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Haematological and serum biochemical responses of pullet chicks fed diets containing single and combined levels of turmeric and clove

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Abstract

The increased pressure on the poultry industry to encourage organic livestock and poultry production has stimulated increased interest in natural consumer accepted growth promoters. Most of the information on the benefits of turmeric and clove concentrate on broiler chickens and there is no documentation on the synergistic effect of turmeric and clove. This study was conducted to assess the effect of Turmeric, Clove and Turmeric + Clove on haematological and serum biochemical indices of pullet chicks. A total of 504, day old pullet chicks (Isa brown) were used for the experiment. The Chicks were divided into 36 groups of 14 each weighed and allotted into experimental units. A total of nine experimental diets were formulated such that they contained 0, 1 and 2% turmeric, 0, 1 and 2% clove, and 0, 1 and 2% turmeric + clove combination on a 1:1 basis, respectively. The chicks in the different experimental units were randomly assigned to the 9 experimental diets in a 3 x 3 factorial arrangement (turmeric x clove x turmeric + clove: 0 x 1 x 2), replicated four times. The experiment lasted 56 days. Blood samples were collected from the wing vein at the 56th day of the experiment and evaluated for their haematological and serum biochemical indices. Birds fed turmeric and clove diets had significantly ($p<0.05$) higher PCV value than those fed turmeric + clove diet while those fed turmeric had significantly ($p<0.05$) higher Hb and RBC than the other treatment groups. The other parameters measured were unaffected by the treatments except for the significantly ($p<0.05$) higher MCV value in birds fed clove diet when compared with turmeric diet group. Birds fed diets containing 2% turmeric, clove or turmeric + clove had significantly ($p<0.05$) higher Hb and RBC, and lower MCV values than those fed 0 and 1% diets. Inclusion of clove in the diet significantly ($p<0.05$) decreased the creatinine value of the birds when compared with the turmeric and turmeric + clove diet groups but all the other parameters determined were unaffected. Birds fed 0% turmeric, clove or turmeric + clove had significantly ($p<0.05$) lower total protein, albumin, globulin, ALP and higher glucose, AST, uric acid, triglyceride, LDL and VLDL values than those fed 1 and 2% diets. Birds fed the 1% turmeric diet recorded the least cholesterol value, across the treatment groups. It was concluded that up to 2% turmeric, clove and TUM+CLV can be included in pullet chick's diet without any detrimental effect on their health status.

Keywords: Clove, turmeric, haematology, serum biochemistry, pullet chicks

Les Réponses biochimiques hématologiques et sériques des poussins de poulet nourris régimes contenant des niveaux simples et combinés de curcuma et de clou de girofle

Résumé

La pression accrue exercée sur l'industrie avicole pour encourager l'élevage biologique et la

production avicole a stimulé un intérêt accru pour les promoteurs de sa croissance acceptés par les consommateurs naturels. La plupart des informations sur les avantages du curcuma et du clou de girofle se concentrent sur les poulets de gril et il n'y a aucune documentation sur l'effet synergique du curcuma et du clou de girofle. Cette étude a été menée pour évaluer l'effet du curcuma, du clou de girofle et du curcuma + clou de girofle sur les indices biochimiques hématologiques et sériques des poussins pullet. Un total de 504 poussins de poulet d'un jour (Isa brown) ont été utilisés pour l'expérience. Les poussins ont été divisés en 36 groupes de 14 chacun pesés et attribués en unités expérimentales. Un total de neuf régimes expérimentaux ont été formulés de telle sorte qu'ils contenaient 0, 1 et 2% de curcuma, 0, 1 et 2% clou de girofle, et 0, 1 et 2% de curcuma + combinaison de clous de girofle sur une base 1:1, respectivement. Les poussins des différentes unités expérimentales ont été assignés au hasard aux 9 régimes expérimentaux dans un arrangement factorial de 3 x 3 (curcuma x clou de girofle x curcuma + clou de girofle : 0 x 1 x 2), répliqué quatre fois. L'expérience a duré 56 jours. Des échantillons de sang ont été prélevés dans la veine de l'aile au 56e jour de l'expérience et évalués pour leurs indices biochimiques hématologiques et sériques. Les oiseaux nourris au curcuma et aux clous de girofle avaient une valeur de PCV significativement ($p<0,05$) plus élevée que ceux nourris au curcuma + clou de girofle, tandis que ceux nourris au curcuma avaient significativement ($p<0,05$) plus élevé Hb et RBC que les autres groupes de traitement. Les autres paramètres mesurés n'ont pas été affectés par les traitements, à l'exception de la valeur significativement ($p<0,05$) plus élevée de MCV chez les oiseaux nourris au régime de clou de girofle par rapport au groupe de régime de curcuma. Les oiseaux nourris avec un régime alimentaire contenant 2 % de curcuma, de clou de girofle ou de curcuma + clou de girofle avaient des taux de Hb et de RBC considérablement ($p<0,05$) plus élevés, et des valeurs de MCV inférieures à celles des régimes nourris à 0 et 1 %. L'inclusion du clou de girofle dans le régime alimentaire de manière significative ($p<0,05$) a diminué la valeur créatinine des oiseaux par rapport aux groupes de régime de curcuma et de curcuma + clou de girofle, mais tous les autres paramètres déterminés n'ont pas été affectés. Les oiseaux nourris à 0 % de curcuma, de clou de girofle ou de curcuma + clou de girofle avaient des valeurs significativement ($p<0,05$) inférieures au total des protéines, de l'albumine, de la globuline, de l'ALP et du glucose plus élevé, de l'AST, de l'acide urique, du triglycéride, du LDL et du VLDL que ceux nourris à 1 et 2 %. Les oiseaux nourris au régime de 1 % de curcuma ont enregistré la valeur de cholestérol la moins élevée dans les groupes de traitement. Il a été conclu que jusqu'à 2% de curcuma, clou de girofle et TUM +CLV peuvent être inclus dans le régime alimentaire du poussin pullet sans aucun effet néfaste sur leur état de santé.

Mots-clés: *Clou de girofle, curcuma, hématologie, biochimie sérique, poussins*

Introduction

Haematology and biochemical indices are helpful tools in animal production. They are used in determining health status, metabolic diseases, nutritional deficiencies and welfare of animal (Menon *et al*., 2013). Incidence of disease and malnutrition are diagnosed from the normal reference values of various serum biochemical indices and haematological parameters measured depending on the case being investigated. The emergence of drug resistance microorganisms, side effects of antimicrobials and the harmful residual toxicity effects of drugs observed in the food chain as well as the ban of the use of antibiotics in many countries have put pressure on the poultry industry to encourage organic livestock farming. This has stimulated increased interest in the usage of consumer accepted natural alternatives such as phytogenic feed

additives (PFA) to improve productivity. The PFA are plant-derived natural bioactive compounds known to exhibit antioxidant, anti-proliferate, anti-carcinogenic, anti-inflammatory, immunomodulatory, anti-diarrheic, hypolipidemic, detoxifying, digestion-stimulating, and flavoring properties (Grashorn, 2010). They have also been reported to enhance performance, feed conversion ratio, carcass meat safety and quality in animals (Stanacev *et al*., 2011; Dhama *et al*., 2014, 2015), immune enhancement and health protection (Alagawany *et al*., 2015a and b). Likewise, inclusion of 10 to 20 g of *Withania somnifera per liter of water* improved hemoglobin, packed cell volume, white blood cells count and antibody titre against viral disease such as infectious bursal disease and infectious bronchitis in broiler chickens (Mushtaq *et al.,* 2012; Pant *et al.,* 2012). PFA can therefore be incorporated into animal feed to enhance livestock productivity. Turmeric (*Curcuma longa*) and clove *(Syzygium aromaticum)* are among many beneficial plants known as Phytogenics that are widely available and used as condiments in many countries. The biologically active ingredient, curcuminoids, is reported to be anti-inflammatory, hypocholestraemic, choleratic, antimicrobial, insect repellent, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic, anticancerous and antihelminthic (Chattopadhyay *et al*., 2004; Xu *et al*., 2006; Voravuthikunchai, 2007; Singh *et al*., 2011b). Turmeric has been reported to have beneficial effects on health status of broiler chickens. Zhongze *et al*., 2008 reported that turmeric inclusion at 0.35 g/Kg stimulated the production of Serum High-density lipoproteins (HDL), reduced total cholesterol, LDL (Low-density lipoproteins) and VLDL (very low density lipoprotein) concentrations. Sugiharto *et al*. (2011) also reported a significant increase in erythrocyte with 600 mg/kg live body weight turmeric meal inclusion in the drinking water of broiler chickens. Riasi et al., (2012), reported that turmeric powder inclusion in diets of Hy-Line W-38 laying hens decreased the level of triglyceride, total and LDL-cholesterol and increased the serum HDL-cholesterol. Malekizadeh et al. (2012), also reported reduced ALT and AST in blood serum of single comb white leghorn (W-36) laying hens with dietary inclusion of turmeric powder from 10.0-30.0 g/kg. Clove is reported to be a natural antiviral, antimicrobial, anti-inflammatory, antiseptic, and anti-fungal agent. Clove is one of the highest sources of manganese which is vital for metabolism, enzyme activation, bone strength, and also adds to clove's high Oxygen Radical Absorbance Capacity (ORAC) antioxidant value. Dalkilic and Guler (2009), documented that supplementation of clove extract at 400ppm have positive effects on performance and digestion process of broiler chickens and can be considered as an alternative natural growth promoter for poultry. Oni et al., (2018) reported that dietary supplementation of mixture of garlic, ginger and chaya leaf up to 10.0 g.kg-1 had positive effects on health status of pullet chicks. Therefore, evaluating the blood profile of pullet chicks fed diets containing turmeric, clove and their combination with the aim of finding alternative to antibiotic growth promoter (AGP) is germane.

Materials and methods

Ethical approval

The study protocol and procedures were carried out in accordance with Babcock University Health Research Ethics Committee guidelines and regulations.

Location of the study

This trial was conducted at a private farm in Ilorin, Kwara State, Nigeria under the supervision of the Animal Research Unit of the Faculty of Agriculture and Industrial

Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria. The site is located in the middle belt of Nigeria on Longitude 4°35"E of the Greenwich and Latitude 8° 29"N of the equator.

Collection and preparation of experimental materials

Fresh turmeric and dried clove buds used in this study were purchased from Mandate market Ilorin, Kwara State. Fresh Turmeric rhizomes were manually cleaned, peeled and cut into thin pieces. Thinly cut turmeric rhizomes were air dried under shade at temperature between 25 – 29°C for 10-15 days until crispy. Dried clove buds were cleaned and air dried for 24 hours prior to milling. Thereafter, they were milled individually in kitchen blender to fine particle sizes and stored in air-tight bags until incorporation into the formulated diet.

Management of birds and diets

A total of five hundred and four (504), day-old ISA Brown pullet chicks were purchased from a reputable hatchery in Ibadan, Nigeria. They were randomly allocated on weight equalization basis to nine dietary groups of 56 birds. Each treatment group had four (4) replicates with 14 birds per replicate in a 3×3 factorial experimental arrangement. Dried wood shavings were used as litter materials in pens measuring 1m x 1.5m x 2m. Nine experimental diets were formulated such that the diets contained 0, 1 and 2% Turmeric, 0, 1 and 2% Clove, and 0, 1 and 2% Turmeric + clove on 1:1 ratio, respectively (Table 1).

Table 1: Ingredient Composition of Pullet Chicks Diets containing Turmeric, Clove and their combination

	TURMERIC			CLOVE			TURMERIC+CLOVE		
Ingredients	0%	1%	2%	0%	1%	2%	0%	1%	2%
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Fish meal (72%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soybean meal	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Wheat offal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Turmeric	-	+	++	-	-	-	-	-	-
Clove	-	-	-	-	+	++	-	-	-
Turmeric+Clove	-	-	-	-	-	-	-	+	++
Total	100	100	100	100	100	100	100	100	100
Calculated		(%)							
ME (Kcal/kg)	2800.05	2800.05	2800.05	2800.05	2800.05	2800.05	2800.05	2800.05	2800.05
Crude Protein	20.80	20.80	20.80	20.80	20.80	20.80	20.80	20.80	20.80
Ether Extract	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09
Crude Fibre	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56
Ash	3.58	3.58	3.58	3.58	3.58	3.58	3.58	3.58	3.58

Chick diet premix (Composition per kg diet) : Vit. A (I.U.) 2,800,000; Vit E (mg) 16,000; Vit. K (mg) 800; Vit. B_1 (mg) 1,200; Vit. B_2 (mg) 1,600; Vit. B_6 E.E4 (mg) 30; Folic Acid (mg) 0.4; Niacin (mg) 20,000; D Cal Pan (mg) 4,400; Co (mg) 120; Cu (mg) 3,200; I (mg) 600; Se (mg) 48; Zn (mg) 24,000; Fe (mg) 16,000; Mn (mg) 40,000; Choline Chloride (mg) 120,000; Antioxidant (mg) 48,000. TUM : Turmeric; CLV: Clove*; TUM + CLV: Turmeric + Clove; 0% (exclusion levels), + = 1%, ++ = 2%,

Haematology and serum biochemistry indices

At day 56 of the birds and 41 day of the feeding trial, blood samples (2.0 ml each) were collected with needle and syringe through the brachial wing vein of three growing chicks (weighing averagely 593.80g) per replicate into EDTA-containing bottles and plain bottles (2 mls per bottle) directly for the determination of haematological and serum biochemical indices using standard procedures, as described by Weiss and Wardrop (2010). Blood samples were collected in the morning. Haematological indices determined were Packed cell volume (PCV), Haemoglobin (Hb), Red blood cells (RBC), White blood cells (WBC), Lymphocyte, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). The serum metabolites determined were Total protein, Albumin, Globulin, blood glucose, uric acid, creatinine, cholesterol, liver enzymes (Alanine transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate transaminase (AST)), and the lipoproteins.

Statistical analysis

Data were arranged in a 3×3 factorial layout and subjected to a completely randomized design. Significant ($p<0.05$) differences among treatment means were determined using Duncan Multiple Range Test as contained in SAS (2010) package.

Results and discussion

Haematology

The main and interactive effects of different levels of turmeric, clove and turmeric + clove on the haematological values of pullet chicks are presented in Tables 2 and 3. Pullet chicks fed turmeric + clove diet had significantly ($p<0.05$) lower PCV value than those fed turmeric and clove diets. Values for Hb and RBC were significantly ($p<0.05$) higher in birds fed turmeric diet when compared with those of the other diet groups. Birds fed clove diet recorded the highest MCV value that compared favourably with that of birds fed turmeric + clove diet but differed significantly($p<0.05$) from those of birds fed turmeric diet. The other parameters determined were unaffected by the treatments. The inclusion of 2% dietary level of the different additives significantly ($p<0.05$) increased the RBC, Hb and MCHC but decreased the MCV value when compared with the control. Birds fed 0 or 1% of the additives had statistically similar RBC, Hb MCHC and MCV values. There were significant interactive effects on PCV, Hb, RBC and MCV values of pullet chicks fed different dietary levels of the different feed additives. Birds fed 1% Turmeric diet had significantly ($p<0.05$) higher PCV value than the other treatment except those fed 1% clove diet while those fed 2% had the highest Hb and RBC but lowest MCV values. The highest values of PCV (39.78%), Hb (12.51g/dl) and RBC (3.05 x 10^{12}/l) were obtained in birds fed diet containing turmeric. However, the values obtained in birds fed turmeric, clove and turmeric+clove diets were within the normal physiological range (PCV: 24.9 - 45.2%, Hb:7.40-13.10g/dl and RBC: 1.58 - 3.8x 10^{12}/l) for healthy birds as reported by Mitruka and Rawnsley (1977). This result indicates normal production of red blood cells (erythropoiesis) as reported by Okpuzor et al., (2009). The observed MCV values in birds fed turmeric diets was within the reference range (90-140fL) reported by Bounous and Stedman (2000). However, the MCV values obtained from birds fed clove and turmeric + clove were higher than the range reported by Bounous and Stedman (2000) but within the range (89–203fl) reported by Aiello and Mays (1998) . These variations could be attributed to the environmental conditions in which the birds were reared or reduced RBC values.

There were significant increases in Hb, RBC and MCHC with increasing dietary of feed additives. Birds fed 2% of the feed additives recorded the highest Hb (12.20g/dl), RBC (3.01 x 10^{12}/l) and MCHC (30.60g/dl) values when compared with birds fed the 0% diets (9.64g/dl, 2.43 x 10^{12}/l and 24.93g/dl). The obtained values were within the reference range (Hb: 7.40-13.10g/dl; RBC: 1.58x 10^{12}/l-3.8 x 10^{12}/l) for healthy chicken reported by Mitruka and Rawnsley (1977). The MCV values of birds decreased with increasing dietary inclusion level, with 2% (131.17fL) recording the lowest value. The obtained MCV value at 2% inclusion level was within the range reported by Bounous and Stedman (2000) indicating no regenerative anaemia/ blood destruction. MCV value at 0 and 1% were higher than the reference value reported by Bounous and Stedman 2000 but within the normal range (89– 203fl) reported by Aiello and Mays, (1998). High MCHC was recorded at 2% and it was within the reference range (26-35 g/dl) reported by Bounous and Stedman, 2000. This is an indication of sufficient iron in the blood.

PCV, Hb, RBC and MCV were significantly ($p<0.05$) influenced by interaction between feed additives (TUM, CLV, TUM + CLV) and levels of inclusion (Table 3). PCV of birds at 1% inclusion of turmeric recorded the highest value (43.05%) compared to the combined effect of turmeric + clove which recorded the least value (32.65%) at 1% inclusion level. This suggests that turmeric at 1% inclusion will improve oxygen carrying capacity of the cells as reported by Isaac *et al.* (2013). Values obtained for Hb and RBC increased as the level of inclusion of the single effect of turmeric, clove and combined effect of turmeric + clove increased. However, the Hb and RBC values (16.25g/dl, 3.89 x 10^{12}/l) of birds fed 2% turmeric diet were highest when compared to 2% clove (10.25g/dl, 2.59 x 10^{12}/l) and turmeric + clove (10.10g/dl, 2.55 x 10^{12}/l) diets. This marked influence of turmeric on haematological parameters is an indication that it can improve the health status of pullet chicks. Okpuzor *et al.,* (2009) reported that an increase in the count of RBC, Hb and PCV is suggestive of polycythemia and positive erythropoiesis. MCV at 2% inclusion of turmeric recorded the lowest (99.57fl) and highest at 0% inclusion of turmeric + clove. Low MCV value obtained at 2% inclusion of turmeric was not worrisome as it falls within the reference range reported by Bounous and Stedman (2000). However, the observed WBC values among birds placed on control diet and feed additives in this study were below the reported range of 9 – 28.6×10^{9}/L as reported by Mitruka and Rawnsley (1998). Odesanmi *et al.*, (2010) documented that decrease in the count of WBC indicates suppression of leucocytes and their production from bone marrow which suggests presence of infection or a regenerative anaemia.

Serum Biochemistry

The main and interactive effects of turmeric, clove and turmeric + clove on serum biochemical indices of pullet chicks are shown in Tables 4 and 5.

There were no significant differences in all the biochemical blood parameters of pullet chicks fed turmeric, clove and turmeric + clove diets except for the significantly ($p<0.05$) low creatinine value observed in birds fed clove diets. This is an indication that turmeric, clove and turmeric + clove do not contain factors that are deleterious to serum biochemical parameters of the chicks. However, the creatinine values of pullet chicks fed turmeric (0.52mg/ml), clove (0.46mg/ml) and turmeric + clove (0.52mg/ml) were less than the reference range (0.90mg/dL- 1.85mg/dL) reported by Mitruka and Rawnsley (1977). Low creatinine values recorded indicates no muscular wastage in the birds which goes in

Table 2: Main effects of Turmeric, Clove, their combination and Levels of inclusion on haematological parameters of pullet chicks (16 – 56 days)

Feed Additive					Level of Inclusion of Additive (%)				
Parameters	**TURMERIC**	**CLOVE**	**TURMERIC + CLOVE**	**SEM**	**0**	**1**	**2**	**SEM**	**FA x LI**
PCV (%)	39.78 [a]	38.72 [a]	35.67 [b]	1.01	37.60	38.52	38.05	0.71	*
Hb (g/dl)	12.51 [a]	9.96 [b]	9.76 [b]	0.88	9.64 [b]	10.40[ab]	12.20 [a]	0.62	*
RBC (x 10^{12}/l)	3.05 [a]	2.54 [b]	2.43 [b]	0.10	2.43 [b]	2.58 [b]	3.01[a]	0.07	*
WBC (10^9/l)	6.00	6.28	5.90	0.24	6.03	6.02	6.13	0.17	NS
LYM (%)	73.33	73.32	72.97	2.51	71.40	75.50	72.72	1.78	NS
MCV (fl)	136.31 [b]	152.64 [a]	146.03[ab]	6.50	155.65 [a]	149.07 [a]	131.17 [b]	4.59	*
MCH (Pg)	41.16	39.02	40.42	3.05	39.78	40.17	40.64	2.16	NS
MCHC (g/dl)	30.24	25.81	26.76	2.30	24.93[b]	27.28[ab]	30.60[a]	1.63	NS

a, b Means on the same row having different superscript were significantly (P<0.05) different. SEM: Standard Error of Mean, PCV: Packed Cell Volume, Hb; haemoglobin, RBC: Red Blood Cell, WBC: White Blood Cell, LYM: Lymphocytes, MCV: Mean corpuscular volume , MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, FA: Feed additive; LI: level of inclusion

Table 3: Interaction effects of Turmeric, Clove, their combination and Levels of inclusion on haematological parameters of pullet chicks (16 – 56 days)

	TURMERIC			**CLOVE**			**TURMERIC + CLOVE**			
Parameters	0%	1%	2%	0%	1%	2%	0%	1%	2%	SEM
PCV (%)	37.70[b]	43.05[a]	38.60[b]	37.70[b]	39.85[ab]	38.60[b]	37.40[b]	32.65[c]	36.95[b]	1.23
Hb (g/dl)	9.74 [b]	11.55 [b]	16.25 [a]	9.74 [b]	9.90 [b]	10.25 [b]	9.45 [b]	9.74 [b]	10.10 [b]	1.08
RBC (x 10^{12}/l)	2.51 [b]	2.74 [b]	3.89 [a]	2.47 [b]	2.55 [b]	2.59 [b]	2.30 [b]	2.45 [b]	2.55 [b]	0.13
WBC (10^9/l)	6.05	5.80	6.15	6.03	6.65	6.15	6.01	5.60	6.10	0.30
LYM (%)	71.70	74.60	73.70	70.80	75.60	73.55	71.70	76.30	70.90	3.08
MCV (fl)	151.71 [a]	157.67 [a]	99.57 [b]	152.63[a]	156.27 [a]	149.03 [a]	162.61 [a]	133.27[a]	144.90 [a]	7.96
MCH (Pg)	38.83	41.94	42.71	39.43	38.82	39.58	41.09	39.76	40.40	3.73
MCHC (g/dl)	25.84	27.07	37.81	25.84	24.94	26.65	25.27	29.83	27.33	2.82

line with the findings of Umit *et al.,* (2011). Inclusion of the phytogenic feed additives at 0, 1 and 2% levels significantly ($p<0.05$) affected all the serum indices measured except for ALT (Table 4). Pullet Chicks fed the 1 and 2% feed additives had significantly ($p<0.05$) higher total protein, albumin, globulin, ALP and creatinine, but lower glucose, AST, uric acid, triglyceride, and VLDL values than those fed 0% feed additive diets. Cholesterol and LDL values were significantly ($p<0.05$) decreased in pullet chicks feed 1% feed additive diets while HDL increased at the 2% level. Except for cholesterol and HDL values that were decreased at 1% inclusion level, there were no significant differences between birds fed the 1 and 2% feed additive diets. Serum total protein is made up of Albumin and globulin. It's assessment in poultry is important as they play vital role in maintaining colloid osmotic pressure, mobility of dietary nutrients, minerals and hormones, as well as biosynthesis of enzymes and immune system (Piotrowska *et al.,* 2011). Values for total protein increased with increasing level of inclusion with the highest value obtained at 2%. This could be attributed to high protein digestibility since high protein in serum is an indicative of protein adequacy (Ahamefule *et al.,* 2006). A high concentration of albumin, usually denotes dehydration while a low concentration may be due to inadequate function of the liver due to malnutrition and infection (Esubonteng, 2011). In this study, the albumin value was increased with increasing inclusion levels, which indicates normal synthetic activity of the liver as its values fall within the reference range (2.10g/dL - 3.45g/dL) reported by Mitruka and Rawnsley (1977). Globulin of birds increased with increase in inclusion levels. Globulin value at 1% (3.16g/dL) and 2% (3.46g/dL) inclusion were higher than the reference range (2.13g/dL-3.02g/dL) reported by Adeyemo, (2008). These elevated values could be attributed to improved host's immune system and enhanced hepatic function since the liver is the site of protein synthesis. Glucose was influenced ($p<0.05$) by levels of inclusion. Birds at 0% level of inclusion recorded the highest (184.40mg/dL) compared to 1% inclusion level which had the least (164.44mg/dL) but similar to that obtained at 2% (166.89mg/dL) inclusion level. Decreased serum glucose observed in 1 and 2% levels of inclusion could indicate normal energy metabolism due to stimulation of endogenous enzymes by phytogenic feed additive to the release of adequate and stable substrate (glucose) needed for mechanical work and body maintenance. However, serum glucose value at 1 and 2% inclusion were within the reference range (152mg/dL-182md/dL) reported by Mitruka and Rawnsley (1977) when compared to the value at 0% inclusion which was higher than the reference. Phytogenic feed additive could therefore be considered to have anti-hyperglycemic activity without affecting basal plasma glucose concentration.The inclusion of the feed additive at 2% level in pullet chicks' diets increased the ALP but decreased the AST values of the chicks. These liver enzymes are important in the determination of the proper functioning of the liver (Ambrosy *et al.,* 2015). An increase in the concentration of these enzymes may be as a result of damaged or diseased liver. However, the increased ALP values of birds fed the 1 and 2% feed additive diets may not be attributed to hepatic necrosis but could be indicative of relationship between ALP and performance traits of pullets. According to Wilcox *et al.,* (1983), a positive correlation exists between ALP activity and egg production. Singh *et al.,* (1983) also reported that ALP activities were higher in pullets selected for higher production. The decreased AST

concentration with increasing levels of inclusion demonstrates proper functioning of the liver of birds fed phytogenic feed additive. The AST and ALP recorded in this experiment are within the normal range; AST (70- 220U/l) and ALP (24.50-44.40U/l) reported by Meluzzi *et al,* 1992 and (Mitruka and Rawnsley, 1977.

The uric acid contents of the birds decreased with increasing levels of the feed additives. Uric-acid in the blood is produced as a result of protein metabolism. Increased protein metabolism, stress and dehydration influence the concentration of Uric acid in the blood (Chernecky and Berger, 2008). Values obtained for Uric Acid in birds fed 1% (3.48mg/dL) and 2% (3.48mg/dL) levels of the feed additives were similar and lower when compared to 0% (3.97 mg/dL) level of inclusion. Oduguwa and Ogunmodede (1995) reported that high serum uric acid concentrations could be due to inefficient protein utilization. Szabo *et al.,* (2005) also documented high serum uric concentration to be typical of feeding nutritionally imbalanced dietary amino acids. However, the values fall within the reference range (2.47mg/dL -8.08mg/dL) reported by Mitruka and Rawnsley, (1977), indicating the test materials and their levels of inclusion had no negative effect on the uric acid content of the birds. Result of Uric acid from this study demonstrated efficient protein utilization by birds exposed to diets containing phytogenic feed additive. The inclusion of 2% of the phytogenic feed additives in pullet chick diets significantly increased the creatinine content of the blood. Creatinine is used to determine the status of the kidney. Kidney functions to excrete waste products resulting from protein metabolism and muscle contraction (Ileke *et al.,* 2014). Esuboteng, (2011) stated that creatinine is excreted by the kidney as a by-product of creatinine phosphate metabolism which is produced as a result of energy production by the skeletal muscles. The high creatinine values reported for birds fed the 1 and 2% diets are within the normal range (Umit *et al.,* 2011) and could not be attributed to muscle damage. The total blood cholesterol of birds on fed 0% (121.43 mg/dL) and 2% (121.96 mg/dL) feed additive diets were similar ($p>0.05$) but significantly different ($p<0.05$) from that of birds fed 1% (93.40 mg/dL) diet. However total blood cholesterol was within the reported range (52.00mg/dL- 148.00 mg/dL) of Mitruka and Rawnsley, (1977). The triglyceride contents of the birds decreased with increasing dietary levels. Triglycerides are synthesized in the liver from fatty acids, protein and glucose when they are above the body's current need and are stored in adipose tissue (Esubonteng, 2011). The significantly ($P<0.05$) decreased triglyceride value with increasing dietary levels may be attributed to regulation of lipid metabolism in a favourable manner. Birds fed 2% diets had significantly ($p<0.05$) higher HDL than the other treatment levels. This could be attributed to enhaced hypocholesterolaemic mechanism and the hypolipidemic action of PFA at this level. Birds fed the 1 and 2% diets had significantly ($p<0.05$) decreased LDL and VLDL values when compared with the control. This effect can be explained by the possible mechanism of antioxidant and anti-peroxide lowering action on LDL or the decrease in hepatic production of very low density lipoprotein (VLDL) which serves as the precursor of LDL in the blood circulation (Kim *et al.,* 2009).

Table 4: Main effects of Turmeric, Clove, their combination and levels of inclusion on Serum Parameters of pullet chicks (16-56 days)

Feed Additive					Level of Inclusion of Additive (0%)				
Parameters	**TURMERIC**	**CLOVE**	**TURMERIC + CLOVE**	**SEM**	**0**	**1**	**2**	**SEM**	**FA x LI**
Total Protein (g/dl)	5.77	5.67	5.80	0.25	4.71 [b]	6.10 [a]	6.43 [a]	0.36	NS
Albumin (g/dl)	2.83	2.84	2.83	0.13	2.58 [b]	2.95 [ab]	2.97 [a]	0.18	NS
Globulin (g/dl)	2.94	2.83	2.97	0.28	2.13 [b]	3.16 [a]	3.46 [a]	0.40	NS
Glucose (mg/dl)	171.59	171.04	173.10	3.29	184.40 [a]	164.44 [b]	166.89 [b]	4.66	NS
ALT (u/l)	21.66	21.38	21.93	1.11	21.55	21.79	21.63	1.57	NS
ALP (u/l)	31.21	32.79	33.20	0.90	30.63 [b]	34.08 [a]	32.50 [ab]	1.28	NS
AST (u/l)	94.99	96.28	93.96	1.67	103.78 [a]	90.96 [b]	90.49 [b]	2.36	NS
Uric Acid (mg/dl)	3.77	3.65	3.52	0.11	3.97 [a]	3.48 [b]	3.48 [b]	0.15	NS
Creatinine (mg/ml)	0.52 [a]	0.46 [b]	0.52 [a]	0.02	0.44 [b]	0.53 [a]	0.54 [a]	0.03	NS
Cholesterol (mg/dl)	123.82	108.10	104.87	6.79	121.43 [a]	93.40 [b]	121.96 [a]	9.61	*
Triglyceride (mg/dl)	101.14	98.97	93.75	6.48	117.55 [a]	92.33 [b]	83.97 [b]	9.17	NS
HDL (mg/dl)	76.66	70.25	74.04	3.41	67.56 [b]	67.02 [b]	86.37 [a]	4.83	*
LDL (mg/dl)	26.93	18.05	12.08	5.80	30.36 [a]	7.91 [b]	18.79 [ab]	8.21	NS
VLDL (mg/dl)	20.23	19.79	18.75	1.30	23.51 [a]	18.47 [b]	16.80 [b]	1.83	NS

ab Means on the same row having different superscript were significantly ($P<0.05$) different. SEM: Standard Error of Mean, ALT: Alalnine transaminase, ALP: Alkaline phosphatase, AST :Aspartate transaminase, HDL: High density lipo-protein, LDL: Low density lipo-protein, VLDL: Very low density lipo-protein. FA: Feed additive; LI: level of inclusion

Table 5: Interaction effects of Turmeric, Clove, their combination and levels of inclusion on serum parameters of pullet chicks (16-56 days)

	TURMERIC(%)			CLOVE(%)			TURMERIC + CLOVE(%)			
Parameters	**0%**	**1%**	**2%**	**0%**	**1%**	**2%**	**0%**	**1%**	**2%**	**SEM**
Total Protein (g/dl)	4.72	5.69	6.89	4.74	6.19	6.07	4.66	6.42	6.32	0.44
Albumin (g/dl)	2.51	3.27	2.71	2.76	2.58	3.18	2.48	2.99	3.02	0.22
Globulin (g/dl)	2.21	2.42	4.18	1.99	3.61	2.89	2.18	3.43	3.30	0.49
Glucose (mg/dl)	184.75	157.85	172.17	184.78	171.33	157.01	183.66	164.13	171.50	5.70
ALT (u/l)	21.08	22.17	21.75	21.50	21.62	21.01	22.08	21.59	22.13	1.93
ALP (u/l)	30.21	34.42	29.02	30.48	34.50	33.39	31.19	33.31	35.09	1.56
AST (u/l)	102.87	91.32	90.79	105.94	91.84	91.06	102.52	89.74	89.63	2.89
Uric Acid (mg/dl)	3.99	3.65	3.66	3.92	3.43	3.60	3.99	3.38	3.19	0.19
Creatinine (mg/ml)	0.44	0.56	0.57	0.45	0.48	0.46	0.42	0.55	0.60	0.03
Cholesterol (mg/dl)	151.64^{a}	77.12^{d}	142.69^{ab}	99.96^{cd}	105.61?	118.72^{abc}	112.69^{bcd}	97.46^{cd}	104.46^{cd}	11.77
Triglyceride (mg/dl)	127.38	90.07	85.96	108.29	111.62	77.00	116.99	75.31	88.96	11.23
HDL (mg/dl)	74.81^{b}	48.62^{c}	106.55^{a}	58.31^{bc}	76.45^{b}	75.98^{b}	69.55^{b}	75.99^{b}	76.57^{b}	5.91
LDL (mg/dl)	51.36	10.49	18.95	19.99	6.83	27.34	19.74	6.41	10.09	10.05
VLDL (mg/dl)	25.48	18.02	17.19	21.66	22.32	15.40	23.40	15.06	17.80	2.25

a, b, c Means on the same row having different superscript were significantly ($P<0.05$) different. SEM: Standard Error of Mean, TUM: Turmeric, CLV: Clove, TUM+CLV: Turmeric+ Clove, ALT: Alalnine transaminase, ALP: Alkaline phosphatase, AST:Aspartate transaminase, HDL: High density lipo-protein, LDL: Low density lipo-protein, VLDL: Very low density lipo-protein. FA: Feed additive; LI: level of inclusion

There were no significant Interaction effects on the serum biochemical parameters of pullet chicks fed turmeric, clove and turmeric + clove at different dietary levels except for cholesterol and HDL (Table 5). Birds fed 1% diet turmeric diet had the lowest cholesterol (77.12mg/dL) value followed by those fed 1% turmeric + clove (97.46mg/dL). Similarly, HDL was significantly highest at 2% (106.55mg/dL) inclusion of turmeric and lowest at 1% (48.62mg/dL) level of inclusion of turmeric. This result is in agreement with the findings of Riasi *et al*. (2012) which showed that turmeric powder inclusion in diets of Hy-Line W-38 laying hens decreased total cholesterol level and increased the serum HDL-cholesterol.

Conclusion

It is concluded that phytogenic feed additives such as turmeric, clove and turmeric + clove could be fed to pullet chick's diet without any detrimental effect on their health status. It is recommended that up to 2% turmeric can be included in pullet chick's diet for best performance.

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