IJAAAR4 (1&2): 67-76,2007 *International Journal of Applied Agricultural and Apicultural Research*
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Replacement Value of fermented millet *(Pennisetum americanum)* for maize in the diets of African Cat fish *(Clarias gariepinus)* fingerlings

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Abstract

The replacement value of fermented millet for maize in the diets of Clarias gariepinus fingerlings reared in a recirculation system was determined. Five isonitrogenous diets were formulated to contain graded levels of fermented millet meal replacing 0, 20, 40, 60 and 80% of maize and fed to triplicate groups of fingerlings weighing 1.28± 0.2g.
The feeding lasted for 12 weeks. Although, the feeds containing fermented millet were acceptable to the fish, the results showed that the diets significantly P<0.05 affected fish performance. There were significant reductions in feed conversion ratio and nutrient utilization efficiency with increasing fermented millet meals. However, highest percentage weight gain and lowest feed conversion ratio were observed on fish fed 20% replacement level, even though, growth depression occurred with increasing dietary levels of fermented millet (P>0.05).

Keywords: Pennisetum americanum, Clarias gariepinus, Fermentation, Performance, blood.

Introduction

Protein is the most expensive component in fish feed and one way of reducing the protein levels in the diets to minimal levels is by adding suitable energy sources (Falaye and Oloruntuyi, 1998). Maize has been a traditional energy source in formulated feeds. However, rising costs and accompanying scarcity is making it increasingly uneconomical as feed grains to animals including fish. Therefore, there is an intensive search for other suitable ingredients that can be used as protein saving energy sources in fish production. A number of unconventional feedstuffs have been investigated as potential energy sources in the diets of cultured fish. Cassava root meal has been incorporated up to 60% as an energy source in pelleted feeds for *Oreochromis niloticus* (Wee and Ng, 1986).

Cocoa pod husk meal has been shown to replace maize in the diet of cichlid, *O. niloticus* and catfish *Glorias isheriensis* (Fagbenro, 1992). Likewise, plantain peel meal has been shown to replace up to 25% of maize in the diet of *C. gariepinus* without adversely affecting the

growth and health status of the fish (Falaye and Oloruntuyi, 1998).

Millet is grown extensively around the world and its protein content is higher than other cereals grown under similar conditions (Railey, 2004). Despite, the higher level of amino acid, the antinutritional factor-phytic acid present in millet requires elimination by appropriate processing technique. Some micro organisms like *Aspergillus niger* have been found as suitable agents for the removal of toxic components and subsequently increasing the protein levels (Essers et al, 1994; Tewe *et al*, 1999).

In view of the increasing demand for fish and high cost of conventional feed ingredients, it is necessary to investigate the replacement value of fermented millet for maize in the diets of *C. gariepinus* fingerlings. The study is therefore aimed at providing information on replacement value of fermented millet for maize in the diets of *Clarias gariepinus* fmgerlings.

Materials and Methods

An average weight of 1.28±0.2g fingerlings were obtained from a commercial fish farm in Ibadan and transferred to the University of Ibadan Fisheries Laboratory and acclimatized to laboratory conditions for two weeks. The experiment was carried out in a water recirculating unit consisting of 25L tanks. The tanks were supplied with aerated circulated water flowing at a rate of O.SLmin"¹, Effluent was passed through a filter designed to remove particles and reduce the level of metabolities. Fifty percent of the water in the system was replaced weekly and water parameters were evaluated regularly.

Experimental Diets

The ingredient and proximate composition of the experimental diets as presented in Table 1. The diets were prepared with fermented millet meal progressively replacing maize at 0, 20, 40, 60 and 80%. Diets were isocaloric and isonitrogenous, Millet were subjected to fermentation by inoculation technique according to the procedure of Abu and Tewe (1996) and Athapol et al (1992).

The millet seeds were sterilized in an autoclave sterilizer for 30 minutes and later inoculated with water containing the Nitrogen sources (10gN as ammonium sulphate and 10gN of urea per kg substrates), spores *of Aspergillus niger and* sulphuric acid to obtain an initial pH of 3.5-4.0. The inoculated millet seeds were then spread on wire mesh trays l.5inch in thickness and incubated in the humidity chamber with the temperature and relative humidity fixed at 35°C and 95 percent respectively. The experiment was left for 84 hours after which it was sundried for 48hours. The fermented millet seeds were grounded into fine powder to obtain the millet meal. All other ingredients were obtained from a local supplier. The ingredients were mixed thoroughly with oil, processed into a paste by adding hot water and pelleted through a metal plate with a die of 2.0mm. The resulting pellets were air-dried after which they were broken up and sieved through graded sieves to obtain pellets with an average diameter of 543µ

Experimental Fish

Clarias gariepinus fingerlings were distributed randomly at a stocking density of 15 fish per tank. Treatments were in triplicate and arranged at random. A total

of 15 tanks were used. The fishes were fed at 3% body weight. This corresponded to the amount of feed consumed during the acclimatization period. They were fed twice daily at 0900hours and 1700hours. Fish from each tank were weighed bi-weekly and corresponding adjustments made in the amount of feed fed, feacal samples for digestibility determination were collected by siphoning out feacal material three hours after tank had been cleared of paniculate materials.

Analytical Procedure

Proximate analysis of the ingredients, diets and fish carcass before and at the end of the experiment were carried out. Crude protein content was determined by using the microkjeldahl method (A.O.A.C., 1990). Crude lipid was determined by ashing in a muffle furnace at 550°C. Moisture by oven-drying to constant weight at 85°C and crude fibre by the acid-base digestion method as described by the A.O.A.C (1990). Apparent protein digestibility were also determined.

Analysis of Growth response and Nutrient Utilization

Weight gain was calculated as the difference between the initial and final body weight of fish.

Specific Growth Rate (SGR) was determined as described by Brown (1957).

$$SGR = \frac{\log W_2 - \log W_i}{T-t}$$

Where

W_1 = Initial weight (g)

W_2 = Final weight (g)

T-t = Time interval between initial and final weight (days) Feed Conversion Ratio (FCR) was determined as described by Hepher (1988).

$$FCR = \frac{\text{Weight gained by fish}}{\text{Weight of feed consumed}}$$

Protein Efficiency Ratio (PER) was determined as described by Mazid *et al*, (1972).

$$PER = \frac{\text{Weight gained}}{\text{Protein fed}}$$

$$\text{Protein fed} = \frac{\% \text{ protein in diet} \times \text{Total diet consumed}}{100}$$

Apparent protein digestibility was also determined

Apparent Protein digestibility

$$= \frac{\% \text{ protein in faeces}}{\% \text{ protein in feed}}$$

Haematological Examination

Fish was sampled at the end of the feeding trials for haematological and plasma biochemical studies. After decapitation of the experimental fish, blood samples were collected from the caudal peduncle of each of 15 randomly selected fish from each treatment group and pooled into ethylenediamine tetracetic acid (EDTA) bottle for haemotological studies. Plasma samples were frozen on dry ice stored at -4°C until analysed. Packed cell volume (PCV), haemoglobin (Hb) concentration, Red blood cell (RBC) and White blood cell (WBC) counts were determined by Blaxhell and Daistey (1973). Mean corpuscular volume (MCV), Mean corpsular haemoglobin (MCH) and Mean Corpuscular haemoglobin

concentration (MCHC) were calculated as described by Jain (1986). Total protein was determined by the biuret method (Reinhold, 1953).

Statistical Analysis

Data obtained were statistically analyzed by applying the analysis of variance (ANOVA) at 5% level of significance and correlation analysis.

Results

The obtained values of water quality parameters in the tanks for the duration of the experiment were stable and within the tolerance range of C. gariepinus (Table 2). All treated fish showed positive weight changes which varied from one treatment to another. Fish fed diet 1 (control diet) had the highest final weight gain followed by fish fed with 20% replacement (Table 3). The differences in weight gain of fish fed control diet and diet 1 were significantly different (P0.05) from fish fed diets with 40, 60 and 80% replacement levels. Highest average daily weight gain was observed for fish fed with control diet and diets with 20% replacement level and lowest values of 4.52 and 5.71 mg/day were recorded for fish fed diets with 60% and 80% replacement levels.

The average daily weight gain of fish fed diet containing 40% replacement level compare favourably with fish fed 60% and 80% replacement level and significantly lower PO.05 than fish fed diet containing 20% replacement and control diet. High percentage weight gain was observed in fish fed control diets and highest value in fish fed diet containing 20% replacement with percentage weight gain of 176.67%. Percentage weight gain for fish fed diets, 40%, 60% and 80% replacement levels were significantly lower (PO.05) than fish fed control diet.

The specific growth rate (SGR) of fish in treatment 3 and 4 decreased -with increased fermented millet content of. the diets. The changes in specific growth rate was significantly negatively correlated ($P<0.05$) with treatments. The feed conversion ratio (FCR) resulting from the dietary treatment range between 1.51 and 3.79. The best FCR was recorded in fish fed control diet while the poorest value of FCR w#s displayed by fish receiving higher level of fermented millet (40%, 60% and 80% replacement level). However, diet 2 (20% replacement level) produced slightly improved FCR among the fermented millet supplemented diets, with a little higher significant ($P<0.05$) difference. The mean protein efficiency ratio of the treated fish decreased as fermented millet increased in the diets even though the significant difference among the treatments was very low ($P<0.05$) compare with fish fed control diet.

Fish fed 20%, 40%, 60%, and 80% replacement levels of fermented millet produced significantly ($P<0.05$) lower apparent protein digestibility values than the fish fed 0.% replacement level of fermented millet (control diet).

The carcass composition of the experimental fish at commencement and end of the experiment is presented in Table 4. All the fish fed test diets showed increased crude protein content and decreased carcass lipid. Correlation analysis were presented in Table 5. Blood parameters of fish fed control and test diets were presented in Table 6.

Table 1: Composition and Analysis of Experimental Diets containing Fermented Millet

	Diets				
Components	1	2	3	4	5
Fish meal	39.89	39.89	39.89	39.89	39.89
Ground -nut cake	23.93	23.93	23.93	23.93	23.93
Yellow maize	19.79	15.83	11.87	7.92	3.96
Fermented Millet	-	3.96	7.92	11.89	15.83
Rice Bran	9.89	9.89	9.89	9.89	9.89
Oyster Shell	2.00	2.00	2.00	2.00	2.00
Vegetable oil	2.50	2.50	2.50	2.50 .	2.50
Vit/mineral premix	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Moisture%	10.03	10.06c	10.89d	8.03b	7.20 [a]
Crude protein %	60.80[d]	60.42[C]	56.15[b]	61.82[d]	55.51[a]
Crude lipid %	1.02[a]	1.05[b]	1.23[C]	1.01 [a]	1.56[d]
Ash %	12.45[3]	12.53[b]	12.71[C]	12.89[e]	2.77[d]
Crude fibre %	1.12[C]	1.36[d]	1.58[e]	0.12[a]	0.37[b]
Nitrogen free extract	2.60[b]	2.69[d]	2.73[e]	2.41[a]	2.63[C]
Gross Energy (Kcal/100g)	249.28	247.72	230.22	253.4[b]	227.59
Protein: Energy ratio	1:4	1:4	1:4	1:4	1:4

Mean in the same row with the same superscripts are not significantly different (P>0.05).
SEM ± Standard Error of means.

Table 2: Water Quality within Experimental Tanks for the duration of the Experiment

Parameter	Range	Mean	+S.D
Temperature °C	27.0-29.0	27.70	±1.1
pH	6.4 -7.0	6.70	±0.3
Dissolved Oxygen (mg/1)	7.5-10.0	8.90	±1.4
Alkalinity (mg/1) $CaC0_3$	75.0-100.0	87.80	±13.3
Nitrate (mg/1)	1.4-5.8	3.60	±2.24
Nitrite (mg/1)	0.09-1.12	0.11	±0.10

Table 3: Growth and Nutrient Utilization of *Clarias gariepinus* fingerings fed Fermented Millet based diets.

	Diets					
Components	1	2	3	4	5	SEM
Initial mean weight(g)	1.28	1.28	1.28	1.28	1.28	
Final mean weight(g)	2.86	2.69	2.01	1.66	1.76	0.11
Mean weight Gain(g)(NWG)	1.58^{b}	1.41^{b}	0.73*	0.38^{a}	0.48 ^{ab}	0.13
Weight Gain (%)	123.44^{d}	110.16^{d}	57.03^{C}	26.69 ^{a}	37.50^{b}	1.44
Mean daily weight gain (mg/day)	18.81^{C}	16.79^{b}	8.69^{a}	4.52^{a}	5.71^{a}	1.55
Specific Growth Rate (%/day)	0.44^{C}	0.4^{C}	0.21^{b}	0.13^{a}	0.48^{C}	0.04
Total Food Consumption/fish/Day	2.39^{b}	2.57^{C}	2.77^{C}	1.39^{a}	1.20 ^{a}	0.12
Food Conversion Ratio	1.51^{a}	1.82^{a}	3.79^{d}	3.61^{C}	2.25^{b}	0.13
Food Conversion Efficiency	66.11^{C}	54.86^{d}	26.35 ^{a}	27.34^{b}	40.00^{C}	0.11
Protein intake	0.96^{b}	1.03^{bc}	1.11^{c}	0.56 ^{ab}	0.48^{a}	0.04
Protein Efficiency Ratio	1.65^{C}	1.37 ^{ab}	0.66^{a}	0.68^{a}	1.00^{b}	0.01
Protein Productive Value (%)	2.08^{a}	3.34^{c}	2.70^{b}	2.27*	2.65^{b}	0.02
Apparent Protein Digestibility (%)	83.3^{d}	82.5^{C}	74.7^{b}	67.6^{a}	65.0^{a}	0.13

Mean in the same row with the same superscripts are not significantly different (P>0.05). SEM ± Standard Error or means

Table 4: Carcass Composition of *Clarias gariepinus* fed fermented Millet diets at the beginning and end of the feeding trial and faecal crude protein

		Diets					
Components	**Initial**	**1**	**2**	**3**	**4**	**5**	**SEM**
Moisture %	77.39^{d}	76.44^{C}	73. 12^{c}	74.07^{a}	74.93^{b}	74.58^{b}	±0.26
Crude Protein %	8.27^{a}	10.27 ^{bc}	11.71^{d}	11.27^{C}	9.54^{b}	9.41^{b}	±0.34
Crude Lipid %	4.31^{a}	4.27^{a}	7.10^{b}	9.54c	4.00^{3}	7.20^{b}	±0.14
Ash%	4.00°	3.24^{b}	2.60 ^{ab}	5.41^{d}	2.3 l^{a}	2.38^{a}	±0.03
Faecal Crude Protein %	-	0.21	0.23	0.38	0.40	0.33	±0.02

Mean in the same row with the same superscripts are not significantly different (P>0.05). SEM ± Standard Error of means.

Table 5: Growth and Nutrient Utilization Parameters *of Clarias gariepinus* Fingerlhtgs fed fermented Millet *(Pennisetum americanuni)* Diets

Y	r	Remark
NWG	0.83	*
TFC	0.70	*
PI	1.00	*
DPI	1.00	*
PER	0.73	*
PPV	0.63	*
APD	0.03	NS

r - correlation Coefficient
* - Significance ($p<0.05$)
NS - Not Significant
NWG - Net Weight Gain
TFC - Total Food Consumption
PI - Protein Intake
DPI - Daily Protein Intake
PER - Protein Efficiency Ratio
PPV - Protein Productive Value
ADP - Apparent Protein Digestibility

Discussion

Optimum growth and feed conversion efficiency were obtained in *C.gariepinus* fingerlings fed 20% replacement millet diet (Diet 2). At this same level of replacement value, fermented millet was best utilized by the fish to enhance weight gain hence they attained the highest specific growth rate except for fish fed 80% replacement millet (Diet 5) where shooters were noted. The depressed growth in fish fed diets beyond 20% replacement level was similar to those reported for C. *gariepinus* reared on diets with substituted plantain peel meal.

Falaye and Oloruntuyi, (1998); Falaye et al (1999) also observed lower growth rates in *C. gariepinus* fed high level of cassava leaf meal. This is contrary to the work of Burtle and Newton (1995) which stated that channel cat fish fed diets containing pearl millet maize ratio of 1:2 or 2:3 gave significantly better gain and efficiency than either grain alone.

Despite the inferior growth produced by high level of fermented millet diets as compared to the control, the diet with 20 % replacement level compared favourably with the latter in terms of weight gain, specific growth rate and feed conversion ratio (FCR) with no significant differences ($P>0.05$).

The significantly ($P>0.05$) lower protein efficiency ratio (PER) offish fed (40%, 60% and 80%)replacement fermented millet diets compared to control (Diet 1) and 20%, attests to the fact that maximum utilization of nutrients were not obtained at higher level of fermented millet in the diets. This is contrary to the work of Abd-Elrazig-SM et al (1998) that no significant differences were found in

egg production, feed intake, feed conversion efficiency or egg weight after the laying hen was fed with pearl millet.

A high apparent protein digestibility coefficient with increased fermented millet in the diets is ascribed to the reduced crude fibre resulting from fermentation i.e total digestion of complex polysaccharides of the millet by fish. This is comparable to the work of Sharma and Kapoor (1996) and contrary to Smith (1979). Falaye et al (1999) observed a lower digestibility coefficient with increased cocoa husk in the diets due to elevated crude fibre resulting from the complex polysaccharides of the husk being poorly digested. More so, Fagbenro (1992) associated the digestibility in *C. isheriensis* fed cocoa husk rations with cellulose activity in the fish gut. Branckaert et al (1973) found that fine milling would suffice to increase digestibility. The final fish carcass composition was generally affected by fermented millet dietary treatments. The slight increase in carcass protein and in verse trend of carcass lipid was consistent with observations on C. *isheriensis* after cocoa husk feeding trial (Fagbenro, 1992). The present trend of tissue nutrient deposition also provides evidence of protein sparing by non-protein energy. Fermented *P. americanum* as a dietary ingredient was acceptable to *C.gariepinus* fingerlings which exhibited positive growth when fed the diets. The absence of deleterious effects on fish and water quality indicates the safety of the dietary fermented *P. americanum* at 20% replacement levels.

There were declines in PCV, RBC counts, Hb concentration and elevation of MCV and MCHC in this study compared with normal values of PCV 37%; RBC 2.4 X 16%m Hb concentration lO.Omg/dl; TPP 4.6 mg/dl; MCV 155.2fl and MCHC 27.1 % established by Adedeji et al, (2000). The reduction of these erythrocytic parameters were more pronounced in the fish fed fermented millet beyond 20% replacement level. The reduced erythrocytic parameters and elevated MCV and MCHC are indications of macrocytic anaemia emanating from increase destruction and subsequent enhanced erythroporesis in the liver. This is comparable to reports by Falaye *et al,* (1999) and Jain, (1986). The decrease in specific growth rate and erythrocytic parametersw with increasing replacement level of fermented millet however indicates that fermented millet is not as efficiently utilized for growth at high concentration as maize. This study revealed that fermented millet could replace up to 20% of maize in the diet of *C. gariepinus* without adversely affecting the growth and health status of the fish.

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