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Research

Effect of Anthocyanin and Ascorbic Acid in Graded Levels of Roselle (*Hibiscus sabdariffa* linn.) Calyx Extract on Blood Profile of Broiler Chickens

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Abstract

A total of 200 day old Abor acre broiler chicks were randomly assigned to five treatments and replicated four times in a completely randomized design to evaluate the effect of anthocyanin and ascorbic acid in graded levels of the red variety of roselle calyx extract on haematological indices and serum biochemistry of broiler chickens. Treatment 1 served as control, while treatment 2, 3, 4 and 5 represented the various graded levels of the extract. The experiment lasted 42 days in which feed was supplied ad libitum. All data obtained were subjected to ANOVA and means separated using Duncan's New Multiple Range Test. Haematological indices namely PCV, haemoglobin and RBC showed no significant difference (P>0.05) in the values obtained among treatment means. However, there was statistical difference (P<0.05) in the values obtained for WBC and neutrophils, other factions of WBC showed no significant difference. Total protein, albumin and AST obtained showed no significant difference (P>0.05) except for globulin and ALT. Anthocyanin and ascorbic acid present in the aqueous extract boosted immunity and fought infections when administered to broiler chickens. A graded level of 4g of the calyx boiled in one litre of water for 30minutes is however recommended for optimum result.

KEY WORDS: Roselle calyx extract, serum biochemistry, haematology, broiler chickens

Introduction

Roselle (*Hibiscus sabdariffa* LINN) belongs to the family Malvaceace and is a popular vegetable in Indonesia, India, West Africa and many tropical regions. In Nigeria, two botanical varieties are recognized, the calyx of the red variety are used for the preparation of "Zobo" drink and soup, while the calyx of the green variety are used to cook soup, stew and sauces. The calyx of Roselle is very rich in vitamin C and riboflavin with some major minerals present (Babalola, 2000). Roselle calyces are used as a digestive and purgative agent and a folk remedy for abscesses, billows, cancer, hypertension etc (Duke, 1985).

Roselle calyx has been shown to contain phytic acid, tannin and glucosides such as delphinidin-3-monoglucosides and delphinidin which are toxic to animal and human tissue at high concentration (Ojokoh *et al*., 2002, Morton, 1987). Tannins have been described as phenolic compounds whose degree of hydroxylation and molecular size are sufficient to form complexes with protein (Goldstein and Swain, 1963). Due to the presence of these anti-nutritional factor, Roselle calyx are usually prepared by steeping it with wood ash overnight or parboiled with wood ash and washed thoroughly prior to it been used (Adanlawo and Ajibade, 2006).

The dry calyxes contain the flavonoids-gossypetin, hibiscetin, anthocyanins and sabdaretin (Pietta, 2000). Certain amounts of delphinidin-3-monoglucoside and cyanidin-3-monoglucoside which constitute the anthocyanin are also present (Lagenhoven *et al*., 2001). There are indications that extracts from the red calyxes of Roselle contains antioxidant principles (Ologundudu *et al*., 2010). It is therefore conceivable that the consumption of the extract may provide natural agents against oxidative tissue damage and other free radicals induced disease conditions (Wolff *et al.,* 1986).

Roselle is an interesting herb ingredient because its petals consist of anthocyanin pigment which has many properties corresponding to biological activities such as antioxidant activity and inhibition of pathogenic bacteria (Wang *et al*., 2000).

Anthocyanins are a group of flavonoid derivatives and natural pigment present in the dried flowers of Roselle and their colours varies with pH. Anthocyanins are polyphenols with known antioxidant activity which may be responsible for some biological activities including the prevention or lowering the risk of cardiovascular disease, diabetes, arthritis and cancer (Miguel, 2011).

Anthocyanins are generally accepted as the largest and most important group of water soluble pigments in nature (Harborne, 1998). According to the pH, they can change from intensely dark red or orange under acidic conditions (pH<2) due to the presence of eight conjugated double bonds carrying a positive charge (Horbowicz *et al*., 2008).

Esonu *et al.*, (2001) had stated that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding. Animashahun *et al.*, (2006) also affirmed that the comparison of blood chemistry profile with nutrient intake might indicate the need for adjustment of certain nutrients upward or downward for different population groups. This study is therefore aimed at evaluating the effect of graded level of red variety of Roselle calyx extract on the blood chemistry of broiler chickens.

Material and methods

Experimental site

This study was carried out at the Teaching and Research Farm of the University of Ibadan, Oyo State. University of Ibadan is located five miles from the city of Ibadan which is geographically located on latitude 7°27´N and longitude 3°53´E. The city is characterized by a tropical wet and dry climate, the wet season runs from March through October while November to February forms the city's dry season. Temperature vary between 27-31°Celcius, mean annual rainfall of 1038mm and relative humidity of 79%.

Experimental Birds and their Management

Two hundred Abor acre day old broiler birds were used for the experiment. These birds were obtained from CHI Farms Limited in Ibadan, Oyo state. The chicks were brooded for seven days on a deep litter system using 200watts electricity bulbs and coal pots. After brooding, the chicks which had already been randomly assigned to five (5) treatments (40 birds per treatment), were replicated four times and ten (10) birds were alloted to each replicate. Vaccination programme necessary which include; NDV (intraoccular), Gumboro (Infectious Bursal Disease) and Newcastle Disease Vaccine (Lasota) were carried out at appropriate times. Anti-stress was also administered to the birds on arrival and after each weighing.

Experimental Material and Diet

Feed ingredient used were obtained from Adom feedmill in Ibadan, these were used to formulated the experimental diet to meet the NRC (1994) nutrient requirement for broiler chickens. The starter diet consisted of 22.60% crude protein and 3101.2kcal/kg Metabolisable energy while the finisher diet had a crude protein of 20.21% and 3101.20kcal/kg Metabolisable energy.

Dried Roselle calyces used were purchased from Oja Oba market also in Ibadan. The calyces were measured into respective graded levels and these were boiled in water for 30 minutes (Chumsri *et al.*, 2008; Bolade *et al*., 2009; Gartaula and Karki, 2010; Unigwe, 2011) and allowed to cool. The extract obtained were served to the broiler chickens *ad libitum* in lieu of water except for the control throughout the experiment.

Experimental Treatments

The birds were randomly assigned to five treatments and replicated four times as follows:

Treatment 1: Broiler chickens without roselle calyx extract but on water only (control)

Treatment 2: Broiler chickens on 2 g of roselle calyx boiled in 1litre of water

Treatment 3: Broiler chickens on 4 g of roselle calyx boiled in 1litre of water

Treatment 4: Broiler chickens on 6 g of roselle calyx boiled in 1litre of water

Treatment 5: Broiler chickens on 8 g of roselle calyx boiled in 1 litre of water

On daily basis, solution gotten as described above was served *ad libitum* in lieu of water except the control throughout the experiment and leftover of the solution was measured every morning to determine the fluid intake of the birds, the anthocyanin and ascorbic acid intake from the extract which was therefore calculated from the fluid intake.

Determination of Anthocyanin and Ascorbic acid from Roselle Calyx extract

- Total anthocyanin content in the extract was determined colorimetrically according to the procedure described by Du and Francis (1973) where a known volume of the filtered extract was diluted to 100 ml with the extracting solvent. The colour intensity was measured at wave length of 520nm for water extracts using Spectrophotometer (model T80 x UVNIS Spectrometer). The total anthocyanins content referred to cyanidin-3-glucoside was calculated using the following equation:

Total anthocyanin (mg/L) =

$$\frac{\text{Absorbance X dilution factor X 100}}{\text{Mls of extract used X 55.9.}}$$

- Ascorbic acid content determination was done using the Redox titration method.

Using a pipette, 20mls of the extract was place hbbd into a 250ml volumetric flask, 150mls of distilled water and 1ml of starch indicator solution were also added into the flask. The samples were titrated with 0.005mol/L iodine solution; the end point of the titration was identified as the first permanent trace of a dark-blue colour due to the starch-iodine complex. The titration was repeated with further aliquots of sample solution until a concordant result was obtained. The average volume of iodine solution used form the concordant titres and moles of iodine reacting were calculated. Using the equation of the titration below, the number of moles of ascorbic acid reacting was determined

Ascorbic acid + I_2 =2 I^- + dehydroascorbic acid

The concentration in mg/100g of ascorbic acid in the sample was also calculated.

To prepare the starch indicator used, 0.25g of soluble starch was weighed into 100mls conical flask, 50mls of boiling water was added, and it was stirred to properly dissolve the starch. The starch indicator solution was cooled before use.

Blood Sample Collection fron experimental birds

At the end of the feeding period (6 weeks), feed was withdrawn 12 hours prior to blood collection. Two birds were selected randomly from each replicate, the birds were bled using a sterilized disposable syringe and needle from puntured vein to aspirate 10mls of blood samples from each bird. 5mls of blood samples were collected into bottles treated with Ethylene Diamine Tetra Acetic acid (EDTA) for haematological assay. The reminder of each blood sample was allowed to coagulate to produce sera for blood chemistry measurements.

Haematological indices measured were carried out as stated in the Practical Haematology Manual (2008)

- ✓ Haemoglobin concentration: using Spectrophotometric method

10µL of the blood sample was intoduced into a clean test tube with the aid of a micro pipette, 2.5mls of haemoglobin reagents was added, mixed and allowed to stand for 3minutes. Another test tube was labelled blank and in it, the reagent was mixed with 10µL of distilled water. A third test tube was labelled standard and 10µL mixed with 2.5mls of the reagent was introduced into it. The absorbance was read at 540nm. To get the concentration of haemoglobin, this formular was used:

Haemoglobin concentration (g/dl) =

absorbance of test sample x concentration of standard
Absorbance of standard

- ✓ Packed cell volume (PCV): using the Wintrobe's microhaematocrit

Since the sample was collected in anti-coagulant bottles, plane capillary tube was used. the capillary tube was filled to three-quarter and sealed at the end with a sealer. It was placed in a centrifuge to spin for 5minutes at 2500rpm (rotation per minute). When the centrifuge stopped, the capillary tube was removed and placed on the microhaematocrit reader. The value was recorded in percentage.

- ✓ Red blood cell: using the improved Neubauer haematocytometer

4mls of diluting fluid (Reagent) was introduced into a test tube using a micro pipette, 20µL of anticoagulated blood was also introduced into the tube (that is 1 in 200 dilution factor). This was mixed continuously for about 2-3minutes, the haematocytometer was prepared and covered with the cover slide. With the aid of a capillary tube, the mixture (blood sample and reagent) was introduced to the side of the haematocytometer. The haematocytometer was placed on the microscope stage and the condenser lowered. The x10 objective lens was focused on the central square. This square was ruled into 25 small squares, each of which was further divided into 16 smaller squares only the four corner squares and the middle squares are counted for red blood cell. The lens was switched to x40 and the squares were counted.

Total red blood count ($x10^{12/L}$) =

N x Dilution factor x Depth Factor
Area counted (mm^2)

Where N is the total of red cells counted.

- ✓ White blood cell: also using the improved Neubauer haematocytometer

1ml of diluent and 50µL of blood sample were mixed in a clean test tube (1 in 20 dilution), it was allowed to stand for 2-3 minutes to ensure complete red blood cell lyse. The haematocytometer was cleaned and covered with the designated cover slip, one side of it was loaded with the aid of a capillary tube, it was allowed to sit for a few minutes to allow the white blood cell settle in the counting chambers. The haematocytometer was placed on the microscope stage and objective lens at x10. The white blood cells were counted in the corner squares.

Total white blood count ($x10^{9/L}$) =

N x Dilution factor x Depth Factor
Area counted (mm^2)

The White blood cell differential count to asses the percentage of various classes of white blood cells present was done by focusing the film under x10 lens, and scanned to check cell distribution. A drop of oil was added and the film was moved to the x100 oil immersion lens. A suitable area where cells are evenly distributed without appreciable overlapping was chosen, the white blood cells were counted using tracking pattern. Each cells identified was immediately tallied as Neutrophil, Lympocyte, Monocyte, Eosinophil and Basophil. These cells have staining characteristics expressed by colour;

Neutrophil – orange pink

Lymphocyte – deep blue

Monocyte – grey-blue

Eosinophil – red

Basophils - purple

Absolute count is obtained by multiplying the percent differential of the cell type in concern by the total white blood cell count.

The procedure for serum biochemistry was according to Laboratory Procedure manual, Biochemistry profile (1999).

Serum biochemistry indices measured include:

- ✓ Total protein: using Spectrophotometric method

20µL of serum was introduced into a test tube, using a micro pipette, 1ml of the reagent was added, the mixture was allowed to incubate at room temperature for 30minutes, meanwhile the blank and standard solution were prepared. The blank solution was a mixture of distilled water and 20µL of the serum while the standard solution was 20µL of the serum and total protein standard. The absorbance was read at 540nm on the spectrophotometer.

Total protein g/L =

Absorbance of test sample x Concentration of standard
Absorbance of standard

- ✓ Albumin: using Spectrophotometric method

10µL of the serum was decanted into a test tube using a micro pipette; 3mls of albumin reagent was added. It was mixed and allowed to incubate for 5minutes at room temperature. The blank and standard solutions were prepared like that of total protein. Absorbance was read at 630nm.

Albumin g/L =

Absorbance of test sample x Concentration of standard
Absorbance of standard

- ✓ Globulin: this was derived by subtracting the value of albumin from total protein.
- ✓ Alanine amino transferase (ALT) and Aspartate amino transferase (AST):

In a test tube, 0.5ml of buffer and 10µL of sample were mixed thoroughly and incubated at 37°C for 30minutes, 0.5ml of ALT/AST substrate was added, this was mixed thoroughly again and left for 20minutes. 0.5mls of 0.4M NaOH was added and then the absorbance read at 540nm.

ALT/AST (IU/L) =

Absorbance of test sample x Concentration of standard
Absorbance of standard

Statistical analysis

Data generated were subjected to Analysis of Variance using SAS (2011) package and means were further separated using Duncan Multiple Range Test of the same software.

Results and Discussion

Anthocyanin and ascorbic acid of the Roselle calyx extract for each graded level is presented in Table 2, both component increased as the graded level of the calyx increased.

Fluid intake of the birds administered the graded levels of the calyx extract is shown in Table 3 and this showed significant difference ($P<0.05$) among the treatment means. Birds in treatment 3 had the highest value for fluid intake (7.71L/bird) while those in treatment 4 had the lowest fluid intake with a value of 6.60L/bird. The average daily fluid intake however showed no significant difference ($P>0.05$) among the treatment means.

Anthocyanin and ascorbic acid intake of broiler chickens administered graded levels of roselle calyx extract from 0-6 Weeks is presented in Table 4.

The results of haematological indices of the birds are shown in Table 5 while the serum biochemistry is presented in Table 6. Haematological indices showed no significant difference in the values of the Packed Cell Volume, Haemoglobin Concentration and Red Blood Cell count of the birds among the treatment means. The White Blood Cell count and Neutrophils showed statistical difference ($P< 0.05$) among the treatment means. However, the other factions of white blood cells namely; Lymphocytes, Monocytes, Eosinophils and Basophils showed no significant difference among treatment means. There was no statistical difference in the values of Total Protein, Albumin and Aspartate amino transferase (AST), but the values obtained for globulin and Alanine amino transferase (ALT) showed significant difference.

The Ascorbic acid from the extract of the dried Roselle calyx are 2.63mg/L for treatment 2, 3.15mg/L for treatment 3, 3.70mg/L for treatment 4 and 3.98mg/L for treatment 5. These values are lower than those obtained by Babalola *et al.*, (2001) in a study on the compositional attributes of roselle calyx, the value of ascorbic acid obtained for the red variety of the Roselle calyx was 6.35mg/L and 8.65mg/L for the green variety. These differences might be due to the concentration/graded level of the calyx in the solution extracted.

Extraction technique for Roselle anthocyanins plays a major role in the antioxidant activity of the extract. Water extraction of calyx has considerable economic potential and produces a brilliant red color extract, rich in anthocyanins and hibiscus acid (Al-Kahtani and Hassan, 1990). The total anthocyanin of the extract of the graded levels was found to vary between 16.67mg/L and 28.67mg/L; this is lower than the value

of 31.05mg/L obtained by Gartaula and Karki (2010) in the optimization of extraction of anthocyanins from Roselle in aqueous medium but higher in value than those obtained by Chumsri *et al.,* (2008) in the studies on the optimum conditions and extraction of Roselle calyx. This difference might be due to variations in concentration of Roselle calyx used.

According to the results obtained, it was observed that there was no significant difference in the values of packed cell volume, haemoglobin concentration and red blood cells among the treatment means. The values fell within the normal range of (23-58%) for packed cell volume as reported for healthy birds (Maxwell *et al*., 1990). Similarly, the values for red blood cell count (2.80-3.17x10^{12}/L) are also within the range for chickens (Jain, 2000). The haemoglobin values (10.07-11.20g/dl) are within the range reported for broilers by Maxwell *et al.,* (1990). These confirm that a principle in Roselle supports haemopoiesis since the value of red blood cell depends on those of haemoglobin and packed cell volume. This is in tandem with the work of Mahadevan and Pradeep (2009) who discovered the presence of iron, minerals and vitamins in Roselle calyx. The study revealed that there was significant difference (P<0.05) in the values of white blood cell (WBC). Treatment 1 had the least value (19.28x10^{9}/L) and treatment 4 had the highest value (28.75x10^{9}/L). The values fell within the normal physiological range for a healthy broiler bird (Talebi *et al.,* 2005) (20.09-30.04x10^{9}/L) except for birds in treatment 1 which was slightly lowers.

Decrease in number of WBC below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism or presence of foreign body in the circulatory system (Ahamefule *et al.,* 2008). According to the findings of Olusola (2011), there was statistical significant increase in White blood cells which are known for defense against invading pathogens and xenobiotics. Aqueous extract of Roselle and anthocyanin isolate also caused higher red blood cell counts when compared with the water control.

Lymphocytes, eosinophils, basophils and monocytes are statistically similar while neutrophils showed significant difference (P<0.05) among treatment means. Treatment 4 had the highest percentage of neutrophils, and the birds in this treatment recorded the lowest fluid intake throughout the experiment though significantly similar to treatment 1, 3 and 5. Neutrophils are the primary component of white blood cells that respond to bacteria infection. Most likely cause of neutrophilia is infection, inflammation and necrosis. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan *et al*., 2013) and enhance adaptability to local environmental and disease prevalent conditions (Isaac *et al.,* 2013).

The result of serum biochemistry obtained among the treatment means, showed no significant difference in the values of Total protein, Albumin and Aspartate amino transferase (AST). This implies that the treatment did not significantly influence these serum biochemistry parameters. This is in accordance with the findings of Onu and Aniebo (2013) which reported no significance difference in total protein and albumin value of broiler chicks administered neem leaf extracts.

Alanine amino transferase (ALT) showed significant difference (P<0.05) among treatment means. This enzyme is known to be found in highest concentration in the liver. Elevated levels of ALT were observed in treatment 4 (29.15IU/L) which had the lowest fluid intake (309.59ml/bird/day) 0-6 weeks. Results also showed that levels of ALT observed in treatment 5 (28.55IU/L) which showed a strong relationship existed in the level of anthocyanin and ascorbic acid intake and values obtained, was statistically comparable to values obtained in treatment 4. Increased levels of transaminases can indicate myocardial infarction, hepatic disease, muscular dystrophy or organ damage. Serum elevations of ALT activity are rarely observed except in parenchyma liver disease (Ponka, 1997). Treatment 1 had the lowest ALT value (22.65IU/L) followed by treatment 2 (23.35 IU/L). Both are statistically comparable to treatment 3(27.60IU/L) which also showed significant similarity between treatments 4 and 5. This further explains why the percent value of neutrophils observed for treatments 4 and 5 were high, as earlier stated. Neutrophils are increased in many inflammatory processes and with tissue necrosis.

Conclusion

Roselle calyx extract contain Anthocyanin and Ascorbic acid and these have erythropoietic, antioxidant and hepatoprotective abilities. These principles present in the extract boosted immunity and fought infections when administered to broiler chickens at a graded level of 4g of the calyx boiled in one litre of water for 30 minutes.

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Table 1: Dietary Composition of Experimental Diets.

Ingredients	Starter phase	Finisher phase
Maize (%)	57.00	55.00
Soyabean meal (%)	26.70	22.00
Full fat soyabean (%)	10.00	9.30
Wheat offal (%)	0.00	9.00
Fishmeal (%)	2.25	1.00
DiCalcium Phosphate (%)	2.00	2.00
Limestone(%)	1.00	1.00
Salt (%)	0.25	0.25
*Premix (%)	0.25	0.25
Methionine (%)	0.20	0.20
Lysine (%)	0.10	1.00
Total	**100.00**	**100.00**
Calculated Analysis		
Crude protein (%)	22.60	20.21
Metabolisable energy (kcal/kg)	3219.60	3101.20
Crude fibre (%)	3.84	4.06
Calcium (%)	1.06	0.97
Available Phosphorus (%)	0.50	0.50
Methionine (%)	0.58	0.53
Lysine (%)	1.30	1.33
Calorie:Protein ratio	142.24:1	153.45:1
Chemical analysis		
Moisture (%)	8.80	8.93
Crude protein (%)	22.93	19.60
Ether extract (%)	13.13	13.07
Ash (%)	5.32	7.07
Crude fibre (%)	7.77	9.03
Nitrogen free extract (%)	42.14	42.30

Composition of Vitamins/1.25kg:A-12,000,000iu D3-3000,000iu E-30g K3-2.5g B1-2g B2-2.5g,Niacin-40g calpan-10g B6-3.5g B12-0.02g Folic Acid-1g Biotin-0.08g Antioxidant-125g.
Composition of minerals/kg:Sodium chloride-8g Potassium chloride-50gsodium biocarbonate-30g Sodium Acid Phosphate-8gSodium Citrate-65g Calcium lactate-17g Lactose-250g.

Table 2: Anthocyanin and Ascorbic acid in Graded levels of Roselle Calyx Extract

	Graded levels of Roselle calyx boiled in 1Litre of water			
Parameters	2g (T_2)	4g(T_3)	6g(T_4)	8g(T_5)
Anthocyanin (mg/L)	16.67	19.50	24.17	28.67
Ascorbic acid (mg/L)	2.63	3.15	3.70	3.98

Table 3: Fluid Intake of Broiler Chickens Administered Graded Levels of Roselle Calyx Extract from 0-6 Weeks

	Treatments						
Parameters	1	2	3	4	5	SEM	P-value
T.Fl.I (L/bird)	7.46^a	7.40^a	7.71^a	6.60^b	7.35^a	217.90	0.03
A.D.Fl.I.(ml/bird/day)	358.10	400.38	366.57	309.59	345.94	45.45	0.72

T.Fl.I = Total Fluid Intake
A.D.Fl.I = Average Daily Fluid Intake
SEM = Standard Error of Mean
a,b: Rows with different superscripts are statistically different ($P<0.05$).
P-value = Probability value

Table 4: Anthocyanin and Ascorbic Acid Intake of Broiler Chickens Administered Graded Levels of Roselle Calyx Extract from 0-6 Weeks

	Treatments				
Parameters	1	2	3	4	5
Total Anthocyanin Intake (mg/L)	0.00	123.25	150.37	159.33	210.56
Total Ascorbic acid Intake (mg/L)	0.00	19.45	24.29	24.37	29.21
Average Daily Anthocyanin Intake (mg/L)	0.00	6.67	7.15	7.48	9.92
Average Daily Ascorbic acid Intake (mg/L)	0.00	1.05	1.17	1.15	1.39

Table 5: Haematolgical Indices of Broiler Chickens Administered Graded Levels of Roselle

	TREATMENTS						
Parameters	1	2	3	4	5	SEM	P-value
PCV (%)	33.00	31.25	29.25	31.50	33.25	0.71	0.43
Haemoglobin (g/dl)	11.10	10.60	10.07	10.38	11.20	0.26	0.64
RBC($x10^{12}$/L)	2.85	2.97	2.80	3.03	3.17	0.11	0.85
WBC($x10^{9}$/L)	19.28^{b}	23.53^{ab}	23.53^{ab}	28.75^{a}	22.70^{ab}	1.24	0.20
Neutrophils (%)	20.00^{ab}	19.25^{b}	23.00^{ab}	32.25^{a}	31.00^{ab}	2.00	0.09
Lymphocytes (%)	73.50	74.75	71.75	63.50	65.25	1.92	0.24
Monocyte (%)	2.50	4.00	2.75	2.50	2.00	0.50	0.81
Eosinophils (%)	3.25	1.75	2.25	1.25	1.00	0.38	0.39
Basophils (%)	0.75	0.25	0.25	0.50	0.75	0.17	0.82

PCV = Packed cell volume
RBC = Red Blood Cell
WBC = White Blood Cell
a,b: Rows with different superscripts are statistically different ($P<0.05$).
SEM = Standard Error of Mean
P-value = Probability value

Table 6: Serum Biochemistry of Broiler Chickens Administered Graded Levels of Roselle Calyx Extract

	TREATMENTS						
Parameters	1	2	3	4	5	SEM	P-value
Total protein(g/L)	59.23	55.63	57.85	52.70	51.95	1.22	0.26
Albumin (g/L)	33.98	33.40	33.20	31.88	32.90	0.55	0.85
Globulin (g/L)	25.25a	22.23ab	24.65a	20.83ab	19.05b	0.84	0.08
AST (IU/L)	115.10	110.65	107.03	104.60	97.28	3.35	0.57
ALT (IU/L)	22.65b	23.35b	27.60ab	29.15a	28.55a	0.57	0.03

AST mean Aspartate amino Transferase
ALT mean Alanine amino Transferase
a,b: Rows with different superscripts are statistically different ($P<0.05$).
SEM = Standard Error of Mean
P-value = Probability value