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Testicular morphometry and sperm reserves of local turkey toms fed varying levels of protein in the diet

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Abstract

The morphometry and sperm reserves of the testis, epididymis and vas deferens of three groups (n=5/ group) of sexually active adult local turkey toms fed isocaloric diet with varying levels (12 %, 16 %, 20 %) of protein were studied for sixteen weeks. The weights of the toms before treatment were between 3.5 – 4.5 kg, while at the end of the experiment the mean ± SD live weight were 5.29 ± 0.65, 5.39 ± 0.45 and 5.63 ± 0.49 kg for groups 1, 2 and 3 respectively. The mean ± SD weights of the paired tunics, testis, epididymis and vas deferens, respectively, were 0.41 ± 0.11 g, 8.27 ± 2.37 g, 0.28 ± 0. 07 g and 0.36 ± 0.11 g (group 1): 0.43 ± 0.02 g, 8.50 ± 0.65 g, 0.33 ± 0.11 g and 0.40 ± 0.11 g (group 2) and: 0.49 ± 0.16 g, 9.83 ± 3.08 g, 0.40 ± 0.13 g and 0.50 ± 0.18 g, (group 3). The mean ± SD lengths of the testes were: 3.72 ± 0.34 cm, 4.40 ± 0.47 cm and 4.48 ± 1.14 cm; the epididymis: 3.12 ± 0.56 cm, 3.17 ± 0.67cm and 3.48 ± 0.49 cm, and the vas deferens: 17.27 ± 1.10 cm, 17.33 ± 0.93 cm and 17.49 ± 1.10 cm, for groups 1, 2 and 3, respectively. Mostly, the parameters of the left organs were greater than those of the right. The mean ± SD weight of the testes positively correlated with that of the epididymis in all the groups (r = 0.72, 0.65 and 0.87 for groups 1, 2 and 3 respectively) and the vas deferens (r = 0.54, 0.72 and 0.75 for groups 1, 2 and 3 respectively). The gonadal sperm reserves were 0.19 ± 0.00 x 10^9 cells/ml, 0.21 ± 0.00 x 10^9 cells/ml and 0.21 ± 0.00 x 10^9 cells/ml for groups 1, 2 and 3 respectively. The mean ± SD extragonadal sperm reserves were, epididymis: 0.08 ± 0.00 x 10^9 cells/ml, 0.12 ± 0.01 x 10^9 cells/ml, 0.18 ± 0.00 x 10^9 cells/ml, and vas deferens: 2.00 ± 0.13 x 10^9 cells/ml, 2.82 ± 0.50 x 10^9 cells/ml and 3.75 ± 0.60 x 10^9 cells/ml for the three groups respectively. The vas deferens had about 88 %, of the extragonadal sperm reserve in group 1 and 90 % in groups 2 and 3. Sperm reserve was positively correlated to body weight and to the length of the testis. The results suggest, therefore, that morphometry and sperm reserves were better in turkey toms fed 16 % and 20 % than 12 % protein diets.

Keywords: Crude protein, Fertility, Morphometry, Reproduction, Sperm reserves, Turkey tom

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Introduction

Reproduction is a function of nutritional state, and also a director of nutrient flux, and both have genetically inherited elements of control. These three systems function integrally in the animal, and as such there is no way that nutrition, reproduction, and genetics can be separated in research (McNamara, 2010). The main function of the testis is sperm production. The epididymis aids in transport and the vas deferens serves both for storage and maturation of sperm (Deviche *et al*., 2011).

Scrotal circumference measurement which is widely used as an index of fertility in mammals is not readily available in birds because of the location of the testis. Attempts are currently underway to use technologies, such as ultra-sonography for accurate determination of the sizes of internal structures, which includes the reproductive organs (Sreesujatha *et al*., 2016), in the avian species, but until such a time when these technologies become readily accessible, the size of the reproductive organs and their sperm reserves are used as indicators of fertility (Kristen *et al*., 2012; Okpe *et al*., 2010)

The reproductive organs of male birds are intra-abdominal, located on either side of the mid-line attached to the dorsal aspect of the abdomen. The reproductive organs consist of a pair of testis, epididymis and vas deferens which terminates in the local copulatory apparatus (Tuncer, 2008). Testicular asymmetry is a common feature of avian testis, studies abound in different species of birds (Graves, 2004; Bull *et al.*, 2007; Calhim & Birkhead, 2007; Gunn *et al.,* 2008). In exotic turkey, studies have also been conducted on the reproductive capacity and morphometry of the reproductive organs and their sperm reserves (Hess *et al.,* 1976).

The morphometry and sperm reserve of the local turkey toms and whether or not they are affected by dietary crude protein levels have not been adequately studied. Therefore this work provides data on the morphometry and sperm reserves of the reproductive organs of the Nigerian local turkey tom, it also gives the possible relationships between various organs, sperm reserve and nutrition.

Materials and Methods

Three (3) groups of indigenous turkey Toms (n = 5) aged 30-34 weeks and weighing 3.5 – 4.5 kg were used for this study. Toms were caged individually in improvised cubicles (1.5 metres x 1.5 metres) and fed 12 %, 16 % and 20 % protein diets in groups 1, 2 and 3, respectively, for 16 weeks. The feed was formulated in collaboration with the Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Nigeria. Compounding and proximate analysis were done at the National Animal Production Research Institute, Ahmadu Bello University, Zaria

Morphometry of the reproductive organs

Five (all) toms from each group were sacrificed and both testicles carefully removed and trimmed of extra tissues. Then organs were partitioned following the anatomical description of McLelland (1991), weighed using electronic balance (Mettler Toledo) and their volumes recorded indirectly by water displacement in a graduated centrifuge tube.

With the help of a thread and a measuring tape, length of the epididymis, vas deferens and testicles were measured. The diameter of the epididymis, vas deferens and the width and thickness of the testicles were measured with vernier calipers and recorded.

Determination of gonadal and extra gonadal sperm reserves

Testicular, epididymal and vas deferens sperm reserves were determined using the homogenization haemocytometric technique (Obidi *et al.,* 2008) with modifications. After careful removal of the *tunica albuginea* with a scalpel blade, each testicle was weighed again and homogenized in 20 mls of physiological saline with an antibiotic (Streptomycin 1:20 v/v), using a sonicator. The epididymis and vas deferens were minced with a pair of scissors and each was also homogenized in 10 mls of physiological saline containing antibiotic. The homogenate volume was recorded and stored at 5°C for 24 hours. They were mixed by agitation at intervals during the period of storage. The homogenates were diluted 1:50 v/v using physiological saline. They were agitated for about a minute and filtered. Volumes of the filtrates were recorded and the sperm/spermatids reserves in the suspension were determined using Neubauer haemocytometer. Two counts were made for each suspension at a magnification of x 40. The number of spermatozoa counted was then multiplied by the dilution factor to obtain the number of sperm cells/ml of the homogenate; this was multiplied by the volume of the homogenate to obtain the total number of sperm cells in the homogenate.

Statistical analysis

The total gonadal and extragonadal sperm count was determined by multiplying sperm concentration by the respective volumes of the organs. Sperm concentration is the product of the number of sperm cells counted, the multiplying factor and the dilution factors used. The data collected were summarized as mean ± SD, correlation between the variables were calculated and tested for significance at 5% confidence level, and a one way analysis of variances was used to compare values between groups.

Results

The mean ± SD live body weights of the turkey toms were 5.29 ± 0.65, 5.39 ± 0.45, 5.63 ± 0.49 for groups

Table 1: Mean ± SD weights of testicles, epididymis and vas deferens of turkey toms fed varying levels (12 %, 16 %, 20 %) of protein in the diet

	Group 1 (12 %)	Group 2 (16 %)	Group 3 (20 %)
No of Animals	5	5	5
Mean Live Weights(kg)	$5.29^{a} \pm 0.65$	$5.39^{b} \pm 0.45$	$5.63^{c} \pm 0.49$
Tunica albuginea (g)	$0.41^{a} \pm 0.11$	$0.43^{a} \pm 0.02$	$0.49^{b} \pm 0.16$
Testicles (g)	$8.27^{a} \pm 2.37$	$8.50^{b} \pm 0.65$	$9.83^{c} \pm 3.08$
Epididymis (g)	$0.28^{a} \pm 0.07$	$0.33^{b} \pm 0.11$	$0.40^{c} \pm 0.13$
Vas deferens (g)	$0.36^{a} \pm 0.11$	$0.40^{b} \pm 0.11$	$0.50^{c} \pm 0.18$

values with different superscripts (across rows) differ significantly ($P < 0.05$)

Table 2: Mean ± SD lengths of testicles, epididymis and vas deferens of turkey toms fed varying levels (12 %, 16 %, 20 %) of protein in diet

	Group 1 (12 %)	Group 2 (16 %)	Group 3 (20 %)
No of Animals	5	5	5
Testicles (cm)	$3.72^{a} \pm 0.34$	$4.40^{b} \pm 0.47$	$4.48^{b} \pm 1.14$
Epididymis (cm)	$3.12^{a} \pm 0.56$	$3.17^{b} \pm 0.67$	$3.48^{b} \pm 0.49$
Vas deferens (cm)	17.27 ± 1.10	17.33 ± 0.93	17.49 ± 1.10

values with different superscripts (across rows) differ significantly ($P < 0.05$)

Table 3: Mean ± SD gonadal (testicular) and extra gonadal (epididymal & vas deferens) sperm reserves of turkey oms fed varying levels (12%, 16 % and 20%) of protein in diet

	Group 1 (12 %)	Group 2 (16 %)	Group 3 (20 %)
No of Toms	5	5	5
Testicles ($x10^9$/gm)	$0.19^{a} \pm 0.00$	$0.21^{b} \pm 0.00$	$0.21^{b} \pm 0.00$
Epididymis ($x10^9$)	$0.08^{a} \pm 0.00$	$0.12^{b} \pm 0.01$	$0.18^{c} \pm 0.00$
Vas deferens ($x10^9$)	$2.00^{a} \pm 0.13$	$2.82^{b} \pm 0.50$	$3.75^{c} \pm 0.60$

values with different superscripts (across rows) differ significantly ($P < 0.05$)

1, 2 and 3 respectively as shown in Table 1. There was significant difference ($P < 0.05$) in the live body weights between groups. The mean ± SD weights of the testicles, epididymis and vasa deferentia are presented in Table 1. The weights of the testicles (in grams) for groups 1, 2 and 3 respectively were 8.27 ± 2.37g, 8.50 ± 0.65g and 9. 83 ± 3.08g. For epididymis, the respective weights (in grams) were 0.28 ± 0.07, 0.33 ± 0.11 and 0.40 ± 0.13. The mean weights of the tunicae albuginea were 0.41 ± 0.11g, 0.43 ± 0.02g and 0.49 ± 0.16g for groups 1, 2 and 3 respectively. Significant differences ($P < 0.05$) were found between the groups with regards to weight of the organs measured, interestingly, there variability of the data as indicated by SD is clear and goes to show that the in all the morphometry values, where groups 2 and 3 had value significantly higher than group 1. The mean ± SD lengths of the testicles were 3.72 ± 0.34cm, 4.40 ± 0.47cm and 4.48 ± 1.14cm for groups 1, 2 and 3 respectively. Those of the epididymis were 3.12 ± 0.56cm, 3.17 ± 0.67cm and 3.48 ± 0.49cm for groups 1, 2 and 3 respectively and vasa deferens had 17.27 ± 1.10cm, 17.33 ± 0.93cm and 17. 49 ± 1.10cm for groups 1, 2 and 3 respectively (Table 2). The lengths of the testicles and epididymis in groups 2 and 3 did not differ significantly ($P > 0.05$) from each other but differed significantly ($P < 0.05$) from those in group 1. Sperm reserves per gram of testicles ($0.19 \pm 0.00 \times 10^9$ cells/ml, $0.21 \pm 0.00 \times 10^9$ cells/ml and $0.21 \pm 0.00 \times 10^9$ cells/ml), epididymis ($0.08 \pm 0.00 \times 10^9$ cells/ml, $0.12 \pm 0.01 \times 10^9$ cells/ml and $0.18 \pm 0.00 \times 10^9$ cells/ml) and the vas deferens ($2.00 \pm 0.06 \times 10^9$ cells/ml, $2.82 \pm 0.5 \times 10^9$ cells/ml and $3.75 \pm 0.6 \times 10^9$ cells/ml) were significantly ($P < 0.05$) different between groups 1 and 2, and groups 1 and 3 (Table 3). However, groups 2 and 3 did not present significantly different results ($P > 0.05$) for testicular sperm reserve. However, the epididymal and vas deferens sperm reserves were significantly different ($P < 0.05$). Correlation between the weights of testicles and that of the epididymis and vas deferens were all positive and there was no significant difference ($P > 0.05$) between their values (Table 4).

Table 4: Correlation between the weights of the testis and epididymis and vas deferens

Correlation between the weights Testes and Epididymis		Correlation between the weights of Testes and Vas deferens	
Group 1 (12%)	r = 0.72	Group 1 (12%)	r =0.54
Group 2 (16%)	r = 0.65	Group 2 (16%)	r = 0.72
Group 3 (20%)	r = 0.87	Group 3 (20%)	r = 0.75

Discussion

Reproductive efficiency is improved with increase in the quantity and quality of diet supplied until an optimum point is reached (Hahn *et al*., 2005).

While it can be argued that the variability within groups is an indication that organs weight and morphometry respond to the treatment slowly, the significant difference between the groups shows that the level of crude protein in the various diets played a role in the appearance of this difference.

Unlike organs' weights and morphometry, sperm reserves were significantly different between groups. Interestingly, the variability in the case of sperm reserves is much less than it is in the case of organs' weights and morphometry as can be seen from the various standard deviations, consequently, repeatability in the case of sperm reserves becomes evidently higher than it is in the case of organs' weights and morphometry. This is perhaps due to the fact that the residual effect is much smaller in sperm reserves than it is in organs' weights and morphometry.

The data on correlation further elucidate the fact that although there was within group variability in the weights and morphometry, there was positive correlation between the variables which further supports the proposition that the weights and morphometry responded slowly to the crude protein level and that the protein level had influence on the between groups performance in both the morphometry and sperm reserve. It can therefore be suggested that the testicular, epididymal and *vas deferens* sperm reserves of the Toms improved significantly with increased level of protein in diet. This is so because although the same amount of feed was supplied, the protein content was varied leading to the variation observed. Although some authors that worked in different species reported results dissimilar from those of this work, Perry (1960) and Etches (1996) in cockerels and Jibril *et al.* (2011) in rams, other authors have however reported similar results like in the rabbit (Ladokun *et al*., 2006), bull (Rekwot *et al.*, 1988) and drakes (Ghonim *et al.,* 2010). These apparent differences and similarities are perhaps because optimal crude protein requirement differs between species or even between breeds of same species (Deviche *et al*., 2011). The reserves are similar to the result of Cecil *et al*. (1988) who reported a mean sperm reserve of 0.2-0.28 ($x10^9$cells/gram) 0.1-0.2 ($x\ 10^9$cells/gram) and 3.2 - 3.95 ($x\ 10^9$cells/gram) for testicular, epididymal and *vas deferens* sperm reserves respectively in exotic breed of turkey. The similarity between the results of this work and those of Cecil *et al*. (1988) suggest that with optimum environmental conditions such as adequate feed supply, the Nigerian indigenous turkey breed can parallel their exotic counterpart in reproductive capacity. Furthermore, in some of the cases the feeds containing 16 % and 20 % protein showed no significant differences between their results, but they both differ significantly from the results obtained from the 12% diet. This suggests that optimum protein level in the diet for reproductive health in the indigenous Toms is obtained at a point between 16% and 20%.

In conclusion, diet protein content influenced the development of the gonadal and extragonadal tissues and semen production of the Nigerian indigenous turkey toms at an optimum protein level of 16 % to 20 %, and this was indicated by positive changes in both the morphometry and sperm reserves of the gonadal and extra gonadal organs.

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