Nigerian J. Anim. Sci. 2018, 20 (1): 173-182

Haematological and biochemical responses of starter broiler chickens fed copper and probiotic supplemented diets

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Target audience: *Nutritionist, researchers, feed millers and poultry farmers*

Abstract

A study was conducted to investigate the haematological and biochemical responses of starter broiler chickens fed copper and probiotics supplemented diets. A total of 180-day old Marshal broiler chicks were randomly allotted to six treatment groups of 30 birds each. The treatments were divided into three replicates of ten birds each; starter diet was formulated and fed for a period of twenty-eight days. The dietary treatments were subjected to complete randomized design arranged in a 2×3 factorial arrangement was made up of probiotics (0 and 500ppm) and copper (0, 125 and 250mg). Data were collected on haematological and serum biochemical indices. Data obtained were analysed using analysis of variance (ANOVA) and, Duncan's Multiple Range Test was used to separate significant means. Dietary supplementation of copper and probiotics showed significant influence (P < 0.05) on haematological and biochemical indices. Biochemical analyses revealed higher significant (P < 0.05) copper × probiotics interaction values of 42.81 g/l, 19.50 g/l and 23.31 g/l for total protein, albumin and globulin respectively in the birds fed 250 mg/kg copper × 500 ppm probiotics /kg. Birds fed control diet recorded lower significant (P < 0.05) values of 33.85 g/l and 15.40 g/l for total protein and albumin. In contrast, birds fed diet supplemented with 250 mg/kg copper × 500 ppm probiotics /kg recorded least significant (P < 0.05) copper x probiotics interaction values of 149.20 mg/dl, 49.00 mg/dl, 46.48 mg/dl and 81.56 mg/dl for cholesterol, triglyceride, high density lipoprotein and low density lipoprotein respectively. Birds on control diet recorded highest values for cholesterol, triglyceride, high-density lipoprotein and low density lipoprotein (respectively). Dietary copper and probiotics supplementation influenced haematological parameters. Copper x probiotics interaction significantly (P < 0.05) influenced white blood cell, glucose, lymphocyte and eosinophil. Broilers fed diet containing 250 mg/kg copper x 500 ppm probiotics /kg had significantly higher white blood cell ($30.65x10^9$/L) than the birds on control diet (26.15×10^9/L). No significant (P > 0.05) copper × probiotic interaction influence on packed cell volume, haemoglobin, red blood cell, neutrophil and monocyte. It can be concluded that dietary copper and probiotics supplementation had significant interaction influence on birds at 250 mg/kg copper × 500 ppm probiotics /kg supplemental level. It could be inferred that the supplementation of both copper and probiotics to starter broiler diet significantly improved the immune-competence of the broiler starters.

Keywords: *haematological; biochemical; copper; probiotics; broiler chicken.*

Description of problem

The extensive uses of antibiotics in poultry to promote growth rate, increase feed efficiency and prevent intestinal infections have led to the development of antibiotic-resistant bacteria in the gastrointestinal tract and drug residuals in meat (1). The use of probiotics in order to competitively exclude the colonization of intestinal pathogens has been embraced for

poultry, especially after some countries banned certain antibiotics being frequently included in rations as growth promoters. According to the currently adopted definition by Food and Agriculture Organization and World Health Organization (2), probiotics are: live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics are defined as viable microorganisms (bacteria or yeasts) that exhibit a beneficial effect on the health of the host when they are ingested (3). The most important advantage of a probiotics is that it neither has any residues in animal production nor exerts any antibiotic resistance by consumption.

Therefore, a lot of researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agents and it was reported that probiotics have a good impact on the poultry performance (4, 5), improve microbial balance, synthesize vitamins (6), decrease pH and release bacteriocins (7). Maruta *et al.* (8) reported that administering probiotic (*Bacillus subtilis* C - 3102) to chickens reduced the level and incidence of campylobacter and salmonella in the intestinal tract of broilers.

Copper is an essential mineral, which serves as co-factor in many enzyme systems in the body. Copper sulphate ($CuSO_4.5H_2O$) is the most commonly used dietary Cu supplement. Copper in the form of Cu-sulphate improves growth rate and feed efficiency in broilers (9, 10). Growth promoting effect of dietary Cu has been attributed to its antimicrobial action (11, 12). Improved availability of Cu from organic Cu complexes compared with the commonly used Cu salts recently has been suggested. Chelates, complexes or proteinates are the organic form of Cu and are usually considered for use in animal diet as alternatives to inorganic Cu source. More bioavailability of Cu is probably due to better absorption, which enhances its efficiency (13, 14). Copper supplementation in diets for chicken could result to an improvement in the digestibility of proteins (15) and retention of nitrogen (16). It has been shown that probiotic could protect broilers against pathogens by colonization in the gastro intestinal tract (17) and stimulation of systemic immune responses (18). This study, therefore, was conducted to investigate the haematological and biochemical responses of starter broiler chickens fed dietary copper and probiotics supplementation.

Materials and Methods

Experimental site

The research was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta. It lies within the forest vegetation zone of western Nigeria at latitude 7ºS132 49.462 N, longitude 3º26' 11.98'E with a mean annual rainfall of 1037 mm and average temperature of 34.7 ^{0}C (Google Earth, 2017).

Experimental Birds and Management

A total of 180 – day old broiler chicks of commercial strain (Marshal broiler ®) were purchased from Nu-Breed Hatchery in Abeokuta. The birds were allotted in to six treatment groups of thirty birds each which were further divided into three replicates of ten birds using completely randomized design. The house and equipment were thoroughly washed and disinfected before the arrival of the chicks. The birds were brooded for two weeks and

they were reared on deep litter system. The feeding trial lasted for twenty-eight days (4 weeks). All recommended vaccination and medications were administered. Feed and water were given *ad libitum* and all the routine management practices were duly followed.

Test Ingredients

Copper sulphate ($CuSO_4.5H_2O$) was obtained from Sigma Chemical Company (St. Louis, U.S.A). The copper was supplemented at 0, 125 and 250mg Cu/kg basal diet. Probiotics (Gro up™) was obtained from Bio Ingredient Nig. Ltd and supplemented at 0 and 500ppm.

Dietary Treatments

Dietary treatments consisted of the basal diet (D1) and basal diet supplemented with, 125mg Cu/kg (D2), 250mg Cu/kg (D3), 500ppm probiotics/kg (D4), 125mg Cu/kg + 500ppm probiotics/kg (D5), 250mg Cu/kg +500ppm probiotics/kg (D6) resulting in 6 dietary treatments (Table 1).

Data Collection

At the end of 4th week, blood samples for haematological indices and serum biochemistry analyses were collected from the wing vein of broiler chicken using sterilized syringe. For haematological parameter such as; Packed cell volume (PCV), Heamoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), Neutrophil, Lympocyte, Monocyte, Eosinophil, 2ml of blood was collected from one bird per replicate into vials containing ethylene diamine tetra-acetic acid (EDTA). Another set of 2ml of blood was collected into bottles without anticoagulant for serum biochemistry. Blood samples for serum biochemistry were placed in an ice bath and transported to the laboratory to determine the following parameters; Total protein, Albumin, Globulin, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic pyruvic Transaminase (SGPT), Creatinine, serum cholesterol, high density lipoprotein and low density lipoprotein.

Results and Discussion

The main effect of different levels of copper and probiotics supplementation on biochemical indices of starter broiler chickens is shown in Table 2. The main effect of copper on albumin, creatinine and SGPT were not significantly different ($P>0.05$). Total protein, globulin, uric acid, SGOT, ALP, triglyceride, cholesterol, HDL and LDL were however, significantly ($P<0.05$) influenced by dietary copper supplementation. Values obtained for total protein and globulin ranges from 34.86-42.36 g/l and 17.69-22.87 g/l. The values increased with increasing level of dietary copper. This improvement could be associated with efficient utilization of dietary crude protein. Addition of Cu improved nitrogen retention, which can be compared with results from Dove (19). High globulin level help the immune system to fight against infection and low level could lead to mortality. Dietary copper supplementation recorded low value range of 10.15 mg/dl for uric acid, which is an indication of efficient absorption and utilization of dietary protein by birds. Castel *et al.* (15) reported that copper supplementation improve protein digestibility. Also it was reported that copper aid nitrogen retention by stimulating hormone and growth factors (16). High significant ($P<0.05$) value range of 98.38 IU/L obtained for SGOT could be linked to liver response to dietary treatment. Similarly, increased value 16.04

IU/L recorded for ALP could be associated to metabolic change in the liver. The lowest values of 88.35 mg/dl, 173.35 mg/dl, 54.80 mg/dl and 98.41 mg/dl were obtained for triglyceride, cholesterol, HDL and LDL respectively. Values recorded for these parameters decreased with increasing level of copper except HDL. This indicated that copper intake reduced cholesterol and triglyceride concentration. The observation in this study could be associated to possibility of high copper concentration to reduce hepatic glutathione through the stimulation of the enzyme 3-hydroxyl-3-methylglutaryl coenzyme reductase (20).

Table 1: Percentage composition of broiler starter diets supplemented with copper sulphate and probiotics (0-4 weeks)

	Dietary treatments					
	Probiotic (0ppm)			**Probiotic (500ppm)**		
Copper (Cu)	**0ppm Cu$^+$**	**125ppmCu$^+$**	**250ppmCu$^+$**	**0ppmCu$^+$**	**125ppmCu$^+$**	**250ppmCu$^+$**
Ingredients:	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Maize	50.0	50.0	50.0	50.0	50.0	50.0
SBM	22.0	22.0	22.0	22.0	22.0	22.0
GNC	12.5	12.5	12.5	12.5	12.5	12.5
Fish meal	2.50	2.50	2.50	2.50	2.50	2.50
Wheat offal	8.20	8.20	8.20	8.20	8.20	8.20
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50
Salt	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
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Supplementation						
$CuSO_4.5H_2O$(ppm)	-	491	982	-	491	982
Probiotic (ppm)	-	-	-	500	500	500
Calculated analysis:						
ME (kcal/kg)	2862	2862	2862	2862	2862	2862
Crude protein (%)	23.47	23.47	23.47	23.47	23.47	23.47
Crude fibre (%)	4.65	4.65	4.65	4.65	4.65	4.65
Ether extract (%)	4.65	4.65	4.65	4.65	4.65	4.65
Ca (%)	1.49	1.49	1.49	1.49	1.49	1.49
P (%)	0.82	0.82	0.82	0.82	0.82	0.82
L-Lysine (%)	1.14	1.14	1.14	1.14	1.14	1.14
DL-Methionine (%)	0.56	0.56	0.56	0.56	0.56	0.56
Cu (mg/kg)	9.41	134.41	259.41	9.41	134.41	259.41

*Premix to supply kg/diet; 9,000IU of Vit A; 2,135IU of Vit D3; 20,mg of Vit E; 1.34mg of Vit B1; 5.34mg of Vit B2; 1.67mg of Vit K3; 12.00mg of Pantothenate; 2.67mg of Vit B6; 0.013mg of Vit B12; 30.00mg of Niacin; 0.10mg of Biotin; 0.10mg of Folic acid; *0.10mgof Copper*; 63.34mg of Iron; 78.00 of Zinc; 117.34mg of Manganese; 0.77mg of Iodine; 0.18mg of Selenium and 10.00mg of antioxidant.

Probiotic supplementation revealed no significant (P>0.05) effect on total protein, albumin, globulin, creatinine and SGPT. However, probiotic supplementation showed higher values of 39.70 g/l, 19.20 g/l and 20.50 g/l compared to the birds fed diet without probiotics. This is in agreement with findings of (21) who reported that probiotic supplementation had no significant effect on total protein and albumin. However, increased uric acid was observed with probiotic supplementation. Uric acid is a measure of dietary protein utilization and lower value indicated efficient protein utilization. Decreased uric acid of 0.93 mg/dl was observed in birds fed diet with probiotics supplementation. The decreased value observed in this study corroborate the range value of 0.90-2.00 mg/dl reported by (22). This result could be an indication of no muscle wastage, high creatinine indicate muscle wastage in livestock and implies that animal survives at expense of body reserve. Increased SGOT in birds with probiotics could be as a result of the liver response to dietary treatment. Triglyceride, cholesterol, HDL and LDL recorded decreased values with probiotics supplementation. Reduced cholesterol and triglyceride may be associated to ability of microbes (*Lactobacillus acidophillus*) to conjugate bile salt in the intestine and thereby preventing synthesis of cholesterol. Also, this observation could be attributed to ability of Probiotics to inhibit hydroxymethyl-glutaryl-coenzyme A, which plays significant role in reducing cholesterol synthesis. This decreased effect of probiotics was in agreement with (23, 24).

Copper × probiotic interaction effect was as presented in Table 3. Copper × probiotic interaction revealed significant (P<0.05) effect on all the parameters measured with the exception of creatinine and SGPT. Birds fed diet supplemented with 250 mg/kg copper × 500 ppm probiotics /kg (D6) recorded higher significant values of 42.81 g/l, 19.50 g/l, 23.31 g/l and 15.70 mg/dl for total protein, albumin globulin, and uric acid respectively. Values obtained for albumin were statistically similar across the dietary treatments. Improved values for total protein, albumin and globulin observed in this study reveal that copper × probiotic interaction contribute to effective protein utilization. Albumin, globulin and creatinine are measures of adequacy in protein quality, quantity and degree of protein utilisation (25). Significant (P<0.05) effect obtained for SGOT was a reflection of the birds responding to dietary treatment. Significant (P<0.05) elevated values range of 92.70 IU/L (D4) to 127.50 IU/L (D1) were obtained for ALP across dietary treatments. Elevated ALP results obtained could be associated with increased osteoblastic activities including traumatic and neoplastic (26). ALP is expected to be high in young chicks due to bone formation. However, lower values recorded for ALP in birds fed 125mgCu/kg diet with or without probiotics supplementation compared to the birds on 0mgCu/kg without probiotics. This observation suggested an improvement in mineral absorption and utilization which could have had a cushion effect and reduction in traumatic and neoplastic activities with copper × probiotic interaction. Significant (P<0.05) differences were obtained among dietary treatments for triglycerol, cholesterol, HDL and LDL. Copper × probiotic interaction resulted in low triglycerol, cholesterol, HDL and LDL in the birds

particularly those fed diet containing 250 mg/kg copper × 500 ppm probiotics /kg (D6). The result in this study could be linked to synergetic influence of copper × probiotic interaction in the birds.

Table 2: Main Effects of dietary copper and probiotic supplementation on serum chemistry of starter broiler chickens Mean±SE

	Level of Copper			Probiotic inclusion	
Parameter	**0 mgCu/kg**	**125mgCu/g**	**250mgCu/g**	**-ve**	**+ve**
Total protein (g/l)	34.86±1.65^{c}	38.26±1.53^{b}	42.36±1.31^{a}	37.28±2.02	39.70±1.47
Albumin (g/l)	17.18±2.17	18.93±0.99	19.48±0.19	17.85±0.66	19.20±1.50
Globulin (g/l)	17.69±0.80^{c}	19.34±0.99^{b}	22.87±1.47^{a}	19.42±1.30	20.50±1.21
Uric acid (mg/dl)	10.83±0.89^{b}	10.15±1.60^{b}	13.33±0.77^{a}	9.47±0.98^{b}	13.4±0.42^{a}
Creatinine (mg/dl)	0.85±0.02	1.00±0.03	0.98±0.03	0.95±0.03	0.93±0.02
SGOT (IU/L)	85.03±2.27^{b}	98.38±0.66^{a}	86.98±1.10^{b}	89.08±1.74^{b}	91.17±2.86^{a}
SGPT (IU/L)	10.62±0.26	10.65±0.4	9.15±0.35	10.23±0.14	10.40±0.42
ALP (IU/L)	110.20±8.10^{b}	94.59±5.41^{c}	116.04±0.33^{a}	116.48±2.53^{a}	97.34±5.54^{b}
Triglyceride (mg/dl)	145.43±11.56^{a}	123.22±9.77^{b}	88.35±13.74^{c}	149.15±11.88^{a}	88.84±9.73^{b}
Cholesterol (mg/dl)	202.7±3.16^{a}	180.37±13.21^{b}	173.35±13.72^{c}	205.12±6.08^{a}	165.82±7.85^{b}
HDL (mg/dl)	54.8±1.64^{a}	54.35±1.76^{a}	49.55±3.70^{b}	56.54±2.19^{a}	49.26±1.99^{b}
LDL (mg/dl)	120.73±4.40^{a}	100.68±7.89^{ab}	98.41±12.22^{b}	121.77±5.07^{a}	91.44±4.59^{b}

^{abc} Mean on the same row having different superscripts were significantly (P<0.05) different.
-ve = probiotic not included +ve = probiotic included
SGOT= Serum glutamate oxaloacetate transaminase SGPT= Serum glutamate pyruvate transaminase. ALP= Alkaline Phosphate HDL= High density lipoprotein LDL= Low density lipoprotein

Table 3: Interaction effects of dietary copper and probiotic supplementation on serum chemistry of starting broiler chickens Mean±SE

	Without probiotics			With probiotics		
Parameters	**0mgCu/kg D1**	**125mgCu/kg D2**	**250mgCu/kg D3**	**0mgCu/kg D4**	**125mgCu/kg D5**	**250mgCu/kg D6**
Total protein (g/l)	33.85±1.24^{d}	36.10±2.42^{c}	41.90±1.32^{b}	35.87±2.07^{c}	40.42±1.58^{b}	42.81±0.49^{a}
Albumin (g/l)	15.40±0.75^{b}	18.70±0.31^{a}	19.45±0.51^{a}	18.95±3.44^{a}	19.15±0.20^{a}	19.50±0.46^{a}
Globulin (g/l)	18.45±78^{b}	17.40±2.74^{b}	22.43±1.84^{a}	16.92±1.41^{c}	21.27±1.79^{a}	23.31±0.02^{a}
Uric acid (mg/dl)	8.95± 0.37^{c}	8.50±0.28^{c}	10.95±0.43^{bc}	12.70±0.52^{b}	11.80±0.40^{b}	15.70±2.65^{a}
Creatinine (mg/dl)	0.85±0.03	1.00±0	1.00±0.06	0.85±0.03	1.00±0	0.95±0.03
SGOT (IU/L)	80.00±0.63^{c}	99.50±0.29^{a}	87.75±1.24^{bc}	90.05±0.08^{b}	97.25±2.16^{a}	86.21±0.29^{bc}
SGPT (IU/L)	10.70±0.52	11.00±0.58	9.00±0.58	10.55±0.26	10.30±0.40	10.34±0.03
ALP (IU/dl)	127.50±3.17^{a}	94.70±0.46^{c}	127.25±1.59^{a}	92.70±3.92^{c}	94.50±0.58^{c}	104.83±4.27^{b}
Triglyceride (mg/dl)	168.80±0.9^{a}	150.96±3.03^{ab}	127.70±15.65^{b}	122.05±11.57^{b}	95.48±10.30^{c}	49.00±0.81^{d}
Cholesterol (mg/dl)	216.40±6.70^{a}	201.47±13.74^{a}	197.50±3.06^{b}	189.00±4.73^{b}	159.27±9.00^{be}	149.20±1.15^{c}
HDL(mg/dl)	58.75±34^{a}	58.25±1.36^{a}	52.62±1.9^{ab}	50.85±2.91^{b}	50.45±2.62^{b}	46.48±1.41^{c}
LDL (mg/dl)	132.36±9.47^{a}	117.71±8.54^{b}	115.25±7.82^{b}	109.10±2.31^{bc}	83.65±0.50^{c}	81.56±3.52^{c}

^{abcd}Mean on the same row having different superscripts were significantly(P<0.05) different.
SGOT= Serum glutamate oxaloacetate transaminase SGPT= Serum glutamate pyruvate transaminase
ALP= Alkaline Phosphate HDL= High density lipoprotein LDL= Low density lipoprotein.

Results of main effects of copper and probiotics supplementation on haematological parameters are summarised in Table 4. The results obtained revealed significant (P<0.05) influence on WBC, glucose, lymphocyte and monocyte with copper supplementation. Elevated value of 29.43 x 10^9 recorded for WBC in the birds fed copper supplemented diet was an indication that the birds reacted to one or more factors in the diets (27). Improved glucose value of 205.75 mg/dl observed in this study with copper supplementation could be attributed to adequate utilization of soluble carbohydrate in the diet. Significant (P<0.05) effect on lymphocyte and monocyte with copper supplementation revealed that birds were not allergic to dietary treatments. Similarly, birds fed diet containing probiotics supplementation showed significant (P<0.05) influence on WBC, lymphocyte, eosinophil and monocyte. Birds had significant (P<0.05) elevated values of 29.98 x 10^9, 67.54%and 2.22% for WBC, lymphocyte and monocyte with probiotics supplementation. This observation could be associated with reaction of birds to dietary treatment. PCV. Haemoglobin, red blood cell and neutrophils were not significantly (P>0.05) influenced by probiotics supplementation. The result agreed with the study carried out by (21) who reported that probiotics supplementation had no effect on blood constituent comprising haemoglobin concentration.

The interaction effect of copper and probiotics supplementation is as presented in Table 5. Copper x probiotics interaction showed significant (P<0.05) influence on WBC, glucose, lymphocyte and eosinophils. Increased values of 30.65 x10^9, 69.54 % and 2.41 % were recorded for WBC, lymphocyte and eosinophil respectively. This observation could be as a result of birds reacting to foreign substance in the body. Low levels of WBC and lymphocyte are an indication of susceptibility to diseases (28). However, PCV, haemoglobin, red blood cell, neutrophil and monocyte were not significantly (P>0.05) affected by the copper x probiotics interaction. Values of 31.27-33.57 % and 10.31-11.30 g/dl recorded for PCV and haemoglobin respectively in this study were within the range for healthy chicken (29). Daramola *et al.* (30) reported that PCV value below 21-35 % indicate that animal is anaemic and is due to poor quality of protein in the diet. High concentration of haemoglobin in the cytoplasm of red blood cell gives an indication of effective oxygen carrying capacity of the blood. According to Oyawoye *et al.* (31) ranged value of 7-15 g/dl indicate that the animal had sufficient blood pigment for efficient transportation of oxygen.

Table 4: Main effect of dietary copper and probiotic supplementation on haematological parameters of starter broiler chickens (4 weeks) Mean±SE

Parameter	Level of Copper			Probiotic inclusion	
	0mgCu/kg	125mgCu/kg	250mgCu/kg	-ve	+ve
Packed cell volume (%)	31.81±0.52	32.34±0.61	33.05±0.58	32.35±1.51	32.44±1.82
Haemoglobin (g/dl)	10.69±0.31	10.50±0.37	10.90±0.32	10.78±0.72	10.61±0.91
White blood cell (x 10^9/L)	27.86±68.51^{b}	29.43±58.42^{a}	29.09±62.01^{a}	27.61±33.14^{b}	29.98±28.21^{a}
Red blood cell (x 10^{12}/L)	2.80±0.06	2.84±0.07	2.92±0.06	2.88±0.31	2.82±0.22
Glucose (mg/dl)	178.00±25.01^{ab}	160.95±23.14^{b}	205.75±24.30^{a}	179.4±33.14^{b}	183.73±28.21^{a}
Lymphocyte (%)	66.44±0.41^{a}	66.73±0.32^{a}	63.43±0.38^{b}	63.53±0.61^{b}	67.54±0.73^{a}
Neutrophil (%)	30.21±0.22	31.79±0.21	30.89±0.24	31.49±0.32	30.44±0.16
Eosmophil (%)	1.81±0.30	1.71±0.27	1.43±0.25	2.02±0.26^{a}	1.28±0.31^{b}
Monocyte (%)	1.88±0.34^{b}	1.67±0.32^{b}	2.15±0.28^{a}	1.58±0.32^{b}	2.22±0.25^{a}

^{ab} Mean on the same row having different superscripts were significantly($P<0.05$) different.
-ve = probiotic not include +ve = probiotic included

Table 5: Interaction effects of dietary copper and probiotic supplementation on haematological parameters of starter broiler chickens (4 weeks) Mean±SE

	WITHOUT PROBIOTIC			WITH PROBIOTIC		
Parameters	**0mgCu/kg D1**	**125mgCu/kg D2**	**250mgCu/kg D3**	**0mgCu/kg D4**	**125mgCu/kg D5**	**250mgCu/kg D6**
Packed cell volume (%)	32.21±1.86	31.27±1.35	33.57±2.82	31.41±2.23	33.4±2.38	32.52±2.36
Haemoglobin (g/dl)	10.73±0.23	10.31±1.72	11.3±2.31	10.64±0.70	10.68±1.57	10.5±1.67
White blood cell(x10^9/L)	26.15±42.78^{b}	28.56±40.82^{ab}	28.12±39.50^{ab}	29.56±43.91^{ab}	30.30±40.1^{a}	30.65±42.72^{a}
Red blood cell(x10^{12}/L)	2.87±0.32	2.79±0.22	2.99±0.54	2.73±0.92	2.8950±0.23	2.85±0.35
Glucose (mg/dl)	163.90±23.4^{bc}	152.50±30.36^{c}	221.80±51.7^{a}	192.10±53.1^{b}	169.40±45.6^{bc}	189.70±52.9^{b}
Lymphocyte (%)	68.52±0.30^{a}	63.92±0.21^{ab}	58.14±0.51^{b}	64.36±0.41^{ab}	69.54±0.33^{a}	68.72±0.5^{a}
Neutrophil (%)	30.41±0.20	32.53±0.05	31.52±0.33	30.01±0.41	31.04±0.16	30.26±0.12
Eosmophil (%)	2.31±0.31^{a}	2.41±0.26^{a}	1.33±0.19^{b}	1.31±0.18^{b}	1.00±0.21^{b}	1.52±0.10^{b}
Monocyte (%)	1.42±0.41^{b}	1.33±0.41^{b}	2.00±0.31^{a}	2.34±0.24^{a}	2.01±0.32^{a}	2.31±0.65^{a}

^{abc}Mean on the same row having different superscripts were significantly($P<0.05$) different.

Conclusions and Applications

It was concluded that:

1. Supplementation at 250 mg/kg copper × 500 ppm probiotics /kg levels improved dietary protein which resulted in improved serum total protein and globulin and had no allergic effect on the birds.
4. Copper and probiotics supplementation in the ration of starter broiler chickens significantly reduced serum cholesterol and triglyceride levels particularly at 250mg Cu/kg diet either alone or in combination with probiotics.

References

1. Caprioli, A., Busani, L., Martel, J.L. and Helmuth, R. (2000). Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. *International Journal of Antimicrobial Agents*, 14: 295–304.
2. WHO (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and

Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report Cordoba/Argentina, pp. 1-34.

3. Salminens, S., Wright, A., Morelli, L., Matean, P., Brassart, D., Vos, M. W., Foden, R. and Matills-sandholm, T. (1998). Demonstration of safety of probiotics. A Review. *International Journal Food Microbiology*. 44: 93-106.
4. Mountzouris, K. C., Tsirtsikos, P., Kalamara, E., Nitsch, S. Schatzmayr, G. and Fegeros, K. (2007). Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus and Pediococcus* strains in promoting broiler performance and modulating cecalmicroflora composition and metabolic activities. *Poultry Science.* 86: 309-317.
5. Koenen, M. E., Karmer, J., Van der Hulst, R., Heres, L., Jeurissen, S. H. and Boersma W. J. (2004). Immunomodulation by probiotic *Lactobacilli* in layer and meat-type chickens. *British Poultry Science*, 45: 355–366.
6. Fuller, R. (1989). Probiotics in man and animals. *Journal of applied Bacteriology.* 66: 365-378.
7. Rolfe, R. D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *Journal of Nutrition* 130: 3965–4025.
8. Maruta, k., Miyazaki, H., Masuda, S. H., Takashi, M., Marabashi, t., Tadano, Y. and Takahashi H. (1996). Exclusion of intestinal pathogens by continuous feeding with Bacillus subtilis C-3102 and its influence on intestinal microflora in broilers. *Animal Science Technology* 67 (3): 273-280.
9. Choi, Y. J. and Paik, I. K. (1989). The effect of supplementing copper sulfate on the performance of broiler chicken. Korean. *Journal Animal Nutrition*. Feed. 13:193-200.
10. Baker, D. H., Odle, J., Funk, M. A. and Wieland, T. M. (1991). Bioavailability of copper in cupric oxide, cuprous and in copper-lysine complex. *Poultry Science*. 70: 177-179.
11. Bunch, R., Speer, V. C., Hays, V. W., Hawbaker, J. H. and Catron, D. V. (1961). Effects of copper sulfate, copper oxide and chlorotetracycline on pig performance. *Journal Animal Science.* 20:723726.
12. Burnell, T. W., Cromwell, G. L. and Stahly, T. S. (1988). Cited by Gohl, J. in Bolltomline of Nutrition. Feedstuff, June 13, pp. 16-18.
13. Downs, K. M., Hess, J. B. Macklin, K. S. and Norton, R. A. (2000). Dietary zinc complex and vitamin E for reducing cellulities incidence in broilers. *Journal Applied Poultry Research*. 9:319-323.
14. Guo, R., Henry, P. R., Holwerda, R. A., Cao, J., Littell, R. C., Miles, R. D. and Ammerman, C. B. (2001). Chemical Characteristics and relative bioavailability of supple-mental organic copper sources for poultry. *Journal of Animal Science*. 79: 1132-1141.
15. Castell, A. G. and Bowland, J. P. (1968). Supplemental copper for swine: Growth, digestibility and carcass measurements. *Canadian Journal Animal Science*. 48:403-411.
16. Braude, R. (1965). Copper as a growth stimulant in pigs. In Cuprum pro vita. Trans. Symp. Copper Development Association. London. pp. 55-57.
17. Pascuel, M., Hugas, M., Badiola, J.I., Monfort, J.M. and Garriga, M. (1999). *Lactobacillus salvarius* CTC2197 prevent *Salmonellla enteritidis* colonization in chicken. *Applied Environmental Microbiology*. 65: 4981-4986.

18. Quere, P. and Girard, F. (1999). Systemic adjuvant effect of cholera toxin in the chicken. Vetenarian immunology and immunopathology. 70: 135- 141.
19. Dove, C. R. (1995). The effect of copper level on nutrient utilization of weanling pigs. *Journal Animal Science.* 73:166-171.
20. Kim, J. W., Chao, P. Y. and Allen, (1992). Inhibition of elevated hepatic glutathione abolishes copper deficiency cholesterolemia. FASEB J. 6:2467-2471.
21. Dimcho, D., Boicheva, S., Simeonova, T. and Vlaikova, T. (2005). Effect of feeding Lactina® probiotic on performance, some blood parameters and caecalmicroflora of mule ducklings. *Trakia Journal of Sciences*, 3: 22–28.
22. Oyeyemi, M. O., Ekanade, A. O and Ogunsemoyin, A. (2014). Haematology, Serum chemistry and Hormonal assay of Yankasa ewe fed concentrate upplementation. Proceeding of 39th Annual Conference of Nigerian Society of Animal Production held at Babcock University Ilishan-Remo. March 16th – 19th 2014.Pp 68-71.
23. Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, M. (1995). Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers *British Poultry Science*. 36: 799–803
24. Mohan, B., Kardirvel, R., Natarajan, A. and Bhaskaran, J. (1996). Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. *British Poultry Science.* 37(2): 395-403.
25. Okorie, K. C., Nkwocha, G. A. and Ndubuisi, E. C. (2011). Implications of feeding varying dietary levels of cassava leaf meal on finisher broiler: Performance carcass, haematological and serological profiles. *Global research journal of science*. 1: 58-66.
26. Kendal, E. H. (2006). Diagnostic value of biochemistry. Clinical Avian Medicine – Volume II, 611-630.
27. Oduguwa, O. O. (2006). Utilization of whole pods of Albizia saman in diets of growing rabbits. *Nigeria Journal of Animal Production* 33(2): 197-202.
28. Akinmutimi, A. H., Oke, U. K. and Abasiekong, S. F. (2004). Observations on blood constituents of broiler finisher birds fed toasted Lima bean (Phaseolus lunatus). Proceeding 9th Annual Conference Animal Science Association of Nigeria (ASAN) September 13th-16th 2004. Ebonyi State University Abakaliki. Pp 55-57.
29. Odoemalam, V.U., Ndelekwute, E.K., Igbonaeme, U.J. and Ogbuewe, I.P. (2014). Comparative effect of Basil leaf (Ocimum gratissimum) meal and antibiotic growth promoter (Oxytetracycline HCL) on haematology and biochemical indices of broiler chickens. *Nigerian Journal of Animal Science.* 16(2): 235-243.
30. Daramola, S. O., Adeloye, A. A., Fatoba, I. A. and Soladoye, A. O. (2005). Haematological and biochemical parameters of West African Dwarf Goats. Livestock Reaearch for Rural Development. 17 (8).
31. Oyawoye, E.O., and Ogunkunle, M. (1998). Physiological and biochemical effect of raw jack beans on broiler. *Proceedings of 23rd conference of Nigerian Society for Animal Production* held at Gateway Hotel, Abeokuta Nigeria. September, 1998. Pp 141-142.