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Serum biochemical indices of Finisher Broiler Chickens fed diets containing unprocessed and variously processed Roselle(*Hibiscus sabdariffa* L.) seeds

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Target audience: Researchers, animal nutritionists, feed millers

Abstract
A 4 week feeding trial was conducted using 180 five-week old Hubbard broiler chickens to determine the effect of feeding variously processed roselle(Hibiscus sabdariffa) seeds on serum biochemical indices with a view to determining the potential of roselle seed as an alternative to soybeans. Five broiler finisher diets were formulated and fed to the chickens in groups of 36 birds per diet, subdivided into 3 replicates of 12 chickens each in a completely randomized design. Diet 1 (control) was maize - soybean based. In diets 2, 3, 4 and 5, 50% of soybean was replaced weight by weight with unprocessed, soaked, sprouted and boiled roselle seed meals respectively. Haemoglobin concentration was depressed (P<0.05) by boiling without affecting red blood cell and other red blood cell dependent parameters, but it depressed red blood cell indicators that are haemoglobin dependent. Packed cell volume did not indicate anaemic tendency among treatments, and white blood cell count did not indicate infection due to treatments. Serum protein was adequate, and liver and kidney function were not compromised. Health and nutrient absorption of birds were not compromised by replacing 50% of soybeans with unprocessed, soaked, sprouted and boiled roselle seeds in broiler chickens.

Key Words: Broiler chicken, blood parameters,roselle.

Description of Problem
Faced with the challenge of competitive demandfor conventional grain feeds by man and animals, and the attendant high cost of grain legumes, it has become imperative to source for alternative feedstuffs to retreat this trend.Roselle seed has been reported to contain 35.90% crude protein (1) and a good mineral profile (2). They are rich in dietary fiber (39 – 42%) (3) and a considerable good ratio of soluble to insoluble fractions (4), and has been recommended for inclusion in broiler diets.

Blood parameters have been reported to be good indicators of physiological, pathological and nutritional status of an animal (5), hence, are being used to infer the impact of nutritional factors in diets on living creatures, andaiding in disease diagnosis. The aim of the study was to determine the effect of feeding differently processed roselle seeds on serum biochemistryof broiler chickens.

Materials and Methods

Experimental site

The study was carried out at the Livestock Farm Complex, Plateau State College of Agriculture, Garkawa, Nigeria. Garkawais on latitude 8° 53'N, longitude 9°32'E, and 853metersabove sea level (6).

Preparation of test ingredients

Roselle seeds were purchased from the market in Mangu, Mangu Local Government Area of Plateau State. The seeds were thoroughly cleansed of dirt by winnowing and sieving. Some seeds were milled without processing of any kind [Unprocessed Roselle Seed Meal, (URM)]. Some were soaked in water for 24 hours, drained, and sun-dried until crispbefore milling [Soaked Roselle Seed Meal (SRM)]. Some seeds were added to water already boiling over fuel wood and cooked for 30 minutes after returning to the boil according to method described by(7). The water was drained and the seeds were sun-dried until crisp, and then milled [Boiled Roselle Seed Meal (BRM)]. Some of the seeds were soaked in water for 24 hours, drained and packed into jute sacks and left at room temperature for 48 hours to sprout. The sprouted seeds were then sun-dried until crisp [Sprouted Roselle Seed Meal (SPRM)]. All the seeds were crushed using a burr mill to the feed particle size and incorporated into experimental diets.

Experimental diets

Five isonitrogenous and isocaloric broiler finisher (20% crude protein and 3000kcal/kg metabolizable energy) dietswere formulated (Table 1). Treatment1(control) was based on full-fat soybean meal. In treatments 2, 3, 4 and 5, 50% of the soybeans were replaced by unprocessed roselle seed meal, soaked roselle seed meal, sprouted roselle seed meal, and boiled roselle seed meal respectively.

Experimental birds and management

One hundred and eighty (180) healthy 5 week-old broiler (Hubbard classic) chicks were balanced for weight and randomly assigned to five treatmentsin a completely randomized design. Each treatment was divided into three replications of 12 birds each.The study lasted for 28 days. Standard management procedures were adhered to.

Blood analysis

Two birds from each replicate were randomly selected and slaughtered by slitting their throats (8). 2.0ml of blood was collected from each bird into separate bijou bottles containing ethylene diaminetetraacetic acid (EDTA) anticoagulant at the rate of 2mg/ml for haematological analysis (9). The analysis for total erythrocyte (RBC) count, total white blood cell (WBC) count, haemoglobin (Hb) concentration, and packed cell volume (PCV) were done using the methods described by(10). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were also calculated according to (10).2.0ml of blood was also collected fromeachbird into separate bijou bottles without EDTA for serum biochemistry (9).Total serum protein, serum albumin, aminotransferase, alanine phosphatase, creatinine, urea, potassium, sodium and chloride were determined according to

methods described by (12). Globulin was calculated as the difference between total protein and albumin (13).

Chemical analysis

Unprocessed and processed roselle seeds, and the experimental diets were analyzed for proximate composition, calcium and phosphorus content according to methods described by (14). Gross energy contents of the diets were determined using a Gallenkamp bomb calorimeter according to the method described by (15).

Statistical analysis

Data generated were subjected to analysis of variance using the (16) software. Significant differences in the treatment means were separated using the Duncan's Multiple Range Test (16).

Table 1: Ingredients and nutrient contents of broiler finisher diets containing unprocessed and processed roselle seeds

	Dietary treatments				
Ingredients	**Control**	**URM**	**SRM**	**SPRM**	**BRM**
Yellow maize	49.12	48.12	48.12	48.12	48.12
Full-fat soybeans	33.08	16.54	16.54	16.54	16.54
Roselle seed*	0.00	16.54	16.54	16.54	16.54
Fish meal	2.00	5.00	5.00	5.00	5.00
Wheat offal	12.00	10.00	10.00	10.00	10.00
Bone ash	3.00	3.00	3.00	3.00	3.00
Iodized salt	0.25	0.25	0.25	0.25	0.25
Vit-Min premix **	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25
TOTAL	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude protein (%)	20.00	20.35	20.78	20.47	20.41
ME (kcal kg^{-1})	3059	3050	3050	3055	3054
Ca (%)	1.33	1.51	1.51	1.53	1.50
Available P (%)	0.79	0.76	0.76	0.76	0.77

**BIOMIX™ supplying per kg diet: Vit. A 5000 IU; Vit. D3 8000 IU; Vit. E 12,000mg; Vit. K3 15000mg; Vit. B1 1000mg; Vit. B2 2000mg; Vit . B6 1600mg; Niacin 12000mg; Pantothenic acid 2000mg; Biotin 1000mg; Vit B12 3000mg; Folic acid 15000mg; Choline chloride 60000mg; Manganese 10000mg; Iron 15000mg; Zinc 800mg; Copper 400mg; Iodine 80mg; Cobalt 40mg; Selenium 8000mg.

*Metabolizable Energy (ME) (kcal/kg) = 37 X %CP + 81 X %EE + 35.5 X %NFE (11).

Results and Discussion

Serum biochemistry indices

Effects of the experimental diets on haematological and serum biochemical indices of the broilers are presented in Table 2. There were significant ($P<0.05$) differences among the various processing methods for all the haematological parameters except packed cell volume (PCV). Birds fed diets containing unprocessed, soaked and sprouted roselle seeds significantly ($P<0.05$) reduced red blood cell (RBC) count. This may be as a result of higher tannin content in the respective diets. Highest reduction of tannin by boiling

(29.90%) might have effectively improved protein digestion and utilization (17) and hence, RBC count. RBC of birds fed boiled roselle seeds was similar to the control. Duwa *et al*. had reported significant (P<0.05) increase in RBC of birds fed boiled roselle seed diets. On the contrary, (19) reported that sprouting produced the most significant (P<0.05) increase in RBC value. RBC values obtained were similar to that reported by (19) though lower than reported by (18). The range obtained was in line with 2 – 4x10^{12}/l (20) for domestic chickens, indicating that the birds were not anaemic. Haemoglobin (Hb) concentration was highest (P<0.05) in birds fed the sprouted seed meal and lowest (P<0.05) in birds fed the boiled seed meal diets. This finding agrees with the report of (19) and (18) for broilers of similar age. Haemoglobin values obtained were within normal range (7.0 – 13.0g/dl) for domestic chickens (20; 22). Values of PCV obtained fall in the range (27 – 41%) reported by (23) for healthy broiler chickens. PCV was not significantly (P<0.05) different among the various processing methods, implying that the processing methods did not affect the basic status of the erythron, hence the birds were not anaemic. Mean corpuscular volume was significantly (P<0.05) higher in birds fed the unprocessed and soaked roselle seed diets. Birds fed sprouted and boiled roselle seeds had similar MCV with the control. Values obtained fall within the reference range (21). Kwari *et al*. (19) found significantly (P<0.05) higher MCV in broiler birds fed the boiled seeds, but a significantly (P<0.05) lower value in birds fed the sprouted seeds. MCH was significantly (P<0.05) increased in birds fed diets containing soaked and sprouted roselle seeds but reduced (P<0.05) in birds fed boiled roselle seeds.

Table 2: Haematological indices of the finisher broiler chickens.

	Dietary treatments					
Parameters	**Control**	**URM**	**SRM**	**SPRM**	**BRM**	**SEM**
RBC (x10^{12}/l)	3.14^{a}	2.61^{bc}	2.41^{c}	2.84^{b}	3.06^{a}	0.17^{*}
Hb (g/dl)	10.43^{ab}	8.63^{bc}	9.50^{ab}	10.77^{a}	7.43^{c}	0.80^{*}
PCV (%)	34.03	29.23	28.80	30.07	33.23	2.25^{NS}
MCV (fl)	109.14^{b}	112.00^{ab}	119.30^{a}	106.05^{b}	108.33^{b}	3.73^{*}
MCH (pg)	33.33^{b}	33.07^{b}	39.31^{a}	37.97^{a}	24.13^{c}	1.61^{*}
MCHC (g/dl)	30.67^{c}	29.53^{c}	33.01^{b}	35.82^{a}	22.24^{d}	0.89^{*}
WBC (x10^{9}/l)	26.87^{bc}	34.17^{ab}	32.83^{ab}	36.20^{a}	22.60^{c}	3.40^{*}

a, b, c, Means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard error of means; NS=Not significantly different (P> 0.05); *=Significantly different (P<0.05). RBC=Red Blood Corpuscules, Hb=Haemoglobin, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration.

MCH values fall within normal range (20). Mean corpuscular haemoglobin concentration (MCHC) was significantly (P<0.05) high in birds fed sprouted and soaked seeds contrary to the findings of (19) and (18). Boiling depressed (P<0.05) MCHC in the birds. The depressive effect of boiling on MCH and MCHC may be attributed to the low haemoglobin value. Haematological parameters within normal range are an indication of adequate nutritional status of the birds.

Serum biochemical indices are presented in Table 3.Total serum protein and globulin were not significantly affected by the processing methods. Albumin level was significantly (P<0.05) decreased in birds fed diets containing sprouted roselle seeds. Boiling and soaking increased (P<0.05) albumin values. (18) reported that broilers fed boiled roselle seeds gave the highest albumin value. Except for birds fed SPRM diet, birds in other treatments had albumin values within the range (1.7 – 2.0g/dl) reported by (23). Aspartate aminotransferase (AST) is significantly (P<0.05) different among the processing methods. Effect of sprouting and boiling reduced (P<0.05) AST while soaking (P<0.05) increased it. However, (19) had found higher AST values in cockerels fed soaked, sprouted, boiled and fermentedroselle seeds. AST levels obtained fall within 88 – 208 iu/l reported by (24). ALT was also significantly (P<0.05) different with higher values in birds fed unprocessed roselle seed meal and soaked roselle seed meal diets, but lower in birds fed sprouted and boiled roselle seeds. ALT: AST did not exceed 1.0 indicating no pathological alterations in the liver (25). Processing methods significantly (P<0.05) reduced alkaline phosphatase. The highest reduction was in birds fed boiled roselle seeds indicating improved liver function. Alkaline phosphatase values were

Table 3: Serum biochemical indices of the finisher broiler chickens.

	Dietary treatments					
Parameters	**Control**	**URM**	**SRM**	**SPRM**	**BRM**	**SEM**
Total protein (g/dl)	4.36	4.05	4.22	3.80	4.20	0.32^{NS}
Albumin (g/dl)	1.96^{a}	1.72^{bc}	1.86^{ab}	1.62^{c}	1.85^{ab}	0.10^{*}
Globulin (g/dl)	2.40	2.36	2.69	2.85	2.35	0.26^{NS}
Aspartate aminotransferase (iu/l)	95.00^{a}	85.33^{c}	93.17^{ab}	85.33^{c}	87.17^{bc}	3.04^{*}
Alanine aminotransferase (iu/l)	16.67^{b}	22.37^{a}	20.17^{a}	12.17^{c}	9.60^{d}	1.34^{*}
Alkaline phosphatase (iu/l)	209.00^{b}	221.67^{a}	199.50^{c}	197.00^{c}	182.67^{d}	1.41^{*}
Creatinine (mmol/l)	46.50^{a}	49.00^{a}	54.00^{a}	52.83^{a}	25.17^{b}	6.12^{*}
Urea (mmol/l)	2.68	3.97	3.15	3.87	3.70	0.58^{NS}
Potassium (mmol/l)	4.30	3.38	3.67	4.43	3.42	0.76^{NS}
Sodium (mmol/l)	79.67	84.17	84.17	78.00	74.14	10.61^{NS}
Chloride (mmol/l)	101.83	101.67	94.67	100.50	109.83	6.34^{NS}

a, b, c, Means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard error of means; NS=Not significantly different (P>0.05); *=Significantly different (P<0.05).

within the range of 167 – 305 iu/l recommended by (26). Corroborating this finding, (19) and (18) reported lowest alkaline phosphatase values for cockerels and broilers fed soaked and boiled, and boiled roselle seed meal diets respectively. Urea was not significantly affected by feeding diets containing the roselle seeds. This result agrees with the findings of (19). Creatinine level was significantly ($P<0.05$) low in birds fed boiled roselle seed diet group. This is a classical sign that the glomerular filtration rate of the kidney was not compromised by feeding the boiled seeds.Duwa *et al.* (18) corroborated the findings of this study by reporting least serum creatinine level for birds fed boiled roselle seeds. Serum electrolytes were not significantly ($P>0.05$) affected by the processing methods, hence kidney function was not affected.

Conclusion and application

1. Haematological parameters of finisher broilers showed no adverse effect on the health status of the birds due to the experimental diets. Boiling was most effective at improving the health status of the birds.
2. Serum biochemical indices indicate that processing roselle seeds did not affect protein availabilitytofinisher broiler chickens.

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