HEMATOLOGY AND SERUM BIOCHEMISTRY OF FINISHER BROILERS FED RAW AND COOKED TURMERIC (*Curcuma longa*) RHIZOME.

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ABSTRACT
This study was conducted to evaluate the effect of different levels of raw and cooked turmeric rhizome (*Curcuma longa*) on some blood and serum parameters of finisher broiler chickens in a complete randomized design. Turmeric rhizome was washed with water and divided into two batches of 20kg each. The first batch was crushed, sundried for 3 days. The second batch was cooked for an hour, crushed with a roller and sundried for 3 days. Both the raw and cooked sundried turmeric rhizomes were then ground using a hammer mill to produce raw and cooked turmeric rhizome meal and bagged respectively. Seven (7) broiler finisher diets were formulated to contain raw or cooked turmeric rhizome meal at 0% (common control diet), 1.0%, 1.5% and 2.0% levels, respectively. The diets were offered ad libitum to 189 Cobb broilers which were randomly divided into 7 dietary treatment groups, each containing 3 replicates of 9 birds per replicate. The experiment lasted for 21days. All the routine management practices were duly observed. The results revealed that addition of raw and cooked turmeric rhizome meals to broiler finisher diet, did not significantly affect ($p>0.05$) most blood parameters and serum biochemical constituents. Significant differences ($p<0.05$) were observed in red blood cell counts and packed cell volume, although no consistent trends were established. It was evident that the different processing methods had no effect on broiler performance based on the results obtained in this study and within the circumstances of the experiments. It can be concluded that sun-dried raw and cooked turmeric rhizome meal did not significantly affect broiler performance except packed cell volume and red blood cell count.

Keyword: Turmeric, broiler, blood parameters, serum constituents.

INTRODUCTION

The economic and nutritional demand of our modern society for food from poultry necessitates the raising of poultry under intensive production system. A lot of Research and Production Strategies have been employed, including the use of antibiotics to achieve this aim (Emadi and Kermanshahi, 2007). Although antibiotics achieved good performance, their potential side effects became a real public health concern globally and eventually led to the ban of the products especially in the western world (Toghyani et al, 2010). This triggered an increased interest in the use of herbs and spices and their products as supplements in animal rations (Owen, 2011). Herbs and spices are currently in use in livestock production because of their positive properties including anti-inflammatory, antiseptic, sedative, and anti-fungal activities, the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral, and antioxidant actions (Toghyani *et al.*, 2010, 2011). A variety of these herbs and spices including turmeric have been widely used as alternatives to synthetic antimicrobial growth promoter in livestock and poultry production.

Turmeric (*Curcuma longa*) is a tropical plant native to southern Asia which is known as golden spice of India and has existed for more than 500 years (Plant Cultures, 2005). India being the largest producer of turmeric supplies 94% of the World's demand (Plant Cultures, 2005). In Nigeria it is cultivated mostly in the homestead gardens in about 19 states where they bear different names and serve different purposes. In Ebonyi and Enugu states, it is used for treatment of malaria and for circumcision, in Benue state, it is used fresh for making yam meals, while in Katsina State inhabitants use it for decoration (Olojede *et al.*, 2000). Turmeric has been shown to have several biological effects, exhibiting anti-inflammatory (Holt *et al.,2005*), anti-oxidant (Iqbal *et al.*, 2003) and hypolipidaemic (Ramirez Tortosa *et al.*, 1999) activities. It has also been suggested that turmeric possess hepato-protective, antitumor, antiviral and anticancer activities (Polasa *et al.*, 1991). Keeping in view the significant importance of Turmeric *(Curcuma longa)*, this research was conducted to evaluate the effect of raw and cooked Turmeric *(Curcuma longa*) rhizome meal on the physiological traits and hematology of the broiler chicken.

MATERIALS AND METHODS

Experimental site:

The experiment was carried out in the Poultry Unit of Teaching and Research Farm and the Animal Science Laboratory in the School of Agriculture and Agricultural Technology (SAAT) of the Federal University of Technology, Owerri, Imo State, Nigeria.

Processing of turmeric rhizome:

Turmeric rhizomes were procured fresh from National Root Crops Research Institute, Department of Minor Root Crops, Umudike, Umuahia, Abia State, Nigeria. The turmeric rhizomes were washed with tap water and divided into two batches of 20kg each. One batch was processed raw and the other

batch was cooked. The first batch (processed raw) was crushed and sun-dried for 3 days. The second batch was cooked (poured into boiling water and was allowed to boil) for 1hr, the water drained off, crushed and sun-dried for 3 days. The raw and cooked sun-dried turmeric were then ground using a hammer mill to produce raw and cooked sundried turmeric rhizome meals. (Table 1).

Experimental diets:

Seven experimental broiler finisher diets were formulated incorporating the turmeric meal at seven dietary levels of 0.00%, 1.00%, 1.50%, and 2.00% raw turmeric and cooked turmeric meals, respectively (Table 2).

Feeding trial:

The processed turmeric rhizome powder milled into the broiler maize-based diets in a feeding trial using a total of 189 (one hundred and eighty nine) five weeks old unsexed broiler chicks of Cobb-strain were used in the study. The birds were divided into 7 groups of 27 birds each. Each group was further subdivided (replicated) into 3 groups of 9 birds each and randomly assigned to one of the 7 experimental diets of 0.00% common control, 1.00%, 1.50% and 2.00% of raw and cooked turmeric respectively, during experimental period.

Management of experimental birds:

The birds were housed in a 1.4 x 1.4m pen with wood shavings of 2cm height as litter material. Feed and water were provided ad-libitum for all treatment groups throughout the experimental period. Also adequate prophylactic medications and vaccinations were administered. The birds were weighed at the beginning of the experiment and weekly thereafter. Daily feed intake was recorded as the difference between weight of feed offered and the left over the next morning.

The study data collected included initial body weight, final body weight, weekly body weight, daily feed intake, weight gain, feed conversion ratio (g feed/g gain). The feeding trial lasted 21 days.

Carcass and organ weight determination

At the end of the feeding trial, five birds were randomly selected from each treatment, starved overnight of feed but not water, weighed and slaughtered by severing their neck and eviscerated for carcass and organ analysis. The weight of heart, liver, kidney and gizzard were measured and expressed as percentage of live weight.

Table 1 Proximate composition of raw and cooked turmeric meal (%)

Composition	Raw turmeric	Cooked turmeric
Moisture Content	12.0	7.0
Ether extract	5.0	4.82
Ash	6.0	5.53
Crude fiber	12.0	10.0
Crude protein	14.54	13.72
Nitrogen free extract	52.46	60.28

Table 2 Ingredient and nutrient composition of the experimental broiler finisher diets

		Dietary levels of Turmeric (%)					
		Raw			Cooked		
Ingredients %	0.00	1.00	1.50	2.00	1.00	1.50	2.00
Maize	55.00	55.00	55.00	55.00	55.00	55.00	55.00
SBM	25.00	25.00	25.00	25.00	25.00	25.00	25.00
PKC	5.00	4.00	3.50	3.00	4.00	3.50	3.00
Turmeric	0.00	1.00	1.50	2.00	1.00	1.50	2.00
BDG	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Wheat Offal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25.	0.25	0.25	0.25	0.25
Vit/minpremix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutrient	Compositi	(%)					

	on						
CP	20.06	20.09	20.04	20.06	20.43	20.11	20.24
CF	5.30	5.34	5.50	5.80	5.40	5.80	5.60
EE	4.37	6.75	6.77	7.05	6.76	6.79	7.1
Ca	1.31	1.4	1.41	1.43	1.4	1.41	1.43
P	1.03	1.1	1.11	1.13	1.1	1.11	1.13
NFE	67.54	63.91	64.56	62.60	63.17	63.09	62.36
*ME (Kcal/kg)	2957.85	2959.70	2961.00	2965.20	2945.80	2940.91	2941.35

Data collection procedure:
Data was collected at the end of the end of the experiment. Five birds per treatment were bled and blood collected from the basilica vein in the wing and analysed. The basilica veins in the wings were punctured with a 5ml scalp vein needle set and 12ml of blood was collected from each bird into sample bottles containing Ethylene Diamine Tetra acetic Acid (EDTA) for haematological determinations. The sample bottles were gently shaken to mix up the blood with EDTA to prevent clotting. Blood samples for biochemistry determinations were collected into clean sample bottles without anticoagulant. Serum was obtained by allowing the blood sample to clot at room temperature for 30 minutes, after which it was centrifuged for ten minutes at 3,000 revolutions per minute using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated and stored.

Determination of Packed Cell Volume
The packed cell volume (PCV) was determined by the micro-haematocrit method (Coles, 1986). A micro-capillary tube was nearly filled with blood sample and sealed at one end with plasticine. It was centrifuged at 12,000 revolutions per minute for 5 minutes with a micro-haematocrit centrifuge. After centrifugation, the PCV was read using a micro-haematocrit reader.

Determination of haemoglobin concentration
The haemoglobin concentration (HB) of the blood samples was determined by the cyano methaemoglobim method (Kachmar, 1970). Solution (5ml) was added to a clean test tube. Then 0.02ml of blood sample was added to the solution and mixed properly. The mixture was allowed to react for 20 minutes and the absorbance was read at 540nm against a blank reagent on a digital colorimeter (Lab Tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the calibration factor derived from the absorbance and concentration of the standard.

Red blood cell count (RBC)
Red blood cell (RBC) count was determined by the haemo-cytometer method (Schalm, et al., 1975). Blood (0.02ml) was pipette from the blood sample and added to 4ml of the red blood cell diluting fluid in a clean test tube to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded into a Neubauer counting chamber and all red blood cells in the five groups of 16 small squares in the central area of the Neubauer counting chamber were enumerated using a light microscope at 40X objective. The number of cells enumerated for each sample was multiplied by 10,000 to obtain the red blood cell count per microlitre of blood.

Total white blood cell count (WBC)
The total white blood cell (WBC) count was determined by the haemocytometer method (Sachalm, et al., 1975). Blood (0.02ml) was pipette into a small test tube containing 0.38ml of white blood cell diluting fluid to make a 1:20 dilution of the blood sample. The diluted sample was filled onto the Neubauer counting chamber, and all the cells on the four corner squares were counted using a light microscope at 10x objective. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood.

Biochemical parameters
Determination of total protein
Total protein was determined by direct Burette method (Lubian, 1978) for in-vitro determination of total protein in serum as plasma. Determination of serum cholesterolThe serum cholesterol was determined by enzymatic colorimetric method (Allian *et al*., 1974) for the in-vitro determination of cholesterol in serum using a QCA enzymatic cholesterol test kit (QuimicaClinicaAplicada, Spain).

Determination of Serum Urea
The serum urea was determined by the modified Berthelot-secrecy method for the in-vitro determination of urea in serum (Fawcett and Scott, 1960), using QCA enzymatic urea test kit.

Determination of Serum Glucose
The blood glucose level (mg/dl) was determined using the Accu-check active diabetes monitoring Kit based on the glucose oxide method (Roche diagnostic, 2005).
Experimental design
The experiment was conducted in a Completely Randomized Design and all data collected were subjected to analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978). Where

significant differences were observed, treatment means were compared using Duncan's Multiple Range Test as outlined by Obi (1990).

Haematological values of broilers fed raw and cooked turmeric rhizome meal

The haematological values of broiler chickens fed different levels of raw and cooked turmeric turmeric rhizome meal are shown in table 2 below. The red blood cell count recorded the following values in the result obtained from analyzing their blood, 3.2, 1.04, 2.79, 1.87, 3.26, 3.74, and 2.66 x 10^6/ul for 0.0%, 1.0%, 1.5%, 2.0% raw turmeric meal and 1.0%, 1.5%, 2.0% cooked turmeric meal groups, respectively. The packed cell volume had 35.5%, 31.0%, 21.0%, 36.5%, 41.5% and 29.5%, respectively. White blood cell count had 18.84, 24.3, 13.88, 18.72, 26.22, 17.06 and 17.08 x 10^5/ul respectively. Haemoglobin concentration recorded the following values 5.7 g/dl, 5.9 g/dl, 6.2 g/dl, 4.9 g/dl, 7.1 g/dl, 5.3 g/dl and 6.0 g/dl respectively. Percentage heterophils had 17.0%, 36.0%, 22.5%, 21.0%, 30.0%, 18.5% and 42.0% respectively. Percentage lymphocyte recorded the following values 81.5%, 64.0%, 76.5%, 76.5%, 78.0%, 79.5% and 56.0% respectively. Eosinophils had 1.5%, 0.0%, 1.0%, 2.5%, 2.0%, 2.0% and 2.0% respectively. Monocyte and basophil recorded nothing respectively.

The red blood cell count, pack cell volume, haemoglobin concentration, and heterophils concentration varied significantly ($p<0.05$) in the results obtained while white blood cell, lymphocyte, eosinophils, monocyte and basophil had no significant ($p>0.05$) variation in their values.

The hemoglobin values were significantly different ($p<0.05$) among the treatment groups with (1.5% cooked turmeric rhizome meal) having the highest value (13.83g/dl) while the lowest hemoglobin concentration (5.90g/dl) was recorded in the treatment group with (1.0% raw turmeric rhizome meal) compared with control. However, the Hemoglobin values observed in this work fell within the range (7-12g/dl) reported by Iheukwumere and Herbert (2012) except for 1.0% raw turmeric rhizome meal. A significant impact was made on the red blood cell and packed cell volume when the cooked turmeric was added to 1.5% level. This agrees with the results obtained by Egbunike *et al.* (2009).The result obtained in red blood cell count

RESULTS AND DISCUSION

for 1.5% cooked turmeric meal recorded the highest value compared to control. However, the haemoglobin, packed cell volume and red blood cells of (1.0% and 2.0%) raw turmeric group were below the ranges given by Campbell, (2013). This suggests that the birds may be at the risk of suffering anemia and dehydration but there no consistent trend among the entire groups.

The birds fed diets containing 1.5% cooked turmeric had the highest PCV (41.50%) compared to control group which recorded (35.50%) though the values in all the treatments appeared to be within the normal range of 30-35% as reported by Campbell *et al.*, (2013), 22.0-35.0%as reported by Jain (1986), and 35.0-50.0% as reported by Jeannine Miesle, (2016) except for the birds fed diets containing 1.0% and 2.0% raw turmeric which did not fell within the normal range reported by Jain (1986) and Campbell *et al*., (2013).The low PCV maybe as a result of anemia as reported by Campbell (2013) and dehydration considering 1.0% raw turmeric meal although no consistent trend. There were significant increase in heterophils concentration at 2.0%cooked turmeric having the highest value compared with control groups and a significant reduction among raw turmeric groups. This is not in agreement with Raghdad and Al-Jaleel (2012) who reported significant ($p<0.05$) reduction in heterophil in blood of broiler fed diet containing turmeric powder compared to control. Heterophils constitute the first line of defense with efficient chemotactic response. It was suggested that birds of treated group were better equipped for the non-specific cellular response when invaded by foreign agents viable or innate (Raghdad and Al-Jaleel, 2012). The normal proportion of lymphocytes is 20-50% (Jeannine Miesle, 2016), but this varies among species. They are an important part of the immune system. Increased numbers (lymphocytosis) are seen in chronic infections and lymphoid leukemia. Treatment 2.0% cooked turmeric had the least value (56.0) while control group recorded the highest value (81.50) there were numerical reduction values among the treated groups compared to control.

Table 2: Effect of the experimental diets on the haematological indices of finisher broiler

	Dietary levels of Turmeric (%)							
	Raw				Cooked			
Parameters	**0.0**	**1.0**	**1.5**	**2.0**	**1.0**	**1.5**	**2.0**	**SEM**
RBC(*10^6/ui	**3.20^{a}**	**1.04^{c}**	**2.79^{a}**	**1.87^{bc}**	**3.26^{a}**	**3.74^{a}**	**2.66^{ab}**	**0.50**
PCV (%)	**35.50^{b}**	**17.70^{d}**	**31.00^{c}**	**21.00^{d}**	**36.50^{b}**	**41.50^{ac}**	**29.50^{cd}**	**1.32**
Hb (g/dl)	**11.83^{ac}**	**5.90^{b}**	**10.33^{ac}**	**7.00^{bc}**	**12.17^{ac}**	**13.83^{a}**	**9.83^{c}**	**1.32**
WBC *10^5/ul	**18.84**	**24.30**	**13.88**	**18.72**	**26.22**	**17.06**	**17.08**	**5.18**
Heterophils (%)	**17.00^{b}**	**36.00^{ab}**	**22.50^{ab}**	**21.00^{b}**	**30.00^{ab}**	**18.50^{b}**	**42.00^{a}**	**6.61**
Lymphocyte (%)	**81.50**	**64.00**	**76.50**	**76.50**	**78.00**	**79.50**	**56.00**	**14.86**
Eosinophils (%)	**1.50**	**0.00**	**1.00**	**2.50**	**2.00**	**2.00**	**2.00**	**0.99**
Monocyte (%)	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Basophil (%)	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

a, b, c means being different superscripts in the same row are significantly different (P<0,05).
RBC=Red blood cell; PCV= Pack cell volume; Hb= haemoglobin; WBC= White blood cells

Serum Biochemical Parameters of Broilers fed raw and cooked turmeric rhizome meal.

The serum biochemistry of broilers fed diets supplemented with turmeric is presented in Table 3 below. The total protein of the birds in common control, 1.0%, 1.5% and 2.0% raw and cooked turmeric meals respectively, recorded 3.42, 3.65, 3.48, 4.11, 3.29, 3.39, and 3.52 g/dl. Globulin.

Table 3: Effect of the experimental diets on serum biochemical indices of finisher broiler birds

	Dietary levels of Turmeric (%)							
Parameters		**Raw**			**Cooked**			
(mg/dl)	**0.0**	**1.0**	**1.5**	**2.0**	**1.0**	**1.5**	**2.0**	**SEM**
Protein	**3.42**	**3.65**	**3.48**	**4.11**	**3.29**	**3.39**	**3.52**	**0.25**
Albumin	**1.54**	**1.80**	**1.86**	**1.64**	**1.95**	**1.78**	**1.75**	**0.24**
Globulin	**1.88**	**1.85**	**1.62**	**2.47**	**1.34**	**1.61**	**1.77**	**0.25**
Creatinine	**1.41**	**1.52**	**1.09**	**1.50**	**1.16**	**1.39**	**1.14**	**0.25**
Urea	**21.26**	**29.52**	**33.16**	**37.0**	**40.36**	**33.77**	**52.11**	**21.59**
Glucose	**124.00**	**108.50**	**103.00**	**106.00**	**121.00**	**83.00**	**82.00**	**42.61**
Cholesterol	**67.65**	**65.46**	**80.48**	**70.52**	**63.50**	**78.22**	**69.05**	**9.92**
TGL	**59.42**	**48.39**	**49.40**	**49.74**	**50.06**	**55.08**	**47.27**	**7.50**
HDL-c	**51.00**	**47.94**	**47.24**	**44.97**	**47.74**	**49.82**	**47.51**	**2.00**
LDL-C	**43.66**	**39.62**	**55.69**	**48.16**	**36.43**	**52.05**	**43.26**	**3.40**

TGL= Triglyceride, HDL-c= High density lipoprotein concentration, LDL-c= Low density lipoprotein concentration

yielded 1.88, 1.85, 1.62, 2.47, 1.34, 1.61 and 1.77 mg/dl respectively. HDL-c recorded 51.00, 47.94, 47.24, 44.97, 47.74, 49.82 and 47.51 mg/dl respectively. LDL-c recorded 43.66, 39.62, 55.69, 48.16, 36.43, 52.05 and 43.26 mg/dl respectively.

Total protein, globulin, HDL-c, LDL-c, albumin, creatinine, urea, glucose, choresterol and TGL had no significant ($p>0.05$) variation in their values. The results obtained for total protein, glucose and cholesterol is in agreement with Ukoha and Onunkwo (2016) who reported no significant ($p>0.05$) variation among the treatment groups for protein, glucose and cholesterol. There were numerical increase in total protein of 2.0 raw turmeric (4.11) compared to common control (3.42) and there were numerical decrease in glucose, TGL and HDL-c values among the treated groups compared with control. The normal reference value given by Church *et al.* (1984) included Total protein: 3.0- 4.9g/dl, Glucose: 197 – 299mg/dl, Urea 1.9-12.5mg/dl and Cholesterol: 129-297mg/dl. In other words, the inclusion of raw and cooked turmeric up to the 2.0% levels respectively, in the diets of the broilers did not affect the total protein, glucose, urea, createnine, TGL, LDL-c, HDL-c and cholesterol. The values obtained for glucose, urea, and cholesterol in this study were not well within the normal range of values by Church *et al. (1984)*. However, the value obtained tor total protein were well within the normal range of values. Eggum (1970) reported that serum urea depends on both the quality and the quantity of the protein supplied in the diet. Decrease in Urea content might be due to anabolic hormonal effects and liver failure.

This result agreed with Namagirilakshmi (2005) who stated that supplementation of turmeric in broiler diet at 0.25, 0.5, 0.75 and 1.0% levels had no significant effect on total cholesterol. Noori *et al.* (2011) reported the different dietary levels of turmeric at 42 days of age had no significant effect on total cholesterol and albumin of the chickens. Mehala and Moorthy (2008) similarly stated that supplementation of turmeric in broiler diet at 0.1 and

0.2% levels had no significant effect on total cholesterol. These results contradicts Emadi and Kermanshahi (2007a) who reported that supplementation with turmeric at 0.25, 0.5 and 0.75% levels in broiler diets significantly decreased blood albumin but had no significant effect on total protein. Results from this present study are in agreement with the result reported by Emadi and Kermanshahi (2007a) who observed that turmeric supplementation into the basal diet of broiler chickens did not affect total triglyceride and not in agreement with the result reported by Emadi and Kermanshahi (2007b) who observed that turmeric supplementation into the basal diet of broiler chickens significantly increased total cholesterol and HDL-c and decreased LDC-Cholesterol. Ordinarily one would expect that supplementation of poultry diets with turmeric at the levels used in this study would significantly reduce the serum levels of these lipids. However, this was not the case. A possible explanation is the manner of processing or the length of storage after processing. Another possible cause of discrepancy in results from various authors may be due to difference in cultivars, time of planting, nature of the soil and time of harvesting and processing. Hopefully future research will throw more light and help to explain the underlying cause of these variations.

CONCLUSION:

Interestingly, some authors did not find beneficial effects of supplementing diets with turmeric meal at the rate of 1.0g/kg (Akbaria *et al.*, 2012) or 2.0g/kg (Mehala and Moorthy, 2008). These reports, including the present study suggested that a lot is yet to be understood on the exact effect and mechanism of turmeric on poultry performance.

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