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Cassava Fiber Meal and Roxazyme® G2 Supplementation on the Performance and Haemato-Biochemical Profile of Broiler Chickens

Research Article

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ABSTRACT

This study assessed the effects of cassava fiber meal (CFM) and Roxazyme® G2 supplementation on performance and haemato-biochemical indices of broiler chickens in a feeding trial that lasted for 56 days. A batch of 360 day-old male Arbor Acres chicks of mean weight 390±8.04 g was allotted to 12 dietary treatments of 5 replications of 6 birds to a replicate in a completely randomized design of 4 × 3 factorial treatments. The CFM was substituted for maize at 0, 20, 40, and 60% levels. Each level was supplemented with Roxazyme® G2 at 0, 100, and 200 mg kg^{-1}. The growth performance, carcass traits, relative organ weights, and haemato-biochemical profile of the broiler chickens were analyzed using General Linear Model (GLM) procedures. Results showed that CFM up to 40% did not decrease weight gain but at 60% substitution level, weight gain decreased significantly ($P<0.05$) during the starter and finisher period. Broiler chickens fed diets containing 60% CFM had lower eviscerated weight but higher liver and kidney weights compared with those fed the control and up to 40% CFM in place of maize. Eosinophil and aspartate aminotransferase (AST) are the haemato-biochemical parameters that were influenced ($P<0.05$) at higher CFM substitution with or without enzyme supplementation. The non-significant interaction in the performance and haemato-biochemical parameters of the birds showed the independency of the two factors (CFM and Roxazyme® G2 supplementation) under consideration. CFM up to 40% substitution for maize with or without Roxazyme® G2 is safe as an energy source in broiler chicken diet.

KEY WORDS blood indices, broilers, cassava waste, exo-enzyme, serum, weight gain.

INTRODUCTION

The search for novel feed resources as alternatives to the competing conventional feed resources in livestock nutrition and value addition has become the focus of animal scientists and other related professionals, particularly in developing countries (Ayaşan, 2010; Agbede, 2019). Many of the conventional key feed ingredients are directly competing with humans. Aside from this, the key feed ingredients are seasonal in supply, inadequate, and expensive, making their economic use as sole energy or protein sources a challenge in broiler production (Agbede, 2019). However, the use of the novel feed resources is plagued with anti-nutritive properties which apart from limiting their utilization in livestock diet also contribute to health challenges in livestock as well as humans. Some alternative feed ingredients are rich in non-starch polysaccharides (NSPs), often designated as fiber, and these NSPs have the inherent property to lock up nutrients, making those nutrients unavailable for animal utilization (Oyewole *et al.*

2020). Aside from this, they form viscous substances in the gut limiting the uptake of nutrients by the intestinal villi. The fibrous nature of some alternative plant sources makes it bulky and ultimately indigestible, particularly by simple stomached animal resulting in constipation. To mitigate these challenges, Animal Scientists had developed various nutrients enhancing technologies such as fermentation (Aro *et al*. 2013; Oloruntola *et al*. 2018a), enzyme supplementation (Ogunsipe, 2014; Ogunsipe *et al*. 2015; Oloruntola *et al*. 2018b; Adeyeye *et al*. 2019), prebiotics, probiotics and organic acids (Al-Saad *et al*. 2014) to enhance the nutritive values of these novel feed resources and promote the better performance of livestock.

Cassava is a major staple food crop in Nigeria. It is also a major raw material in starch production. As at 2017, the world cassava production stands at 291 million tonnes with Africa producing about 177 million. Nigeria is the largest cassava producer with about 59 million tonnes followed by countries like Congo DR, Thailand, Indonesia (FAO, 2019). Cassava pulp, also called cassava fiber is the solid fibrous residue (up to 17% of the tuber) that remains after the flour or starch content had been extracted (Aro *et al*. 2010). The quality and appearance of these residues vary with plant age, time after harvest, industrial equipment, and method used (Cereda and Takahashi, 1996). Cassava fiber contains cyanogenic glucosides, which when hydrolyzed yields hydrogen cyanide (HCN) by the enzyme linamarase. In addition to the cyanide content, cassava fiber is rich in non-starch polysaccharides and phytate but low in protein content (Aro *et al*. 2013; Ogunsipe, 2017). To utilize this waste as an energy source in broiler chicken nutrition, there is a need to unlock the nutrients trapped in the cell wall matrix using bio-degradable technology. The use of exogenous enzyme supplementation in livestock diet has been reported to enhance the nutrient status of feed with consequent improvement in growth rate without compromising the health of the animal (Ayodele *et al*. 2016; Ogunsipe, 2017; Adeyeye *et al*. 2019). Thus, the thrust of this study is to assess the performance and health status of broiler chickens fed high fibrous CFM with or without Roxazyme® G2 supplementation.

MATERIALS AND METHODS

Ethical approval and experimental location

The present study was conducted under the guideline of the Research and Ethics Committee of the Department of Agricultural Science, Adeyemi Federal University of Education, Ondo, Nigeria, and the trial was executed at the Poultry Experimental Unit of the Teaching and Research Farm, Adeyemi Federal University of Education, Ondo, Nigeria. Ondo lies between 07˚ 15'N, 05˚ 05'E with annual rainfall of 1800-3600 mm, 54-91% relative humidity and a mean daily temperature 22-35 ˚C throughout the year (Maps-Street View, 2015). The feeding trial was conducted between the months of February 2021 and March 2021 when the average daily temperature was 30.6 ˚C and the relative humidity was 76%. In the experimental house (pen), the average daily temperature-humidity index (THI) was 30.1 ± 1.64 ºC. The animals were managed under the standard management conditions.

Procurement of cassava fiber meal and Roxazyme® G2

Cassava fiber used in the study was collected fresh from Cassava Processing Factory at km 7, Ondo-Ore Road, Ondo, Nigeria and sun-dried for 5 to 7 days to reduce the moisture content to 11.40% with constant turning to prevent fermentation. The sun-dried cassava fiber was made to pass through 0.55 mm diameter, bagged and kept in store prior to use. The milled CFM was analyzed for its proximate composition, mineral and phytochemical properties.

Roxazyme® G2 is a product of DSM Nutritional Product Europe Ltd., Kaiseraugst, Switzerland. Roxazyme® G2 is a cellulose enzyme complex produced by the activity of microorganisms *Trichoderma longibrachiatum*, stabilized and standardized lignin sulphonation, and calcium sulphate dihydratome. The basic activity of the enzyme comes from cellulases, endo- 1, 4-beta-glucanase (glutamate) and xylanases. Roxazyme® G2 is a complex enzyme derived from *Trichoderma longibrachiatum*, free of smells and soluble in water, having density 0.86 g ml^{-1}. Roxazyme® G2 is a cellulose enzyme complex produced by activity of microorganisms *Trichoderma longibrachiatum.*

Determination of the chemical composition of cassava fiber meal

The CFM and experimental diets were analyzed for the proximate compositions according to AOAC (2002) methods. Maize, soybean meal, groundnut cake, and fish meal were analyzed for their feed composition values. This was to prevent large variations in dietary nutrients after mixing. The mineral constituents were analyzed using AOAC (2002) methods. Phytate was quantified as described by Young and Greaves (1940) while tannin was according to Makkar and Goodchild (1996). Flavonoids and alkaloids were determined according to the methods of Bohani and Kocipai-Abyazan (1994) and Henry (1971), respectively. Cyanide determination was according to the method by Rao *et al*. (1997) while oxalate was by the method of Baker and Silverton (1985) (Table 1).

Experimental diets

The design of the experiment was completely randomized in a 4 × 3 factorial arrangement of treatments. The basal diet was formulated for the starter and finisher phases to meet the NRC (1994) requirements for broiler chickens.

Table 1 Chemical composition (% DM) of cassava fiber meal (n=3)

Proximate composition (g 100 g^{-1})	
Parameters	**Mean±SD**
Dry matter	88.63±1.46
Crude protein	3.95±0.14
Crude fiber	20.02±2.27
Crude fat	3.35±0.19
Ash	4.12±0.46
Mineral content (mg kg-1)	
Calcium (Ca)	0.3530±0.032
Phosphorus (P)	0.2621±0.025
Magnesium (Mg)	0.2000±0.016
Sodium (Na)	0.1048±0.021
Potassium (K)	0.1147±0.023
Copper (Cu)	0.0009±0.0001
Manganese (Mn)	0.0012±0.0001
Phytochemical components	
Cyanide CN^- (mg kg^{-1})	23.97±2.32
Tannin (g 100 g^{-1})	0.08±0.02
Oxalate (mg g^{-1})	269.04±19.72
Phytate-P (mg g^{-1})	4.29±0.57
Phytate (mg g^{-1})	15.23±1.69
Flavonoid (mg 100 g^{-1})	5.69±0.23
Alkaloids (mg g^{-1})	6.18±0.38

SD: standard deviation.

The basal (diet 1) had its maize content replaced with CFM at 20, 40 and 60% on weight for weight basis. The basal diet, which is 0, 20, 40 and 60% CFM-based diet were each mixed thoroughly in one lot and divided into three parts, making a total of 12 diets in all. Thus, each lot of the basal diet was supplemented with Roxazyme® G2 at 0, 100 and 200 mg kg^{-1} and designated diets 1, 2 and 3, respectively. The 20% CFM-based diet was supplemented with Roxazyme® G2 at 0, 100 and 200 mg kg^{-1} and designated diets 4, 5 and 6, respectively, the 40% CFM-based diet was supplemented with Roxazyme® G2 at 0, 100 and 200 mg kg^{-1} and designated diets 7, 8 and 9, respectively while the 60% CFM-based diet was supplemented with Roxazyme® G2 at 0, 100 and 200 mg kg^{-1} and designated 10, 11 and 12, respectively. The gross compositions for the diets for the two phases are as presented in Table 2 while the analyzed phytochemical components of the diets are as presented on Table 3.

Bird’s arrangement and management

A total of three hundred and sixty day-old male broiler chicks of Arbor Acres of mean weight 390 ± 8.04 g were randomly allocated to the twelve (12) experimental diets in a completely randomized design of 4 × 3 factorial arrangements of treatments. Thirty (30) broiler chicks were assigned to each dietary treatment of 5 replications of six (6) birds per replicate. Adequate housing and brooding conditions were maintained throughout the experimental period. The floor space per broiler chick was 0.19 m^2 making a floor space of 1.14 m^2 for 6 chicks. Feeds were supplied *ad libitum* with clean cool water throughout the experimental period.

Data collection

Performance characteristics

Data were collected on daily feed intake. Weekly weight gain was calculated as the difference between the weight of the present week and the initial body weight.

Carcass and organ measurements

On day 56 of the experiment, three birds from each replicate were selected at random, tagged, starved for 12 hours, weighed, stunned and sacrificed by severing the jugular vein with sharp surgical knife and bled. The dressed birds were eviscerated for carcass and organ weight determination. The organs measured were heart, lung, liver, spleen, kidney and gizzard while intestinal length was measured using a tape rule. All organs measured were expressed in g kg^{-1} body weight, except the intestinal length that was expressed in mm. The dressed carcass and eviscerated weights were expressed in % body weight.

Determination of haematological parameters and serum indices

Blood collected were determined by Shenzhen Mind ray Auto Haematology Analyser, Model Bc-3200 (Shenzhen Mind ray Biochemical Electronics Co. Hamburg 20537, Germany) while the serum was analyzed for serum biochemical parameters using commercial kits Reflectron® Plus 8C79 (Roche Diagnostic, GombH Mahnheim, Germany).

Data analysis

The General Linear Model (GLM) was used in analysing the data collected on various parameters:

$$X_{rt} = \mu + \alpha_r + \beta_{rt}$$

Where:
X_{rt}: response variables.
μ: overall mean.
α_r: effect of the *r*th treatment (*r*=diets 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12).
β_{rt}: random error due to experimentation.

The data were subjected to one way analysis of variance using the Statistical Package for Social Sciences (SPSS, 2006) version 15.0. Where differences in means were significant, Duncan multiple range was used for the mean separation (Duncan, 1955).

Table 2 Composition of experimental diets for broiler chickens (g 100 g^{-1}) in which maize was substituted with cassava fiber meal

Ingredients	Levels of cassava fiber meal substitution (%)*							
	Broiler starters				Broiler finishers			
	0	20	40	60	0	20	40	60
Maize	52.53	42.03	31.53	21.01	55.59	44.47	33.35	22.24
CFM	-	10.50	21.00	31.52,	-	11.12	22.24	33.35
SBM	22.50	22.50	22.50	22.50	21.42	21.42	21.42	21.42
GNC	14.20	14.20	14.20	14.20	11.94	11.94	11.94	11.94
Fish meal	5.00	5.00	5.00	5.00	4.50	4.50	4.50	4.50
Bone meal	2.00	2.00	2.00	2.00	2.50	2.50	2.50	2.50
Oyster shell	0.50	0.50	0.50	0.50	0.60	0.60	0.60	0.60
Premix[1]	0.25	0.25	0.25	0.25	0.20	0.20	0.20	0.20
Lysine	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10
DL-methionine	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.35	0.35	0.35	0.35
Veg. oil	2.50	2.50	2.50	2.50	2.70	2.70	2.70	2.70
Calculated value (g 100 g^{-1})								
Crude protein	22.85	22.71	22.55	22.34	20.26	20.18	20.07	20.02
Crude fiber	4.53	4.71	5.26	6.09	5.02	5.87	6.30	6.85
ME (kcal kg^{-1})	3112.07	3007.60	2998.38	2911.73	3129.13	3118.84	3082.61	3013.73
Ca	1.31	1.31	1.32	1.31	1.31	1.30	1.31	1.31
Av. P	0.54	0.54	0.53	0.53	0.56	0.57	0.56	0.56
Analyzed value (g 100 g^{-1})								
Crude protein	22.81±1.27	22.76±1.06	22.57±1.34	22.41±1.02	20.29±1.09	20.24±1.01	20.19±1.11	20.07±1.07
Cruder fiber	4.46±0.25	4.46±0.18	5.31±0.22	6.11±0.51	5.05±0.63	5.79±0.33	6.35±0.49	6.83±0.76
ME (kcal kg^{-1})	3112.07±107.83	3007.60±117.08	2998.38±102.76	2911.73±125.73	3129.13±138.16	3118.84±119.52	3082.61±101.38	3013.73±128.01
Ca	1.31±0.17	1.31±0.21	1.32±0.19	1.31±0.21	1.31±0.18	1.30±0.16	1.31±0.14	1.31±0.13
P	0.540±0.04	0.54±0.07	0.53±0.06	0.53±0.08	0.56±0.11	0.57±0.08	0.56±0.07	0.56±0.09

[1] vitamin A: 8000000 IU; vitamin D_3: 2000000 IU; vitamin E: 8000 mg; vitamin K_3: 2000 mg; vitamin B_1: 1500 mg; vitamin B_2: 4000 mg; vitamin B_6: 1500 mg; vitamin B_{12}: 10 mcg; Niacin: 15000 mg; Pantothenic acid: 5000 mg; Folic acid: 500 mg; Biotin: 20 mcg; Choline chloride: 100000 mg; Mn: 75000 mg; Zn: 45000 mg; Fe: 20000 mg; Cu: 4000 mg; Iodine: 1000 mg; Se: 200 mg; Co: 500 mg and Antioxidant: 125000 mg.
CFM: cassava fiber meal; SBM: soybean meal and GNC: groundnut cake.
* Each level of cassava fiber meal was substituted with 0, 100 and 200 mg kg^{-1} Roxazyme® G2.

Table 3 Phytochemical properties of experimental diets for broiler chickens

Diets	CFM (%)	Tannin (g 100g^{-1})	Oxalate (mg g^{-1})	Phytate-P (mg g^{-1})	Phytate (mg g^{-1})	Cyanide CN^{-1} (mg kg^{-1})
				Broiler starters		
1	0	0.05	0.17^b	2.99^b	10.61^b	2.47^b
2	20	0.06	0.28^a	3.27^a	11.61^a	9.03^a
3	40	0.06	0.30^a	3.35^a	11.89^a	9.24^a
4	60	0.06	0.30^a	3.33^a	11.82^a	9.37^a
SEM		0.10	0.07	0.11	0.68	0.32
P-value		0.09	0.002	0.02	0.03	0.002
				Broiler finishers		
1	0	0.05^b	0.21^b	3.09^c	10.97^b	2.10^c
2	20	0.08^a	0.33^a	3.45^b	12.25^a	8.71^b
3	40	0.08^a	0.34^a	3.56^a	12.64^a	9.18^a
4	60	0.09^a	0.34^a	3.59^a	12.74^a	9.46^a
SEM		0.19	0.12	0.07	0.31	0.11
P-value		0.02	0.002	0.002	0.001	0.002

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

RESULTS AND DISCUSSION

Table 1 shows the proximate composition, mineral content and phytochemical components of cassava fiber meal. The crude protein, crude fiber, metabolizable energy, calcium and phosphorus contents of the experimental diets were not influenced by the levels of cassava fiber meal substitution for maize in the diets (Table 2).

Result on the phytochemical components of the experimental diets (Table 3) shows that oxalate, phytate-P, phytate and cyanide concentrations were significantly (P<0.05) higher in broiler starter diets containing 20, 40 and 60% CFM compared to maize-based diet while in broiler finisher diets all the phytochemical components determined were significant (P<0.05) in CFM-based diets compared to maize-based diet.

The results on broiler chickens (starter and finisher phases) show a significant (P<0.05) decrease in weight gain and feed conversion ratio of birds on diets with 60% CFM-based diets with or without enzyme supplementation when compared with the improved performance of those on the control and up to 40% with or without enzyme supplemented CFM-based diets. On CFM substitution for maize, broiler starter and broiler finisher performances (Tables 4 and 5) show that 40 and 60% replacement of maize with CFM led to significant (P<0.05) decrease in weight gain of between 1.75 and 5.51% when compared to birds on the control diet. At starter phase, enzyme supplementation at 100 mg kg^{-1} led to an improvement in weight gain by 0.42% compared to those on non-enzyme supplemented diet while at the finisher phase, enzyme supplementation resulted in an improvement in weight gain by 0.32-0.37% compared to broiler chickens on the non-enzyme supplemented diet. Average feed consumption and interaction effect were not significantly (P>0.05) influenced by enzyme or non-enzyme supplemented diets at the two physiological growth phases.

Table 6 shows no definite pattern of significant difference in the carcass evaluation of broiler chickens fed enzyme and non-enzyme supplemented diets. Among the organs determined, only the heart weight of broiler chickens on CFM substitution with or without enzyme supplementation was significantly (P<0.05) higher compared to those on 0% CFM with or without enzyme supplementation. Broiler chickens fed diets containing 60% CFM had lower eviscerated weight but higher heart, liver, kidney and gizzard weights as against those fed the control. The effect of enzyme supplementation did not influence the carcass and organ weights of the birds. Also, the dependency of one factor over the other was not significant.

Table 7 shows that the blood composition of broiler chickens were not significantly (P>0.05) influenced with or without enzyme supplementation. Although, numerical improvement was observed in the packed cell volume (PCV), red blood counts (RBC) and haemoglobin concentration (HbC) of broiler chickens on enzyme supplemented diets particularly at 100 mg kg^{-1} dose. The eosinophil decreased (P<0.05) significantly at 40 and 60% with or without enzyme supplemented CFM-based diets. However, CFM substitution for maize led to higher production of eosinophil, but enzyme addition did not significantly influence eosinophil synthesis. The effect of interaction was not significant, an indication that blood synthesis is independent of the factors under consideration.

It is a known fact that an increase in enzyme activity in serum is an indication of problem in the cell population from which the enzyme is derived. The stability of the serum metabolites and serum enzymes, except the aspartate aminotransferase in this study shows the adequacy of the text diets to support the growth of broiler chickens thus, suggesting that enzyme supplemented and non-enzyme supplemented diets promoted the syntheses of serum lipids (Table 8). Although, aspartate aminotransferase (AST) was significant (P<0.05) with or without enzyme supplemented CFM-based diets, but the values were within what cannot cause liver or muscle degeneration. However enzyme supplementation was able to attenuate AST secretion as revealed in the similar values recorded. The dependency of one factor over the other was not noticed as there was no effect of interaction on all the serological variables.

The question whether a high fibrous CFM with or without enzyme supplementation could replace maize as energy source in broiler chicken diets is the central focus of this study. This is because maize as sole energy source in broiler chicken diet is plagued with stiff competition among the multifarious utilizers. Proximate analysis showed that CFM contained appreciable levels of nutrients that could support the healthy growth of broiler chickens. The chemical composition of cassava fiber used in this study showed slight variation from the values reported for cassava wastes by Aro and Aletor (2012) and Fernandes *et al.* (2015). The variations could be due to the time of harvest, stage at harvest, climatic conditions, soil types and other inherent and extraneous factors.

The lower weight gain and feed conversion ratio recorded for birds on higher substitution of CFM with or without enzyme supplementation could be attributed to the effect of high fiber. Dietary fiber's ability to attenuate weight gain could be that soluble fiber when fermented in the large intestine produces two gut hormones; glucagon-like peptide (GLP-1) and peptide YY (PYY) which play significant role in inducing satiety (Keenan *et al.* 2006) and thus increase digesta viscosity thereby limiting nutrients absorption and utilization. The role of dietary fiber to significantly decrease energy intake (Tucker and Thomas, 2009) is a pointer to the decreased weight gain of broiler chickens on high CFM-based diets irrespective of enzyme supplementation. Baer *et al.* (1997) observed that an increased consumption of dietary fiber resulted in a decrease in the ME of the diet with consequent decrease in broiler growth. This may be attributed to the fact that fat digestibility and absorbability decreased as dietary fiber increased. Also, as dietary fiber intake increases, the intake of simple carbohydrates tends to decrease resulting in low glucose availability.

All these could ultimately affect nutrient utilization and decrease weight gain. The similar feed intake of broiler chickens fed enzyme and non-enzyme supplemented CFM could be that birds were not averse to the fibrous CFM-based diets.

Table 4 Performance of broiler-starters fed CFM supplemented with Roxazyme® G2

Diets	CFM (%)	Enzyme (mg kg^{-1})	Initial wt g/b	FLW g/b	AWG g/b/d	AFC g/b/d	FCR
1	0	0	50.29	553.16^{a}	23.94^{a}	42.76	1.79^{b}
2	0	100	50.27	548.69^{ab}	23.73^{a}	40.10	1.69^{a}
3	0	200	50.32	559.20^{a}	24.23^{a}	42.32	1.75^{b}
4	20	0	50.54	561.87^{a}	24.35^{a}	40.81	1.67^{a}
5	20	100	50.58	566.62^{a}	24.57^{a}	43.38	1.76^{b}
6	20	200	50.50	544.53^{ab}	23.52^{ab}	41.37	1.75^{b}
7	40	0	50.68	547.07^{ab}	23.64^{ab}	43.33	1.83^{c}
8	40	100	50.29	552.82^{a}	23.93^{a}	40.19	1.68^{a}
9	40	200	50.53	535.26^{b}	23.08^{b}	40.91	1.77^{b}
10	60	0	50.32	527.23^{c}	22.71^{c}	43.38	1.90^{c}
11	60	100	50.41	529.94^{c}	22.83^{c}	41.61	1.82^{c}
12	60	200	50.48	521.04^{c}	22.41^{c}	41.96	1.87^{c}
SEM			1.12	15.74	0.50	0.37	0.08
P-value			0.85	0.02	0.02	0.25	0.03
Diets	CFM (%)						
1	0		50.29	553.68^{a}	23.97^{a}	41.73	1.74^{a}
2	20		50.54	557.67^{a}	24.15^{a}	41.85	1.73^{a}
3	40		50.50	545.05^{b}	23.55^{b}	41.48	1.76^{a}
4	60		50.40	526.07^{c}	22.65^{c}	42.32	1.86^{b}
SEM			0.52	10.31	0.43	0.54	0.21
P-value			0.91	0.03	0.03	0.37	0.03
Diets	Enzyme (mg kg^{-1})						
1	0		50.46	547.33	23.66	42.57	1.80
2	100		50.39	549.52	23.76	41.32	1.73
3	200		50.46	540.01	23.31	41.64	1.79
SEM			0.52	10.31	0.43	0.54	0.21
P-value			0.77	0.42	0.68	0.41	0.49
CFM (%) × Enzyme (mg kg^{-1})							
SEM			1.12	17.03	0.85	0.78	0.83
P-value			0.85	0.87	0.82	0.49	0.55

CFM: cassava fiber meal; FLW: final live weight; AWG: average weight gain; AFC: average feed consumption and FCR: Feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The role of exo-enzyme (Roxazyme® G2) supplementation in improving weight gain and nutrient utilization in this study could not be ascertained. The plausible reason for this could be due to the efficacy of the enzyme to break down non-starch polysaccharides and release nutrients entrapped in the cell matrix for broiler chicken utilization and growth.

The non-significant trend in the dressed and eviscerated weights of broilers on enzyme and non-enzyme supplemented CFM could not be attributed to dietary effect, as the changes did not follow a particular trend. Although, Ravindran (1995) and Agwunobi (1999) reported that changes in gut transit time of diets and feed efficiency may have effect on dressing weight and weight of the gastrointestinal tract. Although heart weight was significant in broiler chickens fed high CFM irrespective of enzyme supplementation but the increase might not be a cause of worry or indication of hypertrophy, since the development of other organs were not influenced in broiler chickens fed the various dietary treatments. Increased liver weight in this study could also not be attributed to an insufficient oxygen supply in metabolism as the weight might not be an indication of the increased activity of the liver to pump blood. The results on the organs of the birds suggest that CFM with or without enzyme supplementation did not adversely affect organ development of broiler chickens, and as such the health of the birds were not compromised when fed CFM for maize as energy source in broiler chicken diet. Thus, the levels of hydrogen cyanide (HCN) and other phytochemical components in the diets did not trigger the increased activity of these organs.

Haematological indices are useful markers to ascertain the health status of animals.

Table 5 Performance of broiler-chickens fed CFM supplemented with Roxazyme® G2

Diets	CFM (%)	Enzyme (mg kg^{-1})	Initial wt g/b	FLW g/b	AWG g/b/d	AFC g/b/d	FCR
1	0	0	50.29	2339.87^{ab}	40.88^{ab}	101.74	2.49^{a}
2	0	100	50.27	2369.11^{a}	41.41^{a}	102.28	2.47^{a}
3	0	200	50.32	2389.46^{a}	41.77^{a}	100.74	2.41^{a}
4	20	0	50.54	2379.28^{a}	41.58^{a}	101.57	2.44^{a}
5	20	100	50.58	2377.92^{a}	41.56^{a}	101.64	2.45^{a}
6	20	200	50.50	2352.85^{ab}	41.11^{ab}	103.16	2.57^{b}
7	40	0	50.68	2301.48^{b}	40.19^{b}	101.35	2.52^{b}
8	40	100	50.29	2297.99^{b}	40.14^{b}	103.24	2.57^{b}
9	40	200	50.53	2317.82^{ab}	40.49^{b}	101.79	2.51^{b}
10	60	0	50.32	2267.49^{c}	39.59^{c}	103.36	2.61^{c}
11	60	100	50.41	2271.01^{c}	39.66^{c}	102.92	2.60^{c}
12	60	200	50.48	2269.36^{c}	39.62^{c}	103.24	2.61^{c}
SEM			1.12	21.02	0.46	2.49	0.09
P-value			0.38	0.03	0.02	0.52	0.02
Diets	CFM						
1	0		50.29	2366.15^{a}	41.35^{a}	100.23	2.42
2	20		50.54	2370.01^{a}	41.42^{a}	101.53	2.45
3	40		50.50	2305.76^{a}	40.27^{a}	102.13	2.54
4	60		50.40	2269.29^{b}	39.62^{b}	103.17	2.60
SEM			0.52	61.36	2.46	1.99	0.21
P-value			0.91	0.03	0.03	0.69	0.35
Diets	Enzyme (mg kg^{-1})						
1	0		50.46	2322.03	40.56	103.17	2.51
2	100		50.39	2329.01	40.69	102.52	2.52
3	200		50.46	2332.37	40.71	102.23	2.52
SEM			0.52	49.11	2.46	1.99	0.06
P-value			0.77	0.21	0.19	0.67	0.41
CFM (%) × Enzyme (mg kg^{-1})							
SEM			1.12	27.33	0.87	3.78	0.35
P-value			0.85	0.34	0.74	0.59	0.63

CFM: cassava fiber meal; FLW: final live weight; AWG: average weight gain; AFC: average feed consumption and FCR: Feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

They contribute immensely to detection of some changes in the health condition of birds which may not be obvious at the time of physical examination but undoubtedly affect the fitness of the birds (Lakurbe *et al*. 2018). The non-significant difference in the blood composition of birds fed up to 60% CFM with or without enzyme supplementation suggests that CFM as energy source supported similar haematological *milieu* and thus promoted blood building capacity in broiler chickens. Worthy of note was that Roxazyme® G2 supplementation particularly at 100 mg kg^{-1} dose numerically improved the PCV, RBC and HbC of broiler chickens. The improvement observed for these blood parameters might be due to possible increase in the absorption of nutrient within the cells or that cyanogenic glucosides were degraded by the enzyme Roxazyme® G2, thus promoting erythrogenesis and haemopoiesis of broiler chickens.

The erythrocyte sedimentation rate (ESR) of the broiler chickens in this experiment is within the range 2.37-2.69 mm/hr, and thus within the recommendations of 2-4 mm/hr for broiler chickens (Ukorebi *et al*. 2019; Arogbodo *et al*. 2020).

The Low ESR in this study suggests an increase in total plasma protein or glucose in the birds and also indicates good health of the birds.

The PCV range of 31-33% recorded for the birds in this study conformed to the values reported for broiler chickens at 8 weeks of age (Jain, 1993; Nanbol *et al*. 2016; Lakurbe *et al*. 2018; Ilo *et al*. 2019; Arogbodo *et al*. 2020). The haemoglobin range of 10.11-11.09 (g/dL) in the present study was slightly higher than the 9-10 (g/dL) reported by Lakurbe *et al*. (2018), Ilo *et al*. (2019) and Arogbodo *et al*. (2020) but falls within the recommendations of 7-13 (g/dL) for avian species (Sunmola *et al*. 2019).

Table 6 Carcass weight (% body weight) and organ description (g kg^{-1} body weight) of broiler chickens fed CFM with or without Roxazyme® G2 supplementation

Diets	CFM (%)	Enzyme (mg kg^{-1})	Dressed weight	Eviscerated weight	Heart	Lung	Liver	Spleen	Kidney	Gizzard	Intestinal length (mm)
1	0	0	86.35^{a}	76.22^{ab}	0.56^{c}	0.46	2.36	0.16	0.51	1.19	2079.43
2	0	100	85.74^{ab}	78.62^{a}	0.53^{c}	0.41	2.19	0.18	0.47	1.22	2064.17
3	0	200	86.87^{a}	78.85^{a}	0.54^{c}	0.43	2.93	0.14	0.44	1.21	2137.23
4	20	0	84.94^{ab}	78.04^{a}	0.73^{a}	0.47	2.99	0.21	0.57	2.05	2169.16
5	20	100	86.26^{a}	76.71^{ab}	0.60^{bc}	0.48	2.85	0.19	0.44	2.03	2231.05
6	20	200	84.53^{ab}	75.82^{b}	0.58^{c}	0.42	2.36	0.12	0.46	1.93	2211.21
7	40	0	86.03^{a}	75.80^{b}	0.72^{a}	0.45	2.63	0.18	0.52	2.01	2275.18
8	40	100	83.70^{c}	74.62^{b}	0.64^{b}	0.49	2.27	0.12	0.59	1.97	2166.46
9	40	200	84.18^{b}	75.14^{b}	0.63^{b}	0.41	2.99	0.20	0.56	1.96	2258.28
10	60	0	82.38^{c}	73.98^{c}	0.75^{a}	0.43	2.75	0.16	0.52	2.12	2276.08
11	60	100	85.06^{ab}	74.73^{b}	0.63^{b}	0.45	2.92	0.13	0.55	2.11	2197.42
12	60	200	86.41^{a}	72.97^{c}	0.64^{b}	0.42	2.62	0.18	0.57	2.10	2215.54
SEM			2.03	2.17	0.11	0.04	0.54	0.06	0.11	0.43	3.22
P-value			0.03	0.02	0.03	0.17	0.26	0.09	0.13	0.18	0.37
Diets	CFM										
1	0		86.32	76.23^{a}	0.54^{b}	0.43	2.49^{c}	0.16	0.47^{b}	1.21^{c}	2093.61
2	20		85.24	76.85^{a}	0.64^{a}	0.46	2.73^{a}	0.17	0.49^{b}	2.00^{b}	2203.81
3	40		84.64	75.19^{a}	0.66^{a}	0.45	2.63^{b}	0.17	0.56^{a}	1.98^{b}	2233.31
4	60		84.62	73.89^{b}	0.67^{a}	0.43	2.76^{a}	0.16	0.55^{a}	2.11^{a}	2229.68
SEM			0.81	0.72	0.04	0.03	0.19	0.01	0.05	0.62	6.03
P-value			0.29	0.02	0.03	0.14	0.04	0.11	0.02	0.02	0.31
Diets	Enzyme (mg kg^{-1})										
1		0	84.93	76.01	0.69	0.45	2.69	0.18	0.53	1.84	2199.63
2		100	85.19	76.17	0.60	0.46	2.56	0.15	0.51	1.83	2164.77
3		200	85.50	74.44	0.60	0.42	2.72	0.16	0.51	1.80	2205.56
SEM			0.81	0.72	0.04	0.03	0.19	0.01	0.05	0.62	6.03
P-value			0.26	0.14	0.59	0.58	0.21	0.25	0.13	0.27	0.24
CFM (%) × Enzyme (mg kg^{-1})											
SEM			2.61	1.04	0.81	0.42	2.03	0.17	0.26	2.84	3.09
P-value			0.73	0.38	0.63	0.09	0.21	0.13	0.29	0.52	0.65

CFM: cassava fiber meal.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

PCV and haemoglobin reflect increased oxygen transportation to the body tissue for the release of energy for different body function through oxidation of nutrients as well as transportation of carbon dioxide out of the body. The values for RBC of experimental birds ranged from 3.21-3.55 (10^9/mL) and falls within the range reported in literature (Banerjee, 2012; Lakurbe *et al.* 2018; Sunmola *et al.* 2019). MCH is an indicator of blood carrying ability of the RBC. Their values suggest the efficiency of the animal in respiratory function. Mean cell haemoglobin (MCH) values of range 29.09-32.05 pg in this study was lower than the range 38-47 pg reported by Arogbodo *et al.* (2020) but within the recommendations of 23-47 pg for avian species (Sunmola *et al.* 2019). Mean cell volume (MCV) is a haematological index to determine the type of anaemia in animal. When it is high, it may indicate anaemia due to nutritional deficiency, bone marrow abnormalities, liver disease, chronic lung disease and when it is low, it may indicate iron deficiency, chronic disease, pregnancy, haemoglobin disorder such as thalassaemia (Ameen *et al.* 2007).

The MCV range 90.82-99.37 fL in this study was within the range 90-140 fL reported by Mirtuka and Rawnsley (1997) and Arogbodo *et al.* (2020) but lower than the range 104-140 fL reported by Bounous and Stedman (2000). The mean cell haemoglobin concentration (MCHC) values of 30.55-33.28 g/dL in the present study was slightly lower than the range 33.30-33.37 reported by Arogbodo *et al.* (2020) but conformed to the range 30.20-36.20 g/dL reported by Gulland and Hawkey (1990), Laburke *et al.* (2018), Ilo *et al.* (2019) and Sunmola *et al.* (2019) for avian species.

Immune defence system is the role of white blood counts (WBC) and the differential counts. The lymphocytes range of 60.94-62.02% in this study was in agreement with the ranges 45-75%, 58.10-71.70% and 59.33-63.00% reported by Gylstorff (1983), Nemi (1993) and Arogbodo *et al.* (2020) respectively. Heterophil range 21.41-22.87% in this study was within the range 19.80-32.60% and 21-26% reported by Gylstorff (1983) and Arogbodo *et al.* (2020), respectively.

Table 7 Haematological profile of broiler-chickens fed CFM supplemented with Roxazyme® G2

Diets	CFM (%)	Enzyme ($mg\ kg^{-1}$)	ESR (mm/hr)	PCV (%)	RBC (10^9/mL)	HbC (g/dL)	MCH (pg)	MCV (fL)	MCHC (%)	Het (%)	Lym (%)	Mon (%)	Eos (%)	Bas (%)
1	0	0	2.37	32.57	3.48	10.59	30.43	93.59	32.51	21.41	62.02	11.93	1.55^{c}	2.99
2	0	100	2.41	33.32	3.53	11.09	31.41	94.39	33.28	22.72	61.25	12.26	1.41^{c}	3.03
3	0	200	2.32	33.41	3.50	10.92	31.20	95.46	32.68	22.87	61.36	12.03	1.62^{c}	2.87
4	20	0	2.36	31.23	3.25	10.22	31.44	96.09	32.72	22.22	60.98	12.38	1.69^{bc}	3.00
5	20	100	2.45	32.48	3.55	10.48	29.52	91.49	32.26	22.19	61.55	12.11	1.84^{b}	3.11
6	20	200	2.48	32.55	3.53	10.42	29.52	92.21	32.01	22.05	60.72	11.82	1.77^{bc}	3.06
7	40	0	2.51	31.15	3.21	10.16	31.65	97.04	32.62	21.98	61.61	12.17	1.98^{a}	3.01
8	40	100	2.49	31.97	3.52	10.32	29.32	90.82	32.28	22.12	60.94	11.82	2.02^{a}	3.08
9	40	200	2.63	33.09	3.33	10.11	30.36	99.37	30.55	22.08	61.56	12.64	1.95^{a}	3.12
10	60	0	2.54	32.58	3.51	10.62	32.05	92.82	31.67	22.22	61.48	11.83	1.83^{b}	3.02
11	60	100	2.48	33.15	3.53	10.77	29.09	93.91	30.98	22.59	61.27	12.04	1.94^{a}	3.11
12	60	200	2.69	32.84	3.42	10.51	29.85	96.02	31.09	22.36	61.51	12.16	2.01^{a}	3.05
SEM			0.29	0.99	0.21	0.29	1.36	4.01	0.25	0.52	0.49	0.08	0.23	0.21
P-value			0.13	0.11	0.17	0.24	0.24	0.19	0.13	0.37	0.26	0.19	0.03	0.18
Diets	CFM													
1	0		2.37	33.10	3.50	10.86	31.01	94.57	32.82	22.33	61.54	12.07	1.53^{c}	2.96
2	20		2.43	32.09	3.44	10.37	30.16	93.28	32.33	22.15	61.08	12.10	1.77^{b}	3.05
3	40		2.54	32.07	3.35	10.20	30.44	95.73	31.82	22.06	61.37	12.21	1.98^{a}	3.07
4	60		2.57	32.85	3.49	10.63	30.33	94.25	31.25	22.39	61.42	12.01	1.93^{a}	3.06
SEM			0.06	0.36	0.21	0.26	0.53	1.99	0.18	0.45	0.33	0.41	0.07	0.25
P-value			0.27	0.54	0.15	0.31	0.36	0.19	0.24	0.47	0.41	0.75	0.02	0.06
Diets	Enzyme ($mg\ kg^{-1}$)													
1	0		2.44	31.88	3.36	10.40	31.39	94.88	32.38	21.96	61.52	12.08	1.76	3.01
2	100		2.46	32.73	3.53	10.66	29.84	92.65	32.20	22.41	61.25	12.06	1.80	3.07
3	200		2.53	32.97	3.44	10.49	30.23	95.76	31.58	22.33	61.29	12.16	1.84	3.02
SEM			0.06	0.36	0.21	0.26	0.53	1.99	0.18	0.45	0.33	0.41	0.07	0.25
P-value			0.19	0.41	0.22	0.27	0.30	0.21	0.32	0.39	0.52	0.61	0.31	0.11
CFM × Enzyme ($mg\ kg^{-1}$)														
SEM			0.31	0.99	0.27	0.38	2.06	3.31	0.21	0.77	0.50	0.13	0.33	0.28
P-value			0.23	0.01	0.11	0.37	0.24	0.26	0.29	0.52	0.31	0.66	0.38	0.31

CFM: cassava fiber meal; ESR: erythrocyte sedimentation rate; PCV: packed cell volume; RBC: red blood counts; HbC: haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume and MCHC: mean cell haemoglobin concentration.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Although, significant differences in eosinophils were observed in broiler chickens at higher substitution of CFM, but the values obtained were within the recommendations for broiler chickens (Jain, 1986; Talebi *et al*. 2005; Etim *et al*. 2014). This implied that the increase might not be due to toxin or bacterial infection.

The adequacy of the diets is also shown by the stability of the serum enzyme values of the birds on the different diets regardless of enzyme supplementation and cassava fiber levels. The position by some authors (Addass *et al*. 2012; Aro *et al*. 2013; Aya *et al*. 2013; Etim *et al*. 2014) that nutrition, particularly agro-based wastes affect blood composition and blood viscosities of broiler chickens did not hold in this study. The normal range values of the haematological indices obtained from this study suggest no evidence of erythrocytopenia or erythrocytosis.

Among the biomarkers to measure liver status, alanine transaminase (ALT) and AST play significant role.

ALT is an enzyme primarily found in the liver, that helps in chemical reactions. It plays a role in changing stored glucose into usable energy. When there is liver damage or disease, then ALT enters the blood stream. Higher amounts of ALT in the blood typically indicate liver or muscle damage.

AST is an enzyme primarily found in the liver, and also in the heart, muscle tissue, kidneys, brain, and red blood cells. AST helps to metabolize amino acids. With optimal AST levels, there is more energy and food is metabolized more effectively.

Liver damage will cause the liver to spill AST into the blood causing AST to rise. The similar serum biochemical and liver enzymes of broiler chickens fed dietary treatments could be an indication of the adequacy of the nutritive value of the diets to support the normal syntheses of these enzymes or possible indication of no liver or muscle dysfunction (Valchev *et al*. 2014).

Table 8 Serum chemistry of broiler-chickens fed CWM with Roxazyme® G2 supplementation

Diets	CFM (%)	Enzyme (mg kg^{-1})	TSP (g/dL)	Alb (g/dL)	Glo (g/dL)	Chol (mg/dL)	HDL (mmol/L)	LDL (mmol/L)	LDH (U/L)	ALT (U/L	AST (U/L)	ALP (U/L)
1	0	0	3.90	2.02	1.88	131.28	54.02	34.86	3.88	18.72	237.34^{b}	162.32
2	0	100	3.88	1.99	1.89	131.76	54.85	33.71	4.03	18.77	234.89^{b}	157.62
3	0	200	3.84	2.01	1.83	132.11	55.92	34.14	4.11	18.73	240.52^{b}	159.84
4	20	0	3.77	1.92	1.85	131.60	54.77	34.67	4.00	18.71	243.39^{b}	158.75
5	20	100	3.69	1.91	1.78	131.42	54.04	33.33	4.08	18.74	248.03^{ab}	157.91
6	20	200	3.73	1.94	1.79	132.04	55.39	34.49	3.78	18.79	249.93^{ab}	159.03
7	40	0	3.62	1.83	1.79	132.21	55.05	34.35	3.97	18.80	257.03^{a}	156.87
8	40	100	3.83	1.89	1.74	130.94	54.52	35.38	4.10	18.75	251.81^{a}	163.35
9	40	200	3.74	1.86	1.76	131.14	54.65	34.51	3.99	18.73	255.66^{a}	158.90
10	60	0	3.79	1.83	1.78	131.46	54.68	33.98	3.99	18.75	254.87^{a}	160.27
11	60	100	3.82	1.91	1.85	131.99	54.74	34.26	4.11	18.81	257.16^{a}	161.23
12	60	200	3.94	1.93	1.80	131.71	55.02	34.43	4.02	18.72	255.09^{a}	158.94
SEM			0.11	0.31	0.28	2.61	1.72	0.29	0.17	0.17	5.13	3.83
P-value			0.35	0.21	0.18	0.36	0.22	0.16	0.11	0.09	0.03	0.28
Diets	CFM (%)											
1	0		3.87	1.96	1.89	131.72	54.93	34.24	4.01	18.74	237.58^{b}	159.93
2	20		3.73	1.91	1.79	131.69	54.73	34.16	3.95	18.75	247.12^{ab}	158.56
3	40		3.73	1.86	1.76	131.43	54.74	34.75	4.02	18.76	254.83^{a}	159.71
4	60		3.85	1.89	1.81	131.72	54.81	34.22	4.04	18.76	255.71^{a}	160.15
SEM			0.11	0.18	0.12	1.21	2.07	0.54	0.14	0.12	4.06	3.02
P-value			0.42	0.10	0.09	0.63	0.33	0.48	0.36	0.87	0.03	0.72
Diets	Enzyme (mg kg^{-1})											
1	0		3.77	1.88	1.83	131.64	54.63	34.47	3.96	18.74	248.16	159.55
2	100		3.81	1.92	1.81	131.53	54.47	34.17	4.08	18.77	247.97	160.03
3	200		3.81	1.92	1.80	131.75	55.24	34.39	3.98	18.74	250.30	159.18
SEM			0.11	0.18	0.12	1.21	2.07	0.54	0.14	0.12	4.06	3.02
P-value			0.28	0.64	0.41	0.59	0.42	0.48	0.20	0.15	0.52	0.66
CFM × Enzyme (mg kg^{-1})												
SEM			0.17	0.12	0.14	1.09	0.22	0.09	0.43	0.36	1.13	0.47
P-value			0.31	0.26	0.31	0.41	0.28	0.23	0.15	0.19	0.30	0.24

CFM: cassava fiber meal; HDL: high density lipoprotein; LDL: low density lipoprotein; LDH: lactate dehydrogenase; ALT: alanine aminotransferase, AST: aspartate aminotransferase and ALP: alkaline phosphatase.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Significant increase in the AST concentrations of birds fed on CFM-based diets could be due to the residual cyanide in the blood serum. It has been reported that increased activity of AST in the liver and other organs and corresponding increase in the serum of cyanide exposed chicks may be due to *de novo* synthesis and is a likely indication of increased amino acid metabolism, occasioned by increased energy demand (Kadiri and Asagba, 2015). However, increase in the AST of birds on CFM-based diets in this study might not be a cause of worrisome as the levels were within the range recommended for healthy chickens (Meluzzi *et al*. 1992; Kececi and Col, 2011). Also, increase in the AST of the birds could not be due to liver damage because albumin; the main protein produced by the liver with a variety of functions and ALT; a dependable biomarker to determine liver health are not compromised by the dietary treatments.

The observed increase in AST could be attributed to the effect of diet composition to affect metabolism resulting in changes of plasma metabolite levels in poultry or possible excitement by birds before slaughtering (Buyse *et al*. 2002; Swennen *et al*. 2005). AST is associated with the mitochondria and cytoplasm hence alteration in its activity could imply alteration in the cytosolic content (Owen and Amakiri, 2012). Since, there was no detrimental change in the activity of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), it could be inferred that there would be no impairment of the hepatic function of the birds.

CONCLUSION

On the basis of the performance and haemato-biochemical properties of broiler chickens, it could be submitted that

CFM up to 40% substitution for maize with or without Roxazyme® G2 is safe as energy in broiler chicken diet.

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