

Full Length Research Paper

Nutritional enhancement of cocoa pod husk meal through fermentation using *Rhizopus stolonifer*

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This study evaluated the effect of fermentation period on the proximate composition, anti-nutritional content, fibre fractions and amino acid profile of cocoa pod husk meal (CPHM). Cocoa pod husk was taken through a solid state fermentation process involving *Rhizopus stolonifer* as its starter culture for a period of two weeks. The fermented CPHM was dried and analyzed for its proximate composition, anti-nutritional factors, fibre fractions and amino acid profile. The results of the study revealed that the crude protein content of CPHM significantly ($P \leq 0.05$) improved during fermentation by 48.59%, while crude fibre and crude lipid decreased significantly ($P \leq 0.05$) by 14 and 22%, respectively after 2 weeks of fermentation. The theobromine, tannin and phytate of the fermented samples decreased by 77.3, 94 and 27% after 14 days fermentation, respectively. Also, the neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose (HEMM) and cellulose (CELL) decreased progressively as the days of fermentation increased and total essential amino acid (TEAA) of the fermented CPHM increased significantly ($P \leq 0.05$) as days of fermentation increased showing that *Rhizopus stolonifer* may both enhance CPHM protein quantity and improve its quality. It could be concluded that fermentation with *R. stolonifer* for 14 days could improve the nutritive value of CPH and thus increase its inclusion in the formulation of diets for animals.

Key words: Cocoa pod husk meal, fermentation, *Rhizopus stolonifer*, nutritive, anti-nutritive composition.

INTRODUCTION

Countries in West and Central Africa account for 71.4% of the total world production of cocoa (*Theobroma cacao*) beans, primarily for the manufacture of chocolate and cocoa powder (International Cocoa Organization, 2012). An estimate of 6.7 million metric tonnes of cocoa pod husk, a by-product of cocoa cultivation is often generated from these cocoa products. According to Tijani et al.,

(2016), cocoa pod husk contains protein (6.8-10%), gross energy ($10.7 \text{ MJkg}^{-1} \text{ DM}$), fibre (24-35.4%), fat (1.6-2.4%) and non cellulose carbohydrate (46.6%).

Given the critical shortfall in livestock production in most cocoa-producing countries in Africa, attributable largely to the prohibitive cost of animal feed, the utilization of the vast quantities of discarded cocoa waste

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products as affordable accessory animal feed would be of tremendous benefit to millions (Campos-Vega et al., 2018).

Previous works revealed their potential use as an unconventional low-cost feed ingredient for livestock nutrition, reducing feed costs by replacing some of the expensive conventional feed ingredients used in ration formulation (Ozung et al., 2017; Adeyeye et al., 2018). Ashade and Osineye (2013) also reported an increase in weight gain and profit margin when CPH was used to substitute 100% maize in the diet of *Oreochromis niloticus*. However, the replacement value for CPH in monogastric nutrition is limited by its poor nutrient composition which causes slow growth rate of livestock due to poor feed intake and digestibility (Adeyeye et al., 2018). Its low protein value coupled with the presence of high amounts of lignin as well as non-starch polysaccharides (NSPs) including hemicellulose and cellulose, which are poorly utilized by farm animals (particularly monogastrics) constitute major limitation to its replacement value in animal diets (Ozung et al., 2017).

The advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology might offer potential economic utilization of cocoa pod husk. The application of effective bio-treatment approaches such as fungal biotechnology is worth considering for the improvement of the nutritional value of CPH as higher fungi have the ability to bio-transform fibrous agro-residues into value-added products through their extracellular enzyme activities (Oduro-Mensah et al., 2018).

Solid state fermentation was carried out on CPH using *Rhizopus stolonifer*, at the end of fermentation, the substrate (CPH meal) was studied for value-addition, in terms of improved nutritional qualities and reduction in theobromine content. The value-addition may expand the scope of the utilization of CPH in animal husbandry in regions where cocoa is produced on a large scale. This study was therefore carried out to assess the effect of a solid state fermentation treatment involving *R. stolonifer* on the composition of CPH.

MATERIALS AND METHODS

Experimental site

Microbial analysis of CPH meal was conducted at the Microbiology Laboratory and solid-state fermentation of CPH meal was carried out at the Nutrition Laboratory of the Department of Animal Production and Health, the Federal University of Technology Akure (FUTA), while all chemical analyses were carried out at the Centre of Excellence on Food Security, Professor Julius Okojie Central Research Laboratory, FUTA.

The study location lies between latitude 7°15' North and longitude 5°12' East of the equator of the Greenwich in the humid tropical rainforest region. It has an average annual rainfall of about 2378 mm with temperature ranging between 28 and 30°C and a relative humidity of about 80% (Climatedata, 2018).

Collection and processing of cocoa pod husk

Freshly discarded cocoa pod husks were collected during the harvest season from plantation around Idanre and Ondo town, Nigeria. The surface of the pods was cleaned, grossly chopped to pieces and sun-dried to a moisture content of ca. 10%. Dried cocoa pod husks were ground in a hammer mill (Model 912, Winona Attrition Mill Co., Winona, MN) to produce the CPH meal, which was later stored in polyethylene bags and kept under moisture free conditions pending solid state fermentation and chemical analysis of its nutrient.

Isolation of microorganism and preparation of inoculum

R. stolonifer commonly known as black bread mold and sometimes used in preparing fermented foods was isolated from decomposing bread using potato dextrose agar (PDA). Dark patches were scrapped with sterile scalpel to inoculate the medium and incubated at 30 ± 1°C for 5 days. The pure culture was subsequently stored in PDA slants at 4°C. It was identified conventionally according to its macroscopic and microscopic features following the scheme of Domsch et al., (1980). Inoculum was developed by transferring a loopful of mycelium into the inoculum medium (1% sucrose, 0.2% yeast extract, pH 5.50). The flasks were incubated at 30°C on a shaker at 100 rev/min for 24 h. For use as inoculum, the spore suspensions were standardized to 2 × 10⁶ spores/ml. A hemacytometer (Neubauer-ruled Bright Line counting chambers; Hausser Scientific, Horsham, Pa.) was used to count the spores (*n* = 4). Spore suspension of *R. stolonifer* was prepared in distilled water after incubation for up to 5 days in Potato Dextrose Agar nutrient broth at room temperature (25-29°C) following the procedures of Wolk et al., (2000).

Fermentation of cocoa pod husk meal with starter *R. stolonifer*

Dried and finely ground CPH meal (100 g) placed in aluminium foil was sterilized by autoclaving. 1 g of urea was dissolved in 100 ml of sterile water which was used to moisture the sterilized CPH meal. 10 ml of the prepared inoculum of the starter culture *R. stolonifer* was used to inoculate the urea treated CPH meal and kept in an incubation room. The fermentation of the cocoa pod husk meal was terminated on the 3rd day, 5th day, 7th day and 14th day followed by sun drying the substrates for two days to inactivate the microorganism. The dried CPH meal was subsequently kept in air tight plastic container in readiness for proximate analysis (Aro et al., 2008; Laconi and Jayanegara, 2015).

Chemical analyses

The proximate composition of raw and *R. stolonifer* fermented CPH meal (moisture, crude protein, ash and fibre) was determined as described by AOAC (2012). Dry matter (DM) content was based on the weight loss after 24 h in an oven at 104°C; nitrogen (N) content by the macro Kjeldahl method, where crude protein (CP) was calculated as N × 6.25. The ash content was determined as the residue left after incinerating the sample at 600°C for 3 h in a muffle furnace. The analyses for proximate fractions were done in triplicate for each sample. The metabolizable energy (ME) was calculated by methods described by Ponzenga (1985): ME = (37 × %CP) + (81.8 × %FAT) + (35.5 × % nitrogen free extract [NFE]) and amino acid profile was determined as described by Benitez (1989). 20 g of the sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer. Theobromine was

Table 1. Nutrients composition of raw and fermented CPH Meal at various days of fermentation with *Rhizopus stolonifer* (g/100 g).

| Proximate Composition (g/100 g DM) | Unfermented | | Fermented | | | SEM | P value |
|------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|---------|
| | Raw | 3rd day | 5th day | 7th day | 14th day | | |
| Crude protein | 11.27 ^c | 15.58 ^b | 16.32 ^b | 18.20 ^b | 21.92 ^a | 0.28 | 0.05 |
| Ash | 11.37 | 9.73 | 9.52 | 9.23 | 9.03 | 0.15 | 0.67 |
| Crude Fiber | 9.60 ^b | 9.09 ^b | 8.93 ^b | 8.91 ^a | 8.19 ^a | 0.20 | 0.05 |
| Crude Fat | 7.15 ^a | 6.96 ^b | 6.65 ^a | 6.39 ^b | 5.54 ^b | 0.08 | 0.05 |
| NFE | 60.61 | 58.64 | 58.58 | 57.27 | 55.32 | 0.30 | 0.61 |
| M.E (Kcal/kg) | 3153.52 | 3227.51 | 3227.40 | 3229.19 | 3228.07 | 0.35 | 0.49 |

Values represent means of triplicate. ^{ab}Means within a row with different letters are significantly different ($p < 0.05$). SEM: Standard error mean, ME: metabolizable energy = $(37 \times \%CP) + (81.8 \times \%FAT) + (35.5 \times \%NFE)$. Source: Pazuenga (1985).

Table 2. Anti-nutritional composition of raw and fermented CPH Meal at various days of fermentation with *Rhizopus stolonifer* (%DM).

| Anti-nutrient | Unfermented | | Fermented | | | SEM | P value |
|--------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|---------|
| | Raw | 3rd day | 5th day | 7th day | 14th day | | |
| Theobromine (g/100 g DM) | 1.32 ^c | 0.70 ^b | 0.33 ^a | 0.33 ^a | 0.30 ^a | 0.01 | 0.04 |
| Tanin (g/100 g DM) | 0.50 ^c | 0.37 ^{bc} | 0.10 ^b | 0.10 ^b | 0.03 ^a | 0.02 | 0.05 |
| Phytate (mg/100 g) | 30.49 ^c | 28.87 ^b | 23.90 ^a | 23.90 ^a | 22.25 ^a | 0.02 | 0.05 |

determined using the protocol developed by Janna, (2011), while the phytate content was determined by the method of Young and Greaves (1994) based on the ability of standard ferric chloride to precipitate phytate in dilute HCl extracts of fermented CPH meal. The Folin-Ciocalteu method according to Makkar et al., (1993) was employed to determine the levels of tannins in the CPH meals. Fibre fraction was determined as described by Van Soest et al., (1991). The levels of lignin was determined by solubilization of cellulose with sulphuric acid; neutral detergent fiber (NDF) assayed with a heat stable amylase and expressed as inclusive of residual ash and acid detergent fiber (ADF) also expressed as inclusive of residual ash fractions were determined according to procedures described by Robertson and Van Soest, (1981), Mertens (2002) and AOAC (1990) method 973.18, respectively. The values of these fiber fractions were subsequently used to estimate levels of hemicellulose (that is, difference between NDF and ADF values) and cellulose (that is, difference between ADF and lignin-sa values) in the samples.

Statistical analysis

Data generated from the trial were subjected to analyses of variance using SAS (version 9.2) where significant difference were observed, difference between means was tested using Duncan's multiple range test outlined in the SAS statistical package. All analyses were carried out in three replicates ($n=3$).

RESULTS

Proximate composition of unfermented and fermented cocoa pod husk meal

The proximate composition of raw and *R. stolonifer*

fermented CPH meal summarized in Table 1 revealed that crude protein and metabolizable energy increased progressively as the days of fermentation increased from 11.27 (g/100 g DM), 3153.52 (kcal/kg) in the raw to 21.92 (g/100 g DM), 3228.07 (kcal/kg) at 14th day of fermentation with *R. stolonifer*, respectively. However, progressive decreases were observed in ash content ($P \geq 0.05$), crude fibre ($P \leq 0.05$), fat ($P \leq 0.05$) and nitrogen free extract ($P \geq 0.05$) as the days of fermentation increased (that is, ash: 11.37 (g/100 g DM) to 9.03 (g/100 g DM); CF: 9.60 (g/100 g DM) to 8.19 (g/100 g DM); crude fat: 7.15 (g/100 g DM) to 5.54 (g/100 g DM); NFE: 60.61 (g/100 g DM) to 55.32 (g/100 g DM).

The anti-nutrient composition summarized in Table 2 revealed a significant ($P \leq 0.05$) reduction in theobromine concentration of CPH meal during fermentation from 1.32 (g/100 g DM) to 0.33 (g/100 g DM) as the period of fermentation increased. Also, the tannin and phytate concentration of the CPH meal during fermentation significantly ($P \leq 0.05$) reduced from 0.50 (g/100 g DM) to a very minimal level of 0.03 (g/100 g DM) and 30.49 (g/100 g DM) to 22.25 (g/100 g DM), respectively.

Table 3 also shows a significant ($P \leq 0.05$) reduction in all fibre fractions measured. The nitrogen detergent fibre reduced significantly ($p < 0.05$) from 91.89 to 65.89 g/100 g DM after 14 days of fermentation, while acid detergent fibre significantly ($p < 0.05$) reduced after 14 days of fermentation from 45.25 to 61.29 g/100 g DM. Also, the lignin content of the fermented CPH meal significantly ($p < 0.05$) reduced by 53.75% from 23.33 to 10.79 g/100 g DM. Cellulose significantly ($p < 0.05$) reduced from 38.19 g to 29.79 g/100 g DM with a 22% reduction and the

Table 3. Fibre fractions composition of raw and fermented CPH meal at various days of fermentation with *Rhizopus stolonifer* (%DM).

| Fibre fraction | Unfermented | | Fermented | | | SEM | P value |
|----------------|--------------------|---------------------|--------------------|--------------------|--------------------|------|---------|
| | Raw | 3rd day | 5th day | 7th day | 14th day | | |
| NDF | 91.89 ^e | 81.59 ^d | 73.89 ^c | 69.59 ^b | 65.89 ^a | 0.01 | 0.05 |
| ADF | 61.29 ^c | 54.17 ^{bc} | 49.95 ^b | 48.45 ^a | 45.25 ^a | 0.01 | 0.05 |
| ADL | 23.33 ^c | 21.81 ^{bc} | 20.15 ^b | 17.91 ^b | 10.79 ^a | 0.01 | 0.04 |
| HEMM | 30.59 ^c | 27.41 ^{bc} | 23.93 ^b | 21.13 ^a | 20.63 ^a | 0.01 | 0.05 |
| CELL | 38.19 ^c | 34.45 ^{bc} | 33.35 ^b | 30.53 ^a | 29.79 ^a | 0.01 | 0.02 |

Values represent means of triplicate, ^{ab}Means within a row with different letters are significantly different ($p < 0.05$). SEM: Standard error mean, NFE: nitrogen free extract, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, HEMM: hemicellulose, CELL: cellulose.

hemicellulose content significantly ($p < 0.05$) reduced from 30.59 to 20.63 g/100 g DM by 32.56%.

The quantitative composition of amino acids profile of CPH meal fermented with *R. stolonifer* at different periods is shown in Table 4. The duration of fermentation significantly ($p < 0.05$) led to progressive increase in the values of Leucine and Valine while other amino acid profile improved numerically as the days of fermentation increased. Total amino acids increased significantly ($p < 0.05$) as the days of fermentation increased from 0 to 14 days. The values recorded on the 7 and 14th days of fermentation were 50.72 and 59.71 g/100 g protein, respectively, while the value obtained from the untreated CPH meal was 44.06 g/100 g protein. The total essential amino acids (TEAA) increased significantly ($p < 0.05$) and was the highest on days 14 (33.31 g/100 g protein), while the lowest value was obtained in the untreated CPH meal (25.04 g/100 g protein). The non-essential amino acids (TNEAA g/100 g protein) values were the highest on 14 days fermentation period (26.40 g/100 g protein), while the unfermented CPH meal had the lowest (19.02 g/100 g protein). However, the TEAA: TNEAA values were 57:43, 56:44 and 56:44 for 0, 7th and 14th fermentation period, respectively.

DISCUSSION

The result obtained from this study implied that the crude protein (CP) content of the *R. stolonifer*-fermented CPH meal was higher than the one in raw sample by about 27.66 to 48.59% and those earlier reported by Adeyeye et al. (2017) and Ozung et al., (2017). Implying that fermented CPH meal can be used to replace some conventional feed ingredient within the same protein content range. Adeyeye et al. (2017) reported 13.66 g/100 g CP in ash-treated cocoa pod husk meal while Ozung et al., (2017) observed crude protein values ranging between 7.70 and 8.94 g/100 g DM CP as against 21.92 g/100 g DM CP that was obtained in this current study. This is indicative of the efficacy of *R. stolonifer* and the fermentation process employed in this

study to increase the crude protein content of the CPH and this confirms the studies reported by Balagopalan (1996), Leifa et al., (2001) and Alemawor et al., (2009) on the ability of fungi to enhance the nutritive values of agro-residue on coffee husk, cassava by-products and cocoa pod husk. The increase in growth/biomass of the fungus on the fermented cocoa pod husk (FCPH) might account for the increase observed in the protein contents with the fungal hyphae serving as single cell protein. The fungus in view contains a relatively high protein content of high biological value (Waliszewska et al., 1983).

The crude fiber content of the raw CPH meal obtained declined by 5.31 and 14.7% after fermentation (3 to 14 days), implying a better digestibility when used as ingredients in animal nutrition. This supports the intended aim of fermentation which was meant to improve the CP content and lower the crude fiber content of CPH meal so as to enhance the usability of the test ingredient for monogastric animal nutrition. The crude fiber contents reported by Nortey et al., (2015) and Adeyeye et al., (2017) after fermentation were 7.04 and 14.83%, respectively whereas Ozung et al., (2017) reported significantly higher values: 57.42 and 53.37% from fermentation and hot-water treatment of the CPH meal, respectively. Lateef et al., (2008) and Alemawor et al., (2009) reported 7.2 and 17.08% reduction of CF in CPH meal, respectively. The reduction in the crude fibre content may be an indication of *R. stolonifer* having enzymatic system, that is, secretion of cellulose/hemicellulose-degrading enzymes for degradation of polymeric lignocelluloses of CPH (Alemawor et al., 2009). The results herein reported showed that the fungal strain in use can effectively reduce the crude fibre content of CPH and this may have positive effect on its digestibility by animals.

The values for ash and crude fat were partly in consonance with the reports of Ozung et al., (2016, 2017) and Adeyeye et al., (2017). The observed reduction in crude fat was in agreement with the report of Oliveira et al., (2011) who also observed that crude fat content of fungal fermented whole rice bran decreased significantly and might be as a result of lipid use by the filamentous

Table 4. Amino acid profile of raw and fermented CPH meal at various days of fermentation with *Rhizopus stolonifer* (g/100g protein) (DM).

| Amino acid | Unfermented | | Fermented | SEM | P value |
|----------------------|--------------------|--------------------|--------------------|------|---------|
| | Raw | 7th day | 14th day | | |
| Essential | | | | | |
| Arginine | 3.87 | 4.13 | 5.16 | 0.29 | 0.65 |
| Histidine | 1.24 | 1.40 | 1.66 | 0.09 | 0.44 |
| Isoleucine | 3.53 | 4.14 | 4.51 | 2.60 | 0.35 |
| Leucine | 3.56 ^c | 4.49 ^b | 4.96 ^a | 0.34 | 0.04 |
| Lysine | 3.02 | 3.63 | 4.69 | 0.36 | 0.22 |
| Methionine | 0.29 | 0.34 | 0.37 | 0.11 | 0.55 |
| Phenylalanine | 2.57 | 2.84 | 3.72 | 0.30 | 0.87 |
| Threonine | 3.11 | 2.99 | 3.38 | 0.13 | 0.68 |
| Valine | 3.59 ^c | 4.15 ^b | 4.44 ^a | 0.23 | 0.04 |
| Tryptophan | 0.26 | 0.26 | 0.42 | 0.04 | 0.38 |
| TEAA | 25.04 ^c | 28.37 ^b | 33.31 ^a | 1.35 | 0.04 |
| %TEAA | 56.83 | 55.93 | 55.79 | 0.55 | 0.06 |
| Non-Essential | | | | | |
| Alanine | 1.78 | 2.05 | 2.39 | 0.13 | 0.24 |
| Aspartic acid | 4.00 | 4.59 | 5.27 | 0.38 | 0.96 |
| Cysteine | 1.09 | 1.21 | 1.21 | 0.03 | 0.10 |
| Glutamic acid | 4.24 | 5.30 | 6.36 | 0.44 | 0.41 |
| Glycine | 2.04 | 2.30 | 3.11 | 0.26 | 0.77 |
| Proline | 2.13 | 2.64 | 2.84 | 0.16 | 0.07 |
| Serine | 2.19 | 2.54 | 2.64 | 0.10 | 0.10 |
| Tyrosine | 1.55 | 1.72 | 2.58 | 0.23 | 0.49 |
| TNEAA | 19.02 ^c | 22.35 ^b | 26.40 ^a | 0.10 | 0.04 |
| % TNEAA | 43.17 | 44.07 | 44.21 | 0.30 | 0.06 |
| TAA, g/100 g DM | 44.06 ^c | 50.72 ^b | 59.71 ^a | 0.10 | 0.03 |
| TEAA:TNEAA ratio | 57:43 | 56:44 | 56:44 | - | - |

EAA = Essential amino acids; TEAA = total essential amino acids; NEAA = non-essential amino acids; TNEAA = total non-essential amino acids; TAA = total amino acids; SEM = pooled standard error of means. ^{a,b}Mean values within a row without a common lowercase superscript differ at $P < 0.05$.

fungi, possibly in the synthesis of phospholipid constituents of the cell membrane of fungal tissue. It has been reported that during fungal growth, some lipolytic strains assimilate lipids from substrates for biomass production leading to a general reduction of the overall lipids content of the substrate (Das and Weeks, 1979). The nitrogen free extracts value obtained in this study negates the report of both Ozung et al., (2016, 2017) and Adeyeye et al., (2017) who reported 14.56 and 39.31%, respectively for post-fermentation CPH meal.

The result from this current study also showed a remarkable decline in the theobromine content of the FCPH meal. There was a 46.97 to 77.2% decline in theobromine content of the cocoa pod husk meal post-fermentation which is indicative of the ability of *R. stolonifer* to degrade the methylxanthine backbone of theobromine in the substrate. This reduction coefficient in theobromine agrees with the reports of Adamafio et al.,

(2011), Bentil et al., (2015), Amorim et al., (2017) and Oduro-Mensah et al., (2018) who recorded significant decline in theobromine concentration after microbial fermentation. A significant reduction in coefficient by 72% was reported for theobromine by Oduro-Mensah et al. (2018) after employing a solid state fermentation of cocoa pod husk for 7 days using two strains of fungi, namely: *Aspergillus niger* and *Talaromyces* species which is still lower than 77% reported in this current study for the same fermentation period. The degradation of theobromine by *R. stolonifer* could have been made possible by using theobromine as a sole carbon and energy source via the demethylase oxidase, xanthine dehydrogenase, xanthine oxidase, urease and uricase pathway (Yamaoka-Yano and Mazzafera, 1999; Dash and Gummade, 2006; Huq, 2006). The reduction in theobromine content could lead to improved palatability with resultant better feed intake and utilization of FCPH

by animals.

The tannin and phytate contents were also observed to be highly reduced from 0.50 to 0.03 g/100 g DM and 30.49 to 22.25 g/100 g DM, respectively which translate into reduction coefficients of 94.00 and 27.00%, respectively after 14 days fermentation period. The tannin reduction coefficient agrees with the report of Adeyeye et al., (2017) while the phytate reduction coefficient agrees with the report of Bentil et al., (2015). The complexing of phytic acid with nutritionally essential elements and the possibility of interfering with proteolytic digestion have been suggested as responsible for antinutritional activity (Agbede et al., 2009a, b). The decrease in the phytate content of FCPH meal could possibly be attributed to the secretion of the enzyme phytase by the fungi. This enzyme is capable of hydrolyzing phytate (Obboh and Akindahunsi, 2003) and therefore could lead to better accessibility to phosphorus by the animals. The fibre fractions obtained in the FCPH meal in this study reveals a decline in values which disagrees with the report of Ozung et al., (2016) who recorded an increase in values of the fibre fractions after fermentation. Lignin, cellulose and hemicellulose fractions form the bulk of CPH fibre. During the fermentation process, the changes observed in the levels of these fibre fractions indicated the degree of lignocellulose biodegradation exhibited by *R. stolonifer* on CPH meal. The importance of dietary fibre fractions in animal feeding is shown by its ability to influence the rate of passage, mucosal functionality and its role as substrate for gut microbiota which in turn improves performance and digestive health (Gidenne, 2015). Minimal dietary fibre supply is essential to prevent digestive troubles. The cellulose degradation is mostly facilitated by the synergistic action of hydrolytic enzymes (cellulases) secreted by the filamentous fungus during the fermentation (Chesson, 1993; Datta and Chakravarty, 2001). Pothiraj and Eyini (2007) also observed that *R. stolonifer* showed the highest and fastest utilization of cellulose in the solid state fermentation of cassava waste which resulted in 51.24% utilization of cellulose on the 2nd day.

The degradation of the hemicellulose could be as a result of extracellular hemicellulases such as xylanases secreted by the fungus during the fermentation process (Chesson, 1993). This result strongly supports a study by Brimpong et al., (2009) who observed a 41% decrease in hemicellulose after complete colonization of corn cobs by the mycelia of the oyster mushroom. The degradation of lignin could be as a result of the production of lignin-degrading extracellular enzymes such as peroxidases and the laccases that oxidize both the aromatic rings and the aliphatic side chains to produce compounds more absorbable by the current fungi used (Youri, 2004).

The levels of essential amino acids (EAAs) in the fermented meals were substantially higher than the raw CPH meal. The additive effect of *R. stolonifer* during fermentation as single cell protein might have contributed to the observed increase because they are richer in EAAs

particularly lysine and methionine. Data on amino acid profile of fermented CPH meal is limited hence, importance of this study. Donkoh et al., (1991) reported 10 to 15% levels of TEAA and 56% of TAA which was lower than that reported in the current findings. Interestingly, Ramos et al., (2008) reported that enzymatic treatment of cocoa bean husk reduced the negative effects of dietary-induced hypercholesterolemia in an animal model. The lysine/arginine ratio, a determinant of the cholesterolaemic and atherogenic effects of protein was observed to be low for CPH protein. In turn, the TEAA of the fermented CPH meal showed that *R. stolonifer* may both enhance the protein quantity and improve the quality which agrees with the findings of Kutlu et al., (2000), Muhammad and Oloyede (2009) and Dairo et al., (2017) that fungi species are a rich source of proteins and also contain all the essential amino acids.

Conclusion

Results from this study showed that the nutritive values of FCPH meal greatly improved as days of fermentation increased till the 14th day when fermented with *R. stolonifer*. Increase in crude protein, reduction in crude fibre and anti-nutrient content of the CPH meal fermented with *R. stolonifer*, could lead to improved palatability, better feed intake and utilization which would make the meal more suitable for use as alternative feed ingredient for animals especially monogastrics in regions which depend on imported feed ingredients where cocoa pod husk are predominant but left to waste on the farm. The use of the FCPH in animal diets in place of the import dependent ingredients could help to stem the cost of finished feeds for monogastric and rabbit production and increase meat consumption among the resource poor.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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