

Haematological Responses and Serum Biochemical Indices of Broilers Fed Differently Processed Sorrel Seed (*Hibiscus Sabdariffa*) Meal in Semi-Arid Region of Nigeria

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Abstract: Seven weeks feeding trial was conducted to investigate the effect of differently processed sorrel seed (*Hibiscus sabdariffa*) meal on the haematology and serum biochemistry of broiler chicken. Five experimental diets were formulated using differently processed sorrel seed meal. One hundred and sixty (160) 7 days old chicks (Anak 2000) were used. The birds were randomly allotted to four dietary treatments with 40 birds per treatment and ten (10) birds per replicate. Results of serum biochemistry showed significant differences ($P < 0.05$) among treatments except for blood cholesterol and hydrogencarbonate which showed no significant difference ($P > 0.05$). However, significant difference ($P < 0.05$) were observed for the haematological parameters though MCHC, MCH and MCV did not differ among treatments. The result showed that incorporation of differently processed sorrel seed meal has no negative effect on the blood biochemical indices and haematology.

Key words: Haematology • Blood Chemistry • Sorrel Seed Meal • Broilers

INTRODUCTION

The poultry industry in Nigeria, in the last decade, has been greatly affected by high cost of feed [1]. The provision of feed alone has been reported to account for 60 - 80% of the total cost in most livestock production in developing countries such as Nigeria [2, 3] and this emphasize the interest to develop local feedstuffs. In view of this, there is increased interest by Nigerian livestock farmers to harness unconventional feed ingredients such as sorrel seed meal.

Hibiscus sabdariffa locally known as “Yakuwa” is a well-adapted crop in the semi-arid zone of West Africa including Nigeria and it is generally planted as a border crop. The leaves are used as vegetable and the floral parts in the preparation of “Sobo”, a local drink. The stem provides fibre and the seeds are eaten by scavenging poultry [4]. The seed of *Hibiscus sabdariffa* is said to have high protein value and it is on account of its protein content that it is well priced for human consumption in French West Africa (Mali, Guinea, Burkina Faso, Cote D’Ivoire). However, in Nigeria larger quantities of the seeds of this crop are wasted on the farm annually and just enough being collected and stored for planting.

The use of blood examination as a way of assessing the health status of animals has been documented [5]. This is because it plays a vital role in physiological, nutritional and pathological status of organisms [5]. Haematological parameters are those parameters that are related to the blood and blood-forming organs [6]. They range from giving the level of the blood to detecting ailment or disorders through them. It had been reported that biochemical changes as a result of toxins have effects on haematological parameters [7]. The objective of this study was to investigate the effect of sorrel seed meal on the blood components of broiler finisher birds under semi-arid environment.

MATERIALS AND METHODS

Experimental Site: The study was conducted at the Poultry Production Unit (PPU) of Borno State Ministry of Agriculture and Natural Resources Maiduguri. The farm had standard deep litter houses. The study area lies between Latitude 11°-15° inch North and longitude 30° - 05 inch East and had an altitude of 354m above sea level [8]. It is characterized by hot and dry climate and short duration of erratic rainfall (3-4) months per annum

and a long period of dry season. Ambient temperatures are low in December to January ranging from 15-19°C and high in March to June, ranging from 33-44°C and low relative humidity ranging from 5-43.5% [8].

Experimental Stock/plant

Source and Processing of Sorrel Seeds: The sorrel seeds used in this study were procured locally around Maiduguri and processed as follows:

- 25kg of raw sorrel seeds were cleaned and milled and incorporated into the diets of broilers.
- 25kg of sorrel seeds were cleaned and roasted for 40 minutes in frying pan at 100°C and cool under shed for a day, then milled and incorporated into the diets of broilers.
- 25kg of sorrel seeds were cleaned and boiled in water for 30 minutes at 100°C in an aluminum pot, sun-dried for three days, then milled and incorporated into the diets of cockerels.
- 25kg of sorrel seeds were cleaned and soaked in water for 12 hours in a plastic basin, sun dried for three days then milled and incorporated into the diets of cockerels.

Experimental Birds and Management: One hundred and sixty (160) 7 days-old Anak 2000 broiler chicks were used for the study in a completely randomized design. The chicks were individually weighed and randomly selected and allocated to each of the four different types of processing methods. Each type of processing method consists of forty birds with 10 birds per replicate. Chicks were brooded under 100watts electric bulbs. The experiment lasted for seven weeks. All management were adhered to. The birds were vaccinated against Gumboro, at two and five weeks of age while New castle disease vaccine (Lasota) was administered at three weeks of age. Antistress was given each time after administration of drugs. Feed and water was given *ad libitum*.

The processing methods comprised of four diets which contain raw sorrel seed, (RSSM) group1[gp1], roasted sorrel seed (ROSS)group2[gp2], boiled and dried sorrel seed (BDSS)group3[gp3] and soaked and dried sorrel seed (SDSS)group4[gp4] in diets as shown in Table 1.

Chemical Analysis: The experimental diets were analyzed for dry matter (DM), Crude protein (CP), crude fibre (CF), ether extract (EE), ash according to [9] while tannin was determined using the method of Polshettiwar *et al.* [10].

Table 1: Proximate composition, Tannin proportion and reduction level of differently processed sorrel seed meal

Parameter (%)	gp 1	gp 2	gp 3	gp 4
Dry matter	94.30	94.20	90.40	91.55
Moisture content	5.70	5.80	9.60	8.45
Crude protein	26.00	25.40	27.10	25.85
Crude fibre	15.00	14.00	13.10	15.50
Ether extract	16.00	19.00	23.00	16.00
Ash	7.00	8.00	7.00	9.00
Nitrogen free extract NFE)	36.00	33.60	29.80	33.65
Tannins level%	2.50	2.00	0.80	1.60
% Reduction of tannins	-	20.00	68.00	36.00

RSS=Raw sorrel seed; ROSS=Roasted sorrel seed; BDSS=Boiled and dried sorrel seed; SDSS=Soaked and dried sorrel seed

Haematological Studies and Blood Chemistry: At the end of the trial, three birds per replicate were randomly selected. A set of blood samples was collected into EDTA bottles for haematological evaluation while another set of blood samples were collected without anticoagulant for blood chemistry evaluation. Parameters measured under haematological studies included packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC) count. The blood constants, mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH) were computed using appropriate formulae as described by Jain [11]. The bottles were kept in cooled cotton prior to analysis. Haematological parameters and blood chemistry were evaluate according to the procedure described by Davis and Lewis [12]. The serum biochemical assay was carried out using standard chemical procedures: Total serum protein by Golberg refractometer method [13], albumin by Bromocresol green (BCG) method [14], creatinine [15], urea nitrogen [16], serum glucose [17], Sodium ions and Potassium ions by flame photometry [18] serum enzymes by spectrophotometric method [19].

Statistical Analysis: Data obtained was subjected to analysis of variance. Where significant differences occurred, the means will be separated using Duncan multiple range F-test of the SAS [20] options.

RESULTS

Blood Biochemical Indices: Table 3, showed the values for all the serum biochemical indices.

The serum biochemical indices indicated significant ($P<0.05$) differences in favour of birds fed on boiled sorrel seed meal [gp3] but blood calcium and alkaline phosphate indicated significance ($P<0.05$) difference in birds fed on raw sorrel seed meal [gp1]. The blood cholesterol, HCO_3

Table 2: Proximate composition of treatment diets containing differently processed sorrel seed meal at the finisher phase

Parameter (%)	Types of processing methods			
	gp 1	gp 2	gp 3	gp 4
Dry matter	92.80	93.40	92.60	93.40
Moisture content	7.20	6.60	7.40	6.60
Crude protein	19.40	19.50	19.60	19.00
Crude fibre	7.00	8.50	6.00	7.10
Ether extract	5.00	5.50	7.00	6.50
Ash	3.50	2.00	2.00	2.50
Nitrogen free extract (NFE)	65.10	64.40	65.40	64.90

RSS= Raw sorrel seed; ROSS= Roasted sorrel seed; BDSS=Boiled and dried sorrel seed;SDSS:Soaked and dried sorrel seed

Table 3: Blood chemistry of broiler chickens fed raw and differently processed sorrel seed meal (SSM)

Parameters	Types of processing methods				
	gp 1	gp 2	gp 3	gp 4	SEM
Blood Protein (g/dl)	4.20 ^b	2.82 ^b	7.72 ^a	5.65 ^b	0.327*
Blood albumin (g/dl)	2.70 ^b	2.60 ^b	4.07 ^a	2.32 ^b	0.157*
Globulin (g/dl)	2.80 ^c	2.40 ^c	5.35 ^a	4.00 ^b	0.295*
Blood Cholesterol (mg/dl)	3.64	3.23	3.64	3.10	0.237
Urea (mg/dl)	9.62 ^c	17.22 ^b	29.00 ^a	11.00 ^{bc}	1.965*
Creatinine (mmol/l)	42.27 ^a	45.51 ^{ab}	38.50 ^a	55.00 ^c	5.923*
Sodium (Na ⁺) (mmol/l)	1.31 ^b	1.31 ^b	1.38 ^a	1.34 ^{ab}	1.474*
Potassium (K ⁺) (mmol/l)	2.36 ^b	2.42 ^b	3.75 ^a	2.15 ^b	0.303*
Calcium (Ca ²⁺) (mmol/l)	10.55 ^a	9.65 ^c	9.82 ^b	9.55 ^c	2.2396*
HCO ₃ (mmol/l)	23.00	21.25	25.75	22.00	1.490
Bilirubin (mg/dl)	0.82 ^a	0.51 ^b	0.85 ^a	0.40 ^b	0.813*
Bilirubin conjugated (mg/dl)	0.20 ^a	0.20 ^b	0.38 ^a	0.23 ^b	0.035*
Alkaline phosphate (μl)	66.60 ^b	58.82 ^b	17.75 ^a	45.52 ^c	2.953*
Glutamic oxaloacetic transaminase (SGOT) (μl)	10.50	11.50	10.70	9.75	0.635
Glutamic pyruvic transaminase (SGPT) (μl)	10.75 ^c	11.50 ^b	10.97 ^c	70.75 ^a	1.935*

a, b, c, Means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard Error of Means; NS=Not significant (P>0.05); *=Significant difference (P<0.05); RSS=Raw sorrel seed; ROSS=Roasted sorrel seed; BDSS=Boiled and dried sorrel seed; SDSS=Soaked and dried sorrel seed

Table 4: Blood parameters of broilers chickens fed raw and differently processed sorrel seed meal

Parameters	Types of processing methods				
	gp 1	gp 2	gp 3	gp 4	SEM ±
RBC x 10 ⁶ /mm ³	3.00 ^c	3.21 ^c	5.25 ^a	4.10 ^b	0.114*
WBC x 10 ³ /mm ³	14.00 ^c	14.25 ^c	19.25 ^a	18.00 ^b	0.381*
Hb (g/dl)	8.22 ^b	12.45 ^a	12.37 ^a	9.25 ^b	0.328*
MCHC (%)	21.80 ^a	22.55 ^a	23.72 ^a	22.52 ^a	0.388 ^{NS}
MCH (pg)	25.50 ^b	25.50 ^{ab}	27.00 ^a	25.75 ^{ab}	0.578 ^{NS}
MCV (ft)	126.25 ^a	127.25 ^a	124.25 ^a	127.75 ^a	1.037 ^{NS}
PCV (%)	25.00 ^c	31.50 ^a	36.50 ^a	32.25 ^b	0.589*

a, b, c, Means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard Error of Means; NS=Not significant (P>0.05); *=Significant difference (P<0.05); RSS=Raw sorrel seed; ROSS=Roasted sorrel seed; BDSS=Boiled and dried sorrel seed; SDSS=Soaked and dried sorrel seed

and glutamic oxaloacetic transaminase indicated no significant (P>0.05) difference among the types of different processing methods. The result indicated that no effect of tannin in birds fed on boiled sorrel seed meal [gp3] compared to birds fed on raw, roasted and soaked sorrel seed meal [gp1,2&4] respectively.

Haematological Parameters: Data on the haematological parameters are shown in Table 4. There were significant (P<0.05) differences among the types of different processing methods for all the parameters. The haematological indices were red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume, (MCV) and packed cell volumes (PCV). The significant (P<0.05) difference were indicated in birds fed on boiled and soaked sorrel seed meal [gp3&4]. The MCHC, MCH and MCV indicated no significant (P<0.05) difference among the types of different processing methods. These parameters were used to measure the size and haemoglobin content of the erythrocytes and values are useful in diagnosing various forms of anaemia.

DISCUSSION

Blood Biochemical Indices: Blood protein values showed significant difference among the types of processing methods. The significantly higher value of blood protein was indicated in birds fed on boiled sorrel seed meal. The values obtained in this study were within the normal range of serum protein (5.00-7.00 g/dl) reported by Anon [21] and 4.55 to 6.46 g/dl reported by Udoyong *et al.* [22] but lower than 8.32 to 8-70 g/dl reported by Njidda *et al.* [23]. Higher values indicate that there is enzyme hydrolysis of dietary proteins and explained that the blood pool serves as a major source of aminoacids needed for the synthesis of proteins [24, 23]. This observation showed that the protein level in the diet was sufficient to sustain or support the normal protein levels in the blood. It should be noted that the protein levels in the diets were similar.

Blood albumin values indicated significant difference among the different processing methods with birds fed on boiled sorrel seed meal [gp3] having the highest value over birds fed on raw, roasted and soaked sorrel seed meal [gp1,2&4]. This could be the reason why they have comparable total protein content among the different groups. This observation agreed with the observation of

Anon [21] and Allison [25] who reported changes in protein reserve in animal as indicated by serum total protein to be associated with alteration in the albumin fraction.

Blood globulin values also indicated significant difference among the different processing methods with higher significant values in birds fed on boiled sorrel seed meal[gp3]. The result of the blood globulin which was the value obtained after the subtraction of albumin from total serum protein showed that the values obtained could be attributed to the comparable utilization of protein in diets as reflected by the feed conversion ratio (Table 3). Furthermore, the proteins in the diet may be of comparable quality. For instance in pigs fed raw mucuna beans meal; [26] observed the dependence of blood proteins on the quantity and quality of dietary proteins.

Blood cholesterol showed no significant difference among the processing methods. The observed similarity in blood cholesterol was expected because of the comparable dry matter intake of the different diets. The diets contained similar crude fat levels which may invariably lead to comparable dietary fat intake. The values were lower than those reported by (Ogbuewu *et al.* [1] but within the range reported by Njidda *et al.* [23]. Prolonged high levels of blood cholesterol may result in its deposition on the walls of the blood vessels and these deposits may eventually harden to atherosclerotic plaque, these may block important blood vessels and result in a myocardial infraction.

Blood calcium values revealed significant difference among the different processing methods. The blood calcium levels are the most important factors in the formation and maintenance of the bones. Minerals are known to be part of hormones and as activators of enzymes [27]. The values recorded in this study were similar to those recorded by Anon [21]. The reasons for the significant difference may be attributed to calcium ratio of the diet. The evidence of calcium and phosphorus deficiency may be obtained from an analysis of blood serum, which would show content lower than normal [28]. The values of sodium and potassium recorded in this study ranged from, 131.73 to 138.00 mmol/l and 2.15 to 3.75 mmol/l respectively and this differed significantly among the different processing methods, the significant difference could be as a result of the processing method which boiling appear to be the best for sorrel seeds. Sodium and potassium are known to regulate osmotic pressure, maintain membrane potentials and acid-base balance and transmit nerves impulses. Sodium and potassium deficiency affect the tubes of kidney resulting

in an inability to concentrate urine. It also causes alterations of gastric secretions and intestinal motility [29, 30].

Serum creatinine revealed significant different among the types of different processing methods. Birds fed on boiled sorrel seed meal[gp3] had the lowest value. The highest value was in birds fed on soaked sorrel seed meal [gp4] followed by birds fed on roasted and raw sorrel seed meal [gp2&1]. The level of serum creatinine signals the extent of muscle wastage [1]. The significant increase in serum creatinine of birds fed on raw, roasted and soaked sorrel seed meal gives evidence to the fact that the birds were not surviving at the expenses of body reserves. Hence there was no weight losses observed in the study, while the lower value in birds fed on boiled sorrel seed meal [gp3] may be as a result of processing method.

The similarities on conjugated bilirubin value of birds in all the groups ruled out the possibility of liver damage which is usually associated with increased serum conjugated bilirubin [31]. The results of this study is similar to the findings of Ogbuewu *et al.* [1] who reported values ranging from 0.60 to 0.70 mg/dl of CBL. Other serological indices such as blood cholesterol, HCO₃ and glutamic oxaloacate transaminase indicated no significant difference among the different processing methods. The significant difference observed for most of the serological indices are indication of less anti-nutritional factors in the diet formulated with boiled sorrel seed meal [32].

Haematological Parameters: There were significant differences among the different processing methods for all the haematological parameters except mean corpuscular haemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) which did not differ significantly among of different processing methods. The range for PCV (25.00-36.50%) in the different types of processing methods fell within the normal range of values (30–40%)[33] and 28.00 to 30.70% reported by Ogbuewu *et al.* [1]. The lowest value of (25.00%) obtained in birds fed on raw sorrel seed meal[gp1] was inferior to the other processing methods. This could be attributed to the high-tannin content of the diets raw sorrel seed meal. Tannins are known to lower dry matter and protein digestibility [34]. The range 8.22-12.45 g/dl of Hb values were in line with the range 8-13 g/dl [35] for all the different processing methods. Awomiya *et al.* [36] reported that haemoglobin and heamatocrits values are influenced by temperature. The range (3.00-5.25 x 10⁶/dl) of RBC was within the normal range of (3-6 x 10⁶/dl) for chicken [37]. The RBC values

were higher than the values reported by Njidda *et al.* [23] for broilers of similar ages in the same environment. The WBC values range (14.00-19.25 x 10³/dl) tally with the range (9 - 58 x 10³/mm) reported by Oluremi and Sridhar [34]. According to Freeman [38] about 90% of the total leucocytes in the blood of chickens are lymphocytes and heterophyls. The heterophyls, lymphocytes ratio in chickens increase linearly as stressor order increases [38, 39]. The total WBC counts have been reported to increase with stressor applied on the chickens [39].

The range 21.8 - 23.72 g/l of MCHC, 24.50-27.00 µg of MCH and 126.25-127.75 ft of MCV for birds fed on raw, roasted, boiled and soaked sorrel seed meal respectively agreed with the report of Swenson [39] that normal erythrocyte indices for chickens ranged between 21 - 23g/l for MCHC, 25.00-27 µg for MCH and 115 - 127Ft for MCV. These parameters were used to measure the size and haemoglobin content of erythrocytes and values are useful in diagnosing various forms of aneamia.

CONCLUSION

The major anti- nutritional factors identified in the sorrel seed meal are tannins which are known to impair feed intake, nutrient digestibility and growth of poultry and young animals. Boiling as a processing method appears to be a more effective method of tannins reduction in sorrel seed than roasting and soaking in water. The result of this study also reveals that inclusion of sorrel seed meal using different processing methods have no negative effect on the health status of the broiler chickens.

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