Iranian Journal of Fisheries Sciences 19(4) 1889-1910 2020
DOI: 10.22092/ijfs.2018.119856.

Use of solid state fermented bambara nut meal as substitute of fishmeal in the diets of African catfish *Clarias gariepinus*

Enyidi U.D.[1*]; Etim E.O.[1]

Received: November 2017 Accepted: December 2018

Abstract

The use of plant proteins in substituting fishmeal (FM) is mitigated by several anti nutritional factors (ANF) like phytic acid, non starch polysaccharides and protease inhibitors. Fermentation of the plant ingredients can reduce the ANF and improve feed utilization and growth rate of fish. We produced five isonitrogenous and isocaloric diets using solid state fermented bambara nut meal (BNM). Fermented BNM substituted FM in diets of African catfish *Clarias gariepinus.* The FM: BNM inclusion percentages of the diets were, F1, 50:5; F2, 35:20; F3, 20:35; F4, 5:50 and F5, 0:56. There was a control feed labelled as F6. Feed F6 was a variant of F1 but with non fermented BNM. Fingerling African catfish *C. gariepinus* with average weight 5.14±0.05 g were fed with the diets for 56 days. Specific growth rate (SGR) was best for the catfish fed with F1, 7.82±0.25 % day^{-1}, followed by those fed with F6, 7.35±0.24 % day^{-1}. There were, however, no differences in SGR of F2, 7.26±0.18 % day^{-1} and F6. The food conversion ratio was lowest and best for the catfish fed with F1, 1.24±0.19 and F2 1.34±0.06. The growth and nutritional performance of fish fed with F2 were as good as F1 and cost-effective. Feeds made from fermented BNM had better FCR than those from raw BNM. Fermentation increased the protein and amino acid content of the BNM and the catfish gained more weight than from raw BNM. The effects of ANF seem to be highly reduced in solid-state fermented BNM thereby enhancing catfish growth.

Keywords: Solid state fermentation, Fishmeal substitution, Food conversion ratio, Plant proteins, Specific growth rate

1-Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria
*Corresponding author's Email: enyidiuche@yahoo.com

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Introduction

The high cost of fishmeal especially in developing world has necessitated search for plant protein alternatives (Hardy and Tacon, 2002; Lunger *et al*., 2006; Hardy, 2010). Leguminous plants are among the plausible protein sources but majority of them are underutilised or poorly known (Bhat and Karim, 2009). This situation necessitates the search for nutritive leguminous plant, especially those with lesser competition as human food, as fish meal alternatives (Lunge *et al*., 2006; Gatlin *et al*., 2007). Many plant feedstuffs have received attention in recent years. However, due to amino acid imbalances and low percent protein content their usages are limited (Hemre *et al.,* 2009; Enyidi, 2012). Moreover, use of plant proteins as fishmeal substitute is limited by the presence of anti nutritional factors (ANF) inherent in the ingredients (Krogdahl and Holm, 1981; Francis *et al*., 2001; Krogdahl *et al*., 2010). There are many ANFs in plant meals such as proteinases inhibitors, oligosaccharides, and phytic acids (phytate) (Raboy and Dickinson, 1993; Francis *et al*., 2001; Gatlin *et al.,* 2007; Krogdahl *et al*., 2010), trypsin inhibitors, amylase inhibitors (Liener and Kakade, 1980; Robinson and Li, 1996; Hemre *et al*., 2009), tannins, saponins alkaloids, non starch polysaccharides, (Duke, 1981; Yoon *et al*., 2003; Francis *et al*., 2005; Krogdahl *et al*., 2010). ANF interferes with digestion, assimilation and utilization of the nutrients in plant proteins (Storebakken *et al.,* 1998; Refstie *et al*., 1999). Some plant proteins contain gossypol (Dorsa *et al*., 1982 Yildirim-Aksoy *et al*., 2004; Imorou Toko *et al*., 2008) which interferes with fish reproduction. In some instances carbohydrates, such as α-galactosyl derivatives of sucrose (GOSs) and non-starch polysaccharides (NSP) are also classified as ANF (Choct *et al*., 2010). Some NSP like pectins, galactans, cellulose and lignin can cause high faecal water (Olli and Krogdahl, 1994; Refstie *et al*., 1999). NSPs also interfere with digestion and assimilation. Some NSP including arabinan, arabinogalactan and acid polysaccharides can bind minerals in the intestine and hinder digestibility of fat (Storebakken *et al*., 1998). Many plant ingredients that are plausible fishmeal substitutes have NSP (NRC, 1993). Naturally, the NSPs are degraded mainly through enzymatic complex associated with cellulolytic bacteria belonging to the phylum *Actinobacteria*. The phyla *Actinobacteria* are sometimes found in the gut of termites and fungal organisms (Wenzel *et al*., 2002; Wei *et al*., 2009; Wilson, 2011). Among the identified cellulolytic bacteria, the major phylum is the *Actinobacteria* genus *Streptomyces*. The *Actinobacteria* are associated with enzymatic and bioactive substances including cellulase, pectinase and hemicellulase and also in the excretion of various antibiotics or bacteriocins (Killham and Prosser, 2007; Brijwani *et al*., 2010; Dharmaraj, 2010).

Solid state fermentation is known to reduce or remove ANF from plant-based ingredients (Mukhopadhyay and Ray, 1999; Yoon *et al.,* 2003; Servi *et*

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

al., 2008; Shi *et al*., 2015). This can be very useful in aquafeed production using plant proteins. Fermentation is also known to increase the availability of nutrients, minerals and available protein (Lio and Wang, 2012; Khodanazary *et al*., 2013; Shi *et al*., 2015). The effect of fermentation depends on the type of organism used in the process and the type of fermentation carried out (Khodanazary *et al*., 2013). Solid-state fermentation (SSF) is a type of fermentation where the microorganisms are mixed with appropriate ingredients in a dry state and microbe ferments the plant matter in very low water activity or the absence of water completely (Papagianni *et al*., 2001; Pandey, 2003). SSF is not commonly used for microorganisms that require high water activity to develop or grow (Babu and Satyanarayana, 1996; Hölker and Lenz, 2005). There are few organisms that can be used in SSF due to the difficult fermenter-substrate complex. The complex is such that fungi must possess hyphae that can grow on the substrate particle and into the interparticle spaces locally fermenting the substrate (Santos *et al*., 2004). A few bacteria like *Clostridium thermocellum* (Chinn *et al*., 2007), *Bacillus subtilis* (Gupta *et al*., 2008; Mukherjee *et al*., 2008), *Streptomyces, Cohnella* and *Cellulosimicrobium* (Opazo *et al*., 2012) have been used for SSF.

Unfermented BNM has been used as FM substitute in the diets of African catfish (Enyidi and Mgbenka, 2000; Enyidi, 2012; Enyidi *et al*., 2017). bambara nut has been also used in feeding finisher broiler chicks (Ekenyem and Onyeagoro, 2006; Obih and Ekenyem, 2010). In all cases, BNM was a good diet component that produced higher weight gain compare with the control diets. Some effects of fermented BNM as feed ingredient have been examined by Nwanna *et al*. (2005), the authors did not specify the type of fermentation and organism used. Fermentation of BNM with *Rhizopus oligosporus* significantly reduced ANF like trypsin inhibitors from 3.18-0.49 mg/g after 72h (Olanipekun *et al*., 2015). Processing of bambara nut meal for 24 hours through soaking, boiling and fermentation also reduced ANF from about 7.3 mg g^{-1} to 4.2mg g^{-1} (Fadahunsi, 2009). However, Nwanna *et al*., (2005) noted some mineral composition of BNM increased after fermentation with fungi. Fermentation with *S. cerevisiae* had been noted to increase protein content of diets of fermented soy meal from 47% to 58% (Song *et al*., 2008).

Bambara nut (*Voandzeia subterranea*) is underutilized and neglected secondary food crop is grown all over sub-Saharan Africa (Karunaratne *et al*., 2008, Bhat and Karim, 2009). The crude protein content of bambara nut is about 24% (Dakora and Muofhe, 1995; Basu *et al*., 2007, Enyidi, 2012). It is cheap, abundant and easily accessible. Moderate protein content and low market price make BNM as a suitable alternative to both FM and soybean meal in the diets of African catfish (Enyidi *et al*., 2013).

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

This research is aimed at finding the growth and nutritional effects of feeding African catfish *C. gariepinus* with diets made from FM substituted with solid state fermented BNM for 56 days.

Materials and methods

Procurement and pre-treatment of bambara nut meal Grains of white bambara nut were purchased from open grains market at Enugu, Nigeria. The grains were sorted and milled using a locally made attrition mill. The meal was sieved with 0.1 mm sieve to obtain the initial white endosperm of bambara nut. The BNM was autoclaved at 100^{o}C for 10 min, cooled and then stored for use during solid-state fermentation and feed production.

Solid state fermentation of bambara nut meal

Solid state fermentation of BNM was done per treatment feed. Approximately 1.56 kg of BNM was measured with an electronic weighing balance (Metler Toledo analytical balance) and was transferred into a plastic bowl. Common yeast *Saccharomyces cerevisiae* was purchased from a dealer in feed ingredient and bakery products within Enugu, Nigeria. The BNM was vigorously mixed with 200 g of the dried *S. cerevisiae* in an electric mixer for 10 min. The mixture of BNM and yeast was placed inside 15 litre opaque plastic bioreactor equipped with a stirrer on the lid cover. There was no addition of water to the BNM/ *S. cerevisiae* mixture. The container was tightly closed and kept in the laboratory at room temperature fluctuating between 29 -32°C in a well-ventilated area till 96 hours (4 days) for fermentation to take place. The mixture was stirred every 6 hours using a stirrer fixed on top of the bioreactor.

Monitoring of Saccharomyces cerevisiae

The growth and activity of the *S. cerevisiae* were monitored at the end of the second day of fermentation. Approximately, 10 g of the fermenting BNM was taken and placed in an 80 ml stomacher bag. Approximately, 45 ml of buffered peptone water (BPW) was added into the stomacher bag. The mixture of BNM and BPW were homogenized for 2 min and serial dilution with 0.2% peptone water was carried out in ten 100 mL test tubes. About 7 mL of the mixture was spread plated on nutrient agar. The plates were incubated at 32°C for 48 h and biochemical test was done for the *S. cerevisiae* growth. The fermentation process was arrested after four days. The fermented bambara nut meal (FBNM) were blended and stored in a cool dry place for 24 h, after which it was used for feed production. The solid-state fermentation and monitoring was carried out at the Microbiology Laboratory, Department of Microbiology Godfrey Okoye University Enugu, Enugu State, Nigeria.

Feed preparation

The principle of mixture design was employed in designing all the feed used in this experiment (Ruohonen *et al*.,

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

2003; Enyidi *et al*., 2017). There were two major protein sources in the diets [fishmeal (FM) and fermented bambara nut meal (FBNM)]. The five substitution percentage of FM: FBNM per treatment feed were F1: 50:5%, F2: 35:20%, F3: 20:35%, F4: 5:50% and F5: 0:56%. There was a control feed labelled as F6, which was a variant of F1 but its BNM was not fermented. F6, contained 50% FM and 5% raw BNM. There were similar inclusion levels of soybean meal and poultry by-products in all the feeds. The compositions of the experimental feeds are presented in Table 1. The diets were made to be isonitrogenous and isocaloric.

Known weights of all ingredients per treatment listed in Table 1 were measured using an electronic weighing balance, Huaying Electronic Scale (ACS-789), Yongkang Huaying weighing apparatus Co., Ltd. China. The weighed ingredients were mixed with an electric mixer using Kenwood chef mixer (Thorn Eni Harrant England). The ingredients were mixed till a homogenous blend was achieved. Approximately, 200 ml of water was added and then mixed to get the homogenous dough. The dough was preconditioned for 30 min and then pelleted using a 1.8 mm die. The feeds were dried at 45^{o}C in an electric oven Samsung-5.8 Cu. Model: NE58F9710WS.

Experimental design

The experiment was carried out as a completely randomized design. There were three replicates aquarium per treatment feed. The fish were stocked at a density of 20 fish per aquarium making 60 fish per treatment feed. The feeds were labelled as F1 to F5 for the fermented diets while there was a control diet made without fermented bambaranut meal labelled as F6.

Proximate analysis

Crude protein was analyzed by the Kjeldahl method from dried fish samples. Analyses were made using a Tecator Kjeltec model 1002 system using block digestion and steam distillation and crude protein were calculated as %N x 6.25. Amino acid levels were measured according to the methods of the European Commission (1998). The total lipids of fish muscles were measured by chloroform-methanol extraction at a ratio of 2:1. The lipid extraction followed the method described by Enyidi *et al*. (2017). Total lipid was calculated as the weight difference in non-extracted and extracted muscle samples (Parrish, 1999; Kainz *et al*., 2004). The moisture content of the feeds was determined by oven drying feed samples at 105°C. Ash content was determined by incineration samples in a muffle furnace at 550°C for 24 hrs. The ash content was calculated as the weight of ash/weight of sample x 100.

Experimental fish

Fingerlings of African catfish *C. gariepinus* with an average weight of 5.08 g were obtained from a commercial fish farm in Umuahia. The fingerlings were transported in airbags to the wet laboratory of Department of Fisheries and Aquatic Resources

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Management Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The fish were acclimatized for 7 days, during which time they were fed with 32% protein diet. The catfish were distributed into three replicates aquaria per treatment feed. The initial weights of the fish were recorded and subsequent weight increases were taken every two weeks. The catfish were deprived of feed 15 hours prior to start of weight taking experiment. The aquaria were subjected to 12D:12L photoperiod. The water temperature was 28.5±0.09 °C, the phosphorus content of the aquarium water was 0.09 mg L^{-1}, dissolved oxygen content was 5.15±1.09 mg L^{-1} (YSI oxygen meter model 550A, YSI Inc. Yellow Springs, Ohio, USA) and pH was 7.35±0.77. Also the concentrations of nitrate and ammonia were 128.33±1.02 m g^{-1} and 0.7±0.6 mg L^{-1}, respectively (Tetra ammonia kit, Malvern, PA, USA).

Table 1: Feed composition and proximate analysis of experimental feeds varying in fishmeal and solid state fermented fermented bambara nut meal used in feeding African catfish for 56 days.

¤	F1	F2	F3	F4	F5	F6NFB
Fish meal	50	35	20	5	0	**50**
FBNM	5	20	35	50	56	**5**
Soybean meal	30	30	30	30	30	**30**
Poultry meal	11.7	11.7	11.7	11.7	11.7	**11.7**
Bone meal	0.1	0.1	0.1	0.1	0.1	**0.1**
Vit. & Premix	0.1	0.1	0.1	0.1	0.1	**0.1**
Vit C	0.1	0.1	0.1	0.1	0.1	**0.1**
Total	100	100	100	100	100	**100**
Proximate analysis of the experimental feed						
Crude protein	32.77	31.56	31.38	33.78	31.64	**32.78**
Crude lipid	3.86	4.10	3.81	3.84	3.61	**4.18**
Starch	55.32	55.51	53.62	53.08	53.51	**50.78**
Crude fiber	5.27	5.73	6.17	6.2	5.27	**5.73**
Ash	4.78	5.17	5.4	4.79	4.78	**5.17**
Moisture	8.92	8.84	9.63	8.67	8.92	**8.84**
Dry matter	91.13	91.16	90.38	91.33	91.13	**91.16**

F BNM stands for fermented bambara nut meal, SBM is soybean meal, PBP is poultry by-products or Poultry slaughter waste.

Table 2: Proximate analysis of the amino acid contents of bambara nut meal and fishmeal and the recommended values for catfish.

Amino acids	BNM	FBNM	FM	Recommended.
Arg	19.0	24.4	48.3	13.4
Hist	6.7	7.3	18.5	4.7
Ile	9.4	9.3	38.3	8.1
Leu	19.4	22.4	62.7	10.9
Lys	11.7	16.8	52.2	15.9
Met	4.2	6.4	20.8	7.2
Cys	3.0	3.1	7.4	
Phe	12.7	10.4	27.1	15.6
Tyr	11.5	13.9	22.0	
Thr	8.2	8.6	29.0	6.3
Val	9.6	11.3	43.0	9.4

The values of the amino acids are as fed basis in g kg^{-1}Arg. (arginine), His (histidine), Ile (isoleucine), Leu,(leucine), Lys, (lysine), Met, (methionine), Cys (cystine), Phe, (phenylalanine), Tyr (tyrosine), Thr (threonine), Val, (valine). BNM (bambara nut meal), SM (soybean meal), FM fish meal).[a] corresponds to methionine + cystine, [b] corresponds to phenylalanine + tyrosine. Recommended values are g kg^{-1} of dietary protein (NRC, 1993).

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Calculation and statistical analysis

The following calculations were made for each aquarium, which served as the observational unit in the experiment: Specific growth rate (SGR, % day^{-1}) was calculated as 100×(Ln w_2 – Ln w_1)×t-1, where w_1 and w_2 were average weights in g at the start and the end of the experiment, respectively, and t was the length of the experiment in days. Food conversion ratio (FCR) of the catfish was calculated as (food consumed in g)×(change in tank biomass in $g)^{-1}$. Hepatosomatic index (HSI)=was measured as 100×liver weight (g)×fish weight $(g)^{-1}$. Also, peritoneal fat somatic index (FSI) was calculated as 100×intraperitoneal fat (g)×fish weight $(g)^{-1}$.

The other growth parameters were measured as follows:

Survival=100×final number of individuals x initial number of $individuals^{-1}$.

Daily feed intake (DFI) (g^{-1} fish on as fed basis)=Cumulative feed intake–rejects/ number of fish.

Protein efficiency ratio (PER)=(w_2-w_1) (g) ×protein fed $(g)^{-1}$.

Protein retention=100×(final protein content of fish in g - initial protein content of fish in g)×protein fed $(g)^{-1}$.

Cost (Naira kg fish $produced^{-1}$)=Food conversion ratio x feed cost (Naira kg^{-1}) where bambara nut costs ca. 1000.00 kg^{-1}, and fishmeal 3000 kg^{-1}.

Cost per unit protein is = Cost of making (kg) of diet×$protein^{-1}$.

Results were analyzed using one way ANOVA and least significant difference (LSD) 0.05 was used in separating possible differences of treatment means. The statistical package used for analyses was SPSS 14.0.

Feeding and catfish culture management

The fish were fed to satiation three times daily. The period of feeding was in the morning 8.am, afternoon 1-2:30 pm and evening 6-7 pm. Feeding was carefully done to avoid over feeding of the fish. The weights of the feed administered to the fish were recorded and the weights of uneaten feed were also recorded. Prior to feeding the fish, the aquariums were usually washed and uneaten food and faecal materials were removed. In cleaning aquarium, ¾ of the water was usually removed and replaced to avoid stressing the fish. The water supply to the aquarium was from university borehole water. The water was passed though a filter before being pumped into the aquariums. The physico-chemical parameters of the aquarium water were analyzed every other day and the averages were recorded. The results of the physico-chemical parameters analyses are illustrated in table 2. The catfish weights increases were measured every 2 weeks. Before weighing the fish they were denied feed for 15 hours to evacuate their stomach of the feed and faeces. Fish were measured in batches according to treatment feed and replicates.

Results

The catfish were quick in accepting experimental diets and grew with optimal SGR of 7.82±0.25 % day^{-1}

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

achieved by those fed with feed F1. There was a significant difference (p<0.05) between the SGR of the catfish fed with F1, 7.82±0.25 % day^{-1} and those fed with F2, 7.26±0.18 % day^{-1}. The catfish fed with control diet (non fermented BNM) did equally well with a SGR of 7.35 ± 0.24% day^{-1}. There were no significant differences between SGR of the catfish fed with either feed F2 or those of control non fermented BNM diet (p>0.05) (Table 3).

Alternating the percentage FM: FBNM inclusion levels of F2 as in F3, reduced the SGR of the catfish from 7.26±0.18 % day^{-1} of F2 to 6.77±0.38 % day^{-1} of F3. Feed made from complete supplementation of fishmeal with fermented bambara nut meal Feed 4 (F4), produced the least SGR 4.88±0.11 % day^{-1}. It seems that SGR of the catfish was decreasing with increasing inclusion of FBNM (Fig. 1). There was negative correlation between the catfish SGR and inclusion of FBNM (R^2=0.931, Fig.1).

Table 3: Growth performance and feed utilization of African catfish *Clarias gariepinus* fed for 3 months (90 days) with diets substituting fish meal with solid state fermented bambara nut meal at FM:BNM substitution levels of 50/5, 35/20, 20/35,5/50,0/56,50/5non fermented and 0/56non fermented.

Feed	F1	F2	F3	F4	F5	F6nfm	F7 comm
Initial av wt	5.04 ±0.07^{ns}	5.48±0.05^{ns}	5.37±0.02^{ns}	4.89±0.03^{ns}	4.83±0.04^{ns}	5.32±0.06^{ns}	4.89±0.02^{ns}
Final Av wt	85.16 ± 4.3^{a}	76.61±3.1^{b}	56.52±2.1^{d}	27.05±0.3^{e}	21.79±0.32^{e}	76.19±0.13^{b}	67.76±0.56^{c}
Av wt gain	80.12±0.06^{a}	71.13±0.03^{b}	51.15±0.34^{d}	22.16±0.25^{e}	16.96±0.54^{e}	70.87±0.23^{b}	62.87±0.33^{c}
FCR	1.24±0.19^{a}	1.34±0.06^{ab}	1.51±0.16^{b}	2.03±0.19^{c}	2.50±0.08^{d}	1.52±0.08^{b}	1.20±0.90^{a}
SGR	7.82±0.25^{a}	7.26±0.18^{b}	6.77±0.38^{b}	5.34±0.19^{c}	4.88±0.11^{d}	7.35±0.24^{b}	7.14±0.07^{a}
PER	1.70±0.11^{a}	1.57±0.18^{a}	1.10±0.06^{b}	0.50±0.12^{b}	0.37±0.03^{c}	1.66±0.23^{a}	1.50±0.75^{a}
HSI	0.413±2.11^{b}	0.472±1.55^{b}	0.473±1.00^{b}	0.746±1.93^{a}	1.089±1.02^{a}	0.394±2.02^{b}	1.180±1.34^{a}
GSI	0.09 ± 0.07^{b}	0.65± 0.34^{a}	0.17± 0.54^{a}	0.20± 0.76^{a}	0.13± 0.43^{ab}	0.10± 0.54^{b}	0.20± 0.32^{a}
PCR	0.28±0.23^{d}	0.28±0.34^{d}	0.32±0.03^{c}	0.48±0.21^{b}	0.54±0.35^{a}	0.22±0.67^{d}	n/a
Daily feed int.	15.78± 0.04^{a}	15.05±0.07^{a}	11.38±0.09^{b}	7.08±0.05^{c}	6.16±0.11^{d}	6.11±0.32^{d}	11.52±0.41^{b}
Costs/kg ($)	570.30±0.04^{b}	342.7±0.50^{c}	358.6±0.01^{c}	374.5±0.30^{d}	381.3±0.02^{d}	326.8±0.01^{c}	1200±0.05^{a}
Cost/U.protein	12.09±0.07^{b}	7.50±0.08^{c}	7.70±0.04^{c}	8.44±0.03^{d}	8.27±0.01^{d}	7.66±0.08^{c}	28.56±0.07^{a}

Initial av wt=initial average weight (g), final av wt=final average weight (g), Av wt gain=average weight gain, FCR=food conversion ratio, SGR=specific growth rate % day^{-1} , PER =protein efficiency ratio, , HSI=hepatosomatic index, GSI=gonadosomatic index, PCR=protein conversion ratio, Daily feed int.=daily feed intake, Costs kg^{-1} ($)=cost of making a kg of the feed in Nigeria Naira, 1 USD ($) equals 450 Nigerian Naira, Cost/U.protein=cost per unit protein. The formulas for deriving these parameters are written on page. Means not followed by same superscript are significantly different (p<0.05).

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

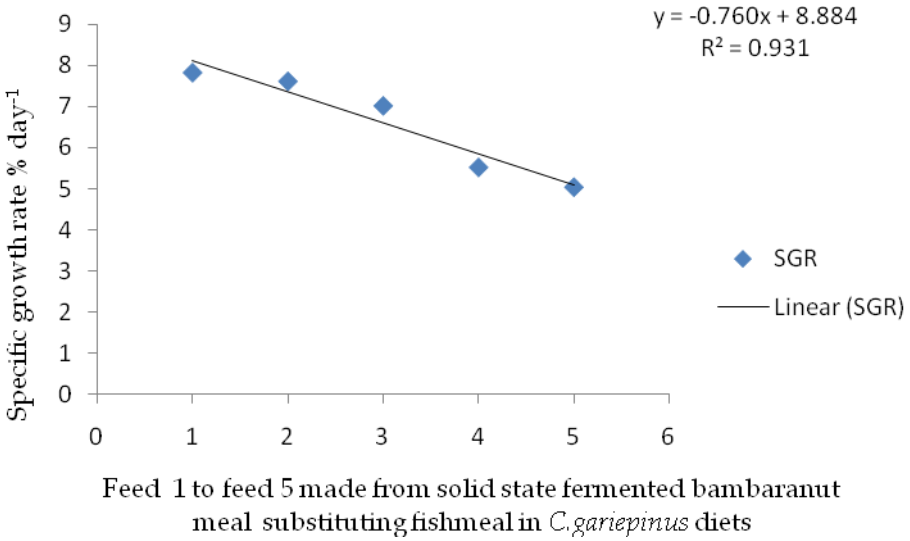

Figure 1: The specific growth rate per diets of fish fed with diets made from solid state fermented bambara nut meal used as substitution of fishmeal in diets of African catfish *Clarias gariepinus.*

The FCR) of the catfish were lowest for those fed with F1 and F2 (1.24±0.19 and 1.34±0.06 respectively). The control diet, (non fermented BNM) had higher FCR than the catfish fed with F1 (p<0.05). The catfish FCR was similar for those fed with 20% FBNM diet F2 and 5% non fermented BNM diet (control) (p>0.05). Total substitution of FM with FBNM increased FCR from 2.03±0.19 of catfish fed with F4 to 2.50±0.08 of F5 (Table 3). There was however a significant difference (p<0.05) in the FCR of catfish fed with feed 4 and feed 5. Generally, it seems that increasing FBNM inclusion increases FCR of the catfish. The catfish FCR was positively correlated to the increasing inclusion of FBNM, (R2 =0.917, Fig. 2).

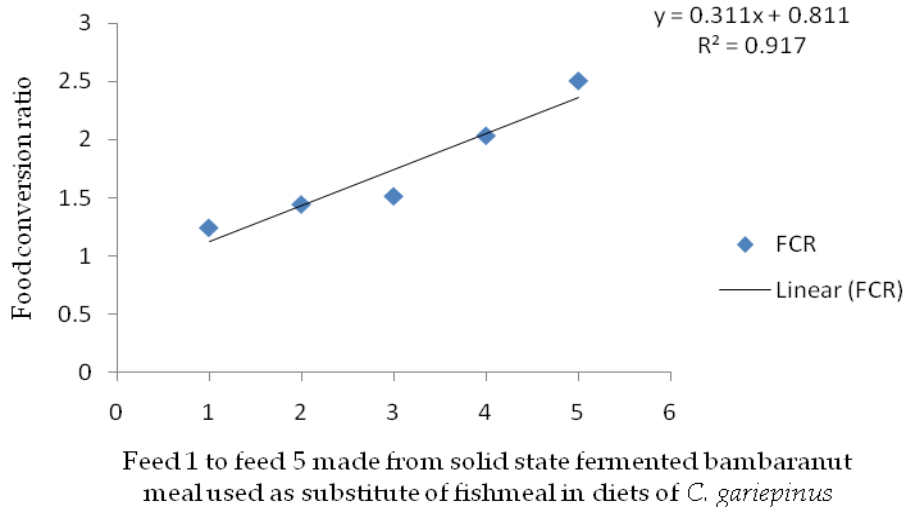

Figure 2: Food conversion ratio of African catfish fed with treatment diets varying solid state fermented bambara nut meal with fishmeal after 56 days feeding experiment.

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

The final average weight gain of the catfish was highest for the catfish fed with F1 (85.16±4.3 g) followed by that of catfish fed with F2 (76.61±3.1 g) and those of control diet made from non fermented BNM (70.87±0.23 g). There was no significant difference (p>0.05) in the final weight of catfish fed with F2 (20% FBNM diet) and control (5% non fermented BNM). Alternating the percentage inclusions of FM: FBNM in F2 and F3, reduced the final weight of the catfish to 56.52±2.1 g from 76.61±3.1 g of F2. The final average weight of the catfish was negatively correlated with the increasing inclusion of FBNM (R^2=0.956, Fig. 3). The catfish fed with diets F1 had significantly higher weight gain (80.12±0.06 g) than those fed with F2 (71.13±0.03 g) and the control diet (70.87±0.23 g) (p<0.05). The complete substitution of FM with FBNM as in F5, produced the lowest AWG of 16.96 ± 0.54 g. Similarly alternating the FM: FBNM ratio of F2 and F3 reduced AWG from 71.13±0.03 g of those fed F2, to 51.15±0.34 g of the catfish fed with F3. The AWG of catfish fed with F2 was significantly different from those fed with F3 (p<0.05). The catfish AWG was negatively correlated to the increasing inclusion of fermented FBNM (R^2=0.967 Fig. 4).

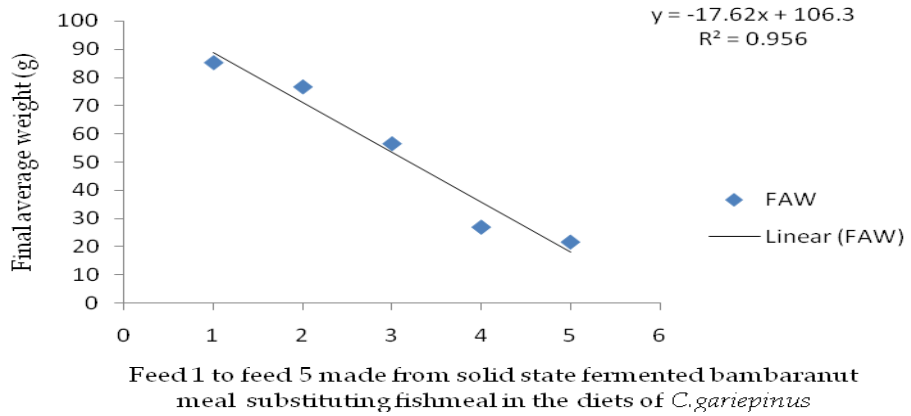

Figure 3: The final average weight of African catfish *Clarias gariepinus* fed diets varying solid state fermented bambara nut meal with fishmeal for 56 days.

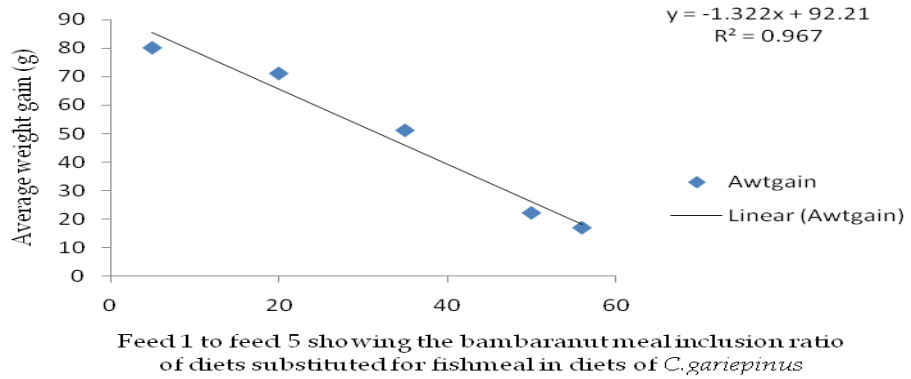

Figure 4. The average weight gain of African catfish *Clarias gariepinus* fed with diets varying solid state fermented bambara nut meal with fishmeal for 56 days.

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

The catfish PER were best but not significantly different (p>0.05) for the fish fed with feed F1 (1.70±0.11), F2 (1.57±0.18) and the control unfermented diet (1.66±0.23). Alternation of the FM: FBNM ratio of the diets F2 and F3 reduced PER from 1.57±0.18 of feed 2 to 1.10±0.06 of feed 3. The lowest PER was recorded with the catfish fed with total substitution of FM as in F5 (0.37±0.03). There was no significant difference between the PER of catfish fed diets substituting FM with either fermented or non fermented bambara nut meal (Table 3). The PER of the catfish was negatively correlated to increasing inclusion of FBNM (R^2=0.958, Fig. 5). The PER of the catfish was also positively correlated to the catfish SGR (R2=0.954, Fig. 6).

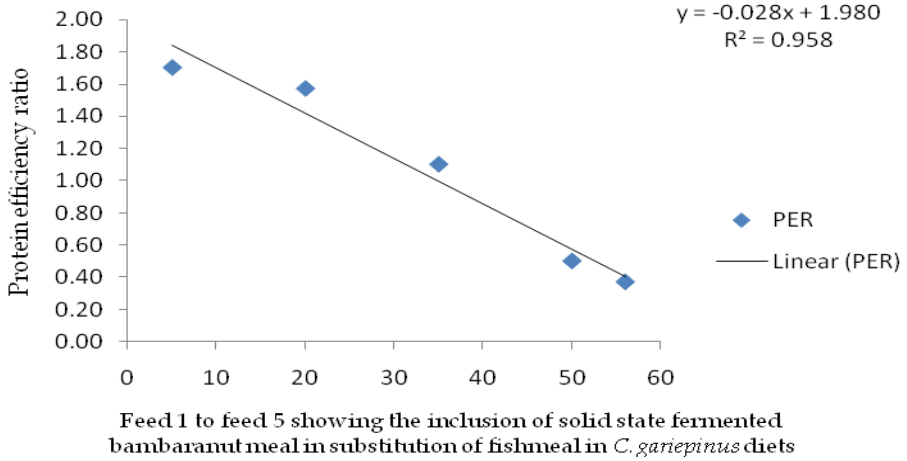

Figure 5: The effects of feeds made from solid state fermented bambara nut meal and used as substitute for fishmeal on the catfish protein efficiency ratio after 56 days feeding period.

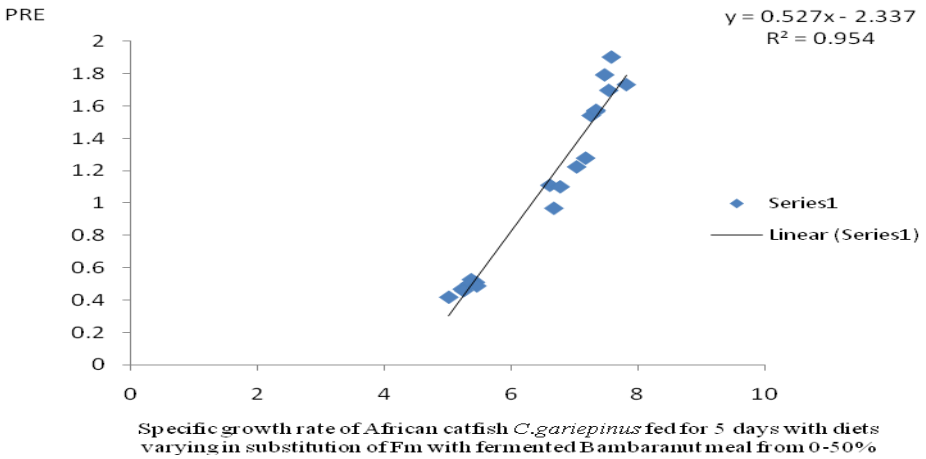

Figure 6: Relationship of protein efficiency ratio and specific growth rate of African catfish fed diets varying in inclusion levels of FM and BNM (as illustrated in Table 1).

The catfish fed with feed 1 had the lowest protein conversion ratio of 0.28±0.23. This means that it will take 0.28±0.23 of the feed to make 1 g of the

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

protein of the fish. On the other hand, it will take 0.54±0.35 of the feed 5 F5 to make a 1 g of the fish protein. There was no significant difference (p>0.05) in the PCR of catfish fed with F2, F3, and the control diet (non fermented BNM diet).

The cost per unit protein measures the cost of producing 1 kg protein of the fish. The diet that produced the highest cost per unit protein in the catfish was feed F1, USD 0.031 (₦12.09±0.07). The cost per unit protein of the catfish fed with F1 was significantly different from that of all other feeds (p<0.05). The cost per unit protein of catfish fed with F2, F3 and the F6, control diet were similar (p>0.05). Similarly, there was difference (p>0.05) in the cost per unit protein of catfish fed with 5% fishmeal diets USD 0.022 (₦8.44±0.03) and 0% fish meal diets USD 0.021 (₦8.27±0.01).

The catfish showed gonadosomatic index which was highest for those fed with F2. The GSI of catfish fed with F2 was significantly (p<0.05) higher than that of catfish fed with F1 (GSI, 0.09). There was higher GSI for the catfish fed raw BNM than the fermented at their inclusion level (p<0.05). Similarly, the catfish fed with F3, F4 and F5 had similar GSI (p>0.05). The visceral fat somatic index measures the quantity of fats deposited in the visceral cavity of the fish against the body weight. The highest quantity of visceral fat was taken from catfish fed diets with complete substitution of FM (F5). The VSI was also high for the catfish fed 5% FM diet (F4) with VSI 1.59%. There was no significant difference (p>0.05) between the VSI of catfish fed with the commercial diet and those on 5% FM diets. The catfish VSI was positively correlated to increasing FBNM (R^2=0.528, Fig. 7).

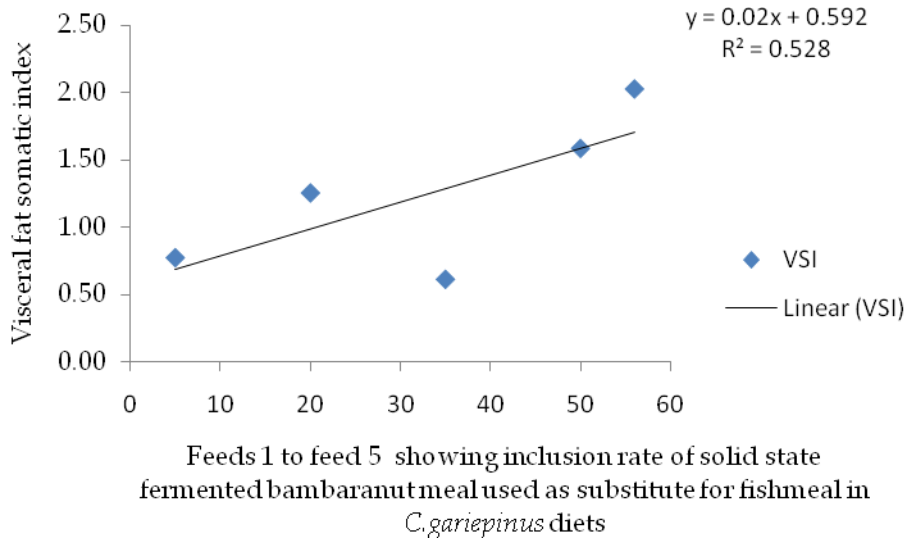

Figure 7: The effects of increasing inclusions of solid state fermented bambarabut meal on the visceral fat somatic index of African catfish fed diets substituting fishmeal with BNM.

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Waste production ratio was lowest for the catfish fed with F1 (WPR, 0.25±0.08). This was significantly lower than all other WPR of the treatment feeds (p<0.05). Alternating the substitution of FM with FBNM as in F3 elevated the WPR from 0.35±0.66 in F2, to 0.48 ± 0.11 in F3, However there was no differences in the waste production ratio of F2 and F3. Similarly, complete substitution of FM with FBNM elevated the waste production ratio to 1.33±0.65. The commercial diet had the least waste production ratio of 0.10±0.08.

The cost of making the diets was highest for feed 1, USD 1.46 (₦570.3, at ₦ 390 USD^{-1}). The cost of making F1 was significantly higher (p<0.05) than cost of making F2, USD 0.87 (₦ 342.7 at ₦ 390 USD^{-1}). However, cost of producing the feed increased by alternating the ingredients of F2 to produce feed 3 to costing USD 0.91 (₦ 358.6 at 390 USD^{-1}).

Cost per unit protein presents a resolution of cost of producing 1 g of the fish protein. Feed F1 had the highest cost per unit protein of USD 0.031 (₦ 12.09±0.07). Cost per unit protein was the same (p>0.05) for F2 USD 0.019 (₦7.50±0.08) and F3, USD 0.019 (₦ 7.70±0.04). Cost per unit protein increased as more fishmeal are replaced peaking at USD 0.022 (₦ 8.44±0.03) for F4 and USD 0.021 (₦ 8.27±0.01) for F5.

Discussion

Based on the results, use of solid state fermented BNM as FM substitute was beneficial in the diets of African catfish. The growth rate of the catfish was significantly improved by incorporation of FBNM instead of the raw meal. Although the incorporation of non fermented or raw BNM also produced good growth rate, the fermented variant was better. A consideration of the SGR of F1 and control F6 shows a difference in performances due to the use of FBNM. However, when FBNM was used alone in 100% substitution of FM the difference was minimal. Fermentation of BNM with yeast has been previously reported to increase protein content (Lio and Wang, 2012). There was high SGR in all treatment diets made from solid state fermented BNM. Previously, Enyidi *et al*. (2017) utilized raw BNM in diets of fingerling African catfish and had maximum SGR of 5.5% day^{-1}. This record was surpassed in this research using FBNM in feed production with maximum SGR of 7.82±0.25% day^{-1}. Higher SGR and weight gain were recorded for Indian shrimp *Fenneropeneaus indicus* fed with diets contained fermented prawn shell (Amar *et al*., 2006).

The explanation could be that fermentation conditioned the ingredients positively to be utilised by the fish. The feed intake was also improved by the high inclusion of FM which provided much needed amino acids. There was a positive correlation between FM and feed intake (R^2=0.952). In Table 2 we noted improved amino acid content of FBNM. It seems that the FBNM together with the FM could have contributed to better utilization of the diets than the raw

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

BNM. In a previous experiment fermentation of bambara nut meal with a mixture of *Rhizopus oligosporous* and *R.nigricans* increased crude protein from 18.66 to 22.60% (Olanipekun *et al.,* 2012). Fermentation of soybean meal with *S. cerevisiae* had protein content increased from 47% to 58% (Omafuvbe *et al*., 2002; Song *et al*., 2008). The increased protein content could have been due to the fungal action in breaking down the proteins to simpler forms making them easily utilized by the catfish by increase proteolytic enzyme production from the microflora (Heseltine, 1983).

Fermentation reduces anti nutritional factors in plant diets. This could also have accounted for better SGR of the catfish in this paper. In previous works, fermentation had been noted to reduce the anti nutritional factors that interact with nutrient utilization such as phytic acid and other anti nutritional factors (Egounlety and Aworth, 2003; Song *et al*., 2008; Lio and Wang, 2012). Fermentation of bambaranut meal has been known to reduce anti nutritional factors like tannins (Obizoba and Egbuna, 1992; Nwanna *et al.,* 2005, Fadahunsi, 2009; Olanipekun *et al*., 2015). Bambara nut has a lower content of phytic acid (0.84%) (Enyidi and Mgbenka, 2015) compared to soybean 1.4% (Liu, 1997). Some of the ANF in the feed may be because of the non starch polysaccharides that are non digestible by the fish. It seems that during fermentation microorganisms digest the carbohydrates in bambara nut meal while using it for their own growth. Consequently, the decreased dry matter and elevated microorganism's weight ratio causes the increased protein content (Hong *et al.,* 2004; Chen *et al.,* 2010).

The weight gain of the catfish was also affected by diets. Although the final average weight of the catfish was best at highest FM inclusion 50% for the catfish diet F1, alternating FBNM with unfermented BNM in diet F6, significantly reduced the weight gain. This goes to show the importance of using FBNM instead of unfermented in the diets of African catfish. However weight gain reduced with increasing substitution of FM. There was a major difference in the catfish weight gain when FBNM was substituted for FM than when raw bambara nut was used. In a previous research, increased weight gain was noted when fermented sesame seed was used in substituting fishmeal in the diets of *Labeo rohita* (Mukhopadhyay and Ray, 1999). Fermentation causes elevation of vitamins including riboflavin, niacin, vitamin B6 and β-carotene. This elevation is due to processing of the carbohydrates by the fermenting microorganism. This may be among the reason for better performance of fish fed with fermented feed than the non fermented control diet. It seems that nutrient interactions with FM and other dietary components, amino acids and carbohydrates broken down during fermentation, could have accounted for the weight gain differences between using FBNM and non fermented BNM.

Fermentation also improved the FCR when FBNM was used in substitution of FM. In substitution of FM with

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

FBNM 5% BNM substitution level compared favorably with 20% BNM. The reason for similar FCR when 5% BNM and 20% BNM were used in substituting FM in the diets of *C. gariepinus* could be because of the fermentation. The performances of the catfish on the FBNM substitute could also be attributed to increasing in the mineral content of the BNM after fermentation. In previous work, Nwanna *et al*. (2005) noted an increase in mineral content of yeast fermented fermentation BNM. Moreover, fermentation with yeast removed hard to digest carbohydrates like galactose, polysaccharides and α, α tetra-halose (Yoon *et al*., 2003), therefore making the feed easily utilizable by the catfish with a low FCR. Alternation of the feed mixes of F2 and F3 increased FCR of the catfish. The increase in FCR of F3 could be due to the higher starch content of F3 compared to F2.

The high cost of fishmeal could make utilization of diet F1 more costly than the F2. This makes it cost effective to use F2, considering also the cost of production per kg of feed USD 1.46, (₦570.3) for F1 and USD 0.88, (₦342.7) for F2. Also cost per unit protein is favorable to F2 as against use of F1. Cost per unit protein of using F1 was USD 0.031 (₦ 12.09) for F1 and USD 0.019 (₦ 7.50) for F2, respectively. The protein efficiency ratio (PER) of the catfish was negatively correlated with increasing BNM and increasing (R^2 0.958). Conversely, PER was positively correlated to SGR (R^2 0.954). This could be because the growth of the fish is based on accretion of more biomass which is protein based. Consequently, the more efficient protein the higher growth rate. Increasing inclusion of FBNM may not have increased PER, but increased the visceral somatic fat. Its seems that the catfish was able to utilize the FBNM in production of more visceral fat. There was positive correlation between the visceral fat and increasing inclusion of FBNM (R^2=0.528). It seems that dietary carbohydrate increases accumulation of body fat. In previous researches, Lin and Shiau (1995) and Hemre *et al*. (2002) noted that dietary carbohydrate increases the efficiency of lipid biosynthesis from dietary lipid. BNM contains small quantity of lipid, but predominantly unsaturated fatty acids like oleic, palmitoleic and caprylic acid.

The carbohydrate content of BNM seems to have affected the hepatosomatic index (HSI) of the catfish. Increasing BNM could have increased carbohydrate in feed and more lipigenic activity of liver (Walton and Cowey, 1982; Hemre and Storebakken, 2000; Hemre *et al*., 2002). The HSI of the catfish increased with increasing BNM inclusion. This could be due to the fact that the liver acts on the carbohydrate breaking it down to fatty acids and glycerol, therefore affecting the HSI. Feed acceptance was however increased more by the inclusion of fishmeal than BNM. The feed did not seem to have any definite effects on the GSI. However, this may be because of the duration of the experiment. However, the catfish fed with the fermented diets and those on

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

non fermented had similar GSI. Further rearing of the catfish on the diets may change the GSI as the catfish develops.

Solid state fermentation of BNM is a significant means of improving nutritional values of feed stuff. Growth and nutritional performance of the African catfish can be enhanced by utilising solid state fermented ingredients in their diet formulation. Solid state fermentation seems to reduce ANF and improving the amino acids profile of the ingredient thereby improving food conversion ratio and supporting fast growth. The technique for solid-state fermentation is relatively easy and can be adapted especially by farmers in sub-Saharan Africa where farms made feed are regularly used to arguments costly imported feed. The reduction in usage of fishmeal can cost saving judging from costs of using F1, F2 and F3 feeds. Moreover, the needs for incorporation of exogenous enzymes like phytase (which is not easy to buy in the developing nations) in feeds can be curtailed using SSF ingredients.

References

Amar, B., Philip, R. and Singh, B., 2006. Efficacy of fermented prawn shell waste as a feed ingredient for Indian white prawn *Fenneropenaeus indicus*. *Aquaculture Nutrition*, 12(**6**), 433-442.

Babu, K.R. and Satyanarayana, T., 1996. Production of bacterial enzymes by solid state fermentation. *Journal of Scientific and Industrial Research*, 55, 464-467.

Basu, S., Roberts, J.A., Azam-Ali, S.N., and Mayes, S., 2007. Bambara groundnut. In: Kole C (ed) Pulse, sugar and tuber crops. Genome mapping and molecular breeding in plants. Vol. 3. Springer-Verlag, Berlin Heidelberg, pp 147-157.

Bhat, R. and Karim, A.A., 2009. Exploring the nutritional potential of wild and underutilized legumes. *Comprehensive Reviews in Food and Food Safety*, 8, 305 -332.

Brijwani, K., Oberoi, H.S. and Vadlani, P.V., 2010. Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry*, 45, 120−128.

Chen, S., Paengkoum, P., Xia, X. and Na-Lumpang, P., 2010. Effects of dietary protein on ruminal fermentation, nitrogen utilization and crude protein maintenance in growing Thai-indigenous beef cattle fed rice straw as roughage. *Journal of Animal and Veterinary Advances*, 8, 1131 -1136

Chinn, M.S., Nokes, S.E. and Strobel, H.J., 2007. Influence of process conditions on end product formation from Clostridium thermocellum 27405 in solid substrate cultivation on paper pulp sludge. *Bioresource Technology*, 98, 2184–2193.

Choct, M., rsjant-Li, Y., McLeish, J. and Peisker, M., 2010. Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. *Asian-*

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Australasian Journal of Animal Sciences, 23, 1386–1398

Dakora, F.D. and Muofhe, L.M., 1995. Nitrogen fixation and nitrogen nutrition in symbiotic bambara groundnut (*Vigna subterranea* (L.) Verdc.) and Kersting's bean (*Macrotyloma geocarpum* (Harms) Marech. et Baud.) In: Hekler J, Begemann F, Mushonga J (ed) Proceedings of the workshop on conservation and improvement of bambara groundnut (*Vigna subterranea*) (L.) Verdc.), Harare Zimbabwe IPGRI Gatersleben/Department of Research & Specialist Services, Harare/International Plant Genetic Resources Institute, Rome, Italy. pp. 72-78.

Dharmaraj, S., 2010. Marine streptomyces as a novel source of bioactive substances. *World Journal of Microbiology and Biotechnology*, 26, 2123-2139.

Dorsa, W. J., Robinette, H. R., Robinson, E. H., Poe, W. E., 1982. Effects of dietary cottonseed meal and gossypol on growth of young channel catfish. Transactions American Fisheries Society, 111: 651–655

Duke, J.A., 1981. Handbook of legumes of world importance. Premium press, New York, USA. 345 P.

Egounlety, M. and Aworth, O.C., 2003. Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa*) Harm. *Journal of Food Engineering*, 56, 249-254.

Ekenyem, B.U. and Onyenagoro, C.P., 2006. Replacement value of bambara nut *Voandzeia subterranea* sievate for soybean meal *Glycine max* on the performance of finisher broiler chicken. *International Journal of poultry Science*, 5, 381-384

Enyidi, U.D., 2012. Production of feeds for African catfish *Clarias gariepinus* using plant proteins. Jyvaskyla Studies in Biological Sciences, 251 P. ISBN:978-951-39-4925-9, ISSN: 1456-9701

Enyidi, U., Kiljunen, M., Jones, R., Vielma, J. and Pirhonen, J., 2013. Nutrient assimilation by first-feeding African catfish (*Clarias gariepinus*) assessed using stable isotope analysis. *Journal of the World Aquaculture Society*, 44, 161-172.

Enyidi, U. and Mgbenka, B.O., 2000. Growth and nutritional effects of bambaranut waste meal on the first feeding frys of African catfish (*Clarias gariepinus*) (Burchell, 1822). Aquaculture Canada 2000 University of Moncton New Brunswick Canada. http://www.aquacultureassociation.ca/abst2000/fishnut.html accessed 12.21.2018

Enyidi, U.D. and Mgbenka, B.O., 2015. Replacement of fish meal with bambara nut waste Meal in the diets of larval African catfish *Clarias gariepinus* Burchell (1822). *British*

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Journal of Applied Science and Technology, 5(**6**), 526-537. DOI: 10.9734/BJAST/2015/12886

Enyidi, U.D., Pirhonen, J., Kettunen, J. and Vielma, J., 2017. Effect of feed protein: Lipid ratio on growth parameters of African catfish *Clarias gariepinus* after fish meal substitution in the diet with bambara nut (*Voandzeia subterranea*) meal and soybean (*Glycine max*) meal. *Fishes*, 2, 1. DOI: doi:10.3390/fishes2010001

European Commission, 1998. Commission directive 98/64/EC. Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC. *Official Journal*, 257, 14-28.

Fadahunsi, I.F., 2009. The effect of soaking, boiling and fermentation with *Rhizopus oligosporus* on the water soluble vitamin content of bambara groundnut: *Pakistan Journal of Nutrition*, 8(**6**), 835 – 840

Francis, G., Makkar, H.P.S. and Becker, K., 2001. Anti nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, 199, 197-227.

Gatlin, III D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, G.T., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. and Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*, 38, 551-579.

Gupta, S., Kapoor, M., Sharma, K.K., Nair, L.M. and Kuhad, R.C., 2008. Production and recovery of an alkaline exo-polygalacturonase from *Bacillus subtilis* RCK under solid-state fermentation using statistical approach. *Bioresource Technology*, 99, 937–945.

Hardy, R.W. and Tacon, A.G.T., 2002. Fishmeal: Historical uses, production trends and future outlook for supplies. In: Responsible marine aquaculture Eds. R.R.. Stickney, J.P. MacVey, CABI publishing, New York. pp. 311-325

Hardy, R.W., 2010. Utilization of plant protein in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Resources*, 41, 770-776

Hemre, G.I and Storebakken, T. 2000. Tissue and organ distribution of C-14 activity in dextrin adapted Atlantic salmon after oral administration of radiolabelled C-14(1)-glucose. *Aquaculture Nutrition*, 6, 229–234.

Hemre, G.I., Mommsen, T.P. and Krogdahl, Å., 2002. Carbohydrate in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, 8, 175-194

Hemre, G.I., Amlund, H., Aursand, M., Bakke, A.M., Olsen, R.E., Ringo, E. and Svihu, B., 2009. Criteria for safe use of plant ingredients in diets for aquaculture fish. Opinion of the panel of animal feed of the Norwegian Scientific

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Committee for Food Safety pp. 1-173.

Heseltine, C.W., 1983. The future of fermented foods. *Nutrition Review*, 41, 293-301.

Hölker, U. and Lenz, J., 2005. Solid-state fermentation—Are there any biotechnological advantages? *Current Opinion in Microbiology*, 8, 301–306

Hong, K.J., Lee, C.H. and Kim, S.W., 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *Journal of Medical Food*, 7, 430–5

Imorou Toko, I., Fiogbe, E.D. and Kestemont, P., 2008. Mineral status of African catfish (*Clarias gariepinus*) fed diets containing graded levels of soybean or cottonseed meals. *Aquaculture*, 275, 298-305

Kainz, M., Arts, M. and Mazumder, A., 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic level. *Limnology and Oceanography*, 49, 1784-1793

Karunaratne, A., Azam-Ali, S., Sasey, A., Adu-dapaah, H. and Crout, N., 2008. Modelling the effect of temperature, soil moisture and photoperiod on growth and development of bambara groundnut (*Vigna subterranea* (L.) Verdc.): BAMGRO model. Global issues Paddock Action. In: Unkovich MJ (ed) Proceedings of the 14th Australian Agronomy Conference Adelaide South Australia, Australian Society of Agronomy. 4 P.

Khodanazary, A., Hajimoradloo, A. and Ghorbani, R., 2013. Influence of solid-state fermentation on nutritive values and enzymatic activities of Anchovy kilka (*Clupeonella engrauliformis* Svetovidov, 1941) meal by using different microorganisms. *International Research Journal of Applied and Basic Sciences*, 4(**8**), 2357-2367

Killham, K. and Prosser, J., 2007. The prokaryotes. In: Eldor P, editor. Soil microbiology ecology, and biochemistry. Third ed. Burlington: Elsevier. pp. 120–143

Krogdahl, Å. and Holm, H., 1981. Soybean proteinase inhibitors and human proteolytic enzymes: selective inactivation of inhibitors by treatment with human gastric juice. *Journal of Nutrition*, 111, 215-219

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S. and Bakke, A.M., 2010. Important antinutrients in foodstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquaculture Research*, 41, 333-344

Liener, I.E. and Kakade, M.L., 1980: Protease inhibitors In: Liener I.E. edited toxic constituents of plant foodstuffs. Academic Press, New York, USA 7-71.

Lio, J. and Wang, T., 2012. Solid-state fermentation of soybean and corn processing coproducts for potential feed improvement. *Journal of Agricultural and Food Chemistry*, 60, 7702–7709.

Lin, J.H. and Shiau, S.Y., 1995. Hepatic enzyme adaptation to

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

different dietary carbohydrates in juvenile tilapia (*Oreochromis niloticus* × *O. aureus*). *Fish Physiology and Biochemistry*, 14, 165-170

Liu, K., 1997. Soybeans: Chemistry, technology, and utilization. New York: Chapman and Hall Press 532 pp

Lunger, A.N., Craig, S.R. and Mclean, E., 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. *Aquaculture*, 257, 393 -399.

Mukherjee, K., Adhikari, H. and Rai, S.K., 2008. Production of alkaline protease by a thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition using Imperata cylindrical grass and potato peel as low-cost medium: characterization and application of enzyme in detergent formulation, *Biochemical Engineering Journal*, 39, 353–361.

Mukhopadhyay, N. and Ray, A.K., 1999. Effect of fermentation on the nutritive value of sesame seed meal in the diets for rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquaculture Nutrition*, 5, 229-236

NRC, (National Research Council), 1993. Nutrient requirements of fish. National Academy Press, Washington, DC, 114 P.

Nwanna, L.C., Enujiugha, V.N., Oseni, A.O. and Nwanna, E.E., 2005. Possible effects of fungal fermentation on bambara groundnut (*Vigna subterranean* (L) as a feedstuff resource. *Journal of Food Technology*, 3(**4**), 572-575

Obih, T.K.O. and Ekenyem, B.U., 2010. Performance and cost evaluation of substituting bambara seed *Vigna subterranean* (L) Verdc. Offal for soybean meal in the diets of broiler starter chicks. *International Journal of Poultry Science*, 9, 349-351

Obizoba, I.C. and Egbuna, H.I., 1992. Effect of germination and fermentation on the nutritional quality of bambara nut (*Voandzeia subterranea* L. Thouars) and its product (milk). *Plant Foods for Human Nutrition*, 42, 13–23.

Olanipekun, B.F., Otunola, E.T., Adejuyitan, J.A. and Adeyanju, J.A., 2012. On proximate and fatty acid composition of bambara groundnut (*Voandzeia subterranean* L. Thouars) a influenced by fermentation with a combination of *Rhizopus oligisporous* and *R. Nigricans*. *Transnational Journal of Science and Technology*, 2, 77-87.

Olanipekun, B.F., Otunola, E.T. and Oyelade, O.J., 2015. Effect of fermentation on antinutritional factors and *in vitro* protein digestibility of bambara nut (*Voandzeia subterranean* L.). *Food Science and Quality Management*, 39, 99-110

Olli, J.J. and Krogdahl, Å., 1994. Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in fresh water. *Acta Agricultura Scandinavica*, 44, 50-60

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Omafuvbe, B.O., Abiose, S.H. and Shonukan, O.O., 2002. Fermentation of soybean (*Glycine max*) for soy -daddawa production by starter cultures of Bacillus. *Food Microbiology*, 19, 561-566.

Opazo, R., Ortu´zar, F., Navarrete, P., Espejo, R. and Romero, J., 2012. Reduction of soybean meal non-starch polysaccharides and a-galactosides by solid-state fermentation using cellulolytic bacteria obtained from different environments. *PLoS ONE*, 7(**9**), e44783. DOI:10.1371/journal.pone.0044783.

Pandey, A., 2003. Solid-state fermentation. *Biochemical Engineering Journal*, 13, 81–84.5.

Papagianni, M., Nokes, S.E. and Filer, K., 2001. Submerged and solid-state phytase fermentation by *Aspergillus niger*: Effects of agitation and medium viscosity on phytase production, fungal morphology and inoculum performance. *Food Technology and Biotechnology*, 39(**4**), 319–326

Parrish, C.C., 1999. Determination of total lipid classes and fatty acids in aquatic samples. In: Wetzel RG, Art MT, Wainmann BC (ed)Lipids in Freshwater Ecosystems. Springer-Verlag, New York, NY, USA. pp. 4-20

Raboy, V. and Dickinson, D.B., 1993. Phytic acid levels in seeds of - and G soja as influenced by phosphorus status. *Crop Science*, 33, 13000-1305

Refstie, S., Svihus, B., Shearer, K.D. and Storebakken, T., 1999: Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non starch polysaccharide content in different soyabean products. *Animal Feed Science and Technology*, 79, 331-345

Robinson, E.D. and Li, M., 1996. A practical guide to nutrition, feeds, and feeding of catfish. Bulletin 1041 -- March Delta Branch Experiment Station Stoneville, Mississippi USA. Pg1-71

Ruohonen, K., Koskela, J., Vielma, J. and Kettunen, J., 2003. Optimal diet composition for European whitefish (*Coregonus lavaretus*): analysis of growth and nutrient utilization in mixture model trials. *Aquaculture*, 225, 27-39.

Santos, M., Rosa, A.S., Dal'boit, S., Mitchell, D.A. and Kriger, N., 2004. Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes. *Bioresource Technology*, 93, 261−268

Servi, S., Ozkaya, H. and Colakoglu, A.S., 2008. Dephytinization of wheat bran by fermentation with baker's yeast, incubation with barley malt flour and autoclaving at different pH levels. *Journal of Cereal Science*, 48, 471-476

Shi, C., He, J., Yu, J., Yu, J., Huang, B., Mao, Z.X., Zheng, P.P. and Chen, D., 2015. Solid state fermentation of rapeseed cake with *Aspergillus niger* for degrading glucosinolates and upgrading nutritional value. *Journal of Animal*

[DOR: 20.1001.1.15622916.2020.19.4.9.7]

Science and Biotechnology, 6(**13**), 2-7

Song, Y.S., Frias, J., Martinez-Villaluenga, C., Vidal-Valdeverde, C. and deMejia, E. G., 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chemistry*, 108, 571-581

Storebakken, T., Shearer, K.D. and Roem, A.J., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein concentrate based diets to Atlantic salmon *Salmo salar*. *Aquaculture*, 161, 365-379

Walton, M.J. and Cowey, C.B., 1982. Aspects of intermediary metabolism in salmonid fish. *Comparative Biochemistry and Physiology*, 73, 59-79.

Wei, H., Xu, Q., Taylor, L.E., Baker, O.J., Tucker, M.P., Ding, S-Y., 2009. Natural paradigms of plant cell wall degradation. *Current Opinion in Biotechnology*, 20, 330–338

Wenzel, M., Schonig, I., Berchtold, M., Kampfer, P. and Konig, H., 2002. Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. *Journal of Applied Microbiology*, 92, 32–40

Wilson, D.B., 2011. Microbial diversity of cellulose hydrolysis. *Current Opinion in Microbiology*, 14, 259–263

Yoon, S.H., Mukerjea, R. and Robyt, J.F., 2003. Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation. *Carbohydrate Research*, 338, 1127-1132

Yildirim-Aksoy, M., Lim, C., Dowd, M.K., Wan, P.J., Klesius, P.H. and Shoemaker, C., 2004. *In vitro* inhibitory effect of gossypol from gossypol-acetic acid and (+)- and (-)-isomers of gossypol on the growth of *Edwardsiella ictaluri*. *Journal of Applied Microbiology*, 97, 87-92.