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EFFECT OF FULLZYME ® SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS OF GROWING RABBITS FED BOILED SORREL SEED (*HIBISCUS SABDARIFFA L.*) MEAL BASED DIETS

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ABSTRACT

This study was carried out to investigate the effect of dietary enzyme (Fullzyme ®) supplementation on haematological parameters of growing rabbits fed boiled sorrel seed meal (BSSM) based diets. A total number of thirty six (36) weaner rabbits of mixed breeds and sexes aged 7-8 weeks (450 – 500 ± 50g) old were randomly allotted into four (4) dietary treatments with three (3) replicates of three rabbits each in a completely randomized design. Four experimental diets were formulated with enzyme supplementation at 0, 0.15, 0.25 and 0.35%, respectively. The experiment lasted for 56 days. Blood samples were collected from three rabbits randomly selected from each treatment at the end of the study for haematological evaluations. Results revealed no significant differences (P>0.05) in all parameters measured except for neutrophils (P< 0.05). Rabbits fed 0.25% enzyme supplementation had 27% lower values of neutrophils than other treatment groups. It was hence concluded that the higher the number of neutrophils present in the blood, the more the level of infection while the lower the number, the lesser the level of infection or may be absent in the blood.

Keywords: Boiled Sorrel Seed Meal, Haematological Parameters, Fullzyme, Rabbits,

INTRODUCTION

The use of unconventional feed ingredients in compounding livestock feed in order to reduce cost of production is documented in literature (Abeke, 2008 and Duru, 2010). Non-conventional feedstuffs offer the best alternatives in our environment for reducing feed cost and therefore a reduction in the cost of meat production (Dafwang *et al*., 2001). Ogundipe *et al*. (1992) and Abeke (1997), reported that solution to high cost of poultry feed is the discovery, processing and harnessing of non- conventional sources of poultry feedstuffs for which there is little or no competition from humans. Sorrel (*Hibiscus sabdariffa*) seed is one of the alternative feed resources that has been found to thrive on a wide range of tropical soil conditions, and can perform well on relatively poorer soil (Adanlawo and Ajibade, 2006). *Hibiscus sabdariffa* is widely grown in the North-Eastern and middle belt regions of Nigeria (Akanya *et al*., 1997). Sorrel seeds contain high amount of protein, dietary fibre, and minerals such as phosphorus (P), calcium (Ca) and magnesium (Mg) (Ismail *et al*., 2008). The seeds contain about 35.90% crude protein (CP), 10.14% ether extract (EE), 10.09% ash and 15-17% crude fibre (CF) (Dashak and Nwanegbo, 2002). Kwari *et al.* (2011) reported raw sorrel seeds to contain 5.18% arginine, 16.5% CF, 13.5% EE and 38.57% CP. Abdu *et al*. (2008) reported 23.46% CP value. However, Nyameh *et al*. (2012) reported that boiled sorrel seeds contain 22. 84% CP, 8.50% CF, 6.50% EE, 6.50% ash, 45.66% NFE and 91.70% DM. while Maikano *et al.* (2014) reported a value of 21.84% CP, 3.60% CF, 5.85% EE, 5.39% ash, 90.40% DM, 53.72% NFE, 1.12% Ca and 0.56% P, respectively. Sorrel seeds in their raw state are

known to have bitter taste which is attributed to anti-nutritional factors present in them. The unprocessed seeds have been reported to contain total phenols, tannin and phytic acid as common anti-nutrients and these have been shown to have detrimental effects on the health and performance of broilers (Dairo *et al*., 2011; Keyembe, 2011). Effective utilization of sorrel seeds by non-ruminant animals will necessitate processing (fermentation, boiling, sprouting etc.) to inactivate the anti-nutritional factors (Soetan and Oyewole, 2009). The adverse effect of some anti-nutrients in feed can be overcomed by repeated washing with water which makes the feed more palatable by reducing its bitterness. Though by washing, little quantity of the dry matter is lost (Joshi *et al*., 2012). It has been reported that enzyme supplementation permit the use of wide range of ingredients without compromising animal's performance and hence provides the least cost formulation (Obadina, *et al*., 2006). Some beneficial effects of applying feed enzymes to monogastric diets include, enhancement of digestion and absorption of nutrients especially protein and fat, increased feed intake, weight gain and feed conversion ratio (Wang *et al*., 2005; Bedford, 2000; Marquardt *et al*., 1996). Recent studies in poultry (Bedford, 2000; Chesson and Steward, 2001) have demonstrated that enzyme addition in the diet improves the feed utilization in the small intestine, changing the substrate that arrives to the hindgut. This change affects the development of the micro biota population in this area and seems to favour the health status of the animals. Enzyme supplementation also improved fibre digestibility in rabbits (Gutiérrez *et al.*, 2002). Since the European Union banned most of the antibiotic growth promoters in animal nutrition due to cross and multiple resistance (Neu, 1992), much research has been conducted to explore the use of multi-enzyme as effective substitutes. Fullzyme is a complex enzyme that contains Amylase, Protease, Cellulose, Lipase, Pectinase, Xylanase, β-Glucanase and Phytase. Although some studies in Nigeria reported effects of unconventional feedstuff and enzyme supplementation on growth performance, nutrient digestibility and blood biochemical profile of broiler chickens (Duru, 2010; Duwa *et al* 2012), there is paucity of information in growing rabbits. The objective of this study was to investigate the effect of fullzyme® supplementation on the haematological parameters of growing rabbits fed boiled sorrel seed (*Hibiscus sabdariffa L*.) meal based diets

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Rabbitary Unit of the Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Zaria. The place is within the Northern Guinea Savanna zone of Nigeria, with Latitude 11^0 09′ 01.78″ N and Longitude 7^0 39′ 14.79″ E at an altitude of 671m above sea level (Ovimaps, 2015).

Sources of Rabbits and Sorrel Seeds

The rabbits of mixed breeds and sexes aged 7 – 8 weeks (450 – 500g ± 50g) were sourced from commercial rabbit farms within Zaria metropolis. The sorrel seeds and other ingredients were purchased from an open market in Sabon Gari, Zaria.

Processing of Sorrel Seeds

The raw sorrel seeds were sorted to ensure cleaned grains. The method adopted by Ari *et al.* (2012) was used for processing. The cleaned sorrel seeds were poured into 20litres of already boiled water per batch of 10kg at 100^0C i.e. in ratio of 2:1 at 45 minutes, respectively. The products were then sun dried by spreading on trays until well dried (at least 75-80%DM), after which it was milled, bagged and stored for experimental diet formulation purpose.

Experimental Diets

Four experimental diets were formulated to meet the requirements of growing rabbits according to NRC (1994).These include T_1

(Boiled Sorrel seed meal diet without enzyme and used as control); T_2 (boiled Sorrel seeds meal based diet with Fullzyme at 0.15%); T_3 (boiled Sorrel seeds meal based diet with Fullzyme at 0.25%) and T_4 (boiled Sorrel seeds meal based diet with Fullzyme at 0.35%) as shown in Table 1

Table 1: Composition of experimental diets

	Boiled sorrel seeds with Fullzyme® supplementation			
Ingredients (kg)	**T_1+0.00%**	**T_2+0.15%**	**T_3+0.25%**	**T_4+0.35%**
Maize	38.33	38.33	38.33	38.33
Soya bean cake	4.17	4.17	4.17	4.17
Boiled sorrel seed meal	15.00	15.00	15.00	15.00
Groundnut Haulms	40.00	40.00	40.00	40.00
Bone meal	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
Crude protein (%)	18.52	18.52	18.52	18.52
Crude fiber (%)	8.54	8.54	8.54	8.54
Ether Extract (%)	5.72	5.72	5.72	5.72
Metabolizable energy (Kcal/kg)	2781	2781	2781	2781
Calcium (%)	0.68	0.68	0.68	0.68
Phosphorus (%)	0.57	0.57	0.57	0.57
Lysine (%)	0.78	0.78	0.78	0.78
Methionine + cysteine (%)	0.66	0.66	0.66	0.66
Ca:p	1.4	1.4	1.4	1.4
Cost/kg diet (₦)	110.22	117.72	122.72	127.72

**Bio-premix supplied per kg of diet: Vit A, 12500 I.U; Vit D_3, 2500 I.U; Vit E, 50mg; Vit K_3, 2.5mg; Vit B_3, 3.5mg; Vit B_6, 6mg; Niacin, 40mg; Pantothenic acid, 10mg; Biotin, 0.8mg; Vit B_{12}, 0.25mg; Folic acid, 1mg; Choline chloride, 300mg; Manganese, 100mg; Iron, 50mg; Zinc, 45mg; Iodine, 1.55mg; Selenium, 0.1mg; Copper, 2mg; Cobalt, 20mg.
**Fullzyme: a complex enzyme that contains Amylase, Protease, Cellulose, Lipase, Pectinase, Xylanase, β-Glucanase and Phytase

Experimental Design and Management of Animals

Thirty – six (36) weaner rabbits of mixed breeds and sexes aged 7-8 weeks (450 – 500g ± 50g) were randomly allotted into four (4) treatments with three (3) replicates per treatment; each replicate had three (3) rabbits in a Completely Randomized Design (CRD).

Before the commencement of the experiment, they were dewormed using ivermectin® dewormer. The rabbits were housed in galvanized wire cages of 40 x 60 x 60cm dimension. Each cage was equipped with a small rubber bowl drinker and a clay/earthen pot feeder. Feed and clean water were supplied *ad-libitum* for a period of 56 days after an adjustment period of a week.

Data Collection

Feed offered and left over were weighed to determine feed intake of the animals. After the initial weight, weekly weights were recorded. These records were used to monitor and determine the performance parameters in terms of average feed intake (AFI), average weight gain (AWG), feed conversion ratio (FCR), final body weight and feed cost/kg gain. Mortality was also recorded as it occurred.

Determination of haematological parameters

Blood samples were collected from the ear vein of three rabbits randomly selected using a sterilized disposable needle and syringe. Prior to bleeding, a cotton swab soaked in 70% ethanol was used to dilate the vein to prevent infection. Two mls (2.0mls) of blood was collected into a labelled sterile bottle containing ethylene diamine tetra acetic acid (EDTA) as anti- coagulant. Haematological parameters; packed cell volume (PCV), Haemoglobin count (Hb), Red blood cell (RBC), White blood cell (WBC); Neutrophils, Eosinophils, Monocytes and Lymphocytes were determined following standard procedures described by Davice and Lewis (1991) at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Chemical analysis

The proximate composition of the boiled sorrel seed was determined by the method of AOAC (2006) at the Biochemical Laboratory of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. Metabolizable energy was calculated using the method of Pauzenga (1985). ME (kcal/kg) = 37 x % CP + 81.1 x % EE + 35.5 x % NFE

Statistical Analysis

All data generated from the study were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) Procedure of SAS (2008) software package. Significant difference between treatment means were separated using Dunnett (Steel and Torrie, 1998).

The Linear Model of the experiment is as follows:

$Y_{ij} = \mu + T_j + e_{ij}$

Where:

Y_{ij} = observation

μ = overall mean

T_j = effect of diet on animals

e_{ij} = random error

RESULTS AND DISCUSSION

The proximate analysis of boiled sorrel seeds shown in Table 2 showed that BSS contain 90.15% dry matter, 29.22% crude protein, 14.50% crude fibre, 10.45% ether extract, 5.47% Ash and 40.36 nitrogen free extract. The calculated metabolizable energy was 3361.42 kcal/kg (Pauzenga, 1985). The higher Crude Protein (CP) level of boiled sorrel seeds may be attributed to the processing method. Similar result was observed by Akinmutimi (2001) who subjected legume seeds to moist-heating and reported a slight increase in the CP levels of the feed. The Crude Fibre (CF) value obtained was slightly lower than the values reported by Samy (1999). The Ether Extract (EE) value was within the range given by FAO (1997). This is non-comparable with the report of Duwa *et al.* (2012) that higher level of EE in the boiled sorrel seeds could be attributed to the decrease in the non-lipid component of the sorrel seeds during cooking. The Ash value agrees with the findings of Ari *et al.* (2013) who reported that

crushed seeds of sorrel ranged from 5.39-6.94% but below the value of 10.09% reported by Dashak and Nwanegbo, (2002). The value observed fell within the range (5.39-6.50%) reported by Nyameh *et al.* (2012) and Maikano *et al.* (2014). The lower level of Nitrogen Free Extract (NFE) in the seeds may be due to the high loss of NFE in water during boiling as previously reported by Samy (1999). This is not in line with the result obtained for this study. The results here showed that boiled seeds could also have high NFE values as observed in 30 and 45 minutes duration of boiling.

Table 2: Proximate composition of boiled sorrel seeds

Nutrients	**Composition**
Dry Matter	90.15
Crude Protein	29.22
Crude Fibre	14.50
Ether Extract	10.45
Ash	5.47
Nitrogen Free Extract	40.36
*Metabolizable energy (Kcal/kg)	3361.42

*Pauzenga (1985)

Haematological Parameters

The result showed no significant differences (P>0.05) for all parameters measured except neutrophils (Table 3). Neutrophils were significantly affected (P<0.05) by enzyme supplementation. Rabbits fed 0.25% enzyme had 27% lower neutrophils than other groups which were at par. Rabbits fed the control diet had the highest result for neutrophils. Haematological indices are reflections of the effect of dietary treatments on an animal in terms of type, quality and amount of ingested feed available to meet the animals physiological, biochemical and metabolic necessities (Ewuola *et al.,* 2004). The best result for neutrophils was observed for rabbits fed diets supplemented with enzyme at 0.25%. Frandson (1986) reported that the number of neutrophils in the blood increases rapidly when acute infection was present, hence a blood count showing this increase was useful in diagnosis of infections. Frandson (1986) reported further that eosinophils which normally are scarce increase in numbers in certain chronic diseases, such as infection with parasites and also in allergic reactions. Although there were no significant differences (P>0.05) in PCV values between all treatment groups, the values obtained were within the range (27.0-50.0%) for healthy rabbits as reported by Onifade *et al*. (1999) and Fanimo *et al*. (2003). Normal values of PCV values indicated the absence of toxic factors such as haemagglutinin which could have adverse

effect on blood formation (Oyawoye and Ogunkule, 1998). A non-significant (P>0.05) effect was observed in white blood cell count WBC across all treatment groups, which was in agreement with the study of Igwebuike (2001) who recorded a range of 5.28- 9.05 x 103 mm^3. Ocheja *et al*. (2011) reported that high white blood cell count was associated with microbial infection or the presence of a foreign body antigen in the circulating system.

Table 3: Haematological parameters of grower rabbits fed boiled sorrel seed meal with Fullzyme ® supplementation

Parameters	T1+0.00%	T2+0.15%	T3+0.25%	T4+0.35%	SEM	LOS
PVC (%)	27.33	34.33	43.67	37.67	3.75	NS
Hemoglobin concentration (g/dl)	14.53	10.97	11.43	12.53	1.38	NS
Red blood cells ($10^6/mm^3$)	7.37	5.60	5.73	6.33	0.66	NS
White blood cells ($10^3/mm^3$)	6.73	8.00	7.53	9.50	1.22	NS
Neutrophils (%)	40.00^a	38.00^a	30.67^b	39.00^a	4.17	*
Eosinophils (%)	0.00	0.67	0.67	0.00	0.47	NS
Monocytes (%)	0.33	0.67	2.00	0.00	0.80	NS
Lymphocytes (%)	59.33	60.33	66.67	60.67	3.93	NS

ab: Means with different superscript on the same row differ significantly at P<0.05
SEM: standard error of mean LOS: level of significance NS: Not significant

CONCLUSION AND RECOMMENDATION

It can be concluded from this study that fullzyme ® supplementation in boiled sorrel seed meal diets could lower neutrophils production by 27% in growing rabbits thereby minimizing cases of disease infection. Hence fullzyme ® can be included in boiled sorrel seeds meal diets at 0.25% for growing rabbits in Northern Guinea Savannah of Nigeria.

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