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RESEARCH ARTICLE

Seasonal Variations in Histomorphology of Testes and Bursa, Immune Parameters and Serum Testosterone Concentration in Male Guinea Fowl (*Numida meleagris*)

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A B S T R A C T

The objective of this study was to investigate the effect of photoperiod on histomorphological changes in testes and bursa, immune function parameters like total leukocyte count and lymphocyte count and serum testosterone level of guinea fowl (*Numida meleagris*) during different breeding seasons. Bursa and testes were collected from 10 mature male guinea fowls aged 6-8 months during full breeding (summer), low breeding (autumn) and non-breeding (winter) seasons (n=10). Tissues were stained with H&E and Image J® software was used for histometric analysis. Statistical analysis revealed that breeding seasons affected significantly (P<0.01) all morphological parameters of testes and bursa. The parameters of testes were found significantly higher during full breeding season which decreased significantly during non-breeding season through low breeding season. A similar trend was shown by diameter of testicular seminiferous tubules. However, the parameters of bursa showed highly significant (P<0.01) value during non-breeding season but this value declined non-significantly (P>0.05) during low breeding and full breeding seasons. Immune parameters exhibited a significantly higher value during non-breeding season with a significantly declining trend during low breeding and full breeding seasons. Serum testosterone levels were also significantly (P<0.01) different during all breeding seasons. Testosterone showed a negative correlation (r =-0.946) with immune function parameters. It is conceivable that increased steroid hormone synthesis enhances testicular activity during full breeding season but depresses the immune function. This trend however, reversed in non-breeding season, which assists the survival in the hard climatic conditions.

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INTRODUCTION

Rural poultry production plays important role in human nutrition, gross domestic production, employment and income generation in developing countries (Conan *et al.*, 2012; Mashkoor *et al*., 2013; Javeed *et al*., 2013). Guinea fowls are domesticated more or less over the entire world because of being an important part of rural poultry (Boko *et al.*, 2011). They are suitable, particularly, to tropical regions because they can easily survive under poor conditions of management than the commercial chickens (Moreki and Seabo, 2012). Guinea fowl is a long day breeder and their reproductive physiology besides the immune status fluctuates seasonally. In birds, neuroendocrine, immune and reproductive systems are interrelated and a functional correlation exists between their lymphoid organs and gonads (Kharwar and Haldar, 2011; Akbar *et al.*, 2012).

Cloacal bursa is a central lymphoid organ in birds responsible for generation, differentiation and maturation of B lymphocytes. These lymphocytes not only maintain the normal immunological functions in birds but also enhance resistance against different diseases (Song *et al.*, 2012). Total leukocyte and lymphocyte counts are the important measures to judge the immune status of an individual (Singh and Haldar, 2005). This status is modulated by many environmental factors like annual changes in light duration, temperature fluctuations, alteration in relative humidity etc. Among all factors light duration and temperature play important role in modulation of lymphatic organs like thymus, spleen and cloacal bursa in terms of total leukocyte and lymphocyte count in birds and mammals (Bilbo *et al.,* 2003; McGuire *et al*., 2011).

Gonadal hormone (testosterone) also influences the immune status or function although it does not stamp down all components of immunity. In mammals, it can impair B-lymphocyte development and differentiation in bone marrow in response to antigen while in birds it can decrease antibody production (Sakiani *et al.,* 2012; Gettler *et al.,* 2014). In addition, ecological factors may significantly modulate both testosterone production and its influences on immune responses (Klein, 2000).

Several researchers have positively correlated the daily changes in general immunological responses of birds with melatonin but negatively with serum testosterone (Singh and Haldar, 2007; Yadav and Haldar, 2013) which in the winter season enhances the immune status (Majewski *et al.,* 2012) and depresses the gonadal activities (Reiter *et al.,* 2009; Dardente, 2011). But no such variations have been studied previously in guinea fowl. So, the present study was carried out to determine the variations in histomorphology of testes and bursa, immune function tests (total lymphocyte count and leukocyte count) and serum testosterone concentration during different breeding seasons in male guinea fowl.

MATERIALS AND METHODS

Experimental design: A total of thirty clinically healthy mature male guinea fowls of 6-8 months age, having average body mass of 1 kg, were collected directly from backyard poultry houses in Faisalabad. The birds were acclimatized by keeping at open poultry shed for one week at the start of each breeding season before slaughter. The birds were subjected to full natural conditions of environment such as sunlight, temperature, humidity and rainfall and offered feed and clean water ad libitum during each specified period.

Climatological data of the study area during each season was taken from the Climatology Laboratory, University of Agriculture, Faisalabad, Pakistan.

Parameters studied: Ten birds in each season were slaughtered and blood was collected in two different test tubes. The test tube containing anticoagulant (EDTA) was used to perform total leukocyte count (TLC) and lymphocyte count (LC). The other test tube without anticoagulant was used for estimation of serum testosterone concentration by radioimmunoassay (RIA) using a commercially available test kit. All serum samples were analyzed for concentration of testosterone by validated radioimmunoassay (RIA). The sensitivity of the testosterone assay was 0.1/ml (defined as the lowest). The inter- and intraassay CVs are 9 and 13.5% for reference.

Samples of testes and bursa were collected. Morphological parameters such as length, width, thickness and circumference of each sample were measured in centimeter with the help of Vernier's caliper. Volume of each testis was determined by the water displacement technique. The specimens were cut, washed and preserved. Slides were prepared by the paraffin tissue preparation technique. Photomicrographs of each testis and bursa were captured using Nikon Optiphot 2 microscope at 200X. These images of testes were used to determine the diameter of seminiferous tubules with the help of automated image analysis system Image J® version 1.43n (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Statistical analysis: One way analysis of variance (ANOVA) was used to compare the means of parameters while least significance difference (LSD) test helped to compare the group means at 5% level of significance.

RESULTS

Seasonal morphological changes in testis and bursa: The mean values of all morphological parameters of testes during each breeding season are given in Table 1. Statistical analysis of all parameters of testes showed significantly ($P<0.01$) different values in all seasons. The highest value was found in full breeding (summer) season which then decreased significantly during low breeding (autumn) and non-breeding (winter) seasons. Moreover, there was found statistically non-significant ($P>0.05$) difference between right and left testis (Fig. 1A).

The mean values of all morphological parameters of bursa during each breeding season are given in the Table 1. Contrary to the testes, statistical analysis showed significantly higher values of all parameters of bursa during non-breeding (winter) season which declined significantly in low breeding (autumn) season but non-significantly in full breeding (summer) season (Fig. 1B).

Seasonal histometric changes in testis and bursa: Statistical findings of histometrical analysis of right and left testis seminiferous tubules (ST) presented a significantly ($P<0.01$) rapid rise in the diameter during full breeding (summer) season when compared with low breeding (autumn) and non-breeding (winter) seasons (Table 2). In addition, there was found a non-significant difference in ST diameter during all breeding seasons when right and left testis were compared as shown in Fig. 2: A, B and C.

The findings of histological structures of bursa indicated the highest number of follicles with high density of lymphocytes during non-breeding (winter) season and follicles along with lymphocyte count were regressed gradually during low breeding (autumn) and full breeding (summer) seasons as shown in Fig. 3: A, B and C.

Immune and hormonal parameters: The TLC and LC showed statistically significantly ($P<0.01$) higher values during non-breeding (winter) season which declined significantly ($P<0.01$) from low breeding (summer) to full breeding (winter) seasons as described in Table 2. The result impression of serum testosterone analysis exhibited statistically significantly ($P<0.01$) higher value in full breeding (summer) season in contrast to low breeding (autumn) and non-breeding (summer) seasons as described in Table 2. Moreover, serum testosterone was negatively correlated ($r=-0.946$) with the immune function parameters.

Table 1: Morphological parameters of testes and bursa of guinea fowl (*Numida meleagris*) during different breeding seasons

	Seasons					
	Full breeding		Low breeding		Non-breeding	
	Testis	Bursa	Testis	Bursa	Testis	Bursa
Weight (g)	1.60±0.164^{a}	0.150±0.042^{a}	0.74±0.043^{b}	0.271±0.059^{a}	0.14±0.023^{c}	0.699±0.043^{b}
Length (cm)	2.24±0.132^{a}	0.989±0.082^{a}	1.61±0.103^{b}	1.347±0.168^{a}	1.02±0 .058^{c}	2.240±0.113^{b}
Width (cm)	1.85±0.164^{a}	0.338±0.066^{a}	1.08±0.068^{b}	0.557±0.082^{ab}	0.62±0.030^{c}	0.762±0.141^{b}
Thickness (cm)	1.20±0.072^{a}	0.221±0.032^{a}	0.82±0.030^{b}	0.346±0.049^{a}	0.50±0.037^{c}	0.581±0.110^{b}
Circumference (cm)	4.12±0.207^{a}	1.111±0.084^{a}	3.13±0.190^{b}	1.711±0.182^{b}	1.67±0.095^{c}	2.434±0.266^{c}
Volume (cm^3)	2.01±0.211^{a}		0.91±0.075^{b}		0.20±0.019^{c}	

Mean (±SEM) values bearing the same superscripts in a row do not differ significantly (P<0.05)

Table 2: Diameter (µm) of seminiferous tubules, immune parameters and hormone level of guinea fowl in different breeding seasons

Seasons	Diameter of seminiferous tubules (µm)		Total Leukocyte Count, TLC (k/µL)	Lymphocyte Count, LC (k/µL)	Serum Testosterone (ng/mL)
	Right testis	Left testis			
Full breeding (Summer)	381.07±30.41^{a}	401.11±21.76^{a}	30.030±1.203^{a}	23.045±0.936^{a}	5.37±0.072^{a}
Low breeding (Autumn)	220.31±16.03^{b}	252.78±4.70 ^{b}	37.610±2.012^{b}	30.849±1.611^{b}	2.15±0.098^{b}
Non-breeding (Winter)	160.53±11.63^{c}	161.21±13.30^{c}	50.020±1.354^{c}	42.884±1.144^{c}	0.62±0.869^{c}

Mean (±SEM) values bearing the different superscripts in a column differ significantly (P>0.01)

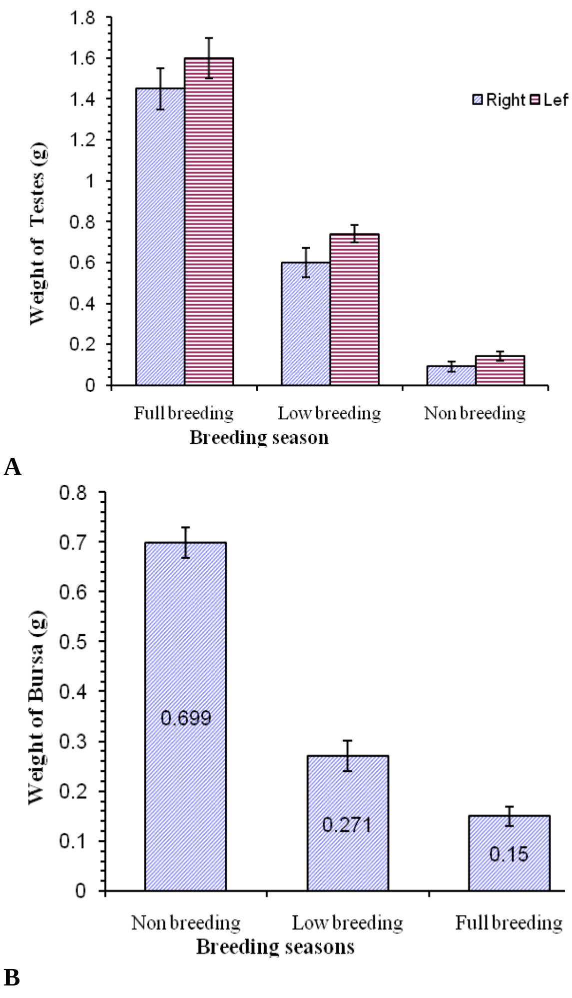

Fig. 1: Effect of breeding seasons on (A) weight of testes and (B) weight of Bursa in full breeding, low breeding and non-breeding seasons of guinea fowl (*Numida meleagris*).

DISCUSSION

Guinea fowl has a significant role in local poultry raised under extensive production systems. It permits farmers to bring in cash mainly for eggs and sometimes meat. Its meat has higher protein contents of approximately 28 % compared to 20 % of domestic fowl (Moreki and Seabo, 2012). Guinea fowl production represents therefore a commercial opportunity for rural and peri-urban farmers.

In this study *N. meleagris* males showed significant rise (P<0.01) in all morphological parameters of testis as well as histological diameter of seminiferous tubules during full breeding (summer) season in contrast to low breeding (autumn) and non-breeding (winter) seasons which are in accordance with the previous reports in guinea fowl (Hien *et al.*, 2011), in domestic pigeon (Madhu and Manna, 2009) in jungle crow (Islam *et al.*, 2010), in European swan (Breucker, 1982), in tropical species (Wikelski *et al.*, 2000) and in domestic quail (Atroni *et al.*, 1997; Haldar and Singh, 2001; Akbar *et al.*, 2012;). The increased diameter of seminiferous tubules contributed to increased values of morphological parameters. Moreover, the left testis was seen non-significantly (P>0.05) heavier than the right in 80% of the birds (Tyler and Gous, 2008). The basis for testicular asymmetry remains unknown but may be due to an unequal number of primordial germ cells incorporated into the embryonic gonads.

The effect of breeding seasons on histomorphological characteristics of bursa was dissimilar to the gonads. Contrary to testes results, morphological parameters like weight, length, width, thickness and circumference of bursa showed significantly (P<0.01) higher values during non-breeding (winter) season which decreased significantly during low breeding season but non-significantly (P>0.05) during full breeding season. Results of histological features of bursa reflected very abundant regressed bursal follicles during full breeding (summer) season suggesting an antiparallel relationship of immune system with the breeding season. The highest number of bursal follicles with high density of lymphocytes observed in bursa during non-breeding (winter) season describing the maintenance of immune system which keeps the birds healthy even in the harsh cold weather (Folstad and Karter, 1992) as explained in Indian jungle bush quail, *Perdicula asiatica* (Haldar and Singh, 2001), European starlings (Bentley *et al.*, 2000) and in Japanese quail, *Coturnix japonica* (Mase and Oishi, 1991; Akbar *et al.*, 2012).

The findings of serum testosterone analysis exposed a parallel relationship between testosterone level and breeding season. The maximum concentration of testosterone was found during full breeding (summer)

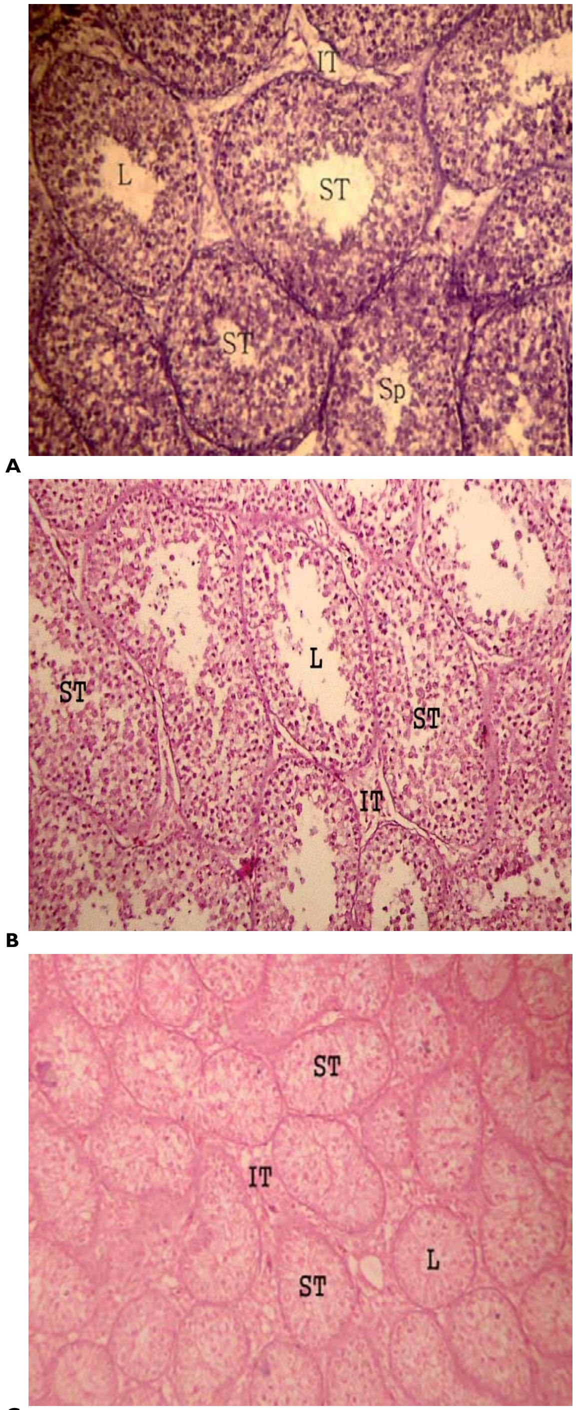

Fig. 2: Photomicrographs of testis of guinea fowl (*Numida meleagris*) (A) in full breeding season showing larger diameters of seminiferous tubules (ST) and inter tubular tissue (IT) along with some blood vessels; (B) in low breeding season showing medium diameters of (ST) along (IT) and seminiferous tubules lumen (L) and (C) in non-breeding season showing lower diameters of (ST) and (IT). H&E; X200.

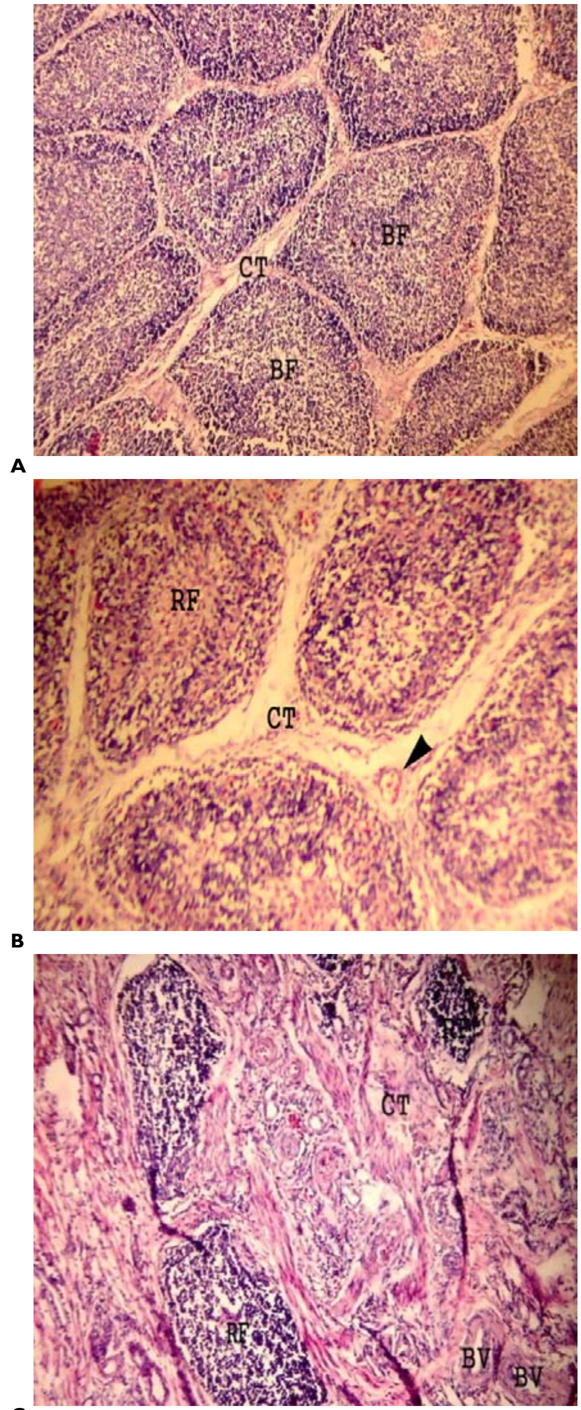

Fig. 3: Photomicrographs of bursa of guinea fowl (*Numida meleagris*) (A) in full breeding season showing regressing follicles (RF) fill with connective tissue (CT) along some blood vessels (BV); (B) in low breeding season showing high density of (RF) along (CT) and (C) in non-breeding season showing high density of bursal follicles (BF) have lymphocytes. H&E; X200.

season which declined significantly (P<0.01) to bottom line from low breeding (autumn) to non-breeding (winter) seasons in this male bird. Similar results have been shown by Haldar and Singh (2001) and Akbar *et al.* (2012) in other bird species.

In the present study, data on total leukocyte count (TLC) and lymphocyte count (LC) showed a significantly higher (P<0.01) value during the short, winter like days (non-breeding season) compared to the autumn (low breeding season) and summer (full breeding season). Evidences of short-day increase in TLC and LC in Japanese quail, *Coturnix japonica* (Akbar *et al.*, 2012); in Indian jungle bush quail, *Perdicula asiatica* (Kharwar and Haldar, 2011; Yadav and Haldar, 2013) also support the present findings. Suppression in immune parameters (TLC and LC) may be due to high testosterone level in circulation (physiological mechanism is unclear as proposed by Singh and Haldar, 2005) which has an inhibitory influence on immune system as reported in adult barn swallows (Evans *et al.,* 2000).

Conclusion: The present findings revealed that photoperiod changed the histomorphology of the testes and bursa and influenced the immune function parameters (TLC and LC) as well as the hormonal profile (serum testosterone). Increased steroid hormone synthesis enhances breeding activity during full breeding season but depresses the immune function. This trend however, reversed in non-breeding season, which assists the survival of tropical birds in the hard climatic conditions. It can be concluded that variations in photoperiod help the seasonal reproducing birds to adapt their physiology accordingly.

Authors' contribution: ASQ, SR and AM designed the project, supervised the work and contributed in the preparation of manuscript. MZA and ZA performed laboratory sampling, statistical analysis and write up.

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