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Performance Characteristics and Feed Utilization of African Cat Fish (*Clarias gariepinus*) Fed Varying Inclusion Levels of Fermented Mulberry Leaf

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A laboratory experiment was carried out, for 54 days, to evaluate the effects of diet, containing different levels of fermented mulberry (*Morus alba* S54) leaf meal (MLM) as dietary protein sources for replacing fish meal protein, on the growth performance, feed utilization and survival of *Clarias gariepinus* juveniles. Five iso-nitrogenous (30%) experimental diets were prepared at various levels of MLM inclusion of 0%, 10%, 20%, 30% and 40% designated as diets A, B, C, D and E respectively. The final weight gain, daily weight gain, percentage weight gain of the fish fed diets A, B and C were not significantly different ($P<0.05$) from one another, but these were significantly different ($P>0.05$) compared to fish fed with diets D and E. The specific growth rate (SGR) and food conversion ratio (FCR) of the fish fed the trial diets were however, not significantly different ($P<0.05$) from one another. The present findings show that MLM diets, replacing 20% fishmeal (FM) protein, is a good potential protein source, in terms of survival rate (SR), feed conversion ratio (FCR) and specific growth rate (SGR). The use of fermented mulberry (*Morus alba*) leaf meal of up to 20% level, is without adverse effects on the growth of *Clarias gariepinus* juveniles.

Key words: Catfish, growth, mulberry, sericulture, survival.

INTRODUCTION

In order to reduce production cost, make fish farming attractive to both private and commercial investor and bridge the already existing wide gap between fish demand and supply in the sub-Saharan Africa, the vital role of locally produced fish feed cannot be over emphasised. Fisheries play a vital role in the food and nutritional security of people, especially in rural areas (Sugunan, 2002). But the production of inland fish is far less than the demand, and naturally, the cost of the fish is high in this continent. So, there is necessity for augmentation of inland fish production. For optimum production of fish, supplementary diet is required, in addition to natural food present in the aquatic environment. Market available fishmeal is quite expensive and is in short supply.

Replacement of fishmeal by cost effective protein source is a priority research in aquaculture throughout the world because of growing scarcity of quality fishmeal supply and escalation of its cost as a result of high demand in the animal production industry. The nutritive value of fish feed depends largely on the quality of protein of the ingredients used in the formulation of feed (Glencross *et al.*, 2007; Li *et al.*, 2009; Hu *et al.*, 2013). Various animal sources are not sufficient to meet the growing demands of fish raising industry and the continued dependence on traditional feed ingredients like rice bran, oil cakes and fish meal has led to increase in the prices of those components (Kumar, 2000). Therefore, there is a growing concern to identify other alternative protein sources which can minimize the use of FM in fish feed formulation. Various less expensive alternative protein sources of animal and plant origin have been tested to replace FM in the formulation of feed for fish; but none has been established as superior or parallel to FM due to characteristic limitations of each ingredient (Mohanta *et al.,* 2013). However, terrestrial animal by-products are considered as most potential alternative protein sources in aquatic animal feed due to their high protein content, low carbohydrate, optimal amino acid profiles and lack of anti-nutritional factors (Hu *et al.,* 2013; Mohanta *et al.,* 2013). The increasing price of feed is considered one of the most important factors that limit profitability in aquaculture systems, caused mainly by the cost of fish meal used as a primary source of protein (McCoy, 1990) and non-availability of some other terrestrial animals in large quantity. Hence, there is a constant search for good quality, cheaper and readily available alternative protein sources for aquaculture diets (Hardy, 2008; Yosulf *et al*., 1994).

Plant proteins are considered among the most viable alternatives to replace fish meal, since their production is only limited by the availability of land, assuming that the environmental conditions for the crops are adequate. The efficacy of various plant sources for partial or complete replacement of fishmeal in aquatic diets has been investigated by a number of workers (Tacon, 1993; Yosulf *et al*., 1994, Ray and Das, 1995; Adeparusi and Agbede, 2005; Adewolu, 2008). Leaf meals are among the unconventional and cheapest sources of plant proteins that may tend to reduce the high cost of feed (De Silva and Andersons, 1995). Hence, several studies had been already conducted utilizing various sources of leaf meal proteins (Ng and Wee, 1989); Eusebio and Coloso (2000); on cassava leaf meal; Olaniyi *et al.* (2013); Adewumi, (2014); Ganzon-Naret (2014), on *Moringa oleifera* leaf meal, *Ipomoea batatas* leaf meals; Bairagi *et al.* (2002), on duckweed (*Lemna polyrhiza*) leaves, Reyes and Fermin (2003), on papaya *Carica papaya*), as partial or complete replacement for FM.

Mulberry is a popular medicinal plant belonging to family *Moraceae* and genus *Morus*. The leaves of mulberry are mainly used as food for the silkworms and they are sometimes eaten as vegetable or used as cattle fodder in different parts of the world. Mulberry (*Morus alba*, Linn.) is a plant cultivated in sericulture industries and Bag *et al.* (2012), reported that their leaves contain a good quantity of protein (21.1%) which can be used as a total substitute (100%) for dietary fish meal.

This plant is resistant to drought and is commonly cultivated for leaves in sericulture industry for feeding the silkworm (*Bombyx mori*). Excess leaves can be obtained, at a cheaper cost, from any sericulture farm for the preparation of fish feed.

Although plant proteins are cost effective, their use is limited by deficiencies in essential amino acids and minerals, the presence of anti-nutritional factors (ANFs) and complex carbohydrates (National Research Council, NRC, 1993; Vielma *et al*., 2003). Fermentation is a simple and cheap method to decrease the anti-nutritional factors and crude fibre contained in plant by-products (Bairagi *et al*., 2002). Fermentation is an environment friendly, cost effective biotechnological tool to overcome many of the inherent problems associated with the utilization of wastes in fish feed formulation.

Mulberry leaf meal (MLM), used in this study was prepared from the leaves of mulberry. Mulberry is a shrub or tree belonging to the order *Urticales*, the family *Moracea*e and the genus *Morus*. More than a hundred species of *Morus* exist, with the majority of them occurring in Asia (Datta, 2001), and in China, there are over 1000 varieties under cultivation (Sanchez, 2001a). In taxonomy, species generally are identified not by the color of the fruits (berries) but by the color of flower buds and leaves. So, a *morus* plant can have different colored berries (black, purple, red, white etc) in the same plant. Three species are recognized for their economic importance; *M. alba; M. rubra and M. nigra. M. alba leaves were used for this study*.

The use of mulberry leaf tea goes back centuries to the traditional Chinese medical system. Mulberry foliage is highly digestible, rich in crude protein (reaching levels of 20 – 24%) and mineral elements (Datta, 2001). Mulberry leaf tea is reported to have powerful antioxidant properties and has been found to lower cholesterol and triglycerides and reduce inflammation. Some scientists concluded that mulberry could be useful both in the treatment of diabetes and in its prevention.

After evaluating the nutritive value of this mulberry foliage, Boschini (2002), concluded that the leaf and cell wall contents, together with structural carbohydrates and ash, indicate that mulberry is an excellent feed for high yielding animals and can be offered fresh or dried in compound feeds. Sanchez (2001b), consequently, proffered that mulberry foliage can be used as a supplement to poor quality forage based diets or as the main component of a ration in livestock production systems. Incorporation of mulberry leaves in the diet of poultry (Narayana and Setty, 1977) resulted in better egg production of poultry and growth of rabbit. Few studies exposed to the nutritive potential and feeding value of mulberry leaves for fish species. Mondal *et al.* (2007) reported that fermented mulberry leaf meal was introduced as a protein source in the diet of the Asian air breathing catfish, *Heteropneustes fossilis*, and found the fish to accept the diet well. But there is no record about introduction of mulberry leaves as a diet supplement for Indian carps, while several aquatic plants have been successfully utilized as protein supplement in the formulation of diet for these groups of fish (Nandeesha *et al.,* 2001; Bairagi *et al*., 2002). Reports are scanty on the use of mulberry leaf for fish feed, especially for the African catfish, *C. gariepinus*. The main objective of this study is to evaluate the effect of the diet supplemented with fermented mulberry leaves, on the growth and nutrient utilization of *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental fish

The experiment was carried out in the animal house of Zoology Department, Ekiti State University, Ado-Ekiti, Nigeria. One hundred and eighty juveniles of *Clarias gariepinus*, (wt 30.2 g) were purchased from the Fiso Fish farm at Adehun, Ado Ekiti, Ekiti state, Nigeria. Fish were reared and acclimatized to the same experimental conditions for two weeks, prior to start of the experiment. The juveniles were fed with the experimental diets twice daily at 9.00-10.00 h a.m. and 18.00-20.00 h p.m. for a period of 54 days, at a fixed dieting rate of 3% of body weight per day. After the period of acclimatization, the fish were randomly distributed into well labelled bowls in triplicates, for each treatment.

Fermentation of mulberry leaf and MLM preparation

Fresh mulberry (*Morus alba)* leaves (variety S54) were obtained from Sericulture office of the Ministry of Agriculture, Ado Ekiti and soaked inside water for three days, at room temperature, after which the fermented leaves were sun/air dried for 2 days and milled into fine powder (MLM).

Feed formulation

Other feed ingredients; fish meal (72%), yellow maize, soya bean, salt, lysine, methionine, vitamin/mineral premix, groundnut oil were purchased from Metrovet Feed Mill, Ado Ekiti while rice bran was purchased from Igbemo Ekiti, Ekiti state. Vegetable oil was obtained from the Kings market, Ado Ekiti. The feed ingredients, were used to prepare the experimental feed, in which MLM was used to replace fishmeal at 0%, 10%, 20%, 30% and 40% levels, tagged diets A, B, C, D and E respectively, using the Pearson's method and based on the crude protein content of the MLM (Tables 1 and 2). The ingredients were properly mixed together and pelleted to a particulate size of 2 mm at K2 Feed Depot, Akure, Ondo state. The feeds were sundried for a week and kept inside well labelled polythene bags in the deep freezer, until ready for use.

Experimental design

The fish were acclimatized for two weeks before the commencement of treatment. Experiments were designed to monitor the growth parameters of the fish and assess biochemical composition of the fillets of the fish. Fifteen bowls were set up for the experiment using triplicates for each dietary treatment A, B, C, D and E. The bowls were filled with two third volume of water and labelled accordingly. Twenty juvenile fish (wt 30.2 g) were randomly distributed into each bowl. The total weight of the fish biomass per bowl was determined using an electric weighing balance. The survival of the fish was monitored and recorded. Water quality parameters were monitored Cleaning of the system and replacement of water was done every other day, while feed adjustment was done every 14 days.

Feeding and Growth Calculations

To determine the growth response of the fish, the weight of the fish was taken forth nightly, and the quantity of the feed consumed recorded. This data was used to calculate the feed conversion ratio (FCR), mean weight gain (MWG), daily weight gain (DWG), percentage weight gain (PWG) using the following equations:

(a) Weight gain = Final body weight – Initial body weight.
(b) Feed conversion ratio (FCR) = Total dry weight of food consumed/Total wet weight gain (g).
(c) Specific growth rate (SGR) = Final wet weight- Initial wet weight/ No. of days × 100.
(d) Survival rate (%) = No. of fish recovered/No. of fish stocked x100.
(e) Percentage Weight Gain (PWG) = Weight gain/ Initial weight ×100.

Biochemical assay

The proximate compositions of the MLM, the experimental feeds and the fish fillets (before and after the experiment) were done at the Central Science Laboratory, Federal University of Technology Akure (FUTA), Ondo state, Nigeria. Tannin, cyanide and phytic acid in MLM and feed were determined at the Federal University of Technology, Akure using the method of AOAC (2006).

Water quality monitoring

Temperature (°C) of water was recorded daily with a temperature meter at 8.00 h and 14.00 h. Other water quality parameters were determined on weekly basis. pH of water was determined in a direct reading digital pH meter (Cybertronics, Kolkata). Dissolved oxygen (DO mgL^{-1}) following standard procedures of American Public Health Association (APHA, 1995) (Table 5).

Statistical analysis

All biological data generated from proximate analyses

Table 1. Proximate composition (g 100^{DM-1}) of mulberry leaf meal (MLM) used.

Ash	Moisture	Crude Protein	Fat	Fibre	NFE
12.09	6.44	16.95	6.27	21.56	36.69

NFE = nitrogen free extract.

Table 2. Feed formulation and proximate composition (g 100DM-1) of experimental diets of *C. gariepinus* Juveniles.

	Diets				
Ingredients	**A**	**B**	**C**	**D**	**E**
MLM	-	2.25	4.50	6.75	22.60
FM	22.60	20.35	18.10	15.85	-
Soybean	22.60	22.60	22.60	22.60	22.60
Maize	23.90	23.90	23.90	23.90	23.90
Rice bran	27.40	27.40	27.40	27.40	27.40
Groundnut oil	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Vit./Mineral Premix	1.00	1.00	1.00	1.00	1.00
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Proximate Composition					
Ash	11.89^{a}	12.39^{b}	12.11^{c}	12.99^{d}	10.16^{c}
C. P	29.69^{d}	29.15^{c}	29.06^{b}	31.11^{e}	29.45^{a}
Fat	13.23^{b}	14.41^{c}	13.66^{b}	13.07^{a}	14.24^{c}
Moisture	11.36^{d}	15.05^{a}	15.79^{b}	16.57^{a}	11.66^{c}
Fibre	4.43^{d}	4.85^{c}	5.15^{a}	5.32^{d}	5.25^{a}
NFE	29.40^{a}	24.15^{b}	24.23^{c}	20.94^{d}	29.24^{a}

$^{a, b, c, d}$ Different superscripts within the same raw demonstrate significant differences, (P>0.05), MLM = mulberry leaf meal, FM = fish meal, C.P = crude protein, NFE = nitrogen free extract.

Table 3. The proximate composition of the fish fillets.

	Initial	A	B	C	D	E
Ash	5.43^{a}	4.86^{a}	4.39^{b}	4.79^{a}	4.37^{b}	4.76^{a}
Moisture	19.35^{d}	20.48^{a}	19.68^{a}	22.78^{ab}	24.47^{b}	26.21^{c}
C.P	65.80^{d}	67.10^{a}	67.81^{a}	66.68^{a}	61.52^{b}	60.22^{b}
Fat	7.26^{d}	8.21^{a}	8.03^{a}	8.43^{a}	6.30^{b}	5.88^{b}
CHO	2.16^{a}	0.15^{c}	0.09^{c}	2.32^{a}	3.34^{b}	3.79^{b}

$^{a, b, c, d}$ Different superscripts within the same raw demonstrate significant differences.
(p>0.05), C.P = crude protein, CHO= total carbohydrates

were subjected to a non-parametric t- test (Mann-Whitney U-Wilcoxon Rank Sum W – test) for comparison of means within a treatment, while the Turkey–HSD one way Analysis of Variance (ANOVA) was used for between treatments comparison, at the 5% level of significance (Duncan, 1955).

RESULTS

Table 1 shows that the fermented MLM contains crude protein (16.75%), ash (12.09%), fat (6.27%), fibre (21.56%), moisture (6.44%) and nitrogen free extract (36.69). From the proximate analysis of the formulated diet in (Table 2), an increasing trend of the fibre and moisture content of the diets was observed, as the MLM inclusion in the diets increased. No clear trend was observed for the other parameters. The proximate analysis of the fish fillets (Table 3) indicates increase in the crude protein content (C.P) of the fillets, as they were fed with the trial diets, up to diet C (20% MLM substitution). The crude protein content of the fish fed diets A, B and C were not significantly different (P<0.05) from one another, but significantly different (P>0.05) from

Table 4. The growth performance and feed utilization of the catfish fed the experimental diets for 54 days.

	Diets				
Parameters	**A**	**B**	**C**	**D**	**E**
Initial Wt.	42.40[a]	42.92[a]	43.00[a]	43.75[a]	41.05[a]
Final Wt.	126.0[a]	122.10[a]	124.03[a]	100.21[b]	89.01[c]
DWG%	1.55[a]	1.46[a]	1.50[a]	1.04[b]	0.88[b]
Wt. Gain%	197[a]	184[a]	188[a]	104[b]	89[c]
Survival %	100[a]	98[a]	100[a]	100[a]	97[a]
SGR(%d^{-1})	0.86[a]	0.88[a]	1.06[a]	0.73[b]	0.81[a]
FCR	1.33 [a]	1.40[a]	1.41[a]	1.62[a]	1.85[a]

[a, b, c, d] Different superscripts within the same raw demonstrate significant differences (p>0.05) DWG = daily weight gain, SGR = specific growth rate, FCR = food conversion ratio.

Table 5. Water quality parameters (mean of the culture media before and after the feeding trials.

Treatment	pH	Conductivity (µs/cm)	Water Temperature (°C)	Dissolved Oxygen concentration (mg l^{-l})
Initial	6.9	76.3	26.5	7.0
A	7.0	79.3	26.0	6.9
B	7.5	78.4	26.5	7.5
C	7.0	81.2	26.4	7.5
D	7.2	75.7	27.3	6.9
E	7.6	83.3	27.9	7.1

the initial value and from the fish fed diets D and E. The moisture content increased when the fish were fed the trial diets. The moisture content of the fillets of fish fed diets A, B and C were significantly higher than (P>0.05) the initial value and lower than (P<0.05) those fed higher MLM inclusion (diet D and E). The fat content also increased significantly (P>0.05) as the fish were fed the trial diet. As the MLM content increased (diet D and E) however, the fat content of the fillets of the fish decreased. The ash content of the fish fed the trial diets suffered decrease, when compared with the initial value, with no distinct trend. The trend of the carbohydrate (CHO) value is also not distinct, but there was increase in the CHO values as the MLM inclusion increased from diet C (20% MLM inclusion).

Observation of the effect of the trial diets on the fish growth performance and nutrient utilization (Table 4) indicated that all the trial diets caused growth of the fish. The final weight gain, daily weight gain, percentage weight gain of the fish fed diets A, B and C were not significantly different (P<0.05) from one another, but these data were significantly different (P>0.05) from those of the fish fed diets D and E. The specific growth rate (SGR) and food conversion ratio (FCR) of the fish fed the trial diets were however, not significantly different (P<0.05) from one another. The growth curve (Figure 1) also demonstrated the order of acceptability and growth performance of the fish fed with the trial diets. Fish fed with diets D and E showed significantly lower growth performance compared with the fish fed diets A, B and C. The survival of the fish, in all the dietary treatments, were not significantly different (P<0.05) compared with the control diet.

DISCUSSION

The crude protein value of 16.75% falls within the expected range of 15.0 – 28.0% for mulberry leaf meal (Sanchez, 2001b) and is adequate to be used as a supplement for substituting fishmeal in diet. Leaf crude protein content varies according to variety, age of plant and growing conditions (Sanchez, 2001b). Yao *et al.* (2000), found mulberry leaf crude protein values to be slightly higher in spring than in the fall (21.1% vs 20.9%). The MLM is also reported to have a good complement of vitamins and minerals. These components are quite beneficial for formulating the fish diet. As reported earlier (Adewumi, 2014), high fibre content of plant-based diets causes dilution of the nutrients, reduces digestibility, resulting in growth depression, as the diets become in-consistent. Fibre creates bulkiness of feed in the gut, reduces feed consumption of animals and creates regular bowel movement. Fibre depresses utilization of feed energy and essential nutrients. This energy deficit would in turn affect other biological parameters, as well as nutrient retention and thus weight loss. There was no fouling of the medium as an indication that the feedstuffs were good and acceptable to the fish. High percentage survival also justifies this and indicates no poisoning or and validates absence of toxins. However, Adeduntan and Oyerinde, (2009), reported SK54 variety

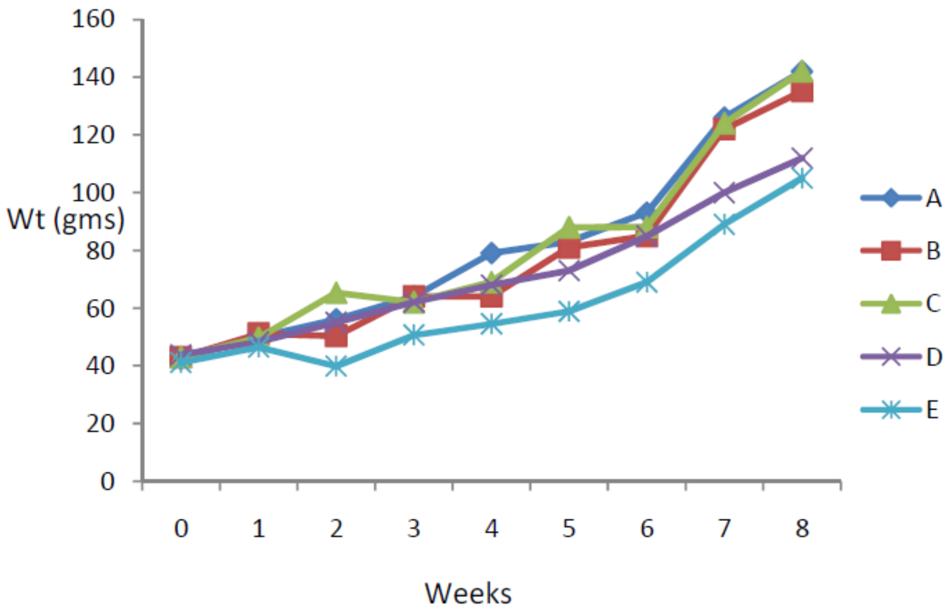

Figure1. The growth performance of the fish fed the trial diets.

of *M. alba* contained 456 mg kg^{-1} phytate; 1.01 mg kg^{-1} cyanide and 3.65 mg kg^{-1} tannin. Mondal *et al.* (2011), reported that fermentation of mulberry leaf must have resulted in a significant decrease in the levels of both the crude fibre and concentration of the anti-nutritional factors, tannin and phytic acid in the ingredient mixture of the diets. Bairagi *et al.* (2002), after using duckweed (*Lemna polyrhiza*) leaf meal fermented with a fish intestine bacterium to rohu (*Labeo rohita* Ham.) fingerlings, also concluded that fermentation reduces the crude fibre and ANF of leaf meal based diets. According to National Research Council, (NRC), (1993) and Vielma *et al*. (2003), no anti-nutritional factors could be detected in the fermented products.

Fermentation must have improved the acceptability and palatability of these diets and have made the fish to be able to tolerate a replacement of fishmeal with a leaf meal of up to 20% (diet C), without adverse effects. This was contrary to the observation of Adewumi (2014), in which the fish could tolerate not more than 10% of *moringa* leaf (unfermented) meal substitution, even though it was reported that *moringa* contains no anti-nutritional factors. This discrepancy can probably be attributed to the effect of fermentation on reduction of crude fibre content of the leaf meal, and thus the feed.

The study indicates that mulberry leaf can be considered as an essential ingredient in feeds for the catfish. The use of such meal as a feedstuff in fish farming is expected to bring down the cost of fish production. As observed in this study, corroborated by other authors (Mondal *et al*., 2011; Mondal *et al*., 2012; Bag *et al*., 2012), MLM enhanced feed efficiency and kept limnological conditions favourable. From various reports on the nutritional value of mulberry on man, as reported above, it can be deduced that the leaf could also have increased the immunity power against common fish diseases. The use of this leaf in fish culture, at a replacement level of 20%, is thus recommended.

Conclusion

This study has investigated the effect of the use of fermented MLM inclusion into the diet of *C. gariepinus*. The experimental diets were acceptable to the fish and there was no detrimental or serious adverse effect on the fish. This investigation revealed that replacement of fishmeal portion of the diet, with up to 20% level, affects good growth and nutrient utilization in *C. gariepinus* diet. The use of this mulberry leaf meal in fish culture, at a replacement level of 20%, is thus recommended. Excess leaves can be obtained at cheap cost, from any sericulture farm, for the preparation of fish feed.

AUTHORS` DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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