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Effect of dietary cassava peel meal supplemented with methionine and multienzyme on hemo-biochemical indices, digestibility, and antioxidants in rabbits

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Abstract

Background: The effects of cassava peel meal (CPM) supplemented with methionine (MET) and multienzyme (ENZ) was studied using 240 5-week-old rabbits in a completely randomized design arranged in 2 × 2 × 2 factorial (2 CPM levels, 0 and 350 g/kg; 2 methionine levels, 5.6 and 8.3 g/kg; and 2 enzyme levels, 0 and 0.5 g/kg).

Results: The CPM inclusion reduced ($P < 0.05$) leucine, lysine, valine, and threonine digestibility, while the digestibility of leucine, lysine, and valine increased ($P < 0.05$) by high methionine level. Multienzyme increased leucine, lysine, and valine digestibility. CPM reduced ($P < 0.01$) red blood cell (RBC) count and the total serum protein. Higher methionine supplementation increased the serum total protein by 9.2%. The CPM inclusion increased bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Methionine and enzyme supplementation increased albumin (ALB) but reduced creatinine (CRE). Superoxide dismutase, glutathione peroxidase, and catalase activity level increased ($P < 0.01$) with methionine and enzyme supplementation.

Conclusion: Cassava peel meal could be included in growing rabbits' diets at 350 g/kg level with methionine at 32.53% higher than requirement and 0.5-g/kg multienzyme supplementation.

Keywords: Cassava, Wastes, Blood, Anti-oxidative status, Rabbit

Background

The role played by cassava and its by-products in alleviating food security challenge is tremendous in Africa (Aro, 2008). For the period covering 2008 and 2012, the African continent produced over 54% of global cassava production, with Nigeria alone contributing 36% (approximately 52 million tons) of the African total production (FAOStat, 2013). This increased cassava production has offered opportunities to intensify the utilization of cassava by-products such as cassava peels, in animal feeding programs. Cassava peels (5–15% of tuber weight), when processed, could be used to replace high input-dependent conventional energy feed ingredients in animal production (Oloruntola et al., 2016). However, the high level of structurally indigestible carbohydrates (cellulose, hemicellulose, pectin, and lignin) and high antinutrients (hydrogen cyanide, tannin, and phytate) coupled with low protein content (Oloruntola et al., 2018) have been identified as the major factors limiting its optimal use in monogastric feeding. The negative effects of these limitations on the animals vary from impaired digestibility to poor feed intake culminating in reduced performance (Adegbola and Okonkwo, 2002). For example, cyanide inhibition of the terminal enzyme of the respiratory chain (cytochrome C oxidase) compromises phosphorylation, and eventual cytotoxic hypoxia is

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expressed in acute cyanide toxicity. In chronic cyanide toxicity, in addition to retarded growth, the increase in serum and urinary levels of thiocyanate is perhaps a continuous cause of sulfur-containing amino acid depletion that takes place in the animal. The thiocyanate also causes inhibition of the intra-thyroidal uptake of iodine, increase in thyroid-stimulating hormone, and a reduction in thyroxine level which is necessary for growth in animals (Lukuyuet al., 2014).

Furthermore, there is a continuous increase in attention given to the preservation of livestock welfare with the aim of enhancing their productivity and preserving them from multi-factorial diseases (Giorgiaet al., 2017). For instance, imbalances in the diet of rabbits have been reported as one of the major causes of deviations from normal physiological functions which usually cause high mortality and low farm productivity (Broom and Johnson, 1993).

Various methods to eliminate or reduce constraints/limitations to cassava peel utilization in animal feeding have been tried and their accomplished success reported. Hyper methionine supplementation (Oladunjoye et al., 2014, Oloruntola et al., 2019) and exogenous enzyme supplementation (Ogunsipe et al., 2015 Oloruntola 2018, Oloruntola et al., 2019) and fermentation (Oloruntola et al., 2015) have been recommended for enhancing the utilization of cassava peels in monogastric animal feeding. Perhaps sundrying is also another possible effective method of improving the potential feeding value of cassava peel meal.

Sulfur-containing amino acids such as methionine are needed for the detoxifying process of cyanide to thiocyanate. Thus, the determination of amino acids' digestibility in animals fed with cassava by-product–based diet consequently becomes necessary to further monitor the animals' well-being while being fed on these by-products. Furthermore, there exists an established link between nutrition and oxidative status of animals (Giorgia et al., 2017) and the physiological stress due to imbalances in diets of animals as this could lead to excessive production of free radicals, with resultant oxidative stress (Khadijah et al., 2009). Blood and its constituents can also provide a valuable medium for nutritional and clinical evaluation of the individual because dietary components affect the blood constituents (Onifade & Tewe, 1993), while serum biochemistry reflects the condition of the organism under the influence of an internal factor such as diet (Akanmu & Adeyemo, 2012).

Therefore, the present feeding trial was conducted to study the effect of cassava peel meal dietary inclusion, varying methionine levels, and multienzyme supplementation on the digestibility of ileal amino acids, blood composition, and oxidative status of growing rabbits with a view to establishing the safe level of cassava peel meal in the diets of growing rabbits.

Materials and methods

The approval of protocol for this feeding trial was given by the Research Ethics Committee of Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria. The study was carried out in a well-ventilated pen at the Teaching and Research Farm of Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria. The site was 437 mm above the sea level and has the mean annual temperature of 26.2 °C and situated at latitudes of 7° 37′ N and 7° 12′ N and longitudes of 5° 11′ E and 5° 31′ E (Oloruntola et al. 2016).

Ingredient preparation and experimental diets

Cassava peels were collected fresh from a cottage cassava processing factory located at Ado Ekiti, Nigeria, washed with clean water, drained, and spread lightly on a tarpaulin for sun-drying for about 2 weeks from the day of collection. Dried cassava peels were thereafter milled with a 3-mm screen hammer mill to obtain cassava peel meal (CPM) and analyzed for its chemical composition (Table 1). The multienzyme (*Biozyme PH*) used in this study, according to the manufacturer (Biomix S.A, Carrera 47C, Sabaneta-Colombia), has a minimum per kilogram of cellulase (700,000 U.A), α-amylase (800,000 U.A), β-glucanase (300,000 U.BG), phytase (1200 F.T.U), protease (8,000, 000 U.P), lipase (20,000 U.I), and xylanase (500 000 U.X). Eight (8) diets were formulated with their gross composition as shown in Table 2. The eight dietary treatments were pelletized (4-mm diameter and 8-mm long) and designated as diets 1, 2, 3, 4, 5, 6, 7, and 8. Diets 1 and 2 had 5.6 g/kg (normal methionine level) but only diet 2 was supplemented with exo-enzyme;

Table 1 Composition (%) of cassava peel meal

Parameters	Quantity
DM	92.17 ± 0.49
Crude protein	5.48 ± 0.13
Crude fiber	10.50 ± 0.12
Ether extract	0.44 ± 0.02
Ash	5.27 ± 0.04
Nitrogen free extract	70.48 ± 0.75
Acid detergent fiber	13.64 ± 0.02
Acid detergent lignin	2.22 ± 0.01
Neutral detergent fiber	21.94 ± 0.11
Methionine	0.04 ± 0.00
Lysine	0.11 ± 0.01
Hydrocyanic acid (mg/kg)	20 ± 0.29

Table 2 Composition of experimental diets

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Multienzyme (0.05%)	-	+	-	+	-	+	-	+
DL-methionine 99%	0.18	0.18	0.48	0.48	0.26	0.26	0.55	0.55
Cassava peel meal	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	36.30	36.30	36.30	36.30	36.30	36.30	36.30	36.30
Wheat offal	7.37	7.37	7.07	7.07	7.36	7.36	7.07	7.07
Soybean 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
L-lysine HCl 78%	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
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Determined analysis (%)								
Crude protein	17.51	17.51	17.46	17.46	16.55	16.55	16.49	16.49
Crude fiber	12.09	12.09	12.07	12.07	16.23	16.23	16.24	16.24
Lysine	0.92	0.92	0.92	0.92	0.90	0.90	0.89	0.89
Methionine	0.58	0.58	0.85	0.85	0.59	0.59	0.85	0.85
NDF	39.96	39.96	39.94	39.94	40.12	40.12	40.14	40.14
ADF	17.54	17.54	17.36	17.36	17.92	17.92	17.95	17.95
ADL	3.61	3.61	3.62	3.62	3.94	3.94	3.96	3.96
Calculated analysis								
Crude protein	17.51	17.53	17.46	17.47	16.55	16.53	16.49	16.47
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
Calcium (%)	0.49	0.49	0.49	0.49	0.57	0.57	0.57	0.57
Available P (%)	0.35	0.35	0.36	0.36	0.39	0.39	0.39	0.39
Energy (kcal/kg)	2581.5	2581.5	2577.6	2577.6	2624.5	2624.5	2620.7	2620.7
*HCN (mg/kg)	0.0	0.0	0.0	0.0	700.0	700.0	700.0	700.0

BDG Brewer's dried grain, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *ADL* acid detergent lignin, *HCN* hydrogen cyanide,
*Calculated based on the determined value on Table 1

diets 3 and 4 had 8.3 g/kg (higher methionine level), but diet 4 was supplemented with exo-enzyme. In diets 5 and 6, dried cassava peel meal was included at 350 g/kg to replace the corresponding amount of maize grain and had 5.6 g/kg (normal methionine level), but diet 6 was supplemented with multienzyme. Diets 7 and 8 had their maize grain being replaced by 350 g/kg of dried cassava peel meal and had 8.3 g/kg (higher methionine level), but diet 8 was multienzyme supplemented.

Animals and experimental design

A total of 240 crossbred rabbits (Chinchilla × New Zealand white) of same sex, at 5 weeks of age and average live weight of 572.99 ± 9.33 g were randomly allotted to the 8 experimental dietary treatments (30 rabbits/dietary treatment; 10 rabbits/replicate; 3 rabbits/replicate). The trial was conducted in a completely randomized design arranged in the form of 2 × 2 × 2 factorial with two CPM levels (0 and 350 g/kg), 2 methionine (MET) levels (5.6 and 8.3 g/kg) and two enzyme (ENZ) levels (0 and 0.5 g/kg). Marker (titanium dioxide) was incorporated into each diet at the rate of 0.5% for the determination of amino acid digestibility.

The rabbits were raised in galvanized wire mesh cages (60 × 55 × 30 cm) kept in a well-ventilated pen, provided with fresh water and their respective experimental diets ad libitum throughout the period of the trial (56 days).

Digestibility study

On day 56 of the feeding trial, one rabbit from each replicate was randomly selected, tagged, weighed, and sacrificed or rather slaughtered. Immediately, the rabbits were dissected; the ileum contents (digesta) were collected in a clean labeled Petri dish for assessment of amino acid digestibility. The digesta samples were thereafter kept in the deep freezer. To obtain dried samples, the frozen digesta were freeze-dried using $VaCO_2$ Zirbus Technology (Hilfe Gottes, 1-37539, Bad Grund, Germany).

Determination of blood parameters

At 56 days of age, 10 rabbits from each treatment were randomly selected and bled following procedures described by Burnett et al. (2003). About 8 ml of blood was collected in to tubes from the marginal ear vein of each rabbit with syringe and needle. Each blood sample was divided into two parts, such that 3 ml blood was placed in a potassium ethylene diamine tetraacetic acid (KEDTA) tube for hematological study and the remaining blood placed in the plain (no anticoagulant) tube for serum chemistry and antioxidant enzyme status determination.

Chemical analysis

The cassava peels and experimental diets were analyzed in triplicates for proximate composition (AOAC, 2000), neutral detergent fiber and acid detergent fiber (Goering and VanSoest, 1970), and cyanide content (Oboh et al., 2002). Amino acid contents of the CPM, diets, and ileal digesta samples were determined as described by Benitez (1989) using *Applied Biosystems PTH Amino Acid Analyzer* (Applied Biosystems Inc., Foster City, CA, 94404, USA, Model 120A). The Cr_2O_3 content of the diets and digesta samples were determined according to the method of Blas et al. (2003), while for the amino acid content, ileal digestibility coefficients were calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta (g/kg)}/\text{digesta}_{Cr2O3}\text{(g/kg)}}{\text{diet (g/kg)}/\text{digesta}_{Cr2O3}\text{(g/kg)}}$$

The hematological indices were determined on the collection day using *Shenzhen Mindray Auto Hematology Analyzer* (Model Bc-3200, Shenzhen Mindray Biomedical Electronics Co., Hamburg, 20537, Germany). The serum chemistry indices were determined with a *Reflectron ® Plus 8C79* (Roche Diagnostic, GonbH Mannheim, Germany) using kits. Glutathione peroxidase (GPx) activity in the plasma sample was determined as described by Rotrucket al. (1973), superoxide dismutase (SOD) as described by Misra and Fridovich (1972), and catalase activity as described by Aebi (1974).

Statistical analysis

Data generated were subjected to general linear model analysis of variance for factorial (2 × 2 × 2) arrangement. First-order and second-order interactions on the main factors were determined. Test for a significant difference between the dietary treatment means was conducted by Duncan's multiple range test at $P \leq 0.05$.

Results

The effects of cassava peel meal, methionine, and multienzyme supplementation on ileal digestibility of seven (7) amino acids are shown in Table 3. The ileal digestibility of leucine, lysine, and valine was the least ($P < 0.05$) in rabbits fed with diet 5 (350 g/kg CPM; 5.6 g/kg MET) but with the highest methionine digestibility. The CPM inclusion (350 g/kg) significantly ($P < 0.05$) reduced the digestibility of leucine (0.92 to 0.85), lysine (0.85 to 0.79), valine (0.85 to 0.81), and threonine (0.80 to 0.79), while methionine and cysteine digestibility increased (0.78 to 0.85 and 0.82 to 0.88), respectively. Also in this study, increasing the methionine from 5.6 g/kg to 8.3 g/kg led to a significant increase in leucine ($P < 0.01$), lysine ($P < 0.05$), and valine ($P < 0.01$) digestibility. Supplemental multienzyme (0.5 g/kg) caused significant ($P < 0.05$) percent increase in leucine, lysine, and valine digestibility by 8%, 6%, and 3.6%, respectively. Interaction of methionine and enzyme was significant ($P < 0.05$) for leucine and lysine.

Table 4 shows that of all the hematological indices measured, only the lymphocyte was affected. The lymphocyte value recorded for rabbits fed with diet 5 was significantly ($P < 0.01$) lower than the rest of the diets. There was significant ($P < 0.03$; 0.01) interactive effects of CPM × ENZ and MET × ENZ on lymphocyte count.

There was a significant ($P < 0.02$) reduction of TPR in rabbits fed with diet 5. In addition, CPM dietary inclusion caused TPR reduction in the rabbits (Table 5), while an increase ($P < 0.05$) in TPR value in the rabbits was recorded due to dietary methionine. There was a significantly ($P < 0.01$) low ALB value recorded in rabbits fed with diet 5 (350 g/kg CPM; 5.6 g/kg MET). However, there was an improvement ($P < 0.05$) in ALB values due to enzyme supplementation.

There was an elevated creatinine level ($P < 0.01$) in rabbits fed with diet 5 (350 g/kg CPM and 5.6 g/kg MET) above other diets, and there was the tendency ($P = 0.09$) of CPM inclusion to numerically increase the creatinine level in this study (Table 5). However, there was a significant ($P < 0.05$) decrease in creatinine levels

Table 3 Effect of cassava peel meal (g/kg), methionine (g/kg), and multienzyme (g/kg) inclusion on ileal digestibility of some amino acids

Diets	CPM	MET	ENZ	Leu	Lys	Phe	Val	Met	His	Thr	Cyst
1	0	5.6	0.00	0.84^{b}	0.78^{b}	0.75	$0.81^{ab\ c}$	0.73^{c}	0.78	0.77	0.76
2	0	5.6	0.50	0.94^{a}	0.85^{a}	0.76	0.86^{a}	0.81^{abc}	0.82	0.80	0.84
3	0	8.3	0.00	0.93^{a}	0.85^{a}	0.78	0.86^{a}	0.80^{ab}	0.82	0.82	0.84
4	0	8.3	0.50	0.95^{a}	0.87^{a}	0.79	0.85^{a}	0.80^{ab}	0.83	0.83	0.83
5	350	5.6	0.00	0.69^{d}	0.70^{c}	0.73	0.76^{c}	0.91^{a}	0.75	0.75	0.93
6	350	5.6	0.50	0.80^{bc}	0.82^{ab}	0.75	0.79^{bc}	0.84^{ab}	0.80	0.78	0.86
7	350	8.3	0.00	0.77^{c}	0.81^{ab}	0.74	0.80^{abc}	0.83^{ab}	0.79	0.78	0.85
8	350	8.3	0.50	0.82^{bc}	0.82^{ab}	0.75	0.84^{ab}	0.83^{ab}	0.80	0.79	0.86
SEM				1.87	1.17	0.86	0.87	1.41	0.87	0.72	1.37
P value				0.01	0.01	0.74	0.01	0.05	0.58	0.11	0.14
Main effect											
CPM	0			0.92^{a}	0.85^{a}	0.77	0.85^{a}	0.78^{b}	0.82	0.80^{a}	0.82^{b}
	350			0.78^{b}	0.79^{b}	0.75	0.81^{b}	0.85^{a}	0.79	0.78^{b}	0.88^{a}
SEM				0.82	0.98	1.31	0.86	1.56	1.27	0.89	1.73
P value				0.01	0.01	0.18	0.01	0.01	0.16	0.03	0.03
MET		5.6		0.82^{b}	0.79^{b}	0.75	0.81^{b}	0.82	0.79	0.78	0.85
		8.3		0.87^{a}	0.84^{a}	0.77	0.85^{a}	0.82	0.81	0.81	0.85
SEM				0.82	0.98	1.31	0.86	1.56	1.73	0.89	0.91
P value				0.01	0.03	0.32	0.01	0.93	0.31	0.07	0.88
ENZ			0.00	0.81^{a}	0.79^{a}	0.75	0.81^{a}	0.81	0.79	0.78	0.85
			0.50	0.88^{b}	0.84^{b}	0.77	0.84^{b}	0.82	0.81	0.80	0.86
SEM				0.82	0.98	1.31	0.86	1.56	1.27	0.89	1.73
P value				0.01	0.02	0.45	0.05	0.88	0.22	0.11	0.89
Interactions											
CPM × MET											
SEM				1.17	1.39	1.85	1.22	2.21	1.79	1.26	2.44
P value				0.92	0.71	0.51	0.31	1.11	0.99	0.35	1.38
CPM × ENZ											
SEM				1.17	1.39	1.85	1.22	2.21	1.79	1.26	2.44
P value				0.32	0.54	0.79	0.55	0.09	0.91	0.84	0.16
MET × ENZ											
SEM				1.17	1.39	1.85	1.22	2.21	1.79	1.26	2.44
P value				0.01	0.01	0.73	0.46	0.88	0.39	0.37	0.89
CPM × MET × ENZ											
SEM				1.64	1.97	2.61	1.72	3.13	2.54	1.78	3.46
P value				0.81	0.41	0.92	0.22	0.10	0.90	0.95	0.13

CPM cassava peel meal, *MET* methionine, *ENZ* enzyme, *Leu* leucine, *Lys* lysine, *Iso* isoleucine, *Phe* phenylalanine, *Val* valine, *Met* methionine, *His* histidine, *Thr* threonine, *Cyst* cystein, ^{a},^{b}, ^{c} Means with a different superscript in the same column are significantly different ($P<0.05$)

of the rabbits by high methionine and multienzyme supplementation.

Bilirubin level was significantly ($P < 0.05$) higher in rabbits fed with diet 5 (350 g/kg CPM and 5.6 g/kg MET) compared to those fed the with other diets. In addition, the bilirubin level increased significantly ($P < 0.01$) with CPM (350 g/kg) inclusion but reduced ($P < 0.01$) with methionine supplementation (Table 5). Reduced ($P < 0.05$) bilirubin level caused by high methionine (8.3 g/kg) supplementation and significant ($P < 0.01$) interactive effect of CPM and MET was also recorded in this study.

Table 4 Effect of cassava peel meal (g/kg), methionine (g/kg), and multi-enzyme (g/kg) inclusion on hematological and erythrocytic indices in rabbits

Diets	CPM	MET	ENZ	PCV	HB	RBC	MCV	MCH	MCHC	WBC	LYM	PLA
1	0	5.6	0.00	35.77	12.68	7.46	50.64	17.70	35.42	7.33	1.59^{bc}	328.33
2	0	5.6	0.50	37.09	13.23	8.66	46.20	16.20	35.55	7.49	1.74^{abc}	323.66
3	0	8.3	0.00	37.14	12.75	8.93	55.16	18.10	34.98	7.78	2.38^{a}	320.32
4	0	8.3	0.50	37.73	12.82	9.02	52.33	16.58	34.41	7.88	1.72^{abc}	317.67
5	350	5.6	0.00	34.17	12.56	4.07	86.54	32.18	36.54	7.08	1.09^{c}	299.00
6	350	5.6	0.50	34.37	12.90	5.27	66.42	24.32	37.39	7.26	2.01^{ab}	324.33
7	350	8.3	0.00	34.49	12.91	5.34	64.94	23.77	37.409	7.28	2.02^{ab}	322.66
8	350	8.3	0.50	34.57	12.94	6.10	61.85	22.48	37.02	7.33	2.01^{ab}	314.00
SEM				0.84	0.44	0.61	4.68	1.59	0.99	0.22	0.11	318.75
P value				0.94	1.00	0.29	0.53	0.15	0.99	0.99	0.01	0.99
Main effect												
CPM	0.00			36.93	12.87	8.52^{a}	17.15^{b}	51.08	35.10	7.62	1.86	322.50
	350			34.40	12.83	5.19^{b}	25.69^{a}	69.94	37.09	6.74	1.78	325.00
SEM				1.35	0.75	0.83	2.02	6.74	1.64	0.37	0.11	13.51
P value				0.21	0.96	0.01	0.01	0.06	0.40	0.47	0.59	0.70
MET		5.6		35.35	12.84	6.36	22.60	62.45	36.22	6.79	1.61^{b}	318.83
		8.3		35.98	12.85	7.34	20.23	58.57	35.95	7.57	2.03^{a}	318.66
SEM				1.35	0.75	0.83	2.02	6.74	1.64	0.37	0.11	13.51
P value				0.74	0.99	0.41	0.42	0.69	0.91	0.15	0.01	0.99
ENZ			0.00	35.39	12.72	6.45	22.94	64.32	36.08	6.87	1.77	317.58
			0.50	35.94	12.97	7.26	19.90	56.70	36.10	7.49	1.87	319.91
SEM				1.35	0.75	0.83	2.02	6.74	1.64	0.37	0.11	13.51
P value				0.78	0.82	0.49	0.30	0.43	0.99	0.24	0.49	0.90
Interactions												
CPM × MET												
SEM				1.91	1.06	1.17	2.86	9.54	2.33	0.52	0.15	19.11
P value				0.84	0.86	0.95	0.34	0.34	0.82	0.49	0.79	0.72
CPM × ENZ												
SEM				1.91	1.06	1.17	2.86	9.54	2.33	0.52	0.15	19.11
P value				0.83	0.95	0.88	0.59	0.68	0.92	0.36	0.02	0.75
MET × ENZ												
SEM				1.91	1.06	1.17	2.86	9.54	2.33	0.52	0.15	19.11
P value				0.91	0.85	0.74	0.57	0.63	0.83	0.31	0.01	0.68
CPM × MET × ENZ												
SEM				2.71	1.50	1.65	4.04	13.49	3.29	0.74	0.21	27.02
P value				0.93	0.96	0.88	0.57	0.69	0.95	0.33	0.83	0.64

CPM cassava peel meal, *MET* methionine, *ENZ* enzyme, *PCV* packed cell volume (%), *HB* hemoglobin concentration (g/dl), *RBC* red blood cells ($\times 10^{12}$/l), *MCH* mean cell hemoglobin (pg), *MCV* mean cell volume (fl), *MCHC* mean cell hemoglobin concentration (g/dl), *WBC* white blood cells ($\times 10^{9}$/l), *LYM* lymphocytes ($\times 10^{9}$/l), *PLA* platelets ($\times 10^{9}$/l), ^{a,b}, ^{c} Means with a different superscript in the same column are significantly different ($P<0.05$)

Alanine aminotransferase (ALT) was stable ($P > 0.05$) across the diets with the highest and the lowest values recorded in rabbits fed with diets 5 and 1, respectively. The CPM inclusion significantly ($P < 0.05$) increased ALT levels in the rabbits. Aspartate aminotransferase (AST) level was higher ($P < 0.05$) in rabbits fed with diet 5 than others (Table 5). In addition, AST level increased ($P < 0.05$) with CPM inclusion in the diet but numerically decreased with high methionine supplementation. CPM × MET interaction was significant for AST.

The levels of antioxidant defense enzymes (superoxide dismutase, glutathione peroxidase, and catalase) studied

Table 5 Effect of cassava peel meal (g/kg), methionine (g/kg), and multienzyme (g/kg) inclusion on serum biochemical indices in rabbits

Diets	CPM	MET	ENZ	TPR	ALB	GLO	CRE	URE	CHO	BIL	ALT	AST
1	0	5.6	0.00	6.61^{a}	2.80^{a}	3.27	73.00^{b}	5.64	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}	5.57	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}	5.99	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}	6.01	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}	4.99	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}	5.47	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}	5.82	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}	6.00	1.15	7.66^{cd}	58.09	52.05^{b}
SEM				0.16	0.06	0.11	5.34	0.14	0.07	0.65	3.75	4.05
P value				0.02	0.01	0.31	0.01	0.71	0.99	0.01	0.54	0.01
Main effect												
CPM	0			6.28^{a}	2.89^{a}	3.35	72.23	5.80	1.12	5.40^{b}	48.07^{b}	49.44^{b}
	350			5.74^{b}	2.64^{b}	3.08	86.08	5.57	1.13	10.24^{a}	64.83^{a}	65.66^{a}
SEM				0.18	0.05	0.16	5.54	0.21	0.11	0.44	5.41	4.21
P value				0.05	0.01	0.26	0.09	0.46	0.94	0.01	0.04	0.02
MET		5.6		5.72^{b}	2.63^{b}	3.07	86.43^{a}	5.41	1.13	8.83^{a}	58.68	62.78
		8.3		6.30^{a}	2.90^{a}	3.36	70.24^{b}	5.95	1.12	6.80^{b}	54.23	52.33
SEM				0.18	0.05	0.16	5.54	5.34	0.11	0.44	5.41	4.21
P value				0.03	0.01	0.22	0.05	0.10	0.95	0.01	0.56	0.09
ENZ			0.00	5.76	2.68^{b}	3.08	87.41^{a}	5.61	1.11	8.23	57.27	62.41
			0.50	6.26	2.85^{a}	3.36	71.14^{b}	5.76	1.14	7.40	55.64	52.69
SEM				0.18	0.05	0.16	5.54	0.21	0.11	0.44	5.41	4.21
P value				0.06	0.05	0.23	0.05	0.62	0.84	0.21	0.83	0.12
Interactions												
CPM × MET												
SEM				0.25	0.08	0.23	7.83	0.31	0.16	0.63	7.65	5.96
P value				0.15	0.15	0.45	0.04	0.65	0.89	0.01	0.46	0.05
CPM × ENZ												
SEM				0.25	0.08	0.23	7.83	0.31	0.16	0.63	7.65	5.96
P value				0.09	0.08	0.32	0.09	0.57	0.69	0.31	0.61	0.06
MET × ENZ												
SEM				0.25	0.08	0.23	7.83	0.31	0.16	0.63	7.65	5.96
P value				0.13	0.08	0.24	0.08	0.86	0.98	0.63	0.98	0.07
CPM × MET × ENZ												
SEM				0.36	0.12	0.32	11.07	0.43	0.23	0.89	10.83	8.43
P value				0.13	0.13	0.24	0.10	0.75	0.63	0.84	0.75	0.31

CPM cassava peel meal, *MET* methionine, *ENZ* enzyme, *TPR* total protein (g/dl), *ALB* albumin (g/dl), *GLO* globulin (g/dl), *CRE* creatinine (μmol/l), *URE* urea (mmol/l), *CHO* cholesterol (mmol/l), *BIL* bilirubin (μmol/l), *ALT* alanine amino transferase (u/l), *AST* aspertate amino transferase (u/l), ^{a,b}, ^{c,d} Means with a different superscript in the same column are significantly different ($P<0.05$)

were least ($P < 0.01$) in rabbits fed with diet 5 (Table 6). In addition, these enzyme levels decreased significantly ($P < 0.05$) with CPM inclusion but increased with high methionine and multienzyme supplementation. Generally, there were significant interactive effects of the CPM, MET, and ENZ.

Discussion

The result of this study showed that the ileal digestibility of leucine, lysine, and valine was least in rabbits fed with diet 5 (350 g/kg CPM; 5.6 g/kg MET) but with the highest methionine digestibility. The cyanogenic activity of CPM-based diets (Table 2) may be responsible for the

Table 6 Effect of cassava peel (g/kg), methionine (g/kg), and multienzyme (g/kg) inclusion on antioxidant status in rabbits

Diets	CPM	MET	ENZ	SOD (%)	GPx (μg/g)	Catalase (mM/ml/min)
1	0	5.6	0.00	82.33^{b}	126.58^{b}	11.38^{b}
2	0	5.6	0.50	88.33^{ab}	165.62^{a}	13.70^{a}
3	0	8.3	0.00	86.67^{a}	166.68^{a}	14.59^{a}
4	0	8.3	0.50	92.33^{a}	168.81^{a}	14.73^{a}
5	350	5.6	0.00	64.67^{c}	100.15^{c}	8.77^{c}
6	350	5.6	0.50	87.00^{ab}	162.74^{a}	13.67^{a}
7	350	8.3	0.00	87.32^{ab}	163.20^{a}	14.53^{a}
8	350	8.3	0.50	90.65^{a}	162.83^{a}	14.71^{a}
SEM				1.80	4.91	0.42
P value				0.01	0.01	0.01
Main effect						
CPM	0			87.91^{a}	156.92^{a}	13.60^{a}
	350			82.41^{b}	147.23^{b}	12.92^{b}
SEM				0.94	1.03	0.17
P value				0.01	0.01	0.01
MET		5.6		80.58^{b}	138.77^{b}	11.88^{b}
		8.3		89.75^{a}	165.38^{a}	14.64^{a}
SEM				0.94	1.03	0.17
P value				0.01	0.01	0.01
ENZ			0.00	80.75^{b}	139.15^{b}	12.32^{b}
			0.50	89.58^{a}	165.00^{a}	14.20^{a}
SEM				0.94	1.03	0.17
P value				0.01	0.01	0.01
Interactions						
CPM*MET						
SEM				1.34	1.45	0.24
P value				0.01	0.00	0.02
CPM*ENZ						
SEM				1.34	1.45	0.24
P value				0.01	0.00	0.02
MET*ENZ						
SEM				1.34	1.45	0.24
P value				0.01	0.00	0.00
CPM*MET*ENZ						
SEM				1.89	2.06	0.33
P value				0.01	0.00	0.02

CPM cassava peel meal (g/kg), *MET* methionine (g/kg), *ENZ* enzyme (g/kg), *SOD* superoxide dismutase, *GPx* glutathione peroxidase, ^{a,b,c} Means with a different superscript in the same column are significantly different ($P<0.05$)

decrease in the digestibility of these amino acids as recorded in this study. This findings agreed with the previous report of Iyayi and Odueso (2003) who recorded low nutrient digestibility following varying cyanide levels in rabbit diets. However, the observed high methionine and cysteine (sulfur-containing amino acids) digestibility recorded in this study following CPM inclusion may be attributed to the fact that sulfur from these amino acids could have been highly used in the process of detoxification of cyanide in the CPM-based diets by the experimental rabbits. According to Morgan and Choct (2016), sulfur-consuming processes involved in the detoxification process of hydrocyanic acid varied from the liberation of hydrocyanic acid by glucosidases being produced by the intestinal microbiota, liver, and other tissues. In the liver, hydrocyanic acid is changed into thiocyanate by rhodanese and thereafter excreted in the urine (Garcia & Dale, 1999). In addition, the use of these sulfur amino acids for the purpose of cyanide detoxification makes them unavailable for protein synthesis in animals. This may aggravate the deficiency of these amino acids, coupled with the thermal stress involved in amino acid catabolism; this could lead to the reduction in digestibility of other amino acids (Iyayi & Odueso, 2003) as experienced in this study. Also in this study, the observed increased digestibility of leucine, lysine, and valine due to increased methionine level from 5.6 g/kg to 8.3 g/kg is in tandem with the report of Oladunjoye et al. (2014) who observed improvement in nutrient utilization in broilers fed with cassava-based diet supplemented with high methionine level. Improvement in amino acid digestibility due to methionine level may also be associated with the detoxifying effect of methionine on hydrocyanic acid (Aniebo, 2012) which could probably reduce the negative impacts of cyanide on nutrient digestibility efficiency. The increase of leucine, lysine, and valine digestibility due to supplemental multienzyme recorded in this study is consistent with the recent reports of Hossain et al. (2016) and Ayodele et al. (2016) who both recorded an improvement in the digestibility of nutrients due to enzyme supplementation. The enzyme enables the animals to degrade antinutrients in feed components (Kiarieet al., 2013; Oloruntola et al., 2019), promotes breakdown of stored proteins by increasing the degree of protein hydrolysis, increasing the proportion of low molecular size proteins, and promoting the availability of protein for uptake (Frietas et al., 2011; Ayodele et al., 2016). This may in part explain the reason behind the improvement in amino acid digestibility due to multienzyme supplementation in this study. The significant interaction effect MET × ENZ on leucine and lysine implies that these two factors would be able to complement one another to bring about improvement in the digestibility of these amino acids in a growing rabbit.

Lymphocytes play a vital role in the body defense of animals against infections. In particular, T cells, which are a group of lymphocyte cells responsible for cell-mediated immunity had been reported to be affected by nutrition and any factor that cause gastrointestinal

malabsorption (Dennis, 2005). Roles of nutrition in preserving or increasing the T-lymphocytes or CD4 cells were reported (Okorie & Ophori, 2013). The decrease in the lymphocyte value in rabbits fed with diet 5 (350 g/kg CPM) may be the resultant effect of a relatively high level of HCN in the diet coupled with low methionine and no multienzyme addition which possibly could lead to the impairment/prevention of essential nutrient digestion and utilization (Prathibha et al., 1995, Lukuyu et al., 2014). This is explained further by the increase of lymphocyte count due to high-level methionine supplementation in this study. Methionine supplementation was reported to improve nutrient utilization and growth performance in rabbits (Job, 1975; Oloruntola et al., 2019). The significant interactive effects of CPM × ENZ and MET × ENZ on lymphocyte count revealed the combining positive effect of these factors on the immunity of the experimental rabbits. However, all the hematological and derived absolute values recorded in this study fall within the normal range (PCV 33–50%, Hb 9.4–17.4 g/dl, RBC $4.7–7.2 \times 10^{12}$/l, MCV 50–75 fl, MCH 16–23 pg and MCHC 280–360 g/l, WBC $5–12 \times 10^{9}$/l, lymphocytes $2–20 \times 10^{9}$/l, platelets $200–1000 \times 10^{9}$/l) as earlier reported (Burnet et al., 2003; Latimer et al., 2003 and Oloruntola et al., 2016).

Total protein (TPR), albumin (ALB), and globulin (GLO) are indicators for measuring dietary protein intake and utilization by animals (Onifade & Tewe, 1993). In this study, the reduction of TPR in rabbits fed with diet 5 and the observed TPR reduction in the rabbits due to CPM dietary inclusions indicate the possible impairment of protein intake in rabbits due to the presence of HCN, the major antinutrient present in CPM. However, the increase in TPR value in the rabbits due to dietary methionine further unveiled the tendency of methionine supplementation to improve the nutritive values of CPM-based diets in rabbit nutrition. In the same vein, low ALB value recorded in rabbits fed with diet 5 (350 g/kg CPM; 5.6 g/kg MET) could be associated with nutritional imbalances precipitated relatively by high dietary HCN concentration caused by the inclusion of CPM in the diet. This could be further explained by the reduction of ALB value in the experimental rabbits due to CPM dietary inclusion. However, the observed improvement in ALB values due to enzyme supplementation further showed the efficiency of the multienzyme supplementation in improving the nutrient utilization and reduction of negative effects of antinutrients in farm animals. In general, the present findings further confirm the toxicity effect of cyanide, which could lead to decrease protein production and increased loss or catabolism of protein with resultant decreased concentration of TPR and decreased production of fibrinogen, immunoglobin, and globulins or increased loss of albumin which also might have resulted in decreased ALB concentration (Lording & Friend, 1991).

In addition, the improvement of TPR and ALB concentrations in rabbits fed with diets containing high methionine and multienzyme supplementation agreed with the reports of Oladunjoye et al. (2014), Ogunsipe et al. (2015), and Oloruntola (2018) who separately reported improvement in the nutritional value of cassava peel meal supplemented with methionine and exo-enzyme. Increased rhodanese activity in kidney and excretion of cyanide metabolites in urine (Wrobel et al., 2004) are among the responses to cyanide poisoning in animals' body systems, meaning that kidneys play some roles in cyanide detoxification. Therefore, the elevation of creatinine levels in rabbits fed with diet 5 (350 g/kg CPM and 5.6 g/kg MET) above other diets and the tendency of CPM inclusion to increase the creatinine level in this study signals a compromise in the integrity of the rabbits' kidney cell due to the presence of cyanide. However, the decrease in creatinine levels of the rabbits by high methionine and multienzyme supplementation in this study further supports the earlier reports on the potentials of methionine (Oladunjoye et al., 2014) and multienzyme (Ogunsipe et al., 2015) in removing the negative effect of cyanide present in cassava by-product–based diet in animals.

Bilirubin level reflects the balance between its production and excretion. Since cyanide detoxification is a function of the liver (Nakajima, 2015), the elevated bilirubin level in rabbits fed with diet 5 in this study may reflect possible liver disorder precipitated by cyanide in the diet due to CPM inclusion. However, the observed reduced bilirubin level caused by high methionine (8.3 g/kg) supplementation in this study could be linked to the activities of rhodanese (the key enzyme involved in sulfur metabolism) which use methionine as a sulfur donor to detoxify cyanide to thiocyanate (Nakajima, 2015). This is further supported by the significant interactive effect of CPM and MET in this study; implying that CPM and MET could interact to produce a positive effect on the health status of the experimental rabbits.

The increased ALT levels in the rabbits due to CPM dietary inclusion also indicate possible hepatocellular injury in the rabbits caused by hydrogen cyanide. However, high methionine and multienzyme supplementation brought the numeric decrease in ALT levels. AST is an important heart status marker. Generally, the result from this study showed that CPM inclusion in rabbits' diets may compromise the liver and heart integrity, but methionine and multienzyme supplementation as shown in this current study may avert this possible health mishap. All the serum chemistry values shown in Table 5 fall within the normal range (total protein 2.8–10.0 g/dl, albumin 3.3–5 g/dl, globulin 1.5–2.7 g/dl, creatinine 70–150 μmol/

l, bilirubin 4.3–12.8 μmol/l, urea 9.3–25.3 mmol/l, cholesterol 0.1–2.0 mmol/l, ALT 55–260 u/l, and AST 33–99 u/l) reported earlier for rabbits (Burnet et al., 2003).

Cyanide caused lipid oxidation in varieties of tissues and imposes oxidative stress on rabbits by inhibition of antioxidant defense enzyme that catalyzes the metabolism of oxygen radicals and peroxides (Shouet al., 2000; Gary & Gary, 2015). It also inhibits metalloenzymes which makes up the anti-oxidation defense (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) (Gary & Gari, 2015). This might suggest the observed decrease in the values of the superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in rabbits fed with diet 5. The enzyme (carbohydrase) can increase the permeability of the aleurone layer and consequently increase the release of unavailable minerals to the animals. For instance, selenium is involved in the active site of enzyme glutathione peroxidase in blood, liver, and edible tissues (Surai, 2002). Therefore, the improvement in the anti-oxidative status of the rabbits due to multienzyme supplementation could be a result of the direct effect of these enzymes or the combined interactive effect of the component enzyme in the multienzyme used in releasing useful minerals that promote the production of antioxidant-protective enzymes in the experimental rabbits. Methionine is a precursor of cysteine, which is required for the synthesis of reduced glutathione and methionine can scavenge for reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical (Metayer et al., 2008).This may be responsible for the increase in the superoxide dismutase, glutathione peroxidase, and catalase in this study. Generally, the significant effect of the CPM × MET × ENZ suggests that the three main effects are interdependent on each other in the determination of the activities of the three antioxidant enzymes studied

Conclusion

The study elucidated the various effects of cassava peel meal when supplemented with methionine and multienzyme on growing rabbits. This study suggested that cassava peel meal could be included in growing rabbits' diet at 350-g/kg level but such diets should be supplemented with methionine at 32.53% higher than the requirement and 5.5-g/kg multienzyme without deleterious effects on the amino acid digestibility, blood, serum, and antioxidant indices. Thus, the nutritive potential of CPM, which is often limited by cyanide toxicity, can be explored for rabbit feeding if methionine couple with appropriate 5.5 g/kg enzyme supplementation level is adopted.

Abbreviations

ANOVA: Analysis of variance; CF: Crude fiber; CP: Crude protein; CPM: Cassava peel meal; MET: Methionine; ENZ: Multienzyme; RBC: Red blood cell; AST: Aspertate aminotransferase; ALT: Alanine aminotransferase; ALB: Albumin; CRE: Creatinine; CHO: Cholesterol; BIL: Bilirubin; URE: Urea; KEDTA: Potassium ethylene diamine tetraacetic acid; AOAC: Association of Analytical Chemists; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; TPR: Total protein; GLO: Globulin; BDG: Brewer's dried grain; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; HCN: Hydrogen cyanide; Leu: Leucine; Lys: Lysine; Iso: Isoleucine; Phe: Phenylalanine; Val: Valine; Met: Methionine; His: Histidine; Thr: Threonine; Cyst: Cystein; PCV: Packed cell volume; HB: Hemoglobin concentration; MCH: Mean cell hemoglobin; MCV: Mean cell volume; MCHC: Mean cell hemoglobin concentration; WBC: White blood cells; LYM: Lymphocytes; PLA: Platelets

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Author's contributions

ODO designed and managed all activities of the experiment. The author searched and gathered referenced materials and the manuscript. The author reviewed and approved the final manuscript.

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Availability of data and materials

The dataset generated and analyzed during the current study is available from the corresponding author on a reasonable request.

Competing interest

The author declares that she have no competing interests.

Ethics approval and consent to participate

The approval of protocol for this feeding trial was given by the Research Ethics Committee of Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria. The experimental animals were managed following the recommendations and guidelines for applied nutrition experiments in rabbits.

Consent for publication

Not applicable

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