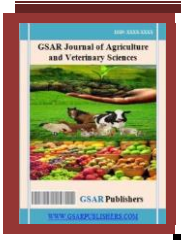

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Haematology and Serum Biochemistry of Broiler Chickens fed *Zingiber officinale*, *Allium sativum* and *Curcumin longa* Blend

By

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Abstract

This study evaluated the effects of processed Zingiber officinale, Allium sativum and Curcumin longa as feed additives on haematology and biochemistry indices of broiler chickens. A total of 240-day-old chicks were used for the study. The chicks were assigned to four diets: Diet 1 (control), Diet 2 (Curcumin longa + Zingiber officinale), Diet 3 (Curcumin longa + Allium sativum), and Diet 4 (Allium sativum + Zingiber officinale), supplemented at 0.50 g/100 kg. Thirty chicks were allocated to each treatment in a completely randomized design with 10 chicks per replicate. The results showed significant effect ($P<0.05$) of the treatments on haematogical parameters (PCV, Hb, RBC, lymphocytes and heterophils). Birds fed diets 4 had higher ($P<0.05$) Hb (11.90g/dl), which was not significantly different from D_3. RBC (3.06 and 3.16 ($x10^6/mm^3$) and lymphocytes (62.33 and 62.33%) levels were significantly higher ($P<0.05$) in birds fed diets 3 and 4. The serum indices of the birds fed diet 4 had significant ($P<0.05$) higher total protein (3.73g/dl) and albumin (1.91g/dl). The urea (19.92mg/dl, 20.05mg/dl and 20.39mg/dl) and ALP (71.39U/L, 70.90U/L and 71.69U/L) content were significantly higher ($P<0.05$) in D_1, D_2 and D_4, while the AST (64.60U/L) was higher ($P<0.05$) in D_1 but similar ($P>0.05$) to D_2 and D_4. The study showed that haematology and serum indices showed no deleterious effect on the birds fed the treatments. However, ginger and garlic blend followed by garlic and turmeric blend at 0.50% is recommended to be use in broiler diets as they showed better influence on blood parameters.

Keywords: *Allium sativum, Curcuminonga, haematological parameters, serum indices, Zingiber officinale*

INTRODUCTION

For many years, antibiotic growth promoters (AGP) have been incorporated into poultry diets because of their favorable effects, however, they are notorious for bacterial resistance and their negative impacts on the consumers' health. With the growing public concern of bacterial resistance to orthodox antibiotics, poultry production industries are looking at AGP alternatives that could maintain the health of the animal. Many types and forms of herbal feed supplements have been used to maintain and improve the health of chickens. The circulatory system in the animal body consists of a fluid called blood (Verma, 2012). The blood contains several metabolites which plays a vital role in physiological nutritional and pathological status of organisms. It has been documented and recommended that blood examination is one of the best ways for medical and nutritional assessments of animals (Egbunike *et al.*, 2009; Adeyemo and Sani, 2013). Furthermore, Etim *et al.* (2014) stated that the use of blood analysis may be a readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials because the ingestion of dietary components has measurable effects on blood composition. Aderemi (2004) posited that haematological study provides the opportunity to clinically study the presence of several metabolites and other constituents in the body of animals and it also plays a vital role in the physiological, nutritional and pathological status of an organism.

The haemato-biochemical profiles are most commonly used in nutritional studies for chickens and other birds (Adeyemi *et al.*, 2000). Factors such as drugs, presence of anti-nutritional factors in diets, variation in breed, gender etc which influence the range values of biochemical and haematological blood components will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction (Akinmutimi, 2004; Oke *et al.*, 2007; Akporhuarho, 2011; Saied *et al.*, 2011; Yanagita *et al.*, 2011). Afolabi et al. (2010) stated that haematological study helps in distinguishing normal state from state of stress.

Odunsi et al. (1999) stated that dietary constituents are reflected in the blood picture of the livestock. Therefore, the

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primary effect of the use phyto-additives in diets of animals can readily be assessed by haematological studies Ginger, garlic or turmeric supplements in broiler chicken diets have been recognized for their strong stimulating effects on the immune and digestive systems in birds, acting as a prebiotic with positive effects on the immune response (Al-Shuwaili *et al.*, 2015; Qorbanpour *et al.*, 2018). It is reported that ginger possesses useful pharmacological potent chemical substances for use in poultry due to its antioxidants, antibacterial, anti-inflammatory, antiseptic, anti-parasitic and immune-modulatory properties (Zhang *et al.*, 2019). Since ginger contains several compounds such as gingerol, shogaols, gingerdiol, and gingerdione (Zhao *et al.*, 2011), that possess antioxidant and pharmacological effects, the addition of ginger to feed might help to promote health status. Wang et al. (1998) stated that phytic materials has been in use as stimulants, spices and additives in human and animal diets. These substances according to Govaris et al. (2004) are known to provide curative, if not remedial actions against pathogenesis of microbes. This implies that Garlic, ginger or turmeric could be potentially useful in improving blood circulation on account of its inhibitory effects on platelet aggregation. This study was designed to determine the effect of ginger, garlic and turmeric blend on haematology and serum biochemistry of broiler chickens.

MATERIALS AND METHODS

ETHICS STATEMENT

This study was in accordance with the guidelines and protocols approved by the Animal Use and Care Committee of Michael Okpara University of Agriculture, Umudike, Nigeria,

EXPERIMENTAL SITE

The experiment was carried out at Sort Out Livestock Farms, Owo, Enugu State, Nigeria. Owo has a tropical wet and dry savannah climate with distinct temperature of 30.2°C and it is 0.74% higher than Nigeria's averages. Owo receives about 169.42 mm of precipitation and has 205.92 rainy days annually (https: // weather and climate.com. 2024) It is situated in Nkanu-east of Enugu state, Nigeria. its geographical coordinates are 6° 30' 0" North, 7° 41' 0" East.

SOURCE AND PREPARATION OF TEST INGREDIENTS

Curcumin longa (Turmeric), *Zingiber officinale* (ginger), *Allium sativum* (garlic) were purchased from Ogbete Market in Enugu State. These medicinal plants were thoroughly washed separately to remove debris. The method of processing adopted for the garlic was such that, it could reduce garlic bulbs to the smallest size. Garlic bulbs were cut with knife and air dried during the harmattan. The moisture content was reduced during the process while retaining the potency and prevent the complete volatilization of the active compound contained therein. This process of drying was carried out for 7-21 days and the dried garlic chips stored in an air-tight containers before being grinded and incorporated into the feed.

The ginger and turmeric were sliced and dried too during the harmattan in a well-ventilated room for short and effective air drying away from direct sunlight. The dried ginger and turmeric chips were stored in an air-tight containers separately before being grinded and mixed with the feed.

EXPERIMENTAL ANIMAL AND MANAGEMENT

A total of Rox-8 120-day-old broiler chicks which were purchased from Agrited farms, Ibadan, was used for this study. The chicks were randomly divided into 4 groups of 30 birds each. Each group was further replicated into 3 replicates with 10 birds per replicate, they were brood separately with a charcoal pot and solar bulbs in their different brooding pen for two weeks. The birds were raised in floor pens with wood shavings as litter material. Each group contains feeders and drinkers for the provision of *ad-libitum* access to feed and water respectively for the 7 weeks period of the experiment. Birds were not vaccinated against new castle disease, infectious bursal disease, Coccidiosis or any kind of poultry disease during the experiments. The study lasted for a period of fourteen (14) weeks. Four experimental broiler diets were formulated such that Diet 1 (Control) is without the medicinal plants, Diet 2 (Turmeric + ginger), Diet 3 (Turmeric + garlic) and Diet 4 (Garlic + Ginger) at 0.50g/ 100kg each. The experimental diets are shown in Tables 1 and 2.

TABLE I: COMPOSITION OF BROILER STARTER DIETS CONTAINING MIXTURES OF *Curcumin longa* (TURMERIC), *Zingiber officinale* (GINGER), *Allium sativum* (GARLIC)

Ingredient (%)	D_1(control)	D_2	D_3	D_4
Maize	48.00	48.00	48.00	48.00
Soya bean	30.00	30.00	30.00	30.00
PKC	8.00	8.00	8.00	8.00
Wheat offal	6.00	5.50	5.50	5.50
Fish meal	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25

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Salt (NaCl)	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Ginger+Tumeric	-	0.50	-	-
Tumeric+garlic	-	-	0.50	-
Garlic+ginger	-	-	-	0.50
Total	100	100	100	100
Calculated Analysis				
ME/Kcal/kg	2854.41	2882.10	2882.20	2881.94
Crude Protein (%)	21.25	21.34	21.34	21.33
NFE (%)	52 45	53.13	53.16	53.20
Crude Fibre (%)	5.46	5.50	5.49	5.49

TABLE II: COMPOSITION OF BROILER FINISHER DIETS CONTAINING MIXTURES OF *Curcumin longa* (TURMERIC), *Zingiber officinale* (GINGER), *Allium sativum* (GARLIC)

Ingredients (%)	**D_1(control)**	**D_2**	**D_3**	**D_4**
Maize	57.00	57.00	57.00	57.00
Soya bean	21.00	21.00	21.00	21.00
P.K.C.	10.00	10.00	10.00	10.00
Wheat offal	4.00	3.50	3.50	3.50
Fish meal	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt (Nacl)	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Ginger+Tumeric	-	0.50	-	-
Tumeric+Garlic	-	-	0.50	-
Garlic+Ginger	-	-	-	0.50
Total	100	100	100	100
Calculate Chemical Analysis				
ME/Kcal/kg	2855.78	2883.47	2883.57	2883.31
Protein (%)	19.25	19.34	19.34	19.33
NFE (%)	53.33	54.01	54.04	54.08
Fibre (%)	6.24	6.28	6.27	6.27

DATA COLLECTION AND ANALYSIS

Three birds per treatment were randomly selected and sacrificed. 6mls each of blood sample was collected from the severed jugular of the birds at 7 weeks; 3mls for haematology while 3mls was for serum biochemistry. The experiments are laid out in a completely randomized design (CRD). The data was subjected to analysis of variance (ANOVA) using Computer Software IBM SPSS Statistics version 25 (IBM Corp (02017).

RESULTS

HAEMATOLOGICAL PARAMETERS

The haematological parameters of broiler chickens (birds) fed diets containing blended constituents of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*) and Tumeric (*Curcumin longa*) is presented in Table III. The WBC, MCV, MCH,

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MCHC, eosinophils and monocytes of the birds were not significantly (P>0.05) influenced by the dietary treatments. The PCV was significantly higher (P<0.05) in birds fed D_4 (28.00%) and D_1 (24.67%) but not significantly different (P>0.05) from those of D_2 (25.33%) and D_3 (25.33%). The Hb concentration (11.90g/dl) of birds fed D_4 was significantly higher (P<0.05) than those of D_1 and D_2 but similar (P>0.05) to the PCV of birds fed D_3 (11.80g/dl). RBC was significantly higher (P<0.05) in birds fed D_4 ($3.16x10^6mm^3$) and D_3 ($3.06 x10^6mm^3$). Similarly, the lymphocytes count (62.33%) of birds fed D_3 and D_4 were significantly higher (P<0.05) than those of D_1 but significantly similarly (P>0.05) to birds fed D_2 (60.00%). The heterophils (35.67%) of birds fed D_2 was significantly higher (P<0.05) when compared to D_3 and D_4 but not significantly different (P>0.05) to D_1 (33.00%).

TABLE III: HAEMATOLOGICAL PARAMETERS OF BROILER CHICKENS (BIRDS) FED DIETS CONTAINING BLENDED CONSTITUENTS OF GINGER (*ZINGIBER OFFICINALE*), GARLIC (*ALLIUM SATIVUM*) AND TUMERIC (*CURCUMIN LONGA*)

Parameters	D_1	D_2	D_3	D_4	SEM
PCV (%)	24.67^a	25.33^{ab}	25.33^{ab}	28.00^a	0.53
Hb (g/dl)	10.93^c	11.33^{bc}	11.80^{ab}	11.90^a	0.13
RBC ($x10^6/mm^3$)	2.83^b	2.91^b	3.06^a	3.16^a	0.04
WBC ($x10^3/mm^3$)	20.73	21.02	20.80	22.00	0.31
MCV (fl)	87.26	86.98	82.77	88.65	1.59
MCH (pg)	38.70	38.93	38.52	37.61	0.24
MCHC (g/dl)	44.33	44.75	44.52	42.64	0.79
Lymphocytes (%)	59.33^b	60.00^{ab}	62.33^a	62.33^a	0.51
Eosinophils (%)	2.00	1.67	2.33	1.67	0.19
Monocytes (%)	5.00	3.67	5.00	5.00	0.28
Heterophils (%)	33.00^{ab}	34.67^a	30.33^b	31.00^b	0.63

$^{a–b:}$ means with different superscripts in the same row are significantly different (p<0.05), SEM= Standard error of mean, Hb=Haemoglobin, RBC=Red Blood Cell, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, D_1 (control i. e. diet without blended constituents of the test ingredients), D_2 (diet containing blended constituents of turmeric and ginger in equal proportion), D_3 (diet containing blended constituents of garlic and turmeric in equal proportion), D_4 (diet containing blended constituents of ginger and garlic in equal proportion)

SERUM INDICES

The serum biochemistry of broiler chickens (birds) fed diets containing blended constituents of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*) and Turmeric (*Curcumin longa*) is presented in Table IV. The total protein, albumin, glucose, cholesterol, urea, AST and ALP were significantly influenced by the dietary treatments. The total protein and albumin were significantly higher (P<0.05) in D_4 (3.73g/dl and 1.91g/dl, respectively). Glucose was significantly higher (P<0.05) in the control group, with the mean value of 224.67mg/dl and lowest in D_3 and D_4. Similarly, the cholesterol was significantly higher (P<0.05) in the control group (D_1) with the mean value of 130.66mg/dl and decreases as the level of inclusion increases. Urea was significantly higher (P<0.05) in birds fed D_4, D_2 and D_1, with the mean values of 20.39mg/dl, 20.05mg/dl and 19.92mg/dl, respectively. AST (64.60U/L) was significantly higher (P<0.05) in birds fed D_1 but significantly similar (P>0.05) to D_2 and D_4. The ALP of birds fed D_1 (71.39U/L), D_2 (70.80U/L) and D_4 (71.69U/L) were significantly higher (P<0.05) when compared to D_3.

TABLE IV: SERUM INDICES OF BROILER CHICKENS (BIRDS) FED DIETS CONTAINING BLENDED CONSTITUENTS OF GINGER (*ZINGIBER OFFICINALE*), GARLIC (*ALLIUM SATIVUM*) AND TURMERIC (*CURCUMIN LONGA*)

Parameters	D_1	D_2	D_3	D_4	SEM
Total protein (g/dl)	3.17^b	3.22^b	3.20^b	3.73^a	0.10
Albumin (g/dl)	1.60^b	1.68^b	1.66^b	1.91^a	0.04
Globulin (g/dl)	1.57	1.54	1.54	1.82	0.06
Glucose (mg/dl)	224.67^a	176.67^b	149.33^c	153.67^c	9.15

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Cholesterol (mg/dl)	130.66[a]	125.52[b]	117.08[c]	107.20[d]	2.70
Creatinine (mg/dl)	0.99	0.89	1.02	0.90	0.02
Urea (mg/dl)	19.92[a]	20.05[a]	16.62[b]	20.39[a]	0.53
Bilirubin (mg/dl)	0.59	0.54	0.51	0.52	0.01
AST (U/L)	64.60[a]	63.13[ab]	60.57[b]	61.04[ab]	0.66
ALT (U/L)	42.58	41.62	40.00	40.91	0.45
ALP (U/L)	71.39[a]	70.80[a]	67.46[b]	71.69[a]	0.61

[a–d:] means with different superscripts in the same row are significantly different ($p<0.05$), S.E.M= Standard Error of mean, ALB=Albumin; AST= Aspartate Transferase; ALP= Alanine Phosphatase, D_1 (control i. e. diet without blended constituents of the test ingredients), D_2 (diet containing blended constituents of turmeric and ginger in equal proportion), D_3 (diet containing blended constituents of garlic and turmeric in equal proportion), D_4 (diet containing blended constituents of ginger and garlic in equal proportion)

DISCUSSION

Okorie *et al.* (2011) posited that the hematological indices offer a means of conducting clinical investigations on the existence of various metabolites and other components in an animal. Rehman and Muhammad (2015) discovered a significant difference ($p<0.005$) in the red blood cell counts in their investigation of the impact of graded levels of ginger and garlic inclusion on broiler diets, which was in line with the findings of this present study. Ademola *et al.* (2009) found no significant change ($P > 0.01$) in PCV, haemoglobin, and RBC, but a significant difference ($P < 0.01$) in TWBC when broiler chicks were fed diets containing ginger, garlic, and their mixture at 1.0, 1.5, and 2.0% inclusion rates. Najafi and Taherpour (2014) found that there was a significant difference ($P < 0.05$) in RBC, hemoglobin, and lymphocyte but not in TWBC or heterophil ($P > 0.05$). However, Mitruka *et al.* (2007) noted that the animal's status affects the number of red blood cells in chickens, which could be a contributing factor in fluctuation. Regretfully, little information is known on how adding herbs to broiler feed affects the blood parameters (Iheanacho *et al*., 2024). The WBC value that this experiment produced was within the range of values that Mitruka *et al.* (2007) reported. It is implied that the chickens were not immunologically challenged since differential leucocytes were employed as sensitive biomarkers essential to immune functions and stress response indicators as stated by Adeyemi and Sani, (2013). The hemoglobin levels throughout the course of the treatment fell falls of the 9–13 g/dl range described by Merck (2012), 7.0–13.0 g/ dl reported by Mitruka and Rawnsley (1977) and higher than 6.5–9.4 g/dl reported by Ameen *et al.* (2007). Haemoglobin measures an animal's ability to tolerate some level of respiratory stress (Sainsbury, 1983). Normal heamoglobin levels indicate efficient transfer of oxygen, glucose, and other feed nutrients throughout the body (Okorie *et al.*, 2011). Lymphocytes were the most common form of white blood cell, followed by heterophills, eosinophils, and monocytes (Afolabi *et al.*, 2011). The readings fell within the typical range of 47.2-83% for healthy chickens, as reported by Riddell (2011). Since lymphocytes are reactive cells in inflammation and delayed hypersensitivity, they are involved in the manufacture of antibodies (Banks, 2014). The birds were healthy, as indicated by normal lymphocyte values.

Higher total recorded in T4 is an indication that the animals were well nourished due to the presence of nutrients in ginger/garlic combinations, similar observation had been reported by Oluwafemi *et al.* (2021). Protein reserve across the treatment is enough to support the growth and general performance of the animal. The outcome of this result is in agreement with the findings of Singh *et al.* (2012). Some other studies have shown that the addition of ginger in broiler diet reduced blood glucose (Akhani *et al*., 2004; Jamel *et al*., 2012), which might be the cause of glucose reduction in this study.

Bamidele and Adejumo (2012) also observed that cholesterol levels were significantly lower ($P<0.01$) in growing pullets fed diets supplemented with combination of ginger and garlic than those fed control diet. Likewise, Oleforuh-Okoleh *et al.* (2015) indicated significant decrease in cholesterol and increase in total protein, albumin, and globulin due to supplementation of ginger and garlic together in broilers, which was in tandem with the report of this present study. Singh *et al.* (2017) also reported that higher level of garlic supplementation significantly reduced the serum glucose and cholesterol level. Similarly, Al Massad *et al.* (2018) also reported lower cholesterol levels in blood and meat of birds supplemented with garlic powder. The possible mechanism of hypocholesterolaemia action of garlic might be through depressing the lipogenic and cholesterogenic activities of liver enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphatase dehydrogenase and 3-hydroxy-3-methylglutaryl-CoA reductase (Mahmoud *et al.*, 2010) and presence of bioactive components such as gingerols and shagols in ginger (Ashani and Verma, 2009). Iheanacho *et al.* (2024) observed no significant difference ($p>0.05$) in cholesterol related to feeding broilers a diet that includes garlic and ginger as additions, which contradicted the report of this work. However, the authors showed that, in comparison to the control food, birds given additions of garlic and ginger had lower cholesterol, which has the lowest cholesterol content and is supplemented with 0.50% garlic and 0.50% ginger, typically produces the best results, which

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was in line with the observation from this study. This could be attributed to the lipolytic properties of the ginger and garlic (Iheanaocho *et al*., 2024). Contrary to this result, Rehman and Muhammad, (2015) discovered in their investigation that adding garlic and ginger as additives at graded levels of 0%, 2%, 6%, and 8% had no influence on the subjects' blood values. While Zomrawi *et al.* (2012) found significant differences between control birds and birds fed ginger diets. On the other hand, research by Bhandari *et al.* (2005), Ademola *et al.* (2009), Zomrawi *et al.* (2012), and Saeid *et al.* (2010) revealed that ginger extracts had an impact on the levels of cholesterol in broiler serum.

The activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in the blood are bioindicators of liver function and damage (Yildirim *et al*., 2011). Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress (Lumeij, 2008). Ibrahim *et al*. (2024) reported that birds fed 0.25%-0.75% ginger turmeric combination had a higher ($P<0.05$) ALT (23.67U/L) when compared to those the control and those fed 0.75%- 0.25%; 0.50%-0.50% and 0%-1.00% ginger-turmeric combinations. This value was lower than the values obtained in this study and within the reference range of 19-107 U/L reported by wikivet, (2023). The authors reported no-significant difference ($P>0.05$) in the AST values of the birds fed the ginger-turmeric combinations when compared to the control, which was in line with the findings of this present study, where the D_2 (turmeric + ginger) was not significantly different ($P>0.05$) from the control. However, Ibrahim *et al*. (2024) reported the range of 16.33-29.67 U/L for AST, which was lower than the range obtained in this study. The range obtained in this study was also higher than the 44-60U/L reported by Bonous and Stedman, (2000) and wikivet, (2023). Ibrahim *et al*. (2024) also observed that birds fed 0.75-0.25% ginger-turmeric combinations had significant ($P<0.05$) mean ALP (138.3U/L), which was not significantly higher ($P>0.05$) to birds fed 0.50%-0.50% ginger-turmeric combinations. The range of ALP obtained by Ibrahim *et al*. (2024) was higher the range obtained in this study but within the reference range reported by Bonous and Stedman, (2000) and wikivet, (2023). It was observed that induced liver damage using aflatoxin in broilers and layers led to an increase in serum ALT (Fernandez *et al*., 1994). Therefore, the lower range obtained in AST and ALT due to the treatments can be deduced as an indication of better liver function. However, all the serum electrolytes obtained in this study were within the normal reference values of a healthy broiler chicken.

CONCLUSION

From the results, it could be concluded that the haematology of the birds showed that birds on ginger + garlic diet had higher Hb, RBC, and lymphocyte, which were comparable with birds fed garlic + turmeric. However, as indicated by the normal reference range, all the birds across the treatment groups were healthy. The serum biochemistry showed that bird fed ginger + garlic had higher TP, albumin, urea, ALP and lower cholesterol level. However, all the serum electrolytes obtained in this study were within the normal reference values of a healthy broiler chicken.

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