

Haematological and serum biochemical indices of finisher broiler chickens fed four differently processed Roselle seed meals (*Hibiscus sabdariffa*) as partial replacement for soybean meal

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Target Audience: Animal nutritionist, Feed miller, Researchers, Farmers.

Abstract

The frequent changes in the prices of conventional protein feed sources have prompted the search for alternative locally feed resources with minimal competition between man and livestock without any deleterious effect on health of the animal this necessitated a 56-day feeding trial to determine the effect of feeding four differently processed Roselle seed meals as partial replacement for soybean meal on haematological and serum biochemical indices of broiler chickens. One hundred and fifty (150) one - day old Arbor acre (+AA) Broiler chicks were randomly allotted to five (5) dietary treatments comprising three replicates and ten chicks per replicate. The experimental diets were formulated with supplementation of differently processed Roselle seed Meal [DPRSM] in the diets of broilers at 15% inclusion levels regardless of the processing methods. The treatments were designated as T₁, T₂, T₃, T₄ & T₅ as control diet (no Roselle seed meal), fermented Roselle seed meal (FRSM), boiled Roselle seed meal (BRSM), lye Roselle seed meal (LRSM) and enzyme Roselle seed meal i.e. (enzyme + raw Roselle seed) (ERSM) respectively. Data obtained were subjected to Analysis of Variance [ANOVA] using (22) and significant means were separated using Duncan's Multiple Range Test of same statistical package at 5% level of probability. The result revealed significant (P<0.05) differences in the heamatological parameters measured except for packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC) and mean Corpuscular Volume (MCV). Birds fed fermented, boiled, lye and enzyme Roselle seed meal had the highest (P<0.05) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells, neutrophils and eosinophil. Furthermore, the serum biochemical indices showed no difference (P>0.05) in all measured parameters except for alanine transaminase (ALT), aspartate serum transaminase (AST) & alkaline phosphatase (ALP). Birds fed enzyme Roselle seed meal had the highest ALT while the least value were observed in T₁, T₂, T₃ and T₄ respectively. In conclusion, the four differently processed Roselle seed meal could be used to partially substitute soybean meal in broilers diet without posing any treat to health of the birds.

Key words: Broiler chicken, Roselle seed, Roxazyme G[®], heamatology and serum biochemical indices

Description of Problem

Blood constituents play a vital role in accessing the physiological, pathological and

nutrition status of an organism; it provides the opportunity to evaluate the presence of several metabolites and other constituent in

the body of an animals (1). The evaluation of the blood profile of poultry may give some insight to the potential of the dietary treatments to meet the metabolic and health needs of the animal (2). Changes in the constituents of blood compounds when compared to normal values could serves as a reflector of the metabolic stage of the animal as well as quality of feed (3). Thus, they are used to determine the systemic relationship and physiological adaptation in the body of animals exposed to toxicants and stress due to environmental, nutritional or pathological factors (4). This is very important because the potential values of the non-conventional feed ingredient (NCF) depend on their nutritive value, availability and safety to animal health. This therefore, necessitated to evaluate the effect of a non-conventional feedstuff called Roselle seed on the health of poultry birds which are of high nutritive value. Roselle seeds for instance have been investigated for its nutritional value in poultry (5). Roselle (*Hibiscus sabdariffa*) seed is a leguminous shrub well adapted to the Guinea and Sudan savannah vegetation's belts of Nigeria (6). The roselle seeds from Nigeria contain about 35.90% crude protein, 10.14% ether extract, 10.09% ash and 15-17% crude fibre (7,8). The seed is a potential source of protein for poultry (9,10). Roselle seed cake (RSC) is not consumed by humans; it is cheap and readily available and therefore poses no threat of scarcity. Unprocessed Roselle seed have been reported to contain anti-nutritional factors which include total phenol, tannin and phytic acid. These factors have been shown to have detrimental effects on the health and performance of animals (10). Processing methods such as boiling water treatment, fermentation and ice water treatment have been reported to be effective to some extent. However, information on lye processing and

the use of feed additives to improve this grain by reducing or eliminating the anti-nutritional factor in these unprocessed seed are drastically scare. Friesen et al. (11) reported that the use of enzyme as feed additives in livestock feed has reported a success as a way of improving the utilization of dietary nutrients and high fibre feedstuff by monogastric animals. Bedford and Schulze (12) documented that supplementation of diets with exogenous enzyme reduced the adverse effects of some of these compounds especially those produced by carbohydrates and proteins. Enzyme had been used to improve fibre digestion and reduced viscosity of digesta (13,14). This research evaluated the effectiveness of four differently processed Roselle seed meals as partial replacement for soybean meal on heamatological and serum biochemical indices of broiler chickens.

Materials and methods

Experimental site

The experiment was conducted at the Poultry Unit, Livestock Teaching and Research Farm, Federal University Dutse, Jigawa State, Nigeria. Dutse is located on longitude 9.34° E and Latitude 11.76°N and has an elevation of 431.36 meters above sea level (15). There is usually a hot diurnal temperature and comparatively cooler at nights during the last 2-3 months of the dry season which is followed by a wet season between the months of June and September (16).

Processing of test ingredient

Roselle seeds were purchased in Dutse ultra-modern market, Jigawa State. The seeds were cleansed by winnowing and hand picking to remove stones and debris. The raw seeds were subjected to four (4) processing methods such as fermentation,

boiling, lye and raw Roselle supplemented with enzyme. Each of these processed Roselle seeds were incorporated in broiler diet at 15% inclusion levels in both starter and finisher of the experiment to replace soybean. The treatments were designated as T₁, T₂, T₃, T₄ & T₅ as control diet (no Roselle seed meal), fermented Roselle seed meal, boiled Roselle seed meal, lye Roselle seed meal and enzyme Roselle seed meal (enzyme + raw Roselle seed) respectively. The processing methods adopted for the Roselle seeds are described below:

Lye-water method: Ash was sieved to remove charcoal and other impurities; hot water was poured over ash in a barrel and a brown liquid dripped at base of container which represents the lye water. Roselle seeds was placed in a muslin cloth and soaked in the lye water for 18 hours, thereafter, it was removed, sun-dried for seven days, crushed and left to be incorporated in the formulation (17).

Hot water treatment: The cleaned Roselle seeds were put inside aluminum tower pot containing 50 litres of clean water in a batch of 50 kg. It was allowed to boil for 30 minutes at 100°C and allowed to cool by spreading on jute bags until stable weight is attained at room temperature. Thereafter, it was dried, crushed and left to be incorporated in the formulation.

Fermented Roselle seeds (FRS): The cleaned Roselle seeds were poured inside a drum of 50 liters of boiling water to boil for 30 minutes at 100°C. The boiled grains were drained, allowed to cool at room temperature and covered in a covered container for 48 hours. Thereafter, the product was sun-dried, crushed and left to be incorporated in the formulation.

Enzyme treatment: The clean raw Roselle seeds were crushed and mixed with enzyme from reputable manufactures and left to be

incorporated in the formulation. The commercial enzyme (Roxazyme G[®]) used in this study is a blend of multi-enzymes consisting of endo - 1, 4 - β -xylanase (EC 3.2.1.8), endo - 1, 3 (4) - β -glucanase (EC 3.2.1.6) and endo - 1, 4 - β -glucanase (EC 3.2.1.4) produced by *Trichoderma reesei*.

Experimental design and animal management

One hundred and fifty (150) day old 'Yammfy, +AA' broiler chicks were randomly allotted to five (5) dietary treatments comprising three replicates and ten chicks per replicate. The experimental diets were formulated with differently processed Roselle seed Meal [PRSM] in the diets of broilers at 15% inclusion levels. The treatments were designated as T₁, T₂, T₃, T₄ & T₅ as control diet (no Roselle seed meal), fermented Roselle seed meal, boiled Roselle seed meal, lye Roselle seed meal and enzyme Roselle seed meal (enzyme + raw Roselle seed) respectively. During brooding, temperature was controlled at 34.5°C for the first 0 to 2 days and then gradually reduced by 2°C per week to a final ambient temperature of 27°C at the last week of brooding. Feed and clean water were supplied *ad libitum*. The birds were reared intensively on deep litter (dried wood shavings) housing system. Normal vaccination program and medication schedule were strictly adhered to.

Experimental diets

Five experimental diets were formulated for the starter and finisher phases of the study. The experimental diets were formulated with 15% inclusion levels of differently processed Roselle seed meal (PRSM) in the diets irrespective of the processing methods to meet the (18) nutrient

requirements for broiler chickens. The chicks were allocated to five different treatments designated as T₁, T₂, T₃, T₄ and T₅ fed with diets containing 0% (control diet), 15% fermented Roselle seed meal, 15% Boiled Roselle seed meal, 15% lye Roselle seed meal and 15% raw Roselle seed meal supplemented with enzyme at 200ppm respectively. The gross compositions of the experimental diets are presented in Table 1 while the proximate composition of the Roselle seed is presented in Table 2

Proximate analysis

The proximate compositions of the diets were determined using the methods of (19). The Kjeldahl procedure was used to determine the crude protein (CP) content. The dry matter was obtained first by eliminating the moisture content using the vacuum oven-dried method. The dry matter was obtained when the weight become constant and moisture was computed as moisture = 100-final weight of sample. Dry matter = 100-% moisture. The ashing procedure as described by (20) was used to determine the ash content. The defatting and boiling methods were used to determine the crude fibre while soxhlet fat extraction method was used to determine the ether extract (EE). The nitrogen – free extract (NFE) was obtained using the formula below: $NFE = 100 - (\% \text{ Moisture} + \% \text{ CP} + \% \text{ CF} + \% \text{ EE} + \% \text{ Ash})$. The energy value ME was calculated using the formula of (21) as follows: $ME \text{ (kcal/kg)} = 37x \% \text{ CP} + 81x \% \text{ EE} + 35.5 x \% \text{ NFE}$.

Data analysis

Data generated were subjected to one-way ANOVA using (22). Significant means at 5% level of probability was separated using Duncan's Multiple Range Test of the same statistical package.

Statistical Model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;

Y_{ij} = the observed response

μ = the overall mean

T_i = the fixed effect of ith treatment (n = fermented Roselle seed meal, boiled Roselle seed meal, lye Roselle seed meal and enzyme Roselle seed meal)

Σ_{ij} = the residual error.

Data Collection and Analysis

Blood sample collection and analysis

At 56 day of the study, blood samples were collected from 6 randomly selected birds per treatment (2 per replicate) to determine the blood serum chemistry. Blood collection was done through brachial vein puncture (23) using needles and syringes. Each blood sample was emptied into 2 sets of well labeled sample bottles; the sample containing anti-coagulant was used for the analysis of hematological traits while the plane bottle without anti-coagulant was used to analyze the serum bio-chemical traits of the birds. The hematological traits analyzed were: Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) and biochemical traits: the total serum protein, albumin and globulin using bromocresol green method (24), Serum creatinine (25) and serum uric acid concentration (26) was determined according to standard procedures. Serum enzymes: alanine transaminase (ALT) and aspartate serum transaminase (AST) were analysed using the commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined using the method of (27)

Table 1: Composition of the experimental diets at finisher phase (kcal/kg Diet)

Ingredients	T ₁	T ₂	T ₃	T ₄	T ₅
Maize	58.00	58.00	58.00	58.00	58.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Soy bean meal	18.00	3.00	3.00	3.00	3.00
G/nut cake	8.00	8.00	8.00	8.00	8.00
Wheat offal	8.00	8.00	8.00	8.00	8.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	1.50	1.50	1.50	1.50	1.50
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Roselle seed	-	15.00	15.00	15.00	15.00
Total	100	100	100	100	100
Determined analysis (%)					
Metabolizable energy	2,986	2,965	2,965	2,965	2,965
(kcal/kg)					
Crude protein	20.56	21.88	20.24	20.56	21.14
Ether extra	8.06	9.84	9.91	10.11	9.56
Crude fibre	8.42	3.28	3.28	3.28	3.28
Ash	7.01	7.43	7.86	6.98	7.44
Nitrogen free extract	54.16	52.68	53.88	53.96	58.84
Dry matter	94.56	96.24	96.70	94.24	95.83

Composition of the premix supplied per 100kg of diet: vit A=1200IU, Vit E=1500, Vit D3=2500IU, Vit. B2 Folic acid=4000mg, Nicotinic acid=4000mg, Pantothenic acid=1500mg, 1=1750mg, Fe=40000mg, Zn=50000mg, Mn=100mg, Boitin=6000mg, Vit. C=30000mg, Cu=1500mg, Co=200mg, Si=100mg

Table 2: Proximate composition of differently processed Roselle seeds

	Raw	Fermented	Boiled	Lye	Enzyme
CP	32.50	34.27	33.48	33.44	33.60
EE	11.26	16.31	15.57	15.46	14.17
ASH	7.54	7.88	7.68	7.78	7.34
CF	10.17	12.49	11.60	11.12	10.82
NFE	48.17	45.83	43.11	43.86	40.18
DM	95.72	95.89	95.17	95.44	94.16

Crude protein, EE=Ether extract, CF= Crude fiber, NFE=Nitrogen free extra, DM=Dry matter

Results

Proximate composition of Roselle seed is presented in Table 2. The crude protein (CP) of the Roselle seed meal ranged between 34.27% for fermented Roselle seed and 32.50% for enzyme Roselle seed meal. The CP was similar for all the processing

methods meanwhile, fermented method recorded higher numerical value. Ether extract (EE) and crude fibre (CF) values for fermented Roselle seed meal were numerically higher compared to values obtained for other processed methods. The CF and EE were numerically higher in

fermented while the lowest was recorded for enzyme Roselle seed meal. The nitrogen free extract (NFE) decreases with the processing methods. Boiled Roselle seed meal recorded higher value of NFE while enzyme Roselle seed meal recorded the lowest value.

Effect of differently processed Roselle seed meal on heamatological parameters of finisher Broiler Chickens is presented in Table 3. There were significant ($P<0.05$) differences in MCH, MCHC and white blood cells. Birds on differently processed Roselle seed meal recorded higher values of these parameters compared to birds on the control diet. The least values of these parameters were observed with birds on control diets. The differential blood counts which are monocytes, eosinophils, neutrophil and lymphocyte were significantly ($P>0.05$) different from each other across the dietary treatments. Birds on differently processed Roselle seed meal have higher values

compared with control diets. The highest neutrophil was recorded for birds on T_3 and T_4 closely followed were birds fed T_2 & T_5 while the lowest value was recorded in birds fed control diet. The eosinophils and lymphocytes increased across the dietary treatments. The value ranged between 3.00 for T_4 & 1.33 for T_5 and 40.00 for T_1 & 30.00 for T_3 respectively. The highest monocytes were recorded for birds on T_1 & T_2 closely followed were birds fed T_4 & T_5 while the lowest value was recorded in birds fed T_3 . The packed cell volume, hemoglobin, red blood cells and MCV measured were not significantly ($P>0.05$) affected by the treatments imposed. The packed cell volume and hemoglobin ranged between 36.67, 35.67 and 12.67, 12.33 respectively. Bird fed T_5 recorded numerically higher red blood cells while the lowest was recorded in T_3 . The MCV ranged from 104.10 (T_2) to 96 (T_5).

Table 3: Effect of differently processed Roselle seed meal on heamatological parameters of finisher Broiler Chickens

Indices	Treatments					SEM
	T ₁	T ₂	T ₃	T ₄	T ₅	
PCV (%)	36.67	36.33	35.67	35.76	36.33	1.23
Hb(g/LI)	12.60	12.67	12.33	12.67	12.43	0.47
RBC($\times 10^6$ /mm ³)	3.67	3.60	3.58	3.63	3.77	0.28
MCV(fl)	97.00	104.10	101.87	101.87	96.73	4.15
MCH (%)	31.83 ^b	34.73 ^a	34.53 ^{ab}	34.37 ^{ab}	34.10 ^{ab}	2.43
MCHC (%)	26.47 ^b	33.47 ^a	33.97 ^a	33.67 ^a	33.70 ^a	2.23
WBC (*10 ³ /mm ³)	17.74 ^b	18.50 ^a	18.93 ^a	18.97 ^a	18.85 ^a	0.35
Deferential Count (%)						
NEUT (%)	58.00 ^c	60.00 ^b	66.67 ^a	63.00 ^{ab}	61.00 ^b	2.53
EOS (%)	2.00 ^b	2.67 ^{ab}	2.33 ^{ab}	3.00 ^a	1.33 ^c	0.36
LYM (%)	40.00 ^a	36.00 ^{ab}	30.00 ^b	38.33 ^{ab}	36.67 ^{ab}	2.84
MON (%)	2.00 ^a	2.00 ^a	1.33 ^b	1.00 ^c	1.00 ^c	0.15

abc=Means of the same row bearing different superscript differ significantly ($p<0.05$). SEM=Standard error of mean. RSM=Roselle seed meal. PCV=Packed cell volume. MCH=Mean corpuscular haemoglobin. MCV=Mean corpuscular volume. MCHC=Mean corpuscular haemoglobin concentration. NEUT=Neutrophils. EOS=Eosinophils. LYM=Lymphocytes. MON=Monophils

Effect of differently processed Roselle seed meal on serum biochemical indices of finisher broiler chickens is as shown in Table 4. The result of biochemical indices revealed significant ($P < 0.05$) influence of differently processed Roselle seed meal on ALP, ALT and AST across the dietary treatments. Birds fed enzyme Roselle seed meal had the highest ALT while the least value were recorded for control diet, T₂, T₃ and T₄ respectively. ALP and AST of birds boiled, lye, enzyme Roselle seed meal were highest compared to birds on control diet and fermented Roselle seed meal which are statistically similar (248.00, 247.33 (u/l)) and

(237.33, 232.67 (u/l)). Nevertheless, the total protein, albumin, globulin, creatinine and uric acid were not significant ($P > 0.05$) across the dietary treatments. The value obtained for total protein (TP) ranged from 34.34- 35.98g/dl while that of albumen ranged from 11.67 - 12.88g/dl. A numerically higher value was recorded for globulin when birds were fed control diets and boiled Roselle seed meal compared to other treatments. The values of the creatinine and the uric acid increased numerically across the treatment although no statistical difference ($P > 0.05$) was observed.

Table 4: Effect of differently processed Roselle seed meal on serum biochemical parameters of finisher Broiler Chickens

Indices	Treatments					SEM
	T ₁	T ₂	T ₃	T ₄	T ₅	
Total protein (g/dl)	35.98	34.67	34.67	34.34	35.00	2.52
Albumin (g/l)	12.88	12.67	11.67	12.67	12.67	1.01
Globulin (g/l)	23.10	22.00	23.00	21.67	22.33	1.70
ALP (u/l)	248.00 ^b	247.33 ^b	286.67 ^a	284.33 ^a	290.00 ^a	52.57
ALT (U/l)	153.67 ^b	150.33 ^b	153.33 ^b	140.00 ^b	227.67 ^a	31.10
AST (u/l)	237.33 ^b	232.67 ^b	280.00 ^a	279.00 ^a	289.00 ^a	69.28
Creatinine (µmol/l)	26.33	27.33	27.00	27.33	27.00	3.63
Uric acid (mmol/l)	541.33	554.33	562.00	567.00	553.67	123.50

ALP=Alkaline phosphatase, ALT=Alanine amino transferase, AST=Aspartate amino transferase. (G/L) gram per liter, (MMOL) millimole per liter, (G/DL) gram per deciliter. (G/L) gram per liter.

Discussion

The crude protein (CP) recorded (34.27%) regardless of the processing method employed were higher compared to (30.45%) reported by (28) and (5) who reported (30.50%) for processed Roselle seed cake. Similarly, it corroborated the works of (7), (29) and (8) that Roselle seed cake contain between 21.40 - 38.75% CP. Ismail et al (9) and (10) attested the seed is a potential source of protein for poultry. In addition, the higher CP observed in all the processing methods especially the fermented

Roselle seed agreed with the earlier report of (30) who noted increased CP (22.44%) in fermented Roselle seed meanwhile the value in recent findings was higher (34.27%). The numerically higher crude fiber observed for fermented Roselle seed agreed with (31) that reported (10.20%) and (11.87%) observed by (30) although the value here were higher than (5) who reported (4.20%). The ether extract (EE) was in accordance with (30) who reported higher EE for fermented Roselle seed in a feeding trial. This suggests the superiority and efficacy of fermented

processing method over its counterpart to sustain life. Furthermore, higher nitrogen free extract (NFE) value recorded for boiled Roselle seed with similarities to other treatments with the lowest numerical value in enzyme Roselle seed correlates trend reported by (30) who recorded a decreasing trend (Raw<soaked<Boiled<Fermented) for Nitrogen Free Extract when Roselle seeds were subjected to different processing methods.

Blood constituents are pertinent factors in accessing the physiological, health status and nutrition status of an organism which are employed in evaluating the presence of several metabolites in the animal body. The evaluation of the blood profile of poultry may give some insight to the potential of the dietary treatments to meet the metabolic and health needs of the animal (2). The blood parameters obtained in this study showed that the birds health status was not hampered by the Roselle seed treatment. The results on hematological parameters of finisher broiler chickens indicated that dietary inclusion of Roselle seed had no significant effect on pack cell volume, hemoglobin, red blood cells measured. The non-significance of pack cell volume, hemoglobin, red blood cells are indications that the birds were not anemic since these parameters are indicators of blood volume and oxygen carrying capacity. It also asserted the seed (Roselle) was better utilized for good nourishment. In addition, the similarity in the various blood constituents of the broiler chickens fed differently Roselle seed meal regardless of the processing methods when compared with basal diets (soybean meal) further substantiated the nutritional efficacy, adequacy and safety of Roselle seed meal in broiler finisher diets. Normal pack cell volume value reflects the absence of toxic factor that could alter the blood formation

process. (32). Colette et al (33) said blood parameters could be used to indicate the physiologic, pathologic and nutrition status of an animal. The report is similar to findings of (28) who observed no significant difference in pack cell volume (PCV), hemoglobin (Hb), red blood cells (RBC) meanwhile the values reported here were higher. Also, (5) asserted no significant difference in PCV, Hb and TP in broilers fed roselle seed cake with or without amino acid supplementation. The work agrees with (34) who noted no differences in PCV, Hb and RBC across the treatment groups when fed broiler chickens with roselle seed meal supplemented with protease enzyme. The values of these parameters reported in this study were higher compared to (34). Although, the result was at variance with (35) who reported significant differences in PCV, Hb, RBC and TP respectively. The non-significant MCV observed across the dietary treatment could be attributed to undiluted nutrient in the feed thus allowing the hepatic cells to perform maximally. MCV are derived from RBC, Hb PCV contents and concentration which are readily used in the assessing the nutritive status of the chickens as a result of the feed ingested (36).

The increased and statistically similar WBC and the differential counts recorded for birds on the differently processed Roselle seed meal compared to basal diet could be attributed to health factors due to presence of anti-nutritional factors (ANF) present in Roselle seed. Roselle seed have been reported to contain anti-nutritional factors which include total phenol, tannin and phytic acid which have detrimental effects on the health and performance of animals (10). It could also mean that none of the processed methods employed was able to totally ameliorate the effect of these ANF content in

Roselle seed. White blood cell is used to build body defense as part of immune system as high WBC count indicates the animals are reacting to one disease (37). Similar observation was given by (28) who noted significant improvement in WBC with higher values. The result agreed with (35) who observed no significant influence of processed Roselle seed meal supplemented with enzyme on WBC. Although, the differential counts reported by (28) showed no significant difference which contradicted the present study. Ocheja et al (38) reported that high white blood cell counts are usually associated with microbial infection antigen in the circulatory system. The lymphocyte and eosinophils recorded in this research were high in birds fed differently processed Roselle seed as basal diets this could be attributed to health problem from the environment rather than the diet since birds on control diet recorded the highest value. High lymphocyte concentration is an indication that there is an inflammatory reaction going on in the system which could likely result into sickness as a result of foreign substances introduced to the body (39). Sembulingam and Sembulingam (40) observed that the number of neutrophils in the blood increases rapidly with acute infection. The lymphocyte values reported here agreed with (41) who obtained (45-70%) but in contrast with (35) that reported (78.66-86%).

Serum chemistry is a concept used to detect abnormalities in physiological processes and pathology of organs in livestock animals (42). Total protein (TP) and its fractions (albumin and globulin) give information needed to interpret the protein quality of a feed. The non-significant effect of differently processed Roselle seed on TP, albumin and globulin could be attributed to the protein quality in Roselle seed. Ismail et

al (9) and (10) have reported Roselle seed as a potential source of protein for poultry. Eggum (43) attributed TP to reflects the quality of protein fed. The work is in line with (28) who reported no influence on TP when birds were fed Roselle seed cake. Report of (35) contradicted this finding, the author observed significant difference in TP of birds fed diets containing fermented Roselle seeds supplemented with enzyme. The numerically increased creatinine and the uric acid observed is similar to report observed by (28) who reported no influence on creatinine of birds fed Roselle seed cake. Creatinine is a nitrogenous waste product formed from the metabolism of creatinine in skeletal muscle. Creatinine determination is used to detect the state of kidney function as high level indicates impaired kidney function. The values reported are within the normal ranged for healthy birds (26.33-27.33 $\mu\text{mol/l}$) which is an indication that there was no muscle wastage. Serum creatinine level is indicative of the extent of muscle wastage occurring in a livestock animal. A low serum uric acid recorded may be implicative of efficient protein utilization. Kumta and Harper (44) and (43) reported a general fall of serum urea concentrations with time in nutritionally balanced amino acids-based diets. Mundow and Bergner (45) also confirmed that the content of blood urea increased when the protein content of the diet increased.

The ALT recorded for lye Roselle seed meal showed reduced serum level of ALT compared to those on other processed methods. The high serum ALT is attributed to serious liver disease and critical conditions most especially the ones that causes extensive cell necrosis, which includes severe viral hepatitis or toxic liver injury (46). Consequently, the reduced AST (U/L) recorded for control diet (T1) and

fermented Roselle seed meal (T2) compared to other processed method implied that birds on T2 were able to adequately condole the ANF in Roselle seed due to the processing method employed. The liver is a prime site for the termination of toxic compounds present in the blood stream.

Conclusion and Application

1. Dietary supplementation of differently processed Roselle seed at 15% inclusion in broiler diet compete well with soybean-based diet without posing any negative effect on health of the birds.
2. Broiler chickens can be raised on processed roselle seed meal in respect to any of the processing methods employed in this experiment as it did not alter the blood profile values measured as compared to a healthy bird on based diet.
3. Regardless of processing methods employed on Roselle seed meal, it did not alter all the measured haematological and serum biochemical indices values obtained as they are within the recommended range for healthy chickens as compared to birds on basal diet.
4. Farmers can incorporate any of the four processed roselle seed most especially the fermented Roselle seed up to 15% inclusion in broiler formulation.

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