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Performance, haemato-biochemical indices and antioxidants status of broiler chickens fed diets supplemented with avocado-bamboo composite leaf mix

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Abstract

Phytogenic feed additives have been suggested to augment nutrient utilization in the gastrointestinal tract by enhancing production of digestive secretions and enzymatic activity. Such effects on gastrointestinal morphology have been postulated to increase the nutrient digestibility in poultry. However, research on avocado—bamboo composite mix on broiler chickens are limited, this thus forms the focus of this study. A six week feeding trial was conducted to evaluate the growth, haemato-biochemical indices and antioxidants status of broiler chickens fed varying levels of composite leaf mix (CLM). The avocado and bamboo leaves used were manually harvested, air-dried and milled to produce leaf meal. The two leaf meals were then mixed in ratio 1:1 to form the CLM. The CLM was included in broiler basal diets at 0, 4, 8 and 12g/kg levels designated diets I – IV, respectively. Ninety six day-old Cobb broiler chicks were randomly allotted to four treatments of three replicates with eight chicks each. At the end of the feeding trial, the result revealed that average final weight of chicken fed diets II (2533.33±66.67g) and III (2406.55±30.59g) were similar but higher than those fed the control (2216.67±29.83g). The same pattern was observed for total weight gain. The feed conversion ratio (FCR) was not affected although birds fed diet II had the best FCR (2.08±0.06).No significant (P>0.05) difference was observed in all the haematological indices save mean cell volume and mean cell haemoglobin which was highest in birds fed 4g/kg CLM supplemented diet (149.87±24.73fL and 49.95±8.25 pg/cell, respectively) as compared to 111.32±16.56fL and 37.12±5.55pg/cell in those supplemented 12g/kg CLM. The same pattern was repeated in percentage lymph .Among the serum biochemistry parameters measured, only Aspartate transaminase was affected and highest in birds fed diet supplemented with 4g/kg CLM (102.52±5.88µ/L) and lowest in those fed 12g/kg CLM (99.33±5.13µ/L). The superoxide dismutase, catalase and glutathione peroxidase increased with increased CLM supplementation. The growth, haematological, serum biochemical and antioxidant parameters obtained from this study suggested that the CLM has no adverse effect on the health status of broiler chickens but on the other hand improved the growth, blood serum and serum antioxidant profiles.

Keywords: *Haematology; serum biochemistry; antioxidant; Cobbs; composite leaf mix*

Performances, indices hémato-biochimiques et statut antioxydant des poulets de chair nourris avec des régimes supplémentés avec un mélange de feuilles composites d'avocat et de bambou

Résumé

Des additifs alimentaires phytogéniques ont été suggérés pour augmenter l'utilisation des nutriments dans le tractus gastro-intestinal en améliorant la production de sécrétions digestives et l'activité enzymatique. De tels effets sur la morphologie gastro-intestinale ont

été postulés pour augmenter la digestibilité des nutriments chez la volaille. Cependant, les recherches sur le mélange composite avocat-bambou sur les poulets de chair sont limitées, c'est donc l'objet de cette étude. Un essai d'alimentation de six semaines a été mené pour évaluer la croissance, les indices hémato-biochimiques et le statut en antioxydants de poulets à griller nourris à des niveaux variables de mélange de feuilles composites (MFC). Les feuilles d'avocat et de bambou utilisées ont été récoltées manuellement, séchées à l'air et broyées pour produire de la farine de feuilles. Les deux farines de feuilles ont ensuite été mélangées dans un rapport 1:1 pour former le MFC. Le MFC a été inclus dans les régimes de base des poulets de chair à des niveaux de 0, 4, 8 et 12 g/kg désignés régimes I à IV, respectivement. Quatre-vingt-seize poussins de chair Cobb âgés d'un jour ont été répartis au hasard dans quatre traitements de trois répétitions avec huit poussins chacun. À la fin de l'essai d'alimentation, le résultat a révélé que le poids final moyen des poulets nourris avec les régimes II (2533,33 ± 66,67 g) et III (2406,55 ± 30,59 g) était similaire mais supérieur à celui des poulets nourris avec le témoin (2216,67 ± 29,83 g). La même tendance a été observée pour le gain de poids total. Le taux de conversion alimentaire (TCA) n'a pas été affecté bien que les oiseaux nourris avec le régime II aient eu le meilleur TCA (2,08 ± 0,06). Aucune différence significative (P>0,05) n'a été observée dans tous les indices hématologiques, sauf le volume cellulaire moyen et l'hémoglobine cellulaire moyenne qui le plus élevé chez les oiseaux nourris avec 4 g/kg de régime supplémenté en MFC (149,87 ± 24,73 fL et 49,95 ± 8,25 pg/cellule, respectivement) par rapport à 111,32 ± 16,56 fL et 37,12 ± 5,55 pg/cellule chez ceux supplémentés en 12 g/kg de MFC. Le même schéma a été répété en pourcentage de lymphe. Parmi les paramètres biochimiques sériques mesurés, seule l'aspartate transaminase était affectée et la plus élevée chez les oiseaux nourris avec un régime supplémenté de 4 g/kg de MFC (102,52 ± 5,88 µ/L) et la plus faible chez ceux nourris avec 12 g/kg de MFC (99,33 ± 5,13 µ/L). La superoxyde dismutase, la catalase et la glutathion peroxydase ont augmenté avec l'augmentation de la supplémentation en MFC. Les paramètres de croissance, hématologiques, biochimiques sériques et antioxydants obtenus à partir de cette étude suggèrent que le MFC n'a pas d'effet néfaste sur l'état de santé des poulets de chair mais améliore en revanche les profils de croissance, de sérum sanguin et d'antioxydants sériques.

Mots-clés: Hématologie, biochimie sérique, antioxydant, Cobbs, mélange de feuilles composites

Introduction

Despite the observed improvement in broiler performance owing to the use of growth promoters, the use of antibiotic growth promoters has been criticized due to its possible role in the occurrence of antimicrobial resistance in humans. This led to the Directive 1831/2003 of the European Parliament, issued on 22nd September 2003, on the ban of the use of all antibiotics and chemotherapeutic drugs as growth promoters in the European Union as of January 1st, 2006. This new context caused an increase in the search for alternative growth promoters in which leaf supplements play a major role. Plants generally contain various active chemical compounds in the entire plant or in specific parts of the plant that confers them therapeutic activity or beneficial effects which can be taken advantage of in improving animal production for increase benefits of mankind. These substances contained in plants have low molecular weight and are derived from the plant secondary metabolism, and these are generally called antioxidants (Huyghebaert, 2003). Blood serves as a

major function of transporting nutrients absorbed from the digestive system or released from storage in adipose tissues or in liver. The haematological parameters are known to affect health, production and adaptability to environmental conditions in livestock (Adenkola *et al*., 2011). Haematological and serum biochemical responses are usually used to assess the clinical and physiological responsiveness and well-being of broiler chickens (Sharma *et al.*, 2015). This is because health status of chickens is paramount in the performance of chickens (Yang *et al.*, 2009) and general well-being of the chickens (Sharma *et al.*, 2015). Haematological and serum biochemical parameters are known to be usually influenced by feed, medication, toxic compounds, infections, age and sex of the birds (Schmidt *et al.*, 2009). The full blood count commonly examines the cellular components of blood while biochemical testing focuses on its chemical constituents (Hrubec *et al.,* 2002). It has been shown that data from haematological profiles could be exploited in the improvement of broiler chicken stocks (Ladokun *et al.,*2008) and in the diagnoses of specific poultry pathologies and might serve as basic knowledge for studies in immunology and comparative avian pathology (Bonadiman, 2009). Plants have the major advantage of still being used as the most effective and cheaper alternative sources of drugs (Latha and Kannabiran, 2006). According to Bibitha *et al*.(2002) the local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa. Many plants are consumed as food, without the in-depth knowledge of their exact chemical composition and contribution to health, although their utilization had passed through several ancestral generations who probably realized from experience that those plant food materials were beneficial (Coe *et al.,*2005). This study therefore aims at underscoring the growth and health implications of utilizing composite leaf mix of avocado and bamboo in broiler chickens production.

Materials and methods

Study area

The study was be carried out at the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure (FUTA), Nigeria and further laboratory analyses carried out at the Central Research Laboratory of FUTA.

Test ingredients preparation

The test ingredients; avocado and bamboo leaves were harvested fresh from maturing stems, air-dried and milled to powder using a commercial feed milling machine. Thereafter, leaf meals were then mixed in ratio one to one (1:1) to produce avocado-bamboo leave composite (CLM).

Experimental birds

A total number of one hundred and sixty (160) day-old broiler chicks of Cobb's breed were used in the experiment. The birds distributed randomly into four dietary treatment groups (0, 4, 8 and 12 g/kg) of composite leaf mix (CLM). Each treatment was replicated in four places, ten birds per replicate labeled diet I-IV, respectivelyin a completely randomizeddesign.

Experimental diets

The gross compositions of the experimental diet are presented in Table 1.

The basal diets were formulated for broiler chickens to meet requirements for broilers and then, divided into four diets. Diet 1, control diet (diet without any supplementation), Diets 2,3 and 4 contained 4, 8 and 12 g/kg of CLM, respectively.

Data collection

At the end of the study, prior to blood collection, the birds were starved of feed, two (2) birds per replicate were randomly selected and bled by severing the jugular vein. About 5 ml of blood samples were

collected from each bird in a two different labeled vacutainer tubes without anticoagulants and were taken to the laboratory for hematological indices analysis, serum biochemistry test and science laboratory for antioxidant enzyme determination. The serum biochemistry indices tested were cholesterol, creatinine, aspartate transaminase, alanine transaminase, albumin, globulins and total protein. The cholesterol was determined as outlined by Roschlau *et al.*(1974), creatinine was assessed by the colometric method described by Newman and Price (1999). Aspartate transaminase and alanine transaminase levels were determined as described by Huang *et al*. (2006). The total protein was determined using the method described by Peters (1968). The antioxidant enzymes such as glutathione peroxidase (GSH), superoxide dismutase (SOD) and catalase (CAT) concentrations were determined using the methods of Beutler *et al*. (1963). Flavonoid was determined using Bohm and Koupai-Abyazani (1994) method, Phytate, phenol and proximate composition of the avocado and bamboo leaf meals and its composites was determined using the method of AOAC (2012).

Statistical analysis

All data collected were subjected to Analysis of Variance (ANOVA) using SPSS statistical package (SPSS 17.0) and Duncan's Multiple Range Test was used to separate significant mean differences which were considered at 95 % level.

Table 1: The gross composition of the basal diet (%)

Ingredients	Quantity (g/kg)
Maize	550.0
Groundnut cake	160.0
Soybean meal	174.5
Wheat offal	35.0
Fish meal	30.0
Premix	2.5
Dicalcium phosphate	10.0
Limestone	15.0
Methionine	1.5
Oil	15.0
Salt	3.0
Lysine	3.5
Total	1000
Calculated analysis	
Crude Protein	21.0
Metabolizable Energy	3000
Calcium	11.0
Phosphorus	9.0

Results

Antioxidant properties of leaf meals and the composite

The antioxidants properties of avocado, bamboo and composite leaf mix is as presented on Table 5. The results show that CLM, avocado and bamboo leaf meals had a mean value of 1.07 mg/100g for phenol content and 37.67% DDPH with the coefficient of variation of 3.74% and 5.90%, respectively. Avocado leaf meal contains the highest phenol (1.12mg/100g) as compared to CLM which recorded the least (1.04mg/100g). DDPH was also highest in avocado leaf meal (39.3%) while bamboo had the least (34.91%) with a mean value of 37.67%.

Performance of broiler chickens fed with supplementary composite

Table 2 shows the performance of broiler

Table 1: Antioxidant property of avocado, bamboo and its composite leaf meal

	Treatment					
Parameter	A	B	CLM	Mean	Stdev.	CV
phenol mg/100g	1.12	1.07	1.04	1.07	0.04	3.74
DDPH %	39.3	34.82	38.91	37.67	2.22	5.89

A: avocado leaf meal, B: bamboo leaf meal, CLM: composite leaf meal, Stdev: standard deviation, CV; coefficient of variation

Table 2: Performance of broiler chickens fed with various levels of composite leaf meal of bamboo and avocad

TREAMENT	Diet				pValue
	1	2	3	4	
	0g/kg	4g/kg	8g/kg	12g/kg	
DAY 1 – 7					
INWT	41.92±2.03	42.11±0.53	41.92±0.45	42.08±0.29	0.999
FWT	342.28±25.19	311.3±15.72	349.11±8.18	356.53±6.58	0.266
TFC	578.4±16.86	517.96±57.88	514.12±17.68	525.88±16.34	0.504
TWG	300.37±24.92	269.2±15.27	307.19±7.81	314.44±6.84	0.254
FCR	1.96±0.23	1.91±0.1	1.67±0.03	1.67±0.02	0.291
DAY 1 – 21					
INWT	41.92±2.03	42.11±0.53	41.92±0.45	42.08±0.29	0.999
FLWT	651.54±55.96	582.41±28.86	611.24±6.98	648.4±12.62	0.427
TFC	1245.48±28.39	1100.54±87.55	1134.3±23.07	1176.19±38.33	0.289
TWG	609.63±54.58	540.3±28.58	569.32±7.09	606.32±12.67	0.410
FCR	2.08±0.23	2.03±0.06	1.99±0.06	1.94±0.04	0.865
DAY 1 – 42					
INWT	41.92±2.03	42.11±0.53	41.92±0.45	42.08±0.29	0.999
FLWT	2216.67±29.83 ^{c}	2533.33±66.67 ^{a}	2406.55±30.59^{ab}	2333.33±11.02^{bc}	0.003
TFC	5654.82±119.72	5180.45±147.57	5417.59±104.99	5480.88±275.70	0.558
TWG	2174.75±28.25^{c}	2491.23±67.1^{a}	2364.63±30.58^{ab}	2291.25±10.75^{bc}	0.003
FCR	2.6±0.02	2.08±0.06	2.29±0.06	2.39±0.12	0.341

INWT; initial weight, FLWT; final weight, TFC; feed intake, TWG; weight gain and FCR; feed conversion ratio.

chickens fed with supplementary composite leaf meal of bamboo and avocado. The results at age 1-7 and 1-21 days shows no significant difference across the dietary treatments but birds fed 8 and 12 g/kg CLM had an improved average final weight (FLWT), average weight gain (TWG) and feed conversion ratio (FCR). At day 42, results reviewed a significant ($p<0.05$) difference among birds fed the test diets. Birds fed diet containing CLM showed the best FLWT (2333.33±11.02 - 2533.33±66.67g) and TWG (2291.25±10.75 - 2491.23±67.1g) as compared to birds fed control diet (2216.67±29.83g and 2174.75±28.25g, respectively). Though there was no significant ($p>0.05$) difference in FCR, birds fed diet containing CLM showed the best FCR (2.08±0.06 – 2.39±0.02) compared 2.60±0.02 of those birds fed control diet

Haematological parameters of broiler chickens fed diets supplemented with CLM

In all the hematological parameters measured, only mean corpuscular haemoglobin, mean cell volume and Lymph were significantly ($p<0.05$) influenced by the dietary treatments (Table 1). Highest mean cell haemoglobin (49.95±8.25pg/cell) was noted in bird fed diet containing 4gCLM/kg feed when compared to those fed diet containing 12gCLM/kg (37.12±5.55 pg/cell). Same pattern was noted in the mean cell volume (149.87±24.73fLand111.32±16.56fL, respectively). The highest percentage lymph (79.67±5.72%) was also recorded in birds fed diet containing 4gCLM/kg feed, and lowest in birds fed diet containing 12gCLM/kg feed (70.67±7.79%)

Serum biochemical indices of broiler chickens fed diets supplemented with CLM

The serum biochemical indices of broiler chickens fed diets containing varying levels of composite leaf mix (Table 2) revealed that among all parameters measured, only aspartate aminotransferase was significantly ($p<0.05$) influenced by the

dietary treatments. Birds fed Diet II (diet supplemented with 4g/kg CLM) recorded the highest aspartate minotransferase (112.52±5.88) than those fed diet IV (diet supplemented with 12g/kg CLM). Cholesterol level was not significantly different but was lowest in birds fed diet with 8g/kg CLM (4.54±0.41) than those fed diet with 12g/kg CLM 5.51±0.29.

Antioxidant properties of broiler chicken fed diets supplemented with CLM

Table 3 shows the antioxidant properties of broiler chicken fed diets containing varying levels of the composite leaf mix. All parameters measured were not significantly ($p>0.05$) influenced by the dietary treatments. Although the highest Superoxide dismutase (0.97±0.4) and Glutathione (0.28±0.02) were observed in birds fed Diet III (8g/kg CLM), while lowest superoxide dismutase (0.68±0.22) and Glutathione (0.21±0.05) were recorded in birds fed Diets I and IV, respectively. Highest catalase (11.88±4.18) was observed in birds fed Diet II, while lowest catalase (6.05±0.61) was observed in bird fed Diet IV.

Table 3: Haematological indices of Cobbs fed diets with various levels of supplementary CLM

Parameter	Diets				pValue
	1 (0g/kg)	2 (4g/kg)	3 (8g/kg)	4 (12g/kg)	
PCV %	37.17±5.88	43.33±3.98	37.17±7.28	37.17±4.07	0.162
RBC (x10 6/L)	2.88±0.47	2.95±0.48	3.03±0.77	3.38±0.47	0.443
MCHC (g/dL)	33.3±0	33.3±0	33.3±0	33.3±0	0.150
MCV(fL)	132.87±34.74^{ab}	149.87±24.73^{a}	125.65±25.7^{ab}	111.32±16.56^{a}	0.015
MCH(pg/cell)	44.3±11.57^{ab}	49.95±8.25^{a}	41.88±8.58^{ab}	37.12±5.55^{a}	0.016
Haemoglobin (Hbg/dL)	12.4±1.95	14.45±1.35	12.38±2.44	12.4±1.36	0.164
WBC (x 10 9/L)	4.9±2.69	5±1.95	6.05±1.55	5.55±2.9	0.817
Granulocytes (x10 9/L)	1±0.8	1±0.68	1.58±0.77	1.3±0.73	0.490
GRA %	19±6.42	18.67±6.41	25.5±8.38	24.83±9.58	0.295
Lymphocytes (x10 9/L)	3.8±1.93	3.93±1.32	4.48±1.22	4.07±2.35	0.916
LYMP %	79±6.51^{ab}	79.67±5.72^{a}	74.33±6.41^{ab}	70.67±7.79^{b}	0.045
Monocytes(x10 9/L)	0.1±0.06	0.08±0.04	0.07±0.08	0.18±0.17	0.236
MON %	2±0.63	1.67±0.82	1.5±1.52	4.5±4.32	0.126

Table 4: Serum indices of Cobbs broiler chickens fed with various levels supplementary CLM

TREATMENT	Diet				pValue
	1 (0g/kg)	2(4g/kg)	3 (8g/kg)	4 (12g/kg)	
Aspartate Aminotransferase (µ/L)	106.52±2.53^{ab}	112.52±5.88^{a}	97.33±2.52^{b}	99.33±5.13^{ab}	0.048
Alanine Aminotransferase (µ/L)	38.42±1.49	36.8±1.46	34.47±0.84	36.57±1.19	0.214
Cholesterol (Mmol/L)	5.16±0.34	4.67±0.17	4.54±0.41	5.51±0.29	0.146
Creatinine (µmol/l)	63.15±37.48	27.99±6.36	31.83±5.71	32.02±5.14	0.556
Total Protein (g/l)	49.37±2.74	52.77±2.78	52.93±1.99	50.38±1.39	0.628
Albumin (g/l)	4.28±0.73	3.83±1.33	4.75±1.47	3.73±0.6	0.907
Globulin (g/l)	45.08±2.86	48.93±2.18	48.18±0.84	46.66±1.35	0.533

Table 5: Serum anti-oxidant properties of Cobbs fed with various levels of supplementary composite leaf meal of bamboo and avocado

TREATMENT	Diet				p Value
	1 (0g/kg)	2 (4g/kg)	3 (8g/kg)	4 (12g/kg)	
SOD	0.68±0.22	0.79±0.38	0.97±0.4	0.95±0.39	0.927
Catalase	7.16±0.78	11.88±4.18	7.52±1.73	6.05±0.61	0.326
GSH	0.25±0.02	0.28±0.05	0.28±0.02	0.21±0.05	0.463

Discussion

Commercial poultry production is associated with various stresses responsible for decreasing productive and reproductive performance of growing chicks, breeders and commercial layers. In broiler chickens, oxidative stress caused by reactive oxygen species (ROS) constitutes an important mechanism that may lead to biological damages, serious health disorders, and impaired growth rates (Fellenberg and Speisky, 2006). Uddin *et al*.(2009) and Jayasri *et al*.(2009) reported that natural antioxidants usually found in plants play a key role in maintaining health status of animals. This is supported by the present study as all the leaf meals used contain some antioxidants. Avocado and bamboo leaf meals in this study contained 1.12 mg/100g and 1.07 mg/100g phenol, respectively. They also contain 39.3% and 34.82% DDPH, respectively. Antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical' in broiler chickens. Antioxidants are also known to protect cells against the damaging effects of reactive oxygen species otherwise called free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006). Antioxidant properties discovered in the CLM used in the present study includes; 1.04 mg/100g phenol and 38.9% DDPH. This agrees with previous studies (Cai *et al*., 2003; Zou *et al*., 2016) that plants contain a wide variety of free radical scavenging molecules including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity. Therefore, the results from the present study suggests that the antioxidants in the leaf meals and their composite could help in scavenging the 'free-oxygen radicals in broiler chickens. At day 42 of the feeding trial, birds fed 4g CLM/kg feed had higher average final weight (2533.33±66.67g) and average total weight gain (2491.23±67.1g) than those fed the control (2216.67±29.83gand 2174.75±28.25g, respectively). This was in line to the results obtained from previous studies (Herawati 2010; Arkan *et al*., 2012) that birds fed diet containing medicinal plants had a more improved body weight than chickens fed diet uncontrolled. It could therefore be suggested that leaf meals increased the production and activities of digestive enzymes as well as improved the intestinal morphology (villi development) of broilers resulting in improved digestibility and utilization of nutrients (Mariana *et al*., 2018; Mustafa, 2019). The improvement of intestinal microbial ecosystem, immune responses and physiological conditions of chickens may also be associated to the improved growth rate in broilers fed with diet supplemented with the CLM used (Rahman and Yang, 2018; Mustafa 2019). The feed intake by birds was not influenced across dietary treatment but birds fed the control diet had the highest feed intake. Again, this is in agreement with the studies of Cabuk *et al.* (2006). Though the FCR was not affected statistically, the birds fed diet with 4gCLM/kg feed had the best feed conversion ratio which also confirms the report of Kamel (2001) that the feed conversion ratio was improved by adding medicinal plant leaf meals or their extracts. Haematological parameters are good indicators of the physiological status of farm animals (Etim *et al.,* 2013). The haematological indices such as red blood count, white blood count, packed cell volume and haemoglobin concentration are usually used for disease diagnosis and feed stress monitoring (Togun and Oseni, 2005). Results from this studies revealed that the supplementation of broiler diets with CLM of avocado and bamboo do not show any alteration in the haematological parameters implying that the diets did not affect the

blood components of the broiler chicken since all the haematological indices were within the normal range reported by Wikihow (2013). All haematological parameter were not affected by the supplementary diets save for MCV, MCHC and the Lymp. This was in line with the report of Olumide *et al*.(2018) who reported no significant difference between broiler chickens fed diet containing various levels of supplementary *O. gratissimum leaf meal*. The present result also agrees with the results by Jimoh *et al.* (2012) which stated that haematological parameters were not significantly affected by garlic supplementation of the broilers diets. Mean cell volume (MCV) is used to calculate the average erythrocyte size, the MCH to measure haemoglobin amount per blood cell and the MCHC to know the amount of haemoglobin relative to the size of the cell per red blood cell. Mean cell haemoglobin and mean corpuscular haemoglobin concentration are also useful in feed toxicity monitoring (Etim *et al*., 2014). When mean cell haemoglobin, mean cell volume is abnormally high and mean corpuscular haemoglobin concentration is abnormally low, it indicates poor quality protein of the test diets (Awoniyi *et al*., 2000). It could therefore be suggested that the CLM used in this study could improve the protein utilization in broiler chicken diets. Normal MCH and MCHC indicated the absence of normocytic anaemia which was reported to be characterized by a decreased number of RBC or PVC (Coles, 1986).The result from this study therefore suggests that the birds were not anaemic. However, the abnormally increase in the mean cell haemoglobin and mean cell value may not certainly be attached to the presence of toxin or anti-nutrients in the feed because the value for mean cell haemoglobin and mean cell value at 0% CLM supplementary rate were 44.3pg/cell and 132.87±34.74fL, respectively which are also outside the normal range. This means that the abnormal high value of MCH could be directed to other factors such as adaptation to adverse weather condition or other things other than the supplemented CLM (Minka and Ayo, 2007). The lymphocytes recorded in this study (70.67±7.79% - 79.67±5.72%) and monocytes (1.5±1.52% - 4.5±4.32%) which are agranulocytes of the white blood cell were in the normal range of the reference values of 47.2 to 85.0 % and 0.06 to 5.0 %, respectively for healthy domestic chickens (Riddell, 2011). This implies that the supplemented CLM in the diet of broiler chicken caused no harmful effects on the health status of the chickens. Biochemical markers are useful tools in diagnosis. The present study revealed that the inclusion of composite leaf mix did not affect the serum biochemical indices of the birds except in the aspartate aminotransferase (AST). The AST was ranged from 112.52±5.88 in birds fed diet containing 4gCLM/kg feed to 97.33±2.52 in birds fed diet containing 8gCLM /kg feed. This study shows that the AST reduces with CLM supplementation but had no regular pattern. The reduction of serum AST in this study suggests that the CLM has protective and therapeutic properties, as abnormally rising AST concentration indicates liver and biliary system disease, skeletal muscle disease, myocardial injury/diseases, haemolytic disorder and haemolysis (Oloruntola *et al.,* 2018). Lack of significant difference in most of blood serum biochemical indices reported in this study is in accordance with the findings of (Yusuf *et al*.(2008) and Oladunjoye *et al*.(2018). Worthy of note is the fact that the non- significant difference between the treatment groups and the control suggests the nutritional adequacy and safety of the test material; the CLM. The antioxidants may be used as defence system to prevent free radicals from damaging the cells and organs of the body,

and against infections and degenerative diseases (Sreelatha and Padma, 2009). No significant difference was observed in the antioxidant property tested for although increase in the activities of the enzymes as revealed by the tested parameter shows that the CLM improved the activity of the enzymes even if not significant.

Conclusion

The study revealed that feeding composite leaf mix of avocado and bamboo at 4g Composite Leaf Meals per kg feed does not have any adverse effects on the health status of broiler chickens fed but improve the growth and total well-being of broiler chickens. Further studies are recommended to ascertain this theory.

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