Effects of Feed Forms and Quantitative Feed Restriction on Blood Profile of Broiler Chickens

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Abstract

The use of different feed forms and nutrient restrictions are usually employed to reduce problems that accompany early-life fast growth rate in broilers, such as increased body fat deposition, high incidence of metabolic disorders, increased mortality, and high incidence of skeletal diseases. Therefore, a 56-day feeding trial was conducted to evaluate the effects of feed forms and levels of feed restriction on blood profile of broiler chickens at starter and finisher phases. One and ninety two 1-day-old broiler chicks (n=192) were divided into eight groups of 24 chicks each. Each group was further divided into three replicates of eight chicks per replicate in a completely randomized design. Birds were fed mash and pellets and restricted at 0, 10, 20 and 30% levels of feed restriction. Blood samples were collected and analysed for haematological and serum biochemical parameters at 4 and 8 weeks. The results showed that at the starter phase birds fed mash recorded significantly higher red blood cells ($2.57x10^{12}$/L) and cholesterol (196.67mg/dL) compared to those fed pellets ($2.12x10^{12}$/L and 188.25mg/dL). Packed cell volume and red blood cell increased with increasing levels of restriction in the finisher phase. The WBC was significantly higher (16.41 $x10^3/mm^3$*) in birds fed mash compared to the value recorded for pellets (15.53* $x10^3/mm^3$*) in the finisher phase. The total protein and albumin decreased with increasing level of restriction in mash treatment group. Serum cholesterol decreased with increasing levels of restriction and varied under pellet group. Birds fed mash ad libitum recorded highest blood cholesterol. It can be concluded that feeding pellets and limiting feed quantity had no deleterious effect on the haematological and serum biochemical parameters of broiler chickens.*

Keywords: blood, broilers, serum, pellets, quantitative

Running title: Effects of feed forms and levels of quantitative feed restriction on blood profile of broiler chickens

Effets des Formes d'Aliment et de la Restriction Alimentaire Quantitative sur le Profil Sanguin des Poulets de Chair

Résumé

L'utilisation de différentes formes d'aliment et la restriction nutritionnelle sont souvent employées pour réduire les problèmes associés à la croissance rapide en début de vie chez les poulets de chair, tels que l'augmentation du dépôt de graisse corporelle, une forte incidence de troubles métaboliques, une mortalité accrue et une prévalence élevée de maladies squelettiques. Ainsi, un essai d'alimentation de 56 jours a été mené pour évaluer les effets des formes d'aliment et des niveaux de restriction alimentaire sur le profil sanguin des poulets de chair aux phases de démarrage et de finition. Cent quatre-vingt-douze poussins de chair âgés d'un jour (n=192) ont été répartis en huit groupes de 24 poussins chacun. Chaque groupe a ensuite été subdivisé en trois répétitions de huit poussins par répétition selon un dispositif complètement randomisé. Les oiseaux ont été nourris avec de la farine et des granulés, et soumis à des niveaux de restriction alimentaire de 0, 10, 20 et 30 %. Des échantillons sanguins ont été prélevés et analysés pour les paramètres hématologiques et biochimiques sériques à 4 et 8 semaines. Les résultats ont montré qu'en phase de démarrage, les oiseaux nourris à la farine présentaient des taux significativement plus élevés de globules rouges (2,57 × 10^{12}/L) et de cholestérol (196,67 mg/dL) par rapport à ceux nourris aux granulés (2,12 × 10^{12}/L et 188,25 mg/dL). Le volume globulaire et le nombre de globules rouges ont augmenté avec l'augmentation des niveaux de restriction en phase de finition. Les leucocytes étaient significativement plus élevés (16,41 × $10^3/mm^3$) chez les oiseaux nourris à la farine que chez ceux nourris aux granulés (15,53 × $10^3/mm^3$) en phase de finition. Les protéines totales et l'albumine ont diminué avec l'augmentation du niveau de restriction dans le groupe traité à

la farine. Le cholestérol sérique a diminué avec l'augmentation des niveaux de restriction et a varié dans le groupe granulé. Les oiseaux nourris à la farine ad libitum ont enregistré le taux de cholestérol sanguin le plus élevé. On peut conclure que l'alimentation en granulés et la limitation de la quantité d'aliment n'ont pas eu d'effet délétère sur les paramètres hématologiques et biochimiques sériques des poulets de chair.

Mots-clés : sang, poulets de chair, sérum, granulés, restriction quantitative

Introduction

Feed processing provides an opportunity to improve broiler performance. Several authors have discovered effects of feed form (mash or pellet) on some blood parameters as total protein, albumin, serum ALT and aspartate aminotransferase (AST) (Andi *et al.* 2011, Corzo *et al*. 2012). Haematological profiles both in human and animal sciences are important indices of the physiological state (Khan and Zafar, 2005). Haematobiochemical indices are of immense importance in poultry production. The blood in the body mainly maintains physiological equilibrium but many physiological conditions may alter this equilibrium. Hence, the haematological values during different physiological situations could be used for diagnosis of various pathological and metabolic disorders, which can adversely affect the productive and reproductive performance of animals (Ahmad *et al*., 2003). Haematological and biochemical indices can be affected by nutrition, age, sex, genetics, physiological status, housing, starvation, environmental factors, stress, disease and transportation (Opara *et al*., 2006). Incidence of disease and malnutrition are diagnosed by the deviation from the normal reference values of the various serum biochemical and haematological parameters measured depending on the case being investigated. Evaluation and interpretation of the results obtained are dependent on the reference value for each animal species, in those regions under existing environmental conditions (Otto *et al*., 2000). Consequently, blood sampling for the assay of biochemical constituents and haematological traits are frequently employed in nutritional studies. Change in the constituent components of blood when compared to normal values could be used to interpret the metabolic state of the animal as well as the quality of feed (Babatunde *et al*., 1992). Wilson (1998) reported that haematological indices may be used to diagnose quantitative, morphologic and physiological alterations that might be associated with heat stress, such as changes in haematocrit, leukocyte counts and haemoglobin. According to Maxwell *et al*. (1990), haemoglobin was reduced when broilers were subjected to the stress caused by feed restriction, whereas the number of erythrocytes increased significantly. The increase in red cells may be due to higher release by the bone marrow, in order to compensate for the reduction in haemoglobin levels. Male broilers have higher erythrocyte counts, haemoglobin and haematocrit, but lower leukocyte counts than females, suggesting that males are more affected when submitted to a stress episode (Garcia-Pestana, 1992). Therefore, this study was conducted to evaluate the effect of feed forms and feed restriction on the haematological and serum biochemistry parameters of broiler chickens

Materials and methods

Experimental site

The experiment was carried out at the Poultry Unit, Directorate of University Farms (DUFARMs), Federal University of Agriculture, Abeokuta, Ogun State. This area is situated in the rainforest vegetation zone of South-western Nigeria on Latitude 7° 13´49.46´´N, Longitude 3° 26´ 11.98´´E and altitude of 98 m above sea level. The climate is humid with a mean precipitation of 1003 mm per annum (Google earth, 2013). Annual mean temperature and humidity ranges from 31.9 to 34.8°C and 79.7 to 90.1%, respectively.

Experimental birds and management

A total of 192, 1-day-old broiler chicks were purchased from a reputable hatchery and were assigned randomly into eight dietary treatments of three replicates with eight birds per replicate. The chicks were brooded for two weeks using charcoal pots. Daily routine management practices were carried out while scheduled vaccinations and medications for broilers were strictly followed. The experiment lasted 56 days.

Experimental diet

A diet of the same nutritional component were processed into mash and pellets. Pelletizing was done using a pellet mill calibrated to 2mm where the feed were conditioned and thermally treated in the fitted conditioners of a pellet mill as described by Aderibigbe *et al*., (2013). The birds were subjected to mash and pelletized feeding at 100, 90, 80 and 70% levels of feed requirement, respectively. Table 1 shows the percentage composition of the experimental diet.

Experimental design

The birds were assigned to two feed forms (mash and pellets) and four restriction levels: 0% restriction (control), 10% restriction (90% of daily feed requirement), 20% restriction (80% of daily feed requirement) and 30% restriction (70% of daily feed requirement) in a 2x4 factorial arrangement of a completely randomised design. Daily feed supply of each chick was predicated on the findings of Aduku (2004) that a broiler chick required 15g feed per day in the first week, 33g in the second week, 56g in the third week, 76g in the fourth week, 93g in the fifth week, 114g in the sixth week, 133g in the seventh week and 151g in the eighth week.

Table 1: Composition (%) of the experimental diet

Feed ingredients	**Percentage**
Maize	47.5
Soyabean meal	9.25
Fish meal (72% CP)	2.25
Groundnut cake	19.00
Palm kernel cake	1.00
Wheat offal	16.00
Oyster shell	1.00
Bone meal	3.00
Lysine	0.25
Methionine	0.25
Common salt	0.25
*Vit./ Min. Premix	0.25
Total	**100.00**
Calculated analysis:	
Crude protein (%)	20.88
Metabolisable energy (MJ/kg)	11.32
Crude fibre (%)	3.91
Ether extract (%)	4.14
L-Lysine (%)	1.13
DL-Methionine (%)	0.54

Calcium (%)	1.29
Phosphorus (%)	0.53

**Premix composition per kg diet: Vit A :400000IU, Vit D:80000IU, Vit E:40000ng, Vit k_3:800mg, Vit B_1:1000MG, Vit B_2:6000mg, Vit B_6:500mg, VitB12:25mg, Niacin:6000mg, Panthothenic acid:2000mg, Folic acid: 200mg, Biotin:8mg, Manganese:300000mg, Iron:8000mg, Zinc:20000mg, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:800000mg*

Data collection

Haematological and biochemical components determination

On the 28th and 56th day of the study, blood samples were collected from two birds per replicate for the determination of haematological, and serum parameters. A volume of 2.5mL of blood was collected into sample bottles containing ethylene diamine tetra acetic acid (EDTA). This was used to determine the haematological parameters; packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell (WBC) and its differentials and red blood cell (RBC) following standard procedures described by Baker and Silverton (1985). Another 2.5mL of blood was collected into sample bottles without anticoagulant for the determination of serum metabolites- serum total protein (TP), albumin, globulin, cholesterol and glucose as described by Baker and Silverton, (1985).

Statistical analysis

All data were subjected to two-way analysis of variance using SAS, (2010). Significant ($P<0.05$) differences among means were separated using Duncan's Multiple Range Test of the same statistical package.

Results

Effects of feed forms and levels of feed restriction on haematology and serum biochemistry of broiler chickens at the fourth week of age.

The main effects of feed forms and levels of feed restriction on haematology, serum biochemistry of broiler chickens at the fourth week of age are presented in Table 2. Feed forms significantly ($p<0.05$) affected the red blood cell concentration and cholesterol concentration. Birds fed mash recorded higher values for RBC ($2.57x10^{12}$/L) and cholesterol (196.67mg/dL) while birds fed pellets recorded lower values for RBC ($2.12x10^{12}$/L) and cholesterol (188.25mg/dL). However, packed cell volume, haemoglobin, white blood cell, total protein, albumin, globulin, glucose, heterophils, lymphocytes, H:L ratio, eosinophils, basophils and monocytes were not significantly ($p>0.05$) affected by feed forms.

The levels of feed restriction had significant effect ($p<0.05$) on values of packed cell volume, haemoglobin, total protein, albumin, cholesterol, heterophils, lymphocytes and H:L ratio. Packed cell volume values ranged from 31.00-34.44% with birds on *ad libitum* feeding recording the highest value while birds restricted at 20% recorded the lowest mean value. Highest mean values were obtained for total protein and heterophils in birds on feed restriction at 20%. Lowest PCV, Hb and lymphocytes values were obtained for broiler chickens on feed restriction at 20%. H:L ratio was highest at 20%, lowest at 30% while *ad libitum* fed birds and 10% restriction level shared similar ($p>0.05$) values. However, values obtained for RBC, WBC, globulin, glucose, eosinophil, basophil and monocytes were not significantly ($p>0.05$) affected by feed forms and levels of restriction.

Table 3 shows the interactive effects of feed forms and levels of feed restriction on haematology, serum biochemistry and stress index of broiler chickens at the fourth week of age. Feed forms and levels of restriction interaction had significant ($p<0.05$) effect on packed cell volume, haemoglobin, red blood cell, total protein, albumin, cholesterol, glucose, heterophils, lymphocytes and H:L ratio with birds fed mash and restricted at 10%

having the highest values for Hb (11.30g/dL), RBC ($2.83x10^{12}$/L), and glucose (260.33mg/dL) while lowest values were obtained for PCV and Hb in birds fed pellet feed restricted at 30%. Total protein and albumin had highest mean values in birds fed pellets *ad libitum* while lowest mean values were obtained in birds fed mash at 10% feed restriction.

Birds fed pellets and restricted at 20% level recorded highest H:L ratio while birds fed mash at 30% level recorded the lowest ratio.

Table 2. Main effects of feed forms and levels of feed restriction on haematology and serum biochemistry of broiler chickens at the fourth week of age

	Feed forms			Levels of restrictions (%)					Reference
Parameters	**Mash**	**Pellets**	**SEM**	**0**	**10**	**20**	**30**	**SEM**	**Value****
Packed cell vol (%)	33.33	31.75	0.76	34.44^{a}	33.50^{ab}	31.00^{b}	31.33^{ab}	1.07	22-35
Haemoglobin(g/dL)	10.72	9.92	0.29	10.90^{a}	11.03^{a}	9.63^{b}	9.70^{b}	0.41	7-13
Red blood cell ($x10^{12}$/L)	2.57^{a}	2.12^{b}	0.13	2.65	2.38	2.08	2.28	0.18	2.5-3.5
White blood cell ($x10^{3}/mm^{3}$)	13.08	14.33	0.79	14.90	13.00	13.52	13.42	1.12	12-30
Heterophils (%)	30.58	32.25	1.38	31.50^{ab}	30.50^{ab}	35.17^{a}	28.50^{b}	1.95	15-40
Lymphocytes (%)	68.25	67.08	1.34	67.67^{ab}	68.50^{ab}	64.00^{ab}	70.50^{a}	1.89	45-70
Eosinophils (%)	0.42	0.33	0.17	0.17	0.50	0.50	0.33	0.24	1.5-6
Basophils (%)	0.33	0.08	0.12	0.33	0.17	0.00	0.33	0.17	Rare
Monocytes (%)	0.42	0.25	0.19	0.33	0.33	0.33	0.33	0.26	5-10
Total protein (mg/dL)	4.77	5.04	0.15	5.13^{a}	4.36^{b}	5.20^{a}	4.91^{ab}	0.21	3-5
Albumin (mg/dL)	2.46	2.64	0.10	2.83^{a}	2.18^{b}	2.65^{a}	2.53^{ab}	1.15	2.1-3.5
Globulin (mg/dL)	2.31	2.40	0.11	2.30	2.18	2.55	2.38	0.15	1.4-3.0
Cholesterol(mg/dL)	196.67^{a}	188.25^{b}	2.91	194.17^{ab}	185.33^{b}	191.67^{ab}	198.67^{a}	4.12	129-297
Glucose (mg/dL)	255.25	243.08	3.57	244.67	248.33	249.00	254.67	5.05	197-299
H:L ratio	0.49	0.46	0.02	0.47^{ab}	0.45^{ab}	0.56^{a}	0.41^{b}	0.04	0.23-0.57

^{a,b,c,d}: Means in the same row within each factor not sharing common superscript are significantly different ($p<0.05$)

SEM: Standard error mean

** Ref Source: Jain (1993)

Table 3. Interactive effects of feed forms and levels of feed restriction on haematology and serum biochemistry of broiler chickens at the fourth week of age

Feed forms	Mash				Pellets					Reference
Levels of restriction	0	10	20	30	0	10	20	30	SEM	Value**
Packed cell vol (%)	35.33^{a}	33.67^{ab}	31.33^{ab}	33.00^{ab}	33.33^{ab}	33.33^{ab}	30.67^{ab}	29.67^{b}	1.52	22-35
Haemoglobin (g/dL)	11.10^{ab}	11.30^{a}	10.03^{abc}	10.43^{abc}	10.70^{abc}	10.76^{abc}	9.23^{bc}	8.97^{c}	0.58	7-13
Red blood cells (x10^{12}/L)	2.73^{ab}	2.83^{a}	2.37^{abc}	2.37^{abc}	2.57^{abc}	1.93^{bc}	1.80^{c}	2.20^{abc}	0.26	2.5-3.5
White blood cells (x10^{3}/mm^{3})	13.13	11.43	13.67	14.00	16.67	14.57	13.57	12.83	1.59	12-30
Heterophils (%)	31.33^{ab}	33.00^{ab}	32.67^{ab}	25.33^{b}	31.67^{ab}	28.00^{ab}	37.67^{a}	31.67^{ab}	2.76	15-40
Lymphocytes (%)	67.67^{ab}	66.00^{ab}	66.00^{ab}	73.33^{a}	67.67^{ab}	71.00^{ab}	62.00^{b}	67.00^{ab}	2.68	45-70
Eosinophils (%)	0.00	0.33	1.00	0.33	0.33	0.67	0.00	0.33	0.33	1.5-6
Basophils (%)	0.67	0.33	0.00	0.33	0.00	0.00	0.00	0.33	0.24	Rare
Monocytes (%)	0.33	0.33	0.33	0.67	0.33	0.33	0.33	0.00	0.37	5-10
Total protein (mg/dL)	4.83^{ab}	4.24^{ab}	5.17^{ab}	4.84^{ab}	5.43^{a}	4.50^{ab}	5.23^{a}	5.00^{ab}	0.29	3-5
Albumin (mg/dL)	2.73^{ab}	2.07^{b}	2.67^{ab}	2.37^{ab}	2.93^{a}	2.30^{ab}	2.63^{ab}	2.70^{ab}	0.21	2.1-3.5
Globulin (mg/dL)	2.10	2.17	2.50	2.47	2.50	2.20	2.60	2.30	0.21	1.4-3.0
Cholesterol (mg/dL)	194.00^{ab}	191.67^{ab}	199.33^{a}	201.67^{a}	194.33^{ab}	179.00^{b}	184.00^{ab}	195.67^{ab}	5.83	129-297
Glucose (mg/dL)	258.67^{a}	260.33^{a}	242.67^{ab}	259.33^{a}	230.67^{b}	236.33^{ab}	255.33^{a}	250.00^{ab}	7.15	197-299
H:L ratio	0.46^{ab}	0.51^{ab}	0.51^{ab}	0.35^{b}	0.47^{ab}	0.40^{b}	0.61^{a}	0.47^{ab}	0.10	0.23-0.57

^{a,b,c,d}: Means in the same row not sharing common superscript are significantly different ($p<0.05$)

SEM: Standard error mean

** Ref Source: Jain (1993)

Effects of feed forms and levels of feed restriction on haematology and serum biochemistry of broiler chickens at the eight week of age.

The main effects of feed forms and levels of feed restriction on haematology, serum biochemistry and stress index of broiler chickens at the eight week of age are presented in Table 4. Feed forms significantly ($p<0.05$) affected the birds' packed cell volume, haemoglobin, red blood cell, white blood cell, albumin and glucose. Birds fed pellets recorded higher PCV, Hb, RBC, albumin and glucose while birds fed mash recorded lower values for these parameters. WBC was higher (16.41 $x10^3/mm^3$) in birds fed mash compared to the value recorded for pellets (15.53 $x10^3/mm^3$).

Levels of restriction significantly ($p<0.05$) affected most parameters analysed with the exception of globulin and basophil with birds fed *ad libitum* having the highest WBC, TP, albumin, cholesterol and eosinophil values and the lowest PCV, Hb, RBC, glucose and monocyte values. Packed cell volume, Hb, RBC and heterophil were highest in birds restricted at 30%. Similar and lowest values were obtained for albumin in birds restricted at 20% and 30%. Eosinophil was also highest and similar ($p<0.05$) for birds fed *ad libitum* and restricted at 10%. Birds restricted at 10% had the highest H:L ratio.

Table 5 shows the results of the interactive effects of feed forms and levels of feed restriction on haematology, serum biochemistry and stress index of broiler chickens at the eight week of age. Feed forms and levels of restriction had significant ($p<0.05$) effect on packed cell volume, haemoglobin, red blood cell, white blood cell, total protein, albumin, cholesterol, glucose, heterophil, lymphocytes, H:L ratio, eosinophil and monocytes. Highest PCV, Hb and RBC values were noted in birds fed pellets at 30% restriction while lowest values of the same parameters were obtained in birds fed mash *ad libitum*. Statistically similar and highest ($p<0.05$) value of 16.67 was obtained for WBC in birds fed mash at *ad libitum* and in birds fed mash and restricted at 10%.

Total protein recorded highest and similar values in birds fed mash at *ad libitum* (5.97mg/dL) and in birds fed pellets and restricted at 10% (5.90mg/dL). Lowest values for TP, albumin, cholesterol and heterophil were obtained for birds fed mash at 30% restriction level. H:L ratio was highest in birds fed mash at 10% levels of restriction but was lowest in birds fed mash at 30% level. Other values varied among treatment groups. However, globulin and basophil values were not significantly ($p>0.05$) affected by the interaction between feed forms and levels of restriction.

Table 4. Main effects of feed forms and levels of feed restriction on haematology, serum biochemistry in broiler chickens at the eight week of age

	Feed forms			Levels of restriction (%)					Ref
Parameters	**Mash**	**Pellets**	**SEM**	**0**	**10**	**20**	**30**	**SEM**	**Value****
Packed cell vol (%)	34.25^{b}	38.50^{a}	0.37	33.83^{b}	36.83^{a}	37.00^{a}	37.83^{a}	0.53	22-35
Haemoglobin (g/dL)	11.51^{b}	12.79^{a}	0.13	11.27^{b}	12.33^{a}	12.30^{a}	12.70^{a}	0.18	7-13
Red blood cells (x10^{12}/L)	1.78^{b}	1.99^{a}	0.02	1.77^{b}	1.90^{a}	1.92^{a}	1.95^{a}	0.03	2.5-3.5
White blood cells (x10^{3}/mm^{3})	16.41^{a}	15.53^{b}	0.12	16.62^{a}	15.63^{b}	15.70^{b}	15.93^{ab}	0.17	12-30
Heterophils (%)	48.33	49.50	0.82	45.00^{b}	60.00^{a}	47.50^{b}	42.67^{b}	1.15	15-40
Lymphocytes (%)	48.83	46.58	0.71	50.67^{a}	37.17^{b}	48.83^{a}	54.17^{a}	1.09	45-70
Eosinophils (%)	1.58	1.92	0.16	2.17^{a}	2.17^{a}	0.83^{b}	1.83^{a}	0.22	1.5-6
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Rare
Monocytes (%)	0.83	0.42	0.16	0.17	0.50	1.00	0.83	0.22	5-10
Total protein (mg/dL)	5.67	5.78	0.04	5.90^{a}	5.85^{a}	5.46^{c}	5.61^{b}	0.06	3-5
Albumin (mg/dL)	3.02^{b}	3.14^{a}	0.03	3.28^{a}	3.07^{b}	2.98^{b}	2.98^{b}	0.05	2.1-3.5
Globulin (mg/dL)	2.67	2.64	0.05	2.62	2.78	2.58	2.63	0.70	1.4-3.0
Cholesterol(mg/dL)	206.25	204.42	0.82	214.67^{a}	200.33^{b}	204.83^{ab}	201.50^{b}	1.15	129-297
Glucose (mg/dL)	165.08^{b}	170.00^{a}	0.36	163.67^{b}	172.50^{a}	166.50^{b}	167.50^{b}	0.51	197-299
H:L ratio	1.11	1.12	0.08	0.90^{b}	1.62^{a}	1.01^{b}	0.91^{b}	0.07	0.23-0.57

^{a,b,c,}: Means in the same row not sharing common superscript are significantly different ($p<0.05$)

SEM: Standard error mean** Ref Source: Jain (1993)

Table 5. Interactive effects of feed forms and levels of feed restriction on haematology and serum biochemistry in broiler chickens at the eight week of age

Feed forms	**Mash**				**Pellets**					**Reference**
Levels of restriction	**0**	**10**	**20**	**30**	**0**	**10**	**20**	**30**	**SEM**	**Value****
Packed cell vol (%)	30.33^{d}	36.33^{bc}	35.33^{c}	35.00^{c}	37.33^{bc}	37.33^{bc}	38.67^{ab}	40.67^{a}	0.74	22-35
Haemoglobin (g/dL)	10.20^{d}	12.20^{bc}	11.73^{c}	11.90^{c}	12.33^{bc}	12.47^{bc}	12.87^{ab}	13.50^{a}	0.26	7-13
Red blood cells ($x10^{12}$/L)	1.58^{c}	1.88^{bcd}	1.84^{cd}	1.82^{d}	1.96^{bc}	1.92^{bcd}	2.00^{ab}	2.09^{a}	0.04	2.5-3.5
White blood cells ($x10^{3}$/mm^{3})	16.67^{a}	16.67^{a}	16.33^{a}	15.97^{a}	16.57^{a}	14.60^{b}	15.07^{b}	15.90^{b}	0.25	12-30
Heterophils (%)	49.00^{c}	61.00^{a}	54.33^{b}	29.00^{e}	42.00^{d}	59.00^{ab}	40.67^{ad}	56.33^{ab}	1.63	15-40
Lymphocytes (%)	49.00^{c}	35.33^{e}	43.00^{d}	68.00^{a}	52.00^{ab}	39.00^{de}	54.67^{b}	40.33^{d}	1.55	45-70
Eosinophils (%)	2.33^{a}	2.33^{a}	0.00^{b}	1.67^{a}	2.00^{a}	2.00^{a}	1.67^{a}	2.00^{a}	0.31	1.5-6
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Rare
Monocytes (%)	0.00^{b}	0.67^{ab}	1.33^{a}	1.33^{a}	0.33^{ab}	0.33^{ab}	0.67	0.33	0.31	5-10
Total protein (mg/dL)	5.97^{a}	5.80^{a}	5.50^{b}	4.47^{b}	5.84^{a}	5.90^{a}	5.64^{b}	5.77^{a}	0.08	3-5
Albumin (mg/dL)	3.30^{a}	3.00^{cde}	2.90^{de}	2.87^{e}	3.27^{ab}	3.13^{abc}	3.07^{bcde}	3.10^{abcd}	0.07	2.1-3.5
Globulin (mg/dL)	2.67	2.80	2.60	2.60	2.57	2.77	2.57	2.67	0.10	1.4-3.0
Cholesterol (mg/dL)	227.33^{a}	203.33^{c}	198.00^{de}	196.33^{e}	202.00^{cd}	197.33^{de}	211.67^{b}	206.67^{c}	1.63	129-297
Glucose (mg/dL)	157.33^{f}	173.33^{a}	163.67^{e}	166.00^{d}	170.00^{bc}	171.67^{ab}	169.33^{c}	169.00^{c}	0.73	197-299
H:L ratio	1.00^{d}	1.74^{a}	1.27^{c}	0.43^{f}	0.80^{de}	1.51^{ab}	0.75^{e}	1.40^{bc}	0.09	0.23-0.57

^{a,b,c,d}: Means in the same row not sharing common superscript are significantly different ($p<0.05$) SEM: Standard error mean

** Ref Source: Jain (1993)

Discussion

In this study, at the starter phase birds fed mash recorded higher red blood cells and cholesterol compared to those fed pellets. Other blood indices although not significantly different, fell within the normal range of haematological and serum biochemical values of poultry showing that there was no incidence of anemia (Waugh and Grant, 2007). Red blood cell was reduced in the finisher phase compared to the starter phase. Packed cell volume and red blood cell increased with increasing levels of restriction in the finisher phase. Haemoglobin concentration was higher in birds fed mash although not significantly different in starter phase but was higher in pellets in the finisher phase. It varied across levels of restriction in both starter and finisher phases. The concentration increased with increasing levels of restriction in pellets at the finisher phase. This could be as a result of reduced number of RBC per unit of blood according to Khan and Zafar (2005). The improvement in the PCV and Hb contents of the birds could be an indication of an increment in the oxygen-carrying capacity of the animal's blood as reported by Fasuyi and Arire (2015). The major functions of the white blood cells and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to transport and distribute antibodies for immune purpose. Animals with high counts of WBC are capable of generating antibodies and have high degree of resistance to diseases (Soetan *et al*., 2013) and enhance adaptability to local environment and disease prevalent conditions (Kabir *et al*., 2011; Okunlola *et al*., 2012 and Isaac *et al*., 2013).

White blood cell counts were not significantly affected by feed form and level of restriction in the starter phase but were affected in the finisher phase where birds fed mash had higher WBC counts compared to pellets. This indicates the capability of birds to generate antibodies for their defense mechanism.

Heterophil and lymphocyte counts were not significantly affected by feed form but varied across level of restriction and were within range as stated by Jain (1993). Feed form also had no effect in the finisher phase but values varied across levels of restriction. Heterophil count values were higher than the normal range. According to Talebi *et al*. (2005), heterophils and lymphocytes and their ratio had been used as serious indicators of stress response and sensitive biomarkers crucial to immune function. Lymphocyte values were within normal range (Jain, 1993) except for birds restricted at 10% that recorded 37.17% as against the minimum of 45.00% as stated by Jain (1993).

Biochemical parameters generally can be used to monitor protein quality of feeds, they also contribute to the viscosity and maintenance of normal blood pressure and pH (Ladokun *et al.*, 2008). Total protein (TP) and albumin were higher at finisher phase compared to starter phase. Values obtained were within normal range (Jain, 1993). Corzo *et al*. (2012) reported that blood glucose was not significantly affected but TP and albumin were higher in broilers fed pellets than mash. In the finisher phase, feed form had no effect on TP and globulin while albumin was affected. Birds fed pellets recorded higher albumin. Levels of restriction also had effect on TP and albumin, with albumin decreasing with increasing level of restriction. The interaction effect gave higher TP, albumin and globulin than the standard range. TP and albumin decreased with increasing level of restriction in mash treatment group. Cholesterol, a metabolic precursor of steroid hormones is synthesized in the liver or absorbed directly from the diet (Wilson, 1998). Several metabolic disorders are as a result of increase in cholesterol levels due to fatty foods (Harcourt-Brown, 2004). The blood cholesterol values were higher in birds fed mash compared to pellets. This is in contrast to the report of Attia *et al*. (2014) that higher cholesterol in birds fed pellets compared to mash. Santoso (2001) reported low cholesterol in restricted birds compared to birds fed *ad libitum*. This agrees with present results. This is in contrast to the findings of Church and Pond (1988), who indicated that meal-fed or starved chickens

have been shown to have higher plasma cholesterol than control birds Blood glucose values varied across levels of restriction under mash and pellets but reduced in finisher phase as compared to starter phase.

In the finisher phase, serum cholesterol decreased with increasing levels of restriction and varied under pellet group. Birds fed mash *ad libitum* recorded highest blood cholesterol which may be connected to higher feed intake leading to higher weight gain and fat deposition (Shaidullah *et al*., 2008 and Rezaeipour *et al*., 2012). Attia *et al*. (2014) reported higher glucose in birds fed pellets compared to mash which contradicts present results.

Conclusion

Arising from improvement in most haematobiochemical indices, changing the form of feed to pellets and limiting feed quantity to up 70% had no deleterious effect on the haematological and serum biochemical parameters of finisher broiler chickens.

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