



## Haematological, Biochemical and Hormonal Profile of Guinea Fowl (*Numida meleagris*) Layers Fed Different Crude Protein Diet in the Seasons in Ghana

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### ABSTRACT

A study was conducted to investigate the effect of varying dietary protein level and season on haematological characteristics, biochemical and hormonal profiles of Indigenous Guinea fowl (*Numida meleagris*). Sixty Pearl Guinea fowls of 12 weeks of age were subjected to four treatment groups of diets containing 16%, 18%, 20% and 22% crude protein and reared in three seasons (Dry: December-March, Major rains: April-July and Minor rains: August-November) in a 3x4 factorial experiment. Data were analyzed using General Linear Model procedure of SAS. Dietary crude protein had no significant effect ( $p>0.05$ ) on all haematological parameters. Total serum protein and albumin increased ( $p<0.05$ ) with increasing dietary protein, but not globulin and cholesterol. Progesterone and oestrogen were significantly ( $p<0.05$ ) influenced by dietary protein level. Haemoglobin and MCHC were highest ( $p<0.05$ ) in the major rainy season whereas PCV and platelets increased ( $p<0.05$ ) in the dry season. WBC and monocytes increased ( $p<0.05$ ) in the rainy seasons and lymphocytes levels were lowest ( $p<0.05$ ) in the major rainy season. Albumen was highest ( $p<0.05$ ) in the hot dry season and lowest in the minor rainy season. Globulin increased ( $p<0.05$ ) from the dry season to the highest in the minor rainy season. Oestrogen, progesterone, luteinizing hormone and prolactin were significantly ( $p<0.05$ ) influenced by season. Results indicate that 16% crude protein is adequate and that seasonal variations influence haematological, biochemical and hormonal profiles of Guinea fowl during laying period.

**Keywords:** Guinea fowl, Season, Dietary protein, Haematological, Biochemical, Hormonal

Poultry production is widespread in Africa and is regarded as a cheap way of alleviating poverty amongst resource poor rural communities (Saina, 2005). In most developing countries, poultry species include chickens, Guinea fowls, turkeys, ducks and pigeons in their order of importance (Kusina and Kusina, 1999). The production of Guinea fowls has been on the increase in small holder farming areas (Microlivestock, 1991) especially in tropical environments. Guinea fowls are members of the family *Numidia*, with *Numidia meleagris* and *Numidia ptilorhyncha* being the common species (Binali and Kanengoni, 1998). Guinea fowl originated from the west coast of Africa and it is currently reared as a delicacy in many countries outside Africa. China currently, consumes the highest value of

Guinea fowl eggs and meat and the bird has assumed a worldwide interest (Global Poultry Trends, 2012). Guinea fowls have traditionally been raised in colonies in the free range system with perches and roosters provided in the household of small holders where the animals return after scavenging (Moreki and Seabo, 2012). The recent interest in the bird had increased its rearing in enhanced intensive houses and a cage system which has resulted in the use of improved protein diet. The breeding of Guinea fowl occurs during the rainy season (Kabera, 1997; Embury, 2001) and in the tropical environment the number of eggs laid varies according to season (Nwagu and Alawa, 1995; Binali and Kanengoni, 1998).

Haematological, biochemical and hormonal parameters have been used as indicators in birds and have been studied extensively in ruminants (Bryner *et al.*, 1990) and poultry (Holst-Schumacher *et al.*, 2010). The values have commonly been used as indicators of health in intensive management to determine physiological variations caused by environmental, nutritional and disease conditions.

The influence of dietary protein and season has been studied in many avian categories. Despite large number of publications that deal with reference values of haematological, biochemical and hormonal indicators in poultry, there is lack of knowledge about these parameters in Guinea fowls. The most current information on these parameters has been published on the effect of day length (Korankye *et al.*, 2018). There is no information about haematological characteristics, biochemical indices and hormonal profiles as influenced by dietary protein levels and season of breeding in Guinea fowls.

This study aims at investigating the changes in the values of some selected haematological, biochemical and hormonal parameters of Guinea fowls fed different level of dietary protein during the laying seasons.

## MATERIALS AND METHODS

The experiment was performed at the poultry section of the Animal science Department of the University of Education of Winneba, Mampong Campus from December 2016 to December, 2017. The area is in the Transitional Zone between the Guinea Savanna Zone of the north and Tropical Rain Forest of the south of Ghana. The climatic condition is wet semi-equatorial type, with a bi-modal rainfall of 1224mm per annum, temperature range of 22.3°C-30.6°C. Rainfall occurs in April to July (Major Rainy Season), August to November (Minor Rainy Season) and December to March (Dry Season) (GMA, 2016)

### Animals, management and design

Twelve (12) males and forty-eight (48) females Pearl Guinea fowls of 12 weeks old were randomly selected from a large flock at the Research Department. Soya bean, wheat bran, maize, tuna fish, Russia fish, premix vitamin, oyster shell, Diacalcium phosphate and salt were used to formulate a diet containing 16%, 18%, 20% and

22% Crude protein levels and 2750 kca/kg metabolizable energy (Annor *et al.*, 2013). Feed and water were offered *ad libitum* in a removable feeding and water troughs. Birds were kept in deep litter floored house of 49.9m × 8.17m × 2.4m. The weather record during the study period is shown in Table 2.

Four (4) Guinea hens and one (1) cock were each subjected to 16% CP, 18% CP, 20% CP and 22% CP. 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.

**Table 1:** Ingredient and Proximate Composition (%) of experimental diets

| Attributes                       | Diet 1<br>(16% CP) | Diet 2<br>(18% CP) | Diet 3<br>(20% CP) | Diet 4<br>(22% CP) |
|----------------------------------|--------------------|--------------------|--------------------|--------------------|
| <i>Ingredients</i>               |                    |                    |                    |                    |
| Maize                            | 61                 | 60                 | 58                 | 55.5               |
| Fish meal<br>(Russia)            | 2.5                | 5                  | 6                  | 9.5                |
| Fish meal (Tuna)                 | 7                  | 8                  | 10                 | 11.0               |
| Soya bean                        | 7                  | 8.5                | 10                 | 10.0               |
| Wheat bran                       | 18.5               | 14.5               | 12.5               | 10.5               |
| Oyster shell                     | 2.5                | 2.5                | 2                  | 2.0                |
| Dicalcium<br>phosphate           | 0.5                | 0.5                | 0.5                | 0.5                |
| Vitamin Premix                   | 0.5                | 0.5                | 0.5                | 0.5                |
| Salt                             | 0.5                | 0.5                | 0.5                | 0.5                |
| Total                            | 100                | 100                | 100                | 100                |
| <i>Proximate Composition (%)</i> |                    |                    |                    |                    |
| Moisture content                 | 10.5               | 10.5               | 10.0               | 11.0               |
| Protein content                  | 15.76              | 17.78              | 19.75              | 21.49              |
| Ether extract                    | 4.0                | 5.5                | 4.0                | 5.0                |
| Ash content                      | 11.0               | 8.5                | 10.0               | 9.5                |
| Crude fibre                      | 5.42               | 4.91               | 4.37               | 4.28               |

### Blood sampling and analysis

Two birds were randomly selected from each replicates in the seasons for 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 Guinea hens using needles and syringes. No sedation was used and only minimum restraint was required. Three (3) ml of the

**Table 2:** Weather Records during the Study Period for the Municipality

| Variables       | Jan | Feb  | Mar  | April | May  | Jun | July | Aug | Sep | Oct | Nov | Dec |
|-----------------|-----|------|------|-------|------|-----|------|-----|-----|-----|-----|-----|
| Temperature(°C) | 33  | 33   | 32   | 33    | 32   | 29  | 27   | 26  | 26  | 28  | 28  | 29  |
| Rainfall (mm)   | 6.1 | 20.2 | 42.4 | 70.1  | 75.7 | 149 | 225  | 138 | 159 | 114 | 86  | 43  |
| Humidity (%)    | 58  | 66   | 71   | 73    | 78   | 86  | 88   | 88  | 87  | 84  | 80  | 70  |
| Cloud cover (%) | 25  | 43   | 44   | 37    | 51   | 74  | 78   | 74  | 71  | 58  | 61  | 56  |
| Sun Hours (hrs) | 118 | 100  | 109  | 115   | 112  | 78  | 67.8 | 62  | 64  | 66  | 62  | 89  |

**Source:** World Weather Online (2017).

blood sample was dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulated tube and the other 3 ml into vacutainer plain tubes. Samples were placed in a Thermo- Cooler box for transportation to the laboratory. The haematological parameters were determined by using Mindray 5 parts haematology analyzer BC-5300.

Serum samples were stored at -20°C after centrifugation at 500g 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). Mindray microplate reader MR 96 A (Shenzhen Mindray Bio-medical electronics Co., Ltd, China) was used for the analysis of hormones whilst Total protein, protein fractions (albumin and globulin) and cholesterol concentrations were analyzed on Mindray BA-88A Biochemistry auto-analyzer (Shenzhen Mindray Bio-medical electronics Co., Ltd, China).

### 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 (PDIF) procedure of SAS.

## RESULTS AND DISCUSSION

### Effect of different dietary protein level on haematological characteristics

The effect of different dietary crude protein levels on haematological characteristics are presented in Table 3. The results of this study demonstrated that different dietary crude protein level showed no significant effect ( $p>0.05$ ) on the values of erythrocytes, platelets and

leukocytes. The haematological values obtained in this study compare favourably to standard values in birds (Mitrika and Rawnsley, 1977; Schalm's *et al.*, 2000). This study showed no significant effect on all erythrocytic indices and the outcome seems to suggest that 16% crude protein level was sufficient to guarantee sufficient physiological status of the birds. It has been reported that haematological parameters did not vary when layer hens were fed diets containing 14%, 15%, 16% and 17% (Adeyemo, 2012). Polat *et al.* 2003 had demonstrated haematological parameters of layer ostriches fed 20% and 23% crude protein levels resulted in no significant effect. The results demonstrated that birds could be predicted to be healthy and underwent the experiment without any stress and pathologically in stature (Hauptonanova *et al.*, 2006). Haematological parameters in birds have been shown to be influenced by various factors such as age, sex, season and nutrition. Erythrocytic indices used to evaluate the health status of different bird species include PCV, HB and RBC that reflect the individual's nutritional status (Nalubamba, 2014).

### Effect of different dietary protein level on biochemical profile

The result of the influence of varied dietary protein level on biochemical parameters are presented in Table 4. Different dietary crude protein levels showed significant effect ( $p<0.05$ ) on total serum protein and albumin. The results demonstrated no significant difference ( $p>0.05$ ) in the values of globulin and cholesterol. Total serum protein was highest in layers fed 20% crude protein and lowest in 22% crude protein. Values for 16% and 18% crude protein diets were similar. Albumin was highest ( $p<0.05$ ) in 18% and lowest in 22% crude protein diets. Similar values were obtained for 16% and 20% crude protein diets.

**Table 3a:** Effect of different dietary crude protein levels and season on haematological characteristics

| <i>Variables</i>                | <i>Haemoglobin (g/dL)</i> | <i>RBC (x10<sup>12</sup>/L)</i> | <i>PCV (%)</i>     | <i>MCV (fL)</i> | <i>MCH. (pg)</i> | <i>MCHC (g/dL)</i> | <i>Platelets (x10<sup>9</sup>/L)</i> |
|---------------------------------|---------------------------|---------------------------------|--------------------|-----------------|------------------|--------------------|--------------------------------------|
| <b>Dietary protein level</b>    |                           |                                 |                    |                 |                  |                    |                                      |
| 16% CP                          | 18.67                     | 3.42                            | 49.67              | 158.33          | 127.36           | 40.54              | 38.22                                |
| 18% CP                          | 18.13                     | 3.20                            | 48.97              | 162.44          | 59.07            | 41.48              | 40.44                                |
| 20%CP                           | 17.81                     | 2.91                            | 48.18              | 171.00          | 65.08            | 39.83              | 31.00                                |
| 22% CP                          | 17.71                     | 3.09                            | 45.70              | 162.44          | 65.38            | 43.36              | 42.56                                |
| Standard Error                  | 0.79                      | 0.28                            | 1.70               | 8.84            | 36.38            | 1.80               | 3.86                                 |
| P-value                         | 0.82                      | 0.63                            | 0.40               | 0.78            | 0.51             | 0.55               | 0.40                                 |
| <b>Season</b>                   |                           |                                 |                    |                 |                  |                    |                                      |
| Dry Season                      | 16.47 <sup>c</sup>        | 3.43                            | 49.83 <sup>a</sup> | 168.17          | 57.21            | 34.14 <sup>c</sup> | 51.00 <sup>a</sup>                   |
| Major Rainy Season              | 21.10 <sup>a</sup>        | 2.84                            | 44.90 <sup>c</sup> | 171.42          | 136.83           | 50.24 <sup>a</sup> | 24.00 <sup>c</sup>                   |
| Minor Rainy Season              | 16.68 <sup>b</sup>        | 3.20                            | 49.65 <sup>b</sup> | 151.08          | 43.63            | 39.53 <sup>b</sup> | 38.50 <sup>b</sup>                   |
| Standard Error                  | 0.68                      | 0.14                            | 1.48               | 7.66            | 31.51            | 1.56               | 4.30                                 |
| P-value                         | 0.01                      | 0.24                            | 0.04               | 0.15            | 0.10             | 0.01               | 0.01                                 |
| <b>Dietary Protein x season</b> |                           |                                 |                    |                 |                  |                    |                                      |
| P-value                         | 0.75                      | 0.77                            | 0.02               | 0.23            | 0.39             | 0.89               | 0.67                                 |

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

RBC= Red Blood Cells; PCV= Packed Cell Volume; MCV= Mean Cell Volume MCH= Mean Cell Haemoglobin; MCHC= Mean Cell Haemoglobin Concentration.

**Table 3b:** Effect of different dietary crude protein levels and season on haematological characteristics

| <i>Variables</i>                | <i>WBC (x10<sup>9</sup>/L)</i> | <i>Neutrophil (x10<sup>9</sup>/μl)</i> | <i>Lymphocytes (x10<sup>9</sup>/μl)</i> | <i>Monocytes (x10<sup>9</sup>/μl)</i> | <i>Eosinophil (x10<sup>9</sup>/μl)</i> | <i>Basophil (x10<sup>9</sup>/μl)</i> |
|---------------------------------|--------------------------------|--|---|---------------------------------------|--|--------------------------------------|
| <b>Dietary protein level</b>    |                                |  |   |                                       |  |                                      |
| 16% CP                          | 87.69                          | 59.56                                  | 38.67                                   | 2.67                                  | 1.88                                   | 1.67                                 |
| 18% CP                          | 77.29                          | 54.89                                  | 43.56                                   | 3.44                                  | 3.00                                   | 1.56                                 |
| 20%CP                           | 76.16                          | 62.33                                  | 34.11                                   | 3.44                                  | 2.78                                   | 1.67                                 |
| 22% CP                          | 72.39                          | 54.33                                  | 41.56                                   | 2.88                                  | 2.11                                   | 1.55                                 |
| Standard Error                  | 4.30                           | 9.48                                   | 8.86                                    | 0.61                                  | 0.45                                   | 0.18                                 |
| P-value                         | 0.10                           | 0.92                                   | 0.89                                    | 0.73                                  | 0.44                                   | 0.93                                 |
| <b>Season</b>                   |                                |  |   |                                       |  |                                      |
| Dry Season                      | 61.73 <sup>c</sup>             | 2.08 <sup>c</sup>                      | 53.92                                   | 50.75 <sup>a</sup>                    | 1.58                                   | 2.92                                 |
| Major Rainy Season              | 86.90 <sup>a</sup>             | 2.83 <sup>b</sup>                      | 71.42                                   | 22.42 <sup>c</sup>                    | 1.58                                   | 1.67                                 |
| Minor Rainy Season              | 86.50 <sup>b</sup>             | 4.42 <sup>a</sup>                      | 48.00                                   | 45.25 <sup>b</sup>                    | 1.67                                   | 2.75                                 |
| Standard Error                  | 3.73                           | 0.52                                   | 8.21                                    | 7.68                                  | 0.15                                   | 0.47                                 |
| P-value                         | 0.01                           | 0.01                                   | 0.13                                    | 0.04                                  | 0.91                                   | 0.15                                 |
| <b>Dietary Protein x season</b> |                                |  |   |                                       |  |                                      |
| P-value                         | 0.25                           | 0.00                                   | 0.42                                    | 0.81                                  | 0.43                                   | 0.01                                 |

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

WBC= White Blood Cells.

Total protein and albumin reflects the availability of protein and their concentration decline in the instinct of reduced protein intake. Albumin serves as the major amino acid pool (Kaneko *et al.*, 1997). The catabolism of albumin provides protein precursors needed for physiological and that higher protein intake has been reported to increase serum albumin (Skyes and Field, 1973; Hallford *et al.*, 1982; Shetaawi and Ross, 1991).

Increasing dietary protein enhanced albumin concentration, but decreased when dietary protein level was 22% in broilers and this may be attributed to possible excess dietary protein (Liu *et al.*, 2015). The level of albumin in serum significantly reduced as dietary protein level was decreased from 18% to 16% crude protein. These results might be related with the deficient in amino acid intake by the animals (Corzo *et al.*, 2009).

Polat *et al.* (2003) found that biochemical parameters of ostriches fed diets of 20% and 23% crude protein did not differ significantly ( $p>0.05$ ). Ding *et al.* (2016) found no effect of protein on total protein and albumin when Fengda-1 layers were fed 14.50%, 15.00% and 15.50% crude protein diets.

#### Effect of different dietary protein level on hormonal profile

The effect of dietary protein level on hormonal profile of Guinea fowl is presented in Table 4. Different dietary protein level resulted in significant difference ( $p<0.05$ ) in progesterone and oestrogen, but not in testosterone ( $p>0.05$ ). Oestrogen was highest ( $p<0.05$ ) in 22% dietary crude protein level followed by 18% and 16%. The lowest value was observed in 20% diet.

The report of this study suggested that dietary crude protein intake enhanced significantly oestrogen and progesterone secretion and is corroborated by earlier report that dietary intake of proteins may affect the circulating levels of progesterone and oestrogen, however, different studies led to opposite results and others showed no changes (Jordan *et al.*, 1979). Earlier studies have demonstrated that serum progesterone and estrogen were not affected by dietary treatment in ovariectomized cows fed 24% crude protein. Saitok and Takahashi (1977) found that ovaries from rats at pregnancy fed 30.0% CP produced four times progesterone as rats fed 39.9% CP. No significant ( $p>0.05$ )

differences was observed in testosterone during the laying seasons. This is supported by earlier report of Mumford (2015) that dietary protein consumption had no direct relationship with testosterone concentration in female in the reproductive stage.

The influence of dietary crude protein level on plasma Follicle Stimulating Hormone and Luteinizing Hormone secretion is presented in Table 4. Increasing dietary crude protein demonstrated no significant effect ( $p>0.05$ ) on plasma Follicle Stimulating Hormone and Luteinizing Hormone secretion. The values of this study were similar to the report of Renema *et al.* (1999) on broiler hens under *ad libitum* feeding. Endocrine functions of the pituitary LH and FSH secretion have been greatly depressed in cows consuming feed high in protein (Jordan and Swanson, 1979). Mean luteinizing hormone was not different between dietary treatments (Blauwiel *et al.*, 1996).

**Table 4:** Effect of dietary protein and season on biochemical

| Variables                       | TSP (g/L)          | Alb (g/L)          | Glob (g/L)         | Chol (mg/dl) |
|---------------------------------|--------------------|--------------------|--------------------|--------------|
| <b>Dietary protein level</b>    |                    |                    |                    |              |
| 16% CP                          | 58.41 <sup>b</sup> | 22.22 <sup>b</sup> | 17.22              | 3.97         |
| 18% CP                          | 57.81 <sup>b</sup> | 25.02 <sup>a</sup> | 18.22              | 3.84         |
| 20%CP                           | 61.25 <sup>a</sup> | 22.10 <sup>b</sup> | 16.22              | 4.04         |
| 22% CP                          | 51.62 <sup>c</sup> | 21.17 <sup>c</sup> | 17.11              | 3.70         |
| Standard Error                  | 1.67               | 0.49               | 2.29               | 0.13         |
| P-value                         | 0.01               | 0.01               | 0.94               | 0.34         |
| <b>Season</b>                   |                    |                    |                    |              |
| Dry Season                      | 39.08              | 25.67 <sup>a</sup> | 13.42 <sup>c</sup> | 3.87         |
| Major Rainy Season              | 41.17              | 23.58 <sup>b</sup> | 17.58 <sup>b</sup> | 3.77         |
| Minor Rainy Season              | 37.39              | 16.83 <sup>c</sup> | 20.58 <sup>a</sup> | 4.00         |
| Standard Error                  | 2.20               | 1.29               | 1.97               | 0.11         |
| P-value                         | 0.48               | 0.01               | 0.05               | 0.39         |
| <b>Dietary Protein x season</b> |                    |                    |                    |              |
| P-value                         | 0.82               | 0.92               | 0.88               | 0.67         |

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

TSP=Total Serum Protein; Alb=Albumin; Glob= Globulin; Chol= Cholesterol.



**Table 5:** Effect of dietary protein and season on hormonal parameters

| <i>Variables</i>                | <i>Progesterone (ng/dl)</i> | <i>FSH (IU/ml)</i> | <i>LH (IU/ml)</i> | <i>Prolactin (ng/ml)</i> | <i>Oestrogen (pg/ml)</i> | <i>Testosterone</i> |
|---------------------------------|-----------------------------|--------------------|-------------------|--------------------------|--------------------------|---------------------|
| <b>Dietary protein level</b>    |                             |                    |                   |                          |                          |                     |
| 16% CP                          | 75.09 <sup>d</sup>          | 1.77               | 4.44              | 27.08                    | 505.52 <sup>c</sup>      | 1.89                |
| 18% CP                          | 81.53 <sup>a</sup>          | 2.26               | 4.54              | 31.76                    | 514.39 <sup>b</sup>      | 1.91                |
| 20%CP                           | 81.45 <sup>b</sup>          | 2.02               | 4.29              | 32.74                    | 460.51 <sup>d</sup>      | 1.47                |
| 22% CP                          | 78.69 <sup>c</sup>          | 2.01               | 3.91              | 31.53                    | 532.70 <sup>a</sup>      | 1.80                |
| Standard Error                  | 1.69                        | 0.36               | 0.43              | 2.60                     | 29.89                    | 0.25                |
| P-value                         | 0.04                        | 0.82               | 0.73              | 0.43                     | 0.01                     | 0.59                |
| <b>Season</b>                   |                             |                    |                   |                          |                          |                     |
| Dry Season                      | 80.94                       | 2.10               | 5.90 <sup>a</sup> | 23.50 <sup>c</sup>       | 473 <sup>b</sup>         | 2.27 <sup>a</sup>   |
| Major Rainy Season              | 79.31                       | 2.13               | 4.15 <sup>b</sup> | 32.77 <sup>b</sup>       | 587 <sup>a</sup>         | 1.13 <sup>c</sup>   |
| Minor Rainy Season              | 77.31                       | 1.82               | 2.85 <sup>c</sup> | 36.09 <sup>a</sup>       | 449.75 <sup>c</sup>      | 1.90 <sup>b</sup>   |
| Standard Error                  | 1.46                        | 0.31               | 0.37              | 2.25                     | 25.88                    | 0.22                |
| P-value                         | 0.23                        | 0.74               | 0.01              | 0.01                     | 0.01                     | 0.01                |
| <b>Dietary Protein x season</b> |                             |                    |                   |                          |                          |                     |
| P-value                         | 0.99                        | 0.65               | 0.23              | 0.01                     | 0.64                     | 0.42                |

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

FSH= Follicle Stimulating Hormones; LH=Luteinizing Hormone.

This study demonstrated no increase in FSH and LH in increasing dietary protein and may be ascribable to high energy expenditure in protein metabolism which may antagonize leptin action (Kratz *et al.*, 2002). The physiological significance is that leptin acts on hypothalamic cells to stimulate release of luteinizing hormone (LH)-releasing hormone (LHRH), thereby triggering gonadotropin release. This process is stimulated by high energy and energy restriction reduces concentration of leptin (Nakamura *et al.*, 2013).

The effect of experimental diet on prolactin secretion has been shown on Table 4. Statistical analysis revealed that dietary crude protein had no significant (p>0.05) effect on prolactin secretion. Hawrylewicz *et al.* (1986) demonstrated that dietary protein had no effect on serum prolactin in rats. The study showed that results from high protein were similar to those injected with saline which showed lack of any effect on serum hormone activities throughout the period of administration. The present study contradicts report that dietary protein is probably the agent responsible for prolactin secretion induced by meal (Harold *et al.*, 1983).

#### Effect of season on haematological characteristics

The mean values of haemoglobin (Hb), Red blood cells (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell haemoglobin Count (MCHC) as influenced by the three seasons in Indigenous Guinea fowl is presented in Table 5. The major rainy season had the highest (p<0.05) Hb, whilst lowest Hb was recorded in the dry season. Mean values for PCV and MCHC were significantly (p<0.05) different. The highest (p<0.05) PCV value was recorded in the dry season whereas major rainy season had the lowest. Mean MCHC was highest (p<0.05) during the major rainy season and lowest in the dry season. Mean concentrations for RBC, MCH and MCV were statistically (p>0.05) not different in all the three seasons.

Higher values for Haemoglobin during the major rainy season may be ascribable to increased feed intake during the period so the animals did not become anemic. Reduction of PCV, RBCs and HB has been reported to occur during egg production and has been shown to persist into later stages of incubation and chick rearing (Wagner *et al.*, 2008; Nalubamba *et al.*, 2014). This report has

demonstrated lower ( $p < 0.05$ ) values of PCV and RBC in the minor rainy season. It is likely that the reduction of erythrocytic indices in this study was due to increased egg production during the major rainy season.

The highest ( $p < 0.05$ ) WBC was observed in the Major rainy season. This was followed by the minor rainy season. The dry season recorded significantly lower value. Monocytes was higher in the minor rainy season and lowest in the dry season. The mean values for the total white cell count and differentials; lymphocytes, monocytes and neutrophil had been observed to be higher in the wet season (Okebe *et al.*, 2016). Although the experimental birds were clinically well, these higher values of WBC and monocytes and lack of significant effect in neutrophil and lymphocytes may be due to subclinical infections during the wet season (Mackenzie *et al.*, 2010) and therefore mobilization of lymphocytes and neutrophil were not necessary.

#### Effect of season on biochemical profile

The mean biochemical values for this study are presented in Table 5. Values for Total serum protein were highest in the rainy season than in the dry season, but they were not statistically ( $p > 0.05$ ) different. Albumin recorded the highest ( $p < 0.05$ ) value in the dry season followed by the major rainy season. The lowest value was observed during the cold minor rainy season. Season had significant ( $p < 0.05$ ) effect on globulin. Highest value of globulin was observed in the minor rainy season whilst dry season was the lowest. Season had no significant ( $p > 0.05$ ) effect on the cholesterol levels of the experimental birds.

The result of this study dissents the earlier report that highest total serum protein was observed during the rainy season (Nalubamba, 2014). During the cold minor rainy season feed available is mobilized to generate energy for the survival of the animals and proteins are not found in the peripheral blood.

The dry season of Ghana is characterized by high ambient temperature. Overall means of total protein, albumin, and globulin had been found to be significantly lower due to exposure of the animals to high temperature (Habeeb *et al.*, 1993; Hattingh, 1982; Morera *et al.*, 1991). It has been found that plasma total proteins were decreased significantly when hens were exposed to above 36°C environment than moderate temperature regime. The

results of albumin and globulin of this study contradicts earlier report that at higher temperatures, plasma albumin decreased significantly while globulin concentration increased significantly during hot seasons and may be attributable to changes in body temperature that causes a shift in tissue fluids and thus cause a change in the concentration of plasma proteins (Elaroussi, 1981).

The level of cholesterol was not significant ( $p > 0.05$ ), however, lower values were obtained in the hot dry season and major rainy season where temperatures were relatively high. This is supported by earlier report that relative lower plasma cholesterol level during the hot dry season seems to be linked to increases in temperature and/or physical activity of the animal in the period (Ockene *et al.*, 2004).

#### Effect of season on hormonal profile

Hormonal results are shown in Table 5. Sex steroid hormones showed strong association ( $p < 0.05$ ) with seasonal variation except progesterone. It is noted that mean oestrogen level was highest ( $p < 0.05$ ) during the major rainy season and lowest during the dry season. Seasonal variations resulted in significant ( $p > 0.05$ ) increase in testosterone levels. Testosterone level was highest in the dry season and lowest in the rainy season.

Physiological and behavioral strategies to cope with environmental changes as well as to manage life activities in the face of seasonal variations have been reported (Buchanan, 2000; Romero, 2004). The increases in oestrogen level during the laying period in the major rainy season is supported by Modesto and Canario (2003) who reported similar trends in the spawning season in Lusitanian toadfish. The period of increased oestrogen is associated with ovarian recrudescence and promotion of the synthesis of hepatic yolk precursors (vitellogenin) and oocyte size during the period of vitellogenesis (Crim and Idler, 1978; Schulz, 1984).

Progesterone did not increase with varying season. Seasonal variation of progesterone had been reported to be synchronous with laying activity (Jalme *et al.*, 1996). Plasma progesterone decreased in hens reared under heat stress (Attia *et al.*, 2016) and the expectation of this outcome was not observed.

Testosterone peaked in the dry season suggesting a possible impact season on gonadal activity (Jalme *et al.*, 1996). In

many seasonally breeding birds, circulating testosterone levels increase sharply early in the breeding season and drops when the female lays eggs which correspond with dry and major rainy seasons of this study. The administration of exogenous testosterone in female spotless starlings before egg laying caused negative effects on reproductive performance (López-Rull and Gil, 2009).

The effect of season on the LH and FSH and Prolactin of female pearl Guinea fowls is presented in Table 6. Season showed significant ( $p < 0.05$ ) effect on LH and Prolactin but not on FSH. LH was highest ( $p < 0.05$ ) during the hot dry season. This was followed by the Major rainy season and the lowest was observed in the minor rainy seasons. It was demonstrated that the minor rainy season was associated with highest ( $p < 0.05$ ) level of prolactin. Result indicates that increasing hot dry season resulted in decreasing prolactin secretion in guinea fowls. The values of FSH, LH and Prolactin were within the reference range of birds.

FSH and LH levels had been shown to be significantly different during the laying period and in Guinea fowls mostly in the hot dry season and major rainy season. The results of the current study contradicts earlier report that FSH and LH levels of ostriches increased during the winter and summer (Pandian and Selvan, 2017) which coincides with the minor rainy season and the dry season in our study area. It can be proposed that the seasonal differences in LH and FSH secretion are linked to the external factors of daylight length and nutrition which results in an increase in ovarian activity and more follicles during the laying (Niekerk and Niekerk, 1997).

The results of this study showed a significant increase of prolactin level in the minor rainy season where there was decrease in laying performance. Circulating prolactin level has been linked to the reproductive efficiency of hens (David *et al.*, 2003). This report is complemented by earlier report that prolactin secretion is photo-stimulatory dependent (Proudman and Siope, 2005) and that low photo-stimulation induced increased prolactin secretion. This is so because the minor rainy season observed the lowest photoperiod and supports prolactin secretion. It is amply evident that level of LH and Prolactin hormone are subject to seasonal variations and support seasonality of breeding in the avian world (Degen *et al.*, 1994).

## CONCLUSION

Results indicate that season play significant role in guaranteeing the optimum pathological and reproductive performance. There is ample indication that a diet containing 16% crude protein is adequate to improve the haematological, biochemical and hormonal profiles to achieve optimum reproductive performance and fertility of Guinea hens without any health threats during laying period. Further study should be undertaken to determine the actual amino acids levels that influenced the pathological status of Guinea fowls.

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