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Effect of Day Length and Seasonal Variation on Haematological, Biochemical and Hormonal Traits of Indigenous Guinea fowl *(Numida meleagris)* in Ghana

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ABSTRACT

This study was conducted to investigate the effect of day length and season on haematological characteristics, biochemical and hormonal profiles of laying Guinea fowls *(Numida meleagris)*. Four hens and 1 male were each subjected to 12 hours of light and 12 hours of darkness (12L: 12D), 14hours of light and 10 hours of darkness (14L: 10D), 16 hours of light and 8 hours of darkness (16L: 8D) and 18hours of light and 6 hours of darkness (18L: 6D). Each group was replicated three times and reared in three seasons (Dry-December-March, Major rains-April-July and Minor rains-August-November) in a 3×4 factorial experiment. Data were analyzed using General Linear Model procedure of SAS. Significantly ($p<0.05$) higher Packed cell volume (PCV), lymphocytes and eosinophil, total serum protein and prolactin values were observed in 16L: 8D as 14L: 10D resulted in highest ($p<0.05$) neutrophil and albumin. PCV and Platelets were highest ($p<0.05$) in major rains whiles red blood cells and neutrophil were highest ($p<0.05$) in dry and minor rainy seasons, respectively. Total serum protein increased ($p<0.05$) from the dry season to minor rainy seasons. Oestrogen and luteinizing hormones were highest ($p<0.05$) in major rains and prolactin levels were higher ($p<0.05$) in minor rains and lowest in major rains. Interaction effect was not significant ($p>0.05$) in all parameters except prolactin. The findings of this study suggest that daylength of 14-16 hrs and major rainy season in Ghana supports adequate haematological, biochemical and hormonal profiles of Guinea fowls.

Keywords: Guinea fowl, Season, Day length, Haematology, Biochemistry, Hormonal

Guinea fowl (*Numida meleagris*) originated from Guinea coast of West Africa and has been domesticated worldwide including Ghana (Annor *et al*., 2013). In many countries of Western Africa, where the bird is ubiquitously distributed, Guinea fowls are the second most important source of meat and eggs after chicken (Bernacki *et al.,* 2013). Currently, there are different varieties of Guinea fowls which include white, black, lavender and pearl. Guinea fowl production is one of the most important and probably most profitable forms of poultry production in the three Northern regions of Ghana (Issaka and Yeboah, 2016). The importance of Guinea fowl production in the national economy of developing countries and its role in improving the nutritional status and income of many small communities has been very significant. In Ghana, it contributes about 7.1% of total poultry production (FAO, 2014). Nutritionally, it has high protein content (28%), tasty meat and eggs, high meat to bone ratio, low in calories, rich in vitamins, minerals and essential fatty acids (Annor *et al*., 2013).

Challenges to commercialization of Guinea fowl have been studied (Gono *et al.,* 2013) and these have included challenges in breeding, inadequate feeding and health management, poor housing and environmental influences (Annor *et al.,* 2013). Environmental factors such as temperature, rainfall, humidity and photoperiod have had significant impact on the haematological, blood chemistry and hormonal profiles in as much as these indices reflect the physiological responsiveness of the animal to its internal and external environment and also related to blood forming organs which influence production and reproduction traits of poultry (Bamishaiye *et al.*, 2009). However, very few data have been found through literature with respect to these values for Guinea fowls. The aim of this study was to investigate the influence of season and day length on haematological characteristics, biochemical indices and hormonal profile of the indigenous Helmeted Guinea Fowl in the middle belt of Ghana.

MATERIALS AND METHODS

The study was conducted at the Poultry Unit of the Department of Animal Science Education, University of Education, Winneba, Mampong campus from September, 2015 to December, 2016.

Mampong is found in the Transitional Zone between the Guinea Savanna Zone of the north and Tropical Rain Forest of the south of Ghana with wet semi-equatorial type, a bi-modal rainfall of 1224mm per annum and temperature range of 22.3ºC-30.6ºC. Rainfall occurs in April to July (Major Rainy Season), August to November (Minor Rainy Season) and December to March (Dry Season) (Ghana Meteorological Agency, 2016).

Animals and management

Twelve (12) males and 48 females Pearl Guinea fowls of 34 weeks old were selected from a flock at the Research Department with an average weight of 1.8kg and 1.5kg for females and males, respectively. Formulated diet containing 16.5% crude protein and 2750 kca/kg metabolizable energy (Annor *et al*., 2013) was compounded from soya bean, wheat bran, maize, tuna fish, Russia fish, premix vitamin, oyster shell, dicalcium phosphate and salt. Feed and water were supplied *ad libitum.* Birds were kept in deep litter floored house of 49.90 m × 8.17 m × 2.40 m and LED bulbs of 50W were used to supply light at an intensity of 5.8lx. The weather record during the study period is shown in Table 1. Four hens and 1 male were each subjected to 12 hours of light and 12 hours of darkness (12L: 12D), 14 hours of light and 10 hours of darkness (14L: 10D), 16 hours of light and 8 hours of darkness (16L: 8D) and 18hours of light and 6 hours of darkness (18L: 6D). Each group was replicated three times and reared in three seasons (Dry-December-March, Major Rains-April-July and Minor Rains-August-November) in a 3×4 factorial experimental design.

Blood sampling

Six (6) ml blood samples were obtained by puncturing the brachial vein of the underside of the web of the wing of the hens using needles and syringes for analysis. Three (3) ml of the blood sample was dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulated tube and the other 3 ml into vacutainer plain tubes. Serum samples were stored at -20ºC after centrifugation at 500 *g* for 15 minutes until assay was performed.

Assay parameters included Serum enzyme-linked immunosorbent assay (ELISA) Progesterone, Estradiol, Prolactin, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). The hormones were performed on Mindray® microplate reader *MR* 96 A (Shenzhen Mindray Bio-medical electronics Co., Ltd, China). Total proteins and the protein fractions (albumin and globulin) and cholesterol concentrations were analyzed on Mindray *BA*-88A Biochemistry auto-analyzer (Shenzhen Mindray Bio-medical electronics Co., Ltd,

Table 1: Monthly Weather records during the study period for Mampong Municipality

Variables	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Temperature (ºC)	33	33	32	32	31	28	27	27	28	29	31	32
Rainfall (mm)	8.4	16	175	128	103	161	73	89	222	108	42	16
Humidity (%)	50	60	77	80	80	86	86	86	89	87	78	67
Cloud cover (%)	28	36	47	51	56	77	81	83	89	69	53	38
Sun Hours	115	106	112	104	106	66.3	54	50	54.5	59.3	82	94

Source: World Weather Online (2016).

China). Similarly, the full blood count was determined by using Mindray 5 parts haematology analyzer *BC*-5300.

Statistical analysis

Data collected were analyzed using General Linear Model (GLM) procedure of Statistical Analysis System (SAS for Windows, version 7). The means were separated by using the probability of difference (PDIFF) procedure of SAS.

RESULTS AND DISCUSSION

Effect of Day length on haematological indices

The effect of day length on haematological parameters is presented in Table 2. Significant ($p<0.05$) effect were observed in Packed Cell Volume, Neutrophil, Lymphocytes and Eosinophil. The highest ($p<0.05$) Packed Cell Volume was observed in 16L: 8D, followed by 18L: 6D with the lowest in 12L: 12D. Day length effect on neutrophil was highest in 14L: 10D with 16L: 8D being the lowest observed value. Highest lymphocyte value was observed in 16L: 8D with 14L: 10D recording the lowest. Eosinophil was highest in 16L: 8D and lowest in12L: 12D.

The haematological parameters of guinea fowl recorded in this study were within ranges (Pandian *et al.* 2012). Higher values of Packed Cell Volume (PCV) obtained in this study had been reported in similar study (Nazifi *et al.*, 2003) and is significant in the diagnosis of anaemia and also serves as useful indices of bone marrow capacity to produce red blood cells (Chineke *et al*., 2006). The increase of lymphocytes and eosinophil values with increasing day length is supported by earlier report by Hauptmanova *et al.* (2006) and Gbolabo *et al*. (2015) who posited that there is mobilization of stress defense mechanism to sustain its life activities in response to increasing day length which changes the environmental of the bird.

Day length effect on serum biochemistry is presented in Table 3. Day length had significant ($p<0.05$) effect on Total serum protein and albumin but not globulin and cholesterol ($p>0.05$) (Table 3). Values for Total serum protein increased with increasing day length from 12L: 12D and 16L: 8D and reduced drastically to the lowest at 18L: 6D. Albumin recorded highest value in 14L: 10D and lowest in 18L: 6D. Globulin levels were similar in 12L: 12D, 14L: 10D, 16L: 10D and 18L: 6D. Increasing day length did not show any beneficial effect on cholesterol levels ($p>0.05$). Day length revealed no significant

Table 2: Effect of Day length on haematological indices

Variables	T0 (12L:12D)	T1 (14L:10D)	T2 (16L:8D)	T3 (18L:6D)	Stand Error	P-value
Haemoglobin *(g/dL)*	16.40	15.13	16.22	17.33	0.67	0.17
Red Blood Cells ($x10^{12}/L$)	2.36	2.51	2.33	2.31	0.16	0.81
Packed Cell Volume *(%)*	51.83 ^{c}	48.58 ^{d}	59.46^{a}	53.63 ^{b}	1.85	0.03
Mean Cell Volume (*fL*)	71.80	77.05	68.48	71.69	4.96	0.67
Mean Cell Haemog (*pg*)	55.11	58.05	67.03	66.07	4.73	0.22
MCHC *(g/dL)*	33.40	69.72	33.60	33.76	17.73	0.39
Platelets ($x10^9/L$)	50.44	65.66	59.33	55.44	3.86	0.06
White Blood Cells ($x10^9/L$)	7.66	5.72	7.45	6.62	0.65	0.16
Neutrophil ($x10^9/\mu l$)	61.56^{b}	75.78 ^{a}	42.11^{c}	53.55 ^{b}	6.64	0.01
Lymphocytes ($x10^9/\mu l$)	55.67^{c}	52.00 ^{c}	81.78 ^{a}	64.44^{b}	6.76	0.02
Monocytes ($x10^9/\mu l$)	2.11	2.33	2.33	1.89	0.33	0.74
Eosinophil ($x10^9/\mu l$)	2.00 ^{c}	3.55^{b}	3.67^{a}	2.44 ^{c}	0.46	0.04
Basophil ($x10^9/\mu l$)	1.67	1.56	1.67	1.55	0.18	0.93

Means bearing different superscripts in the same row are significantly different ($P<0.05$)

12L: 12D=12 hours of light: 12 hours of darkness, 14L: 10D=14 hours of light: 10 hours of darkness, 16L: 8D=16 hours of light: 8 hours of darkness and 18L: 6D= 18 hours of light: 6 hours of darkness.

Table 3: Effect of day length on biochemical and hormonal profiles

Variables	T0 (12L:12D)	T1 (14L:10D)	T2 (16L:8D)	T3 (18L:6D)	Standard Error	P-value
Effect on biochemical profile						
Tot Serum Protein (*g/L*)	58.41[b]	57.81[b]	61.25[a]	51.62[c]	1.67	0.01
Albumin (*g/L*)	22.22 [b]	25.02[a]	22.10 [b]	21.17 [c]	0.49	0.01
Globulin (*g/L*)	5.92	5.75	5.99	5.31	0.21	0.13
Cholesterol (*mg/dl*)	3.77	3.75	3.66	3.55	0.21	0.64
Effect on hormonal profile						
Progesterone *(ng/dl)*	75.02	81.25	79.21	76.35	1.96	0.13
Follicle Stimulating Hormone *(IU/ml)*	4.48	4.57	4.18	4.37	0.52	0.96
Luteinizing Hormone *(IU/ml)*	4.22	4.44	4.22	4.49	0.52	0.34
Prolactin *(ng/ml)*	49.78[d]	56.55 [c]	66.66[a]	61.44[b]	3.26	0.01
Oestrogen (*pg/ml*)	505.52	514.39	460.51	532.70	29.89	0.38

Means bearing different superscripts in the same row are significantly different (P<0.05)

12L: 12D=12 hours of light: 12 hours of darkness, 14L: 10D=14 hours of light: 10 hours of darkness, 16L: 8D=16 hours of light: 8 hours of darkness and 18L: 6D= 18 hours of light: 6 hours of darkness.

(p>0.05) difference in all hormonal parameters measured except prolactin (Table 3). Prolactin maintained increasing (p<0.05) tendency with increasing day length from 12L: 12D up to 16L: 8D. The highest value was obtained in 16L: 8D and 12L: 12D was lowest.

The biochemical parameters were within ranges as reported in means and reference range of values (Obinna *et al*., 2011). The significant differences in Total serum protein detected among groups may be attributed to day length effect on feed metabolism (Leveille and Sandber-lich, 1961) even as birds were on the same diet, and that the low value for animals raised under 18 hrs daylength might have been induced by stress level which resulted in decreased metabolism (Mack *et al.*, 2013) as birds subjected to heat stress conditions spend less time feeding, more time drinking and panting, resting and daylength above 17 hrs – 20 hrs induce photo refractoriness in layers (Lewis *et al.*, 2006). Lack of any difference in cholesterol level may be due to the same diet and same usage of energy in the intensive system of the experimental animals (Obinna *et al.*, 2011). Progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) did not increase with day length variation and disagrees with the report of Lewis *et al*. (1999) that increasing day length results in photo stimulation which induces the hypothalamus to release GnRH which facilitates the production of Follicle Stimulating Hormone and Luteinizing Hormone. Results of prolactin is supported by Opel and Proudman (1982) that increasing day length increases prolactin secretion, however, Rozemboim *et al.* (2014) reported of no significance difference. Prolactin secretion is nocturnally controlled and increases in darkness and photo refractoriness resulting in ovarian regression and broodiness (Kagya-Agyemang *et al.*, 2012).

Effect of season on haematological characteristics

Haematological values are shown in (Table 4). Red blood cells were highest during dry season while the highest (p<0.05) value of Packed Cell Volume was recorded in major and minor rainy seasons and were similar. The highest (p<0.05) values of platelets were recorded in major and minor rainy seasons and lowest in the dry season. Neutrophil increased (p<0.05) from dry season through the major rains and peaked in the minor rainy season. White blood cells, monocytes, lymphocytes, basophil and eosinophil, values were not significantly different (p>0.05). Lymphocytes values were, however, higher (p<0.05) during the major and minor rainy seasons.

The values for leukocytes were within range of birds (Hauptmanova *et al.,* 2002). Gattani *et al.* (2016) reported that season has significant effect on haemoglobin concentration, Red Blood Cells, and Packed Cell Volume in female turkeys. And the erythrocyte values obtained in this study is supported by earlier studies of Guinea fowl (Oyewale, 1991) and turkey (Olayemi and Ojo, 2007).

Table 4: Effect of season on haematological characteristics

Variables	December-March (Dry Season)	April-July (Major Rainy Season)	August-November (Minor Rainy Season)	Stand Error	P-Value
Haemoglobin *(g/dL)*	15.65	16.72	16.44	0.58	0.42
Red Blood Cells *(x10^9/µl)*	2.81[a]	2.16[b]	2.16[b]	0.14	0.01
Packed Cell volume (%)	48.97[b]	55.81[a]	55.35 [a]	1.60	0.01
Mean Cell Volume *(f/L)*	72.65	72.93	71.04	4.27	0.94
MCH *(p/g)*	62.74	62.04	59.91	4.10	0.87
MCHC (p/g)	60.54	34.16	33.16	15.35	0.37
Platelets *(x10^9/µl)*	37.5[b]	69.08 [a]	66.58 [a]	3.34	0.01
Wh. Blood Cells *(x10^9/µl)*	62.84	72.12	70.89	5.65	0.46
Monocytes *(x10^9/µl)*	1.67	2.33	2.50	0.28	0.11
Neutrophil *(x10^9/µl)*	43.42[c]	62.83 [b]	68.50 [a]	5.75	0.01
Lymphocytes *(x10^9/µl)*	56.25	66.17	68.00	5.85	0.32
Basophil *(x10^9/µl)*	1.58	1.58	1.67	0.15	0.91
Eosinophil *(x10^9/µl)*	2.75	3.00	3.00	0.39	0.09

Means bearing different superscripts in the same row are significantly different ($P<0.05$)

MCH=Mean Cell Haemoglobin; MCHC=Mean Cell Haemoglobin Concentration

Thermal stressor, influences livestock physiological activities which subsequently frustrate overall performance of feed intake, thus reduces Packed Cell Volume during dry season. Yahav *et al*. (1997) found no change in haemoglobin concentration with increased temperature. Packed cell volume increased when birds were exposed to lower temperature in winter (Atta, 2002). Seasonal changes in rates of parasitism have been shown to affect seasonal changes in the level of anti-parasitism defenses (Lochmiller *et al.,* 1994). Layers in the dry season appeared to be safer in disease prevalence compared to rainy season where disease prevalence is higher as they are compelled towards mobilization of body defense mechanism such as increase in neutrophil and lymphocytes percent in the blood during rainy seasons to fight diseases (Davis *et al.,* 2008).

Effect of season on Biochemical and hormonal profile

Results of effect of season on biochemical and hormonal profiles are presented in Table 5. The results indicate that minor rainy season had highest ($p<0.05$) value of Total serum protein endowment as compared to dry season and major rainy season. Season had no significant ($p>0.05$) effect on albumin, globulin and total cholesterol level. Total serum protein value was statistically highest ($p < 0.05$) in the minor rainy season which is the coldest period in the year indicating the seasonal influence on biochemical values. Mean peripheral plasma concentration of oestrogen, luteinizing hormone and prolactin were significantly ($p<0.05$) influenced by seasonal variations. Luteinizing Hormone and oestrogen values were highest during the dry seasons and major rains, respectively. Prolactin level was highest ($p<0.05$) in the minor rainy season and lowest in the major rainy season. Season variation had no significant ($p>0.05$) effect on follicle stimulating hormone and progesterone.

Al-Heeti *et al.* (1985) found that the highest plasma total protein levels were recorded under moderate temperature and the lowest ones were recorded under high temperature. However, this is contradicted by the study of Silva *et al.* (2011) which reported no difference in hot and cold seasons. The decrease in total serum protein during the dry season may be due to reduced protein consumption and consequently decreased supply of essential amino acids from feed, accompanied by reduced protein digestibility because of exposure of birds to high environmental temperature as birds reduce feed intake (Gattani *et al*., 2016).

Table 5: Effect of Season on biochemical and hormonal profile

Variables	December-March (Dry Season)	April-July (Major Rainy Season)	August-November (Minor Rainy Season)	Stand Error	P-Value
Effect on biochemical profile					
Total serum protein *(g/dL)*	55.32^{b}	56.34^{b}	60.16^{a}	1.44	0.01
Albumin *(g/dL)*	22.62	23.24	22.02	0.42	0.14
Globulin *(mg/dL)*	5.55	5.88	5.80	0.18	0.44
Cholesterol *(mg/dL)*	3.52	3.81	3.72	0.11	0.20
Effect on hormonal profile					
Oestrogen *(pg/ml)*	473^{b}	587^{a}	449.75^{b}	25.88	0.01
Progesterone *(ng/dl)*	78.72	79.31	75.83	1.69	0.31
FSH *(IU/ml)*	4.42	4.64	4.14	0.44	0.74
LH *(IU/ml)*	4.76^{a}	4.59^{b}	3.61^{c}	0.13	0.01
Prolactin *(ng/ml)*	53.95^{b}	40.20^{c}	81.67^{a}	2.82	0.01

Means bearing different superscripts in the same row are significantly different (P<0.05)

FSH = Follicle stimulating Hormone; LH = Luteinizing Hormone

A significant reduction in plasma progesterone and estrogen had been detected after exposing birds to heat stress and significant increase in these sex hormones was observed when temperatures were reduced (Rozemboim *et al.,* 2014). Seasonal changes in reproductive activities are generally correlated with gonadotropin secretion (Ubakka *et al*., 2013) and rainy seasons stimulate gonadotropin secretion.

Effect of season and day length interaction on haematological characteristics, biochemical and hormonal profiles

Season and daylength Interaction effect on all haematological characteristics were not significant. Only prolactin was significant (p<0.05) among biochemical and hormonal profiles as both higher temperature and increased daylength results in prolactin supply (Ubuka *et al.,* 2013). There is currently no work on interaction effect of season and day length in Guinea fowls. Photoperiod and light intensity has been studied in broiler and was significant only in cholesterol and total protein (Fidan *et al.,* 2017) and some production traits in layers (Proudfoot *et al.,* 1984).

CONCLUSION

The findings of this study suggest that season and day length had significant effect on haematological characteristics, and biochemical and hormonal profiles of Guinea fowls and therefore must be considered when planning the breeding program for commercialization of Guinea fowls as these values reflects the physiological responsiveness of the animal to influence production and reproductive traits.

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