Impact of Processing on Cassava Root Tuber and Use as a Replacer of Maize in the Diet of Nile Tilapia, *Oreochromis niloticus*

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Abstract

This study was carried out to investigate the effects of processing methods on the nutritive quality of cassava root tuber and effects on the growth performance, nutrient utilization and nutrient digestibility in the diets of *Oreochromis niloticus*. Cassava tubers (IITA-TMS-I982132) were subjected to four different processing methods namely: sun-drying (SDC), soaking (SC), solid-state fermentation (SSF) and anaerobic fermentation (AFC). Five iso-nitrogenous (35% crude protein) diets were formulated in which maize was replaced at 50% level by each of the processed cassava root meals. Diet 1 (CTR), Diet 2 (SDC50), Diet 3 (SSF 50), Diet 4 (SC 50), and Diet 5 (AFC 50) were fed to 120 fingerlings (3.20±0.50g) *ad libitum* in three replicates. Ether extract (2.00±0.05) was significantly higher ($p<0.05$) in AFC, while cyanide content (3.02±0.03) was significantly higher ($p<0.05$) in SDC. Feed conversion ratio (1.49±0.20) in fish fed Diet AFC was significantly different ($p<0.05$) from the highest (1.61±0.02) in fish fed diet SC. Likewise, percentage weight gain (286.82±3.77%), protein efficiency ratio (1.94±0.24) and apparent net protein utilization (86.85±0.45) were significantly ($p<0.05$) better in fish fed AFC than other treatments. Protein digestibility was statistically higher in fish fed diet AFC50. The study concluded that processing had a significant impact on the nutritive value of cassava tuber while anaerobically fermented cassava root tuber (AFC) at 50% maize replacement among others exhibited better performance, feed utilization and digestibility in the diet of *O. niloticus* fingerlings.

Keywords: Digestibility, Fermentation, cassava root tuber, Nile tilapia

Introduction

One of the conventional major ingredients used in commercial aquafeed is maize which is included between 10-40% by weight in feed production as an energy source (FAO, 2012). This ingredient, however, is not one in which Nigeria has a competitive production advantage. And on the global arena its production is affected by several factors, the most prominent being the recent climate change. Global cereal supply and demand is forecast to tighten considerably in 2012/2013 and will fall by 2.7% from the previous year's record, leading to a 25 million tones contraction in world stocks (FAO, 2012).

The bulk of the maize produced globally is used as biofuels. In the U.S.A, demand for maize as seed stock for ethanol production is a critical factor. Currently, Brazil, the EU and the USA produce 90% of global ethanol as biofuel. Producing a litre of ethanol requires 2.56kg of corn; the current capacity in the USA is 7.1 billion litres requiring 61,580,000 metric tons of corn (Peter *et al.*, 2014). This in addition to China's heavy importation of grains to feed its ever-increasing swine production has placed an all-time higher-demand-than supply pressure on maize (Hardy, 2000).

A substitute crop of note in which the country has a competitive production advantage is cassava. Nigeria is the highest producer of cassava in the world with an ever-increasing capacity to produce more (Olutosin and Barbara, 2019). World cassava output in 2017 was 291,992,646 metric tons out of which Nigeria produced 59,485,947 metric tons or 20% of the global output (Olutosin and Barbara, 2019). This is so because cassava production in Africa is a strategic crop both for food security and poverty alleviation (FAO, 2012). A hardy plant, cassava is highly tolerant to poor soil conditions, drought and pests (Vongsamphanh and Wanapat, 2004). Cassava root production has been increasing steadily since the 1960s and surged in the 2000s (+40% between 1997 and 2007, from 161 to 224 million tons). Its use in animal feeding also grew from 25% in 1997 to 34% in 2007. In 2010, 52% of cassava was produced in Africa, 33% in Asia and 15% in Latin America (FAO, 2011).

The nutritional attributes of cassava make it a very good energy substitute for maize. Cassava roots contain a large amount of starch, ranging from 70 to 85% DM, which increases with the stage of harvesting (~~Ly 1998~~; Régnier, 2011). However, their protein content (typically < 3%) is lower than that of cereal grains (Heuze *et al.* 2016). Cassava can be substituted for cereals at a high level in rations for all classes of livestock, provided that it is supplemented with a nitrogen source (Heuze*et al.* 2016). The fibre content in cassava, which depends on the variety and age of the root, is also extremely low. Usually, its content does not exceed 1.5% in fresh root and 4% in root flour (Gil and Buitrago, 2002), which makes cassava roots highly digestible in all livestock species. Hydrogen Cyanide content may not be a problem, owing to the current improved varieties, and processing (Sanni *et al.*, 2002).

Processing of products is a device aimed at modifying the product, preserving the vital nutrients and increasing its shelf life. There are various methods such as fermentation, drying, and soaking. Fermentation is one of the oldest methods of applied biotechnology, having been used in food processing and preservation as well as beverage production for over 6,000 years (Motarjemi, 2002). Fermentation increases the nutrient contents of food through the biosynthesis of vitamins, essential amino acids and proteins. It improves protein quality and fibre digestibility (Adewusi *et al*., 1999). It also enhances the availability of micronutrients to organisms for utilization and aids in the degradation of anti-nutritional factors (Achinewhu *et al*., 1998). This work is therefore tailored towards the assessment of the viability of replacing maize with differently processed cassava root tuber

Methodology

Experimental design

The experimental design was completely randomized designs. The feeding trial was conducted in rectangular plastic tanks (60 litres) in the hatchery complex of the Federal University of Agriculture, Abeokuta, Nigeria. The tanks were filled to 2/3 of its volume with

water supplied from the university's water reservoir. The system was a flow-through with water exchange rate of 1.5L/min. to sustain an optimal culture environment.
Experimental Fish *O. niloticus* fingerlings were sourced from a reputable fish hatchery in Lagos State, Nigeria. The fish were acclimatized and fed with commercial feed for two weeks before commencing them on the experimental diets.

Processing of cassava root tuber and experimental diets preparation

Cassava tubers (IITA-TMS-I982132) were procured from International Institute for Tropical Agriculture, (IITA) and whole cassava tubers were processed using the following methods:
Sun-dried cassava was prepared by washing freshly harvested cassava tuber and sundried for 2 weeks. Soaked cassava was prepared by washing freshly harvested cassava tubers, soaked with water in a big plastic bowl for 72 h and the water changed every six hours to prevent fermentation, sundried and stored in airtight containers. Solid-state fermentation was carried out in line with the method of Amey, (1987) and Sauti *et al*. (1987). Freshly harvested cassava roots, washed, drained and cut into big-sized parts. These were exposed to the sun for two hours after which they were covered with plantain leaves and left to ferment in this solid state for 96 hours. The ensuing mouldy cassava was scraped off the mould, and sundried. This was then milled into powder and stored in a clean container.

While anaerobic fermentation followed the method of Obasa *et al*. (2017). Anaerobic fermentation was carried out after milling the sundried cassava tuber to powder (595µm) and fermented for 48hrs in a 10L plastic container at room temperature. Fermented Cassava tuber meal was prepared by mixing the meal with water in ratio 1:1 (wt. /vol.) and allowed to ferment at room temperature (28-30ºC) for 48h, after which the pH decreased to a stabilized level (3.7). The temperature of the fermented cassava meal was taken at 12h intervals using mercury in glass thermometer model 2751-K. The fermented meal was then sundried, milled and sieved using a 595µm sieve.

The differently processed cassavas were then analyzed for proximate composition and hydrogen cyanide level. Each of these was used to replace maize at 50% level in a diet containing 35% crude protein level. Control diet contained 100% maize and 0% cassava flour (Table 1). The diets layout was as follows: Diet 1 contained 0% cassava root meal or Control (CTR), Diet 2, 50% sundried cassava root meal (SDC50), Diet 3, 50% solid-state fermented cassava (SSF50), Diet 4, 50% soaked cassava root meal (SC50) and Diet 5, 50% anaerobically fermented cassava root meal (AFC50).

Experimental procedure

One hundred and fifty fingerlings (mean weight 3.20g±1.77) of *O. niloticus* were stocked at 10 fish per tank with each treatment having three replicates. The fingerlings were fed experimental diet ad-*libitum* twice daily at 9.00 h and 17.00 h for a period of 90days. The fish were batch- weighed bi-weekly using sensitive electronic balance (METTLER TOLEDO, PB602). Water quality was monitored weekly for temperature using mercury-in-glass thermometer; dissolved oxygen (DO) using DO meter; pH with a pH meter (E251); and ammonia using Hach water quality analyzer.

At the beginning of the feeding trial, composite samples of 10 whole fish were analyzed while a random sample of 5 fish per tank were analyzed for proximate composition at the end of the feeding period.

Table 1. Gross Composition of Experimental Diet

Ingredients	CONTROL	SDC50	SSF50	SC50	AFC50
Fish meal (72%)	13.14	13.56	13.52	13.60	13.60
Soya Bean Meal	26.28	27.12	27.04	27.20	27.20
Groundnut Cake	26.28	27.12	27.04	27.20	27.20
Maize	26.8	12.35	12.45	12.30	12.30
Processed Cassava Meal	Nil	12.35	12.45	12.30	12.30
Methionine	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Fish Premix	1	1	1	1	1
Vegetable Oil	5	5	5	5	5
Dicalcium Phosphate	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Radar Vitamin. Premix. Supply /100g Diet. Palmat A: 1000Iu; Cholecalcifero (D): 1000Iu; G-Tocopherolacetate (E): 1.1mg; Menacilione (K): 0.02mg; Thiamine B1: 0.63mg; Riboflavin (B2): 0.5mg; Panthothenic Acid: 1.0mg; Phyridoxine (B6): 0.15mg; Cyanocobalamine (B12): 0.001mg; Nicotinic Acid: 3.0mg; Folic Acid: 0.1mg; Ascorbic Acid (C): 0.1mg; Iron (Fe): 0.05mg; Cu: 0.25mg; Mn: 6.00mg; Co: 0.5mg; Zn: 5.0mg; Sn: 0.02mg.

Nitrogen content (crude protein), fat, fibre, ash and moisture content of the diets and composite fish samples were analyzed using AOAC (2000) method.

The metabolizable energy (ME) of each diet was calculated using Atwater's calculation as described by Foster and Smith (1997). M.E (Kcal/kg) = 10[(3.5*CP) + (8.5*CF) + (3.5 NFE)]

Where CP = % Crude Protein, CF = % Crude Fat, NFE = %Nitrogen Free Extract

Growth performance was expressed as the mean weights gain (MWG), Percentage weight Gain (PWG), Daily Growth Rate (DGR), feed conversion ratio (FCR), Protein Efficiency Ratio (PER) followed the methods of Obasa *et al.* (2013).

The calculation formulas are as follows:

$MWG\ (g) = W_{final} - W_{initial}$

$PWG\ (\%) = (W_{final} - W_{initial}) \times 100/W_{initial}$

FCR = (Total dry feed fed)/(Wet weight gain)

PER=Mean Weight Gain/Mean Crude Protein Fed

DGR = DGR (g) = Final weight(g) –

Initial weight(g)/Number of days

%WG= Final body weight(g) – Initial body weight(g) x 100/Initial weight of fish

Digestibility assessment commenced after they had fed for four days. Feaces were collected in each tank by siphoning out immediately after ejection and oven dried at $105^{O}C$ to a constant weight. Protein contents were analyzed using AOAC (2000) method. Determination of chromium iii oxide of the feed and feacal samples in triplicate followed the method of Furakawa and Tsukahara (1966). Apparent digestibility coefficient (ADC) calculation followed the following formulae:

ADC (%) – 1000(1- (% Cr_2O_3 in diet) (% nutrient in feaces) / (% Cr_2O_3 in feaces) (% nutrient in diet).

Water quality parameters did not differ significantly among the experimental compartments. Temperature ranged between 29.0 - 30.5^oC, pH, 7.6 – 8.2, dissolved oxygen, 6.5 – 7.4 and ammonia as ammonia - N (NH3-N) (0.25 - 0.45mg L^1).

Statistical analysis

The data obtained from the research were subjected to a one-way analysis of variance (ANOVA) to determine whether or not there are significant differences among the means of the various treatments($P<0.05$). Duncan Multiple Range Test was used to separate the means further where there were significant differences among means were also tested for significance ($P<0.05$) using Duncan Multiple Range Test (DMRT).

Results

The result of the proximate and cyanide composition of differently processed whole cassava root is presented in Table 2. Ether extract, crude fibre and calculated metabolizable energy values were significantly higher in AFC while crude protein was significantly higher ($p<0.05$) in SSF and significantly lower in AFC. Likewise, cyanide content was statistically higher ($p<0.05$) in SDC and lowest ($p<0.05$) in SSF.

Table 2. Proximate and anti-nutritional composition of differently processed whole cassava root meal

Parameters (%)	SDC	SSF	SC	AFC
Moisture	11.00±1.25	11.50±2.14^a	11.50±2.04^a	11.50±1.66^a
Ether extract	1.00±0.01^b	0.50±0.01^c	1.00±0.00^b	2.00±0.05^a
Ash	0.09±0.01^a	0.05±0.01^b	0.10±0.01^a	0.10±0.01^a
Crude fibre	0.00±0.00^c	0.00±0.00^c	0.01±0.01^b	0.04±0.01^a
Crude protein	3.38±0.01^b	3.99±0.01^a	2.91±0.01^c	2.83±0.02^d
Cyanide	3.02±0.03^a	2.19±0.02^d	2.45±0.01^c	2.55±0.02^b
NFE	81.51±2.50^b	81.76±2.65^c	82.03±3.40^a	80.98±3.20^b
M E	305.08±3.20^b	304.38±2.65^b	305.8±3.20^b	310.34±4.50^a

Means with different superscripts along the rows were significantly different at ($p<0.05$)

The result of the proximate composition of experimental Diets is presented in Table 2. The crude protein and moisture values were statistically similar ($p>0.05$), ether extract and ash contents were significantly higher ($p<0.05$) in diet SC50 while fibre was significantly higher ($p<0.05$) in the Control diet.

Table 3. Proximate composition of Experimental Diets I

Parameters (%)	CTR	SDC50	SSF50	SC50	AFC50
Moisture	10.51±0.06[a]	10.48±0.05[a]	10.62±0.06[a]	10.55±0.06[a]	10.53±0.06[a]
Fat	6.00±0.82[c]	6.00±0.68[c]	8.00±1.10[a]	7.50±0.65[b]	7.20±1.22[b]
Crude fibre	3.45±0.12[a]	3.21±0.21[b]	3.19±0.24[c]	3.10±0.32[d]	3.11±0.22[d]
Ash	7.50±0.92[c]	7.35±0.73[d]	7.75±0.33[b]	8.00±0.67[a]	7.55±1.10[c]
Crude protein	34.95±0.20[a]	34.90±0.24[a]	34.98±0.22[a]	34.96±0.32[a]	34.98±0.22[a]

Means with the same superscripts along the rows were not significantly different at (p>0.05)

The result of the proximate composition of the experimental fish, *O. niloticus* is presented ion Table 4. Significantly low-fat content was observed in initial fish while the values of fat, protein, moisture and crude fibre were statically similar (p>0.05) in all fish fed the experimental diets.

Table 4. Proximate composition of *Oreochromis niloticus* fed differently processed cassava root meal-based diet

Parameters	Initial	CTR	SDC50	SSF50	SC50	AFC50
Moisture	9.50±0.64[a]	9.80±0.50[a]	10.25±0.34[a]	9.85±0.32[a]	10.50±0.44[a]	10.65±0.55[a]
Fat	9.50±0.44[b]	10.50±0.66[a]	10.85±0.40[a]	10.95±0.42[a]	11.65±0.74[a]	10.35±0.62[a]
Crude fibre	2.75±0.02[a]	2.95±0.07[a]	2.88±0.06[a]	3.14±0.04[a]	2.98±0.05[a]	2.97±0.05[a]
Ash	10.40±1.80[c]	15.50±1.55[ab]	15.75±1.85[ab]	16.10±1.90[b]	17.65±2.45[a]	16.50±1.88[b]
Crude protein	53.45±3.50[b]	57.30±2.01[a]	57.33±1.76[a]	57.51±2.30[a]	57.25±3.45[a]	57.99±2.40[a]

Means with the same superscripts along the rows were not significantly (p>0.05) different.

Growth performance, nutrient utilization and survival of *O. niloticus* fingerlings fed differently processed cassava root meal based he results of the growth performance, nutrient utilization and survival of *O. niloticus* fed with differently processed cassava root meal-based diet are presented in Table 7. Growth performance parameters such as mean weight gain, feed conversion ratio and percentage weight gain were significantly higher (p<0.05) in fish fed diet AFC50 than in fish fed the control diet. Likewise, nutrient utilization parameter like protein efficiency ratio (PER) was significantly higher in fish fed diet AFC50 while values of apparent net protein utilization (ANPU) observed in all fish fed the experimental diets were statistically similar (p>0.05). Apparent protein digestibility value in fish fed diet AFC50 was however significantly higher (p<0.05) than in fish fed other experimental diets.

Table 7. Growth, nutrient utilization and digestibility *Oreochromis niloticus* fed differently processed whole cassava root meal

Parameters	CTR	SDC50	SSF50	SC50	AFC50
Initial mean weight (g)	3.14±0.53	3.25±0.50	3.20±0.37	3.20±0.30	3.29±0.78
Final mean weight (g)	11.57±1.06^{d}	12.14±1.02^{b}	11.97±0.15^{c}	11.57±0.14^{d}	12.48±1.78^{a}
Mean weight gain (g)	8.43±1.36^{c}	8.88±0.53^{b}	8.75±0.25^{bc}	8.37±0.27^{c}	9.19±1.22^{a}
Mean feed intake (g)	12.96±0.16^{c}	13.22±0.24^{ab}	13.86±0.12^{a}	13.43±0.30^{b}	13.55±0.13^{bc}
Feed conversion ratio	1.57±0.28^{b}	1.49±0.06^{c}	1.59±0.05^{b}	1.61±0.02^{a}	1.49±0.02^{c}
(%) weight gain	277.47±60.40^{b}	275.80±27.19^{b}	274.46±36.91^{b}	263.41±34.10^{c}	286.82±61.87^{a}
ANPU (%)	84.94±2.83^{a}	84.24±0.73^{a}	83.78±0.39^{a}	73.83±3.16^{b}	86.85±0.45^{a}
Protein efficiency ratio	1.86±0.31^{b}	1.92±0.08^{ab}	1.81±0.06^{bc}	1.78±0.02^{c}	1.94±0.25^{a}
App. protein digestibility (%)	79.01±2.20^{b}	78.95±2.75^{b}	78.45±2.50^{b}	73.55±1.75^{c}	81.85±1.50^{a}
Daily growth rate (%)	0.12±0.02^{b}	0.13±0.01^{a}	0.13±0.01^{a}	0.12±0.00^{b}	0.13±0.02^{a}
Survival rate (%)	96.67±5.77^{b}	96.67±5.77^{b}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Means with different superscripts are significantly different ($p<0.05$).
ANPU = Apparent net protein utilization

Discussion

Results of this study revealed that the processing methods significantly affected the nutritive value of cassava root tuber. This is similar to result of Adriani *et al.* (2012) who reported an increased value in the crude protein (CP) of cassava root tuber subjected to solid state fermentation. This increase in the CP might be as a result of the growth of mold on the cassava roots which was also in line with the observation of Amey (1987) on cassava. There was also a reduction in the values of ether extract, ash and crude fibre contents which was significantly lower ($P<0.05$) than the other processing methods. This result was in agreement with the report of Andriani*et.al*. (2012) who reported that the enzymatic process of the microbes decreased the content of crude fat, water, ash, hydrogen cyanide (HCN), starch, lignin, cellulose and hemicelluloses. The lowest HCN found in solid state fermentation (SSF) was in agreement with the findings of Andriani *et al.* (2012), they reported a lowering effect of microbial action on the HCN content of cassava. Likewise, Akinyele and Agboro (2007) reported that fermentation increases the nutrient contents of food through the biosynthesis of vitamins, essential amino-acids and proteins. It improves protein quality and fibre digestibility. It also enhances the availability of micro nutrient to organisms for utilization and aids in the degradation of anti-nutritional factors (Achinewhu *et al*., 1998). However, the enzymatic process of the microbes which decreased the starch content of cassava processed through SSF could have been responsible for its significantly low

metabolizable energy (M.E) (Adedoyin, 2014).

The significantly better ($p < 0.05$) performance in growth in fish fed anaerobically fermented (AFC50) diet in this study was expected. This, according to Mehta *et al.* (2012), is because anaerobic fermentation has been observed to contribute several advantages to food which include addition of new tastes, flavours, aromas, enhancement of the nutritional value of food by increasing digestibility and production of vitamins and elimination of toxic substances. This position was also corroborated by Ali *et al.* (2003) who stated that fermentation is one of the processes that decrease the level of anti-nutrient in food and increases the starch digestibility, protein digestibility and nutritive value. Likewise, several studies have reported that the use of fermented vegetable products could enhance non-specific immune responses, growth performances, feed efficiency and digestibility of nutrients in fish as well as terrestrial animals (Ashida and Okimasu, 2005; ~~Kim *et al*., 2007; Yang *et al*., 2007;~~ Min *et al*., 2009). Fermentation has also been suggested as an alternative means to improve the nutritional value of vegetable protein sources for fish feed (Shimeno *et al*., 1994).

The significantly higher apparent digestibility observed in fish fed diet AFC50 than the other treatments indicated a better utilization by fish fed the experimental rations. This was supported by the works of Orire and Ricketts (2013) who reported that *O. niloticus* digested the protein in fermented melon shell meal-based diet as high as 93.2%. This might be as a result of degradation of complex compounds into simpler ones which made the nutrient readily available for the organism and also the removal of anti-nutritional factor by the fermenting microorganisms. This position was corroborated by Mehta *et al.* (2012) who reported that fermentation could enhance the nutritional value of a food product through increased vitamin levels and improved digestibility. They further stated that many fruit and vegetable products contain toxins and anti-nutritional compounds that can be removed or detoxified by the action of microorganisms during fermentation. This detoxification and destruction of undesirable factors such as phytates, tannins, cyanide and polyphenols present in raw foods lead to improved digestibility of such food substances (Sharma and Kapoor, 1996). Likewise, Quan *et al.* (2018) observed increasing content of flavonoids and phenols and antioxidant properties in wampee [*Clausen alansium* (Lour.) Skeel)] leaves as fermentation days increased up to nine days, which was mainly attributed to increase in alcoholic content of the product.

The high survival percentage recorded in this study indicated that feeding *O. niloticus* fingerlings with anaerobically fermented cassava meal did not lead to high mortality of the fish. Although FCR in fish fed Diet SDC50 was better, however, protein digestibility was significantly ($p < 0.05$) higher in fish fed Diet AFC50. These trends were similar to the observations of many workers as Refstie *et al.* (2005) who reported a better performance in Atlantic Salmon, *Salmon salar* when fed fermented white flakes over the fish fed unfermented white flakes. This was equally in agreement with the works of Ogunji *et al.* (2014) who found out that catfish, *C. gariepinus* fed fermented African yam bean *Sphenostylis stenocarpa* at 45% level performed better than the control which had no fermented portion. The significantly better ($p < 0.05$) performance in growth in fish fed anaerobically fermented diet in this study was expected. This, according to Mehta *et al.* (2012), is because anaerobic fermentation has been observed to contribute several advantages to food which include addition of new tastes, flavours, aromas, enhancement of the nutritional value of food by increasing

digestibility and production of vitamins and elimination of toxic substances. This position was also corroborated by Ali *et al.* (2003) who stated that fermentation is one of the processes that decrease the level of antinutrients in food and increases the starch digestibility, protein digestibility and nutritive value. Several studies have reported that the use of fermented vegetable products could enhance non-specific immune responses, growth performances, feed efficiency and digestibility of nutrients in fish as well as terrestrial animals (Ashida and Okimasu, 2005; Min *et al*., 2009).

Conclusion

We therefore conclude that the different processing methods significantly affected the cassava root tuber and the solid-state fermentation method (SSF) significantly improved its nutritive value through significant reduction of cyanide and increase in protein content. Also, anaerobic fermentation was more suitable to replace maize at 50% replacement level, as it sustained significantly better growth, feed utilization and apparent protein digestibility in the diet of Nile tilapia, *O. niloticus* fingerlings.

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