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GROWTH, HAEMATOLOGY AND SERUM CHEMISTRY OF BROILER CHICKENS FED RAW OR DIFFERENTLY PROCESSED SORREL (*HIBISCUS SABDARIFFA*) SEED MEAL IN A SEMI-ARID ENVIRONMENT

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ABSTRACT
The effects of feeding raw or differently processed sorrel seed meal on the growth, haematology and serum biochemistry of broiler chickens were investigated in a-9 week experiment. Six diets were fed to 270 day-old Anak-2000 broilers chicks in groups of 45 chicks per diet with 3 replicates of 15 chicks each in a completely randomized design. The diets consisted of a control based on soyabean meal as major protein source and 5 other diets, in which 50% of the soyabean meal was replaced with raw (RSSM), soaked (SSSM), boiled (BSSM), sprouted (SPSSM), and fermented (FSSM) sorrel seed meal. Data were collected on the growth, haematological and serum biochemical parameters. Results showed superiority of sorrel seed to soyabean in term of essential amino acids. Sprouting however, had a slight reducing effect on the amino acid composition while boiling was the most effective in reducing the tannin content of the seed. There were no significant (P>0.05) dietary effects on final weight and daily weight gain. Feed intake was significantly (P<0.05) increased on the processed sorrel seed meal-based diets compared to the raw seed-based and the control diets. Feed conversion ratio (FCR) and feed cost of meat production were increased (P<0.05) on the fermented and sprouted seed diets. Packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), and haemoglobin (Hb) concentration were reduced (P<0.05) on the RSSM, SPSSM and FSSM-based diets. Serum total protein, globulin and cholesterol were reduced (P<0.05) by sprouting. Serum concentrations of sodium and potassium were not affected (P>0.05) by the diet. The haematological and serological values observed on all the diets were comparable to literature values for broiler chickens. It was concluded that up to 50% of dietary soyabean meal can be replaced with sorrel seed meal either raw or processed without adverse effects on the growth, haematology and serum biochemistry and thus the health of broiler chickens.

KEYWORDS: alternative protein source, processing, broilers, performance

INTRODUCTION

The conventional protein feedstuffs for poultry such as soyabean, groundnut cake and fish meal are scarce and expensive because they are competed for by humans as food and other industrial uses. The development of alternative protein feedstuffs in monogastric animal diets will therefore continue to be a necessity in developing countries. Sorrel (Hibiscus sabdariffa) belongs to the malvaceae family and forms a popular vegetable in Indonesia, India and many tropical regions (Tindal, 1986). It is now widely grown in the North Eastern and middle belt regions of Nigeria (Akanya et al., 1997) mainly for its calyxes used for the preparation of a local drink but the leaves are also used for soup and as a pot herb (Adigun, 2003). There are several reports on the chemical composition of sorrel seeds. Studies by APRC (1999) reported that sorrel seeds contain 28% crude protein, 19.9% ether extract, 5.5% ash and 18% crude fibre. In other studies Dashak and Nwanegbo (2002) and (Isidahomen et al., 2006) reported 35.19 and 25.92% CP respectively and 15% CF in sorrel seeds. The ether extract ranges between 19.90% (APRC, 1999) and 23.00% (Dashak and Nwanegbo, 2002; Isidahomen et al., 2006). However, Purseglove (1969) and Duke (1983) reported the presence of tannins in sorrel seeds which are known to exert detrimental effects on the health of animals (Jansman, 1983). Different processing methods have been reported to reduce the tannin content in plant seeds (Price et al., 1979; Price et al., 1980). This study was therefore designed to determine the effect of feeding raw or differently processed sorrel seed meal on the growth, haematology and serum chemistry of broiler chickens.

MATERIALS AND METHODS

Study Site

The study was conducted at the poultry unit of the Teaching and Research Farm, Department of Animal Science, University of Maiduguri in North Eastern Nigeria. The area, which falls within the semi-arid zone, lies between Latitude 11°05' and 12° North and Longitude 13°05' and 14° East and at an altitude of 354M above sea level (Alaku, 1983). In the study area sorrel is grown by small and medium scale farmers as a border crop. The calyxes are used as a drink and the leaves as vegetable while the seeds are primarily used as planting materials for the next season.

Source and Processing of Sorrel Seed

Sorrel seed was purchased from the market in Maiduguri metropolis, cleaned and processed as follows:

i) Raw sorrel seed meal (RSSM): the cleaned seed was fetched, milled and labeled RSSM;
ii) Soaked sorrel seed meal (SSSM): the cleaned seed was soaked in tap water for 24 hours, sun-dried for 72 hours then milled and labeled SSSM;

iii) Boiled sorrel seed meal (BSSM): the seed was boiled in tap water at 100^{oc} for 30 minutes, sun-dried for 72 hours, milled and labeled BSSM;

iv) Sprouted sorrel seed meal (SPSSM): sorrel seed was soaked for 24 hours and allowed to sprout for 2 days. The sprouted seed was sun-dried for 72 hours, milled and labeled SPSSM ;

v) Fermented sorrel seed meal (FSSM): the seed was boiled at 100^{oc} for 30 minutes and washed and kept in an air-tight container to ferment for 3 days. The fermented seed was sun-dried for 72 hours, milled and labeled FSSM.

Experimental Diets

Six experimental starter (23% crude protein) and finisher (20% crude protein) were formulated (Table 1). Diet 1 (control) was based on soyabean meal as the major source of protein. In diets 2, 3, 4, 5 and 6 fifty percent (50%) of the soyabean was replaced by RSSM, SSSM, BSSM, SPSSM and FSSM respectively.

Experimental Stock and Management

A total of two hundred and seventy (270) mixed sex Anak-2000 broiler chicks were used for the study which lasted 9 weeks (2nd August to 9th October, 2009). The chicks were weighed individually and randomly allotted to six (6) dietary treatments in groups of 45 chicks with 3 replicates of 15 chicks each in a completely randomized design (CRD). Each group received one of the experimental diets and clean drinking water ad-libitum for a period of 9 weeks. The starter diet was fed for the first 4 weeks and the finisher for the last 5 weeks of age.

Data Collection

Data were collected on the growth performance (feed intake, weight gain, final weight and feed conversion ratio), haematological and serum biochemical indices. A weighed quantity of feed was supplied daily and the leftover weighed each morning and subtracted from the quantity supplied to obtain the daily feed intake. The birds were weighed at weekly intervals to determine the weekly and subsequently the daily weight gain. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to weight gain as follows:

$$\text{FCR} = \frac{\text{feed intake}}{\text{Weight gain}}$$

At the end of the experiment (week 9), blood samples were collected from 6 birds in each experimental group (i.e. 2 birds per replicate) for the determination of the haematological and serum biochemical parameters. The birds randomly selected were fasted from 6.00 pm to 6.00 am and bled early in the morning to avoid temporary elevation of blood metabolites by feeding as observed by Bush (1975). Samples were collected from the brachial vein using disposable syringes and needles (21 gauges).

Samples for haematological study were collected into sample tubes containing heparin as anticoagulant while serological samples were collected into sample tubes containing no heparin. Serum was obtained after the blood was allowed to stand for 2 h at room temperature and centrifuged at 2,000 revolutions per minute (r.p.m) for 10 minutes to separate the cells from the serum. Haematological parameters included the packed cell volume (PCV), red blood cells (RBC), haemoglobin concentration (Hb), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCV), and mean corpuscular haemoglobin concentration (MCVC). Total protein, albumin, globulin, urea, cholesterol, sodium, potassium and chloride formed the biochemical data.

TABLE-1. Ingredient and analyzed chemical composition of the experimental diets

Diets	Starter						Finisher					
	Control	RSSM	SSSM	BSSM	SPSSM	FSSM	Control	RSSM	SSSM	BSSM	SPSSM	FSSM
Ingredients (%)												
Maize	48.00	48.00	48.00	48.00	48.00	48.00	56.00	56.00	56.00	56.00	56.00	56.00
Soybean	30.00	15.00	15.00	15.00	15.00	15.00	22.00	11.00	11.00	11.00	11.00	11.00
Sorrel seed	--	15.00	15.00	15.00	15.00	15.00	--	11.00	11.00	11.00	11.00	11.00
Wheat offal	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	8.00	8.00	8.00	8.00	8.00	8.00	7.00	7.00	7.00	7.00	7.00	7.00
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	2.70	2.70	2.70	2.70	2.70	2.70	2.60	2.60	2.60	2.60	2.60	2.60
*Premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition (%)												
Crude protein	23.18	23.04	23.13	23.10	22.98	23.16	20.20	20.41	20.63	20.01	19.56	20.18
Ether extract	5.78	6.12	6.36	6.93	5.89	6.12	8.70	8.79	9.41	9.13	8.73	9.41
Nitrogen free extract	52.97	49.86	49.84	48.91	50.55	49.64	54.34	53.34	52.76	52.89	53.76	50.99

**ME (Kcal/kg)	3206.3	3118.2	3140.3	3152.1	3121.9	3114.9	3381.2	3360.7	3398.5	3357.5	3339.9	3344.0

ME**: Metabolizable Energy calculated according to Pauzenga (1985) as ME (Kcal/kg) = 37 X % CP + 81 X % EE + 35.5 X % NFE.
* Vitamin – Mineral Premix (BIO – MIX) Supply the following per Kg: Vitamin. A 5000Iµ; Vit. D_3, 888, 000IU; Vit. E, 12,000mg; Vit. K_3; 1500mg; Vit. B_1, 1000mg; Vit. B_2, 2000mg; Vit. B_6, 1600mg; Niacin, 12,000mg; Pantothenic acid, 2000mg; Biotin, 1000mg; Vit. B_{12}, 3000mg; folic acid, 15000mg; Choline Chloride, 60,000mg; Manganese, 10,000mg; Iron, 15000mg;Zinc, 800mg; Copper, 400mg; Iodine, 80mg; cobalt, 40mg; Selenium, 8000mg.
RSSM = Raw Sorrel Seed Meal; SSSM = Soaked sorrel seed meal; BSSM = Boiled sorrel seed meal; SPSSM = Sprouted sorrel seed meal; FSSM= fermented sorrel seed meal

Chemical Analysis
The raw and processed sorrel seeds were analyzed for chemical composition (crude protein, fibre, amino acids and tannins). The experimental diets were also analyzed for proximate composition according to AOAC (1990). Samples were analyzed for amino acid composition according to Spackman et al. (1958). The tannin content of the samples was determined spectrophotometrically using the 4-amino antipyrene procedure (APHA, 1985).
Blood samples were analyzed for haematological parameters according to routinely available clinical methods as expounded by Bush (1975) and WHO (1980).

Statistical Analysis
Blood Data generated from were analyzed for variance (Steel and Torrie, 1980) using statistix (Statistix, 2003).

RESULTS
The results of chemical analysis are presented in tables 1 and 2. There was no indication of processing effect on the crude protein of sorrel seed, but the crude fibre was increased in the processed seeds compared to the raw. The levels of phosphorus and essential amino acids were lower in the sprouted seed than the raw and seeds from other processing methods. The highest reduction (34.04%) of tannin was observed in the boiled seed. The percentage tannin reduction was similar in the other processing methods (about 21%).

TABLE- 2. Chemical composition of differently processed sorrel and soyabean meal

Constituents	Processing methods of sorrel seed*					[1]Soyabean meal
	RSSS	SSSM	BSSM	SPSSM	FSSM	
Crude protein (%)	38.57	38.20	37.80	37.40	38.59	37.00
Crude fibre (%)	13.50	15.30	15.14	15.80	15.25	5.50
**ME (kcal/kg)	3500.10	3508.93	3508.93	3486.50	3563.04	3300.00
Calcium (%)	0.33	0.32	0.32	0.31	0.31	0.25
Phosphorus (%)	0.55	0.52	0.52	0.49	0.51	0.58
Arginine (%)	5.18	5.50	5.42	4.80	5.70	2.80
Histidine (%)	1.99	1.73	1.64	1.55	1.70	0.89
Isoleucine (%)	3.30	2.90	2.84	2.34	2.99	2.00
Leucine (%)	4.99	5.97	5.60	4.86	5.57	2.80
Lysine (%)	2.58	2.54	2.73	2.06	2.64	2.40
Methionine (%)	1.33	1.19	1.18	1.04	1.20	0.51
Phenylalanine (%)	4.17	3.73	3.97	3.22	3.88	1.80
Threonine (%)	2.83	3.10	3.06	2.76	3.13	1.50
Tryptophan (%)	0.73	0.66	0.63	0.49	0.67	0.55
Valine (%)	3.19	2.79	2.66	2.44	2.88	1.80
Tannic acid (mg/g)	3.29	2.59	2.17	2.57	2.59	NR
Tannin reduction (%)	-	21.28	34.04	21.88	21.28	-

RSSM = Raw Sorrel Seed Meal; SSSM = Soaked sorrel seed meal; SPSSM = Sprouted sorrel seed meal; FSSM = Fermented sorrel seed meal; BSSM = Boiled sorrel seed meal; *Analyzed
** Metabolizable Energy calculated according to (14) as ME = 37 x % CP + 81 x % EE + 35.50 x % NFE
[1]Source: (21); NR = Not reported.

The growth performance data (Table 3) showed no dietary effects (P>0.05) on the final weight and daily weight gain of the broilers. Daily feed did not differ (P>0.05) between the control and the raw sorrel seed-based diets but was increased (P<0.05) on the processed sorrel seed meal diets. The feed conversion ratio (FCR) and feed cost of meat production were significantly (P<0.05) increased on diets based on the fermented and sprouted sorrel seeds. The cost of the Kg feed was reduced on the sorrel seed meal-based diets compared to the control based on soyabean meal. No mortality was recorded during the period of the experiment.
The results of haematological analysis (Table 4) showed lower (P<0.05) PCV, RBC, Hb and WBC values on the RSSM and BSSM-based diets, but their values did not differ (P>0.05) amongst the control, SSSM, SPSSM and FSSM diets. There were significant (P<0.05) reductions of the MCV on the SPSSM and MCH on the BSSM, SPSSM and FSSM- based diets. The mean corpuscular

haemoglobin concentration (MCHC) was not affected (P>0.05) by the diet.

Results of serum analysis are presented in Table 5. Serum total protein, globulin, and cholesterol were markedly (P<0.05) reduced on the SPSSM. The raw, sprouted, and soaked sorrel seed meal-based diets had significantly (P<0.05) higher chloride (Cl^-) values. The serum concentrations of sodium (Na^+) and potassium (K^+) were not affected (P>0.05) by dietary treatment.

TABLE- 3. Growth performance of broiler chickens fed differently processed sorrel (*Hibiscussabdariffa*) seed meal

Parameters	Diets						
	Control	RSSM	SSSM	BSSM	SPSSM	FSSM	SEM
Initial weight (g/bird)	132	131	130	131	133	132	13.70^{NS}
final weight (g/bird)	2708.37	2776.70	2835.00	2732.31	2627.56	2736.09	176.30^{NS}
Daily feed intake (g/bird)	96.31^{c}	96.71^{c}	112.58^{b}	122.39^{ab}	124.16^{ab}	131.46^{a}	0.19*
Daily weight gain (g/bird)	42.99	40.90	45.00	43.37	40.12	43.43	3.37^{NS}
FCR (Feed:gain)	2.85^{b}	2.86^{b}	2.83^{b}	2.78^{b}	3.16^{ab}	3.24^{a}	1.06*
Cost of feed (₦/Kg)	68.24	68.54	68.99	69.29	68.84	69.14	0.96^{NS}
Feed cost (₦/Kg gain)	194.48^{b}	196.02^{b}	195.24^{b}	192.62^{b}	217.53^{a}	224.01^{a}	1.90*

a, b, c = Means within the same row bearing different superscripts differ significantly (P< 0.05)
* = Significant (P< 0.05); NS = Not significant (P> 0.05); SEM = Standard Error of Means;
RSSM = Raw Sorrel Seed Meal; SSSM = Soaked sorrel seed meal; BSSM = Boiled Sorrel Seed Meal SPSSM = Sprouted sorrel seed meal; FSSM = Fermented sorrel seed meal.
₦1 = $0.007 at the time of the study.

TABLE- 4. Haematological indices of broiler chickens fed differently processed sorrel (*Hibiscus sabdariffa*) seed meal

Parameters	Diets						
	Control	RSSM	SSSM	BSSM	SPSSM	FSSM	SEM
PCV (%)	34.42^{a}	30.01^{b}	34.50^{a}	30.00^{b}	35.25^{a}	32.50^{ab}	1.39*
RBC Count (x10^{6}mm$^{4)}$	3.01^{bc}	2.88^{c}	3.12^{b}	2.91^{c}	4.12^{a}	3.37^{ab}	0.05*
Hb Concentration (g/dl)	8.90^{ab}	7.67^{cd}	8.73^{b}	7.55^{d}	9.68^{a}	7.97^{c}	0.19*
MCV (fl)	99.78^{b}	109.41^{a}	110.37^{a}	103.87^{ab}	84.54^{c}	95.65^{bc}	4.03*
MCH (Pg)	27.00^{a}	27.13^{a}	27.97^{a}	26.06^{b}	23.19^{b}	23.58^{b}	1.06*
MCHC (%)	26.06	25.35	25.34	25.06	27.43	24.65	0.96^{NS}
WBC count (X10^{3}/mm^{3})	49.52^{ab}	40.30^{c}	52.00^{a}	41.60^{c}	53.00^{a}	47.30^{b}	0.90*

a, b, c, d = Means within the same row bearing different superscripts differ significantly (P< 0.05)
* = Significant (P< 0.05); NS = Not significant (P> 0.05); SEM = Standard Error of Means;
RSSM = Raw Sorrel Seed Meal; SSSM = Soaked sorrel seed meal; BSSM = Boiled Sorrel Seed Meal SPSSM = Sprouted sorrel seed meal; FSSM = Fermented sorrel seed meal.

TABLE- 5. Serum biochemical indices in broiler chickens fed differently processed sorrel (*Hibiscus sabdariffa*) seed meal

	differently processed sorrel seed meal based diets						
Parameters	Control	RSSM	SSSM	BSSM	SPSSM	FSSM	SEM
Total Protein (g/dl)	4.40	4.10^{a}	4.25^{a}	4.05^{a}	3.40^{b}	4.50^{a}	0.31*
Albumin (g/dl)	2.00^{ab}	1.80^{b}	1.80^{b}	1.70^{b}	1.80^{b}	2.10^{a}	0.12*
Globulin (g/dl)	2.45^{a}	2.30^{a}	2.45^{a}	2.35^{a}	1.60^{b}	2.40^{a}	0.11*
Urea (mmol/L)	2.05	2.15	2.10	2.20	2.20	2.15	0.15^{NS}
Cholesterol (mmol/L)	3.70^{ab}	3.45^{b}	3.35^{b}	3.30^{b}	2.90^{c}	3.80^{a}	0.18*
Sodium (Na^+) (mmol/L)	140.10	135.00	138.00	133.00	139.00	134.00	2.39^{NS}
Potassium(K^+)(Mmol/L)	4.75	4.70	4.65	4.80	4.60	4.85	0.43^{NS}
Chloride (Cl^-) (Mmol/L)	103.50^{b}	105.00^{a}	107.00^{a}	97.00^{c}	106.00^{a}	99.00^{c}	3.61*

a, b, c,= Means within the same row bearing different superscripts differ significantly (P< 0.05)
* =Significant (P< 0.05); NS = Not significant (P> 0.05); BSSM = Boiled Sorrel Seed Meal; SPSSM = Sprouted sorrel seed meal; FSSM = Fermented sorrel seed meal;
SEM = Standard Error of Means; RSSM = Raw Sorrel Seed Meal; SSSM = Soaked sorrel seed meal.

DISCUSSION

The protein content of the test material (sorrel seed) compares well with the value (37%) reported for soyabean

(NRC, 1994; Smith, 2001). With the exception of lysine sorrel seed had higher values of all the essential amino acids than those reported in soyabean by NRC (1994). The reason for the higher fibre in the processed seeds compared to the raw was not clear, but probably due to the loss of some soluble nutrients in the processing water during soaking and boiling and the utilization of nutrients by fermenting micro-organisms as well as the germinating seed. The increased demand for nutrients, particularly phosphorus by germinating seeds (Reddy et al., 1982) could be a possible explanation for the reduced phosphorus and essential amino acids in the sprouted seed in the present experiment. The maximum reduction of tannin in the boiled seed and the similarity in tannin levels amongst the other processing methods is an indication of the presence of both water-soluble (hydrolysable) and heat labile tannins in sorrel seed. The crude protein (CF) and metabolizable energy (ME) of the diets (Table 1) met the recommendations for broiler chickens in the tropics (NRC, 1994; Smith, 2001).

The similarities in the final weight and daily weight gain amongst the dietary groups suggest that the toxicity of tannin was not manifested. At the present levels of inclusion of the test material the concentration of tannin even in the raw seed meal-based diets (calculated) was 0.86 and 0.79% in the starter and finisher diets respectively. These concentrations are below the 1.30% reported to be tolerated by chicks (Jansman et al., 1989). The levels of most vital nutrients were reduced in the processed seeds compared to the raw. The significantly higher feed intake on the processed seed-based diets might be in an attempt to meet their requirements for these nutrients. The lower FCR values on the fermented and sprouted seed diets translated into a higher feed cost of broiler meat production on these diets. The reduction in the cost of the Kg feed on the sorrel seed meal- based diets compared to the control was attributable to the price difference between sorrel seed (₦37.50/Kg) and soyabean (₦66.67/Kg) at the time of the experiment.

The values for PCV, RBC, and Hb were comparable to the ranges of 30-35%; 2.88-4.12 x 10^6 mm^3 and 7.55-9.68g/dl respectively reported by Swenson (1970) and Campbell et al. (2003) for domestic chickens. The reduced PCV, RBC, WBC and Hb concentration on the raw and boiled sorrel seed meal-based diets despite the highest reduction of tannin by boiling is an indication that these effects were not due to tannin toxicity.

It has been reported that serum biochemical constituents are positively correlated with the quality of the diet (Brown and Clime, 1972; Adeyemi et al., 2000). The reduction of essential nutrients on the SPSSM which was attributed to the increased demand of these nutrients by the germinating seed must have translated into the reduced serum total protein, albumin, globulin and cholesterol in the group fed the SPSSM diet. The values of the blood parameters on all the diets are comparable to those reported in literature for broiler chickens (Swenson, 1970; Campbell et al., 2003) indicating nutritional adequacy of the diets. The reduced serum cholesterol is an added advantage as this will result to lower cholesterol deposition in poultry products (meat and eggs) and thus reduced incidence of coronary diseases in humans following the consumption of these products.

It was concluded that up to 50% of dietary soyabean meal can be replaced with sorrel seed meal either raw or processed without adverse effects on the growth, haematology and serum chemistry and thus the health of broiler chickens. Investigation into higher levels of replacement needs to be carried out as this will further reduce the cost of poultry production.

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