Blood biochemistry and haematology of weaner rabbits fed sun-dried, ensiled, and fermented cassava peel-based diets

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ABSTRACT

Twenty-four New Zealand white × Chinchilla weaner bucks, aged between 7 and 8 weeks and averaging 0.9 kg in weight, were divided into four groups of six 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 randomised. Diet A, the control, was a 16.18 per cent 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 per cent CP in which 10 per cent 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 the study included packed cell volume (PCV), white blood cells (WBC), neutrophil (N), and lymphocytes (L). The 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. The results showed that PCV, WBC, N and L were affected ($P<0.05$) by experimental diets. Also affected ($P<0.05$) were bilirubin, SGPT and SGOT. However, serum creatinine, urea and eosinophil were unaffected ($P>0.05$) by treatment diets. Liver and kidney weights also did not differ ($P>0.05$) among rabbits fed different dietary treatments.

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RÉSUMÉ

AHAMEFULE, F. O., EDUOK, G. O., USMAN, A., AMAEFULE, K. U., OBUA, B. E. & OGUIKE, S. A.: *Biochimie et hématologie de sang de lapins en sevrage, nourris de régime à base de pelure de manioc séchée de soleil, ensilée et fermentée.* Vingt-quatre lapins mâles en sevrage de l'espèce de Nouvelle - Zélande blanc × Chinchilla agé de 7 à 8 semaines et de poids moyen de 0.9 kg étaient divisés en 4 groups de 6 lapins chacun et employés dans un essai d'alimentation de 12 semaines pour évaluer la biochimie et l'hématologie de sang de lapins nourris de régimes à base de pelure de manioc séchée de soleil, ensilées et fermentée. Les régimes d'essai classés A, B, C et D étaient complètement choisis au hasard. Le régime A, le contrôle, était une ration de sevrage avec 16.18% de CP (protéine brute) formulée de maïs, déchets de maïs, farine de graine de soja, farine sanguine, coquille d'huître, engrais de cendres d'os, vitamine prémix et sel ordinaire. Les régimes B, C et D étaient aussi des rations de sevrage, respectivement, avec 16.10, 16.20 et 16.08% de CP enquel 10% de maïs du régime de contrôle était remplacé par les pelures de manioc, respectivement, séchée de soleil, ensilées et fermentée. Les régimes étaient approximativement iso-cloriques. Les éléments hématologiques de l'étude comprenaient la volume de cellule tassée (VCT), le globule sanguin blanc (GSB), le neutrophile (N) et les lymphocytes (L). Les paramètres biochimiques étaient le sérum créatinine, l'urée, la bilirubine (totale et conjuguée), le sérum glutamique transaminase (SGPT), le sérum glutamique oxaloacétique transaminase (SGOT), l'éosinophile et le sucre dans le sang. Les poids de foie et de rein étaient également suivis de près. Les résultats montraient que VCT, GSB, les neutrophiles et les lymphocytes étaient modifiés ($P<0.05$) par les régimes expérimentaux. Egalement modifié ($P<0.05$) étaient bilirubine, SGPT et SGOT. Le sérum créatinine, l'urée et l'éosinophile n'étaient pas toutefois modifiés ($P<0.05$) par les régimes de traitements. Les poids de foie et de rein aussi ne différaient pas ($P>0.05$) parmi les lapins nourris de différents traitements diététiques.

Introduction

Blood is a complex fluid containing large variety of dissolved suspended inorganic and organic substances (Stewart, 1991), or specialised circulating tissues and cells suspended in the intercellular fluid substance (Dellman & Brown, 1976) which circulates in the arteries, vessels and capillaries of man and animals (Kronfield & Mediway, 1975). Its primary function is to transport oxygen from respiratory organs to body cells (Duke, 1975), to distribute nutrients and enzymes to cells, and to carry away waste products (Baker & Silverton, 1992), 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. These blood components are influenced by the quantity and quality of feed, and also by the level of anti-nutritional elements or factors present in the feed (Akinmutimi, 2004). The biochemical components are sensitive to elements of toxicity in feeds. They can also be used to monitor protein quality of feeds. The haematological components are also valuable in monitoring feed toxicity, especially with feed constituents that affect the formation of blood (Oyawoye & Ogunkunle, 1990).

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 (HCN) derivatives. The HCN has been shown to be toxic to livestock (Mcdonald, Edwards & Greenhalgh, 1995) and, therefore, limits the use of cassava peels in the raw state as feed for livestock (Smith, 1988). Cassava peels have been detoxified through sun-drying (Ahamefule, Ibeawuchi & Nwankwo, 2003), ensiling (Okeke, Obioha & Udeogu, 1985), and fermentation (Ijaiya, 2001).

This study, therefore, aimed 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

The site used for the study was the Rabbit Unit of the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike. The geography and location of Umudike is as described by Ahamefule *et al*. (2003).

Experimental procedure

Twenty-four weaner bucks (New Zealand white × Chinchilla) of 7 to 8 weeks of age and weighing averagely 0.9 kg (0.80-0.95 kg) were randomly divided into four groups, with six animals in each group. Each animal was housed individually in a standard hutch provided with a feeder and waterer. The hutch system was of the three-tier model, housed in well-ventilated cement-floored pens and raised 120 cm above the ground. Before the experiment (2 weeks), each animal was dewormed and given an acaricide bath. Four experimental diets (A, B, C, D) were formulated (Table 2). Diet A, the control, was a 16.18 per cent CP weaner ration based on maize and maize offal. Diets B, C, and D were also weaner rations in which 10 per cent 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 randomised (Steel & 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 8 to12 weeks of study from four rabbits randomly selected from the six in each group, using the methods of Uko, Ataja & Tanko (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 10 ml (of first volume) was collected over labelled sterile universal bottles containing 1.0 mg ml^{-1} ethyldiamine tetracetic acid (EDTA) and 0.1 mg ml^{-1} Heparin. This was used to determine the haematological component according to the methods of Ajagbonna, Onifade & Suleman (1999) and Uko *et al.* (2000). Another 10 ml (of 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 & Price, 1997; Uko *et al.*, 2000).

Feed and data analyses

Feed samples were analysed 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 randomised design (Steel & 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 determined by Ahamefule *et al.* (2003), Okeke *et al.* (1985), and Ijaiya (2001) for the respective products elsewhere. Table 2 shows the percentage and 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 determined 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 & 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 & Rawnsley, 1997). Table 4 shows a summary of haematological and biochemical values determined for rabbits fed the control and CPB diets in this study.

All the haematological components of study (PCV, WBC, lymphocytes, eosinophil) for rabbits on control diet (A) fell within normal ranges (Table 3). Among treatment diets, PCV value determined for rabbits on fermented CPB diet (D) (46.50%) 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 value (46.50%) did not differ significantly (P>0.05) from

TABLE 1

Proximate Composition of Sun-dried, Ensiled and Fermented Cassava Peels

Constituent (%)	*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

TABLE 2

Percentage and Proximate Compositions of Experimental Diets

Ingredient (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
Analysed 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, 1500 IU; Vit E, 11.0 mg; Riboflavin, 9.0 mg; Biotin, 0.25; Pantothenic acid, 11.0 mg; Vit k3, 3.0 mg; B2, 2.5 mg; B6, 0.3 mg; B12, 8.0 mg; Nicotinic acid, 8.0 mg; Fe, 5.0 mg; Mn, 10.0 mg; Zn, 4.5 mg; Co,0.2 mg; Se,0.01mg.

that recorded for rabbits (45.75%) on sun-dried CPB diet (B). The PCV values observed 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 detoxifying cassava peels as may have been shown in the normal PCV range of values observed for rabbits subsisting on either sun-dried or ensiled CPB diets. The fermentation method may, however, not have been as effective as either the 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.

The average WBC value ($10^3 \mu l^{-1}$) for rabbits fed fermented CPB diet (7265) was also significantly higher ($P<0.05$) than those of the sun-dried (5800), ensiled (6275), and the control (6000). Although values of the ensiled, like the fermented overshot the normal range (3000-6000), the ensiled 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 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 rabbits fed ensiled CPB diets did not differ ($P>0.05$) from those on control. Animals on sun-dried CPB had values significantly lower ($P<0.05$) than those of either the ensiled or control. However, these observed values (ensiled and control) did not differ

TABLE 3

Mean and Normal Ranges of Haematological and Biochemical Components for Rabbit

Parameter	*Range*	*Mean*
Haematological		
Packed cell volume (%)	25.0 - 45.0	34.0
White blood cell ($10^3\mu l^{-1}$)	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 100 ml^{-1})	0.4 - 3.0	2.0
Conjugated bilirubin (mg 100 ml^{-1})	0.2 - 0.4	0.3
Serum creatinine (mg100 ml^{-1})	0.6 - 0.8	0.7
Urea (mg 100 ml^{-1})	30.0 - 37.3	34.0
SGOT (ml l^{-1})	12.0 - 18.0	15.0
SGPT (ml l^{-1})	9.0 - 15.0	12.0
Sugar (mg 100 ml^{-1})	65.3 - 74.8	70.0

Source: Mitruka & Rawnsley (1977); Kronfield & Mediway (1975).

($P>0.05$). 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; however, it did not differ ($P>0.05$) from either the fermented or control groups. Although blood neutrophil and lymphocyte concentrations showed variation between treatment groups, the values determined 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

Table 4 shows a summary of biochemical values recorded for rabbits on different treatment groups in this study. The control had normal range of values for all biochemical parameters measured. Serum creatinine levels were within normal range and did not differ ($P>0.05$) among treatment groups. The values recorded for animals on Diets B, C and D or the cassava peel-based diets agreed with the findings of Omole & Sonaiya (1981); suggesting that there was no wasting or catabolism of muscle tissues, and that animals were not surviving at the expense of body reserve. This indicated that dietary protein was well used by rabbits.

Blood urea concentration was also normal among treatment groups, suggesting also the effectiveness of the processing methods. Increase in serum urea concentration may suggest an increase in activities of urea enzymes orninthine, carbonyl transferase and orginase, which may also indicate kidney damage (Ajabonna *et al.*, 1999). The normal range of values recorded implied, therefore, that the dietary proteins of the CPB diets and the control were well used (Reinhold, 1953).

Values for total and conjugated bilirubin (TB, CB) showed significant differences ($P<0.05$) among rabbits on different dietary treatments. Although 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 for animals 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 enough or adequate protein in the experimental rations for normal metabolic and physiological activities (Ologhobo *et al.*, 1992).

The significantly higher CB and TB values determined for rabbits on control over those for rabbits on 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. However,

TABLE 4

Summary of Haematological and Biochemical Blood Components of Rabbits Fed Sun-dried, Ensiled, and Fermented Cassava Peel-based Diets

Parameter	*Control*	*Sun-dried*	*Ensiled*	*Fermented*	*SEM*
Haematological	*(A)*	*(B)*	*(C)*	*(D)*	
Packed cell volume (%)	42.75^{c}	45.75^{ab}	43.25^{bc}	46.50^{a}	0.92*
White blood cell (10^3 µl^{-1})	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}	0.40 ns
Biochemical					
Total bilirubin (mg 100 ml^{-1})	1.06^{a}	0.46^{c}	0.85^{b}	0.83^{b}	0.04*
Conjugated bilirubin (mg 100 ml^{-1})	0.33^{a}	0.24^{b}	0.26^{b}	0.27^{b}	0.20*
Serum creatinine (mg 100 ml^{-1})	0.76^{a}	0.68^{a}	0.60^{a}	0.71^{a}	0.05 ns
Urea (mg 100 ml^{-1})	33.00^{a}	33.00^{a}	36.25^{a}	34.50^{a}	1.66 ns
SGOT (ml l^{-1})	12.0^{b}	11.50^{b}	16.0^{a}	7.73^{c}	0.92*
SGPT (ml l^{-1})	9.17^{b}	10.42^{b}	12.80^{a}	12.17^{a}	0.69*
Sugar (mg 100 ml^{-1})	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}	4.24 ns
Kidney (g)	4.92^{a}	5.00^{a}	4.50^{a}	4.55^{a}	0.53 ns
Feed intake					
Average daily feed intake (g)	64.90^{a}	70.86^{a}	74.73^{a}	74.07^{a}	3.15 ns

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

protein availability is associated closely with protein quality. Though the protein components of all the test diets may be of good quality, comparatively, however, the control diet may have shown 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 & Sonaiya, 1981). This inadvertently may have affected the protein quality of the CPB test diets, perhaps negligibly, compared 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 recorded differed significantly ($P<0.05$) among treatment groups. The SGOT concentration (ml l^{-1}) of rabbits on the control diet (12) was lower ($P<0.05$) compared to those of the ensiled (16), similar ($P>0.05$) to the group on the sun-dried (11.50), and higher ($P<0.05$) compared to those on fermented (7.73) CPB diets. The SGPT values (ml l^{-1}) for the control (9.47) and sun-dried (10.42) groups were similar ($P>0.05$). However, these observed values were 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 infaction or response to several toxic factors (Sigma, 1985). Coles (1986) has, however, suggested caution in interpreting serum enzyme activity, because enzyme activities vary greatly among species and even among tissues and

organs.

The blood sugar concentrations of rabbits fed the CPB diets, as in the control, were within normal stipulated range (Tables 3 and 4). However, the mean concentrations recorded differed ($P<0.5$) significantly among groups. Rabbits on the control diet had significantly higher blood sugar concentration compared to rabbits 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 observed 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 and 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 because of metabolism or insulin- related disorder, leads to breakdown of fat and proteins as energy source.

Values determined for kidney and liver weights in this study showed no 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 any toxic element is 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 an attempt to reduce these toxic elements or anti-nutritional factors to non-toxic metabolites. The observation with the reported liver and kidney weights in rabbits of different treatment groups in this study suggests that the CPB test diets did not contain any appreciable toxin within the experimental groups. This indicated that the processing methods were able to detoxify or bring to a non-lethal level, the anti-nutritional factor or HCN associated with cassava peel. This view is corroborated by Okeke *et al.* (1985), Ijaiya (2001), and Ahamefule *et al*. (2002).

The haematological and biochemical values recorded for rabbits fed sun-dried, ensiled and fermented cassava peel-based diets, except for PCV and WBC, fell within normal stipulated ranges. This indicates 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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