Biotechnological Application of Cassava-Degrading Fungal (CDF) Amylase in Broiler Feed Formulation

Article · January 2016

DOI: 10.9734/BBJ/2016/20614

3 authors, including:

Oghenetega Jonathan Avwioroko
Redeemer's University

Nyerhovwo Tonukari
Delta State University, Abraka

British Biotechnology Journal
10(1): 1-12, 2016, Article no.BBJ.20614
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Biotechnological Application of Cassava-Degrading Fungal (CDF) Amylase in Broiler Feed Formulation

Oghenetega J. Avwioroko[1*], Akpovwehwee A. Anigboro[1] and Nyerhovwo J. Tonukari[1]

[1]*Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author NJT designed the study and wrote the protocols. Authors OJA and AAA managed the analyses of the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author NJT supervised the study to ensure accuracy at all steps. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/20614
Editor(s):
(1) Christopher Cullis, Francis Hobart Herrick Professor and Chair of Biology, Case Western Reserve University, USA.
(2) Csilla Tothova, University of Veterinary Medicine and Pharmacy in Kosice, Slovakia.
Reviewers:
(1) Mohamed Hafez Aboubakr, Benha University, Egypt.
(2) Ionica Mihaela Iancu, Banat University of Agronomical Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://sciencedomain.org/review-history/11551

Original Research Article

Received 31st July 2015
Accepted 8th September 2015
Published 27th September 2015

ABSTRACT

In this study, an attempt was made to substitute maize (*Zea mays* L.) content of broiler starter feed with ground cassava (*Manihot esculenta* Crantz) peels enzymatically improved with amylase-producing fungi with a view to having a cost-effective yet nutritious and health-friendly feed. The biochemical components of the formulated feeds were determined as well as the effect of the feeds on some biochemical parameters in the broiler chicks. Six starter feeds tagged Control Feed, 20%CPFG, 40%CPFG, 60%CPFG, 80%CPFG and 100%CPFG were formulated with respect to variations in maize and cassava peel contents. The results showed a significant ($p<0.05$) increase in total polysaccharide contents of Feeds 20%CPFG, 60%CPFG, 80%CPFG and 100%CPFG compared to the Control Feed (191.4±14.5 mg/g of feed). Total soluble protein and reducing sugar contents were statistically different in most of the feeds ($p<0.05$). All the feeds contain relatively high amounts of total phenol (>70 mgCE/g of feed) and most of them comparatively high in anthocyanin relative to the control feed anthocyanin content (225.4±12.2 mg/g of feed). The highest weight gain

**Corresponding author: Email: joavwioroko@gmail.com;*

(108.0±3.0 g) was observed in broiler chicks fed the feed ration containing 60% cassava peels improved with amylase-producing fungi and 40% maize (60%CPFG). Broiler chicks fed the formulated feeds, including the control, exhibited over 40% inhibition against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in birds fed the compounded feeds and control slightly differed except in group 60%CPFG. It was concluded that the maize content in broiler feed can be replaced with cassava peels improved with fungal amylase upto a maximum of 60%. This would significantly decrease the overall cost of broiler feed production without compromising the nutritional, antioxidant and health-friendly potentials of the feed.

Keywords: Broiler starter; maize; cassava peels; fungal amylase; feed formulation.

1. INTRODUCTION

Maize (*Zea mays* L.) plays a very important role in human and animal nutrition in a number of developed and developing countries, worldwide [1]. Plenitudes of feeds used in feeding poultry birds are usually composed of maize as a major constituent amidst many others [2]. The use of maize in feed formulation is believed to have stemmed from a simple and common observation among native birds (fowls) which naturally display a great interest or affinity for maize (corn) while in search for food. Researchers have long confirmed that maize has a rich content of carbohydrates, fats, proteins, important vitamins and minerals [1]. The energy-rich nature of maize makes it a choice for poultry feeds. Aside its use in the poultry, maize and its starch have also gained application over the years in other chemical industries where maize starch is used in production of synthetic polymers and also in some hotels where the cooked, relatively non-jelly starch is consumed as palp, commonly referred to as ‘Akamu’ in the Niger-Delta region of Nigeria. Presently, the cost of maize in Nigeria has vehemently increased due to its high demand both in homes, hotels, poultries, feed producing industries and other chemical industries. This, in turn, has led to the high cost of rearing poultry birds either for subsistence or commercial purposes.

The search for cost-efficient, nutritious and health friendly substitutes for maize in poultry feed production has, for the past two decades, been on-going since the cost of maize constitutes about 70% of the over-all cost of feed production. A cursory consideration of the nutrient composition of cassava (*Manihot esculenta*) peels, epicarps of cassava tubers removed by grating or manual peeling, points to the fact that cassava peels may be good substitutes for maize in poultry feed formulation due to the rich content of starch, protein and some minerals in cassava peels [3]. Also, due to the ready availability of cassava peels in rural communities in which the peels are usually considered as waste from cassava, with no industrial uses, the cost of obtaining cassava peels would be significantly lower than that of equal quantity of maize. However, precautionary steps would need to be taken to enable poultry birds fed with a feed containing cassava peels to efficiently digest or metabolize the starch and other glycosides present in the peels so as to prevent malnutrition resulting from feed indigestion [4,5].

Most saprophytic fungi growing on organic food substances derive nutrient materials from such complex foods by secreting digestive enzymes such as alpha amylases, cellulases, pectinases etc, which aid the fungi in breaking down the food to simpler biomolecules. According to Nouadri et al. [6] and Metin et al. [7], amylases generally cleave glycosidic linkages in starch or cellulose molecules thereby breaking these complex carbohydrates to disaccharides like maltose and or glucose.

In this experiment, an attempt was made to wholly or partly replace maize (*Zea mays*) flour in broiler starter feed with ground cassava peels enzymatically improved with amylase-producing fungi (*Aspergillus* sp.CSA35) with a view to having a cost-effective yet nutritious and health-friendly broiler starter feed. The biochemical components of the formulated feeds were determined as well as the effect of the feeds on some biochemical parameters in the broiler chicks.

2. MATERIALS AND METHODS

2.1 Preparation of YPD Agar

YPD (yeast extract-peptone dextrose) agar was prepared by measuring 2.0 g of glucose monohydrate, 1.0 g yeast extract, 2.0 g peptone and 1.5 g agar-agar powder into a 250 ml conical flask. Little volume of distilled water was added to dissolve the flask contents and thereafter, the solution was made up to 100 ml with distilled water. The solution was sterilized by autoclaving for 15 min at 121℃.

2.2 Growth of *Aspergillus* sp.CSA35

Aspergillus sp.CSA35, a fungus associated with cassava (*Manihot esculenta* Crantz) spoilage in Nigeria, previously identified by [8] and reported to secrete amylase [9], was cultured using YPD agar for 5 days and thereafter sub-cultured for another 7 days in sterilized Cassava Starch Agar (CSA) containing 2% cassava flour as the sole source of carbon, 1% $NaNO_2$ and 1.5% agar powder to ensure that only cassava starch-degrading fungi grew. 5 µl of a 20% antibiotic (ampicillin) was added to each subculture to prevent growth of bacteria.

2.3 Digestion of Cassava Peels with Fungal Amylase

Fresh cassava (*M. esculenta*) peels were collected from farmers within Otorho-Agbon community in Ethiope East Local Government of Delta State, Nigeria. The cassava peels were mixed with biomass of the amylase-secreting fungi (*Aspergillus* sp.CSA35) grown as described above, to orchestrate the process of enzymatic hydrolysis of the cassava peel starch by the fungal amylase. The cassava peel-fungi mixture was enriched with urea as nitrogen source and moistened with little water to facilitate the growth of the fungi. The amylase-secreting fungi were allowed to grow on the cassava peels at room temperature for two weeks and thereafter, the peels were grinded for broiler starter feed formulation. Other broiler starter feed ingredients used in the experiment (Table 1) were purchased from HarmonyPath Farm, Otorho-Agbon, Delta State.

2.4 Experimental Feed Formulation/ Experimental Design

Six broiler starter feed diets were formulated for broiler starters (0-4 weeks old chick) by varying the amount of maize in their feed via substitution of the maize (*Zea mays* L.) with cassava peels improved with amylase-producing fungi. All other feed ingredients were kept constant. The feeds were fed to a total of thirty-six, newly hatched (day-old) broiler chicks, divided in six groups of six birds each, for seven days as follow:

Control Feed: 100% Maize (control);
20%CPFG: 20% Cassava peels improved with fungal amylase and 80%maize;
40%CPFG: 40% Cassava peels improved with fungal amylase and 60%maize;
60%CPFG: 60% Cassava peels improved with fungal amylase and 40%maize;
80%CPFG: 80% Cassava peels improved with fungal amylase and 20%maize;
100%CPFG: 100% Cassava peels improved with fungal amylase and 0%maize.

The combinations above were used, alongside other poultry feed ingredients not varied, to form the gross six (6) different diets for the broiler chicks (Table 1). The rations were formulated according to Pfizer Nutrient Master Plan

Table 1. Gross composition of diets for the broiler chicks

Ingredients	**Formulated diets**					
	Control	**20% CPFG**	**40% CPFG**	**60% CPFG**	**80% CPFG**	**100% CPFG**
PKC (Kg)	1.302	1.302	1.302	1.302	1.302	1.302
BSG or wheat offal (Kg)	2.1	2.1	2.1	2.1	2.1	2.1
Soyabean meal (Kg)	2.52	2.52	2.52	2.52	2.52	2.52
Oil (Litre)	0.021	0.021	0.021	0.021	0.021	0.021
Bone meal (Kg)	0.168	0.168	0.168	0.168	0.168	0.168
Limestone (Kg)	0.126	0.126	0.126	0.126	0.126	0.126
Starter supermix (Kg)	0.063	0.063	0.063	0.063	0.063	0.063
Maize (Kg)	2.1	1.68	1.26	0.84	0.42	-
CPFG (Kg)	-	0.42	0.84	1.26	1.68	2.1

Where PKC = Palm kernel cake; BSG = Brewers spent grain; and, CPFG = Cassava peels improved with fungal amylase.

Specification [10,11]. The broiler chicks were of the traditional white Cornish breed (Cornish White x White Plymouth Rock) and were purchased from a local commercial hatchery (Aromatik Konsult Ent.) in Otorho-Agbon, Delta State.

2.5 Data Collection and Analyses

2.5.1 Weight gain measurement

The weights of the chicks were taken on day 1 of the experiment and also at the end of the seven-day experiment. The difference in weight (g) was recorded as weight gained since all chicks measured weighed higher than the first day.

2.5.2 Estimation of nutrients and phytochemicals in the formulated feeds

2.5.2.1 Total reducing sugar content

This was determined according to the method of [12]. 3 ml of DNS reagent was added to 3 ml of the ethanolic extract of each feed (5%w/v) and then, test tubes were tightly caped to avoid loss of liquid due to evaporation. Test tubes contents were heated at 90℃ for 5 -15 min to develop a red/brown colour and 1 ml of a 40% potassium sodium tartrate (Rochelle salt) was added to stabilize the colour. After cooling to room temperature in a cold water bath, absorbance was read in a spectrophotometer at a wavelength of 575 nm against reagent blank.

2.5.2.2 Total soluble protein content

The Biuret method described by [13] was followed. 1.5 ml of Biuret reagent was added to 0.5 ml of ethanolic extract of each feed (5%w/v) and thoroughly mixed. Thereafter, the test tubes were left to stand for 30 min at room temperature and then the absorbances of sample and serum bovine albumin (standard protein) were read in a spectrophotometer at a wavelength of 540 nm against reagent blank.

2.5.2.3 Total phenol content of feed

This was carried out according to the method described by [14]. 1 ml of Folin C reagent was added to 1 ml of the ethanolic extract of each feed (5%w/v). After 3 min, 1 ml of saturated Na_2CO_3 solution was added and the solution was made up to 10 ml with distilled water. The reaction mixture was kept in the dark for 90 min. The absorbance was read at 725 nm. Catechin was used as standard, following the assay conditions described.

2.5.2.4 Total soluble polysaccharide content

The amount of total soluble polysaccharides was estimated by phenol sulphuric acid reagent method [15]. 500 mg each of feed was homogenized with 10 ml of 80% ethanol. Then each homogenate was centrifuged at 2000 rpm for 15-20 min to obtain a supernatant. 1 ml of the supernatant (alcoholic extract) and 1 ml of 5% phenol solution were added together and mixed. Then 5 ml of 96% sulphuric acid was added. Each tube was gently agitated during the addition of the acid and then allowed to stand in a water bath at 25-30℃ for 20 min. The optical density of the characteristic yellow orange color thus developed was measured at 490 nm in a spectrophotometer. Simultaneously a standard curve was prepared by using known concentrations of glucose. The amount of sugar was expressed as mg/g feed sample.

2.5.2.5 Estimation of total anthocyanin content

The monomeric anthocyanin content of the feeds was measured using a modified pH differential method [16]. The methanol extract of feed (5%w/v) was mixed thoroughly with 0.025 M potassium chloride buffer (pH 1) in 1:2 ratio of extract to buffer. Likewise, the methanol extract of feed was also mixed with a 50 mM sodium acetate buffer pH 4.5 in a separate test tube. A spectrophotometer was used to measure absorbance at 510 and 700 nm against a buffer blank at pH 1.0 and 4.5, respectively. Absorbance readings were converted to total milligrams of cyanidin-3-glucoside (C3G). The anthocyanin content was calculated as follows:

$$\text{Total monomeric anthocyanins (mg/100 g)} = \Delta A \times MW \times 1000/(\varepsilon \times C)$$

$$\Delta A = (A510 - A700)\ pH\ 1.0 - (A510 - A700)\ pH\ 4.5.$$

Where A is absorbance, MW (449.2) is molecular weight for C3G, ε (26,900) is the molar absorptivity C3G and C is the concentration of the feed methanolic extract in mg/ml. The anthocyanin content was expressed as milligrams of C3G equivalents per 100 g of feed in triplicate.

2.5.3 Collection of blood sample

Venous blood was collected using sterile syringe and needle from pronounced veins in the wings and/ or legs of the chicks and transferred into a test tube. The blood was allowed to clot for some time and thereafter, dislodged and centrifuged at 2000 g for 10 min to obtain the serum as supernatant. The supernatants (sera) were safely stored at 4℃ while being used for some biochemical analyses.

2.5.4 Biochemical parameters analyzed in bird's serum

2.5.4.1 Serum ALT and AST activities

In order to assess the impact of the compounded feeds on the delicate organs such as the liver, two liver function tests - alanine aminotransferase activity (ALT) and aspartate aminotransferase activity (AST) - were carried on the sera of birds fed with the different feed rations including control. ALT and AST activities assay were carried out using Randox kits. The assay procedures were diligently followed as described in the manufacturer's manuals.

2.5.4.2 Inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical

This was estimated according to the method described by [17]. To 100 µl of bird serum, 2.7 ml methanolic solution of DPPH radical (6×10^{-5} mol/l) was added. The mixture was shaken vigorously and left to stand for 60 min in the dark until stable absorption values could be obtained. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The standard used was ascorbic acid. The radical scavenging activity was calculated by the formula:

$$\%RSA = [(A_{DPPH} - A_S)/ A_{DPPH}] \times 100.$$

Where %RSA = % DPPH discolouration; A_{DPPH} = absorbance of DPPH solution, and A_S = absorbance of the solution when the sample was added at a particular level.

2.5.5 Statistical analysis

The results were expressed as Means ± Standard Deviation. A one-way analysis of variance was used to analyze result data and a p-value of < 0.05 was considered as statistically significant. Where a significant difference was observed, data were further analysed using a Post-Hoc analysis, Tukey's significant difference (TSD) test, to know the groups that caused the significant differences. The analyses were computed using GraphPad Prism software (version 5.0.1).

3. RESULTS

3.1 Enzymatic Improvement of Cassava Peels with Amylase-producing Fungi

After mixing the cassava peels with amylase-producing fungi and allowing the fungi to grow for 2 weeks, the presence of many white and dark-brown patches of fungal hyphae/biomass was observed in the cassava peels as well as green and brown spores (Fig. 1), indicating that the amylase-producing fungi were able to hydrolyze the starch contents of the cassava peels for growth. The pre-digestion or enzymatic hydrolysis of the cassava peels by the fungi could increase the digestibility of the nutrients and phytochemical contents of the peels by poultry birds.

Fig. 1. Enzymatic improvement of cassava peels with amylase-producing fungi

Cassava peels were mixed with amylase-secreting fungi and left for 2 weeks. Fungal growth was enhanced with urea and little moisture

3.2 Biochemical Constituents of Feeds

3.2.1 Total polysaccharide, reducing sugar and soluble protein contents of the feeds

Some nutritional components of the experimental feeds including the control were determined and the results showed a non significant ($p>0.05$) increase in total soluble polysaccharide contents in Feeds 40%CPFG compared to the control commercial feed (191.4±14.5 mg/g of feed) (Fig. 2). However, a significant ($p<0.05$) increase in total polysaccharide contents was observed in Feeds 20%CPFG, 60%CPFG, 80%CPFG and 100%CPFG when compared to the control. The reducing sugar contents were statistically different ($p<0.05$) in most of the feeds. Total soluble protein contents of all the formulated feeds significantly ($p<0.05$) higher than that of the control feed, except in Feed 60%CPFG in which the protein content did not differ statistically from that of the control feed ($p>0.05$).

3.2.2 Phenolic contents of formulated feeds

Assessment of the compounded feeds for presence of a phytochemical that possesses antioxidant property showed that all the tested feeds including the control contain relatively high amounts of total phenol (>70 mgCE/g of feed) (Fig. 3). The phenol contents of all the formulated feeds appeared to be significantly ($p<0.05$) higher than that of the control feed (62.5±0.5 mgCE/g of feed). The highest content of phenol was observed in Feed 80%CPFG (140.0±2.4 mg CE/g of feed). The phenol contents of Feeds 40%CPFG and 60%CPFG are almost equivalent (92.5±2.2 and 92.5±1.9 mgCE/g of feed, respectively), not significantly different ($p>0.05$).

3.2.3 Anthocyanin content of formulated feeds

Apart from Feeds 40%CPFG and 60%CPFG which had significantly ($p<0.05$) low anthocyanin contents (198.7±7.6 and 71.8±5.4 mg/g of feed, respectively) compared to the control commercial feed (225.4±12.2 mg/g of feed) (Fig. 4), the other feed rations are comparatively high in anthocyanin relative to the control. The highest anthocyanin content was found in Feed 100%CPFG (577.8±10.4 mg/g of feed).

3.2.4 Weight gain (g) by broiler chicks

A corresponding increase in weight gain was observed in birds fed 20 - 60%CPFG compared to the control group; this was later followed by a corresponding decrease in weight gain as concentration of CPFG increased further from 80-100%. The highest weight gain (108.0±3.0 g) was, however, observed in broiler chicks fed with feed ration supplemented with 60% cassava peels degraded with amylase-producing fungi (60%CPFG) and was significantly higher ($p<0.05$) than weight gain by chicks fed the control feed (60.7±1.0 g) (Fig. 5). The weight gain by birds fed with 100%CPFG feed ration was not statistically different ($p>0.05$) from those of the control feed (60.7±1.0 g).

3.2.5 Inhibition of DPPH free radical-induced oxidative stress

Analysis of serum of birds fed with the different feed rations including the control feed indicate that the broiler chicks in each group exhibit

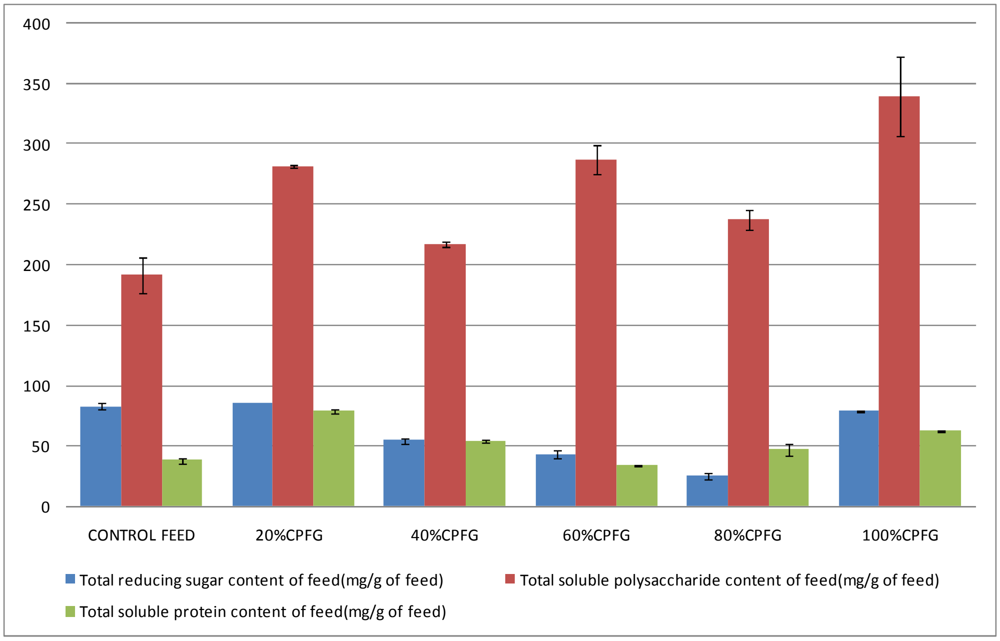

Fig. 2. Total soluble polysaccharide, total reducing sugar and total soluble protein contents of the diet rations (mg /g of feed)

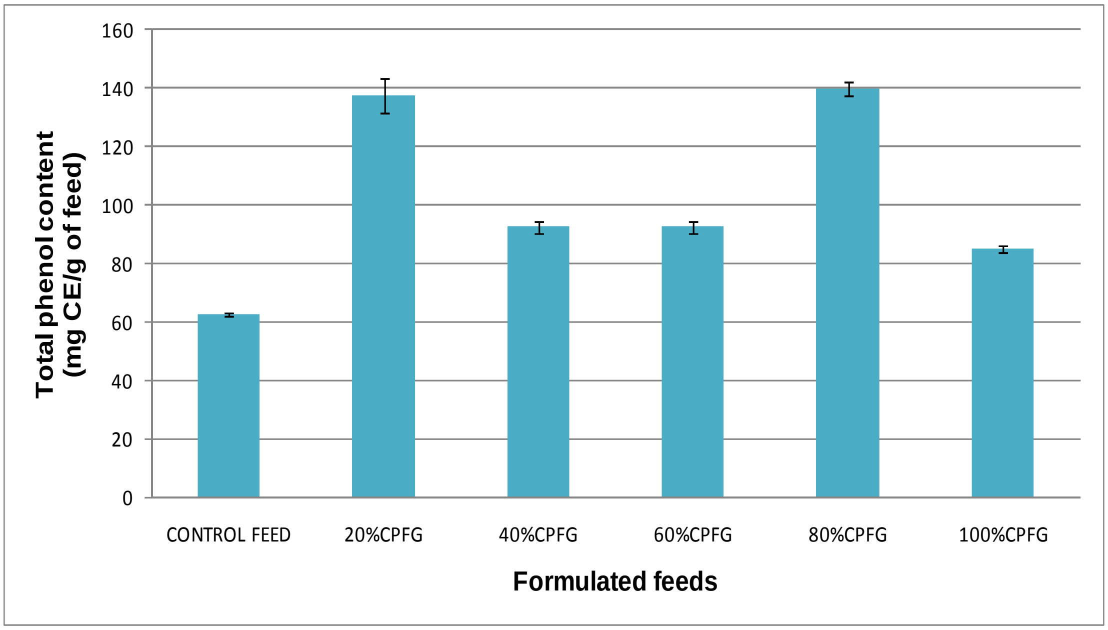

Fig. 3. Total phenol content of the diet rations (mg CE/g of feed)

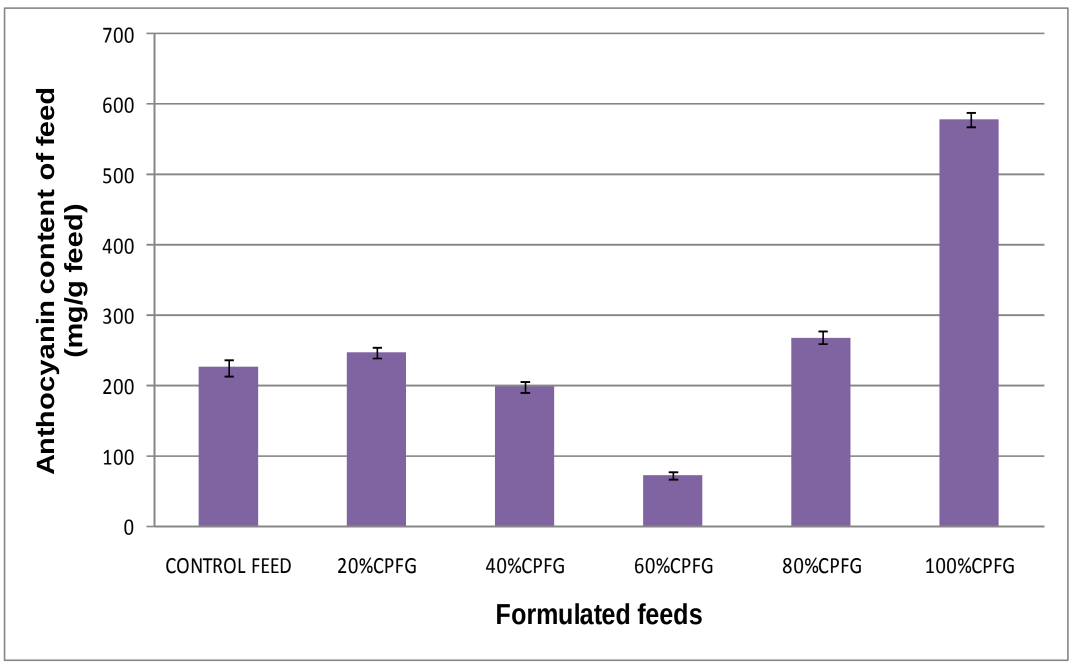

Fig. 4. Anthocyanin content of the diet rations (mg /g of feed)

inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical by over 40% (Fig. 6). Whereas the DPPH radical scavenging activities exhibited by birds from 20%CPFG and 40%CPFG experimental feed groups were not significantly different (p>0.05) from that of birds from the control feed group (62.00±1.97%), that of birds fed 60%CPFG and 80%CPFG was significantly decreased (p<0.05). However, birds fed with Feed 100%CPFG showed a DPPH radical scavenging activity of 74.00±6.33%, significantly higher (p<0.05) than that of the control group birds (62.00±1.97%).

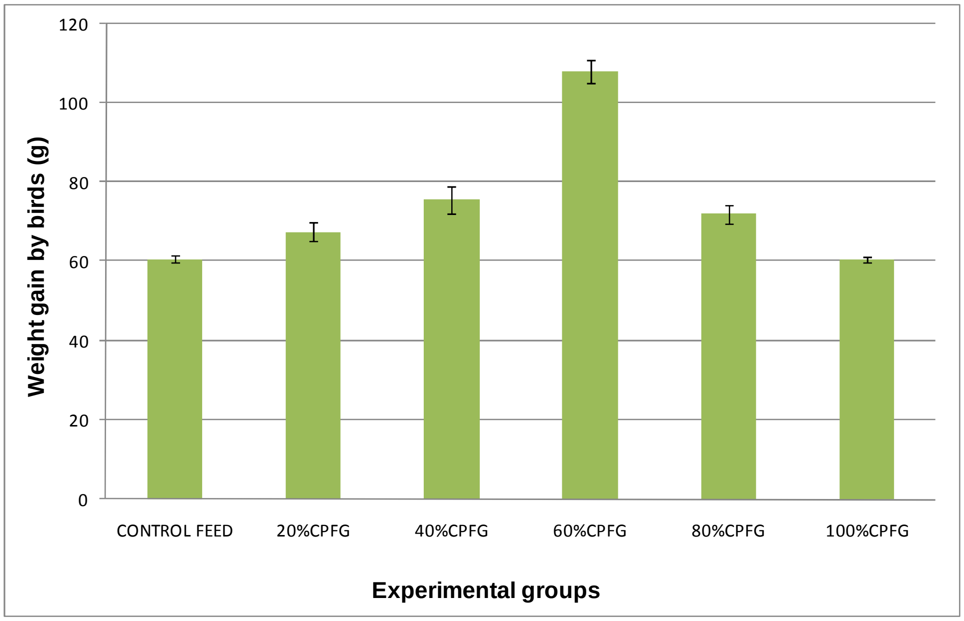

Fig. 5. Weight gains (g) by the chicks

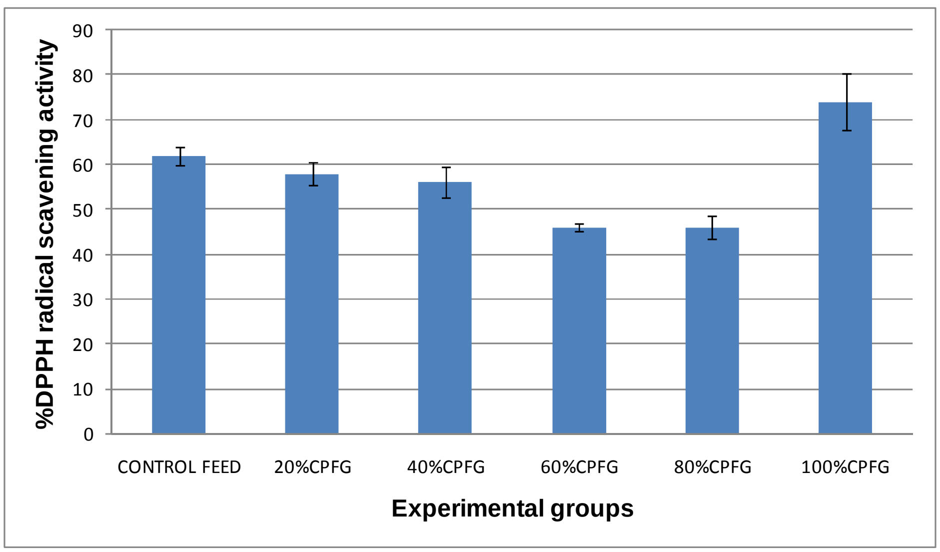

Fig. 6. %DPPH radical scavenging activity in serum of birds

3.2.6 Activities of serum ALT and AST in the broiler chicks

The results shown in Fig. 7 indicate slight differences in the respective serum activities of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in birds fed with the control feed and those fed the compounded experimental feeds in which maize was partly or wholly substituted with ground cassava peels enzymatically degraded with amylase-producing fungi. Apart from birds fed Feed 40%CPFG in which AST activity was not significantly different ($p>0.05$) from that of the control group, AST activities in other experimental groups were slightly increased. Whereas the activity of ALT was significantly decreased ($p<0.05$) in serum of broiler chicks fed Feed 20%CPFG, 40%CPFG and 100% CPFG compared to the control group, the difference in ALT levels between broiler chicks fed Feed 60%CPFG and the control was, however, not significant ($p > 0.05$).

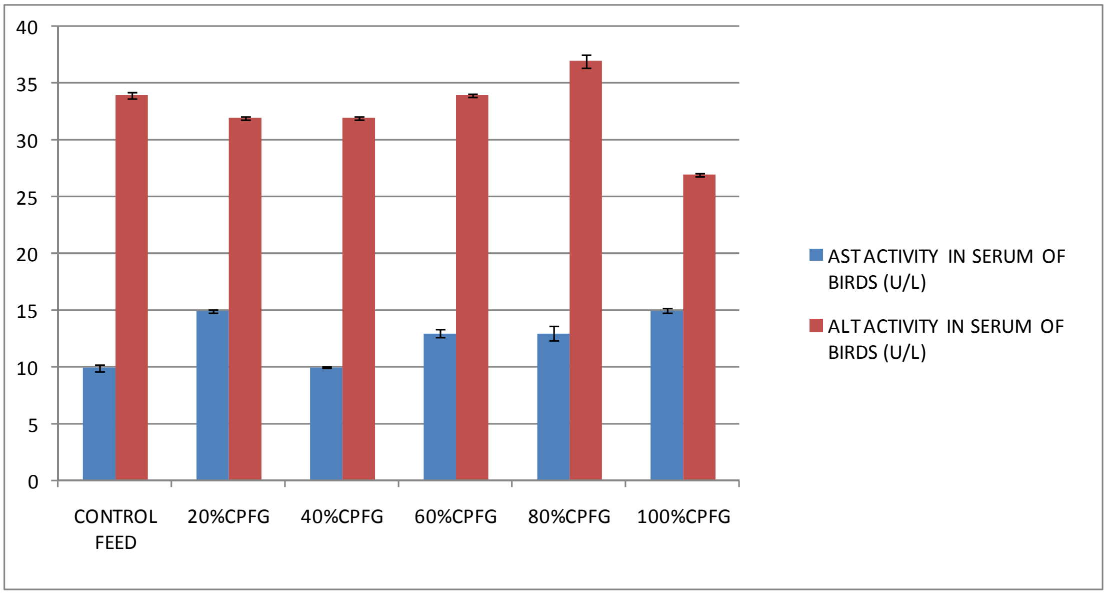

Fig. 7. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (U/L) of birds

4. DISCUSSION

Rearing of poultry birds has now turned into one of the most important divisions of agriculture throughout the world and broiler chicks today are the most widely grown birds in the world as the fastest source of animal protein [18]. However, successful broiler development is dependent on optimal feed intake throughout the growing period. According to Addass et al. [19], a number of factors such as environmental temperature, diet nutrient composition, feed aroma and physical quality/appearance etc, has been considered to have a very significant impact on optimal feed intake and general broiler growth. Hence, a formulated broiler starter feed should, generally, be able to meet the basic carbohydrate, protein, vitamin and mineral requirements of the birds in order to facilitate healthy growth/ meat production.

In the present study, maize (*Zea mays*) flour in a commercial broiler starter feed was wholly or partly replaced with ground cassava peels enzymatically degraded with amylase-producing fungi. The biochemical components of the formulated feeds were determined as well as the effect of the feeds on some biochemical parameters in the broiler chicks.

The results of the analysis showed a non-significant ($p>0.05$) increase in total soluble polysaccharide contents in feeds whose maize contents were replaced with 40% ground fungi-degraded cassava peels (40%CPFG) compared to the control commercial feed (191.4±14.5 mg/g of feed) (Fig. 2). However, a significant ($p<0.05$) increase in total polysaccharide contents was observed in Feeds 20%CPFG, 60%CPFG, 80%CPFG and 100%CPFG when compared to the control feed. Similarly, the total soluble protein contents of all the formulated feeds are significantly ($p<0.05$) higher than that of the control feed, except in Feed 60%CPFG in which the protein content did not differ statistically from that of the control feed ($p>0.05$). These findings indicate that the nutritional components of the formulated feeds are not lower than those of the control commercial feed used, hence consumption of the formulated feeds by the birds would be able to provide the utmost cellular needs of energy and protein thereby preventing malnutrition and protein deficiency-related diseases. According to [20], malnutrition results when animals are subjected to feed rations deficient in some key nutrient requirements like proteins, vitamins, carbohydrate etc or when some major nutrient materials appear to be non-digestible.

Assessment of the compounded feeds for presence of a phytochemical that exhibits antioxidant property showed that all the tested feeds including the control contain relatively high amounts of total phenol [>70 mg catechin equivalent (CE)/g of feed] (Fig. 3). The phenol contents of all the formulated feeds appeared to be significantly ($p<0.05$) higher than that of the control feed (62.5±0.5 mgCE/g of feed). Phenols have been severally reported to possess antioxidant and antimicrobial properties [21-23]. Hence, the presence of appreciable amounts of phenol in the compounded feeds (20 - 100%CPFG) suggests that these feeds will exhibit a good level of antioxidant protection against oxidative stress in birds fed with them [24].

Anthocyanin is a special form of flavonoid which possesses anti-cancer properties [25,26]. It inhibits tumour formation *in vivo*. Hence, the presence of anthocyanin in a diet feed would make the feed more of a nutraceutical than just a source of nutrients or energy. In the present study, apart from Feeds 40%CPFG and 60%CPFG which had significantly ($p<0.05$) low anthocyanin contents (198.7±7.6 and 71.8±5.4 mg/g of feed, respectively) compared to the control commercial feed (225.4±12.2 mg/g of feed) (Fig. 4), the other feed rations are comparatively high in anthocyanin relative to the control. Feed supplemented with 80 - 100% of cassava peels degraded with amylase-producing fungi (100%CPFG), however, appeared to have a significantly ($p<0.05$) higher anthocyanin than the control feed (Fig. 4). This implies that, apart from providing the basic nutrient requirements of the birds, these anthocyanin-containing Feed rations, especially Feed 100%CPFG, possess a high potential to prevent induction of tumor formation in cells of birds fed with them than those of birds fed the control feed in which anthocyanin level is relatively low [25,26].

Researches have also shown that successful broiler growth is dependent on optimal feed intake throughout the growing period, which in turn leads to significant weight gain by the birds [19,27]. The highest weight gain (108.0±3.0 g) was observed in broiler chicks fed with feed ration supplemented with 60% cassava peels degraded with amylase-producing fungi (60%CPFG) and was significantly higher ($p<0.05$) than weight gain by chicks fed the control feed (60.7±1.0 g) (Fig. 5). However, the weight gain by birds fed 100%CPFG feed ration was not statistically different ($p>0.05$) from that of birds fed with the control feed (60.7±1.0 g). These findings indicate that the broiler chicks were able to consume and efficiently metabolize the feed ration whose maize content was significantly (60%) replaced with blends of cassava peels degraded with amylase-producing fungi (60%CPFG), much better than the control commercial feed within the duration of the experiment hence, the higher weight gain [4,5]. From an economic point of view, the 60% replacement of maize with cassava peels degraded with amylase-producing fungi in the broiler feed will likewise result to a 60% reduction in the quantity and cost of maize used in feed production thereby decreasing significantly the overall cost of broiler feed production since maize accounts for about 70% total cost of poultry feed production.

Assessment of the potential physiological effect of the consumed formulated feeds on broiler chicks fed with them showed that the broiler chicks in each group, including the control, exhibit over 40% inhibition against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-induced oxidative stress *in vitro* (Fig. 6). According to Deng et al. [28], the ability of a cell to inhibit or scavenge DPPH free radical can be used as a measure of its antioxidant capacity. This indicates that the formulated feeds, to a good extent, possess relatively high antioxidant capacity (>40%RSA). Hence, maize replacement in poultry broiler starter feed (either partly or wholly) with cassava peels degraded with amylase-producing fungi may not pose treat to the ability of the birds to resist oxidative stress or to maintain a healthy status. This finding is also supported by the fact that the compounded feed rations are rich in phytochemicals like phenol and anthocyanin (Figs. 3 and 4) that can boost the antioxidant status of the birds upon consumption of the feeds [29].

In order to assess the impact of the compounded feeds on liver as a delicate organ, the serum activities of alanine aminotransferase activity (ALT) and aspartate aminotransferase (AST) were analyzed in birds fed the different feed rations including control. The results shown in Fig. 7 indicate that there was no drastic change in the respective serum activities of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in birds fed with the control feed and those fed with the compounded experimental feeds in which maize was partly or wholly substituted with ground cassava peels enzymatically degraded with amylase-producing fungi, especially the group fed 60%CPFG. According to [30], intense elevation in activities of serum AST and ALT reflects hepatic injury, some inflammatory disease or hepatic cellular damage. Both aminotransferases (ALT and AST) function as links between carbohydrate and protein metabolism by the interconversion of strategic compounds like α - ketoglutarate and alanine to pyruvic acid and glutamic acid respectively, a process known as transamination [31,32]. Since there is no significant difference (p>0.05) in the serum activity of ALT in birds fed with the compounded feed 60%CPFG and the control, it could be inferred that the compounded feed ration would be as safe to the liver as the control feed.

5. CONCLUSION

The findings of the present study established the possibility of partly substituting maize (*Zea mays* L.) flour in poultry broiler starter feed with ground cassava peels enzymatically degraded with amylase-producing fungi (*Aspergillus* sp.CSA35). Like the control feed, the formulated feeds containing enzyme-degraded cassava peels are rich in energy-yielding compounds, protein, phytochemicals and do not appear to pose threat to liver and general health of broiler chicks. The highest weight gain (108.0±3.0 g) was observed in broiler chicks fed with feed ration supplemented with 60% cassava peels degraded with amylase-producing fungi (60%CPFG) and was significantly higher (p<0.05) than weight gain by chicks fed the control feed (60.7±1.0 g). From an economic point of view, the 60% replacement of maize with cassava peels degraded with amylase-producing fungi in the broiler feed would likewise result to a 60% reduction in the quantity and cost of maize used in feed production thereby decreasing significantly the overall cost of broiler feed production.

ACKNOWLEDGEMENTS

We deeply appreciate the financial and technical supports of the management and personnels of African Research Laboratories, Otorho-Agbon, Ethiope East, Delta State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prasanna BM, Vasal SK, Kassahun B, Singh NN. Quality protein maize. Current Science. 2001;81(10):1308-1319.
2. Amerah AM, Ravindran V, Lentle RG, Thomas DG. Feed particle size: Implications on the digestion and performance of poultry. World's Poultry Science Journal. 2007;63(03),439-455.
3. Montagnac JA, Davis CR, Tanumihardjo SA. Nutritional value of cassava for use as a staple food and recent advances for improvement. Compr. Rev. Food Sci. Food Safety. 2009;8:181-194.
4. Rostagno HS, Pupa JMR, Pack M. Diet formulation for broilers based on total versus digestible amino acids. J. Appl. Poultry Res. 1995;4(3):293-299.
5. Svihus B, Kløvstad KH, Perez V, Zimonja O, Sahlström S, Schüller RB, Jeksrud WK, Prestløkken E. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. Animal Feed Sci. Technol. 2004;117(3):281-293.
6. Nouadri T, Meraihi Z, Shahrazed DD, and Leila B. Purification and characterization of the α-amylase isolated from *Penicillium camemberti* PL21. Afr. J. Biochem. Res. 2010;4(6):155-162.
7. Metin K, Koc O, Ateşlier BB, Biyik HH. Purification and characterization of α-amylase produced by *Penicillium citrinum* HBF62. Afr. J. Biotechnol. 2010;9(45): 7692-7701.
8. Avwioroko OJ, Tonukari NJ. Isolation and molecular identification of *Aspergillus* species associated with the spoilage of cassava in Nigeria. Nig. J. Sci. Environ. 2014;13(1):55-68.
9. Avwioroko OJ, Tonukari NJ, Asagba SO. Biochemical characterization of crude α-amylase of *Aspergillus* spp. associated with the spoilage of cassava (*Manihot esculenta*) tubers and processed products in Nigeria. Adv. Biochem. 2015;3(1):15-23.
10. Pfizer. The Nutrient Master Plan. Livestock Feeds Ltd, Ikeja, Nigeria. 1992;1-2.
11. Brisibe EA, Umoren UE, Owai PU, Brisibe F. Dietary inclusion of dried Artemisia annua leaves for management of coccidiosis and growth enhancement in chickens. Afri. J. Biotechnol. 2008;7(22): 4083-4092.
12. Miller GL. Use of dinitrosalicylic acid their purification, properties, action on starch and reagent for determination of reducing sugar. Anal. Chem. 1959;31:426-429.
13. Gornal A, Bardsmill CT, David MM. Determination of serum protein by means

of biuret reaction. J. Biol. Chem. 1949;177: 751-766.
14. Oyedemi SO, Bradley G, Afolayan AJ. In-vitro and -vivo antioxidant activities of aqueous extract of Strychnos henningsii Gilg. Afri. J. Pharm. Pharmacol. 2010;4(2): 070-078.
15. Dubois M, Gilles KA, Hamilton JK, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances. Analytical chemistry. 1956; 28(3):350-356.
16. Chew YL, Goh JK, Lim YY. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem. 2009; 116(1):13-18.
17. Liyana-Pathiranan CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L) as affected by gastric pH conditions. J. Agric Food Chem. 2005;53:2433-2440.
18. F.A.O. (1995). Directory of Production, Statistics Division, Food and Agriculture Organization (FAO), Rome, Italy. 1994;48.
19. Addass PA, Midau A, Perez IKA, Magaji MY. The effect of type and levels of animal protein supplements on the growth rate of rats. Agric. Biol. J. North Am. 2010;1(5):1-12.
20. Orosz SE. Clinical Avian Nutrition. Vet. Clinics North Am.: Exotic Animal Practice. 2014;17(3):397-413.
21. Proestos C, Boziaris IS, Nychas GJ, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006;95(4):664-671.
22. Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira, E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem. Toxicol. 2008;46(12): 3774-3779.
23. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak. J. Pharm. Sci. 2009;22(3):277-281.
24. Sumazian Y, Syahida A, Hakiman M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J. Med. Plants Res. 2010;4(10):881-890.
25. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer Lett. 2008;269(2):281–290.
26. Wang LS, Carmella S, Keyes R, Kuo CT, Huang YW, Hecht SS, Stoner GD. Anthocyanins and cancer prevention. In Nutraceuticals and Cancer, Springer Netherlands. 2012;201-229.
27. Gous RM. Nutritional limitations on growth and development in poultry. Livestock Sci. 2010;130(1):25-32.
28. Deng J, Cheng W, Yang G. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chemistry. 2011;125(4):1430-1435.
29. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191-203.
30. Asagba SO, Eriyamremu GE. Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet. J. Nutri. Environ. Med. 2007;16(3-4):267-274.
31. Hagopian K, Ramsey JJ, Weindruch R. Caloric restriction increases gluconeogenic and transaminase enzyme activities in mouse liver. Exp. Gerontol. 2003;38(3): 267-278.
32. Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB, Haffner SM. Liver markers and development of the metabolic syndrome: The insulin resistance atherosclerosis study. Diabetes. 2005;54(11):3140-3147.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11551