ASSESSMENT OF CASSAVA PEEL MEAL, METHIONINE AND ENZYME ON SERUM METABOLITES OF GROWING RABBITS

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ABSTRACT

This study evaluated the effect of cassava peel meal (CPM) inclusion, methionine (MET) supplementation and enzyme (ENZ) supplementation on serum chemical indices of growing rabbits. Two hundred and forty 5-weeks old rabbits were randomly distributed into 8 experimental diets/groups (1,2,3,4,5,6,7 and 8) with factorial arrangement (2x2x2) including 2 levels of CPM (0 and 350 g/kg), 2 levels of MET (5.6 and 8.3 g/kg) and 2 levels of ENZ (0 and 0.5 g/kg). Total protein (TPR) and albumin (ALB) concentration in rabbits fed diet 5 (350 g/kg CPM; 5.6 g/kg ENZ) were lower than the rest diets. TPR and ALB concentration were low at 350 g/kg CPM but improved by 8.3g/kg methionine and 0.5 g/kg enzyme supplementations. CPM inclusion tends ($P=0.09$) to increase the creatinine level, while 8.3g/kg methionine and 0.5 g/kg enzyme supplementations ($P<0.05$) reduced the creatinine level. Bilirubin level increased ($P<0.05$) with 350 g/kg CPM inclusion but was reduced by 8.3 g/kg methionine. The alanine amino transferase (ALT) and aspertate amino transferase (AST) levels increased ($P<0.05$) with 350 g/kg CPM inclusion. However, AST level tends ($P=0.09$) to reduce with 8.3g/kg methionine. Production of healthy rabbits with 350 g/kg CPM based diet required raising methionine supplementation to 8.6 g/kg and/or 5.0 g/kg enzyme supplementation.

INTRODUCTION

The acute feed shortage being experienced in intensive rearing systems because of high cost of major feed ingredients such as maize, millet, soybean meal and groundnut cake among others was instrumental to the rising cost of animal protein in developing countries. This situation has geared the focus of animal nutritionists to intensity research into alternative feed resources and in particular, a grain replacement (Onifade and Tewe, 1993). One of the identified ways of reducing animal production cost is the use of agro-industrial by-products for animal feeding. Based on the fact that energy sources contributed to the 45-60% monogastric finished feed and animals eat to satisfy their energy requirements (Egbunike *et al.*, 2009), replacement of maize with processed cassava peels in part or in full in monogastric production will go a long way in reducing the production cost. Although, cassava peels are highly digestible, its high fibre content, heavy loads of antinutrients likes cyanide, low and caloric value are the major constraints to its use in livestock nutrition (Aro, 2008). Ingestion of lethal dose of cyanide leads to acute toxicity and death caused by the inhibition of cytochrome

oxidase of the respiratory chain while when the sub-lethal doses of cyanide are consumed, the inhibition of cellular respiration are reversed by the removal of HCN by detoxification process via many pathways among which is the reaction of cyanide with blood and later converted to the less toxic thiocyanate (Nwokoro *et al.*, 2000). Methods such as fermentation (Oloruntola *et al.*, 2015; 2016), palm oil inclusion (Omole and Nwudike, 1983), sun-drying and cooking (Aro, 2008) among others had been used in reducing the cyanic level of cassava peels. Recently, the use of methionine supplementation (Oladunjoye *et al.*, 2014) and enzyme supplementation (Ogunsipe *et al.*, 2015) are being recommended. Blood is involved in detoxification process of cyanide, and blood and its constituent provides a reliable medium for nutritional evaluation and clinical diagnosis of individuals (Babatunde *et al.*, 1992; Onifade and Tewe, 1993). Therefore, this study was conducted to determine the effect of cassava peel, high methionine and enzyme supplementation on serum biochemistry indices of growing rabbits.

MATERIALS AND METHOD

Cassava peels collected fresh from cassava processing factories in Ado Ekiti, Nigeria were washed with clean water, drained, sun-dried, milled with 3 mm screen hammer mill to obtain cassava peel meal (CPM). The multi-enzyme (Biozyme PH) and methionine used for this study was obtained from a commercial feed mill in Akure, Nigeria. The multi-enzyme (Biozyme PH) used in this study, according to the manufacturer (Biomix S.A, Carrera 47C, Sabaneta-Colombia) has a minimum/kg of cellulase (700,000U.A), α-amylase (800,000U.A), β-glucanse (300,000U.BG), phytase (1,200F.T.U), protease (8,000,000U.P), lipase (20,000U.I) and xylanase (500 000U.X). Treatments were assigned in a completely randomized design with factorial arrangement (2x2x2) including 2 levels of cassava peels (0 and 350 g/kg), 2 levels of methionine (5.6 and 8.3 g/kg) and 2 levels of enzyme (0 and 0.5 g/kg). Eight diets were formulated for the rabbits and designated as diet 1, 2, 3, 4, 5, 6, 7, 8 (Table 1) The diets were pelletized (4mm diameter and 8mm long).A total of 240, cross breeds (Chinchilla and New Zealand white) of equal sex, at 35 days of age with average size of 572.99±9.33 were randomly allotted to 8 dietary treatments (30 rabbits/diet, 10 replicate/diet). The rabbits were housed in galvanized wire mesh cages (60x49x30 cm) kept in a well ventilated pen. Experimental diets and fresh water were always available for a period of 8 weeks. The cassava peels, experimental diets faeces were analysed for proximate composition AOAC (2000) and cyanide (Rao *et al*., 1997) in duplicate. Lysine and methionine contents of the feed and cassava peels were determined using methods described by Benitez (1989). At the end of the experiment, blood samples for haematological study were collected as described by Burnett *et al*., (2003). The serum chemistry indices were determined with a Reflectron ® Plus 8C79 (Roche Diagnostic, GonbH Mahnheim, Germany) using kits. Statistical analysis was carried out with SPSS version 20. Data generated were analysed as 2x2x2 factorial arrangement with 2 levels of cassava peels, 2 levels of methionine and 2 levels of enzyme and test for significance between the dietary treatment means by Duncan's Multiple Range Test at P≤0.05.

RESULTS AND DISCUSSION

Result of chemical analysis showed that cassava peel meal (CPM) has crude protein (54.79 g/kg), crude fibre (105 g/kg), ether extract (4.35 g/kg), ash (52.74 g/kg), nitrogen free extract (704.81 g/kg), hydrogen cyanide (0.02 g/kg), methionine (0.36 g/kg) and lysine (1.14 g/kg). All the serum chemistry values shown in Table 2 fall within the normal range reported earlier (Mitruka 1977; Burnet *et al*., 2003). Total protein (TPR), albumin (ALB) and globulin (GLO) are indicators for measuring total protein intake (Birth and Schuldt, 1982, Onifade and Tewe, 1993). In this study, TPR and ALB concentration in rabbits fed diet 5 (350 g/kg CPM; 5.6 g/kg ENZ) were lower than the rest diets. This observation might have occurred as a result of relatively high cyanide concentration of diet 5 due to CPM inclusion, which probably caused decreased protein production and increased loss or catabolism of protein with resultant decreased concentration of TPR and decrease production of fibrinogen, immunoglobin and globulins or increased loss of albumin which also might have resulted in decreased ALB concentration (Peter and Susan, 1991).This is further explained by significant (P<0.05) decrease in TPR and ALB concentration with CPM inclusion. The TPR and ALB concentration increased significantly (P<0.05) with high methionine supplementation, while enzyme tends (P<0.06) to increase TPR concentration, it significantly (P<0.05) increased concentration of ALB in the rabbits. These observations in this study agreed with Oladunjoye *et al*., 2014 and Ogunsipe *et al*., 2015 who reported improvement of nutritional value of cassava peel meal by high methionine and enzyme supplementation was reported. Increased rhodanese activity in kidney and excretion of cyanide metabolites in urine (Wrobel *et al*., 2004) are amongst the responses to cyanide poisoning in animals' body system, meaning that kidneys play some roles in cyanide detoxification. Therefore, elevation of creatinine level (P<0.05) in diet 5 (350g/kg CPM and 5.6 g/kg MET) above other diets and the tendency

($P=0.09$) of CPM inclusion in rabbits' diet to increase their creatinine level in this study signalled compromise in the integrity the rabbits' kidney cell due to presence of cyanide in the rabbits' diet as a result of CPM inclusion. However, the significant ($P<0.05$) decrease in creatinine levels of the rabbits by high methionine and enzyme supplementation in this study further supports the earlier reports on potentials of methionine (Oladunjoye *et al.*, 2014) and enzyme (Ogunsipe *et al.*, 2015) in removing the negative effect of cyanide present in cassava by-products based diet in animals. Bilirubin level reflects the balance between its production and excretion. Bilirubin level was significantly ($P<0.05$) higher in diet 5 compared to the rest diets and its level also increased significantly ($P<0.05$) by CPM (350g/kg) inclusion. Since cyanide detoxification is a function of the liver (Nakajima, 2015), the elevated bilirubin level in rabbits fed diet 5 in this study may reflect possible liver disease disorder precipitated by cyanide in the diet due to CPM inclusion. Rhodanese, key enzyme involved in sulphur metabolism use methionine as sulphur donor to detoxify cyanide to thiocyanate (Nakajima, 2015). This may be associated with significant ($P<0.05$) reduction of bilirubin level by high methionine supplementation of rabbit diet and the significant ($P<0.05$) interaction effect of CPM and MET in this study. Alanine amino transferase (ALT) was stable ($P>0.05$) across the diets, although CPM inclusion significantly ($P<0.05$) increased ALT levels in the rabbits while high methionine and enzyme supplementation brought numeric decrease ALT levels. Aspertate amino transferase (AST) level was higher ($P<0.05$) in rabbits fed diet 5 than others. In addition, ALT level increased ($P<0.05$) with CPM inclusion in the diet while the AST level tend to decrease ($P=0.09$) by high methionine supplementation of the rabbits' diet. CPM and MET interaction was significant for AST. ALT is used for evaluating hepato-cellular injury while AST is used for determining the health state of the heart. The result from this study showed that CPM (350 g/kg) in rabbits' diets may compromise the liver and heart integrity, but high methionine and enzyme supplementation may avert this possible health mishap.

CONCLUSION

Commercial production of healthy rabbits with 350 g/kg based CPM required raising methionine supplementation to 8.6 g/kg and/or 5.0 g/kg enzyme supplementation.

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Table 1: Composition of experimental diets

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Multi-enzyme	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Methionine	0.18	0.18	0.48	0.48	0.26	0.26	0.55	0.55
Cassava Peel	0.00	0.00	0.00	0.00	35.00	35.00	35.00	35.00
Maize	37.00	37.00	37.00	37.00	1.93	1.93	1.93	1.93
BDG	36.80	36.80	36.80	36.80	36.80	36.80	36.80	36.80
Wheat Offal	7.37	7.37	7.07	7.07	7.36	7.36	7.07	7.07
Soy bean meal	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Maize Husk	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Bone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vegetable oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Determined analysis								
Crude protein	17.51	17.52	17.46	17.47	16.55	16.53	16.49	16.48
Crude fibre	12.09	12.06	12.07	12.03	16.25	16.23	16.21	16.23
Calculated analysis								
Lysine	0.88	0.88	0.88	0.88	0.87	0.87	0.87	0.87
Methionine	0.56	0.56	0.83	0.83	0.55	0.55	0.81	0.81
Energy (kcal/kg)	2581.5	2581.5	2577.6	2577.6	2624.5	2624.5	2620.7	2620.7

BDG: Brewer dried grain

Table 2: Effect of dried cassava peels inclusion (g/kg), methionine (g/kg). and multi-enzyme (g/kg) supplementation on serum serum chemistry in rabbits

DIETS	CPM	MET	ENZ	TPR	ALB	GLO	CRE	CHO	BIL	ALT	AST
1	0	5.6	0.00	6.61^{a}	2.80^{a}	3.27	73.00^{b}	1.19	5.55^{d}	45.01	49.87^{b}
2	0	5.6	0.50	6.21^{a}	2.85^{a}	3.32	70.10^{b}	1.07	5.20^{d}	49.80	46.50^{b}
3	0	8.3	0.00	6.35^{a}	2.97^{a}	3.38	73.35^{b}	1.08	5.42^{d}	48.87	47.01^{b}
4	0	8.3	0.50	6.40^{a}	2.97^{a}	3.43	72.50^{b}	1.13	5.43^{d}	48.62	54.41^{b}
5	350	5.6	0.00	4.37^{b}	2.14^{b}	2.32	132.71^{a}	1.03	13.26^{a}	73.86	96.92^{a}
6	350	5.6	0.50	6.14^{a}	2.75^{a}	3.39	73.85^{b}	1.21	11.33^{ab}	66.05	57.82^{b}
7	350	8.3	0.00	6.15^{a}	2.81^{a}	3.34	70.58^{b}	1.13	8.72^{bc}	61.35	55.85^{b}
8	350	8.3	0.50	6.29^{a}	2.86^{a}	3.30	68.13^{b}	1.15	7.66^{cd}	58.09	52.05^{b}
SEM				0.16	0.06	0.11	5.34	0.07	0.65	3.75	4.05
P value				0.02	0.00	0.31	0.01	0.99	0.00	0.54	0.01
MAIN EFFECT											
CPM	0.00			6.28^{a}	2.89^{a}	3.35	72.23	1.12	5.40^{b}	48.07^{b}	49.44^{b}
	350			5.74^{b}	2.64^{b}	3.08	86.08	1.13	10.24^{a}	64.83^{a}	65.66^{a}
SEM				0.18	0.05	0.16	5.54	0.11	0.44	5.41	4.21
P value				0.05	0.01	0.26	0.09	0.94	0.00	0.04	0.02
MET		5.6		5.72^{b}	2.63^{b}	3.07	87.41^{a}	1.13	8.83^{a}	58.68	62.78
		8.3		6.30^{a}	2.90^{a}	3.36	71.14^{b}	1.12	6.80^{b}	54.23	52.33
SEM				0.18	0.05	0.16	5.54	0.11	0.44	5.41	4.21
P value				0.03	0.01	0.22	0.05	0.95	0.01	0.56	0.09
ENZ			0.00	5.76	2.68^{b}	3.08	87.41^{a}	1.11	8.23	57.27	62.41
			0.50	6.26	2.85^{a}	3.36	71.14^{b}	1.14	7.40	55.64	52.69
SEM				0.18	0.05	0.16	5.54	0.11	0.44	5.41	4.21
P value				0.06	0.05	0.23	0.05	0.84	0.20	0.83	0.12
INTERACTIONS											
CPM*MET											
SEM				0.25	0.08	0.23	7.83	0.16	0.63	7.65	5.96
P value				0.15	0.15	0.44	0.03	0.89	0.01	0.46	0.04
CPM*ENZ											
SEM				0.25	0.08	0.23	7.83	0.16	0.63	7.65	5.96
P value				0.09	0.08	0.32	0.08	0.69	0.31	0.61	0.06
MET*ENZ											
SEM				0.25	0.08	0.23	7.83	0.16	0.63	7.65	5.96
P value				0.13	0.08	0.24	0.08	0.98	0.63	0.98	0.07
CPM*MET*ENZ											
SEM				0.36	0.12	0.32	11.07	0.23	0.89	10.83	8.43
P value				0.13	0.13	0.24	0.10	0.63	0.84	0.75	0.31

CPM: cassava peel meal; MET: methionine; ENZ: enzyme, TRP:total protein (g/dl); ALB:albumin (g/dl); GLO;globulin (g/dl); CRE:creatinine (μmol/l); CHO:cholesterol (mmol/l); BIL:bilirubin (μmol/l); ALT:alanine amino transferase (μ/l); AST:aspertate amino transferase (μ/l):