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Improving Nutritional quality of Millet (*Pennisetum americanum*) by solid state fermentation and the effects on the growth performance of African Cat Fish.

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Abstract

Millet is becoming prominent in fish feed formulation as energy source but there is need to increase its protein level and remove the antinutrient for its maximum utilization to be effected. The objective of this study to determine the level of improving the nutritive value, removing the antinutrient of millet by solid state fermentation process using Aspergillus niger and effect of the product on the growth performance of African catfish fingerlings. Millet seeds were sterilized for 30 minutes, inoculated with Ammonium sulphate and urea at 10gNKg-1 substrate, spores of Aspergillus niger and sulphuric acid to obtain initial pH of 3.5-40. The mixture was fermented for 84h at 35^0C and 90-95% RH in the laboratory, then sundried for 48hrs, five diets(40% Crude protein) were formulated, containing 0, 4, 8, 12 and 16% inclusion levels of fermented millet . The diets were fed to 225 fingerlings (two week old) weighing 1.28±0.2, stocked at density of 15 fingerlings per tank in triplicate.

Millet protein increased from 10.9% to 17.0%, phytic acid reduced significantly from 0.02mg/100g to 0.014mg/100g. fingerlings fed 4% inclusion has the highest value of Percentage weight gain (PWG) 110.2%, Protein Efficiency Ratio (PER) 1.4 and lowest value of Feed conversion ratio (FCR) 1.8 while fingerlings fed diet containing 8% and 12% inclusion levels had the lowest value of PWG 26.7%, 37.5% and PER 0.66, 0.68 respectively with highest FCR 3.6. Therefore,4% of fermented millet could be included in the nutrition of African cat fish fingerlings any adverse eefect.

Keywords: *Pennisetum americanum*, Fermentation, Haematology, Feed conversion ratio and percentage inclusion level

Introduction

The developing and underdeveloped countries in the world often face the challenges of meeting the food demand for their entire population that is growing in an alarming proportion (Kumar, 2007). As estimated by FAO, (2005), 14% of the world population was undernourished between 2001-2003. This was high in Africa region (37.4%) and least in developed and industrialized countries (<2.5%). These estimates emphasize the urgent need to identify and supply the nutritive food for the people to build up the most valuable human resource. Undoubtedly, Agriculture is the single sector meting the major food requirement all over the world together with livestock and fish from the wild and aquaculture. Although, the indiscriminate fishing has led to the depletion of stock in the ocean as well as many natural water bodies. According to FAO, (2005), aquaculture is increasing at an average rate of about 12% annually with the current production of 42.30 million metric tones during 2003. Even though, there are problems facing aquaculture industry in developing countries but the most crucial one is the scarcity and ever-increasing prices of conventional fish food ingredients. However, feed ingredients are of paramount importance in fish production as adequate feeding is required to promote fast growth and high yields (Falaye et al, 1999). Therefore, this situation has led to intensive search for alternative nutrient sources for fish (Falaye, 1990). Although, there is need to considerand assesses their economics, nutritional value and availability in substantial quantities before their utilization can be regarded as adequate. Recently, the use of millet (Pennisetum americanum) is becoming prominent in fish feed formulation as energy source because of its abundance and relatively low price.

According to Railey, (2004), millet is an attractive feed grain, grown extensively around the world particularly in Africa and is no doubt superior to other cereals (Maize and sorghum etc) with respect to some of the nutrients especially average protein minerals and fat (Sharma and Kapoor, 1996). When compared to maize on a weight basis, pearl millet is 8%-60% higher in crude protein, 40% richer in lysine and methionine and 30%

richer in threonine (Andrew et al, 1996). Despite, all these, millet contains antinutritional factor (Phytic acid). According to Burton et al (1972) 48-70% of the total phosphorus in millet are not available because they are in phytate form, having form complex compounds with phytic acid. Due to the presence of antinutrients and low level of protein content in millet, its maximum utilization is being affected. However, fermentation processes using fungi are known to affect the chemical composition of food, improve the nutritive value and reduce the level of antinutrients (Sharma and Kapoor, 1996). The study was carried out to improve the nutritive value, reduce the antinutrients levels of millet by solid state fermentation, determining the level of inclusion of the product and its effect on the growth performance of *Clarias gariepinus* fingerlings.

2. Materials and methods

2.1Experimental site

2.2Culture of *Aspergillus niger*

Aspergillus niger was obtained from culture collection. The organisms were cultivated at 25^0C on malt extract agar slant containing (g/l): lab malt extract agar, 20.0 lab agar $N0_2$ 10.0; $NaN0_32.0KH_2P0_4$; MgSO4 $7H_20.05$. The spores were harvested by tween 80 solution 10ml, 0.01%v/c which were then adjusted to give 10^7 to spore per ml with sterile water.

2.3Solid state fermentation of Millet by inoculation Technique

The millet seeds were fermented 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 sulphated and 10gN as 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 perforated wire mesh trays 1.5inch in thickness and incubated in the humidity chamber with the temperature and relative humidity fixed at 35^0C and 95% respectively. The experiment was left for 84hours after which it was sundried for 48hours. The product was subjected to proximate analysis and phytic acid content determination.

2.4Experimental Diets

The fermented milled seeds were grounded into fine powder to obtain millet meal. Five diets were formulated to contain 40% crude protein, with varying inclusion levels of fermented millet (0, 4, 8, 12 and 16%), (Table 1). Person's square method was used to obtain the quantity of feed ingredient in the experimental diets. Each of the meal was mixed with maize flour which served as source of energy and a binder, fish meal (animal protein), groundnut cake (plant protein), and vegetable oil/cod liver oil as fatty acids and vitamin/mineral premix. Test diets were prepared by an initial mixing of all ingredients by manual process, which was repeated with the addition of hot water at the rule of 100mls per kg diets until the mixtures becomes a dough form. The homogenous blend was then pelleted manually using pelleting machine and sundried. The dried pellets were stored in air tight polythene bag at – 20^0C until fed.

2.4 Experiemental fish and *procedure*

Two weeks old fingerlings *C gariepinus* (meal weight 1.28±0.2g) were obtained from a commercial fish farm in Ibadan. For two weeks, fish were acclimatized to laboratory conditions and fed with commercial diets. Thereafter, the fish were randomly distributed and stocked in fifteen, 25 litre circular plastic tanks within a single water recirculation system with a continuous supply of aerated water at a flow of (1 litre per min). Fresh water from connected tap was added to the system at 0.5 litre/1min to replace water losses by splashing and evaporation. The temperature of water in the tank was maintained between 27^0C and 29^0C. Water quality parameters (p^H and dissolved oxygen) were monitored throughout the 84days of feeding trials (Table 2). A 12 hour photo was provided and maintained by fluorescent lighting.

2.5Feeding Regime

The fish were fed experimental diet for thirteen days in two weeks, twice daily by 0900hours and 01700hours at the rate of 3% fish biomass per day.

Fish were reweigh every 2 weeks fortnightly and feeding adjusted to reflect the new body weight, feacal matter and feed remains were siphoned out per tank, the faecal samples were dried at 105^0C for 24hours and stored in airtight sample bottles for subsequent crude protein analysis. At the commencement of the experiment, 15 fish were killed, weighed and ovendried at 80^0C and analyzed for proximate composition.

2.6Analytical procedure

Experimental diets, fermented millet meals, initial and final fish carcasses and fish feaces were subjected to proximate analysis using the methods described by A.O.A.C. (1990).

Moisture content was determined by drying in an oven preset at 105^0C for 12 hours. Crude protein content (N x 6.25) of the diets and feedstuffs were determined by the Micro-Kjekdahi method (A.O.A.C, 1990). Lipid content was assessed by the Soxhlet extraction apparatus (AOAC, 1990). Ash content was obtained by incinerating samples in a muffle furnace at 450^0C for 12hours and fibre content according to AOAC, (1990). Water quality parameters (Temperature, dissolved oxygen, pH and Total alkalinity) were also measure according to Boyd, (1981)

2.7Analysis of growth response and feed utilization

Growth response and nutrient utilization calculated includes specific growth rate (SGR), Food Conversion Ratio, (FCR), Protein Efficiency Ratio (PER), Gross Food Conversion Efficiency (GFCE), Protein Intake (PI) and Total Feed Intake. Digestibility was determined apparently.

Weight gain = Final body weight – initial body weight

Specific Growth Rate (SGR) was determined by Brown (1957)

$$SGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

Where W_1 = Initial Weight (g)

W_2 = Final Weight (g)

e = The base of natural logarithm

T_2 = Final (g)

T_1 = Initial (g)

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)

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

$$\text{Protein Intake} = \frac{\%\text{protein in diet X total diet consumed}}{100}$$

$$\text{Apparent protein digestibility was also determined} = \frac{\%\text{protein in faeces}}{\%\text{ protein in feed}}$$

2.8Statistical Analysis

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

3. Results

The changes in percentage crude protein, lipid fibre and phyticacid contents of inoculated millet after 84 hours of fermentation are shown in Table 2. The percentage crude protein of millet increased from 10.93% to 17.33% while the crude lipid and crude fibre decreased significantly from (3.12% and 3.65) to (2.58% and 3.27%) respectivel. Likewise the level of phytate reduced significantly from 0.70mg/0.02 mg/100g.

The mineral composition of raw and fermented millet is presented in Table 3. Fermentation had relatively increased the Ca, mg and P_{o4} present in the millet.

3.1Growth

The mean weight gain were significantly different ($P<0.05$). Fish fed control diet (diet 1) had the highest mean weight gain followed by fish fed 3% fermented millet (FM)inclusion . (Table 5).

Highest average daily weight gain (18.81g/d) was recorded by fish fed control diet and 3% FM inclusion level while the lowest values of 4.25g/d and 5.71/g/day were recorded for fish fed 60% and 80% fermented millet inclusion level respectively. The changes in specific growth rate was significantly negatively correlated (P>0.05) with treatments. FCR resulting from the dietary treatments ranged between 1.51 and 3.79. The best FCR was recorded by fish fed control diet and the poorest value was displayed by fish fed higher level of fermented millet (3%, 6% and 8% incluison values). However fish fed diet 2 (3% inclusion value) produced slightly improved FCR with a little higher significant difference (P<0.05). The PER of the fish fed fermented millet in the diets increased even though the significant differences among the treatments were very low (P<0.05) compared with fish fed control diet.

4.Discussion

Fermentation process had relatively increased the calcium magnesium and phosphorus and these observation is similar to the report of Tewe et al., (1999) who recorded a significant reduction in phytate level millet after fermentation thereby making some minerals available. It is also in line with the findings of Abdallah et al, (1998) who recorded similar things on the effect of traditional process on phytate and mineral content of pearl millet. However study revealed that fermented millet could be included up to3% in the diet of *C. gariepinus* without adversely affecting the growth and nutrient utilization of the fish.

Optimum growth and feed conversion efficiency were obtained in *C. gariepinus* fingerlings fed 3% fermented millet diets (Diets 2). At this level, the fermented millet was best utilized by the fish to enhance weight gain hence they attained the highest specific growth rate recorded among the selected except for diets where shooters were noted. The depressed growth in fish fed diets beyond 3% inclusion level was similar to those reported for *C. gariepinus* reared on diets with substituted plantain peel meal (Falaye and Oloruntunyi, 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). Channel catfish showed equivalent weight gain and feed efficiency when either maize or peal millet was fed at 30% of total diet. However, diets containing peel 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 3% inclusion (lower) level compared favourably with the latter in terms of weight gain, specific growth rate the feed conversion ration (FCR) with no significantly differences (P>0.05).

The significantly (P>0.05) lower protein efficiency ratio (PER) of fish fed 3%,6 % and8 % fermented millet replacement values compare to control and3 %, attests to the fact that maximum utilization of nutrients were not obtained at higher levels of fermented millet in the diets. This is contrary to the work of Abd-Elerazig-SM et al (1998) 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.

The final fish carcass composition was generally affected by fermented millet dietary treatments. The slight increase in carcass protein and inverse trend of carcass protein and inverse 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 3% inclusion level.

5. Conclusion

This study revealed that fermented millet could be included up to 3 % in the diet of *C. gariepinus* without adversely affecting health of the fish

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

	Diets				
Component	**1**	**2**	**3**	**4**	**5**
Fish meal	39.89	39.89	39.89	39.89	39.89
Groundnut 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
Level of maize					
Replacement with					
Fermented millet	0%	20%	40%	60%	80%
Level of fermented					
Millet inclusion	0	3%	6%	8%	12% SEM
Moisture %	10.03^{c}	10.06^{c}	10.89^{d}	8.03^{b}	7.20 $\pm$0.38
Crude protein %	60.80^{d}	60.42^{c}	56.15^{b}	61.82	55.51 $\pm$0.9
Crude lipid %	1.02^{a}	1.05^{b}	1.23^{c}	1.01^{a}	$156^{d}\pm$0.06
Crude fibre %	1.12^{c}	1.36^{d}	1.58^{c}	0.12^{a}	$0.37^{b}\pm$0.05
Ash %	12.45^{a}	12.53^{b}	12.71^{c}	12.89c	$12.77^{d}\pm$0.04
Nitrogen free extract	2.60^{b}	2.69^{d}	2.73^{c}	2.41^{a}	$2.63^{c}\pm$0.03
Gross Energy (Kcal/100g)					
Crude protein %	249.28	247.72	230.22	253.46	227.59
Protein: energy ratio	1:4	1:4	1:4	1:4	1:4

Table 2: Proximate Composition and Phytic acid content of raw and fermeted Millet (Pennisetum americanum)

Component	Raw Millet %Mean Value	± SD	fermented Millet % Mean Value	± SD
Crude protein %	10.93	0.23	17.33	0.20
Crude lipid %	3.12	0.02	2.58	0.20
Crude fibre %	3.65	0.20	3.27	0.22
Phytic acid Content (mg/100g)	0.70	0.02	0.02	0.02

± SD = Standard Deviation

Tables 3: Mineral Composition of Raw and Fermented Millet (pennisetum americanum)

Component	Raw Millet %mean Value	± SD	Fermented Millet %mean Value	± SD
Calcium	0.17	0.01	0.11	0.02
Magnesium	0.059	0.50	0.091	0.01
Phosphorus	0.023	0.001	0.061	0.001

±SD = Standard deviation

Means in the same row with the same superscripts are not significantly different (P>0.05).

± = Standard Deviation

Table 4: Water quality within experimental tanks for the duration of the experiment

Parameter	Range	Mean	± SD
Temperature ^{0}C	27.0-29.0	27.70	1.1
pH	6.4-7.0	6.70	0.3
Dissolved oxygen (mg1/L)	7.5-10.0	8.90	1.4
Alkalinity (mg/1) $CaC0_3$	75.0-100	87.80	13.3
Nitrate (mg/1)	1.4-5.8	3.60	2.24
Nitrite (mg/l)	0.09-1.12	0.11	0.10

± = Standard Deviation

The water quality parameters monitored during the experimental period falls within the range recommended by viveen et al,(1987) and Boyd (1991).

Table 5: Growth and nutrient utilization results of *Clarias gariepinus* fingerlings fed fermented Millet diet (during the experimental period)

	Diets					
Component	**1**	**2**	**3**	**4**	**5**	**SEM**
Initial mean weight (g)	1.28	1.28	1.28	1.28	1.28	1.28
Final mean weight (g)	2.86	2.69	2.10	1.66	1.76	± 0.11
Mean weight gain (g)						
(NWG)	1.58^{b}	1.41^{b}	0.73^{ab}	0.38^{a}	0.48^{ab}	± 0.13
Percentage weight gain (%)	123.44^{a}	110.16^{a}	57.03^{c}	26.69^{a}	37.50^{b}	± 1.44
(mg/day) (ADW)	18.81	16.79^{b}	8.69^{a}	4.52^{a}	5.71^{a}	± 1.55
Specific growth rate	0.44^{c}	0.40^{c}	0.21^{b}	0.13^{a}	0.48^{c}	± 0.04
(%/day) (SGR)						
Total food						
Consumption/ fish/day	2.39^{b}	2.57	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}	22.5^{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^{ab}	2.27^{ab}	2.65^{b}	0.02
Apparatus protein Digestibility (%)	83.27d	82.47^{c}	74.73^{b}	67.63^{a}	65.00^{a}	0.13

Means in the same row with the same superscripts are not significantly different (P>0.05) ± = Standard Deviation

Tables 6: Carcass Composition of Clarias gariepinus fed fermented Millet diets at the beginning and end of the feeding trial and feacal crude protein

Component	**Initial**	**Diets number**					
		1	2	3	4	5	SEM
Moisture%	77.39^{d}	76.44^{c}	73.12^{a}	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}	11.27^{c}	9.54^{b}	± 0.34
Crude lipid %	4.31^{a}	4.27^{a}	7.10^{b}	9.54^{c}	4.00^{a}	7.20^{b}	± 0.14
Ash %	4.00^{c}	3.24^{b}	2.60^{ab}	5.41	2.31^{a}	2.30^{a}	± 0.03
Faecal crude							
Protein	-	0.21	0.23	0.38	0.40	0.33	± 0.02

Means in the same row with the same superscripts are not significantly different (P>0.05)

Parameters on each row with different superscript are significantly different at P < 0.05