Effect of season on testicular morphometry and sperm production in adult guinea cocks

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SUMMARY

A study was conducted to investigate the effect of season on testicular morphometric parameters and sperm production in adult Guinea cocks. Five (5) Guinea cocks (GCs) were randomly selected from a group of thirty homogenous adult pearl Guinea cocks during the major rainy, minor rainy season, and dry seasons of the year. The selected GCs were humanely euthanized and used for determining testicular morphometric parameters, sperm reserve, and sperm production. The results of this study showed that the body weight of Guinea cocks (GCs) and testicular morphometric parameters were significantly (P<0.05) higher in the rainy season than in the dry season, while testicular weight and the gonado-somatic index (GSI) were similar. Sperm reserves and daily sperm production were significantly (P<0.05) higher in the rainy season than in the dry season. The study concluded that the season of the year affected the body weight of the Guinea cocks, testicular morphometric parameters, sperm reserve, and sperm production in adult Guinea cocks.

Key words: Guinea cock – Sperm production – Testicular morphometry

INTRODUCTION

In their natural habitats, avian species exhibit some mating systems including monogamy**,** polygyny, polyandry, and promiscuity (Kempenaers, 2022). Approximately 92% of all birds are socially monogamous (Santos and Nakagawa, 2017).

Guinea fowls (GFs) in their natural habitats are seasonal breeders and monogamous in their sexual behavior (Konlan et al., 2011). The instinct toward monogamy appears to be maintained even under intensive production systems. Consequently, Guinea Fowls under intensive production systems are maintained with a recommended mating ratio of one male to four females (Annor et al., 2012) or one male to two or three females (Soara et al., 2020; Sodjedo et al., 2022). In livestock and poultry production, the selection of breeding males is based on the measurement of body weight and testicular morphometry, which are good indicators of sperm production (Agga et al., 2011). According to Adejoh-Ubani et al. (2022), breeder broiler cocks should be selected for natural mating or artificial insemination, only when they are having optimum semen production. This study investigated seasonal changes in testicular morphometry, sperm reserve, and sperm production in adult Guinea cocks.

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Submitted: March 19, 2024. **Accepted:** May 3, 2024

https://doi.org/10.52083/ILBS5521

MATERIALS AND METHODS

Ethical Approval

The experimental protocols for the present study were approved by the Animal Research and Ethics Committee of the College of Agriculture Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED), Mampong – Ashanti, Ghana.

Study Location

This study was conducted at the Poultry Unit of the Department of Animal Science Education, AAMUSTED. The study area lies in the transitional zone of Ghana and is located within longitudes 0º05′′W and 1º30′′W and latitudes 6º55′′N and 7º30′′N (Ghana Statistical Services,2014) at an altitude of about 457 m above sea level.

Management of Birds

A total of thirty (30) Guinea cocks (GCs) were randomly selected from an established foundation stock on the farm. The selected GCs were Sixty (60) weeks old at the start of the experiment, and were housed on wooden slates in cages measuring 300 m x 100 m x 120 m, with a maximum of six (6) birds per cage. The birds were provided with water *ad libitum* and natural daylight only. The experimental birds were fed a breeder ratio diet containing 16.5% crude protein and 2,800 kcal ME/kg (Annor et al., 2012; Yildirim, 2012).

Gross Testicular Morphometry

In July 2020, five (5) GCs were randomly selected and individually weighed using an electronic scale (Model 14192-233B, Constant Electronics, China), and humanely euthanized by severing the common carotid artery. After euthanization, the testes were dissected and freed from all connective tissue. The length (LT) and width (TWd) of each testis were measured using a digital vernier caliper and recorded to the nearest 0.1 mm. The volume (V) of the testis was estimated as described by Mohammed et al. (2018) as follows:

$V=0.5236\ LW^2$, where L represents the length and W is the width of the testes.

The right and left testes of each GC were weighed using an electronic precision balance (HRB series balance# HR12120049, China). The left testis was processed for the determination of sperm reserves, as described by (Wada et al., 2016), while the right testis was preserved in 10 % formalin for histological examination. These procedures were repeated in October and December 2020, respectively. The gonado-somatic index or relative testis weight was defined as the ratio of total testis weight to the body weight of GC expressed as a percentage (Kouatcho et al., 2015).

Histological Processing

The right testis (RT) from five (5) slaughtered GCs were preserved in 10 % formalin solution for twenty-four hours. Tissue samples were taken from the cranial, medial, and caudal regions of the testes, placed in labeled cassettes, and fixed in formalin for one week. The samples were processed for histological examination as described by de Reviers (Freneau et al., 2016). The samples were dehydrated through increasing concentrations of graded ethanol (70%, 80%, 95%, 100%), cleared in xylene, infiltrated with paraffin wax, and then embedded in molten paraffin wax. Paraffinized tissue was cut into 4-μm-thick sections using a Leica YD315 manual microtome (Jinhua YIDI Medical Appliances Co.Ltd, China) and floated on a tissue floater (YDA13, Jinhua YIDI Medical Appliances Co.Ltd, China), and then placed on clean glass slides. The slides were deparaffinized in xylene, rehydrated in graded ethanol, and rinsed in water. The deparaffinized slides were stained for five (5) minutes in Haematoxylin (Cat no: 00783, Mumbai, India), rinsed in water, and counterstained in Eosin for 30 seconds (Cas no:17372-87- 1, Siheug, South Korea), and mounted with DPX mountant. These procedures were repeated for samples collected in October and December 2020, respectively.

Histometric Testicular Parameters

The seminiferous tubular diameter, the height of the seminiferous epithelium, and the luminal diameter were evaluated from computer-generated images using an Amscope 14 MP digital camera (Model no FMA 050, China) with an associated image the analysis software program (MU1203-FL, China), attached to an Olympus microscope (Olympus BX 43, Tokyo, Japan). This was done in ten (10) randomly selected cross-sections of seminiferous tubules (ST), which were round or nearly round per Guinea cock. The diameter of the seminiferous tubules (STD) was measured across the minor and major axes and the mean diameter was obtained (Leão et al., 2017; Dharani et al., 2018). The height of the seminiferous epithelium was evaluated by the mean of two opposed measurements, taken from the basal membrane to the luminal border in cross sections of the ST (Andreussi et al., 2014; Akhtar et al., 2020). The luminal tubular diameter was obtained by the difference between the total diameter (STD) and the sum of both measurements of the epithelial height (Akhtar et al., 2020).

Testicular Biopsy Score

Spermatogenesis was determined by the semi-quantitative modified Johnsen testicular biopsy score (Gune et al., 2019). The modified Johnsen's score was estimated in ten (10) cross-sections of seminiferous tubules per animal and a mean value was obtained per season.

Sperm Production

Sperm production and sperm reserves were estimated from testicular homogenates. Testicular samples were taken from the left testis of five (5) euthanized GCs and homogenized as previously described with some modifications (Wada et al., 2016). Each testis was homogenized in 25 ml normal saline solution containing antibiotics (sodium penicillin G, 100 IU/mL and streptomycin sulphate, 1 mg/ml) for about one minute. The diluted testicular homogenate solutions were stored overnight in a refrigerator at 5˚C. The resulting solutions were filtered and used for the determination of the spermatozoa concentration in a modified Neubauer hemocytometer (Mohan et al., 2016). The gonadal sperm reserves were estimated by multiplying sperm concentration by the respective volumes of the testis. Daily sperm production was estimated by dividing the number of homogenization-resistant nuclei by 4.5, the average number of days that elongated spermatids remain in the testis before they enter into the excurrent ducts of the testis (Froman and Rhoads, 2012). The association between gross morphometric parameters and sperm production was evaluated using Pearson's coefficient of correlation.

Data Analysis

The results obtained were analyzed using one-way analysis of variance (ANOVA) with the help of the Graph Pad Prism Software (Version 8.00, GraphPad Software, San Diego, California, USA). The results were expressed as mean ± SEM, where SEM is the standard error of the mean, and the means differentiated using Tukey's multiple comparison test at P< 0.05.

RESULTS

Gross Testicular Morphometric Parameters

The present study was conducted during the major rainy, minor rainy, and dry seasons in 2020 to investigate the effect of seasons on testicular morphometric parameters in adult Guinea cocks. The prevailing weather conditions for 2020 in the study area are presented in Table 1. The rainy season started in March, peaked in May, and ceased in December. The lowest temperatures were recorded in August (minimum recorded = 20°C) and the highest in February (maximum recorded = 39°C).

The season of the year significantly (P< 0.05) influenced the body weight (BW) of GCs, length (LT), width (WdT), and volume (VT) of the testis (Table 2). These parameters were highest in the minor rainy season, followed by the major rainy season, and lowest in the dry season. The weight of the left testes (WLT), weight of the right testes (WRT), total testis weight (TTW), and the gonado-somatic index (GSI) were not significantly different (P<0.05) in the seasons.

Testicular Histometric Parameters

The diameter of the seminiferous tubules (STD), the height of epithelium of the seminiferous tubules (EH), and the diameter of the lumen of the seminiferous tubules (LD) were not significantly

Table 1. The effect of season on gross testicular morphometric parameters.

Parameters (Mean ± SE)	Major Rainy Season	Minor Rainy Season	Dry Season	P-value
BW/Kg	1.3a ±0.037	1.50b± 0.045	1.4a ± 0.024	0.011
WLT /g	0.79 ±0.038	0.88 ± 0.11	0.78 ± 0.0076	0.6403
WRT /g	0.56 ± 0.0793	0.65 ± 0.1586	0.54 ± 0.0566	0.7592
LLT /cm	1.90a ± 0.024	2.3b ± 0.04	2.0 a ± 0.20	0.0001
LRT /cm	1.70a ± 0.0400	2.1b ± 0.051	1.70a ± 0.055	0.0004
WdLT/cm	1.2a ± 0.051	1.40b ± 0.045	1.3a ± 0.024	0.0372
WdRT/cm	1.00a± 0.071	1.20ab ± 0.071	1.30b ± 0.032	0.0131
VLT /ml	1.5a ± 0.12	2.4b ± 0.17	1.6a ± 0.074	0.0008
VRT /ml	0.94a ± 0.14	1.6 b ± 0.21	1.5 b ± 0.081	0.0245
TTW/g	1.36 ± 0.0919	1.53± 0.2520	1.33 ± 0.1245	0.6667
GSI/ %	0.103 ± 0.0058	0.101 ± 0.0146	0.098 ± 0.0094	0.9418

Means with different superscripts across rows are significantly different at P< 0.05. BW- body weight, WLT- weight of left testis, WRT- weight of right testis, LLT- length of left testis, LRT- left of right testis, WdLT- width of left testis, WdRT- width of right testis, VLT- volume of left testis, VRT- volume of right testis, TTW- weight of the paired testis, GSI -gonadosomatic index.

Table 2. The effect of season on testicular histometric parameters.

Parameters (Mean ± SEM)	Major Rainy Season	Minor Rainy Season	Dry Season	P-value
Diameter of Sem T (µm)	666.08 ± 30.54	626.49 ± 45.40	547.11 ± 70.98	0.2521
Height of Epithelium of Sem T (µm)	182.04 ± 9.77	231.34 ± 28.32	190.18 ± 26.18	0.2276
Diameter of Lumen of Sem T (µm)	302 ± 48.92	163.75 ± 34.26	165.39 ± 25.07	0.0624
Johnsen's Testicular Biopsy Score	9.4a± 0.16	9.5a ± 0.16	4.9b ± 0.23	0.0000

Means with different superscripts across rows are significantly different at P< 0.05. Sem T- seminiferous tubule.

different (P> 0.05) during the seasons of the year (Table 2). The season of the year significantly (P<0.05) influenced Johnsen's testicular biopsy score.

Spermatozoa Production

The effect of the season on sperm reserves (SR) per testis, sperm reserves per gramme testis (SRG), daily sperm production (DSP) per testis, and daily sperm production per gramme testis (DSPG) are presented in Table 3. The season of the year significantly (P<0.05) affected SR, SRG, DSP, and DSPG. These parameters were significantly higher in the minor rainy season but similar in the dry and major rainy seasons.

The association between gross testicular morphometric parameters and sperm production was evaluated using Pearson's coefficient of correlation (Table 4). The results show that the weight of the left testis (WLT) moderately correlated (r=

Table 3. The effect of season on sperm reserves and sperm production (x 106) in adult guinea cocks.

Parameter	Major Rainy Season	Minor Rainy Season	Dry Season	P-Value
SR	580.0 a ± 47	1055b ± 117	616.0a ± 46	0.0018
SRG	747± 91a	1233 ± 137b	818 ± 95a	0.0180
DSP	128 ± 10a	231 ± 26b	130. ± 10a	0.0018
DSPG	165± 20 a	272 ± 30b	180 ± 21a	0.0180

Means with different superscripts across rows are significantly different at P< 0.05. SR- sperm reserves, SRG- sperm reserves per gram, DSP- daily sperm production, DSPG- daily sperm production per gram.

Table 4. Coefficient of correlation (r) between morphometric parameters and sperm production in adult guinea cocks.

Parameter	DSPG	DSP	SRG	SR	VT	WdT	LT	WT
WT	-0.28	0.41	-0.28	0.41	0.44	0.37	0.45	_-
LT	0.54	0.80	0.54	0.80*	0.86	0.65*	_	
WdT	0.51	0.72	0.51	0.72	0.94*	_-		
VT	0.60	0.85	0.60	0.85	_			
SR	0.76	1.00*	0.76*	_				
SRG	1.00*	0.76*	_-					
DSP	0.76*	_						
DSPG	_-							

Denotes significant difference. SR- sperm reserves, SRG- sperm reserves per gramme, DSP- daily sperm production, DSPG- daily sperm production per gramme, WT- weight of the testis, LT- length of testis, WdT- width of testis, VR- volume of testis.

0.37- 0.45, P<0.001) with its volume (VLT), length (LLT), and width (WdLT), while LLT, WdLT, and VLT were highly correlated (r=0.65-0.94, P<0.001). WLT correlated moderately (r=0.41, P<0.001) with SR and DSP but negatively and lowly (r=-0.28, P<0.001) correlated with SRG and DSPG respectively. On the other hand, LLT and VLT correlated moderately (r=0.54-0.60, P<0.001) with SRG and DSPG but correlated highly (r=0.80-0.85, P<0.001) with DSP and SR, respectively. Similarly, WdLT correlated moderately (r=0.51, P<0.001) with DSPG and SR and correlated highly (r= 0.72, P<0.001) with SR and DSP, respectively.

DISCUSSION

Gross Testicular Morphometric Parameters

The season of the year significantly influenced the body weight (BW) of Guinea cocks (GCs), length (TL), width (TWd), and volume of the testis, but did not affect the weight of the left and right testes, total testis weight (TTW) and gonado-somatic index (GSI). These findings agree with previous reports on Guinea cocks (Mohan et al., 2016; Qureshi et al., 2016; Ahmed et al., 2022). According to Abdul-Rahman et al. (2016), the testicular morphometric parameters of GCs in Northern Ghana were higher during the rainy season than during the dry season. Moreover, Dharani et al. (2018) stated that except for the mean body weight, the testicular morphometric parameters of GCs in the tropical savannah climatic areas in India were the highest during the summer and monsoon I seasons and the least during the monsoon II season. Similarly, the body weight and testicular morphometric parameters in the Japanese quail were higher in the summer and rainy seasons than in autumn and winter (Shil et al., 2015).

The annual reproductive cycle in seasonal breeders, including Guinea fowls is classified into phases as follows: progressive/recrudescence, breeding, regression, and non-breeding (Qureshi et al., 2016; Simoes et al., 2012). The breeding period in avian species is affected by day length, temperature, food availability, and suitable breeding habitat (Cox et al., 2013; Silva et al., 2017). Studies conducted on domestic ducks in the tropics have demonstrated that changes in testicular morphology occur in tandem with the annual phases of reproduction (Simoes et al., 2012). The increase in gross testicular morphometric parameters during the breeding season was due to hypertrophy of the testicular parenchyma and increased spermatogenic activity. The breeding season usually coincides with increasing day length, increased precipitation, lower ambient temperatures, and food availability. The birds responded to increasing daylight via stimulation of the hypothalamus-pituitary -gonadal axis and the resulting secretion of hormones such as GnRH1, FSH, and LH. These hormones regulate the functioning of the reproductive system via a positive or negative feedback mechanism. The dry seasons are unfavorable for avian reproduction due to the existing hot humid conditions, which may lead to heat stress. Similarly, very low or freezing temperatures are detrimental to the growth and proper functioning of the reproductive system.

Histo-morphometric Parameters

The results of the present study show that the histo-morphometric parameters were similar during the different seasons of the year. These findings are contrary to reports on Guinea fowls (Qureshi et al., 2016; Dharani et al., 2018; Ahmed et al., 2022), Muscovy ducks (Gerzilov et al., 2016), and the Japanese quail (Shil et al., 2015). The explanation for this difference may be due to the prevailing weather conditions during the study period. There appeared to be no sharp changes in the weather conditions during the different seasons. The histological changes in the testicular tissue of birds are associated with the restoration of spermatogenesis at the beginning of the breeding season and testicular tissue regression during the non-breeding season (Leska et al., 2012).

The results demonstrate active spermatogenesis of the testes in the rainy season and testicular regression during the dry season. These findings are consistent with previous reports on Guinea fowls (Abdul-Rahman et al., 2017; Ahmed et al., 2022) and ducks (Gumulka and Rozenboim, 2015). Spermatogenic activity in birds is seasonal and depends on the levels of gonadotropins and testosterone. During the breeding season, the levels of these hormones are high, accounting for the proliferative growth of the testes. On the contrary, the levels of these hormones decline in the non-breeding season, resulting in a decline in testicular size due to the inhibition of cellular proliferation and apoptosis of germinal cells (Thurston and Korn, 2000). In seasonal breeders such as wild birds, spermatogenesis is arrested when the concentration of testosterone falls below the basal levels (Santiago-Moreno et al., 2015). However, in chickens and turkeys, low levels of these hormones lead to the production of ejaculates with low sperm concentrations and morphologically abnormal spermatozoa (Santiago-Moreno et al., 2009).

Spermatozoa Production

The season of the year significantly affected sperm reserves and sperm production. These values were the highest in the minor rainy season and the lowest in the dry season. These findings agree with the report by Abdul Rahman et al. (2016), that testicular sperm production per testis and testicular sperm production per gramme testis were significantly higher in breeding than in non-breeding cocks. This is because the breeding season was associated with hypertrophy of testicular parenchyma and active spermatogenesis, while during the non-breeding season, atrophy of the testicular parenchyma and incomplete spermatogenesis occurred (Dharani et al., 2017). The breeding season of Guinea fowls commences with the onset of the rainy and is terminated during the dry season.

The study reported that there was a positive correlation ($r=0.54-0.85$, $P< 0.05$) between testicular morphometric parameters and sperm reserves and sperm production. This finding is consistent with reports in broiler breeder cocks (Uchechukwu et al., 2015) and native Nigerian breeder cocks (Orlu and Egbunike, 2010). A similar observation was reported in turkey toms (Yahaya et al., 2017) and Guinea fowls (Abdul-Rahman et al., 2017).

The gross testicular morphometric parameters, sperm reserves, and sperm production were affected by the season of the year. There was a high positive correlation between, gross testicular morphometric parameters, sperm reserves, and sperm production.

ACKNOWLEDGEMENTS

We wish to acknowledge the laboratory support provided by the staff of the Regional Veterinary Laboratory, Kumasi, and the Histo-Pathology Laboratory, School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology.

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