Biochemical characterization of solid-state fermented cassava roots (*Manihot esculenta* Crantz) and its application in broiler feed formulation

Egoamaka O. Egbune[1,6,*], Eferhire Aganbi[1,2], Akpovwehwee A. Anigboro[1], Theresa Ezedom[3], Ogheneyoma Onojakpor[4], Alex-Ifo Amata[5], Nyerhovwo J. Tonukari[1,6]

[1]Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Nigeria

[2]Georgia State University, J. Mack Robinson College of Business, 3348 Peachtree Rd NE, Atlanta, GA, 30326, USA

[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria

[4]Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa

[5]Department of Animal Science and Fisheries, Faculty of Agricultural Science, Delta State University, Abraka, Nigeria

[6]Tonukari Biotechnology Laboratory, Sapele, Delta State, Nigeria

*Correspondence to Egoamaka O. Egbune. Email: egbuneegoamaka@gmail.com

Abstract

The biochemical parameters of solid-state fermented peeled and unpeeled cassava roots (*Manihot esculenta* Crantz) and their application in broiler feed formulations were investigated. Fermentation occurred at room temperature for 72 h (pH 3—9). The samples utilized for five (5) broiler starter feeds were labeled: control, unfermented unpeeled cassava (UUC), unfermented peeled cassava (UPC), fermented unpeeled cassava (FUC), and fermented peeled cassava (FPC). Formulations were made by substituting fermented/non-fermented cassava roots at pH 7 for maize (w/w%). Fermentation-induced changes included increased soluble and total protein concentrations (69.3 and 334.5 mg/g) and (9.6 and 10.8%), respectively, in cultures prepared with peeled and unpeeled cassava at pH 7 compared to the control ($p < 0.05$), and a reduction ($p < 0.01$) in cyanide concentration from 44.4 to 78.7 mg/kg in the control to 8.5 and 13.7 mg/kg in fermented cassava at pH 7. Birds fed FUC and FPC meal (0.6 and 0.5 kg) gained significantly more weight ($p < 0.05$) than those fed the control (0.3 kg). The biochemical parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and urea levels in broiler serum did not differ significantly ($p > 0.05$) for birds fed with fermented peeled and unpeeled cassava. Conversely, serum albumin and calcium levels were significantly lower ($p < 0.05$) for birds fed with the control feed compared to birds fed with fermented feeds. The results imply that fermented peeled and unpeeled cassava roots could be a safe and nutritionally beneficial replacement for maize in broiler diet.

Keywords: Cassava; Solid-state fermentation; *Rhizopus oligosporus*; Soluble proteins; · Glucose

Introduction

Feed is the most expensive input in poultry farming, accounting for up to 80% of total production expenses, and it is a significant component in determining the feasibility and popularity of the industry (Olugbemi et al. 2010). Maize prices have risen dramatically in recent years due to rivalry with food businesses, increased biofuel production, and scarcity in Africa (Dalheimer et al. 2021). As a result, chicken feed makers have difficulty finding alternative feedstock's without sacrificing quality.

Cassava (*Manihot esculenta*, Crantz) is a promising carbohydrate source since it is high in starch and may replace maize in broiler diets (Okhonlaye and Foluke 2016; Tonukari et al. 2015). However, cassava's food competence is limited compared to other cereal grains due to its high moisture content, fibrous components, and insufficient amount of proteins. Cassava proteins have been described as inferior in terms of quality due to very little or no presence of essential amino acids (Olugbemi et al. 2010). The microbiological method of Solid-state fermentation (SSF) is one approach for enhancing nutrient bioavailability and boosting animal feed's nutritious value (Egbune et al. 2022).

The nutritional makeup of cassava is influenced by the specific tissue (roots or leaves) and by several variables like the plant's age, variety, and environmental circumstances (Okwuonu et al. 2021). Leaves include protein, vitamins, and minerals, while the root is a significant energy source. The root has a crude fat content of 0.1–0.5% on fresh Cassava weight (FW); 1–3% crude proteins and 80–90% carbohydrates on dry weight (DW); 80% of which is starch with minor amounts of sucrose, glucose, fructose, and maltose remaining (Ferraro et al. 2015).

Fermentation increases the nutritional value of foods by improving protein quality, fiber absorbability, and the creation of vitamins, vital amino acids, and protein (Li et al. 2020; Qin et al. 2022). It also helps with vitamin availability and decreases antinutritional substances (Morgan and Choct 2016). Several bacteria and fungal species, most notably *Aspergillus niger* and *Saccharomyces cerevisiae*, have been employed as starting cultures in SSF to create enzymes and other improved by-products (Sadh et al. 2018). The filamentous fungus *Rhizopus oligosporus* is generally recognized as a safe organism that develops quickly between 34 and 45 °C (Aganbi et al. 2020). It assumes various morphological hyphal forms and effectively utilized in the SSF of tempeh (a traditional fermented Indonesian meal produced from soybeans) (Miszkiewicz et al. 2004). This study looked at the viability of *R. oligosporus* as a starting culture for converting peeled and unpeeled cassava samples into a more nutritious component with the potential for use as animal feedstock, as well as the biochemical characteristics of the resultant broiler starter feed. There have been no reports of *R. oligosporus* solid-state fermentation of peeled or unpeeled cassava or its impact on cassava biochemical parameters. We investigated the benefits of completely substituting maize with *R. oligosporus*-fermented peeled and unpeeled cassava root in broiler diets. The overall goal was to produce a cost-effective but nutritious and health-beneficial broiler starting meal. The study also focused on understanding the influence of feed compositions on broiler serum biochemical parameters, plus the cost–benefit analysis of peeled and unpeeled cassava root post-fermentation (*M. esculenta* Crantz) in broiler feed formulations.

Materials and methods

Materials

Cassava (*M. esculenta* Crantz) roots were harvested from Songhai Delta, Amukpe Sapele, Delta State, Nigeria. Peeled and unpeeled cassava root were cleaned and cut into pieces (≈ 110 2 cm × 1 cm), then dried in the sun at a constant weight for 24 h. The dried materials were reduced to a fine powder (particle size 71 m), and stored at 37 °C. Tonukari Biotechnology Laboratory in Sapele, Delta State, Nigeria supplied the *Rhizopus oligosporus* strains sourced from PT Aneka Fermentasi Industri in Bandung, Indonesia. The organism was preserved in a well-corked, sterile vial filled with glycerol and stored in a refrigerator (4 °C). Before inoculation, cells were re-constituted in Potato Dextrose agar medium.

Preparation of substrates for solid-state fermentation

The fungus' estimated colony forming unit (CFU) per gram was calculated as described by Egbune et al. 2022; Ofuya and Nwajiuba 1990. In marked sterile petri dishes one gram (g) of *R. oligosporus* (1.4×10^2 CFU) was homogenized in 10 mL of phosphate and citrate buffers (pH 3—9). Ten grams of the powdered, peeled, and unpeeled cassava root was added to the homogenized mix. Sealed petri dishes containing cells-cassava mix was d allowed to ferment at room temperature for 72 h. Alongside the test samples, control samples were created, comprising dried and crushed peeled or unpeeled cassava that was mold-free, buffer alone added, and cell-free. After fermentation, aliquots (6 g) of the cell-cassava mixtures prepared at the various pH levels was collected for further analysis. Aliquots were processed by homogenizing with 40 mL distilled water using mortar and pestle. 10 mL of the resulting mixture was collected in test tubes, centrifuged for 10 min and supernatant stored as the crude extract. Crude extracts prepared as replicate samples was used in subsequent assays.

Biochemical parameters

Soluble protein determination

The approach outlined by Gornall, et al. (1949) was used to determine the soluble proteins. A graph of absorbance of protein standard (bovin serum albumin) was plotted against concentration (mg/mL) to obtain a calibration curve which was used in estimating the amount of protein present in samples.

Total protein determination

Chang (2003) described the Kjeldahl assay technique for total proteins analysis (total nitrogen multiplied by 6.25 to get protein content). The following formula was used to determine the N_2 content and, therefore, the protein level:

1 mL of 1 NH_2SO_4 = 14 mg

Protein (%) = N_2 (%) × 6.25

$$N2\,(\%) = \frac{100}{W} \times \frac{N \times 14}{W} \times \frac{Vt}{Va} \times T.B$$

W = Weight of sample (0.5 g).

N = Normality of titrant (0.02 N H_2SO_4).

Vt = Total digest volume (100 mL).

Va = Volume of digest analyzed (10 mL).

T = Sample titre value.

B = Blank titre value.

Reducing sugars determination

Reducing sugars was estimated in samples using the 3,5-dinitrosalicylic acid (DNS) colorimetric method (Miller 1959).

Glucose determination

The Randox glucose kit's recommended procedure was used to measure glucose (Randox Laboratories Ltd, UK). The concentration (mg/dl) of glucose in samples was calculated using the formula below.

$$Glucose\,Conc. = \frac{Abs\ sample}{Abs\ standard} \times Standard\,conc.\ i.\ e\,1.02mg/ml$$

Cyanide determination

The cyanide concentration in solid fermented and unfermented cassava samples was measured spectrophotometrically at 585 nm using a cyanide kit (Hughes et al. 1994).

pH Measurement

The pH of the substrate was measured using a Mettler Toledo pH meter. Prior to usage, the pH meter was calibrated using buffer solutions with pH values of 4.0, 7.0, and 10.0.

Feed formulation and trial

Feeding studies were conducted to substitute maize in broiler diets with fermented peeled and unpeeled cassava. Five broiler starter feeds were formulated for chicks (0–4 weeks old) obtained from CHI Farms Ibadan, Oyo State; details of feeds components and composition are shown in Table 1. The diets were fed to twenty-five (25) chicks separated into five treatment groups (n = 5) for four weeks. Throughout the research, feed and water were provided ad libitum; birds were weighed on the first and last day of the feeding experiment. Weight growth was calculated as the difference in weight (g) between weeks 1 and 4.

Table 1. Composition of broilers' diet in percentage weight by weight (%w/w)

Component	Control	Unfermented unpeeled cassava (UUC)	Unfermented peeled cassava (UPC)	Fermented unpeeled cassava (FUC)	Fermented peeled cassava (FPC)
Maize	50	0	0	0	0
Unfermented unpeeled cassava	0	50	0	0	0
Unfermented Peeled cassava	0	0	50	0	0
Fermented unpeeled cassava	0	0	0	50	0
Fermented peeled cassava	0	0	0	0	50
Soybean	30	30	30	30	30
Wheat offal	10	10	10	10	10
Palm kernel cake	5	5	5	5	5
Bone meal	2	2	2	2	2
Limestone	2	2	2	2	2
Salt	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25

Venous blood was drawn and placed in clean-test vials; this was allowed to agglomerate before centrifugation (3500 rpm) to collect the serum as supernatant, which was stored at (4 °C) and utilized for biochemical assays.. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, albulmin, calcium, and cholesterol are among the biochemical indicators measured in blood serum. These tests were conducted following protocols outlined in RANDOX test kits. Ethical use of animals in the present study was approved by the Research and Ethics Committee (Ref: REC/FOS/21/04), Faculty of Science Delta State University, Abraka.

Metabolizable energy and proximate analysis

The compounded meal was evaluated according to the method described by AOAC (1995). Utilizing the AOAC (1995) formula, metabolic energy (ME) (Kcal/kg) was computed as follows:

ME=(37CP)+(81.8CF)+(35.5NFE)

where

NFE represents nitrogen-free extract (carbohydrate), CF represents crude fat, and CP represents crude protein (%).

Proximate analysis of feeds

Determination of percentage of fat

The solvent extraction gravimetric method described by Kirk and Sawyer (1991) was used. The weight of fat expressed as a percentage of the weight of the sample analysed was calculated as follows:

$$\%\text{fat} = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

where

W1 = Weight of empty extraction flask.

W2 = Weight of flask + oil (fat) extract.

W = Weight of sample material.

Determination of percentage of fibre

The Weende method (James 1995) was used. The weight of fibres expressed as the percentage of the weight of the sample was calculated as follows:

$$\%\text{fiber} = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

where

W = Weight of sample material.

W_2 = Weight of crucible + sample material.

W 3 = Weight of crucible + ash.

Determination of percentage of Ash

The percentage of ash was performed using the furnace incineration gravimetric method of James (1995). The percentage of ash content was calculated as follows:

$$\%\mathrm{Ash} = \frac{\mathrm{W2} - \mathrm{W1}}{\mathrm{W}} \times 100$$

where

W = Weight of sample material analysed.

W1 = Weight of empty crucible.

W2 = Weight of crucible + ash.

Determination of percentage of carbohydrate

This was done using the procedure described by James (1995) and calculated using the formula below:

$$\%\mathrm{Carbohydrate} = \frac{\%\mathrm{protein} + \%\mathrm{fat} + \%\mathrm{fibre}}{+\%\mathrm{ash} + \%\mathrm{moisturecontent}}$$

Determination of moisture percentage

This was done on a fresh weight basis using the gravimetric method of AOAC (1990). Calculated and reported as a percentage of the weight of the sample examined, the weight of the moisture lost is as follows:

$$\%\mathrm{Moisturecontent(Mc)} = \frac{\mathrm{W2} - \mathrm{W3}}{\mathrm{W2} - \mathrm{W1}} \times 100$$

where

W_1 = Weight of empty moisture.

W_2 = Weight of can + sample before drying.

W_3 = Weight of the can + dried sample.

Determination of dry matter percentage

This was done by finding the difference between the percentage moisture content and 100 and is calculated thus:

$$\% \text{ Dry matter} = 100 - \% \text{ MC}$$

where MC = Moisture Content.

Analysis of cost–benefit

A cost–benefit analysis of maize, cassava, and the filamentous fungus (*R. oligosporus*) employed in solid-state fermentation was performed.

Statistical evaluation

The data were examined using SPSS software. Analysis of variance (ANOVA) was used to evaluate the experimental data, and a Fischer test of least significance (LSD) was used to compare the group averages. Significant differences were considered at $p < 0.05$.

Results and discussion

Effects of fermentation on cassava biochemical parameters

Fermentation increased the soluble proteins concentration from 44.3 to 167.5 mg/g in unfermented peeled and unpeeled cassava root to 69.3 and 335.8 mg/g respectively, in fermented peeled and unpeeled roots at pH 7 (Fig. 1A). The observed fermentation-induced increase in soluble proteins is comparable to similar findings reported for maize offal following solid-state fermentation with *R. oligosporus* (Anigboro et al. 2020). Plausible explanation for high soluble proteins level is the secretion of extracellular enzymes into the fermentation substrates as microbial cells underwent growth processes while exploiting cassava starch as carbon source (Hawashi et al. 2019). Since the observed increase in soluble proteins level in the fermented cassava samples was significant ($p < 0.05$), we considered these fermented substrates as likely replacement for maize in broiler meals. An important factor for consideration is whether fermentation enhances cassava's appeal and absorptive properties for domesticated animals.

The results of the total protein content determination in *R. oligosporus* fermented peeled and unpeeled cassava roots are shown in Fig. 1B. As expected, fermentation increased total proteins level from 2.3 to 3.8% in peeled and unpeeled cassava, respectively, to 9.6% in fermented peeled at pH 6 and 10.7% in fermented unpeeled at pH 7. In line with this, Ariyo and Ikpesu (2016), reported increases in the range 4.4–29.2% in the total proteins content of flour prepared from inoculated cassava peel; and 4.5–9.8% in flour from naturally fermented cassava peel. Another study described increases in total proteins as high as 61.0% and 41.0% in maize bran and wheat offal following fermentation with *Trichoderma viride* and *A. niger* respectively (Iyayi 2004).

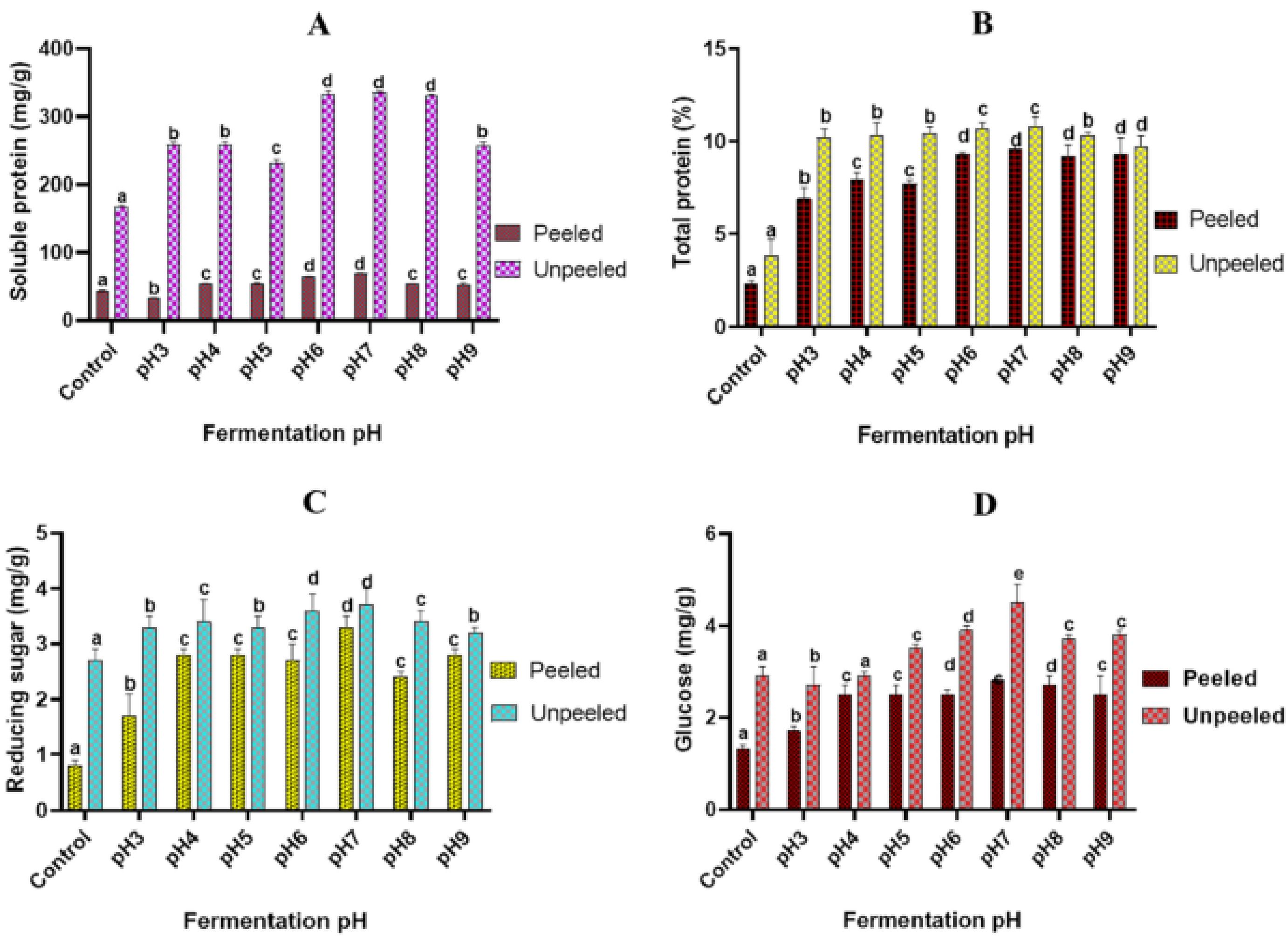

Fig. 1. A Soluble proteins, **B** Concentration of total proteins, **C** Reducing sugar concentrations and **D** Glucose levels in the *R. oligosporus* fermented peeled and unpeeled cassava roots. Values with the letters b, c, and d superscripted ([b, c, d]) are considerably at variance with the control ($p < 0.05$), those with the letter a superscripted ([a]) are not statistically altered ($p > 0.05$)

The impact of fermentation on the concentration of reducing sugars in peeled and unpeeled cassava substrate is depicted in Fig. 1C. Following fermentation, the amount of reducing sugars increased considerably ($P < 0.05$) from 0.8 to 2.7 mg/g in peeled and unpeeled cassava to 3.3 and 3.7 mg/g, respectively. Overall, fermented peeled and unpeeled cassava had more reducing sugars at 72 h incubation period compared with unfermented control. This is likely the outcome of microbial degradation as substrates were utilized as carbon source to activate multiple enzymes (Egbune et al. 2021a).

Glucose concentration in fermented substrates increased significantly ($P < 0.05$), from 1.3 to 2.9 mg/g in peeled and unpeeled cassava samples to 2.7 and 4.5 mg/g post-fermentation (Fig. 1D). Increased glucose levels in *R. oligosporus*-fermented peeled and unpeeled cassava are consistent with recent findings in *R. oligosporus*-fermented maize (*Zea mays*) offal fermentation (Egbune et al. 2021b). The breakdown of complex polysaccharides to glucose and other reducing sugars as a result of fermentation-induced extracellular enzyme secretion might explain the observed rise in glucose levels.

When compared to the control, the cyanide concentration of the fermented, peeled and unpeeled cassava roots dramatically decreased ($p < 0.01$), from 44.4 to 78.7 mg/kg in the control group to 8.5 and 13.7 mg/kg at pH 7 (Fig. 2A). This result is in line with previous studies. According to Naa et al. (2010), microorganisms found in fermented cassava pulp juice can increase the nutritional value of cassava peels by optimizing protein levels and lowering the cyanogenic glycoside content to levels suitable for animal consumption, whereas Muzanila et al. (2000) confirmed that fermentation can successfully reduce HCN levels of cassava from 400 mg/kg in fresh roots to 5.84 mg/kg after submerged fermentation compared to 14.0 mg/kg. Fermentation significantly enhances nutritional digestibility and availability, sensory characteristics, and shelf life, and lowers antinutritional factors (Samtiya et al. 2021). The results of this study indicate that the filamentous fungus *R. oligosporus* has the capacity to degrade cassava, enhance antioxidant properties in solid-state Fermented peeled and unpeeled cassava, and be particularly effective in cyanide detoxification.

The results showed a general increase in pH as fermentation progressed. Fermentation increased the initial pH of the peeled cassava and unpeeled cassava media from 3.0 to 5.3, and a similar trend was observed at initial pH values of 4.0 and 5.0. However, at initial pH 7.0, 8.0, and 9.0 there was an observed decrease in pH of the media for both peeled cassava and unpeeled cassava as fermentation progressed (Fig. 2B). The highest pH recorded was 7.7 for the peeled cassava medium at an initial pH of 5.0. The pH levels were significantly altered during fermentation. Protein degradation in the raw materials into amino acid and peptide fractions and the breakdown of organic acids were used to explain the pH rise (Trigueros et al. 2021). The explanation is that during the fermentation process, organic acids such as citric and lactic acids are released, which causes the pH to fall (Delgado-Fernández et al. 2019). Oyarekua (2013) reported a similar decrease in the pH of the cofermentation process of maize (50%), cowpea (30%) and sweet potato (20% w/w) for the production of complementary infant food. Organic acids are reportedly produced by the metabolic activities of fungi during fermentation (Oduah et al. 2015). However, the observed pH reduction in the fermented medium in this study may be due to increased amounts of organic acids produced by the metabolic activities of the fungi.

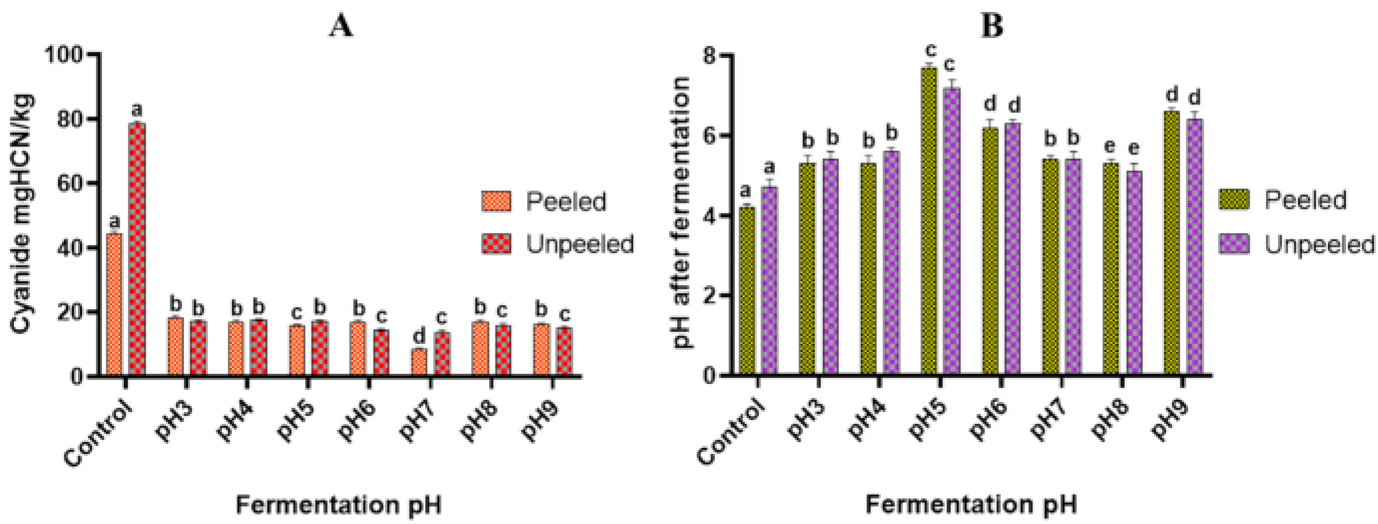

Fig. 2. A Cyanide concentration and **B** pH after fermentation in the *R. oligosporus* fermented peeled and unpeeled cassava roots. Values with the letters b, c, and d superscripted ([b, c, d]) are considerably at variance with the control ($p < 0.01$), those with the letter a superscripted ([a]) are not statistically altered ($p > 0.01$)

Table 2. Proximate composition of formulated feeds

Composition	Control feed	Unfermented unpeeled CASSAVA (UUC)	Fermented unpeeled cassava (FUC)	Unfermented peeled cassava (UPC)	Fermented peeled cassava (FPC)
Protein (%)	19.4 ± 0.3	6.4 ± 0.1	27.5 ± 0.5	4.6 ± 0.1	24.4 ± 0.4
Fat content (%)	3.6 ± 0.1	2.9 ± 0.1	4.7 ± 0.2	1.1 ± 0.4	4.3 ± 0.1
Fiber (%)	21.7 ± 0.4	17.4 ± 0.2	24.2 ± 0.4	3.9 ± 0.1	10.4 ± 0.1
Ash (%)	7.8 ± 0.2	6.5 ± 0.1	6.8 ± 0.1	4.4 ± 0.1	11.7 ± 0.4
Carbohydrate (%)	34.6 ± 0.3	46.8 ± 0.1	37.3 ± 0.2	73.7 ± 0.3	43.3 ± 0.3
Moisture (%)	9.4 ± 0.2	6.7 ± 0.02	4.9 ± 0.1	9.1 ± 0.1	5.9 ± 0.2
Dry matter (%)	95.5 ± 0.2	94.3 ± 0.1	93.9 ± 0.3	91.2 ± 0.4	91.9 ± 0.1
Metabolizable energy (kcal/kg)	2238.8	2127.7	2718.4	2882.8	2789.8

Table 2 depicts the results of the proximate analyses performed on the prepared diets. The composition of broiler starter feed was found to be as follows: crude protein 4.6–27. 5%, crude fat 1.1 – 4.7%, crude fiber 3.9–21.7%, ash 6.5–11.7%, carbohydrate 34.6–73.7%, moisture 4.9–9.4%, and dry matter 91.2–95. 5%. Fermented unpeeled cassava (FUC) had the greatest crude protein content (27.5%) of the formulated diets, whereas unfermented peeled cassava (UPC) had the lowest value (4.6%). The computed metabolizable energy (ME) value of the prepared feed samples ranged from 2127.7 to 2882.8 kcal/kg. The levels of proximate composition changed between the control and experimental meals. The crude protein value of the control feed was somewhat lower (19.4%) than the National Research Council (NRC 1994) suggested value (22.0–24.0% CP) for broiler starter diets, but the experimental feeds, fermented unpeeled cassava (FUC) and fermented peeled cassava (FPC), were higher. For broiler starter feed, the energy levels of the control feed and the experimental feed were higher than the NRC suggested values (2800–3000 kcal/kg ME; National Research Council (NRC 1994).

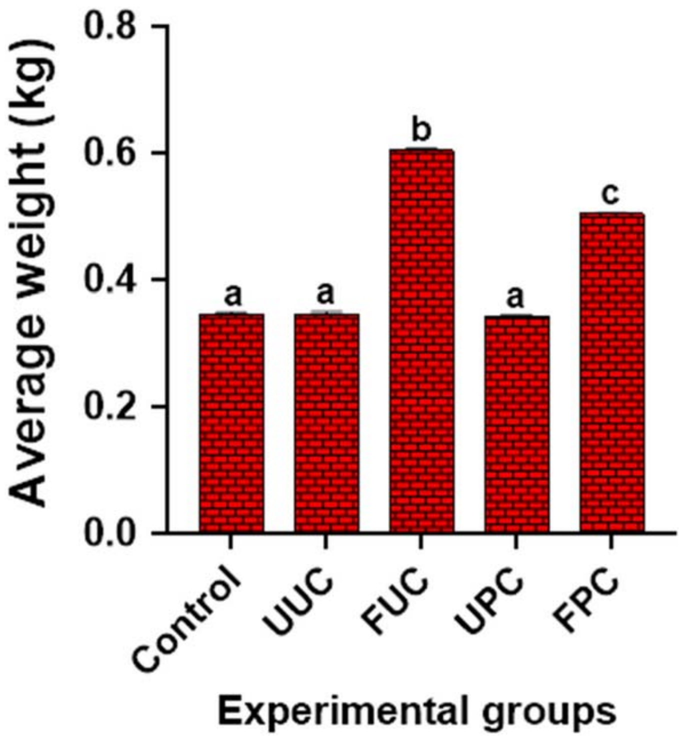

Fig. 3. Weight gained (kg) by the broiler chicks. UUC: Unfermented unpeeled cassava, UPC: Unfermented peeled cassava, FUC: Fermented unpeeled cassava, FPC: Fermented peeled cassava (n = 5). Values with the letters b, c, and d superscripted (b, c, d) are considerably at variance with the control ($p < 0.05$), those with the letter a superscripted ([a]) are not statistically altered ($p > 0.05$)

The effect of feed formulations on broiler chicks' body weight

Birds given unfermented unpeeled cassava (UUC), fermented unpeeled cassava (FUC), unfermented peeled cassava (UPC), and fermented peeled cassava (FPC) had increased weight compared to the control group. Fermented unpeeled cassava and fermented peeled cassava meals increased the chicks weight significantly more ($p < 0.05$) than the control feed. The birds given fermented unpeeled cassava had the highest weight (0.604 ± 0.033 kg)

(Fig. 3). The weight increase indicates that the broilers were able to ingest and swiftly metabolize the feed that was totally replaced with fermented unpeeled cassava (FUC) and fermented peeled cassava (FPC) during the trial period more effectively than the control diet. Tonukari et al. (2016) and Avwioroko et al. (2016) found similar results. Economically, substituting fermented unpeeled cassava (FUC) and fermented peeled cassava (FPC) for maize in the diet would reduce the cost and volume of corn used in broiler feed formulation, as up to 80% of the price of the broiler diet is made up of maize.

Feed compositions' effect on serum biochemical parameters

The blood levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (U/L) in the birds are shown in Fig. 4A. There were no differences in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity between birds fed the control food and those fed the experimental diets. In a manner similar to that of Khempaka et al. (2016), the activities of AST and ALT as markers of liver health in birds fed fermented unpeeled cassava (FUC) and fermented unpeeled cassava (FPC) versus control were investigated. The bioconversion of urea to fungal biomass protein and the removal of hydrogen cyanide HCN during fermentation may play a role in the safe inclusion of fermented unpeeled and fermented peeled cassava in chicken diets (Bayitse et al. 2015). Serum AST and ALT activity increases in response to inflammatory disease, hepatic cellular damage, or hepatic injury (Lala et al. 2021). It is likely that the experimental diets were not damaging to the liver because there was no significant increase ($p > 0.05$) in serum ALT and AST activity in birds fed the experimental diet vs the control diet.

Creatinine levels in the blood of the birds given the prepared experimental diet were not significantly different from those of the control groups ($p > 0.05$) (Fig. 4B). A high creatinine level in the blood serum may suggest a rise in muscle tear and wear as the chicks develop and perform metabolic activities (Okonkwo et al. 2019). All readings in the study fell within the same range of creatinine levels (0.84 to 1.21 mg/dl), showing no discernible rise in creatinine levels (Merck's Manual 1998). The study's findings on creatinine levels concur with those of Ahamefule et al. (2006), who found no significant increase ($p > 0.05$) and values within the physiological range.

Similarly, as compared to the control group, the urea level in the formulated meals did not substantially increase ($p > 0.05$) (Fig. 4B). This shows that no kidney damage was induced by hydrogen cyanide or other antinutritional substances in the meal, which may be attributable to the right quantity produced by fermentation. Its lower level in unfermented peeled cassava (UPC) and fermented peeled cassava (FPC) compared to control might be owing to the action of enzymes in the diet that expedites protein consumption. Serum urea elevations may indicate increased activity of urea enzymes such as ornithine, carbonyl transferase, and arginase, as well as kidney injury or catabolism induced by fever or tissue necrosis (Ajagbonna et al. 1999).

Albumin levels in birds fed fermented unpeeled cassava (FUC) and fermented peeled cassava (FPC) differed significantly ($p < 0.05$) from the control diet (Fig. 4C), but there was no difference in the values obtained for the control, unfermented unpeeled cassava, and unfermented peeled cassava fed groups. The blood albumin concentrations determined in

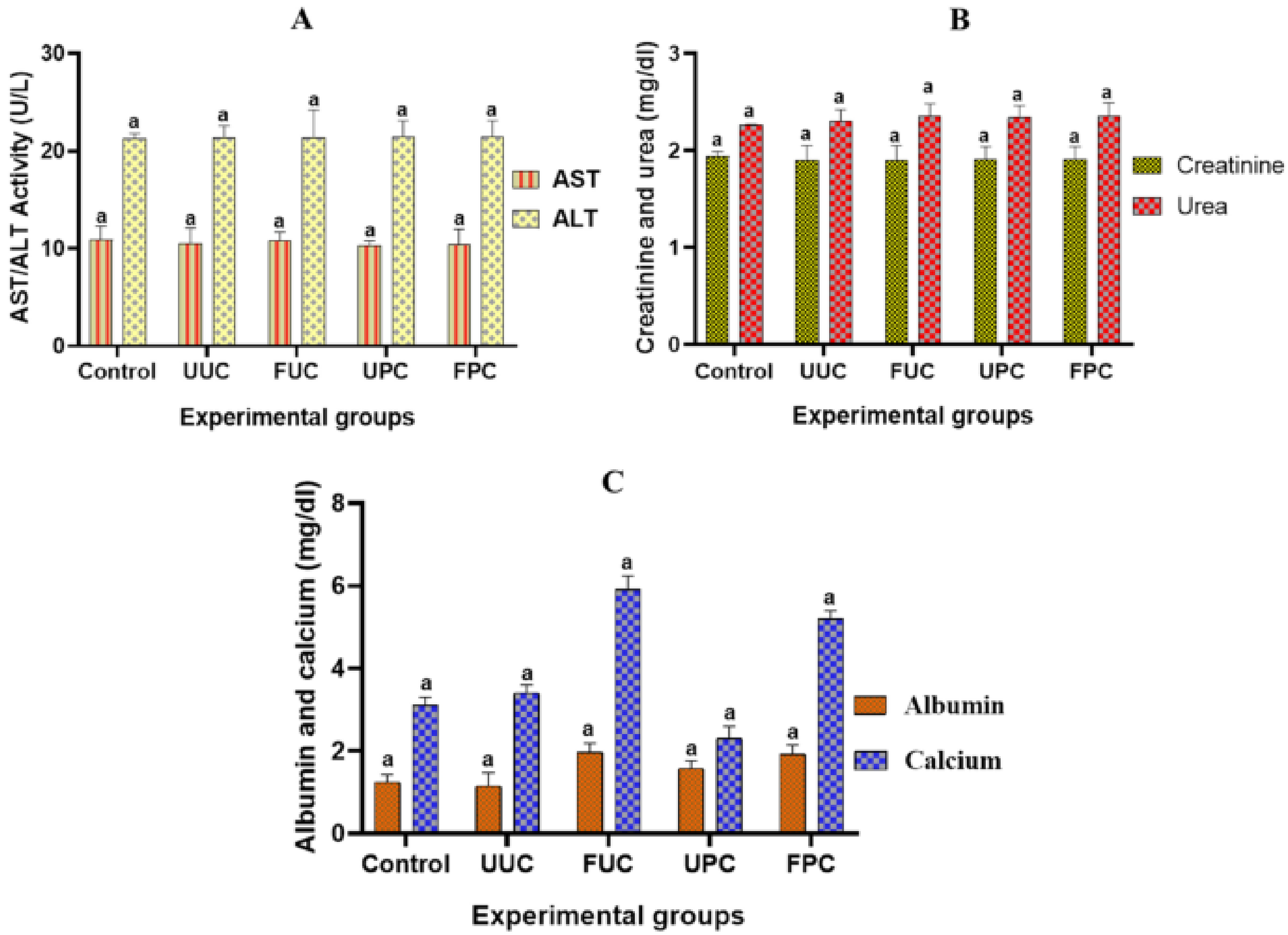

Fig. 4. A Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (U/L), **B** Serum creatinine and urea levels (mg/dl), and **C** Serum albumin and calcium levels (mg/dl) in the broiler chicks UUC: Unfermented unpeeled cassava, UPC: Unfermented peeled cassava, FUC: Fermented unpeeled cassava, FPC: Fermented peeled cassava (n = 5). Values with the letters b, c, and d superscripted ($^{b, c, d}$) are considerably at variance with the control ($p < 0.05$), those with the letter a superscripted (^{a}) are not statistically altered ($p > 0.05$)

this study are comparable to those previously determined in developing pigs fed cassava peel-based diets supplemented with Avizyme® 1300 Fashina (1991). Protein deficit has been shown to diminish the majority of haematological and serum parameters by decreasing or impeding blood cell formation, which is largely proteinaceous (Mafuvadze and Erlwanger 2007). As a consequence of the birds' efficient protein utilization, this finding suggested that the protein levels in the food may sustain their normal protein stores (Zhao et al. 2021). As a consequence, the nutritional profile of the specified food and feed additives was sufficient to sustain the general function of the birds.

Similarly, blood calcium levels in birds fed fermented unpeeled cassava and fermented peeled cassava diets were shown to be considerably higher ($p < 0.05$) than in birds fed control feed (Fig. 4C). The birds given fermented unpeeled cassava (FUC) had the greatest blood calcium levels. Fermentation increases the amount of minerals and ash in cassava peels (Aruna 2019). Increased blood calcium levels might be funneled into bone formation osteogenesis pathways, finally producing birds with healthy and strong bones (Dawson-Hughes 2015). The transport of short-chain free fatty acids from the bloodstream to cells for -oxidation during fasting or to adipose tissues in a well-fed state is made possible by serum albumin in particular. These fatty acids are then stored intracellularly as triacylglycerol molecules or triacylglycerolotides in lipid droplets (Shinawi and Abu-Elheiga 2014). This might explain why birds fed fermented unpeeled cassava (FUC) meals gained more weight than those fed control diets.

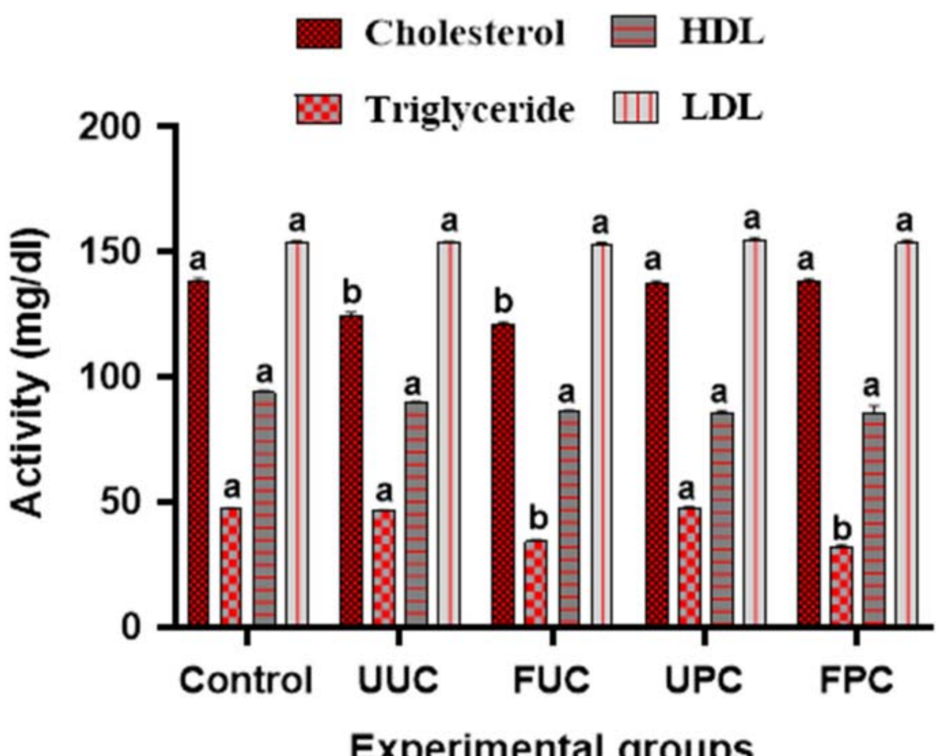

Fig. 5. Serum cholesterol, triglycerides, HDL and LDL levels (mg/dl) in the broiler chicks. UUC: Unfermented unpeeled cassava, UPC: Unfermented peeled cassava, FUC: Fermented unpeeled cassava, FPC: Fermented peeled cassava (n = 5). Values with the letters b, c, and d superscripted ([b, c, d]) are considerably at variance with the control ($p < 0.05$), those with the letter a superscripted ([a]) are not statistically altered ($p > 0.05$)

The cholesterol content of birds fed control meals was higher than that of the other experimental diets (Fig. 5). This however contradicts the findings of Sadeghi and Pourreza (2007), who observed that the fiber content of the animal's food had a negative relationship with the cholesterol level in the blood. The anomaly might be caused by the kind and source of the fiber delivered. The significant decrease in cholesterol content in unfermented unpeeled cassava (UUC) and fermented unpeeled cassava (FUC) meal may indicate the presence of hypocholesterolemic properties. This observation might be connected to a decrease in lipid mobilization. The reduction in blood cholesterol level seen in broilers fed fermented unpeeled cassava (FUC) meal implies that meals can lower cholesterol in the meat.

Serum total triglyceride levels were lower ($p < 0.05$) in fermented unpeeled cassava (FUC) and fermented unpeeled cassava (FPC) than in the control diet (Fig. 5). Feeding broilers a fermented mixture of cassava pulp and Moringa oleifera leaf meal (FCPMO) (fermented distillery by-product) led to a decrease in plasma triglyceride content, according to Sugiharto (2019). Triglyceride levels in the circulatory systems of broilers decreased as a result of enhanced triglyceride hydrolysis and fatty acid oxidation in fermented feed (Sugiharto and Ranjitkar, 2019). However, there was no significant variation in the low-density lipoproteins (LDL) and high-density lipoprotein (HDL) levels of broiler chicks.

According to the data in Table 3, the cheapest feed to achieve 1 kg live weight increase was unfermented unpeeled cassava (₦1977) and the most expensive was the control diet (₦2377). Fermented feeds (fermented unpeeled cassava and fermented peeled cassava) are priced at (₦2077.20) and (₦2177.20), respectively. In comparison to the control group, the cost of compounding 1 kg of feed and the cost of feed utilized per bird were significantly lowered ($P < 0.05$). Maize costs ₦240/kg depending on the seller, whereas fermented unpeeled cassava meal costs ₦180.2/kg.

According to Olugbemi et al. (2010), feed pricing accounts for up to 80% of overall formulation costs and is critical for the survival and profitability of chickens. This surge in breeding expenses has forced many small farmers out of the poultry industry, while major farmers are unable to avoid passing the cost on to customers, making chicken products extremely expensive. Given that cassava is now less expensive than maize, the findings of this study seem to strongly support the use of fermented cassava meal as a financially advantageous alternative to maize in Nigerian broiler diets. This might assist to cut the rising cost of chicken feed and, by extension, poultry in the country.

Table 3. Cost benefit analysis of solid state fermented peeled and unpeeled cassava (*M. esculenta* Crantz) in broiler feed formulation

Ingredient	Quantity (kg)	Unit price (₦/kg)	Control	Unfermented unpeeled cassava (UUC)	Unfermented peeled cassava (UPC)	Fermented unpeeled cassava (FUC)	Fermented peeled cassava (FPC)
Maize	5	240	1200	0	0	0	0
Unpeeled cassava	5	160	0	800	0	0	0
Peeled cassava	5	170	0	0	850	0	0
Fermented unpeeled cassava	5	180	0	0	0	900	0
Fermented peeled cassava	5	200	0	0	0	0	1000
Soybean	3	300	900	900	900	900	900
Wheat offal	1	80	80	80	80	80	80
Palm kernel cake	0.50	50	25	25	25	25	25
Bone meal	0.20	10	2	2	2	2	2
Limestone	0.20	10	2	2	2	2	2
Salt	0.03	100	3	3	3	3	3
Premix	0.03	1500	45	45	45	45	45
Lysine	0.03	1500	45	45	45	45	45
Methionine	0.03	2500	75	75	75	75	75
Rhizopus oligosporus	0.001	200	0	0	0	0.20	0.20
Total cost of feeding (₦)	–	–	2377	1977	2027	2077.20	2177.20
Initial Weight (kg/bird)			0.04	0.04	0.04	0.04	0.04
Final Weight (kg/bird)			0.34	0.35	0.35	0.61	0.50
Weight gain (kg/bird)			0.30	0.31	0.31	0.57	0.46

Conclusion

This research revealed that solid-state fermentation of peeled and unpeeled cassava with *R. oligosporus* boosted the nutritional value (soluble proteins, 69.3 and 335.8 mg/g; total proteins, 9.6 and 10.8%; glucose, 2.8 and 4.5 mg/g and reducing sugars, 3.3 and 3.7 mg/g) at pH 7. The study's findings proved the possibility of completely substituting maize in broiler diets with *R. oligosporus* fermented peeled and unpeeled cassava roots, with birds fed with fermented unpeeled cassava (FUC) having the maximum weight increase of 0.605 kg. Similar to the control diet, the designed meals including fermented cassava are high in energy-yielding metabolites, and do not appear to be harmful to the liver or the overall health of broiler chicks. This study also found that fermented cassava may be added to broiler starter meals with no negative impacts on the birds' blood biochemical profiles. Based on this research the use of solid-state Fermentation (SSF) in a number of bio-technological procedures and the production of improved bioproducts appears to be attractive and promising.

Data Availability

The availability of experimental data would be subject to reasonable request.

Contributions

NJT., AA, AA.A., EA., and EOE: Conceptualization, investigation, formal analysis, methodology, investigation, data curation, visualization, software, writing-original, writing-review, and editing. OO., and TE: Project administration and supervision. All authors read and approved the final version of the manuscript.

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