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Haematology and Serum Biochemical Profile of Laying Chickens Reared on Deep Litter System with or without Access to Grass or Legume Pasture under Humid Tropical Climate

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Abstract**—**There has been a growing interest on the effects of access to pasture on poultry health status. However, there is a paucity of data on the relative benefits of grass and legume pastures. An experiment was conducted to determine the effects of rearing systems {deep litter system (DL), deep litter with access to legumes (LP) or grass (GP) pastures} haematology and serum chemistry of ISA Brown layers. The study involved the use of two hundred and forty 12 weeks old pullets. The birds were reared until 60 weeks of age. Eighty birds were assigned to each treatment; each treatment had four replicates of 20 birds each. Blood samples (2.5 ml) were collected from the wing vein of two birds per replicate and serum chemistry and haematological parameters were determined. The results showed that there were no significant differences between treatments in all the parameters considered at 18 weeks of age. At 24 weeks old, the percentage of heterophyl (HET) in DL and LP were similar but higher than that of GP. The ratio of H:L was higher ($P<0.05$) in DL than those of LP and GP while LP and GP were comparable. At week 38 of age, the percentage of PCV in the birds in LP and GP were similar but the birds in DL had significantly lower level than that of GP. In the early production phase, serum total protein of the birds in LP was similar to that of GP but higher ($P<0.05$) than that of DL. At the peak production phase (week 38), the total protein in GP and DL were similar but significantly lower than that of LP. The albumin level in LP was greater ($P<0.05$) than GP but similar to that of DL. In the late production phase, the total protein in LP was significantly higher than that of DL but similar to that of GP. It was concluded that rearing chickens in either grass or legume pasture did not have deleterious effects on the health of laying chickens but improved some parameters including blood protein and HET/lymphocyte.

Keywords**—**Rearing systems, *Stylosanthes, Cynodon* serum chemistry, haematology, hen.

I. INTRODUCTION

IN recent years, more attention has been given to animal welfare and there has been an increasing consumer interest in pastured poultry production. The growing concern for animal welfare has led to more extensive rearing systems [10]. This may be explained partly by the perception of consumers of the free-range environment or with outdoor access as being natural, environmentally friendly and animal welfare friendly [23]. Consequently, more extensive rearing systems have been proposed [10], [14], [24]. It has been shown that when compared with conventional confined system, outdoor systems can decrease stress conditions and increase bird comfort [12], leading to stronger leg bones and walking ability [13]. However, access to pasture has been reported to be accompanied by variable pasture intake and might have negative effects on weight gain and feed efficiency in broilers [11].

Blood profiling is a helpful tool in detecting the health status, metabolic diseases, nutritional deficiencies, and welfare of animals [9]. Also, determination of the indicators of the internal environment is one of the methods of evaluating the effect of the factors of the housing environment on the health and production of farm animals, as it provides valuable information about the relationship between the internal environment of the organism, nutrition, age and performance [3]. To date, there is a paucity of information on the health status of laying hens raised under either grass or legume based pastures. There is no clear evidence that the health of hens is improved when they are raised under pastured production system. Few studies have been published on this subject and the results are conflicting, due to the great variation in the breeds used, production methods, rations used and pasture availability [10]. The findings [15]-[17] on the use of conventional and free-range raising systems on hen performance has remained inconclusive, as they did not establish the superiority of one system over the other. There are however advantages and disadvantages in both systems. As outdoor systems become increasingly important, the effects on the health status indices needs to be clear. The aim of the present study was to investigate the haematology and serum chemistry of the birds reared in the deep litter systems with or without access to grass or legume pastures.

II. MATERIALS AND METHODS

A. Experimental Site

This study was conducted at the Teaching and Research Farms Directorate (TREFAD) of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

B. Paddock Establishment

Prior to the establishment of the paddock, the land was ploughed and harrowed. The grass species *Cynodon dactylon* was established vegetatively (sprigs). The sprigs were planted

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at 7 m³/ha into a well prepared seedbed. The legume pasture was established using *Stylosanthes hamata* seeds that were sown at the rate of 3 kg of seed/ha. Irrigation was practiced during the dry season. The chemical compositions of the pastures are shown in Table I.

TABLE I
PROXIMATE COMPOSITION OF *CYNODON DACTYLON* AND *STYLOSANTHES HAMATA*

	Cynodon dactylon	*Stylosanthes hamata*
Moisture content (%)	76.78	81.22
Dry matter (%)	23.22	18.76
Crude protein (%)	8.60	16.90
Fibre content (%)	1.02	1.16
Carbohydrate (%)	14.36	10.01
Ash content (%)	1.18	1.07
Sodium (mg/g)	2.12	2.67
Potassium (mg/g)	6.78	4.66
Phosphorus (mg/g)	1.27	2.12
Magnesium (mg/g)	0.73	0.61
Calcium (mg/g)	0.77	0.77
Iron (mg/g)	1.24	1.89
Zinc (mg/g)	0.01	0.02

C. Experimental Birds and Management

A total number of 240 pullets of a commercial strain (Isa Brown) were purchased from a reputable commercial farm. The birds were housed at 12 weeks of age under three experimental groups viz. deep litter (DL), deep litter with access to a grass-based (GP) and deep litter with access to a legume-based pasture (LP). The size of the pasture in each of the replicates was 80 m^2. The birds were allocated to the deep litter houses at 20 birds/pen, with four replicate pens per treatment. Nesting boxes, perches, feed and water troughs were provided in the houses. The composition of the experimental diets of growers and layers are shown in Tables II and III, respectively. Birds were reared until 60 weeks of age when the experiment was terminated.

D. Blood Samples Collection and Analysis

Blood collection was done at 18, 24, 38 and 60 weeks of age. Blood samples (5 ml each) were collected from the *vena basilica* of the left wings with the use of sterile hypodermic syringes. A sample of 2.5 ml of blood was drawn into heparinized tubes in order to prevent coagulation while the remaining 2.5 ml was drawn into plain bottles and left to coagulate. The blood samples were analysed for haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC). Hb estimation was determined by the cyanmethaemoglobin method and PCV by the microhematocrit method [26]. TEC and TLC were determined using Neubaur's hemocytometer and Toluidine blue (0.015%) saline as diluent [27]. The blood films stained with Wright's stain [25] was studied for DLC. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was calculated [29]. Blood obtained from the experimental birds was analyzed for serum metabolites. Total serum protein was measured in serum for individual animal using the biuret method. Serum albumin and globulin were determined using the bromocresol purple method of [7]. Serum creatinine was determined using the principle of Jaffe reaction as described by [8]. Serum urea was determined using the enzymatic colorimetric method as described by [28].

TABLE II
PERCENTAGE COMPOSITION OF GROWER DIET USED IN THE EXPERIMENT

Ingredients	**Percentage**
Maize	40.00
Corn bran	16.00
Wheat offal	10.19
Palm kernel cake	10.00
Soybean meal	10.00
Groundnut cake	6.00
Fishmeal	2.00
Bone meal	3.00
Oyster shell	2.00
Salt	0.25
*Premix	0.25
Lysine	0.20
Methionine	0.11
Total	**100**
Calculated analysis	
Crude protein	16.22
Crude fibre	4.51
Ether extract	3.99
Calcium	2.00
Phosphorus	0.69
Methionine	0.47
Lysine	0.82
Metabolisable energy (Kcal/kg)	2851

*Biomix grower premix supplied the following per kg diet: - Vit. A, 10,000 i.u.; Vit D3, 2000 i.u; Vit E, 23 mg; Niacin 27.5 mg, Vit B1, 1.8 mg; VitB2, 5 mg; Vit B6, 3 mg; Vit B12, 0.015 mg, Vit K, 2 mg; Pantothenic acid, 7.5 mg, Folic acid, 0.75 mg; Choline chloride, 300 mg; cobalt, 0.2 mg, copper, 3 mg; Iodine, 1mg, Iron, 20 mg; Manganese, 40 mg: Selenium, 0.2 mg.

E. Statistical Analysis

All the data collected were subjected to Analysis of Variance using SAS (1999) statistical package while significant means were compared using Duncan's Multiple Range Test.

III. RESULTS

A. Haematology

Table IV shows the effects of rearing systems on the haematology of growing pullets. Rearing systems did not have significant effects on the haematological parameters of the birds at 18 weeks old.

The effects of rearing systems on the haematology of the layers in the early production phase (24 weeks old) is presented in Table V. PCV, Hb, Eosinophil (EOSN), MCV, MCH, MCHC and Basophil (BAS) were not significantly different among the housing systems. White blood cell (WBC) was higher ($p<0.05$) in DL than GP but similar to that of LP. The percentage of HET in DL and LP were similar but higher than that of GP. Also, the level of Lymphocyte (LYM) in GP was similar to those of the LP and DL but GP was significantly higher ($p < 0.05$) than that of DL. H:L ratio was significant higher in birds in DL than those of GP and LP.

TABLE II
PERCENTAGE COMPOSITION OF LAYER DIET USED IN THE EXPERIMENT

Ingredient	Percentage
Maize	48.00
Soybeans	11.00
Groundnut cake	8.75
Fishmeal	1.50
Palm kernel cake	5.00
Wheat offal	14.00
Bone meal	2.50
Limestone	8.50
*Premix	0.25
Lysine	0.10
Methionine	0.15
Total	**100**
Calculated Feed Analysis	
ME (Kcal/kg)	2,489.05
CP (%)	17.13
CF (%)	4.30
Ether extract (%)	4.30
Calcium (%)	4.11
Phosphorus (%)	0.92
Lysine (%)	0.88
Methionine (%)	0.44

*Supplied per kg premix: Biotin = 40 mg; Zn = 58 mg; Fe = 5800 mg; Vit A =1,000,000 i.u Folic acid = 500 mg; Se = 120 mg; I = 60 mg; Nictotinic acid = 2800 mg; Cu = 700 mg; Mn = 4800 mg; Vit K = 1,500 mg; Riboflavin = 500 mg; Co = 300 mg.

TABLE IV
EFFECTS OF REARING SYSTEMS ON THE HAEMATOLOGY OF GROWING PULLETS (18 WEEKS OF AGE)

Parameters	Rearing systems		
	Deep litter	Legume pasture	Grass pasture
PCV (%)	22.75±1.11	24.50±0.96	23.25±0.85
Hb (g/dl)	7.10±0.32	6.78±0.29	6.70±0.82
RBC($x10^{12}$/L)	1.48±0.29	1.58±0.11	1.60±0.13
WBC ($x10^{9}$/L)	14.15±1.49	17.20±0.91	17.23±0.96
HET (%)	43.25±8.30	31.00±3.11	33.75±1.49
LYM (%)	55.75±8.29	67.25±2.87	64.50±1.71
BAS (%)	0.00±0.00	0.50±0.23	1.00±0.57
MON (%)	1.00±0.00	0.50±0.28	1.00±0.00
H:L	0.96±0.41	0.47±0.07	0.53±0.03
MCV (*Fl*)	165.86±19.93	157.54±10.59	147.87±12.36
MCH (Pg)	49.52±6.31	50.62±4.61	42.15±4.91
MCHC (g/%)	29.84±0.75	31.98±0.89	28.54±2.56

Table VI shows the effects of rearing systems on the haematology of layers in the peak production phase (38 weeks of age). HB, HET, LYM, BAS and MON were not significantly different among all the housing systems. The PCV of the birds in LP and GP were lower than that those of the birds in GP while the level of RBC was similar in LP and GP. GP was also similar to DL but LP was significantly higher ($p<0.05$) than DL. The level of WBC was similar in DL and GP but significantly lower than that of LP. The percentage of EOSN in DL was higher ($p<0.05$) than those of LP and GP while LP and GP were similar. The level of MCV and MCH in deep litter was similar to that of the grass pasture but significantly higher than that of the legume pasture. The hens in the grass and legume pasture had similar levels of MCV and MCH.

The effects of rearing systems on the haematology of the layers in the late production phase (60 weeks of age) is presented in Table VII. There was no difference ($p<0.05$) in most of the parameters considered among the rearing systems. However, H:L and was higher in the birds in DL than those of LP and GP. A similar trend was also recorded for HET .

TABLE V
EFFECTS OF REARING SYSTEMS ON THE HAEMATOLOGY OF THE LAYERS DURING EARLY PRODUCTION PHASE (24 WEEKS OF AGE)

Parameters	Rearing systems		
	Deep litter	Legume pasture	Grass pasture
PCV (%)	25.50±0.96	29.50±2.10	23.50±3.59
Hb (g/dl)	8.15±0.83	8.13±1.02	7.93±0.82
RBC($x10^{12}$/L)	1.45±0.10	1.50±0.09	1.50±0.29
WBC ($x10^{9}$/L)	19.53±2.46[a]	12.03±1.13[b]	17.40±1.54[ab]
HET (%)	35.25±3.47[a]	27.75±1.49[ab]	26.50±1.71[b]
LYM (%)	66.50±1.19[b]	70.5±1.71[ab]	71.75±1.11[a]
EOSN (%)	0.50±0.29	0.25±0.25	1.00±0.57
BAS (%)	0.50±0.28	----	0.50±0.28
MON (%)	0.50±0.28	0.50±0.28	0.50±0.28
H:L	0.53±0.06[a]	0.39±0.03[b]	0.37±0.02[b]
MCV (*Fl*)	176.93±5.42	196.86±8.58	165.01±19.36
MCH (Pg)	55.84±2.64	53.51±3.79	57.25±8.32
MCHC (g/%)	31.74±2.23	27.35±2.40	34.42±1.80

[ab] Means within rows with different superscripts are significantly different ($P<0.05$).

B. Serum Chemistry

The effects of rearing systems on the serum chemistry of growing pullets are presented in Table VIII. The total protein, albumin, globulin and urea were not influenced by the rearing system. Creatinine was however significantly lower in DL than in the GP, but similar to that of the LP. GP and LP were comparable. The level of total protein in GP and DL were similar in the early production phase. Albumin level was higher in LP than in DL and GP but the levels were similar in GP and DL. The level of globulin in GP was higher in GP than LP but comparable to that of DL. Globulin in DL and LP were similar. Creatinine and urea levels were not influenced by the rearing systems in the early phase of production. At the peak production phase, the total protein in GP and DL were similar, but significantly lower than in the LP. The level of albumin of the birds in LP was significantly higher than those of the birds in GP. GP and DL were comparable. Globulin level was similar in LP and GP, but significantly lower than that of DL. Moreover, the urea level was similar in DL and LP, but significantly higher than that of the GP.

In the late production phase, the level of serum total protein of the birds in LP and GP were significantly higher than those of DL. Albumin level in DL and GP were similar, but significantly higher than that of LP. The urea level in DL and LP were similar, but significantly lower than that of the GP.

Creatinine and globulin were not influenced by the housing systems.

TABLE VI
EFFECTS OF REARING SYSTEMS ON THE HAEMATOLOGY OF THE LAYERS IN THE PEAK PRODUCTION PHASE (38 WEEKS OF AGE)

Parameters	Rearing systems		
	Deep litter	Legume pasture	Grass pasture
PCV (%)	24.25±0.85^{b}	27.50±0.95^{ab}	30.25±2.01^{a}
Hb (g/dl)	7.88±0.82	8.85±0.09	9.18±0.83
RBC(x10^{12}/L)	1.28±0.14^{b}	2.03±0.11^{a}	1.88±0.29^{ab}
WBC (x10^{9}/L)	13.40±1.01^{b}	20.70±1.37^{a}	13.23±1.17^{b}
HET (%)	33.50±2.63	30.00±2.16	34.00±4.02
LYM (%)	64.00±2.16	67.75±2.87	64.50±3.88
EOSN (%)	2.00±0.00^{a}	0.00±0.00^{b}	0.75±0.47^{b}
BAS (%)	0.50±0.28	0.00±0.00	0.00±0.00
MON (%)	0.50±0.28	0.75±0.75	0.50±0.5
H:L	0.53±0.05	0.45±0.04	0.54±0.09
MCV (*Fl*)	193.09±11.27^{a}	136.26±3.02^{b}	168.86±16.72^{ab}
MCH (Pg)	61.75±3.57^{a}	44.01±1.92^{b}	50.89±4.84^{ab}
MCHC (g/%)	32.23±2.31	32.26±0.75	30.19±0.74

^{ab} Means within rows with different superscripts are significantly different (P<0.05)

TABLE VII
EFFECTS OF REARING SYSTEMS ON THE HAEMATOLOGY OF THE LAYERS DURING LATE PRODUCTION PHASE (60 WEEKS OF AGE)

Parameters	Rearing systems		
	Deep litter	Legume pasture	Grass pasture
PCV (%)	30.25±2.39	38.00±2.16	31.25±2.87
Hb (g/dl)	9.48±1.27	12.28±0.15	9.98±1.23
RBC(x10^{12}/L)	1.90±0.40	2.58±0.28	2.55±0.45
WBC (x10^{9}/L)	19.33±0.92	21.05±2.48	15.35±1.33
HET (%)	38.25±0.85^{a}	29.25±0.8^{b}	32.50±1.94^{b}
LYM (%)	60.75±0.75	60.50±1.89	66.00±3.85
EOSN (%)	0.50±0.28	1.00±0.00	0.50±0.28
BAS (%)	1.00±0.57	0.00±0.00	0.00±0.00
MON (%)	1.00±0.57	0.50±0.28	1.00±0.57
H:L	0.63±0.02^{a}	0.48±0.00^{b}	0.49±0.01^{b}
MCV (*Fl*)	170.62±17.75	149.95±7.14	132.86±19.63
MCH (Pg)	52.11±4.70	49.35±5.04	42.16±6.35
MCHC (g/%)	30.97±2.21	32.63±1.94	31.75±1.64

^{ab} Means within rows with different superscripts are significantly different (P<0.05).

IV. DISCUSSION

Haematological parameters in birds have been shown to be influenced by various factors such as age, sex, diet, climatic conditions and the method of rearing [4]-[6]. In the present study, the haematological parameters were not significantly different in the different systems in the pullets and the values were in harmony with the normal range for healthy birds. PCV and Hb levels remained comparable indicating the birds on the pasture were not facing starvation at this stage of growth.

The variations observed in the haematological parameter of the laying hens is consistent with the previous studies performed with laying hens during the laying period where an alteration of blood biochemistry as well as blood haematology due to egg laying [2], [3] was reported. The enhanced hematological profile in laying hens raised on grass and legume pasture may be due to consumption of some bioactive nutrients in the pasture by the birds. It has been reported that high PCV values indicate an increase in the circulating red blood cells due to good nutrition and welfare [21], [22]. The higher values recorded in the birds on grass pasture at week 38 in the present study therefore suggests a better nutrition in the birds.

TABLE VIII
EFFECTS OF REARING SYSTEMS ON THE SERUM CHEMISTRY OF LAYERS

Parameters	Rearing systems		
	Deep litter	Legume pasture	Grass pasture
(Growing pullets at 18 weeks)			
Total Protein (g/dl)	4.55±0.69	4.05±0.11	3.75±0.46
Albumin (g/dl)	1.80±0.26	1.60±0.04	1.50±0.20
Globulin (g/dl)	2.75±0.41	2.45±0.06	2.25±0.29
Urea (mg/dl)	1.60±0.04	1.61±0.26	1.80±0.20
Creatinine (mg/dl)	0.15±0.02^{b}	0.20±0.04^{ab}	0.35±0.06^{a}
(Early production: 24 weeks old)			
Total Protein (g/dl)	4.78±0.35^{b}	5.63±0.23^{a}	5.05±0.09^{ab}
Albumin (g/dl)	2.53±0.21^{b}	3.43±0.23^{a}	2.43±0.08^{b}
Globulin (g/dl)	2.25±0.15^{ab}	2.20±0.08^{b}	2.63±0.11^{a}
Urea (mg/dl)	1.85±0.29	3.60±0.21	13.25±10.23
Creatinine (mg/dl)	0.68±0.11	0.60±0.13	0.43±0.11
(Peak production: 38 weeks old)			
Total Protein (g/dl)	5.15±0.35^{b}	6.13±0.19^{a}	4.50±0.24^{b}
Albumin (g/dl)	3.10±0.12^{ab}	3.43±0.15^{a}	2.83±0.18^{b}
Globulin (g/dl)	2.98±0.14^{a}	1.73±0.23^{b}	1.68±0.13^{b}
Urea (mg/dl)	3.98±0.19^{a}	4.40±0.12^{a}	2.90±0.27^{b}
Creatinine (mg/dl)	0.33±0.11	0.60±0.12	0.35±0.09
(Late production: 60 weeks old)			
Total Protein (g/dl)	4.20±0.13^{b}	5.35±0.26^{a}	4.75±0.26^{ab}
Albumin (g/dl)	3.15±0.09^{a}	2.08±0.19^{b}	2.70±0.13^{a}
Globulin (g/dl)	2.20±0.22	2.45±0.09	2.13±0.11
Urea (mg/dl)	2.850.11^{b}	2.45±0.09^{b}	4.65±0.56^{a}
Creatinine (mg/dl)	0.580.11	0.38±0.17	0.43±0.09

^{abcd} Means within rows with different superscripts are significantly different (P<0.05).

The RBC counts are influenced among other factors by nutrition, physical activities and volume and its reduction indicates anaemia. In this study, there was no clinical state of anaemic condition. This result is an indication that rearing hens on pasture had no negative effect on the blood parameters, but instead has the ability to improve these parameters. Most of the values obtained for haematological as well as the serum biochemical indices in this study fall within the normal literature values. This indicates that hens can be raised on either grass or legume pasture without a deleterious effect on their health status. The increase in RBC in the hens kept on legume pasture at peak phase of production may be an indication of higher protein intake [19].

The LYMs were not significantly different at the second and the last phases. This is suggesting that the hens in different rearing systems were not at risk of microbial poisoning due to access to runs. This is consistent with the findings of [30] who attributed the production of these granulocytes to stimulation of the reticulo-endothelia system by dietary endogenous toxic substances. These granulocytes are involved in providing the body with a defense against invading microorganisms. They are attracted in large number to any area of the body which has been invaded by microorganisms. Moreover, the non-significance of the WBC across treatments in the pullets and at the late phase of production in the layers indicates that consumption of forage plants did not impair the birds' ability to wade off infection [31]. The higher EOSNs recorded in the birds in the deep litter system at week 38 suggests a higher level of stress in the birds, as it has been reported that EOSNs disappear from circulation and BAS increase in circulation during stress [19].

The H:L ratio has been proposed as a reliable measure of stress in chickens [1]. Previous studies in chickens have suggested that an increased H:L ratio is associated with increased environmental stress [32] based on the supposition that environmental stressors induce an elevation in plasma corticosterone concentrations, which in turn, stimulates a rise in HET concentration. In the present study, hens without access to pasture exhibited significantly higher H:L ratios compared to the free-range hens at the early phase of production indicating some level of stress. This suggests that the hens in the deep litter were more stressed than those kept in the "free range". The similarity in H:L ratios in the hens in legume and grass pasture in this study suggests a similarity in their ability to cope with environmental stress.

The higher serum total protein in birds reared in LP throughout the egg production stages appears to be attributable to the protein utilization in legumes by the birds, as [18] reported that reduced serum total protein and globulin levels manifest as an alteration in normal systemic protein utilization. The improvement in serum protein in the birds in LP indicates a rise in amino acid absorption and utilization. On the contrary, our observation disagrees with the finding of [20] who reported parameters such as serum total protein, albumin and globulin were not significantly different in hens reared in alternative housing systems namely: Partitioned Conventional Cage, Extended Conventional Cage and Deep Litter System. The difference could be as a result of the fact that the birds in the present study had access to pasture. This suggests that the systemic protein utilization of the chickens was unaltered by the housing system but by the intake of pasture.

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