




Evaluation of fermented African mesquite seed (*Prosopis africana*) on growth and nutrient digestibility of Nile tilapia (*Oreochromis niloticus*) fingerlings

Shina Al-Habeeb Aroyehun • Wilfred Olusegun Alegbeleye • Olubunmi Temilade Agbebi • Abiodun Akinde

Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, Ogun State, Nigeria

Correspondence

Shina Al-Habeeb Aroyehun; Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, Ogun State, Nigeria

 saalhabeeb@yahoo.com

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Abstract

Production of cheap and quality dietary protein to replace the expensive conventional protein was studied in an 84-day feeding trial of Nile tilapia *Oreochromis niloticus*. African mesquite (*Prosopis africana*) seeds were subjected to fermentation processes and four iso-nitrogenous diets (35% crude protein) were formulated to contain graded levels of fermented African mesquite seed meal (FMS) to replace soybean meal (SBM) at four inclusion levels (0%, 15%, 30% and 45%) replicated in a completely randomised design. Proximate analysis showed increased in crude protein (35.10% to 41.29%), amino acids concentration, minerals content (calcium and phosphorus) while there were reductions in crude fibre (7.79% to 2.13%), crude lipids (4.02% to 2.67%) and anti-nutritional factors of FMS. The highest protein digestibility of 83.35% was recorded in FMS30 while FMS15 showed the least (77.07%). Carcass compositions were affected by diets composition. The minimum feeding cost of US\$ 0.88 kg⁻¹ was registered in FMS45 while FMS15 recorded the highest value of US\$ 1.37 kg⁻¹. In conclusion, 45% of FMS could replace SBM without compromising growth performance of Nile tilapia.

Keywords: Economic efficiency; leguminous plants; Nile tilapia; processing; proximate analysis

1 | INTRODUCTION

Aquaculture in sub-Saharan Africa has shown a steady growth over the last decade driven by population growth and its important contribution to poverty alleviation (FAO 2017). However, despite remarkable performance observed significant challenges to its development remain in the region (Frimpong and Anane-Taabeah 2017). One of the major problems is the high cost of inputs (especially feed) as the sector involves growing volumes of feed. Fish feed constitutes 50 – 70 % of the operational costs based on the type of fish farming system being practiced (Mohsin *et al.* 2012; El-Sayed 2014), thus determines largely the profitability of fish production. To mitigate the effect of fish feed cost continuous effort is required to

evaluate the replacement value of the costly conventional protein sources such as fish meal and soybean meal owing to unprecedented high price, competitive use as a dietary protein source for human and biofuel production (Moutinho *et al.* 2017). In order to achieve that, it is essential to study the nutrient composition and utilisation of underused leguminous protein sources that could substitute soybean meal as an exclusive feed source. Various studies have been reported on the use of different plants as partial or complete replacements for fishmeal or soybean meal in aqua feeds (Richter *et al.* 2003; Dernekba and Karayucel 2017). In some studies, reasonable feed utilisation and growth performance ratios were achieved (e.g. Yue and Zhou 2008; El-Tawil *et al.* 2020). In others,

growth shown to be insignificant in fish fed substitute diets as a replacement for soybean meal or fishmeal as revealed by Xie *et al.* (2021).

African mesquite seed (*Prosopis africana*) is a leguminous plant mostly found growing in Nigeria and other parts of West African region (Yousif 2003). The pods (fruits) are sickle in shape and flattened with each containing 10 to 20 seeds embedded in sweet pulp (Yousif 2003). Its English names include African mesquite, Iron tree, while its local African names are Kiriya (Hausa), Ayan (Yoruba) and Ubwa (Ibo) (Ogunshe *et al.* 2007). However, there appears to be limited report on the use of African mesquite seed as aqua feed. Hence, research thus aimed to study the effects of fermentation processing technique on the nutrient composition of African mesquite seeds and utilisation of fermented meal (FMS) in the practical diet of Nile tilapia *Oreochromis niloticus* fingerlings.

2 | METHODOLOGY

2.1 African mesquite seed collection and processing

Two kilograms of dried African mesquite seeds were obtained from a local market in Benue, Nigeria. Other feedstuffs such as soybean meal, groundnut cake, fish meal, and maize meal were purchased from a reputable livestock feed store at Fajol area, Abeokuta, Nigeria.

African mesquite seeds were fermented with *Aspergillus niger* and *A. flavus* according to the modify procedure by Balogun and Oyeyiola (2012). It was boiled in distilled water at 100°C for 6 h. The boiled seed coats were removed and poured in 10 L plastic container. Then the seeds were inoculated with starter organisms at 2% (weight basis) and left for 3 days to be colonised by *Aspergillus niger* and *A. flavus* mycelia at the veterinary microbiology laboratory, Federal University of Agriculture Abeokuta (Funaab). The fermented seeds were oven dried at 55°C for 24 h, cooled in air and milled with food blender (model: 3D, China), and sieved to produce fermented African mesquite seed meal (FMS).

Proximate composition of fermented and raw African mesquite seed meals (Table 1) were analysed for chemical composition according to the method of Association of Official Analytical Chemists (AOAC 2005). Amino acid profiles (Table 2) were determined following Benitez (1989). The anti-nutrient analysis such as phytate and tannin were determined by the methods of Graham (1992) and Wheeler and Ferrel (1971) respectively. The initial and final Nile tilapia carcass proximate analyses were determined according to AOAC (2005).

2.2 Experimental diets

One control diet (0% FMS) and three isonitrogenous (35% CP) and isocaloric diets (17 kJ g⁻¹) were compounded using Pearson square method to substitute SBM with FMS at 15%, 30% and 45% graded levels (Table 3). As an indigestible marker to estimate the total digestibility of dry matter and protein in feed, 0.5% chromium oxide (Cr₂O₃)

was added to each treatment diet (Olude *et al.* 2016). The feedstuffs were carefully mixed and 5% vegetable oil was added to each treatment diet. The diets were pelleted using 2.0 mm die hand fabricated pelleting machine. Thereafter, the pelleted feeds were sun dried and stowed in tagged plastic containers before stored in refrigerator.

TABLE 1 Nutritional content and anti-nutritional factor of fermented and raw African mesquite seed (%).

Parameters	Fermented	Raw
Moisture	9.41 ± 0.05 ^b	14.80 ± 0.40 ^a
Crude protein	41.29 ± 1.10 ^a	35.10 ± 1.07 ^b
Crude lipid	2.67 ± 0.66 ^b	4.02 ± 0.01 ^a
Ash	5.25 ± 0.15 ^a	4.00 ± 0.15 ^b
Crude fibre	2.13 ± 0.03 ^b	7.79 ± 0.07 ^a
Nitrogen free extract	39.25 ± 0.62 ^a	34.29 ± 1.27 ^b
Calcium	5.56 ± 0.76 ^a	2.11 ± 0.07 ^b
Phosphorus	3.70 ± 0.06 ^a	2.31 ± 0.04 ^b
Tannin	ND	2.8
Phyate	ND	3.6

ND, not detected; Means ± standard errors on the same row with the different superscript are significantly different ($p < 0.05$).

TABLE 2 Amino acids concentration (g 100g⁻¹ protein) of soybean, raw and fermented African mesquite seed.

Amino acid	Fermented	Raw	Toast soybean ^a
Essential			
Arginine	6.88	5.59	4.48
Histidine	2.62	2.24	3.00
Isoleucine	3.01	3.21	2.32
Leucine	9.75	7.29	6.00
Lysine	6.26	4.56	3.60
Methionine	2.35	1.82	0.88
Phenylalanine	4.97	3.99	3.06
Threonine	3.99	3.38	2.80
Tryptophan	1.21	1.00	-
Valine	3.51	3.67	2.85
Non-essential			
Tyrosine	3.44	3.10	2.63
Proline	3.55	3.25	3.08
Cystine	1.09	0.85	0.70
Alanine	5.00	4.17	3.04
Glutamic acid	14.53	12.26	14.94
Glycine	5.51	4.51	3.35
Serine	3.83	3.46	1.90
Aspartic acid	11.72	9.02	10.49

^a Ari *et al.* (2012).

2.3 Fish and feeding trial setup

The study was carried out at the indoor fish nursery building of Federal University of Agriculture Abeokuta, Nigeria. A total of one hundred and twenty mono sex fingerlings of Nile tilapia (5.54 ± 0.03 g) were procured from a reliable farm in Isheri-Osun, southwestern Nigeria. The fish were transported to nursery building using oxygen bag

and acclimatised in 2500 L circular tank for one week. The fish were placed on commercial feed prior to being starved for 24 h to the start of the experiment. The fingerlings were randomly shared to the 12 experimental tanks (30 fish per treatment replicated thrice) of 40 L capacity in a completely randomised design. Experimental fish in each tank were fed tested diets twice a day (0900 h and 1600 h) for 84 days (between July and September, 2017) at the rate of 3% body weight and the rations were adjusted weekly according to weight gain. Water quality parameters such as pH, temperature and dissolved oxygen were maintained by ensuring twice weekly water exchange throughout the study period. After feeding the fish for 84 days, growth and nutrient digestibility parameters were calculated using the following equations:

$$\text{Mean weight gain (g)} = W_2 - W_1$$

$$\text{Specific growth rate (\% \cdot \text{day}^{-1})} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$$

Where W_1 and W_2 are the initial and final weight of fish respectively (g); \ln is the natural logarithm; and T_1 and T_2 represent beginning and end of the experiment respectively, in days.

$$\text{Survival (\%)} = \frac{\text{Fish stocked} - \text{Mortality}}{\text{Fish stocked}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed fed (g)}}{\text{Weight gain (g)}}$$

$$\text{Feed efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Feed fed (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{protein intake (g)}}$$

$$\text{Protein productivity value (\%)} = \frac{N_b - N_a}{N_i} \times 100$$

Where N_a and N_b are the initial and final carcass protein respectively; N_i is the protein intake (g)

Fish faeces samples were collected (Olude *et al.* 2016) for 14 days toward the end of the feeding trial from the rearing tanks and air dried prior to protein analysis by AOAC (2005) method. Apparent digestibility coefficient (ADC) was determined by acid digestion method of Furukawa and Tsukahara (1966):

$$\text{ADC(\%)} = 100 - \left[100 \times \left(\frac{\% \text{Cr}_2\text{O}_3 \text{ in diet}}{\% \text{Cr}_2\text{O}_3 \text{ in faeces}} \times \frac{\% \text{nutrient in faeces}}{\% \text{nutrient in diet}} \right) \right]$$

2.4 Feed and feeding cost evaluation

The production cost of each treatment was determined according to Moutinho *et al.* (2017) taking to cognisance cost price of the feedstuff during the feeding trial. The cost of each experimental diet was deduced by multiplication of each share of the feedstuff by its respective costs per kilogram. Other parameters such as economic conversion ratio (ECR; feed cost to produce a kilogram of fish)

and economic profit index (EPI) of replacing FMS for SBM were also calculated.

TABLE 3 Proximate analysis and diet formulation of fermented African mesquite seed meal (FMS).

Ingredients	FMS0	FMS15	FMS30	FMS45
Ingredients (%)				
Maize	27.91	27.81	27.81	27.81
Fish meal	16.02	16.04	16.04	16.04
Groundnut cake	16.02	16.04	16.04	16.04
Soybean Meal	32.05	27.28	22.47	17.65
Fermented MSM ^a	-	4.81	9.63	14.44
Vegetable oil	5	5	5	5
Vit/ Min premix ^b	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Starch	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25
Dicalciumphosphate	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5
Proximate analysis (%)				
Moisture	9.29	9.39	9.17	9.22
Crude lipid	10.88	10.04	11.55	11.40
Ash	10.68	10.42	10.33	10.55
Crude protein	36.01	35.78	35.09	35.61
Crude fibre	4.08	4.81	4.79	4.93
Nitrogen free extract	29.08	29.58	29.08	28.30
Gross energy(kj g ⁻¹) ^c	17.42	17.12	17.45	17.38

^a mesquite seed meal;

^b vitamin and mineral premix (per kg of feed): vitamin A, 6000 IU; vitamin D3, 2000 IU; vitamin E, 100 mg; vitamin C, 100 mg; vitamin K3, 10 mg; vitamin B1, 15 mg; vitamin B2, 15 mg; vitamin B6, 15 mg; pantothenic acid, 50 mg; niacin, 60 mg; biotin, 0.2 mg; vitamin B12, 0.025 mg; folic acid, 3 mg; iron, 50 mg; zinc, 100 mg; cobalt, 0.1 mg; copper, 10 mg; selenium, 0.5 mg; manganese, 20 mg; magnesium, 500 mg; chromic oxide, 0.5%, 2 mg; inositol, 400 mg; choline, 2000 mg; anti-oxidant, 100 mg; calcium propionate, 1000 mg.

^c calculated using the factors: carbohydrates, 4.1 kcal g⁻¹; protein, 5.5 kcal g⁻¹ and lipids, 9.1 kcal g⁻¹ (New 1987) and transformed to *kJ* using the factor 4.184

2.5 Statistical analysis

All data on proximate composition, growth, feed and feeding evaluation were subjected to one-way analysis of variance (ANOVA) using IBM SPSS (version 9.0) statistical analysis programme at an α significant level of 0.05. Duncan Multiple Range Test was used to compare differences between means.

3 | RESULTS

The crude protein and ash content of the African mesquite seeds were positively ($p < 0.05$) affected by fermentation treatment (Table 1). Mean values of 41.29% and

5.25% were recorded for protein and ash respectively. The same was observed for calcium and phosphorus contents. However, crude fibre and lipid content were significantly reduced (Table 1). Tannin and phytate were below the detection limits in fermented sample. Generally, a higher value in all essential and non-essential amino acids was observed for the analysed seeds (Table 2).

The twice weekly observed water quality parameters during the feeding trial of Nile tilapia reared in plastic tanks showed a temperature range of 26.81 to 27.84°C, pH of 6.22 to 6.97 and dissolved oxygen of 6.62 to 7.93 mg L⁻¹. The average final weight, weight gain and specific growth rate of fish fed FMS45 were similar ($p > 0.05$) to those fed the FMS30 (Table 4). However, both were significantly different ($p < 0.05$) from control diet. Likewise, feed efficiency and protein efficiency ratio recorded for

FMS45 and FMS30 were similar ($p > 0.05$). The highest feed conversion ratio was recorded for FMS15. Survival rate, however, was not differ significantly ($p > 0.05$) among the dietary treatments. At the end of the trial, carcass composition was significantly affected ($p < 0.05$) and with elevated crude protein and decreased crude lipid as the inclusion level increased (Table 5). The various dietary levels of FMS significantly influenced ($p < 0.05$) diet cost and economic parameters, ECR and EPI (Table 6). FMS was lesser (as US\$ kg⁻¹) than SBM and diet cost reduced as the inclusion of FMS was increased. The ECR of the FMS15 diet was the highest (US\$ 1.37 kg⁻¹) while FMS45 diet recorded the minimum (US\$ 0.88 kg⁻¹). The highest value of EPI of US\$ 0.007 kg⁻¹ was shown in FMS45 group and least in FMS0 diets (US\$ 0.004 kg⁻¹).

TABLE 4 Growth and nutrient digestibility of Nile tilapia fingerlings fed fermented African mesquite seed meal.

Parameters	FMS0	FMS15	FMS30	FMS45
Initial weight (g)	5.53 ± 0.02 ^a	5.52 ± 0.10 ^a	5.54 ± 0.01 ^a	5.56 ± 0.02 ^a
Final weight (g)	10.60 ± 0.70 ^b	10.44 ± 0.35 ^b	13.63 ± 0.06 ^a	13.73 ± 0.14 ^a
Weight gain (g)	5.08 ± 0.60 ^b	4.91 ± 0.35 ^b	8.09 ± 0.06 ^a	8.17 ± 0.15 ^a
Survival rate (%)	100 ± 0.00 ^a	100 ± 0.00 ^a	96.67 ± 5.77 ^a	100 ± 0.00 ^a
Feed intake (g)	7.35 ± 0.26 ^c	7.58 ± 0.40 ^c	8.89 ± 0.35 ^b	9.07 ± 0.83 ^a
Specific growth rate (% day ⁻¹)	1.74 ± 0.00 ^b	1.71 ± 0.06 ^b	2.17 ± 0.02 ^a	2.18 ± 0.03 ^a
Feed conversion ratio	1.45 ± 0.04 ^b	1.54 ± 0.08 ^a	1.10 ± 0.05 ^c	1.11 ± 0.12 ^c
Feed efficiency ratio	0.69 ± 0.02 ^b	0.65 ± 0.04 ^b	0.91 ± 0.06 ^a	0.90 ± 0.14 ^a
Protein efficiency ratio	1.97 ± 0.05 ^b	1.85 ± 0.10 ^b	2.58 ± 0.17 ^a	2.57 ± 0.39 ^a
Protein productivity value (%)	0.66 ± 0.03 ^b	0.55 ± 0.00 ^b	1.37 ± 0.11 ^a	1.60 ± 0.24 ^a
Apparent dry matter digestibility (%)	77.88 ± 0.03 ^a	75.47 ± 0.66 ^c	76.41 ± 0.37 ^b	76.6 ± 0.46 ^b
Apparent protein digestibility (%)	83.23 ± 0.31 ^a	78.83 ± 0.55 ^b	83.35 ± 0.46 ^a	82.91 ± 0.37 ^a

Means ± standard errors ($n = 5$) on the same column with the different superscript are significantly different ($p < 0.05$)

TABLE 5 Proximate composition (%) of Nile tilapia fingerlings fed fermented African mesquite seed meal.

Parameters	Initial	FMS0	FMS15	FMS30	FMS45
Moisture	76.23 ± 0.34 ^a	73.56 ± 0.96 ^a	74.74 ± 0.34 ^a	74.67 ± 0.45 ^a	75.11 ± 0.46 ^a
Protein	52.22 ± 0.04 ^e	53.85 ± 0.05 ^c	53.75 ± 0.05 ^d	55.80 ± 0.0 ^b	56.45 ± 0.1 ^a
Lipid	8.70 ± 0.25 ^a	8.39 ± 0.2 ^a	7.18 ± 0.02 ^b	7.09 ± 0.01 ^b	7.03 ± 0.02 ^b
Ash	10.53 ± 0.08 ^d	11.22 ± 0.02 ^c	11.02 ± 0.01 ^c	11.45 ± 0.12 ^b	11.81 ± 0.06 ^a

Means ± standard errors ($n = 5$) on the same column with the different superscript are significantly different ($p < 0.05$)

TABLE 6 Economic analysis of Nile tilapia fingerlings fed fermented African mesquite seed meal.

Parameters	FMS0	FMS15	FMS30	FMS45
Diet price (US\$ kg ⁻¹)	0.93	0.89	0.84	0.79
Economic conversion ratio (US\$)	1.35 ± 0.50 ^b	1.37 ± 0.94 ^a	0.92 ± 0.15 ^c	0.88 ± 0.43 ^c
Economic profit index (US\$)	0.004 ± 0.03 ^c	0.004 ± 0.01 ^c	0.006 ± 0.02 ^b	0.007 ± 0.02 ^a

Means ± standard errors ($n = 3$) on the same column with the different superscript are significantly different ($P < 0.05$)

4 | DISCUSSION

The treatment techniques applied in this research influenced the nutrient composition. Fermentation resulted in increase in the crude protein content and a decline in the value of crude fibre, and ether extract. This increase in protein value may be due to the biomass of fungal protein or bioconversion of carbohydrates in colonised sub-

strates into mycelia proteins by the growing fungus during the fermentation process (Igbabul *et al.* 2014). The results are harmonious with previous studies (Annongu and Ter Meulen 2000; Yusuf *et al.* 2008) who reported 42.52%, 4.95% and 3.60% of crude protein, ash and ether extract respectively. The decreased lipid content and carbohydrates observed in fermented sample could be due

to the conversion of fatty acid as carbon and energy source by fermenting organism (De-Rue *et al.* 1994). The reduction of crude fibre content of fermented sample could be as result of the degradation by fermenting microbes (Igbabul *et al.* 2014). This is of great nutritional significance particularly to monogastric organism such as fish that cannot tolerate high levels of dietary fibre which result in low nutrient availability.

Some of the amino acid concentrations in fermented samples, such as methionine, leucine tryptophan in this study were on the high side when compared previous study (Annongu and Ter Meulen 2000) which could be due to the type of processing techniques employed. The results of amino acid compositions clearly depict African mesquite seed as a potential protein source comparable with other conventional protein sources especially of plant origin.

The presence of anti-nutritional factors in legume seeds has been interpreted as an expression of the chemical warfare of plants against their predators (Carmona 1996). Therefore, these seeds can best be utilised after some form of processing such as fermentation, toasting, autoclaving etc. to enhance the bio availability of the nutrients (Okomoda *et al.* 2016). The present study demonstrated that fermentation eliminated the anti-nutrient content of the seeds. This observation could have probably resulted from thermal destruction of endogenous phytase of African mesquite seed meal when it was being prepared for fermentation as elicited by Refstie *et al.* (2005).

The water quality parameters observed in this study showed that the condition in the experimental tanks were favourable for normal growth and physiological activities of Nile tilapia. This trial showed that the growth and nutrient utilisation of Nile tilapia were influenced by experimental diets. Generally, the group fed FMS30 and FMS45 showed the best performance in terms of growth and nutrient utilisation as these were revealed by the values of MWG and FCR observed in the present study. There was a direct relationship between performance and FMS inclusion levels. This suggested greater protein utilisation of FMS and an indicator of improved nutritional quality. The increase in apparent protein digestibility observed could probably be attributed to the lower indigestible insoluble non starch polysaccharides in the diets. The superior performance of the FMS30 and FMS45 over the control is noteworthy, since soybean could also be limited by imbalance in amino acid profile, presence of residual trypsin inhibitors (Storebakken *et al.* 2000). It is also known to contain about 20 – 30 % non-starch polysaccharide (NSP; Storebakken *et al.* 2000). Similar observations have also been reported by Alegbeleye *et al.* (2012).

Generally, nutrients are deposited in fish body at a rate proportional to its levels in diets (Imorou Toko *et al.* 2007). Final carcass crude protein was higher than the

initial; suggestive that tested diets favoured body protein deposition as much as the control, and confirmed an adequate protein digestibility in dietary treatments (Alegbeleye *et al.* 2012). This was further indicated by the value of protein productivity value observed in this trial.

Substitution of soybean meal with fermented African mesquite seed seem to be economically viable. The cost of the formulated diets for Nile tilapia was reduced as FMS levels increased and this is in consonance with various authors (e.g. Martinez-Llorens *et al.* 2007; Moutinho *et al.* 2017). Also, it was shown that the economic parameters analysed revealed better utilisation of various dietary level of FMS, resulting in lower economic conversion ratio for the FMS diets, with a higher economic profit index at 45% inclusion of FMS. According to Moutinho *et al.* (2017) EPI is a more appropriate parameter to assess economic profitability, as it considers feedstuff costs and fish selling price, hence, this finding imply that there is a remarkable economic return by substituting 45% FMS with SBM.

5 | CONCLUSIONS

It can be concluded that fermentation employed, improved the quality of African mesquite seed. Therefore, considering nutrient utilisation, cost of production and health status of Nile tilapia, FMS can partly replace soybean up to 45%.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

SAA & WOA designed the research, analysed the data and prepared the manuscript. **OTA** proof read the manuscript and assisted in supervisory role. **AA** assisted in the data collection and manuscript preparation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author.

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SA Aroyehun  <https://orcid.org/0000-0003-0010-470X>

WO Alegbeleye  <https://orcid.org/0000-0003-3944-8382>

A Akinde  <https://orcid.org/0000-0003-1753-655X>