REPLACEMENT VALUE OF BOILED AND FERMENTED SORREL *(HIBISCUS SABDARIFFA*) SEED MEAL FOR SOYA BEAN MEAL ON THE BLOOD PARAMETERS OF GROWING RABBITS

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ABSTRACT
A ten-week feeding trial was conducted to determine the effect of replacing full-fat soya bean meal (FFSBM) with boiled and fermented sorrel seed meal (BFSSM) on the heamatological parameters and serum biochemical indices of growing rabbits. Thirty cross-bred rabbits (New Zealand White X Dutch) between six and seven weeks of age with average initial body weight of 489.80 ± 25g were caged individually and allotted to five (5) dietary treatments. Each treatment was divided into three replicates of two rabbits. The rabbits were given diets and clean drinking water ad libitum throughout the period of the experiment. In diets 1 (control), 2, 3, 4 and 5 BFSSM replaced FFSBM at 0, 25, 50, 75, and 100%, respectively. The heamatological indices measured were packed cell volume (PCV), heamoglobin (Hb) concentration, white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular heamoglobin concentration (MCHC) and differential counts; serum biochemical indices such as total protein, albumin, globulin, glucose, urea and cholesterol were also measured. The haematological parameters indicated that there were no significant (P > 0.05) difference among treatment groups in all the parameters while the serum biochemical indices also indicated no treatments effect (P>0.05) in all the parameters measured except total protein which showed significant (P<0.05) difference among the treatment groups. Treatment 2 had the highest value compared to other treatments with similar values, but did not differ (P>0.05) differ from those fed T3 diet. It was therefore concluded that, 50% of the full-fat soya bean in rabbit diets could be replaced with boiled and fermented sorrel seed meal without deleterious effect on haematological parameters.
Keywords: Boiled and fermented sorrel seed meal, growing rabbits, and blood parameters

INTRODUCTION

The protein consumption of people in developing countries is below the Food and Agriculture Organization's (FAO, 2000) recommendation of 28 g of animal protein/person/day. The British Medical Association (1993) also estimated the average animal protein intake of Nigerians to be about 8.27 g per caput per day. Marguisit (1985) even gave a lower estimate of 5.5 g per person per day. FAO (2000) also reported that about 200 million people in Africa were undernourished at the dawn of the millennium. In recent years, the use of soya bean and groundnut cake (GNC) which used to be the major plant protein of choice in monogastric diet is becoming limited due to the high cost of production and the competitive requirement of soya bean and groundnut cake in human diets. An alternative protein concentrate worth exploring is sorrel seed meal. The crude fibre and 11.63% nitrogen-free extract (Kwari, 2009). Igwebuike *et al*. (2005) and Kwari (2009) successfully used the raw sorrel seed meal in the diets of growing rabbits and broiler tannins, an anti-nutritional factor present in the seed. This paper reports the effect of graded levels of boiled and fermented sorrel seed meal as a replacement for soya bean meal on the haematology and serum biochemistry of growing rabbits.

MATERIALS AND METHODS

Location of the Study

The study was conducted at the Livestock Teaching and Research Farm, University of Maiduguri, Maiduguri. (Sudan savanna region of Nigeria) along Bama Road, Borno State, above sea levels (Encarta, 2007).The area has a semi arid tropical climate with a wide seasonal diurnal range of temperature. The hottest months are April and May with a range between 39.4 and 40.1^{0}C under shade (Wikipedia, 2013). There is a long dry season of 7-8 months between the months of October and May. During the last 2-3 months of the dry season there is hot diurnal temperature and comparatively cooler

nights. This is followed by a wet season between the months of June and September. The average annual rainfall is about 500 mm. The relative humidity is about 45% in August and usually lowers to about 5% in December and January.

Source and Processing of Sorrel Seeds

Sorrel seed were bought from Gamboru market in Maiduguri, Nigeria. The sorrel seed were thoroughly cleaned and subjected to boiling for 30 minutes and washed. The boiled seed were kept in an air-tight container to ferment for three days. The fermented sorrel seeds were allowed to sun-dry for three days, milled and labeled as boiled and fermented sorrel seed meal (BFSSM).

Experimental Stock and their Management

Thirty (30) rabbits between 6 and 7 weeks of age, were used for the feeding trial which lasted for ten (10) weeks. The rabbits were weighed and then randomly assigned to five dietary treatments; i.e six (6) rabbits per treatment. Each treatment was divided into three replicates of two rabbits per replicate in a completely randomised design (CRD). The rabbits were kept in separate cages with dimension of 42 cm x 42 cm x 42 cm (L x W x H). The cages were raised 1m above the ground for easy cleaning. Metallic feeding troughs and plastic drinkers were provided in each cage. Feeds and water were provided *ad libitum* throughout the period of the experiment.

Experimental Diets

Five (5) experimental diets were prepared for the growing rabbits. Full-fat soya bean meal was replaced with boiled and fermented sorrel seed meal (BFSSM) at 0, 25, 50, 75 and 100% levels in diets 1 (control), 2, 3, 4 and 5 respectively. The ingredient composition and the calculated analyses of the experimental diets are presented in Table 1.

Table 1: Ingredients Composition (%) of the Experimental Diets

	Levels of FFSBM Replaced with BFSSM (%)				
Ingredients	T1 (0)	T2 (25)	T3 (50)	T4 (75)	T5 (100)
Maize	40.35	40.35	40.35	40.35	40.35
Wheat offal	12.00	12.00	12.00	12.00	12.00
Groundnut cake	5.00	5.00	5.00	5.00	5.00
Full fat soyabean meal	20.00	15.00	10.00	5.00	0.00
Boiled and fermented sorrel seed meal	0.00	5.00	10.00	15.00	20.00
Groundnut haulms	17.00	17.00	17.00	17.00	17.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Table salt	0.50	0.05	0.50	0.50	0.50
Premix*	0.15	0.15	0.15	0.15	0.15
Total	**100**	**100**	**100**	**100**	**100**

** Premix (grow fast) manufactured by Animal Care Service Consult (Nig) Ltd. Lagos, Supplied the following per kg of premix: Vitamin A, 5000,00 IU; Vitamin D_3 800,000 IU; Vitamin E, 12,000 mg; Vitamin K, 1,5000 mg; Vitamin B_1, 1,000 mg; Vitamin B_2, 2,000 mg, Vitamin B_6, 1,500 mg; Niacin, 12,000 mg; pantothenic acid, 20.00 mg; Biotin,10.00 mg; Vitamin B_{12}, 300.00 mg; folic acid, 150,000 mg; choline, 60,000 mg; manganese, 10,000 mg; iron;15,000 mg, zinc 800.00 mg; copper 400.00 mg; iodine 80.00 mg; cobalt 40 mg; selenium 8,00 mg.* NFE = Nitrogen-free extract; ME = Metabolizable energy: Calculated according to the formula of Pauzenga (1985) as: ME (Kcal/kg) = 37 x % CP + 81 x % EE + 35.5 x % NFE.

Blood Parameters

At week 10 of the experiment, blood samples were collected randomly from three (3) rabbits per treatment for the determination of the haematological and serum biochemical indices. Samples were collected from the ear vein of the rabbits by venipuncture using disposable needle (21–gauge needle) and syringes. The rabbits were fasted overnight (12 hours) and normally bled in the morning (7.00–8.00am) to avoid excessive bleeding. The collection site was cleaned with alcohol and xylene to dilate the veins. Sterile cotton wool was used to cover the punctured vein after collection. The blood samples were collected in sample bottles containing dipotassium salt of ethylene diamine–tetra acetic acid (EDTA–K^{2+}) which served as an anticoagulant for haematology while the bottles for serum biochemical indices were free of EDTA–K^{2+}. The haematological analysis of blood samples were carried out at the Department of Veterinary Public Health, Faculty of Veterinary Medicine, University

of Maiduguri, Nigeria, using the routinely available clinical methods (Bush, 1975). The haematological indices determined were packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and differential counts. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to standard formulae (Jain, 1986). The other blood samples collected without anticoagulant were used to determine the biochemical components such as albumin, total protein, cholesterol, globulin, glucose and urea using the methods described by other workers (Bush, 1975; Uko *et al*., 2000).

Statistical Analysis

All data collected in the course of the experiment were subjected to analysis of variance (ANOVA) using the CRD (Steel and Torrie, 1980) and where applicable, Duncan's multiple range test (Duncan, 1955) was used for mean separation. Computer package (Statistix 9.0) was used for the analysis.

RESULTS AND DISCUSSION

Haematological Indices

The results for the haematological parameters are presented in Table 2 There were no significant (P> 0.05) differences among the treatments for all the parameters evaluated. The PCV values (38.00 to 42.00%) obtained in this study are within the normal range (31 to 50%) reported by Canadian Council on Animal Care (1984) and Medirabbit (2003) as the ideal values for healthy rabbits. Igwebuike *et al.* (2005) obtained PCV values (27.50 to 40.50%) for rabbits fed between 0 and 75% raw sorrel seed meal as a replacement for groundnut cake. The haemoglobin (Hb) values ranged from 12.63 to 13.96 g/dL. These values are within the range (9.40 to 17.40 g/dL) reported by Medirabbit (2003) as the normal Hb range for adequate metabolism and as such there was no sign of anemia in the rabbits observed. The RBC values ranged from 10.40 to 12.33 x 10^6/mm^3 which was higher than 3.85 to 4.65 x 10^6/mm^3 reported by Medirabbit (2003). The WBC values observed in this experiment (8.53 to 11.20 x 10^3/mm^3) are within the range (5 to 13 x 10^3/mm^3) reported by Medirabbit (2003) as the reference values. The values of WBC obtained indicated that the rabbits were in good health and in a state of readiness to combat attack by foreign bodies.

Table 2: Haematological Indices of Rabbits Fed Graded Levels of Boiled and Fermented Sorrel Seed Meal (BFSMM) as a replacement for FFSBM

	Level of FFSBM Replaced with BFSSM (%)					
Parameters	T1 (0)	T2 (25)	T3 (50)	T4 (75)	T5 (100)	SEM
Packed cell volume (%)	41.67	42.00	39.67	38.00	39.00	1.70^{NS}
Haemoglobin concentration (g/dL)	13.87	13.96	12.63	12.63	12.97	0.46^{NS}
Red blood cells (x 10^6/mm^3)	12.33	11.40	10.80	11.47	10.73	0.93^{NS}
White blood cells (x 10^3/mm^3)	9.67	11.20	7.67	8.53	8.60	2.47^{NS}
Mean corpuscular volume (*fl*)	33.85	37.36	37.60	33.80	33.21	3.40^{NS}
Mean corpuscular haemoglobin (pg)	11.33	12.42	11.93	11.23	12.16	0.95^{NS}
Mean corpuscular haemoglobin concentration (%)	33.27	33.25	31.98	33.24	33.24	0.54^{NS}
Differential Counts						
Neutrophil (%)	45.67	42.67	41.67	49.00	44.67	6.26^{NS}
Eosinophils (%)	1.00	0.33	1.33	0.00	0.67	0.54^{NS}
Lymphocytes (%)	53.33	57.00	57.00	51.00	54.67	6.14^{NS}

SEM = Standard Error of Means; NS = Not Significant (P> 0.05); BFSSM = Boil-Fermented Sorrel Seed Meal; FFSBM = Full-fat Soya bean Meal

Similarly there were no significant (P> 0.05) differences among treatment groups with respect to the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and means corpuscular haemoglobin concentration (MCHC) and the values were within the reference values reported by Anon. (1980). These parameters (MCV, MCH, and MCHC) which are usually used to measure the size and haemoglobin content of the erythrocytes are very useful in diagnosing diseases, toxicity level and anaemia (Duwa *et al*., 2012). The values recorded for neutrophil and lymphocyte ranged between

41.67 to 49.00% and 51 to 57%. They all fall within the normal range of 30 to 65% and 28 to 85%, respectively for healthy rabbits (Mitruka and Rawnsley, 1997). The eosinophils concentration were between 0.00 to 1.33% and the values are slightly lower than the values reported by Ahamefule *et al*. (2005) for rabbits fed sundried, ensiled and fermented cassava peel- based diets. The values recorded for basophil and monocyte were too low for analysis. The values observed suggest that the levels of BFSSM in the diets had no adverse effect on most of the haematological parameters.

Serum Biochemical Indices

The results obtained for the serum biochemical indices are presented in table 3. There were significant (P< 0.05) differences among treatment groups for total protein. Treatment T2 had higher values compared to other treatments with similar values, but did not differ (P>0.05) from those fed T3 diet. The total protein values (53.67 to 72.67 g/l) obtained in this experiment were within the reference values of 50 to 75 g/L reported by Medirabbit (2003). Schalm *et al*. (1975) reported that the concentration of serum protein at any given time is a function of the nutritional status, water balance and other factors affecting the state of health of the animal. There were no treatment effects (P>0.05) on all the other serum biochemical indices evaluated. Albumin values (32.00-44.33 g/L) obtained were slightly higher than the reference values (25 to 40 g/L) reported in Medirabbit (2003). It has been reported that low albumin indicates low protein intake and high serum albumin indicate fast growing animals (Schalm *et al*., 1975). The glucose levels (3.77 to 4.53 mmol/L) are close to the values (4.20 to 8.90 mmol/L) reported by Medirabbit (2003) as normal values for growing rabbits. The values of the urea recorded (4.63 to 5.17 mmol/L) were lower than the 9.1 to 25.5 mmol/L reported by Medirabbit (2003). The cholesterol values (0.66 to 1.47 mmol/L) obtained in this study were within the range (0.10 to 2.00 mmol/L) reported by Medirabbit (2003). Since the values for all the serum biochemical indices observed in this study were within the normal ranges reported in Medirabbit (2003), it therefore indicates that boiled and fermented sorrel seed meal had no adverse effect on serum biochemical indices of growing rabbits.

Table 3: Serum Biochemical Indices of Rabbits Fed Graded Levels of Boiled and Fermented Sorrel Seed Meal (BFSSM)

	Level of FFSBM Replaced with BFSSM (%)					
Parameters	T1 (0)	T2 (25)	T3 (50)	T4 (75)	T5 (100)	SEM
Total protein (g/L)	59.00^{b}	72.67^{a}	63.33^{ab}	57.33^{b}	57.32^{b}	3.37^{*}
Albumin (g/L)	34.67	44.33	32.00	36.33	34.33	4.29^{NS}
Globulin (g/L)	24.33	28.33	31.33	21.00	19.33	4.29^{NS}
Glucose (mmol/L)	4.37	3.77	4.53	4.43	4.37	0.28^{NS}
Urea (mmol/L)	4.73	5.17	4.67	4.73	4.63	0.29^{NS}
Cholesterol (mmol/L)	1.47	1.00	0.66	1.30	1.40	0.28^{NS}

SEM = Standard Error of Means; NS = Not Significant (P > 0.05); * = Significant Difference (P < 0.05); ^{a,b} = Means in the same row bearing different superscripts differ significantly (P< 0.05)
FFSBM = Full-fat Soya Bean Meal

CONCLUSION

Based on this finding, boiled and fermented sorrel seed meal (BFSSM) had no deleterious effect on the haematology and serum biochemistry of growing rabbits. Therefore up to 100% of BFSSM can replace full fat soya bean in rabbits diet.

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