

Growth Responses and Nutritional Evaluation of Cassava Peel Based Diet on Tilapia (*Oreochromis niloticus*) Fish Fingerlings

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Abstract: The role of cassava peel as a cheap carbohydrate source capable of supplying adequate calories to Tilapia fish (*Oreochromis niloticus*) fingerlings with improved protein value through fermentation with biomass from palm wine and other protein sources were investigated. The protein content of the cassava peel was enhanced by fermentation using a mixed culture of bacteria (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) and yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*). Microbial population increased with microbial succession after 3 days of fermentation and the crude protein value of the fermented cassava peels increased from an initial value of 5.4 to 17.2%. The decrease in pH from 7.2 to 3.4 enhanced the growth of lactic acid bacteria thus inhibiting proteolysis resulting from the activity of the competing *clostridial* species. The specific growth rates of the fishes appeared to decrease with the progression of the feeding experiment and their weight increased with 70 and 50% for the 8 weeks of the study with respect to soybean and the fermented cassava peel and compared favourably with the control (fish meal) which is 60%. Growth performance results showed that the fishes performed better on soybean meal than on the other diets, though, the observed differences were not statistically significant ($p > 0.05$).

Key words: Palm wine, cassava peels, fermentation and protein, fish fingerlings, growth response

INTRODUCTION

Agricultural processing operations produce waste by-products. Properly managed and utilized agricultural wastes are a natural resource that can produce economic returns. The use of cassava and its wastes in livestock feeding has been limited, due to the presence of toxic cyanogenic glucosides, nutrients deficiency, high fibre and ash content of the peels. Cassava peels hydrolysate could serve as a good substrate for the production of value-added products. There exists a great potential in the use of microorganisms for the production of high quality feedstuffs from the abundantly available agro-industrial wastes. Cassava peels by virtue of its relatively low cost and abundance in developing nations is considered to be suitable substrates for microbial fermentation and protein enrichment (Ubalua, 2007). Processing of cassava wastes to meet minimum requirements for incorporation into commercial livestock feed production, would certainly relieve the pressure on demand for available cereal grains. The high-energy value of cassava makes it a very

attractive carbohydrate ingredient in animal diet. The low protein content of cassava tubers (0.7-1.3% fresh weight) is a disadvantage, restricting the use of cassava as animal feed, but this can be improved on by upgrading the feed with protein additives, such as soybean, or, by using microbial techniques or both.

Fermentation has been identified as one of the less expensive means of increasing the protein quality of cassava and cassava wastes. The use of microorganisms to convert carbohydrates, lignocelluloses and other industrial wastes into foodstuffs rich in protein is possible due to the following inherent nature of microorganisms:

- (a) Ability to multiply rapidly.
- (b) Their amenability to modification genetically for growth on a particular substrate under particular cultural conditions.
- (c) They have high protein content varying from 3.5- 60%.
- (d) They have growth versatility in both slurry and on solids.

- (e) Their nutritional values are as good as other conventional foods rich in protein.

Several organisms and fermentation methods have been investigated to increase the protein content of cassava and cassava wastes using solid-state fermentation (Daubresse *et al.*, 1987). A solid-state fermentation process for the protein enrichment of cassava flour and cassava starch factory wastes using the fungus *Trichoderma pseudokoningii* Rifai was developed by Balagopalan (1996). The highest increase in protein content was observed to be 14.32 g 100 g⁻¹ dry matter from an initial 1.28 g 100 g⁻¹ dry matter. Feeding experiments on poultry showed the potential for protein-enriched cassava feed using microbial techniques (Balagopalan *et al.*, 1991). Careful formulation of the cassava diet is important to nutritionally balance the feed.

Tilapia fish (*Oreochromis niloticus*) is a favourite delicacy in Nigeria (Ubalua and Ezeronye, 2007). It is important in aquaculture due to some good attributes like general hardiness, resistance to diseases, relative fast growth, high tolerance to low water quality, high fecundity and its general acceptability by consumers. Eyo (2003) reported that fish meal is the best protein source for fish feeds due to its high protein content and excellent array of amino acids. However, the ever increasing cost of it in the tropics coupled with its scarcity has spurred diversification of research for alternatives. Furthermore, Eyo (1990) proposed that soybean meal could be used to replace fish meal partially or completely in fish diet. Varying results have been obtained by many researchers on the use of plant protein sources as replacement. Poor results with cotton seed meal has been reported by some authors while, palm kernel meal experiment by Omoregie and Ogbemudia (1993), demonstrated that high crude fibre present in palm kernel meal reduced digestibility at higher inclusion levels.

Palm wine, also called palm toddy or simply toddy, is an alcoholic beverage consumed throughout the tropics and appears as a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri*. The unfermented sap is a sweet, colourless syrup containing about 10-12% sugar, which is mainly sucrose. Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products (Obire, 2005), whereas, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organisms. Several yeasts and bacterial flora have been implicated in its fermentation process and are reported to originate from several

sources including the tapping instruments, containers and the environment (Orimaiye, 1997; Nesler *et al.*, 2004). Uzogara *et al.* (1990) and Iheonu (2000) have reported on the nutritional, medical, religious and social uses of palm wine which have increasingly enhanced the demand for this natural product by over 10 million people in Nigeria.

This study was therefore, designed to investigate the growth and nutritional effect of a cassava peel microbial enrichment technique that could be adapted and upgraded for operation for the production of enriched cassava peel based fish feed. Mixed cultures of *Lactobacillus delbruckii*, *Lactobacillus coryneformis* and *Saccharomyces* sp. isolated from soil, utensils and grating machines associated with cassava mash production as earlier reported by Okafor (1998) and Oboh (2006), were substituted with yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and bacteria (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) all from palm wine..

MATERIALS AND METHODS

Fermentation of Cassava peels/palm wine biomass:

Freshly harvested cassava (NR 8082) roots from National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Nigeria farm were washed with water and peeled. The peels were again washed and dried in a carbolite oven (model S 30 2RR England) at a temperature of 65°C until crispy. They were subsequently pulverized with Thomas Wiley Mill (model ED-5 USA) to a fine powder and stored in an airtight container. A 50 L of fresh palm wine was bought from a local Oye market, Nimo, in Njikoka local government Area, Anambra State, Nigeria. Four different (A, B, C and D) 5 L bottles were 3-quarter filled with the palm wine and 171 g LG⁻¹ of sugar was added to each of the bottles to build up the microbial biomass. Additionally, 32.5 mL LG⁻¹ of vitamin B complex was also added to each of the 5 bottles to supplement the nutrients. To bottles A and B, 150 mg LG⁻¹ of chloramphenicol was added to inhibit bacterial growth. Their temperature, Optical Density (OD) and pH were recorded. The bottles were left standing for 1 day before 120 g of the cassava peel powder were added to each of the 5 fermentation bottles. They were thoroughly agitated to ensure uniform mixing and aeration. Agitations of the bottles were done twice daily for the 14 days the mixtures were allowed to ferment. Temperature, Optical Density (OD) and pH of the fermenting mixtures were determined every day for the 14 days after which the microbial biomass was harvested.

Harvesting and drying: The bottles and the contents were allowed to settle and the supernatants discarded leaving behind the creamy-white slurry at the bottom (biomass). The biomass were each poured into a clean sterilized white cloth bag and squeezed. They were then dried in an oven at 60°C for 48 h. The biomass were separately ground and weighed to determine the yield and stored in a refrigerator. The biomass from bottles (C and D) was used for the experiment.

Diet preparation: Diet preparation was done by the methods of Tacon (1987) with some modifications. Three iso-nitrogenous and iso-calorific diets were prepared with pure white fish meals, soybean meal and fermented cassava peel/palm wine biomass. Each of these served as the main protein source in the diets. The diets were formulated at 21% crude protein level. Bone meal, oyster shell, oil, vitamins (premix), salt and maize were also added before extrusion and pelleted with a table-top manual pelleting machine (Canasta per *alimenti* ACTEA, Italy). The detailed composition of the diets is as shown in Table 1.

Proximate analysis of the experimental cassava based diet: The dried solid paste obtained after washing twice in normal saline (0.6% NaCl) contained the fermented cassava peel and the palm wine biomass. After overnight desiccation at 60°C, this paste (0.2 g) was weighed and its Kjeldahl protein-nitrogen content was measured by the Nessler method Raw *et al.* (1975), after mineralization in the presence of concentrated sulphuric acid (H₂SO₄). The protein content was determined from the amount of ammoniacal nitrogen multiplied by 6.25. The crude fibre, lipid, ash of the experimental diet were determined by the methods described by the Association of Official Analytical Chemists (1995). Dry matter was determined by the air-oven methods as described by Cockerel (1975). Results are as shown in Table 2.

Table 1: Percentage composition of experimental diets

Ingredients	Fish meal diet	Soybean meal diet	Fermented cassava peel/palm wine
Fish meal	26.94	-	-
Bone meal	3.00	3.00	3.00
Soybean	-	39.97	-
Oyster shell	2.00	2.00	2.00
Salt	0.50	0.50	0.50
Fermented cassava peel/palm wine biomass	-	-	84.66
Oil	3.00	3.00	3.00
Premix	0.25	0.25	0.25
Maize	64.31	51.28	6.60

Adapted from Tacon, 1987

Feeding trials: Feeding trials were conducted with the diets on Tilapia fingerlings (*Oreochromis niloticus*) in concrete tanks, each measuring 0.8 m×0.8 m×0.6 m (Beck *et al.*, 1979). All experiments were conducted using the hatchery facilities of the African Regional Aquaculture Centre (ARAC), ALUU, Port Harcourt, Nigeria. The fingerlings were held for 1 week in the tanks for proper acclimatization before the commencement of the experiment. Six tanks each containing 10 fingerlings of Tilapia (*O. niloticus*) were used. Two tanks were used as replicates for each diet. The total length and weight of the test fish were measured before stocking. They were held in fresh water obtained from a borehole in the ARAC compound. The fish were fed on a daily ration of 5% body weight of the respective diets. The daily ration was usually divided into two and dispensed in the morning (about 9.00 h) and late afternoon (about 16.00 h). Water exchange was carried out once a week. Bi-weekly sampling of the test fish was carried out and during this time the total length and weight of the fish were measured to determine growth. The daily ration was then adjusted to accommodate recorded weight gain. The tanks were then thoroughly cleaned and filled with fresh water before re-stocking the fish.

RESULTS

The microbial successions in the fermenting cassava peel/palm wine biomass are as shown in Table 2. The early fermenting organisms (24 h) were identified as *Streptococcus lactis*, *Lactobacillus plantarum*, *Goetrichum candidum*, *Candida tropicalis*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Leuconostoc mesenteroides*. As the fermentation progressed into the second day (24-48 h) there was a rapid increase in the number of lactic acid bacteria.

Proximate composition of experimental feed: The results of the proximate analysis of the experimental diets are as shown in Table 1. The findings showed that the percentage crude protein of fish meal diet was 42.6%; 40.8% for soybean meal and 32.6% for the palm wine biomass/fermented cassava peel. Crude fibre content for

Table 2: Proximate composition of the diets

Component (%)	Fish meal	Soybean	Palm wine biomass/fermented cassava peel
Dry matter	89.3	91.2	90.5
Crude protein	42.6	40.8	32.6
Crude fibre	2.4	2.8	24.2
Lipid	11.9	15.5	2.8
Ash	12.5	10.0	5.9

The results are means of three different determinations

Table 3: Microbial profile of the fermenting cassava peel/palm wine biomass

Fermentation period (h)	Bacteria TV (cfu mL ⁻¹ ×10 ³)	Fungi/yeast TVC (cfu mL ⁻¹ ×10 ³)
0-24	<i>Lactobacillus plantarum</i>	1.6
	<i>Leuconostoc mesenteroides</i>	1.4
	<i>Streptococcus lactis</i>	1.2
24-42	<i>Lactobacillus plantarum</i>	2.72
	<i>Leuconostoc mesenteroides</i>	2.74
	<i>Streptococcus lactis</i>	2.48
48-72	<i>Lactobacillus plantarum</i>	2.69
	<i>Streptococcus lactis</i>	2.48
	<i>Candida tropicalis</i>	1.05

The above results were based on three replications

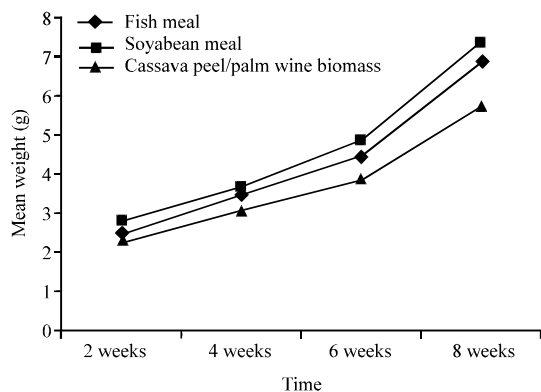


Fig. 1: Effect of different dietary protein sources on the weight of Tilapia (*Oreochromis niloticus*) fingerlings

the fish meal, soybean and palm wine biomass/fermented cassava peel were 2.4, 2.8 and 24.2%, respectively.

Feed performance and acceptability: The results of the eight weeks feeding trials showed that the three different iso-nitrogenous, iso-calorific pelleted diets prepared with pure white fish meal, soybean meal and the palm wine biomass/fermented cassava peel as the main protein sources were readily accepted by the Tilapia (*Oreochromis niloticus*) fingerlings. The mean weight of the test fish fed on a daily ration of 5% body weight based on a bi-weekly sampling are shown in Fig. 1. The mean weights of the fish fed on fish meal diet ranged between 1.84 g (stocking) and 6.94 g. This gave a 64% increase in weight within the 8 weeks period. The mean weight of the fish fed on the soybean meal diet ranged between 1.84 g (stocking) and 7.43 g (69.9% weight increase) while the mean weight of the fish fed on the palm wine biomass/fermented cassava peel diet ranged between 1.84 g (stocking) and 5.80 g (50% weight increase). Based on the above results, the test fish appeared to have accepted the soybean meal diet better than the other diets. The final weight of the fish fed on soybean diet was 7.43 g, that from the fish meal diet was 6.94 and 5.80 g for the fish fed on palm wine

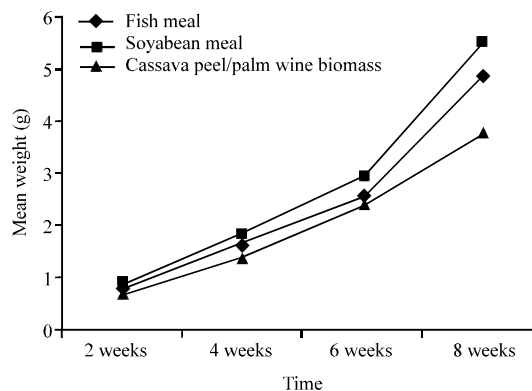


Fig. 2: Growth performance of Tilapia (*Oreochromis niloticus*) fingerlings fed with diets containing different protein sources

biomass/fermented cassava peel diet. The observed differences in mean weight were not statistically significant at 5% probability level.

Growth performance: The growth performance of Tilapia (*O. niloticus*) fingerlings fed on the experimental diets containing different protein sources are shown in Fig. 2. Growth performance was determined as the mean weight gained by the fingerlings during the period of study. Thus, the mean weight gain of the fishes on fish meal diet ranged between 0.77 and 5.1 g, whereas for those fed on soybean meal was between 0.94-5.6 g while, those fed on palm wine biomass/fermented cassava peel diet ranged between 0.64 and 3.96 g. The observed differences in mean weight gain for the fish on the different experimental diets was not statistically significant at 5% probability level.

The effect of the different dietary protein sources on the specific growth rate of the Tilapia fingerlings is as shown in Fig. 3. The highest specific growth rate of 0.68 g day⁻¹ was obtained for fish fed on fish meal diet, 0.66 g day⁻¹ for fish on soybean meal diet and 0.65 g day⁻¹ for fish fed on the palm wine biomass/fermented cassava peel diet. The observed differences in specific growth rate was not statistically

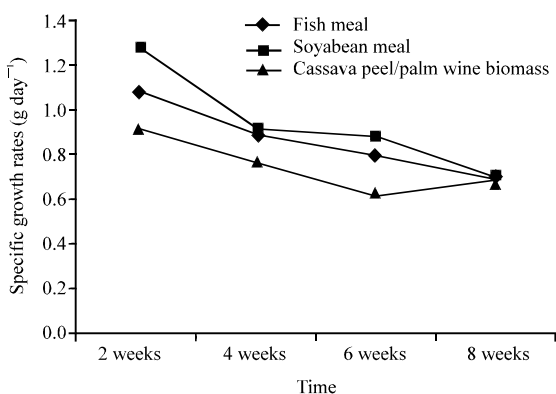


Fig. 3: Effect of different dietary protein sources on specific growth rates (μ) of Tilapia (*Oreochromis niloticus*)

significant ($p>0.05$). The specific growth rates of the fishes appeared to decrease with the progression of the feeding experiment. But no deaths were recorded throughout the experiment as a result of feeding on any of the experimental diets.

Average daily growth rate: The results of the average daily growth rates of the fishes fed on the diets containing different protein sources are as shown in Fig. 4. The fishes fed on fish meal diet recorded an average daily growth rate of 0.055-0.089 g while those on soybean meal diet ranged between 0.067-0.092 g and those on the palm wine biomass/fermented cassava peel diet was between 0.046-0.071 g. There was no statistically significant difference ($p>0.05$) in the observed average daily growth rate between the fishes fed on the different protein rations.

Fulton's condition factor: The results obtained from the determination of fatness (robustness) or well being of the fish as measured by the Fulton's condition factor (k) are as shown in Table 4. The K-factor (Bagenal and Tesch, 1978) was derived as follows:

$$K = \frac{W \times 10^2}{L^3}$$

where, W and L represents the observed total weight and total length of each fish, respectively. The fish fed on soybean meal diet were fatter with a mean K-factor of 1.59 while those fed on fishmeal and palm wine biomass/fermented cassava peel diets were of equal fatness with a mean K-factor of 1.56.

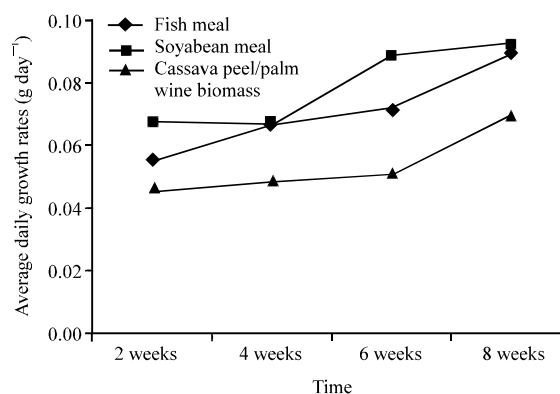


Fig. 4: Average daily growth rate of Tilapia (*Oreochromis niloticus*) fingerlings fed with diets containing different protein sources

Table 4: Fulton's condition factor (k) determinations for the tilapia (*O. Niloticus*) fingerlings on the three different experimental diets

Sampling diet	Fish meal	Soybean	Palm wine biomass/fermented cassava peel
Stocking	1.53	1.53	1.53
1st sampling	1.58	1.60	1.61
2nd sampling	1.54	1.57	1.52
3rd sampling	1.56	1.59	1.57
Final	1.59	1.67	1.55
Mean k-factor	1.56	1.59	1.56

DISCUSSION

The role of cassava peel as a cheap carbohydrate source capable of supplying adequate calories to Tilapia fish (*O. niloticus*) fingerlings with improved protein value through fermentation and other protein sources were investigated. Cassava and cassava wastes are notably low in protein. Thus, the need to fortify the peels for fish feed with cheap microbial protein is therefore a necessity in view of the ever increasing protein demands, the availability of the peels and high prices of soybean and fish meals in most developing nations have further necessitated the search for an alternative. Our interest in using palm wine as our microbial source in this experiment was based on the fact that it contains abundant yeast cells, with reasonable concentration of proteins and its availability. Lactic acid bacteria which appeared at the early stages of the fermentation (Table 3), may have constituted the endogenous microflora of the substrate and thrived on the products of hydrolysis of cassava polymer. A similar observation was noted by Vascoceles *et al.* (1990). These observations were corroborated by Ezeronye (2001), who reported that lactic acid production is usually accompanied with the lowering of pH of the fermenting liquor from 7.2 to 3.4, thus, inhibiting proteolysis resulting from the activity of the

competing *Clostridial* species. At such a low pH, it may have been difficult for the non-lactic bacteria species to thrive, though, the yeast *Candida tropicalis* persisted to the end of the fermentation and the maximum yield of the microbial biomass was observed at a temperature optimum of 28 and 29°C.

The protein value of the palm wine biomass/cassava peel diet increased from an initial crude protein value of 5.4 to 17.2% after fermentation. Expectedly, there was an increased microbial build-up with the consequent conversion of the nutrients in the substrate into microbial biomass. Such must have necessitated the increase in the crude protein value of the palm wine biomass/cassava peel diet. This result conforms with those of Ofuya and Obilor (1993) and Ezeronye (2001) with their documentation of an increase in the crude protein value of cassava peel, a cellulosic substrate, from 5.4 to 16.7% by solid-state fermentation using *Rhizopus* species. The authors found the diet formulated from this protein enriched cassava peel suitable as poultry feed and for monogastric animals, respectively, necessitating the alternative use of cassava peel because it is available and easily degraded and utilized by the cellulolytic microorganisms to produce microbial biomass.

Palm wine biomass/fermented cassava peel, fish meal and soybean all contained crude fibre content of 24.2, 2.4 and 2.8%, respectively with a high crude protein value of 32.6%, 42.6% and 40.8%, respectively (Table 2). The three different iso-nitrogenous and iso-calorific pelleted diets were readily accepted by the Tilapia (*O. niloticus*) fingerlings. Fish fed on soybean meal diet and palm wine biomass/fermented cassava peel had weight increases of 70 and 50%, respectively within the 8 weeks of the study which compared favourably with the control (fish meal) (Fig. 1). Those fed on soybean meal diet were fatter (mean k-factor 1.59) while, those fed on fish meal and palm wine biomass/fermented cassava peel diets were of equal fatness (mean k-factor 1.56) (Table 4). The fishes appeared to have accepted the soybean meal better than the other diets. Growth performance results showed that the fishes performed better on soybean than on the other diets, though the observed differences were not statistically significant ($p>0.05$).

CONCLUSION

In conclusion, though, the soybean meal appeared to be more acceptable to the fingerlings, the palm wine biomass/fermented cassava peel diet compared favourably with the fish meal diet and their nutritional qualities were not at variance with that of the soybean meal. It therefore, suggests that palm wine biomass/fermented cassava peel diet is a viable alternative to soybean meal. Further research is needed in the exploitation of palm wine in fish

feed formulation in view of its availability in this part of the world and its established medicinal and nutritional properties.

ACKNOWLEDGEMENT

We are grateful to the management of Deka Medical Laboratories for their support.

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