02±0.00	7.98±0.48	1.04±0.01	175.43±5.26

PTW: Paired testes weight (g), P.ep.wt: Paired epididymal weight (g), P. Tunica: Paired tunica albuginea weight (g)

Table 2: Volumetric proportions (%) and absolute weights (gm) of testicular elements of White Fulani bulls (Mean±SEM)

Liveweight groups	A-Type spg	B-Type spg	Young 1ospc	Old 1ospc	2ospc	R. spd	El. spd	spz
Group I †	3.33±0.46	2.92±0.57	3.06±0.44	3.05±0.40	1.18±0.16	2.20±0.23	0.83±0.16	1.17±0.15
††	3.89±0.15	3.41±0.13	5.57±0.23	3.56±0.09	1.38±0.10	2.57±0.07	0.97±0.02	1.37±0.03
Group II †	2.25±0.13	2.61±0.20	3.51±0.35	3.74±0.31	0.91±0.10	2.51±0.12	1.20±0.12	1.58±0.24
††	3.38±0.21	3.92±0.30	5.27±0.38	5.62±0.17	1.37±0.07	3.77±0.12	1.80±0.03	2.37±0.04
Group III †	2.26±0.15	2.34±0.20	3.14±0.25	3.71±0.29	1.19±0.08	3.01±0.14	0.96±0.11	1.86±0.16
††	3.76±0.12	3.89±0.11	5.24±0.41	6.17±0.58	1.98±0.01	5.01±0.11	1.60±0.01	3.09±0.21
Group IV †	2.36±0.20	1.99±0.21	2.75±0.22	3.64±0.35	1.62±0.25	2.50±0.24	1.36±0.27	1.54±0.24
††	9.16±0.44	7.73±0.49	10.68±0.38	14.13±0.66	6.29±0.20	13.59±0.39	5.28±0.42	5.98±0.39
Liveweight groups	Setoli Cells	B. Memb.	Leydig Cells	Int. Cells other than leydig		Int. Cell spaces	Cellular cytoplasm	Lumen
Group I †	1.86±0.16	4.72±0.39	0.74±0.08	2.78±0.29		4.80±0.63	60.58±1.31	6.78±1.32
††	2.17±0.02	5.51±0.02	0.86±0.01	3.25±0.03		5.60±0.33	70.73±0.62	7.92±1.81
Group II †	2.22±0.15	5.07±0.20	0.88±0.04	2.38±0.25		5.04±0.41	61.45±0.80	4.65±0.84
††	3.36±0.13	7.62±0.08	1.32±0.02	3.58±0.08		7.57±0.39	92.32±5.21	6.99±0.91
Group III †	2.09±0.14	4.44±0.29	0.94±0.04	2.64±0.19		5.69±0.53	61.59±0.71	4.14±0.65
††	3.48±0.14	7.39±0.08	1.56±0.01	4.39±0.23		9.47±0.88	102.48±11.01	6.89±0.39
Group IV †	2.51±0.19	4.48±0.29	1.11±0.18	1.57±0.24		4.28±0.56	64.78±0.95	2.51±0.80
††	9.75±0.61	17.40±0.77	4.31±0.14	6.10±0.21		16.62±0.80	251.55±4.18	9.75±0.72

†: Volumetric proportions of testicular elements. ††: Absolute weights of testicular elements, spg: Spermatogonia, spc: Spermatocytes, spd: Speronatios, R: Round, El: Elongated, Spz: Spermatozoa, B: Basement, Int: Interstitial

Tunica and epididymal weights: The mean group values of paired epididymal weight and paired tunica albuginea weight are shown in Fig. 2. The mean values of group IV bulls were significantly ($p<0.05$) higher than all the other groups, which did not differ significantly ($p>0.05$) from one another in the two measurements.

Derivations from testicular morphometry: The mean group gonadal index (relative weight of testes to live weight), the relative weight of paired epididymal weight to live weight and the relative paired tunica albuginea weight to paired testes weight are shown in Table 1. The gonadal index did not differ significantly ($p>0.05$) between groups except group IV, which was significantly ($p<0.05$) higher in value than all other groups. There was no significant ($p>0.05$) difference between groups in the relative epididymal weights. However the mean relative weight of paired tunica albuginea to the paired testes weight decreased insignificantly ($p>0.05$) from group I-IV.

Testicular histometry

Seminiferous tubule diameter (STD): Table 1 shows the mean group values of the seminiferous tubule diameter. There was no significant ($p>0.05$) difference between the groups.

Volumetric proportions and absolute weights of cellular elements: Table 2 shows the live weight related changes in the volumetric proportions and absolute weights of cellular elements in the seminiferous epithelium. There was a relative marginal decrease in the spermatogonial volume percent along with increasing live weight groups. The proportion of spermatocytes and spermatids did not show a relative increase with liveweight groups. The volumetric proportion of Sertoli cell did not show a fixed pattern but tended to increase unlike the basement membrane, which increased up to group II and decreased thereafter. The cellular cytoplasm tended to increase with live weight groups but the tubular lumen decreased in volumetric proportion. However, the absolute weights of cellular elements of bulls in group IV with the mean value of group I bulls being significantly higher than in group IV bulls, were significantly higher than in all the other groups.

Stages in the cycle of seminiferous epithelium: There was no significant ($p>0.05$) difference between live weight groups in each of the stagesin the cycle of seminiferous epithelium, which appeared stable irrespective of live weight (Table 3).

Epididymal histometry

Tubular diameter: Figure 4 shows the mean group values of epidiymal tubule diameter for the different segments of the epididymis. There was no significant ($p>0.05$) difference between the groups in this parameter.

Table 3: Live weight related changes in the stages in the cycle of seminiferous epithelium of white fulani bull extensively managed in the humid tropics

	Stage							
Groups	I	II	III	IV	V	VI	VII	VIII
I (171-200 kg)	22.12±1.00	13.24±1.74	7.40±0.62	10.31±0.76	6.28±0.76	21.34±1.31	10.71±0.37	8.60±0.82
II (201-230 kg)	24.23±0.82	13.31±1.04	8.75±0.51	11.29±0.53	5.33±0.40	20.61±1.42	9.81±0.82	6.64±0.56
III (231-260 kg)	20.38±1.11	11.32±0.72	7.15±0.60	11.30±0.57	6.99±0.28	23.32±1.13	11.84±0.86	7.71±0.58
IV (>260 kg)	19.92±1.45	10.29±0.39	5.92±0.72	9.20±0.99	6.23±0.36	26.04±1.26	12.92±1.39	9.49±1.68

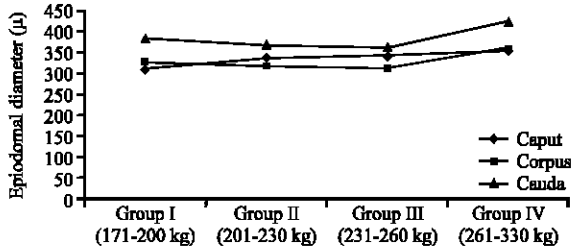

Fig. 4: Epididumal tubule diameter

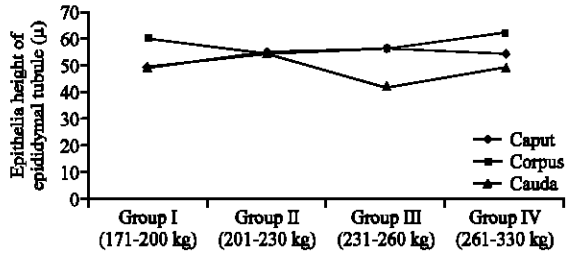

Fig. 5: Mean variation of epithelial height of epididymal tubule diameter among live weight groups

Epithelial height of epididymal tubule: Figure 5 shows the mean group epithelial heights of the caput, corpus and cauda epididymal tubules. Apart from the epithelial height of the caput epididymal tubule, which showed an increasing tendency from group I-III with a decrease in group IV, the epithelial height showed no fixed pattern with increasing live weight groups.

DISCUSSION

The study revealed a positive relationship between scrotal circumference, testes weight and live weight, which could lead to adequate prediction of the reproductive status of the bull in the absence of birth records as long as the live weight is known. Live weight can be estimated in practical terms from hearth girth measurement.

The similar pattern observed for the different morphometric characteristics along with the live weight groups, with a non-significant ($p>0.05$) increasing trend from group I through to group III but with a significant ($p<0.05$) increase in group IV, indicates some consistency in the pattern of growth of these characteristics. That the mean paired testes weight of group III bulls was significantly higher than the group I bulls in line with the scrotal circumference, confirms the reports in literature (Pant *et al*., 2003; Park *et al*., 2003; Vasquez *et al*., 2003; Togun *et al*., 2006; Togun, 2006; Hamilton and Stark, 1997) that scrotal circumference is a basis for estimating testes size as well as selecting breeding bulls. This is important since testis size has been reported as a good indicator of the present and future capacity of spurn production and breeding quality of bulls (Togun *et al*., 2006; Ezekwe, 1998; Perry and Petterson, 2001; Togun and Egbunike, 2006). Testicular weight has been reported to vary with age and body weight (Ezekwe, 1998; Togun *et al*., 2006; Togun and Egbunike, 2006) but according to Hahn *et al*. (1969), the scrotal circumference reaches a maximum at 5-6 years, when it remains relatively stable. It is thus expected that the testis size would also reach its maximum at probably a period not too far from this time in view of the established relationship between scrotal circumference and testicular size (Togun *et al*., 2006; Ezekwe, 1998; Perry and Petterson, 2001; Togun and Egbunike, 2006).

From the report of Olaloku (1972), the White Fulani breed reached its mature weight at about 348 kg on an experimental station, under the semi-intensive management system. Such system would offer a more conductive environment to the animals than the extensive system of management by the nomadic Fulani herdsmen typified in this study. From the observation in this study, the morphometric characteristics of the White Fulani bulls approached the plateau of their growth curve at around the live weight range of 260-330 kg. It would be expected that increases in morphometric measurements would be low or non existent from this stage of growth in the WF bull.

The volumetric proportion of cellular elements studied dealt with the relative volume occupied by each element within the testis. The inconsistency in the pattern of the relative increase in germ cell nuclei volume with increasing live weight would not seem to indicate that heavier bulls in this study have a grater capacity per unit volume of the testis to produce sperm cells than the smaller bulls. This is in spite of the fact that the heavier bulls paraded larger testes and scrotal circumference. The spermatocyte and spermatid population have been positively linked with sperm production rate just as the

Sertoli (Berndtson *et al*., 1987) and Leydig (Abbasi *et al*., 1980) cells in cattle. However, one valid observation is that with the bulls in this study, active spermatogenesis was still taking place with most of the spermatogenic cells attaining maturation to sperm cells. The significantly ($p<0.05$) higher mean values of absolute weight observed for all the cellular elements of the seminiferous epithelium in group IV bulls than all the other groups is a ready explanation for the significantly ($p<0.05$) heavier tests of the group IV bulls. This observation can further be extended to suggest the possibility of lager number of cells to enhance relative total sperm production.

The non_significant difference between groups in the eight stages of the seminiferous epithelial cycle supports earlier observation (Swierstra and Foote, 1963) that the kinetic of spermatogenesis is species specific being, similar in pubertal and adult, irrespective of the site or the testis of sampling (Swierstra, 1968; Egbunike *et al*., 1983; Togun and Egbunike, 2005).

The non significant ($p<0.05$) difference between the epididymal tubule diameters (caput, corpus and cauda) and the epithelial heights of the epidymal tubules, point to the fact that the White Fulani bulls in the live weight range involved in this study, have very similar testicular and epididymal histometry. This suggests that a plateau state in testicular and epididymal functions are imminent at this stage or have been reached, having attained a physiological stable state of development. This is complemented by the result of other parameters studied above.

It is thus obvious from the above results that it was only in the scrotal circumference and the testes weight that there were significant ($p<0.05$) differences between the live weight groups studied. This would point to an apparent superiority of the larger animals in spermatozoa production. However the association of spermatogenic cells, as well as other histomorphometric characteristics, did not indicate a similar conclusion but pointed to the probability that sperm production capacity, quality and efficiency between the groups might not be significantly different. The only possible difference would thus be accounted for through the differences conferred by testicular size and scrotal circumference, both of which have been established to have highly, significant correlation with each other and with total sperm producing capability of an animal.

It can therefore be concluded that the live weight range in this study corresponds to the stage of physiological stability of the bulls.

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