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Blood Biochemistry and Haematology of Weaner Rabbits Fed Sundried, Ensiled and Fermented Cassava Peel Based Diets

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Abstract: Twenty four New Zealand white X Chinchilla weaner bucks aged between 7 and 8 weeks and averaging 0.9kg in weight were divided into 4 groups of 6 each and used in a 12-week feeding trial to evaluate the blood biochemistry and haematology of rabbits fed sun-dried, ensiled and fermented cassava peel based diets. The test diets designated A, B, C and D were Completely Randomized . Diet A, the control, was a 16.18% CP (crude protein) weaner ration formulated from maize, maize offals, soya bean meal, blood meal, oyster shell, bone meal, vitamin premix and common salt. Diets B, C and D were also weaner rations of respectively 16.10, 16.20 and 16.08% CP in which 10% maize of the control diet was replaced respectively with sun-dried, ensiled and fermented cassava peels. The diets were roughly iso-caloric. The haematological components of study included packed cell volume (PCV), white blood cells (WBC), neutrophil (N) and lymphocytes (L). Biochemical parameters were serum creatinine, urea, bilirubin (total and conjugated), serum glutamic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), eosinophil and blood sugar. Liver and kidney weights were also monitored. Results showed that PCV, WBC, neutrophils and lymphocytes were affected ($P<0.05$) by experimental diets. Also affected ($P<0.05$) were bilirubin, SGPT and SGOT. Serum creatinine, urea and eosinophil were however not affected ($P>0.05$) by treatment diets. Liver and kidney weights also did not differ ($P>0.05$) among rabbits fed different dietary treatments.

Key words: Cassava peel based diets, weaner rabbits, blood

Introduction

Blood is a complex fluid containing large variety of dissolved suspended inorganic and organic substances (Stewart, 1991) or specialized circulating tissues and cells suspended in the intercellular fluid substance (Dellman and Brown, 1976) which circulates in the arteries, vessels and capillaries of man and animals (Kronfield and Mediway, 1975), and whose primary function is to transport oxygen from respiratory organs to body cells (Duke, 1975), distributing nutrients and enzymes to cells and carrying away waste products (Baker and Silverton, 1982), thereby maintaining homeostasis of the internal environment (Bentrick, 1974). The various functions of the blood are made possible by the individual and collective actions of its constituents – the haematological and biochemical components. Generally speaking, both the haematological and biochemical blood components are influenced by the quantity and quality of feed and also the level of anti-nutritional elements or factors present in the feed (Akinmutimi, 2004). Biochemical components are sensitive to elements of toxicity in feeds. They can also be used to monitor protein quality of feeds. Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998).

Cassava peel, an energy component of the test diets in this study contains cyanogenic glycosides – lostraulin and linamarin (Smith, 1988). Both compounds are hydrogen cyanide derivatives. The substance (HCN) has been shown to be toxic to livestock (McDonald *et al*, 1995) and therefore limits the use of cassava peels in the raw state as feed for livestock (Smith, 1988). Detoxification of cassava peels have been made possible through sun-drying (Ahamefule *et al.*, 2003), ensiling (Okeke *et al.,* 1985) and fermentation (Ijaiya, 2001). Efforts therefore were made in this study to assess the effect of processing methods on haematological and biochemical blood components, in rabbits fed sun-dried, ensiled and fermented cassava peel based diets.

Materials and Methods

Experimental site: This study was carried out at the rabbit unit of Teaching and Research farm, Michael Okpara University of Agriculture, Umudike. The geography and location of Umudike is as described in Ahamefule *et al.* (2003).

Experimental procedure: Twenty four weaner bucks (New Zealand White X Chinchilla) of 7-8 weeks of age and weighing averagely 0.9kg (0.80-0.95kg) were randomly divided into 4 groups of 6 animals. Each

Table 1: Proximate composition of sun-dried, ensiled and fermented cassava peels

Constituents %	SDCP	ECP	FCP
Dry matter	85.20	95.55	94.76
Crude protein	4.38	3.63	3.50
Crude fibre	15.70	13.64	9.20
Ether extract	1.08	0.94	1.10
Nitrogen free extract	73.84	77.52	82.77
Ash	5.0	4.27	3.43

SDCP = Sun-dried cassava peel. ECP = ensiled cassava peel. FCP = Fermented cassava peel

animal was housed individually in a standard hutch provided with a feeder and waterer. The entire hutch system was of the three-tier model, housed in well ventilated cement floored pens and raised 120 cm above the ground. Prior to the experiment (2-weeks) each animal was dewormed and given an acaricide bath. Four experimental diets (A, B, C, D) were formulated as shown in Table 2. Diet A, the control, was a 16.18% CP weaner ration based on maize and maize offal (Table 2). Diets B, C, and D were also weaner rations in which 10% maize (of the control) was replaced respectively with sun-dried, ensiled and fermented cassava peels. The diets were roughly iso-caloric and iso-nitrogenous. The experiment lasted for 12 weeks. Feeding was completely randomized (Steel and Torrie, 1980).

Fresh cassava peels of variety TMS 30555 were collected from the commercial 'Garri' processing unit of the National Root Crop Research Institute (NRCRI), Umudike. They were subsequently divided into three lots. The first, second and third lots were processed into sun-dried, ensiled and fermented cassava peels, respectively according to methods described by Ahamefule *et al.* (2003).

Blood samples were collected weekly during weeks 8-12 of study from 4 rabbits randomly selected from the 6 in each group using methods of Uko *et al.* (2000) by puncturing the jugular vein and allowing free flow of blood into labelled sterile universal bottles. Pooled sample from each group was divided into two volumes. An initial 10ml (of first volume) was collected over labelled sterile universal bottles containing 1.0mg/ml ethyldiamine tetracetic acid (EDTA) and 0.1mg/ml Heparin. This was used to determine the haematological component according to the method of Ajagbonna *et al.* (1999) and Uko *et al.* (2000). Another 10ml (second volume), was collected over labelled sterile sample bottles without coagulant and used to determine the biochemical components (Doumal, 1972; Sigma 1985; Ajagbonna *et al.,* 1999; Spencer and Price, 1997; Uko *et al.*, 2000).

Feed and data analysis: Feed samples were analyzed using standard methods laid down by the Association of Official Analytical Chemists (AOAC, 1990). All data were subjected to analysis of variance (ANOVA) applicable to a Completely Randomized Design (Steel and Torrie, 1980). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Experimental diets: The proximate compositions of the sun-dried, ensiled and fermented cassava peels used in this study (Table 1) are comparable to values obtained by Ahamefule *et al.* (2003), Okeke *et al.* (1985) and Ijaiya (2001) for the respective products elsewhere. Table 2 shows the percentage / proximate compositions of the experimental diets. The percent dry matter of the sun-dried (93.40), ensiled (93.50) and fermented (94.06) cassava peel based (CPB) diets, the crude protein (16.10; 16.20; 16.08%) and gross energy (2.89; 2.87; 2.90 kcal DE) values did not differ ($P<0.05$) from the control (94.85 DM; 16:18% CP; 2.96 kcal DE). The Nitrogen free extract and ether extract values were also similar ($P>0.05$) for all diets.

The proximate values obtained for crude protein, crude fibre, and gross energy for the CPB diets and the control fell within recommended range (12-17% CP; 10-20% CF; 2390-2500 kcal DE) for optimum growth and performance in rabbits (Aduku and Olukosi, 1990). The fat component of the diets as represented by the ether extract values, were also within the range (20-25%) of recommended level in rabbit nutrition.

Haematological components: Table 3 shows the mean and normal ranges of some haematological and biochemical blood values of rabbit (Mitruka and Rawnsley, 1997). Table 4 shows a summary of haematological and biochemical values obtained for rabbits fed the control and the CPB diets in this study.

All haematological parameters of study (PVC, WBC, lymphocytes, eosinophil) for rabbits on control diet (A) fell within normal ranges (Table 3). Among treatment diets, PCV value obtained for rabbits on fermented CPB diet (D) (46.5%) was higher than the normal range (25-45%) (Table 3), for the control rabbits (42.75%) and for rabbits on ensiled CPB diet (43.25%). However, this figure (46.50%) did not differ significantly ($P>0.05$) from that obtained for rabbits (45.75%) on sun-dried CPB diet (B). The PCV values obtained for rabbits on the control and all the CPB diets, except for the fermented (D), fell within normal range (Table 3). This however would suggest that sun-drying and ensiling processing methods were good enough for detoxification of cassava peels as may have been demonstrated in the normal PCV range of values observed for rabbits subsisting on either sun-dried or ensiled CPB diets. Fermentation method however may not have been as effective as either sun-drying or ensiling processing methods considering the relatively higher PCV value recorded for animals on the CPB diet which nevertheless was above normal range.

Table 2: Percentage and proximate Composition of the experimental diets

Ingredients (kg)	A	B	C	D
Maize	400	300	300	300
Maize offal	300	300	300	300
SDCP	--	100	--	--
ECP	--	--	100	--
FCP	--	--	--	100
Full fat soya	250	245	245	245
Blood meal	15	20	20	20
Oyster shell	10	10	10	10
Bone meal	20	20	20	20
Salt	2.5	2.5	2.5	2.5
Vitamin premix *	2.5	2.5	2.5	2.5
Total	1000	1000	1000	1000
Analyzed content (%)				
Dry matter (DM)	94.55	93.40	93.50	94.06
Crude protein (CP)	16.18	16.10	16.20	16.08
Crude fibre (CF)	10.03	10.16	10.13	10.19
Ether extract (EE)	3.98	2.10	2.09	2.05
Nitrogen free extract (NFE)	57.54	58.94	56.48	59.82
Ash	7.12	6.10	8.60	5.92
Gross energy (MJ/Kcal/DM)	2.960	2.890	2.870	2.900

* To provide the following per kg diet: Vit, A, 1500IU; Vit E, 11.0mg; Riboflavin, 9.0mg; Biotin, 0.25; Pantothenic acid, 11.0mg; Vit k_3, 3.0mg; B_2, 2.5mg; B_6, 0.3mg; B_{12}, 8.0mg; Nicotinic acid, 8.0 mg; Fe, 5.0mg; Mn, 10.0mg; Zn, 4.5mg; Co,0.2mg; Se,0.01mg

Table 3: Mean and normal ranges of haematological and biochemical components for rabbit

Parameter/indices	Range	Mean
Haematological		
Packed cell volume (%)	25.0 - 45.0	34.0
White Blood cell (10^3u/l)	3000 - 6000	4000
Neutrophil (%)	35.0 - 43.2	39.0
Lymphocyte (%)	53.5 - 65.8	60.0
Eosinophil (%)	1.0 - 2.5	2.0
Biochemical		
Total bilirubin (mg/100ml)	0.4 - 3.0	2.0
Conjugated bilirubin (mg/100ml)	0.2 - 0.4	0.3
Serum creatinine (mg/100ml)	0.6 - 0.8	0.7
Urea (mg/100ml)	30.0 - 37.3	34.0
SGOT (ml/l)	12.0 - 18.0	15.0
SGPT (ml/l)	9.0 - 15.0	12.0
Sugar (mg/100ml)	65.3 - 74.8	70.0

Source: Mitruka and Rawnsley (1977)
Kronfield and Mediway (1975).

The average WBC values (10^3u/l) for rabbits fed fermented CPB diet (7265) also was significantly higher (P<0.05) than those of the sun-dried (5800), ensiled (6275) and the control (6000) group. Even though values of the ensiled, like the fermented overshot the normal range (3000-6000), the ensiled however did not differ (P>0.05) from either the sun-dried or control. A significant WBC value for rabbits on diet D may be associated with microbial infection or the presence of antigens or foreign proteins in the circulatory system.

Serum neutrophil and lymphocyte values also differed (P<0.05) for treatment groups. Mean neutrophil values for ensiled CPBD fed rabbits did not differ (P>0.05) from those on control. Animals on sun-dried CPB had values, which were significantly lower (P<0.05) than those of either the ensiled or control. These observed values (ensiled and control) however did not differ (P>0.05). Blood lymphocytes were fairly similar (P>0.05) in concentration in rabbits fed sun-dried and fermented CPB diets and as well as in the control. Animals on ensiled CPB diet had significantly lower (P<0.05) concentration of lymphocytes than the sun-dried group, which however did not differ (P>0.05) from either the fermented or control groups. Even though blood neutrophil and lymphocyte concentrations showed variation between treatment groups, the values obtained for all groups were within normal range (Table 3). In WBC differential count, an abnormally high neutrophil level is synonymous with bacterial infection while higher lymphocyte counts indicate infection of viral origin (Akinmutimi, 2004).

Biochemical components: A summary of biochemical values obtained for rabbits on different treatment groups in this study are summarized in Table 4. The control group had normal range of values for all biochemical parameters measured in this study. Serum creatinine levels were within normal range and did not differ (P>0.05) among treatment groups. The values obtained for animals on diets B, C and D or the cassava peel based diets were in consonance with the findings of Omole and Sonaiya (1981) and suggested that there was no wasting or catabolism of muscle tissues, and that animals were not surviving at the expense of body reserve. This was a good indication that dietary protein was well utilized by rabbits.

Blood urea concentration was also normal among treatment groups, suggesting also the effectiveness of processing methods. Increase serum urea concentration may suggest an increase in activities of

Table 4: Summary of haematological and biochemical blood components of rabbits fed sun-dried, ensiled and fermented cassava peel based diets

Parameter/Indices Haematological	Control (A)	Sun-dried (B)	Ensiled (C)	Fermented (D)	SEM
Packed cell volume (%)	42.75[c]	45.75[ab]	43.25[bc]	46.50[a]	0.92*
White Blood cell (10^3u/l)	6000.0[b]	5800.0[b]	6275.0[b]	7265.0[a]	269.22*
Neutrophil (%)	41.50[a]	37.50[b]	43.25[a]	40.25[ab]	1.17*
Lymphocyte (%)	59.00[ab]	60.75[a]	56.50[b]	57.50[ab]	1.16*
Eosinophil (%)	1.50[a]	2.00[a]	1.25[a]	1.75[a]	`0.40ns
Biochemical					
Total bilirubin (mg/100ml)	1.06[a]	0.46[c]	0.85[b]	0.83[b]	0.04*
Conjugated bilirubin (mg/100ml)	0.33[a]	0.24[b]	0.26[b]	0.27[b]	0.20*
Serum creatinine (mg/100ml)	0.76[a]	0.68[a]	0.60[a]	0.71[a]	0.05ns
Urea (mg/100ml)	33.00[a]	33.00[a]	36.25[a]	34.50[a]	1.66ns
SGOT (ml/l)	12.0[b]	11.50[b]	16.0[a]	7.73[c]	0.92*
SGPT (ml/l)	9.17[b]	10.42[b]	12.80[a]	12.17[a]	0.69*
Sugar (mg/100ml)	77.75[a]	68.50[b]	68.25[b]	70.50[b]	2.06*
Organ weight					
Liver (g)	51.80[a]	54.10[a]	50.02[a]	48.10[a]	4.24ns
Kidney (g)	4.92a	5.00[a]	4.50[a]	4.55[a]	0.53ns
Feed intake					
Average daily feed intake (g)	64.90[a]	70.86[a]	74.73[a]	74.07[a]	3.15ns

SEM= Standard error of the mean. NS = Not significant (P>0.05). * = Significant (P<0.05)

urea enzymes Orninthine, carbonyl transferase and orginase which may also indicate kidney damage (Ajabonna *et al.,* 1999). The normal range of values obtained implied therefore that the dietary proteins of the CPB diets and the control were well utilized (Reinhold, 1953)

Values for total and conjugated bilirubin (TB, CB) showed significant differences (P<0.05) among rabbits on different dietary treatments. Even though serum levels fell within normal range for rabbits on all treatment groups, the animals subsisting on the control diet had serum concentration, which was significantly higher (P<0.05) than those on sun-dried, ensiled and fermented CPB diets. Total and conjugated bilirubin are indicators of protein adequacy. The normal range of values observed for rabbits fed CPB diets and the control suggest sufficient or adequate protein in the experimental rations for normal metabolic and physiological activities (Ologhobo *et al.,* 1992). The significantly higher CB and TB values obtained for rabbits on control over those of sun-dried, ensiled and fermented CPB diets may have to do with protein availability, which though related to protein adequacy or total protein, is in no way synonymous to it. Protein availability however is associated very closely with protein quality. Though the protein components of all the test diets may be of good quality, in comparative terms however, the control diet may have demonstrated dietary protein of superior quality. The protein quality of a diet is a summation of the quality of protein contributed by individual constituents. The amino acid profile of cassava peels shows that it is limiting in methionine (Omole and Sonaiya, 1981). This inadvertently may have affected the protein quality of the CPB test diets, perhaps negligibly, in relation to the control.

The SGOT and SGPT serum concentrations of rabbits fed diets B,C and D, the cassava peel based diets, like the control, were within normal range, but the values obtained differed significantly (P<0.05) among treatment groups. The SGOT concentrations (ml/l) of rabbits on control diet (12) was lower (P<0.05) to those of the ensiled (16), similar (P>0.05) to the group on sun-dried (11.50) and higher (P<0.05) to those on fermented (7.73) CPB diets. The SGPT values (ml/l) for the control (9.47) and sun-dried (10.42) groups were similar (P>0.05), these observed figures were however lower (P<0.05) than those of the ensiled (12.50) and fermented (12.17) groups. An increase in serum SGOT and SGPT has been reported by Fasina *et al.* (1999) to signify necrosis and myocardial in faction or response to the presence of a number of toxic factors (Sigma, 1985). Coles (1986) has however called for caution in the interpretation of serum enzyme activity, since enzyme activities vary greatly among species and even among tissues and organs.

The blood sugar level of rabbits fed the CPB diets, as in the control, were within normal stipulated range (Table 3 and 4). The mean concentrations recorded however differed (P<0.5) significantly among groups. Rabbits on control diet attained significantly higher blood sugar concentration than rabbit fed sun-dried, ensiled or fermented cassava peel based diets. The all maize based energy source of the control diet is thought to be responsible for this. However the normal range of blood sugar level obtained for rabbits fed CPB diets in this study indicated that the animals were not surviving at the expense of body tissues (Ologhobo *et al.,* 1992). Generally, carbohydrate rich food such as maize and cassava peel are broken down in the gastro-intestinal tract (GIT) of ruminant and non-ruminant livestock and absorbed into the blood as glucose. The glucose is then carried to the liver where it is stored as glycogen. By the action of insulin, only enough glucose is left in the blood for normal metabolism. Hence insulin regulates the level of glucose available in the blood for energy. Depletion of carbohydrate reserve, either due to metabolism or insulin related disorder, leads to breakdown of fat and proteins as energy source.

Values obtained for kidney and liver weights in this study showed any significant difference (P>0.05) among treatment groups. It is a common practice in feeding trials to use weights of some internal organs like liver and kidney as indicators of toxicity. Bone (1979) reported that if there is any toxic elements in the feed, abnormalities in weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs in attempt to reduce these toxic elements or anti-nutritional factors to non-toxic metabolites. Our observation with the reported liver and kidney weights in rabbits of different treatment groups suggest that the CPB test diets did not contain any appreciable toxin within experimental groups. This was an indication that the processing methods were able to detoxify or bring to a non-lethal level the anti-nutritional factor or hydrogen cyanide (HCN) associated with cassava peel. This view is collaborated by Okeke *et al.* (1985), Ijaiya (2001) and Ahamefule *et al.* (2003).

In conclusion, the haematological and biochemical values obtained for rabbits fed sun-dried, ensiled and fermented cassava peel based diets, except for PCV and WBC fell within normal stipulated ranges. This is a good indication that sun-drying, ensiling and fermentation could be used to reduce HCN to a non-lethal level in cassava peels for rabbit nutrition in Nigeria.

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