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A novel pig feed formulation containing *Aspergillus niger* CSA35 pretreated-cassava peels and its effect on growth and selected biochemical parameters of pigs

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This study investigated the effects of Aspergillus niger CSA35 pretreated-cassava (Manihot esculenta Crantz) peel feed (CPFG) on the body weight gain and some selected biochemical parameters of pigs. Cassava peels treated with biomass of A. niger CSA35 for a period of three weeks to initiate enzymatic digestion of peels were dried, ground and used in varying proportions to formulate pig rations in combination with other feed ingredients. Twenty 85–95 days old pigs (5.85 ± 0.70 kg) were randomly divided into four experimental groups. Group 1 received 0% CPFG amidst other feed ingredients (control), while Groups 2, 3 and 4 received 40%, 60% and 100% CPFG respectively. At the end of the feeding trial (21 days), the animals were weighed and blood samples collected for biochemical analysis. Results showed that increasing the amount of the fungus-pretreated cassava peels in pig rations increased the protein, fat and carbohydrate contents of the experimental feeds. Conversely, the percentage fibre content was reduced. The weight gain of pigs fed the control diet was significantly (p<0.05) lower than those fed with 60% CPFG and 100% CPFG but did not differ from those fed 40% CPFG. Serum calcium and albumin levels were observed to be significantly lower (p<0.05) in control group than in treatment groups. The highest serum calcium level was, however, observed in 100% CPFG group. Activities of liver function enzymes and serum creatinine level of pigs fed the formulated diets did not significantly differ from those of control unlike their serum urea levels. It was concluded that pig feeds formulated with cassava peels pretreated with A. niger CSA35 enhanced feed's nutritive value and metabolisable energy, boosted serum albumin and calcium levels in pigs, increased pigs body weight and are health-friendly since the feeds did not pose threat of liver damage in the pigs investigated.

Key words: Cassava peels, *Aspergillus niger* CSA35, pig feed formulation, weight gain, biochemical parameters.

INTRODUCTION

The need to provide adequate animal protein for the growing population of third world countries is of major concern. It has been recognized over the years that the development of swine, poultry and rabbit sub sector of the animal industry is the fastest means of bridging the protein deficiency gap prevalent in most tropical countries (FAO, 1990). This is due to their short generation interval and high fecundity (Nkwengulila, 2014; John et al., 2014) but, the major constraint against pig production is the high cost of feeds. In Nigeria, it could be as high as 75 to 80% in the fattening herd and 60 to 65% in the breeding herd (Tewe, 1997; Akinfala and Tewe, 2001).

Commercialized production of these animals' feeds involves use of ingredients that have prohibitive costs due to their comparative use between man and animals. Most of these ingredients are energy-based. Thus, improvement in animal production through the use of non-conventional feed ingredients or energy sources has long been advocated (Nnadi et al., 2010) and efforts to reduce the cost of production are being directed towards the use of affordable and available alternative sources of energy and protein in the diets of pig (Woyengo et al., 2014). The alternative feedstuff, therefore, must be ingredients with less competition by other secondary industrial users and producers which are readily available in commercial quantities and affordable prices. Also, pigs should be capable of converting these alternative feedstuffs (which will normally be discarded by humans) into wholesome animal protein (Adesehinwa et al., 2011; Adesehinwa, 2008).

Cassava, *Manihot esculenta* Crantz, is a major carbohydrate-rich tuber plant cultivated in the tropics (Avwioroko and Tonukari, 2014). Current world total production is about 157 million tons per annum with Nigeria accounting for about 16% (FAO, 1990). The first attempt at substituting cereals with cassava in commercial pig rations began during the Second World War where its use cushioned the effect of post war shortage of grains in Europe (Muller et al., 1974). Manner and Gomez (1973) and Iyayi and Tewe (1994) noted conclusively that cassava might replace maize and cereals without any negative effects. In Nigeria, in spite of cassava availability, its use as sole component of energy source in livestock feed has not been given due recognition (Avwioroko et al., 2016). However, there are many pioneering studies which highlighted on the suitability of cassava tuberous meal for swine feeding and its potential as a good substitute for maize for all classes of pigs (Job, 1975; Adegbola, 1977; Nghi, 1986; Tewe and Egbunike, 1992). Jiménez et al. (2005) had reported that pigs fed diets formulated to contain 40% of cassava root meal with other ingredients showed similar performance and carcass traits with those fed conventional diet. Cassava peel meal has, therefore, become one feed ingredient which has been consistently incorporated into the diets of pigs as alternative energy source (Iyayi and Tewe, 1988; Adesehinwa et al., 1998), but for its high fibrous content (a feature of most locally available agro-industrial by-products and wastes) which has limited its use by monogastric animals (Longe and Fagbenro-Byron, 1990).

Fibrousness of feedstuffs (mostly of by-product of plant origin) is important in relation to their feeding value to pigs (Adesehinwa et al., 1998). The addition of fibre to swine diets decreases the digestible energy (DE) and metabolisable energy (ME) concentration of the diet (Kennelly et al., 1978; Kennelly and Aherne, 1980) and often results in bulk feeds. The influence of crude fibre on organic matter digestibility varies from feed to feed, depending on the special characteristics of the crude fibre in individual feeds (Kidder and Manner, 1978). The fibrous portion of feed, being fairly indigestible to pigs, influences the digestibility of the other constituents by exerting a protective action, encasing these constituents in a digestion-proof shield, as it were, thereby obstructing the access of digestive enzymes (Mitaru and Blair, 1984). Hence, for efficient use of cassava peel in pig feeding, some form of physical treatment is essential to the breaking down of the fibre encapsulating the more soluble constituents so that digestive secretions can penetrate more completely (Kidder and Manner, 1978).

Another constraint to the use of these agro-industrial wastes is the dearth of affordable and sustainable local technologies to modify these products to forms acceptable to our livestock industries. A way out is to source for cost-effective and environmentally-friendly measures as exemplified by the biotechnological option of fermentation using generally recognized as safe (GRAS) microorganisms (US FDA/CFSAN, 2008) for the biological transformations of agro-industrial wastes to yield products with enhanced nutritional values (Israelides et al., 1998; Oboh and Akindahunsi, 2003; Nwafor and Ejukonemu, 2004; Aro, 2008). Microbial fermentation has been reported as an effective means of breaking down non-starch polysaccharides of agro-industrial wastes to increase their metabolisable energy and their nutritive value in general (Aro, 2008).

The concept of using microorganisms in enhancing the nutritive value of plant and animal products is not entirely a new one. Aro (2008) had reported using a number of isolates from rotting cassava to enrich cassava and in recent years, considerable emphasis has been placed on the improvement of fibrous crops by the growing of non-toxic fungi on straw (Shrivastava et al., 2011). The ability of fungi to produce enzymes, which bring about catalytic transformations via a wide range of desirable reactions, makes them interesting to industrialists and agriculturists (Adrio and Demain, 2014). Recent advances in biotechnological applications of this sort are opening new frontiers in the bioconversion of agro-industrial wastes to products of significance in livestock production (Avwioroko et al., 2016). In this study, the effect of pig feeds formulated with cassava peels pre-treated with *Aspergillus niger* CSA35 on body weight gain and on

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Table 1. Composition of formulated experimental feeds.

Ingredients (kg)	Group 1 (0%CPFG)	Group 2 (40%CPFG)	Group 3 (60%CPFG)	Group 4 (100%CPFG)
BSG or wheat offal	20	20	20	20
PKC	50	50	50	50
Fresh cassava peels (Non-treated)	27	16.2	10.8	-
Cassava peels pre-treated with fungi (CPFG)	-	10.8	16.2	27
Bone meal	1.25	1.25	1.25	1.25
Limestone	1	1	1	1
Pig Grower Supermix	0.75	0.75	0.75	0.75
Total (100 kg)	100	100	100	100

BSG, Brewer's spent grain; PKC, palm kernel cake; CPFG, cassava peels pre-treated with fungus.

some selected biochemical parameters of pigs was investigated.

MATERIALS AND METHODS

Preparation of yeast peptone dextrose (YPD) agar

Yeast peptone dextrose agar (YPD) was prepared following the method described by Avwioroko et al. (2015). Briefly, 2.0 g of glucose monohydrate, 1.0 g of yeast extract, 2.0 g peptone and 1.5 g agar-agar powder were measured 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 (H_2O). The solution was sterilized by autoclaving it for 15 min at 121°C, allowed to cool to about 50°C and thereafter poured into sterile petri dishes to solidify.

Growth of *Aspergillus niger* CSA35 on cassava peels

A fungus, *A. niger* CSA35, previously isolated and identified to be associated with cassava spoilage/degradation using 18S rRNA gene sequence (Avwioroko and Tonukari, 2014), was grown in sterile yeast peptone dextrose (YPD) agar in the dark for a period of 7 days. The fungal biomass was later sub-cultured using a cassava finished product (eba) to facilitate its massive growth and sporulation for one week (A sterile forcep was used as inoculating tool and about five scoops of fungal cells was collected from plate and spread all over the surface of the cassava finished product (eba) which weighed approximately 600 g]. At the end of one week incubation at room temperature, there was massive noticeable fungus growth on the 'eba' prior to use for treatment of cassava peels]. The fungus was finally transferred into 52.5 kg of fresh cassava (*M*. *esculenta*) peels and thoroughly mixed together to ensure growth on all the cassava peels. The medium was stirred twice daily to ensure even distribution of nutrients thereby facilitating the release of more digestive enzymes by the fungus to degrade the substrate. After three weeks, the fungus-treated cassava peels were dried, ground and used in varying proportions to produce pig rations in combination with other feed ingredients.

Experimental animals and experimental feed composition

Twenty pigs of the large white breed, between the ages of 85 and 95 days, with average body weight of 5.85 ± 0.70 kg, were used for the study. They were injected with Ivomec® (Ivermectin) subcutaneously against endo-and ecto-parasites and weighed using a cage and a weighing balance, before the experiment began. Table 1 shows the composition of the formulated feeds given to the experimental animals. The pigs were randomly divided into four treatment groups. Each treatment group had five pigs in a completely randomized design. All groups were fed with the same quantity of their respective experimental diet twice daily. The pigs were allowed *ad libitum* access to the diets and water throughout the duration of the study (21 days).

Blood sample collection

At the end of the feeding trial (21 days), the pigs were bled to obtain blood samples. The bleeding was done in the morning of day 22 before feeding and 2 ml of blood was collected into test tube via ear vein puncture method. The blood samples were allowed to clot and then centrifuged at 3000 *g* for 10 min. The supernatant (serum) was separated and used for biochemical analysis.

Biochemical assays

The proximate analysis of the compounded feed was carried out using standard laboratory methods (AOAC, 1995). Serum urea concentration estimation was carried out according to the method described by Weatherburn (1967). The principle is based on hydrolysis of urea in serum to ammonia in the presence of urease; thereafter, the ammonia was measured photometrically by Bethelot's reaction. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Estimation of serum creatinine was done by the principle of Bartels and Bohmer (1972). Creatinine in alkaline solution reacted with picric acid to form a coloured complex which is directly proportional to the creatinine concentration. Serum albumin concentration was determined by the method of Doumas et al. (1971) and serum calcium level was estimated according to the method described by Lothar (1998). All the assays were carried out in accordance with the instructions in their respective RANDOX assay kits.

Estimation of metabolisable energy

Metabolisable energy was calculated from the proximate chemical composition data using the AOAC (1995) formula:

Metabolisable energy (Kcal/kg) = (37 × CP) + (81.8 × CF) + (35.5 × NFE)

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

Statistical analysis

The values obtained from the different experiments were reported as mean ± SD. The significant differences between mean values were obtained by using One-way Analysis of Variance (ANOVA) and least significance test (LSD) procedure as described by Ogbueibu (2005).

RESULTS

Proximate composition of formulated pig diets and energy levels

The proximate compositions of the formulated feeds used in this experiment are presented in Figure 1a. The protein content of the experimental diet increased from 13.3% (in feed 0% CPFG) to 16.15% (in 100%CPFG feed). Similarly, the percentage fat content of the feeds increased from 3.3% in 0% CPFG feed to 3.7% in 100% CPFG feed. Likewise, the percentage carbohydrate content of the feeds also increased with increase in amount of fungus-treated cassava peels included in the feed. Conversely, the percentage fibre content was reduced by increasing the amount of fungus-treated cassava peels in the diets. An increase was observed in the metabolisable energy value of the pig diets as the percentage (%) of fungal-degraded cassava peels in the diet increased (Figure 1b). It ranged from 3112.14 kcal in the control diet (0% CPFG) to 3293.27 kcal in the last group (100% CPFG).

Effect of formulated pig diets on body weight

The weight gain of pigs fed the control diet (0% CPFG) was significantly ($p<0.05$) lower than those fed with 60% CPFG and 100% CPFG in their diets but not significantly ($p>0.05$) different from those fed 40% CPFG. Pigs maintained on diet containing 100% CPFG were, however, observed to have the highest weight gain of 1.23 kg (Figure 2).

Effect of formulated pig diets on some biochemical parameters

As shown in Figure 3, the activities of serum aspartate aminotransferase (AST) in pigs fed feed 60% CPFG and 100% CPFG did not significantly differ ($p>0.05$) compared to that of pigs fed the control diet (0% CPFG). However, the AST activity of the group fed with 40% CPFG diet was significantly ($p<0.05$) reduced compared with that of the control feed group. Also, the serum activities of alanine aminotransferase (ALT) in pigs maintained on diets formulated with the fungi-pretreated cassava peels were not significantly ($p>0.05$) different from that of the control group pigs.

The serum urea level in pigs fed the control diet was significantly lower ($p<0.05$) when compared with all the experimental groups (Figure 4). Serum creatinine level in pigs fed the control diet was, however, not significantly different ($p>0.05$) when compared to those of other experimental groups (Figure 4). Levels of serum albumin in pigs fed the formulated feeds containing the fungus-pretreated cassava peels were significantly higher ($p<0.05$) than those of the control group pigs (Figure 5). The value ranged from 3.4 mg/dl in the control diet group (0% CPFG) to 8.8 mg/dl in pigs maintained on diet formulated with 100% CPFG.

Similarly, serum calcium level was observed to be significantly higher ($p<0.05$) in pigs fed the respective formulated experimental diets compared to the control group (0% CPFG). The highest serum calcium level was observed in pigs fed with feed containing 100% CPFG (Figure 5).

DISCUSSION

The ability of fungi to degrade cassava fiber has been reported in literature (Ofuya and Nwajiuba, 1990; Iyayi and Losel, 2001). The report of Ofuya and Nwajiuba (1990) revealed successful biodegradation of the fibrous by-products of cassava tuber processing (cassava peels) by *Rhizopus* species. In their study, over 35% of the original cellulose content of the substrate was lost in solid state fermentation. *A. niger* grown on rye grass straw was also reported by Han (1978) to produce similar results. The increase in crude protein value of the degraded cassava peels in the present study was partly due to the ability of the fungal digestive enzymes to increase the bioavailability of the protein hitherto encapsulated by the cell. The increased protein content in feeds containing cassava peels pretreated with *A. niger* CSA35 is also in agreement with the findings of Bachtar (2005) who reported an increase in crude protein when *A. niger* was inoculated on sago fibre and cassava fibre, resulting to 16.5 and 18.5% protein increase, respectively. The increase in the energy value of the diets observed in this study may be due to the ability of the fungus to breakdown the starch and non-starch polysaccharide contents of the cassava peels into monomeric sugars, thereby making the diets easily metabolisable (Iyayi and Aderolu, 2004; Balagopalan, 1996). The percentage increase in carbohydrate content and decrease in fibre content observed in the formulated diets was in consonance with the report of Olowofeso et al. (2003) who described the effects of *S. cerevisiae* (yeast) and dietary fiber sources on the diets of growing pigs. Another study by Oboh and Akindahunsi (2003) reported biochemical changes in cassava products (flour and gari) after subjection to *S. cerevisiae* solid media fermentation. A short-term (21 days) feeding trial carried out on 85 to

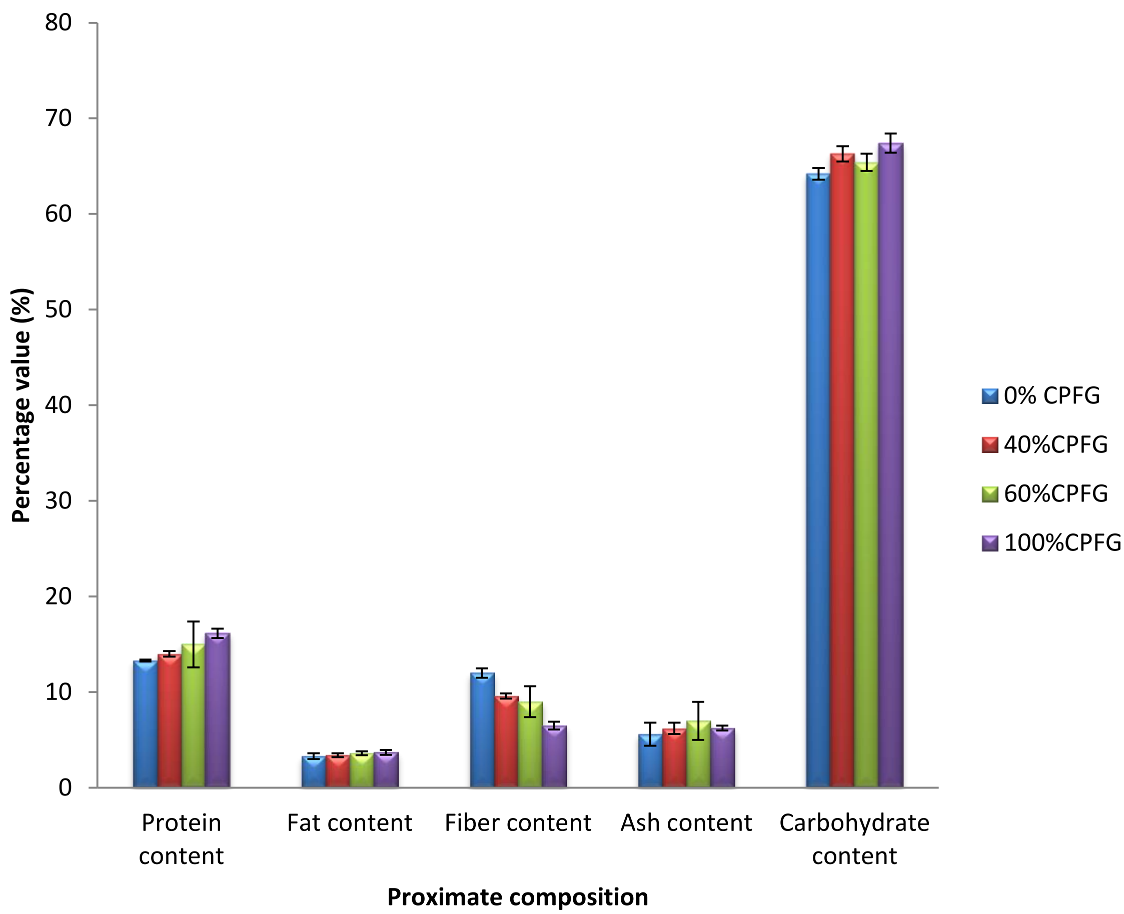

Figure 1a. Proximate composition of the formulated experimental pig feeds. CPFG denotes cassava peels pre-treated with fungus.

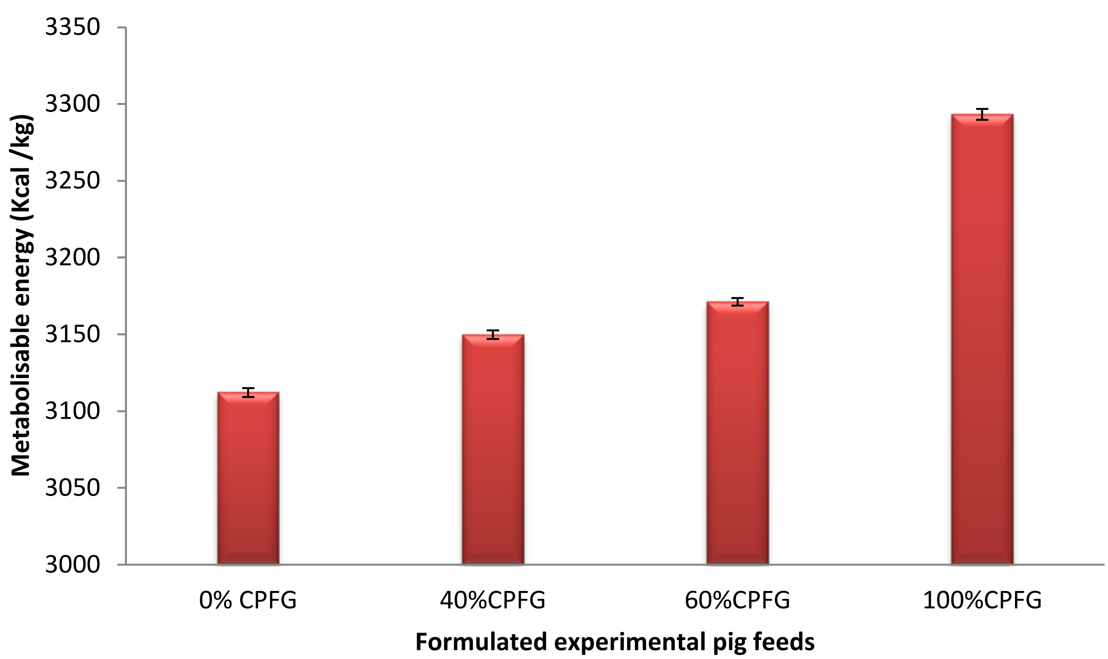

Figure 1b. Metabolisable energy (Kcal/kg) of the formulated pig feeds. CPFG denotes cassava peels pre-treated with fungus.

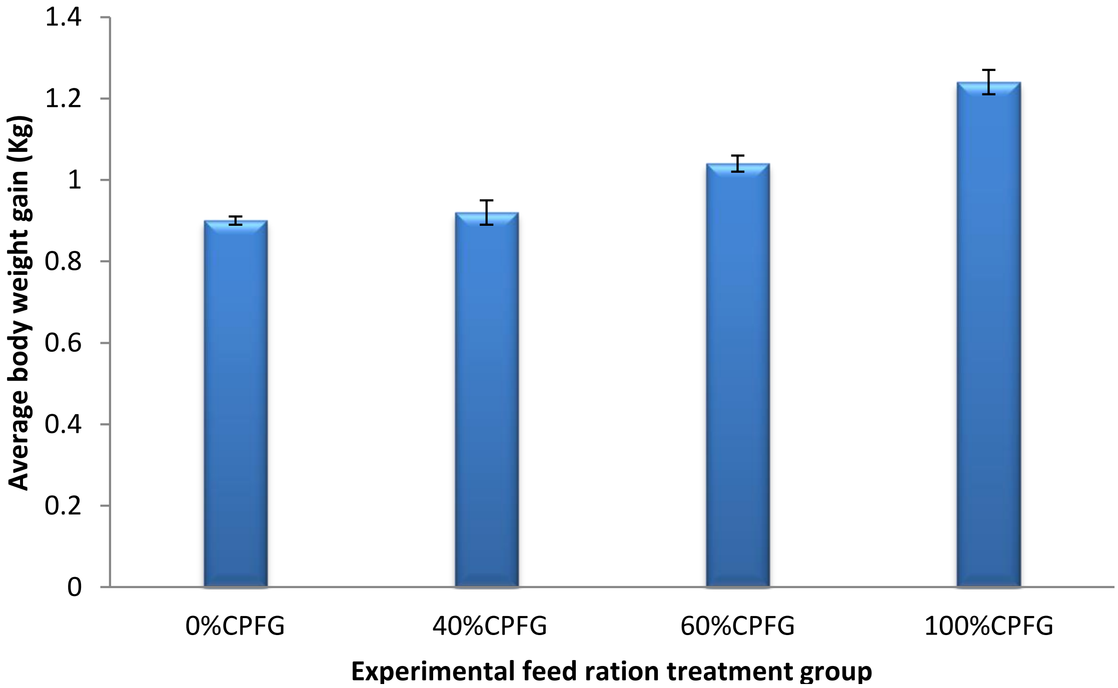

Figure 2. Effect of feed pretreatment on the average body weight gain (Kg) of the experimental pigs. CPFG denotes cassava peels pre-treated with fungus.

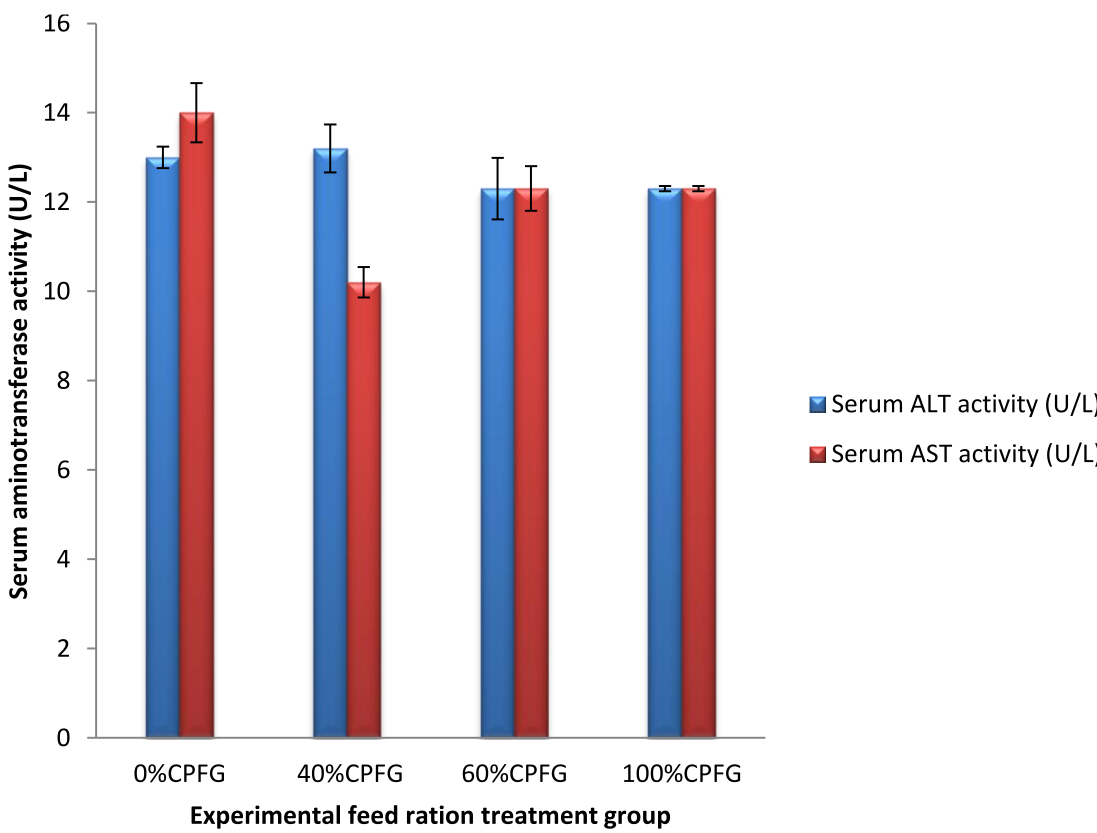

Figure 3. Effect of feed pretreatment on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (U/L) in the experimental pigs. CPFG denotes cassava peels pre-treated with fungus.

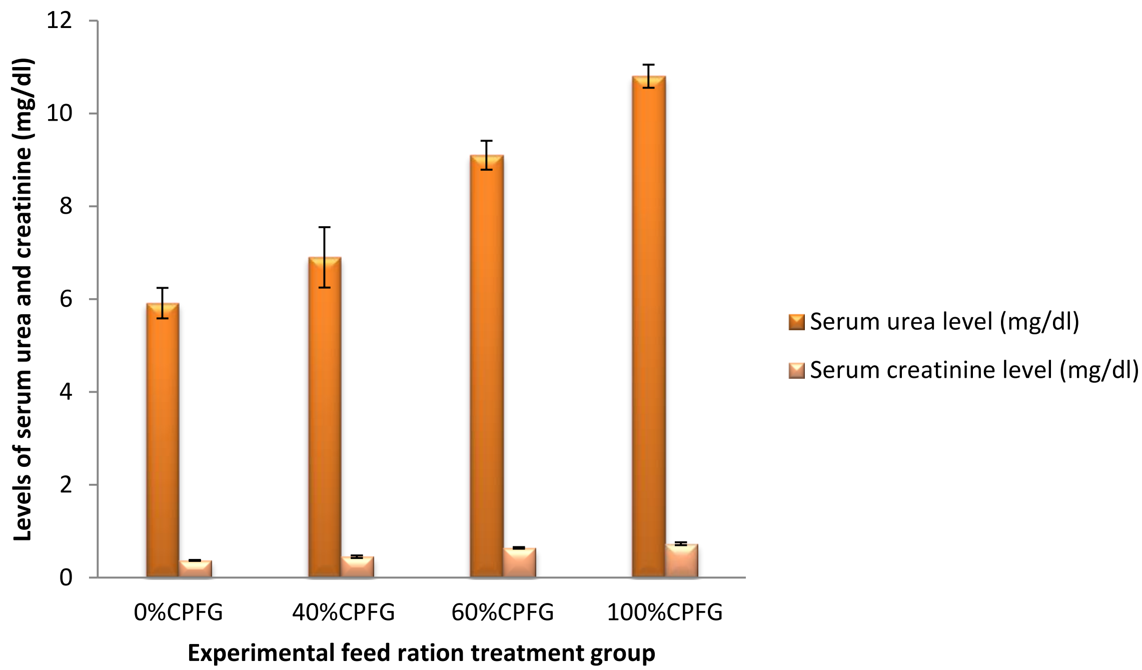

Figure 4. Effect of feed pretreatment on serum urea and creatinine levels (mg/dl) in the experimental pigs. CPFG denotes cassava peels pre-treated with fungus.

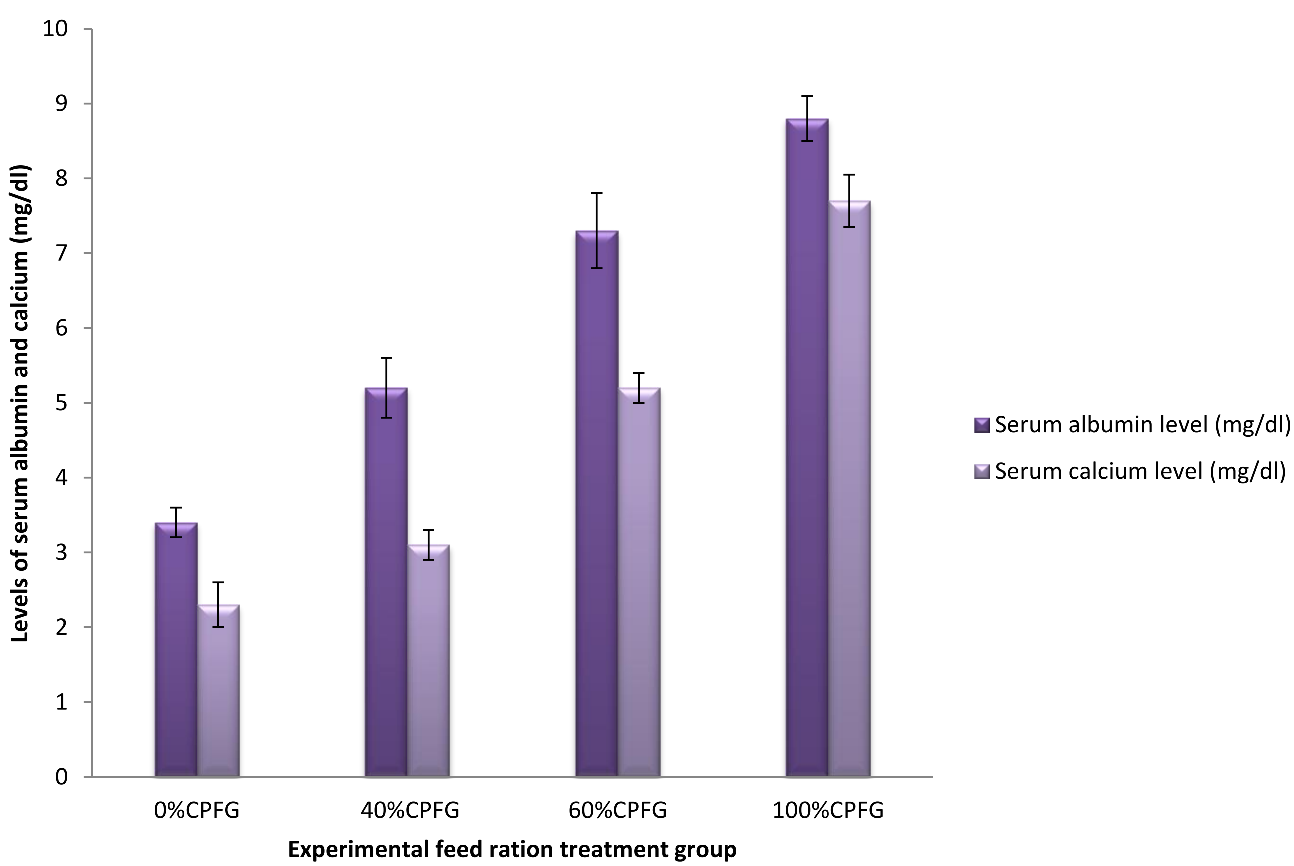

Figure 5. Effect of feed pretreatment on serum albumin and calcium levels (mg/dl) in the experimental pigs. CPFG denotes cassava peels pre-treated with fungus.

95 day-old swine using the formulated feeds showed that pigs fed diets containing cassava peels pretreated with *A. niger* CSA35 had significantly higher weight gain than those fed control diet. The efficiency of protein and feed utilization was reported to decrease with increased fibre content in diets (Adesehinwa, 2007). Thus, the greater weight gain of the pigs maintained on diets formulated with fungal-degraded cassava peels compared to the control in the present study supports this fact since the formulated feeds containing fungus-pretreated cassava peels had lower fibre contents relative to the control feed and that led to their efficient utilization than the control. The increase in weight of the pigs could also be attributed to the fact that the viscosity of the feed along the gastro-intestinal tract of the birds was reduced. Undegraded agro-industrial by-products are able to increase viscosity of the digesta and the transit time in the gastro-intestinal tract which can lead to increase in the size and stability of the unstirred layer at the mucosal surface of the digestive tract (Bedford, 1995). This reduces the contact between the feed and the digestive enzymes and slows the uptake of sugars, amino acids and lipids in the foregut, resulting in the impaired digestibility of the major nutrients (Bedford, 1995; van der Klis et al., 1995). This may result in poor weight gain by livestock fed undegraded feed materials by-products like cassava. There is also cumulative evidence that increasing viscosity of the digesta promotes bacterial proliferation, which is to the detriment of overall digestive efficiency and by implication, may affect the body weight gain of a livestock (Choct et al., 1995). The improvement in the utilization of the biodegraded cassava peels may ultimately have resulted in improvement of the body weights of the fed pigs.

The influence of diets on haematological and serum biochemical variables has been established (Mafuvadze and Erlwanger, 2007). Feeding of pigs with the experimental diets formulated in the present study did not induce elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the pigs. This indicated that the integrity of the liver as a delicate internal organ in the pigs was not jeopardized. Usually, elevated activities of these aminotransferase enzymes (ALT and AST) in the serum are used as indicators or biomarkers of hepatocellular injury or damage (Emenalum et al., 2009; Adeyemo and Sani, 2013). The results of serum aminotransferase activities obtained in the present study showed that the formulated pig diets using *A. niger* CSA35 pre-digested cassava peels were not only able to meet the requirement of the growing pigs but also did not pose toxicity threat to the pigs' internal organs especially the liver within the duration of the study. Further researches on clinical effect would, however, be required since serum urea level in pigs maintained on the experimental diets was observed to be significantly higher ($p<0.05$) compared to control group pigs (0% CPFG).

Serum calcium and albumin levels were observed to be significantly lower ($p<0.05$) in control diet group pigs compared to the respective formulated-diet group pigs. Fermentation has also been shown to increase the mineral (ash) content of cassava peels (Olowofeso et al., 2003; Oboh and Akindahunsi, 2003). The increased serum calcium could be channeled into pathways involving bone formation (osteogenesis) thereby resulting to production of swine with healthy and strong bones over a long period of time (Dawson-Hughes, 2015; Peacock, 2010). High calcium and albumin levels have also been severally reported to be implicated in many physiological reactions required for general wellbeing of living organisms (Roche et al., 2008; van der Vusse, 2009; Bose and Tarafder, 2012; Carmeliet et al., 2015). Specifically, serum albumin is required for transport of short-chain free fatty acids from the blood stream to cells for β-oxidation (during starvation) (van der Vusse, 2009; Shinawi and Abu-Elheiga, 2014) or to adipose tissues (in a well-fed state) where they are stored intracellularly in the form of triacylglycerol molecules or triacylglycerides in lipid droplets, thereby leading to the production of pigs with relatively high adiposity (Dunning et al., 2014). This could be a possible biochemical rationale for the higher body weights observed in pigs fed diets containing cassava peels pretreated with the fungus compared to those fed the control diet.

Conclusion

Pretreating cassava peels with *A. niger* CSA35 could be used to enhance its nutritive value and metabolisable energy. The fermented peels in addition to other feed ingredients could be a source of nutrient for growing pigs which would bring about increase in weight of the pigs without any adverse effect on their serum biochemical parameters. A 60 to 100% inclusion of pretreated cassava peel is recommended given its high effect on the weight gain of growing pigs as recorded in the present study.

Conflict of Interests

The authors have not declared any conflict of interests.

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