PHYSIOLOGY AND REPRODUCTION

Effects of season on the reproductive organ and plasma testosterone concentrations in guinea cocks (Numida meleagris)

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ABSTRACT The physiological basis of seasonal breeding in the guinea fowl (Numida meleagris) still remains unknown, despite the socioeconomic importance of these birds, particularly in Ghana. A study involving a total of 50 local guinea cocks was conducted, and documented gross anatomical and histological differences in the reproductive organs of breeding and non-breeding male guinea fowls. The study also compared peripheral testosterone concentrations in breeding and non-breeding cocks. Seasonal differences in variables measured were determined using two-tailed t-test/Mann-Whitney U-test. All comparisons were made at 5% level of significance. Breeding males had significantly (P = 0.000) higher anatomical biometric parameters than their non-breeding counterparts. Also, breeding birds had thicker (P = 0.000) phalli than their non-breeding counterparts. Histologically, regressing testis was characterized by the presence of sloughed off cells and increased debris in the tubular lumen and within the excurrent duct system, collapsed tubules and reduction in tubular lumen. Germ and Sertoli cell populations and nuclear diameters and actual seminiferous tubular diameter and length in regressing testes were significantly (P = 0.000) lower than in active testes. Leydig cell nuclear diameters and populations were also significantly (P = 0.000) reduced. Relative volume of seminiferous tubules in the testis, testicular sperm production/mg testis and per testis and peripheral testosterone concentrations were all higher (P < 0.05) in breeding than non-breeding testis. The ducts in the epididymal region also saw significant (P < 0.05) reductions in luminal diameters in non-breeding birds. Significant regression in anatomical and histological structures of the guinea cock reproductive tract occurred during the non-breeding season, and lower peripheral testosterone concentrations may be responsible for this phenomenon.

Key words: Guinea fowl, infertility, testis, testosterone, seasonal breeding

INTRODUCTION

Guinea fowls are seasonal breeders (Awotwi, 1987). In Europe, sexual activity of the male guinea fowl is limited to the spring months of April to July. In Africa, however, there are two seasons in areas with bimodal rainfall patterns and only one in those with monomodal rainfall patterns (Awotwi, 1987). In these so-called “opportunistic breeders,” the breeding season is not determined primarily by changes in day length, but by external, unpredictable factors (non-photic cues), such as food availability or rain (Gahr et al., 2005; Small et al., 2007). This has been amply demonstrated in guinea fowls (Numida meleagris) (Awotwi, 1987). Similarly, in small ground finches, only rainfall, but not photoperiod, light intensity during the day, or ambient temperature, correlated with the pattern in gonad development (Hau et al., 2004). The authors further demonstrated that three populations of small ground finches from habitats with different rainfall patterns (Santa Fe lowlands, New Mexico, the United States, and Santa Cruz lowlands and highlands, Bolivia) grew their gonads when the rainy season began at their respective locations. Using captive birds, Hau and associates (Cited by Hau, 2001) demonstrated that it was rainfall and not increase in insect (and later grass seed) abundance that sets a signal for the suitability of environmental conditions for breeding.

The testicular involution that takes place at the end of the reproductive season in seasonal breeders was
originally attributed to tissue necrosis brought about by the failure of androgens to maintain the integrity of the seminiferous tubules (Thurston and Korn, 2000). Presently, there is indication that seasonal testicular regression results from a precisely controlled process of programmed cell death (apoptosis) that is regulated by complex signaling mechanisms (Deviche et al., 2011). Despite the large seasonal variation in testis size observed in many bird species, few studies have examined apoptosis in the avian testes (Deviche et al., 2011). In the non-breeding season, the testes of male guinea fowls regressed in size and seminiferous tubules showed signs of degeneration with almost no spermatogenic activity (Awotwi, 1975). Similarly, Jenkins et al. (2007) reported 19-fold and 9-fold decrease in testis size and spermatogenic activity, respectively, in the American crow *Corvus brachyrhynchos* at the end of the breeding season. The decrease in size likely results primarily from Sertoli cell apoptosis (Rodriguez et al., 1997). In the Japanese quail *Coturnix coturnix japonica*, apoptosis associated with testicular regression was similarly limited to Sertoli and germ cells (Ubuka et al., 2006).

Two anterior pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), play a primary role in the control of testicular endocrine and exocrine functions. The importance of these hormones is underlined by the results from studies involving hypophysectomy, which causes testicular involution in the chicken *Gallus domesticus* (Brown et al., 1975; Tanaka and Fujioka, 1981) and Japanese quail *Coturnix coturnix japonica* (Brown and Follett, 1977), whereas administration of chicken *Gallus domesticus* purified LH, but especially chicken *Gallus domesticus* purified FSH, to hypophysectomized Japanese quail stimulates testicular development and growth (Brown et al., 1975).

Even though some effects of season on the reproductive organ of male guinea fowls have been documented, these were not detailed and involved a small data size (Awotwi, 1975). Also, the endocrine changes associated with these effects are not known. Furthermore, information on testosterone concentrations in these birds, irrespective of the season or physiological state is limited. The present study was, therefore, designed to assess the differences in anatomy and histological structure of the reproductive tracts of sexually mature guinea cocks (*Numida meleagris*) during the rainy (breeding season) and dry (non-breeding season) seasons, and associated endocrine changes.

**MATERIALS AND METHODS**

**Experimental Site**

The study was conducted at the Poultry Unit of the Department of Animal Science, University for Development Studies, Nyanpaka, Tamale (Ghana). Nyanpa lies on latitude 9°69’N and longitude 0°83’W. Temperatures are generally high with minimum and maximum values of 22 and 35°C recorded in March and December, respectively (SARI, 2008). Rainfall is monomodal with mean annual rainfall varying from 1,000 to 1,500 mm and peaks from August to September, with a relatively long dry season extending from November to April. The area lies in the Guinea Savannah zone, and has nearly equal amounts of light and darkness (12L: 12D) throughout the year. Guinea fowls (*Numida meleagris*) in this location generally breed during the rainy season (May to October) and are not fertile during the dry season (November to April) (Awotwi, 1987).

**Animals and Management**

A total of 50 local guinea cocks (*Numidae meleagris*) (comprising 25 each of breeding and non-breeding birds), of the pearl variety, were used for the study. Birds were brooded for 6 weeks (Teye and Gyawu, 2002), and then transferred to a deep litter house (floor spacing: 1.8 sq ft/bird; Lohmann LSL, Germany) until the end of the experiment. They were individually identified using tags placed through their inner wings to prevent detection by other birds and thus avoid pecking. Keets were brooded at 35°C from hatching until 3 wk of age (WOA), and then at 32°C until 6 WOA (Teye and Gyawu, 2002). Birds were then maintained at ambient temperatures of between 22°C and 35°C until the end of the experiment. Feed and water were supplied ad libitum. Day-old keets were fed ground maize in flat feeders followed by a starter ration from day 2 until 6 WOA. This was followed by a grower ration from 6 WOA until 21 WOA and then a layer feed until the end of the experiment. The starter (22% crude protein and 3,000 Kcal ME/kg diet), grower (14% crude protein and 2,800 Kcal ME/kg diet), and breeder (17.5% crude protein and 2,800 Kcal ME/kg diet) rations were obtained from a commercial feed supplier (Agricare Ghana Limited, Kumasi, Ghana).

Information on lighting requirements of the local guinea fowls from hatching are unavailable, and those used for chickens are usually employed. In this case, however, the “golden rule” to follow in designing lighting programs for pullets (Thiele, 2009) was followed. All birds received 24-h light from day-old until 1 WOA, and this was reduced to 16 h until birds were 3 WOA. These longer light periods during the first 3 wk of life were to ensure maximum feed consumption, enough to ensure maximum growth, initially. This was gradually reduced to a minimum of 12 h by 7 WOA, marking the phase of constant light (Thiele, 2009). Thereafter, birds were maintained under natural photoperiods (12L: 12D) until the end of the study.

**Experimental Procedure**

Breeding cocks were sampled in July and August (rainy season), while their non-breeding counterparts were sampled in January and February (dry season). All procedures used followed approved guidelines for ethical treatment of experimental animals.
Gross Anatomical Differences in Reproductive Tracts of Breeding and Non-Breeding Males

Twenty 32-week-old birds (10 per season) were weighed and sacrificed by cervical dislocation. Their testes and reproductive tracts were completely freed from the adjoining ligaments and fascia. Measurements were then taken and organs fixed in 10% formalin overnight for histology.

Testicular length, width and height were measured using calipers and recorded to the nearest 0.1 mm. Testicular volume was estimated using the formula for a prolated spheroid as follows: \( V = \frac{4}{3}\pi(\frac{1}{2}L)(\frac{1}{2}W)^2 \) (Ramirez-Bautista and Gutierrez-Mayen, 2003) and recorded to the nearest 0.1 cm³. Testicular weights were taken with a Mettler electronic scale and recorded to the nearest 0.1 mg. Relative testes weight was also calculated as the ratio of testes to body weight. Length of ductus deferens was measured as the distance from the point of attachment of the ductus to the epididymal region until it entered the connective tissue of the internal part of the cloaca on either sides of the large intestine and ureters, using a ruler and recorded to the nearest 0.1 cm. Finally, qualitative changes including change in color of the testes were observed and recorded. Total reading for a parameter per testis was presented as average for the 2 testes (i.e., left testis reading + right testis reading/2). This applied to both anatomical and histological measurements.

Phallus length and thickness were measured in 50 birds (25 per season). This measurement was made easier if 2 people were available. The bird was tipped upside down with one hand and the tail pushed towards the head. Simultaneously, the first finger and thumb were placed on the opposite sides of the vent and slowly separated with a gentle but firm pressing motion, stretching and everting the cloaca to expose the phallus of the bird, if present. In taking phallic measurements, the pressing was done by the same investigator and in the same manner for all birds so that there were no variations in the degree of exposure of the phallus in different birds. Length of the phallus (longitudinal axis, from the outer rim of the ventral lip of the vent to the tip of the organ) and thickness (dorsventrally, at the thickest section of the organ, and this coincides with the middle of the organ) were taken using calipers.

Histological Preparation, Cell Identification, Stereological Analyses and Cell Counts

The histological techniques used in the present study have been described previously (de Reviers, 1971a; b), therefore only a brief description is given here. The testes (with capsule intact and epididymal region attached) were each divided into 2 halves. One half of each was fixed in 10% formalin, dehydrated in absolute ethanol and embedded in paraffin wax. They were sectioned (5 μm) using microtome (Leica RM2125RT), floated onto poly-L-lysine subbed slides (Polysine; VWR International Leuven, Germany), and stained in eosin and Mayer’s hematoxylin. Germinal cell counts were restricted to preleptotene primary spermatocyte, type I spermatocyte in prophase I and step I spermatids (Aire et al., 1980). Sertoli and Leydig cell nuclei were also counted. The Sertoli cells were identified on the basis of their nuclei following the descriptions given by Zlotnik (1947) and de Reviers (1971b), while Leydig cells were identified by their characteristic location as clusters in the interstitial region and by nuclear diameter. In all cases, the location, relative size, shape and nuclear morphology of germ and somatic cells helped in cell identification. Nuclear diameters of testicular germ and somatic cells were obtained with previously calibrated calipers (this was calibrated using graticule under immersion oil) under immersion oil, using sections from 5 males and counting 20 nuclei/cell type/male. Cell counts/transverse section were determined from 10 sections of individual seminiferous tubules/slide and 10 interstitial areas (surface area determined)/slide for Leydig cells. Germ cell counts were determined for all testes involved. The numbers of fragmented nuclei were relatively high, and partially sectioned nuclei were counted as seen, if their cell type were clearly recognizable. To compensate for possible overestimation of cell numbers under such conditions, initial cell counts were corrected using Abercrombie’s (1946) correction factor as follows: \( N_c = N \times e \div (e + d) \), where:

\[ N_c = \text{The corrected number of cells in the preparation} \]
\[ N = \text{The number of nuclei counted/tubular section} \]
\[ e = \text{The thickness of the histological preparation} \]
\[ d = \text{the diameter of the nucleus of a given cell type} \]

This correction determines the number of cells with nuclei effectively present in the preparation.

Total number of cell (\( N_t \)): Total cell numbers for germ and Sertoli cells per testis were determined using the formula \( N_t = L_t \times N_c \div e \).

Where \( L_t = \text{Length of seminiferous tubules (estimated below)} \), and \( e \) and \( N_c \) defined as in the above. Total Leydig cell numbers were determined in relation to the interstitial area occupied by the cells, and expressed as number of cells per 1000 \( \mu m^2 \) of interstitial area.

Dimensions of Seminiferous tubule (ST)

Total length of seminiferous tube (\( L_t \)) was estimated based on the formula

\[ L_t = V_r \times (100 - C) \times 10^{-1} \div S \]
where: \( Vr = \text{percentage of testicular tissue occupied by the ST as measured by a modification of the Chalkley’s (1943) technique (Attal and Courot, 1963; de Reviers, 1971a)} \). This was determined by taking a picture of an entire cross section of each testis under the light microscope at \( 4 \times \) magnification. Each cross section therefore yielded several pictures depending on the size of the cross section. Each picture was subsequently opened with previously calibrated ImageJ software (National Institutes of Health, Bethesda, MD), and grids \( 50 \mu m \) apart were superimposed on the entire image. With a pencil tool plug-in, the grids on each image were grouped into 25-point grids (as obtained with 25-point grid graticule) and each field labeled, in ascending order, until the entire cross-section was covered. Forty fields were then randomly chosen per cross-section and counted as in the Chalkley’s (1943) technique. Points that fell on the tubes (including the basement membrane) were considered as tubular while those that fell outside the tube were considered as non-tubular. This also represents the ratio of tubular to non-tubular tissue (Brillard, 1986). \( Vr \) is expressed as a percentage of testicular tissue occupied by seminiferous tubules. From this, therefore, \( Vr \) could be determined according to the formula:

\[
Vr = \frac{TW \times \%\text{tubes}}{p}
\]

where \( TW = \text{testis weight (g), p = specific gravity of the testis (in guinea fowls} p = 1.05 \text{g/cm}^3, \text{as in the male chicken} de Reviers, 1971a; \% \text{tubes} = \text{number of ST points within the eyepiece} \div \text{total number of points of the eyepiece} \times 100; C = \text{the histological contraction of the testes, given by (volume of fresh tissue} \div \text{volume of embedded tissue} \div \text{volume of fresh tissue}) \times 100 \text{(Attal and Courot, 1963); S = mean area of a transverse section of ST. For guinea fowls, C in both immature and mature birds was estimated as 33.4 ± 13.1 (Brillard, 1986). The ImageJ software (National Institutes of Health) was used to measure the surface area of the tubules directly instead of deriving it from the diameter. Tubules tended to elongate with age, and diameters may therefore not be accurate when measured directly. Nonetheless, in tubules with minimum and maximum diameter differences not exceeding 20% (Brillard, 1986), diameters and surface areas were measured in order to compare apparent diameters (diameters measured directly) to actual diameters (diameters derived from the surface area using the formula \( D = \sqrt{\text{surface area} \times 4 \div \pi} \)). LT was expressed in meters (m).

**Sertoli Efficiency and Quantitation of Spermatogenesis**

Other parameters estimated were ratio of round spermatids to Sertoli cells, Sertoli efficiency (total number of germ cells beyond the spermatogonia stage, supported by each Sertoli cell) and meiotic index. Meiotic index, which measures the rate of spermatogenesis, was expressed as a theoretical ratio based on the mean ratio for 5 males, and was calculated as follows: given that each type I spermatocyte should provide 4 round spermatids during meiosis (\( MI = 4 \)), and that ultimately, the actual ratio of type I spermatocytes to round spermatids is dependent on the life span of each cell type, \( \%MI \) is therefore given as \( 100 \times \text{number of round spermatids} \div \text{life span of round spermatids} \div 4 \times \text{number of type I spermatocyte} \div \text{life span of type I spermatocyte} \text{(Noirault et al., 2006). The life spans of primary spermatocyte and round spermatid in the guinea fowl (Numida meleagris) are 4.5 and 2.5 days, respectively, as obtained from BrdU observations and reported by Hein et al. (2011).}

**Diameters of the Various Excurrent Ducts**

Using the ImageJ software (National Institutes of Health), the surface areas of the various excurrent ducts, namely: proximal ductuli efferentes (vasa efferentia; ductus efferentes directly draining the rete testes), distal ductus efferentes (ductus efferentes further away from the rete testes), epididymal ducts (connecting ducts/excretory canals) and ductus epididymidis (epididymal canal) were determined. These surface areas were converted to diameters using the formula \( D = \sqrt{\text{surface area} \times 4 \div \pi} \).

**Seasonal Variation in Testicular Sperm Production**

A fragment of testis (of volumes ranging between 28.3 to 265 mm\(^3\)) from each testis was weighed (fwt), homogenized in 0.25 M sucrose (1:200; testes:sucrose), and elongated spermatids (el) and testicular spermatozoa (tsp) were counted using haemocytometer (10 replicates per testes). Results for each male were estimated as follows:

\[
\text{TSP/male} = \text{right TSP} + \text{left TSP} = \frac{(\text{el} + \text{tsp})}{\text{fwt}} \times \text{testicular weight}
\]

(Noirault et al., 2006).

**Testosterone Assay**

Two milliliters of blood was collected into EDTA vacutainer tubes from the wing vein, and spun at 5000 rpm for 3 min at room temperature (18 to 25°C). Plasma was then pipetted into a 1.5-mL microcentrifuge tube and stored at −20°C until subsequently analyzed for testosterone. In all 20 birds were bled, 10 per season.

Before the main assay, parallelism was established by extracting serially diluted pooled guinea fowl plasma samples and serial concentrations of pure testosterone and comparing the slopes of the plasma samples with that of the standard curve. No difference was observed
between the slope of serially diluted pooled guinea fowl plasma sample and that of serial concentrations of pure testosterone samples (standards).

The testosterone assay was a RIA using tritiated tracer (Amersham International, Buckinghamshire, UK) and a procedure as originally described by Sheffield and O'Shaughnessy (1989). The testosterone antibody was obtained from Guildhay Antisera, Surrey, UK. The detection limit was 0.06 ng/mL, and intra-assay coefficient of variation was 9.5%. Cross reactivity with androstenedione and androstanediol were 0.3 and 3.9%, respectively. The assays were performed after sample extraction using diethyl ether in duplicate of 50 μL aliquots. Peripheral testosterone concentrations in all the samples assayed were determined using the standard curve generated by the AssayZap® software (Premier Biosoft, Palo Alto, CA). All samples were evaluated for testosterone in one assay.

### Statistical Analysis

Data were analyzed using the SPSS software, version 20 (IBM, 2011). Seasonal differences in testosterone profiles, gross anatomical and histological variables were determined using two-tailed t-test. Where variances were unequal, this test was replaced with the Mann-Whitney U test. Data were presented either as mean ± standard error of mean or median (interquartile range). All comparisons were done at 5% level of significance.

### RESULTS

Seasonal differences in gross anatomy of the reproductive tract in male guinea fowls are shown in Table 1. Breeding males had significantly (P = 0.000) higher testicular weight, length, height, width and volume than non-breeding males. Similarly, gonadosomatic index in breeding males was higher (P = 0.000) than in their non-breeding counterparts. Length of the ductus deferens, on the other hand, remained unchanged between the 2 seasons. There was no difference (P > 0.05) in phallus length between breeding and non-breeding male guinea fowls (Numida meleagris). However, breeding males had thicker (P = 0.000) phalli than their non-breeding counterparts. Breeding and non-breeding males had similar (P > 0.05) bodyweights at 32 WOA (Table 1).

Generally, some qualitative histological changes were noticed in the seminiferous epithelium of male guinea fowls (Numida meleagris) during the non-breeding season. One major change was the presence of sloughed-off cells in their seminiferous tubular lumen. As a result, most cells were not located within appropriate cellular associations. There were instances when Sertoli cells were seen in the luminal zone. Some of the sloughed-off cells reached the excurrent duct system as well. Generally, fewer germ cells were found in the seminiferous tubules of such birds, and germ cells beyond the primary spermatocyte stage were mostly affected. There was also increased debris in the tubular lumen and in some birds, the lumen appeared to have reduced significantly. Most of the tubules were also collapsed, and a tubule may run along the entire length of a testis. Generally, fewer or no spermatozoa at all were spotted in the luminal zone. Some of the sloughed-off cells reached the excurrent duct system as well. Generally, fewer or no spermatozoa at all were spotted in the seminiferous tubules and lumen of the excurrent ducts system of the non-breeding birds.

Table 2 indicates the histological differences between breeding and non-breeding guinea cocks (Numida meleagris). The number of primary spermatocytes, round spermatids, Sertoli cells, Leydig cells, total germ cells, actual seminiferous tubular diameter and length were significantly (P = 0.000) higher in breeding than non-breeding birds. Similarly, testicular sperm production per mg of testis, testicular sperm production per testis and relative volume of seminiferous tubules were higher (P < 0.05) in breeding than non-breeding testes. No differences (P > 0.05) were found between the 2 groups in apparent tubular diameter, Sertoli efficiency, number of round spermatids per Sertoli cell and meiotic index.

Variations also occurred in germ and somatic cells nuclear diameters between breeding and non-breeding birds. The nuclear diameters of pre-leptotene primary

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### Table 1. Mean testicular anatomical and body weight differences between breeding and non-breeding local guinea fowls.

<table>
<thead>
<tr>
<th>Testicular biometric variables (mm)</th>
<th>Season</th>
<th>Breeding</th>
<th>Non-breeding</th>
<th>P-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular weight (mg)</td>
<td>875.0 (695.0–955.0)</td>
<td>351.0 (238.0–416.0)</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
<tr>
<td>Testicular length</td>
<td>18.2 ± 0.5</td>
<td>13.2 ± 0.4</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Testicular height</td>
<td>7.6 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Testicular width</td>
<td>9.8 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Testicular volume (cm³)</td>
<td>1.0 (0.8–1.1)</td>
<td>0.4 (0.3–0.4)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Length of ductus deferens</td>
<td>11.3 ± 0.2</td>
<td>11.8 ± 0.5</td>
<td>0.238</td>
<td></td>
</tr>
<tr>
<td>Phallus length</td>
<td>6.2 ± 0.3</td>
<td>6.6 ± 0.3</td>
<td>0.350</td>
<td></td>
</tr>
<tr>
<td>Phallus thickness</td>
<td>6.5 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>1193.2 ± 27.8</td>
<td>1190.4 ± 24.3</td>
<td>0.940</td>
<td></td>
</tr>
</tbody>
</table>

*Median (Interquartile range).
The results of the present study revealed that the testes of a non-breeding male guinea fowl (*Numida meleagris*) regressed significantly compared to its breeding counterpart. Testicular weight, length, height, width and volume were much higher in breeding males (Table 4). Similarly, breeding guinea cocks (*Numida meleagris*) had higher (*P* < 0.05) peripheral testosterone concentration than non-breeding cocks (Figure 1).

### Table 2. Histological differences between breeding and non-breeding testes in local guinea cocks.

<table>
<thead>
<tr>
<th>Morphometric characteristics {Median (Interquartile range)}</th>
<th>Breeding</th>
<th>Non-breeding</th>
<th><em>P</em>-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of round spermatids (×10⁸)</td>
<td>8.4 (6.3–12.0)</td>
<td>3.0 (2.0–4.0)</td>
<td>0.000</td>
</tr>
<tr>
<td>Number of type I spermatocytes (×10⁸)</td>
<td>8.2 (6.0–10.9)</td>
<td>2.6 (1.9–3.2)</td>
<td>0.000</td>
</tr>
<tr>
<td>Total germ cell population (×10⁹)</td>
<td>17.1 (12.6–23.0)</td>
<td>5.4 (4.0–7.4)</td>
<td>0.000</td>
</tr>
<tr>
<td>Sertoli cells population (×10⁹)</td>
<td>1.6 (1.3–2.0)</td>
<td>0.5 (0.4–0.7)</td>
<td>0.000</td>
</tr>
<tr>
<td>Round spermatids/Sertoli cell</td>
<td>5.8 ± 0.2</td>
<td>5.8 ± 0.3</td>
<td>0.872</td>
</tr>
<tr>
<td>Total germ cells/Sertoli cell</td>
<td>11.0 ± 0.3</td>
<td>10.7 ± 0.4</td>
<td>0.676</td>
</tr>
<tr>
<td>Meiotic index (%)</td>
<td>377.3 ± 4.8</td>
<td>392.3 ± 7.4</td>
<td>0.084</td>
</tr>
<tr>
<td>Number of Leydig cells/1000 μm²</td>
<td>11.9 (9.9–13.9)</td>
<td>5.4 (4.4–6.8)</td>
<td>0.000</td>
</tr>
<tr>
<td>Apparent diameter of seminiferous tubule (μm)</td>
<td>358.4 ± 6.7</td>
<td>495.1 ± 8.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Actual diameter of seminiferous tubule (μm)</td>
<td>96.7 ± 0.3</td>
<td>95.3 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Relative volume of seminiferous tubules in the testis (%)</td>
<td>23.1 (20.7–24.9)</td>
<td>10.7 (8.7–12.2)</td>
<td>0.000</td>
</tr>
<tr>
<td>Testicular sperm production/milligram testis (×10⁵)</td>
<td>1.4 (0.9–1.8)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>Testicular sperm production/testis (×10⁷)</td>
<td>9.9 (8.5–18.0)</td>
<td>1.2 (0.7–3.5)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Mean ± SEM.*

**DISCUSSION**

The results of the present study revealed that the testes of a non-breeding male guinea fowl (*Numida meleagris*) regressed significantly compared to its breeding counterpart. Testicular weight, length, height, width and volume were much higher in breeding males (Table 4). Similarly, breeding guinea cocks (*Numida meleagris*) had higher (*P* < 0.05) peripheral testosterone concentration than non-breeding cocks (Figure 1).
than non-breeding males. This was consistent with the observations made in an earlier study (Awotwi, 1975) in the same species. The higher anatomical biometric traits in breeding males may be due to higher testosterone synthesis in the breeding than non-breeding males as high gonadotropin and/or testosterone concentrations have been reported in birds with bigger testes during the breeding season (Brown and Follett, 1977; Malecki et al., 1998).

The lack of difference in phallus length between breeding and non-breeding males in the present study was not surprising because it was generally difficult getting an accurate measure of the length compared to the thickness. Even though steps were taken to ensure a more accurate reading by using the same researcher to take all measurements and pressing the cloaca in the same manner to expose the phallus for measurements, inaccurate readings were still possible since the degree of pressing could influence the degree of protrusion of the phallus for length measurements. Perhaps, phallus thickness is a better measure of phallus size in these birds. Lake and Furr (1971) reported of testosterone-mediated development of phallic structure in the cock’s cloaca. The authors noted that a large daily dose (3 mg) of testosterone propionate given to 1-day-old chicks caused a pronounced growth of the ductus deferens and phallic structures after one week. It was, therefore, not surprising that the guinea fowl (Numida meleagris) had thicker phallus during the breeding season when peripheral testosterone concentrations were higher (over two-fold) than in their non-breeding counterparts.

The decreased size of seminiferous tubules, increased amount of cellular debris in the tubular lumen, degenerating germ cells and collapse of seminiferous tubules observed in the present study have also been reported in other non-breeding birds, including Japanese quail (Coturnix coturnix japonica) (Victor and Wilbor, 1974) and gander (Anser anser domesticus) (Gumulka and Rozenboim, 2015). In the present study, with the exception of meiotic index and Sertoli efficiency, all other histological morphometric parameters were much higher in breeding than non-breeding guinea fowls (Numida meleagris). It was, however, surprising that Sertoli efficiency, which is an indication of the rate of spermatogenesis did not differ between the two seasons, even though Sertoli cells tended to support slightly more germ cells in breeding than non-breeding birds. It is possible that most of the samples used in non-breeding birds were not yet at the advanced stage of regression (Victor and Wilbor, 1974). The testosterone concentration found in breeding males in the present study was two-fold higher than that found in their non-breeding counterparts. This may be attributed to higher gonadotropin secretion in breeding birds leading to higher testosterone production, as reported in other breeding birds, e.g., domestic duck (Anas platyrhynchos domesticus) (Yang et al., 2005) and ostrich (Struthio camelus) (Degen et al., 1994).

Major cellular changes were also found in the seminiferous epithelium. Both somatic (Leydig and Sertoli cells) and germ cell nuclear diameters were much higher in breeding than non-breeding males. These findings may be indicative of Leydig cell inactivity and include those previously described for Leydig cells during normal testicular regression in winter (Rohss and Silverin, 1983; Tae et al., 2005). The presence of a lumen within the seminiferous tubule is an indicator of active secretion by Sertoli cells (Dym et al., 1977). Therefore, the reduction of the tubular lumen in regressed testes of the guinea fowl (Numida meleagris) was most likely due to cessation or marked reduction in the production of fluid by the Sertoli cells, which is significant in sperm transport.

The observed changes in Sertoli and Leydig cell nuclei diameters in non-breeding guinea fowls (Numida meleagris) may be a consequence of reduced gonadotropin secretion leading to reduced testosterone levels. Testosterone is required for supporting spermatogenesis (Kirby and Froman, 2000) and its secretion by the Leydig cell is controlled by LH. Lake and Furr (1971) hypothesised that FSH may possibly be required for the production of earlier stages of spermatocytes, while testosterone supports the transformation of secondary spermatocytes to spermatids in galliform birds. Deficient production of these hormones as evidenced in non-breeding animals may therefore be responsible for the loss of germ cells from primary spermatocyte and beyond in these birds. Similarly, the regression of the ducts within the excurrent duct system of the guinea fowl (Numida meleagris) may be attributed to lower testosterone levels recorded in non-breeding birds, since this hormone has been implicated in the maintenance of the excurrent duct system (Kirby and Froman, 2000). Similar to the observations of Ubuka et al. (2006) in the Japanese quail (Coturnix coturnix japonica), the reduction in Sertoli cell population in non-breeding birds is attributable to apoptotic cell deaths occurring during the non-breeding season, due to higher GnIH synthesis and release, and consequently, lower gonadotropin and testosterone release.

The testicular involution observed in the guinea fowl (Numida meleagris) during the non-breeding season is often referred to as a return to an “infantile state” (Reiter, 1980). These assumptions are supported by the significant loss of germ cells from primary spermatocyte and beyond from the seminiferous tubule and reduction of tubular lumen. Vitale and associates (1973), however, demonstrated that immature animals unlike their regressed adult counterparts, lacked a blood-testis barrier. Also, cells of regressed animals were constantly proliferating as evidenced by the meiotic index in the present study, in contrast to those of pre-pubertal animals, which show no commitment of germinal cells beyond the gonocyte phase of development.

Like the rufus-winged sparrows (Deviche and Small, 2005), the guinea fowl (Numida meleagris) (Awotwi,
1987) uses rainfall as a cue to initiate reproductive activities, and LH concentrations were higher 20 days into the rainy season than non-rainy season in the rufous-winged sparrows. Conversely, fewer GnIH perikarya were found in rainy than non-rainy season (Deviche et al., 2006). The injection of GnIH in these birds did not have any influence on plasma LH concentrations, hence it was concluded that GnIH might not play a role in the acute mechanism, but might play a role in the seasonal control of LH through inhibitory effects on the GnRH system (Deviche et al., 2006). In the quail, however, Ubuka et al. (2006) demonstrated that 2-week administration of GnIH lead to decreased LH synthesis and release, decreased peripheral testosterone concentrations and apoptotic cell deaths in germ and Sertoli cells. Rapid deactivation of the GnIH system, therefore, could provide a mechanism by which reproductive activities are reinitiated during the non-breeding season in an opportunistic breeder like the guinea fowl (Numida meleagris).

Significant regression in anatomical and histological structures of the guinea cock reproductive tract occurred during the non-breeding season. Testosterone concentrations in these birds are 2-fold lower during the non-breeding season, and may be responsible for regression of the reproductive tract and reduction in phallus thickness observed in guinea cocks during this time of the year.

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