

HEMATOLOGICAL AND SERUM BIOCHEMICAL CHARACTERISTICS OF COCKERELS FED GRADED LEVELS OF BOILED SORREL SEED MEAL

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ABSTRACT: The effect of feeding graded levels of boiled sorrel seed meal (BSSM) at 0,15,30,45 and 60% as replacement for groundnut cake in the diet of 200 seven day old chicks of Isa brown x Goldline cockerel on heamatological and serum biochemical indices was assessed in a 14week feeding trial. Each treatment consisted of forty birds and was replicated four times with 10 birds per replicate in a completely randomized design. At the end of the experiment, blood samples were randomly collected from four birds in each group for heamatological and blood chemistry studies. BSSM at 60% significantly ($P<0.05$) increased the values of RBC, Hb, WBC, PCV and MCV although they were within normal ranges. Total protein and albumin were significantly ($P<0.05$) increased while creatinine was significantly ($P<0.05$) reduced by higher levels of BSSM in the diet. It was concluded that up to 60% of GNC can be replaced by BSSM in the diet of cockerels without adverse effects on blood characteristics.

Keywords: Cockerels, Boiled Sorrel Seed Meal, Heamatology, Serum Biochemistry

INTRODUCTION

The use of groundnut cake and soya bean meal as the only source of protein in poultry diet is generally becoming economically impracticable in Nigeria. Due to soaring price of these conventional feed ingredients (Ubosi, 1986) in developing countries, commercial poultry enterprises are closing down. So the development of cheap and readily available alternative sources of feed ingredient in poultry nutrition is currently receiving attention. One of such non-conventional feed ingredient that can be used as an alternative to groundnut cake, soya bean cake and fish meal is the sorrel seed meal (*Hibiscus sabdariffa*). It is less competed for by both human and livestock and hence not expensive. The leaves are used as vegetable by humans and the stem produces fibre while the seeds on the other hand are widely eaten by scavenging poultry (Philips, 1977).

In avian species, hematobiochemical studies are almost always performed for research purposes in order to evaluate the effect of rearing technique, feeding regimes, environmental condition and toxicity levels (Vergal *et al.*, 1984; Duwa *et al.*, 2012; Kabir, 2012). Swenson (1993) reported that the erythrocyte indices are used to measure the size and haemoglobin content of erythrocytes and values are useful in diagnosing diseases, toxicity level and anaemia. Awoniyi *et al.* (2000) also reported that haemoglobin and haematocrits values are influenced by temperature. The PCV which measures the cellular concentration of RBC in relation to the plasma volume is usually the first indicator of anaemia. This study was designed to determine the effect of boiled sorrel seed meal as replacement for groundnut cake on blood parameters of broilers under a semi-arid condition.

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° - 51'N and longitude 013° - 05'E and had an altitude of 354m above sea level. 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% (Alaku, 1983).

ORIGINAL ARTICLE



Experimental Stock and Diet

Two hundred 7 days-old Isa brown x Goldline cockerel chicks were used for the study. The cockerel chicks were individually weighed and randomly selected and allocated to each of the five replacement levels of groundnut cake replaced by boiled sorrel seed meal (BSSM) in a completely randomized design. Each treatment consisted of forty birds and was replicated four times with 10 birds per replicate. The sorrel seed meal was incorporated at 0, 15, 30, 45 and 60% level of replacement for groundnut cake respectively as shown in the Tables 1 and 2. The sorrel seeds used in this study were procured locally around Maiduguri and processed as follows; 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.

Each treatment received one of the diets. Feed and clean drinking water were provided *ad libitum* throughout the experimental period of 14 weeks.

Table 1 - Composition of chick mash containing varying levels of boiled sorrel seed meal

Ingredients (%)	Replacement levels of groundnut cake by boiled sorrel seed meal				
	(Control 0%)	15%	30%	45%	60%
Maize	57.47	56.97	55.97	54.97	53.97
Groundnut cake	20.28	17.24	14.20	11.15	8.11
Sorrel seed meal	0.00	3.04	6.08	9.13	12.17
Wheat offal	13.00	13.00	13.00	13.00	13.00
Fish meal	5.50	6.00	7.00	8.00	9.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Premix ¹	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100
Calculated analysis					
Crude protein (%)	19.93	19.91	19.88	19.67	19.56
ME (kcal/kg)	2807.24	2802.70	2795.70	2788.10	2780.0
Crude fibre (%)	3.84	4.14	4.45	4.75	5.06
Calcium (%)	1.08	1.01	1.06	1.12	1.17
Phosphorus (%)	1.01	1.07	1.15	1.13	1.14
Lysine (%)	0.87	0.99	1.15	1.29	1.44
Methionine (%)	0.32	0.36	0.41	0.45	0.49

¹ Bio-mix chicks supplied /kg. Vit. A = 4,000,000.00 IU, Vit. D₃ = 800,000.00 IU, Vit. E = 9,200.00mg, Niacin = 11,000.00mg, Vit B₁ = 720.00mg, B₂ = 2,000.00mg, B₆ = 1,200.00mg, B₁₂ = 600mg, K₃ = 800.00mg, Pantothenic acid = 3,000.00mg, Biotin = 2,400.00mg, Folic acid = 300.00mg, Iodine 400.00mg, Iron = 8,000.00mg, manganese = 16,000.00mg, selenium = 80.00mg, zinc = 12,000.00mg, Anti oxidant = 500.00mg.

Table 2 - Composition of growers mash diet containing varying levels of boiled sorrel seed meal

Ingredients (%)	Replacement levels of groundnut cake by boiled sorrel seed meal				
	(Control 0%)	15%	30%	45%	60%
Maize	67.37	66.87	66.37	65.87	65.37
Groundnut cake	9.88	8.40	6.92	5.43	3.95
Sorrel seed meal	0.00	1.48	2.96	4.45	5.93
Wheat offal	16.00	16.00	16.00	16.00	16.00
Fish meal	3.00	3.50	4.00	4.50	5.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Methionine	0.1	0.1	0.1	0.1	0.1
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Premix *	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100
Calculated analysis					
Crude protein (%)	15.00	14.90	14.90	14.93	14.80
ME (kcal/kg)	2849.52	2845.90	2845.90	2838.64	2835.01
Crude fibre (%)	3.85	3.99	4.15	4.29	4.44
Calcium (%)	1.30	1.37	1.50	1.48	1.46
Phosphorus (%)	1.03	1.04	1.11	1.12	1.13
Lysine (%)	0.61	0.67	0.75	0.82	0.89
Methionine (%)	0.25	0.28	0.3	0.32	0.35

* Bio-mix Grower supplied /kg. Vit. A = 4,000,000.00 IU, Vit. D₃ = 800,000.00 IU, Vit. E = 10,000.00mg, K₁ = 200.00mg, B₂ = 1000.00mg, Pantothenic acid = 3,500.00mg, Biotin = 1,500.00mg, Folic Acid = 200,000mg, Choline Chloride = 120,000.00mg, cobalt = 80.00mg, copper = 800.00mg, Iodine = 100.00mg, Iron = 15,000.00mg, Manganese = 60,000.00mg, Selenium = 400.00mg, Zinc = 15,000.00mg, Anti oxidant = 400.00mg.



Blood Collection and Analysis

At the end of the experiment, blood samples were randomly collected from four birds in each group for hematological and blood chemistry studies. Each blood sample were taken from the wing-vein by means of a sterile disposable (21-gauge) syringe and needle, and then into sample bottles containing dipotassium salts of ethylene diamine tetra-acetic acid (EDTA) for hematological studies while samples for biochemical indices were collected into anticoagulant free tubes and allowed to clot. Sera were obtained after the blood sample has been allowed to stand for two hours at room temperature and centrifuged for ten minutes at 2000 rpm to separate the plasma from the serum.

Microhaematocrit method was used to determine packed cell volume (PCV) and cyanmethaemoglobin method was used to determine haemoglobin (Hb). Red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubauer haemocytometer. Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the standard formula of Schalm et al. (1985). Total protein was determined, using the burette method and urea was by the dimethyl ethyl monoxide method. Creatinine was determined by Jaffe reaction method while serum albumin was obtained using the dye-binding technique with bromocresol green. Serum potassium, Calcium and sodium were determined by the calorimetric method (Daumas, 1975; Varley et al., 1980; Henry et al., 1974; WHO, 2003).

Statistical analysis

Data were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of the Statistical Analysis Software package (SAS Institute, 2002), means were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

The hematological indices of cockerels fed different levels of boiled sorrel seed meal (BSSM) are shown in Table 3. A significant difference ($P < 0.05$) was observed for all the parameters measured. Mean corpuscular hemoglobin values were depressed ($P < 0.05$) by BSSM at levels above 15%. MCHC was significantly lowered ($P < 0.05$) at 60% levels of replacement.

Table 3 - Blood parameters of cockerels fed on diets containing varying levels of sorrel seed meal as a replacement for groundnut cake

Parameters	Replacement levels of GNC by BSSM					±SEM
	(0%)	15%	30%	45%	60%	
RBC x 10 ⁶ / dl	3.45 ^d	4.60 ^c	4.40 ^c	7.32 ^b	9.51 ^a	0.165*
WBC x 10 ³ /mm ³	14.00 ^c	17.75 ^b	17.25 ^b	18.25 ^{ab}	21.50 ^a	1.071*
Hb (%)	15.25 ^d	16.20 ^{cd}	18.45 ^{ab}	17.47 ^{bc}	19.40 ^a	0.434*
MCHC (g/l)	33.52 ^b	33.30 ^b	35.25 ^{ab}	33.27 ^b	25.48 ^c	0.484*
MCH (µg)	28.67 ^a	27.88 ^a	24.65 ^b	24.20 ^b	25.28 ^b	0.558*
MCV (ft)	126.75 ^c	141.90 ^{ab}	141.75 ^{ab}	136.65 ^b	144.85 ^a	1.703*
PCV (%)	26.50 ^b	26.00 ^b	26.00 ^b	27.50 ^a	28.50 ^a	0.418*

a, b, c, d Means within the same row bearing different superscripts differ significantly ($P < 0.05$), SEM - Standard Error of Means, NS - Not significant ($P > 0.05$), * Significant difference ($P < 0.05$)

Table 4 - Blood parameters of cockerels fed on diets containing varying levels of sorrel seed meal as a replacement for groundnut cake

PARAMETERS	Replacement levels of GNC by BSSM					± SEM
	0%	15%	30%	45%	60%	
Protein (g/dl)	4.22 ^c	4.98 ^b	5.11 ^b	6.88 ^a	6.82 ^a	0.472*
Albumin (g/dl)	2.96 ^c	3.78 ^b	4.41 ^a	3.87 ^{ab}	3.98 ^{ab}	1.881*
Globulin (g/dl)	1.24	1.07	1.01	2.99	2.92	0.121 ^{NS}
Glucose (Mmol/L)	9.33 ^b	8.68 ^b	9.85 ^b	9.94 ^b	12.14 ^a	0.148*
Cholesterol (mg/dl)	3.33	3.12	2.98	3.04	2.82	0.220 ^{NS}
Urea (mg/dl)	4.56 ^a	3.11 ^{ab}	2.19 ^c	3.36 ^{ab}	3.29 ^{ab}	1.061*
Creatinine (Mmol/L)	47.37 ^{ab}	44.88 ^b	50.02 ^a	41.18 ^c	40.11 ^c	6.014*
Sodium (Mmol/L)	142.11 ^a	140.10 ^a	135.19 ^b	134.34 ^b	140.56 ^c	11.180*
Potassium (Mmol/L)	4.19 ^a	2.67 ^c	3.81 ^{ab}	5.73 ^a	3.08 ^{ab}	1.011*
Calcium (Mmol/L)	1.37 ^c	2.48 ^a	1.97 ^b	1.88 ^b	2.77 ^a	0.107*
Bicarbonate (Mmol/L)	18.2	17.31	19.07	19.58	19.11	1.010 ^{NS}
Total Bilirubin (mg/dl)	4.12 ^c	6.10 ^a	4.80 ^c	5.71 ^b	7.18 ^a	1.013*
Conjugated Bilirubin (mg/dl)	5.14 ^b	3.26 ^c	5.01 ^b	6.44 ^a	5.19 ^b	1.870*
Alkaline Phosphatase (IU/L)	40.47	41.39	39.79	40.19	40.73	1.347 ^{NS}
AST (IU/L)	11.31	9.18	10.89	11.01	11.89	2.531 ^{NS}
ALT (IU/L)	10.78	10.96	8.91	9.1	10.11	1.499 ^{NS}

a, b, c, d Means within the same row bearing different superscripts differ significantly ($P < 0.05$), SEM - Standard Error of Means, NS - Not significant ($P > 0.05$), * Significant difference ($P < 0.05$)



All other parameters measured were however higher ($P < 0.05$) in the group fed the highest replacement levels compared to the control. The values obtained for all the haematological indices were similar to ranges reported by Kwari et al. (2010) and Duwa et al. (2012) when cockerels were fed dietary sorrel seed meal. Similarly, the values are within the ranges reported by CCAC (1980) and Merck (1986) for normal chicken. This shows that BSSM had no adverse effect on erythropoiesis and is an indication that the diet did not cause nutrient restriction.

Results of the biochemical indices for cockerels fed BSSM are shown on Table 4. No effect ($P > 0.05$) of dietary BSSM was seen on the following parameters; globulin, cholesterol, bicarbonate, Alkaline phosphatase, ALT (Alkaline Amino Transferase) and AST (Aspartate Amino Transferase) which is an indication that BSSM has no toxic effect. This is contrary to the report of Kwari et al. (2010) who fed differently processed sorrel seed meal. The results however showed that total protein value rose with increased levels of dietary BSSM, although a statistical difference ($P < 0.05$) was observed for values of albumin no specific trend was noticed. Urea levels were similar ($P > 0.05$) between the control and other treatments except the group offered 30% replacement which had lower values. Creatinine was however, depressed ($P < 0.05$) by higher levels (45 - 60%) of BSSM compared to the control. It has been reported by Butani et al. (2002) that dietary protein affects serum creatinine by elevating it values. Hence, the difference observed in this work may be as a result of the higher protein in GNC compared to BSSM and not an impairment of renal function. Glucose level was highest ($P < 0.05$) in the group with 60% replacement level. Levels of Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{2+}) all differ among treatments. Sodium at the highest level of replacement (60%) was similar ($P > 0.05$) to the control group. Potassium was depressed at 15% level of inclusion of BSSM but it was similar for all other groups. Generally, calcium levels were higher in the groups fed BSSM. This may be attributed to the higher calcium levels in sorrel seeds compared to groundnut cake (Kwari et al., 2011).

It has been reported that haematological and serum biochemical indices are affected by nutritional status of an individual (Adeyemo et al., 2010; Kwari et al., 2011; Obikaonu et al., 2011)

CONCLUSION

It was concluded that up to 60% of dietary groundnut cake can be replaced with BSSM without adverse effects on haematology and serum biochemical parameters of cockerels.

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