

HAEMATOLOGY AND SERUM BIOCHEMISTRY OF BROILER CHICKENS OFFERED EXTRACTS OF DRIED ROSELLE PLANT (*Hibiscus sabdariffa*) CALYX IN DRINKING WATER

¹Asaniyan, E. K.* and ²Akinduro, V. O.

¹Department of Animal Production and Health, Olusegun Agagu University of Science and Technology, P.M.B.353, Okitipupa. Ondo State, Nigeria.

²Department of Animal Science, College of Agriculture, Osun State University, P. M. B 4494, Osogbo, Osun State, Nigeria.

*Corresponding author's e-mail: ekasaniyan@gmail.com; Tel.: +2348035726703

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ABSTRACT

The challenge of antibiotics resistance and the fatality of its residues in human and animal health led to the ban of antibiotic growth promoters in animal diets. This has prompted the search for alternatives; especially through phytobiotic investigations. Therefore, this study investigated the effects of aqueous dried calyx extract of roselle plant on the haematological and serum biochemical responses of broiler chickens with the aim of establishing its optimal level as nutrient additive. The five varying concentration levels- 0, 5, 10, 20 and 30 ml of 2 litres of calyx extract solution per litre of drinking water were treatments T1, T2, T3, T4 and T5 respectively. Thirty broiler chickens were randomly assigned per treatment of three replicates each. The experiment lasted for eight weeks.

The results revealed significant differences in the haematological parameters. However, the values of white blood cells (WBC) ($11400.00 \times 10^3 / \mu\text{l}$ - $13466.67 \times 10^3 / \mu\text{l}$), lymphocyte (ranged between 48.33 and 61.33%), monocytes (2.00-3.67%), eosinophils (1.67-5.00%) and basophil (0.00 – 1.00%) were within the normal range for healthy chickens. Hence, roselle plant aqueous extract maintained the haematological content of the blood. The level of lymphocyte revealed adequate production of antibodies that prevented the occurrence of bacterial infection or allergic condition throughout experimental period. The influence of aqueous roselle plant on the levels of aspartate transaminase (AST) and albumin (ALB) indicated uncompromised liver function. Therefore, haematological and serum biochemistry parameters showed enhanced health and the wellbeing of the broiler chickens. The 5 ml and 10 ml levels of the extract of dried roselle calyx in drinking water were found to be similarly optimal in terms of lymphocyte levels. Levels above 10 ml could be detrimental to the health and wellbeing of the chickens.

Key words: Blood constituents, Chicken health, Drinking water, Roselle calyx extract

INTRODUCTION

The health and welfare of broiler chicken are of concern in achieving good performance and returns on production. Efforts are constantly ongoing in maintaining their healthy performance without compromising the safety of broiler meat. Nutritional investigations into reduction in production cost through alternative ingredients never compromised the fact that good health is an indicator of excellent performance. The necessity of controlling infectious pathogens and increasing feed efficiencies led to the discovery of antibiotics (Engberg *et al.*, 2000).

Antibiotics are routinely used to treat and prevent infections in humans and animals. However, scientific evidence suggests that the massive use of these compounds led to increased problem of antibiotic resistance (Furtula *et al.*, 2010; Forgetta

et al., 2012), and presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez and Angeles, 2017), compromised human and animal health (Diarra *et al.*, 2010). This consequently led to the ban of antibiotic growth promoters in animal diets in the European Union in 2006. Therefore, there are consistent efforts to find the effective alternatives to prevention and control of spread of resistant bacteria through plant phytobiotic investigations (Diarra and Malouin, 2014).

Black cumin seed (Khalaji *et al.*, 2011; Abd El-Hack *et al.*, 2018), pawpaw seed (Bolu *et al.*, 2009); bitter leaf (Nwaoguikpe, 2010), have all been reported to have a growth promoting effect on different poultry such as chicken and quail which serve varying purposes such as meat and egg production. Furthermore, the beneficial use of

plant seeds and plant extracts in ruminants is well documented (Faniyi *et al.*, 2016; Hernandez *et al.*, 2017). Consequently, plant parts or their extracts are of benefits to livestock performance. Feeding the broiler chickens with the diets containing antibiotic alternatives alleviated the negative effects of removing antibiotics from their diets (Yakhkeshi *et al.*, 2011). Licorice (*Glycyrrhiza glabra*) extract in drinking water can reduce abdominal fat and serum levels of low density lipoprotein cholesterol and total cholesterol without any adverse effects on broilers performance and immune status (Khamisabadi *et al.*, 2015).

Roselle is a tropical shrub belonging to the family *Malvaceae* which produces red, dark red and green form of calyces. The chemical composition of the red calyces revealed that they are good sources of vitamin C, flavonoids, minerals and antioxidants (Babalola *et al.*, 2001; Wong *et al.*, 2002). Roselle aqueous extract is usually processed into a sour tasting refreshing drink called *Zobo* in Nigeria (Fasoyiro *et al.*, 2005). The acid content of the calyces increases during growth but decreases when it reaches maturity or ripens (Frimpong, *et al.*, 2014). The roselle plant is considered an antiseptic, emollient, sedative and tonic agent that produces soothing and invigorating effects in the body (Olaleye, 2007). In human, it aids digestion, promotes kidney function, improves cardiovascular health and helps reduce fever (Fasoyiro *et al.*, 2005). Consequently, the roselle calyx has the potential of being used to supplement or replace synthetic antibiotics in drinking water of broiler chickens as farmers are unrelentingly sourcing for alternative ways to improve their performance.

Blood provides the opportunity to assess the physiological, pathological and nutritional status of animals through the evaluation of its constituents and the presence of metabolites (Nseabasi *et al.*, 2014). The haematological parameters which affect health, production and adaptability to environmental conditions in livestock include the packed cell volume (PCV), haemoglobin (Hb), total protein (TP) and serum globulin (SG) (Medugu *et al.*, 2010; Adenkola *et al.*, 2011). Benerjee, (2008) reported normal blood values for domestic fowl to be a PCV 25-45%, RBC $2-4 \times 10^6$, Hb 7-13g/dl, WBC $9-31 \times 10^3$, and

total serum protein 5-7%. Haematological components are valuable in monitoring feed toxicity especially feed constituents that affects the formation of blood (Amusa *et al.*, 2015). Consequently, effects of aqueous dried calyx extract of roselle plant administered through drinking water on the serum biochemistry and haematological parameters of broiler chickens were investigated. Hence, this study aimed at establishing the optimal level of aqueous dried calyx extract of roselle plant as nutrient additive in broiler chicken production.

MATERIALS AND METHOD

The study was conducted at the Poultry Unit of the Teaching and Research Farm of College of Agriculture, Ejigbo Campus, Osun State University, Osun State, Nigeria. The farm is located on latitude $7^{\circ}54^1\text{N}$ and longitude $4^{\circ}18^1\text{E}$ and $4^{\circ}54^1\text{E}$ at an altitude of 426 m above the sea level.

Housing

The chicks were raised in a standard deep litter system for 8 weeks (56 days). The poultry house and the necessary equipment used were fumigated and disinfected using formalin, potassium permanganate and Dettol.

Dried Calyx Extract The dried calyx from Roselle plant purchased from a local market was measured using a sensitive scale. Two hundred grams (200 g) of the sample was boiled in 2 litres of water for 10 minutes to obtain the extract solution of the flower extract. After boiling, the extract solution obtained was applied into the drinking water with the aid of measuring cylinder.

Management and Experimental Procedure

One hundred and fifty (150) Abor acre day- old broiler chicks were procured from a reputable hatchery in Ibadan, Oyo State. The chicks were randomly assigned to five (5) treatments (30 birds per treatment) replicated three (3) times with ten (10) birds per replicate under a completely randomized design (CRD) experiment. They were brooded on deep litter system using 200 watts electricity bulbs and coal pots for fourteen days. The necessary vaccination program: Newcastle Disease Vaccine (NDV intraocular), Gumboro (Infectious Bursa Disease) and Newcastle Disease

Vaccine (NDV Lasota) were carried out at appropriate times. Anti-stress (solution of glucose and vitamins) was also administered to the birds on arrival. The birds were generally fed starter diet (Table 1) for four weeks without the experimental drinking water. At the finisher phase, the birds were given aqueous extracts of calyx at various levels of inclusion in drinking water at finisher phase and fed finisher diet (Table1) for 4 weeks. Both routine (cleaning of pen, changing of water,

and washing of feeders and drinkers) and occasional (medication and vaccination) management practices were thoroughly carried out with strict hygiene measures. Each of the five treatments represented the concentration of the dried calyx extract from roselle plant per litre of drinking water. The concentrations were 0, 5, 10, 20 and 30 ml of extract solution per litre of drinking water for treatments T1, T2, T3, T4 and T5 respectively.

Table 1: Gross Composition of Broiler Diets

Ingredients	Composition	
	Starter (23% Crude protein)	Finisher (20% Crude protein)
Maize	50.00	50.00
Wheat offal	3.90	10.50
Brewer dried grain	5.00	10.00
Groundnut cake	10.00	12.00
Soya bean meal	24.50	13.00
Fish meal	3.00	-
Oyster shell	0.20	0.20
Bone meal	2.50	3.00
Methionine	0.25	0.30
Lysine	-	0.30
Premix	0.25	0.30
Salt	0.40	0.40
Total	100	100
Fibre level	4.34%	4.87%
Energy	2822.99MEkcal/kg	2717.38MEkcal/kg

Serum and Haematological and Biochemical Analysis

At the end of the experimental period (8 weeks), feed was withdrawn 12 hours prior to blood collection. Three birds were selected randomly from each replicate; the birds were bled using a sterilized disposable syringe and needle from punctured vein to aspirate 10 ml of blood samples from each bird. Five (5) ml of blood samples were collected into bottles treated with ethylene diamine tetra acetic acid (EDTA) for haematological assay. The remainder of each blood sample was allowed to coagulate to produce serum for blood chemistry measurements (Ochei and Kolhatkar, 2008).

The samples were preserved deep-frozen (-20 °C) before being transported to the laboratory for

analysis. The haematological parameters determined were red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC), lymphocytes, basophils, neutrophils, monocytes and eosinophil. The haematological parameters were determined by the use of Neubauer haematocytometer (Lamb, 1991). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were computed using the formulae outlined by Jain (1986). Samples for biochemical parameters were first centrifuged and decanted. Sigma kits were thereafter used to determine protein, globulins and albumins. Serum aspartic aminotransferase (AST), alkaline phosphatase (ALP) and alkaline

aminotransferase (ALT) were determined using the procedure of Baker and Silverton (1985).

Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using the Statistical Analysis Package (SAS (2008) version 9.1, USA). Where significant differences were found, the means were compared using the Duncan's multiple range test of the same statistical package.

RESULTS AND DISCUSSION

The result of haematological and serum parameters are as presented in tables 2 and 3, respectively. There were significant ($P < 0.05$) differences in five out of the ten haematological parameters considered: white blood cell counts (WBC), lymphocytes, monocytes, eosinophil and basophil. There were no significant ($P > 0.05$) differences in packed cell volume (PCV), haemoglobin counts (HBC), red blood cell counts (RBC), blood platelets and heterophils values. This suggests unequal influence of the treatments (varying levels of aqueous extract of dried roselle calyx in drinking water) on the haematological parameters of the broiler chickens (Al-Baghdadi, 2011). However, all the haematological values were within the normal range for healthy chickens (Mirtuka and Rawnsley, 1997; CCAC, 1993).

Therefore, non-significant influence of the treatments on the few of the haematological parameters signified their uncompromised physiological relevance in the broiler chickens. The broiler chickens on T2 (5 ml extract level) had the highest WBC value ($13466.67 \times 10^3 / \mu\text{l}$) and T5 ($11400.00 \times 10^3 / \mu\text{l}$) (30 ml extract level) were significantly ($P < 0.05$) different from one another; but both were similar ($P > 0.05$) to those birds on T1 ($12806.67 \times 10^3 / \mu\text{l}$) (0% without extract), T3 ($13023.33 \times 10^3 / \mu\text{l}$) (10 ml extract level) and T4 ($13000.00 \times 10^3 / \mu\text{l}$) (20 ml extract level) which were statistically insignificant. The white blood cells (WBC) protect the body against infection and disease with the production of antibodies through the process of phagocytosis (Soetan *et al.*, 2013). Therefore, the higher values of WBC in T2, T3 and T4 above treatment T1 indicated that the roselle calyx extract has the potential of enhancing better immune system development in broiler chickens. However, such potential reflected most

at 5 ml extract level (T2) even though similar potential could still be realised at treatments T3 and T4.

This corroborated the report of Olaleye (2007) that roselle calyx extract is considered an antiseptic, emollient, sedative and tonic agent that produces soothing and invigorating effects in the body. All the investigated WBC differentials showed significant ($P < 0.05$) differences across the treatments except heterophils. Generally, WBC differentials are involved in recognising body intruders, killing harmful bacteria and creating antibodies to protect the body against future exposure to some pathogens like bacteria and viruses. The lymphocytes value for the broiler chickens across the treatments ranged between 48.33 and 61.33%. This is in agreement with values of 45-75% and 58.10-71.70% reported by Gylstorff (1983) and Nemi (1993) respectively.

Lymphocyte were significantly ($p < 0.05$) different in values; with decreasing magnitudes as the extract levels increases. Broiler chickens under treatment T5 (48.33%) (30 ml extract level) had the least value of lymphocytes similar to treatment T4 (58.33%) but significantly ($p < 0.05$) different from other treatments. However, treatments T2 (61.00%) and T3 (60.67%) had similar values to T1 (61.33%) (control (0 ml) without extract) of the highest lymphocyte value. The decreasing values of lymphocytes as the extract level increases could be a signal that the extract level might not necessarily be high before realising its physiological relevance to lymphocyte production and effectiveness in broiler chickens. Also, similar lymphocyte values observed in extract-containing treatments with treatment T1 (0 ml without extract) could be as a result of absence of disease condition that could trigger pronounced physiological response of lymphocytes. This was evident in the healthy condition of the experimental birds throughout the duration of the study.

There were significant ($P < 0.05$) differences in the values of monocytes (2.00-3.67%). This value range is lower than the 16% and 8.10 – 16.10% reported for broiler chickens by Sebastian *et al.*, (2012) and Gylstorff, (1983) respectively; but treatments T2 (2.33%), T3 (2.00%) and T4

(2.67%) had their values within normal range 0-3% (Kahn, 2005) while treatments T1 (3.33%) and T5 (3.67%) had their values slightly outside the normal range. Birds on T5 had the highest value of monocytes and was significantly ($p < 0.05$) different from others except T1. Those on T2, T3 and T4 had similar values but T4 was not significantly ($P > 0.05$) different from T1.

Monocytes are the largest member of the white blood cells differentials with migrating capacity to various tissues of the body to eliminate harmful and dead matters. Consequently, the white blood cells values that were virtually high in all the treatments containing the extracts from roselle calyx was not as a result of inflammation or disease which ordinarily should have increased the monocyte count but the antioxidant property of the extract was responsible for maintaining white blood cell counts (Wang *et al.*, 2000; Essa *et al.*, 2006). Eosinophil values were significantly ($P < 0.05$) different from one another across the treatments. T4 (5.00%) was significantly ($P < 0.05$) different from T1 (1.67%). Those on T4 were similar to those on T2 (4.00%), T3 (3.67%) and T5 (3.33%) while those birds on T1 had similar values with T3 and T5. The range of value obtained for eosinophil (1.67-5.00%) was similar to the range of 1.20-3.10% reported by Nemi (1993) for broiler chickens. Basophil values were as well significantly ($P < 0.05$) different across the treatments. Though, treatment T4 (1.00%) significantly ($P < 0.05$) different from T3 (0.00%); but had similar values of basophil with T1 (0.33%), T2 (0.33%) and T5 (0.33%) while those on T3 were also similar to T1, T2 and T5.

The basophil value range of 0.00 – 1.00% obtained falls outside the normal range of 1.5-2.5% reported by Nemi (1993). Eosinophils fight against the activities of larger parasites such as worms and modulate allergic inflammatory responses while basophils establish defence against allergic reactions from parasites and bacteria. The various deviations of eosinophils and basophils values could be attributed to the different experimental conditions under which the studies were conducted. However, the eosinophils and basophils were within the range reported by Nanbol *et al.* (2016). Overall, values of the haematological parameters obtained from this

study pointed to the normal health condition of the experimental broiler chickens.

The serum biochemical parameters as presented in table 3 did not show distinct significant ($P < 0.05$) differences of the serum parameters across the treatments. Aspartate transaminase (AST) and albumin (ALB) were the only parameters that showed significant differences; others (alanine transaminase (ALT), total protein (TP), cholesterol (CHOL), triglyceride and high density lipoprotein (HDL)) were not. Serum biochemical parameters are a reflection of the health, nutrition, climate and management exposure of animals. Hence, serum biochemical parameters can be indicators of the productive performance of the broiler chickens and of metabolic diseases.

The aspartate transaminase (AST) values of broiler chickens under T4 (40.91 U/L) (20 ml extract level) had the highest value that was significantly ($P < 0.05$) different from other treatments. Those birds on treatments T1 (26.82 U/L) (0 ml, without extract), T2 (21.53 U/L) (5 ml extract level) and T5 (22.22 U/L) (30 ml extract level) had similar AST values while those on T3 (11.77 U/L) (10 ml extract level) with the least value was significantly different from all the treatments. AST and ALT are the indicators of hepatic function. Treatment T4 with the highest level of AST (40.91 U/L) that was significantly different from the other treatments, did not show any liver dysfunction along with others that were virtually similar. This could be as a result of the influence of the antioxidant potential of the extract that impacted beneficially on the physiological functions of birds especially the ALT. However, the combined high levels of AST and ALT could be detrimental to liver function and consequently compromise the broiler chickens' health. The albumin level of broiler chickens in treatment T5 had the highest value (1.98 g/dl) which was significantly different from treatment T1 (0.97 g/dl) but both were similar to treatments T2 (1.26 g/dl), T3 (1.34 g/dl) and T4 (1.59 g/dl). The albumin values of 0.97-1.98 g/dl observed in this study did not fall within the range of 25.00 – 45.00 g/dl and 10.80 – 16.00 g/dl reported by Harr (2002) and Ross *et al.* (1978) respectively. These deviations could be associated

with broiler breed and the treatment levels of aqueous extract of roselle calyx. Total serum protein and albumin serve as a measure of biosynthetic production of plasma proteins by the

liver. Therefore, the level of albumin supported the functionality of the liver; hence broiler chickens' health was enhanced by the *Hibiscus sabdariffa* extract (Oladele and Ayo, 1999).

Table 2: Effect of Varying Levels of Aqueous Extracts from Dry Calyx of Roselle Plant (*Hibiscus sabdariffa*) on Haematological Indices of Broiler Chickens

Parameters	T1 (0 ml)	T2 (5 ml)	T3 (10 ml)	T4 (20 ml)	T5 (30 ml)	±SEM
PCV (%)	30.67	23.67	25.33	29.00	24.33	3.70
HBC (g/dl)	10.17	7.45	8.20	9.47	8.00	1.16
RBC ($\times 10^6$ /mm ³)	3.52	2.55	2.38	3.36	2.42	0.48
Platelet ($\times 10^3$ / μ l)	171666.67	183666.67	192000.00	189000.00	116616.67	2825.39
WBC ($\times 10^3$ / μ l)	12806.67 ^{ab}	13466.67 ^a	13023.33 ^{ab}	13000.00 ^{ab}	11400.00 ^b	600.60
Lymphocytes (%)	61.33 ^a	61.00 ^a	60.67 ^a	58.33 ^{ab}	48.33 ^b	3.60
Heterophils (%)	33.33	32.33	34.33	32.33	40.00	2.87
Monocytes (%)	3.33 ^{ab}	2.33 ^c	2.00 ^c	2.67 ^{bc}	3.67 ^a	0.30
Eosinophils (%)	1.67 ^b	4.00 ^a	3.67 ^{ab}	5.00 ^a	3.33 ^{ab}	0.45
Basophil (%)	0.33 ^{ab}	0.33 ^{ab}	0.00 ^b	1.00 ^a	0.33 ^{ab}	0.26

^{ab} Means in the same row with different superscripts are significantly different (P<0.05)

PCV – Packed cell volume, HBC – Haemoglobin concentration, RBC – Red blood cell, WBC – White blood cell, **SEM**- Standard Error of Means

Table 3: Effect of varying levels of Aqueous Extracts from Dry Calyx of Roselle (*Hibiscus sabdariffa*) Plant on Serum Characteristics of Broiler Chickens

Parameters	T1 (0 ml)	T2 (5 ml)	T3 (10ml)	T4 (20 ml)	T5 (30 ml)	±SEM
Aspartate transaminase (AST) (U/L)	26.82 ^b	21.53 ^b	11.77 ^c	40.91 ^a	22.22 ^b	2.06
Alanine transaminase (ALT) (U/L)	4.89	5.37	5.01	2.95	3.32	0.93
Total protein (g/dl)	3.18	3.63	5.55	5.15	4.39	0.82
Albumin (ALB) (g/dl)	0.97 ^b	1.26 ^{ab}	1.34 ^{ab}	1.59 ^{ab}	1.98 ^a	0.27
Cholesterol (mg/dl)	246.54	210.06	211.95	260.38	303.77	61.64
Triglyceride (mg/dl)	65.37	66.88	84.51	91.18	78.03	9.45
High density lipoprotein (mg/dl)	37.50	37.08	41.25	36.49	54.10	5.92

^{ab} Means in the same row with different superscripts are significantly different (P<0.05)

SEM- Standard Error of Means

CONCLUSION

The haematological and serum biochemistry parameters showed that the aqueous extract of dried roselle calyx supported the health and the wellbeing of the broiler chickens. The 5 ml and the 10 ml levels of the extract of dried roselle calyx in drinking water of broiler chickens were found to be most suitable in terms of lymphocyte levels; however levels above 10 ml could be detrimental

to the health and wellbeing of the chickens.

RECOMMENDATION

Further studies on histopathological profile of birds fed extract of dried roselle calyx is recommended to reveal impact on tissues.. Also, pharmacological potency of the extract can be investigated towards harnessing its accurate administration as supplement for livestock use.

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