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Effect of *Moringa olifera* and Methionine as Feed Supplement on Growth and Utilization of *Clarias gariepinus*

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ABSTRACT
An experiment was conducted in the Department of Agricultural Science Education, Federal College of Education (Technical), Asaba, Nigeria to determine the response of the use of *Moringa oleifera* leaf and methionine as partial feed replacement on the growth of African catfish *Clarias gariepinus*. A 4 x 4 factorial experiment in Randomized Complete Block Design (RCBD) with three replications was used for the study. The two factors were Moringa (0%, 10%, 20% and 30%) and Methionine (0%, 10%, 20% and 30%). The following data were collected: Mean Weight Gain, Percentage Weight Gain, Specific growth rate, Feed conversion ratio, Protein Intake, Feed intake and Protein Efficiency Ratio. Data collected were subjected to analysis of variance (ANOVA). The means were separated using Least Significant Difference (LSD). Mean Weight Gain, Percentage Weight Gain, Specific growth rate, Feed conversion ratio, Protein Intake, Feed intake and Protein Efficiency Ratio increased with increase in Moringa and methionine percentage rate. Application of either 30% of moringa leaf or 30% of methionine gave the highest growth and nutrient use parameters. Complementary application of Moringa leaf with methionine as feed replacement was significantly higher than sole application. The combination of 30% Moringa with 30% Methionine had significantly higher effect than other treatment combinations during the experimental period and was found satisfactory for achieving growth of *Clarias gariepinus* fingerlings. Fish farmers are recommended to use treatment combination of 30% of moringa and 30% of methionine supplement rates in fish feed for *Clarias gariepinus* to improve growth and nutrient utilization (protein intake). However, if restricted to moringa leaf as a source, 30% of it as feed replacement is recommended.
Keywords: *Clarias gariepinus*, moringa leaf, feed replacement, methionine, growth

INTRODUCTION

In Nigeria a daily dietary protein intake of 70g comprising of 35g of plant and animal protein respectively has been recommended (FAO, 1996). Even though, animal protein contains a well-balanced amino-acid profile and better utilized compared to protein from plant source, plant protein plays vital role in human physiological functions. However, the minimum recommended daily intake of 6.5g of animal protein per head is yet to be met in Nigeria (Smith, 2001). The inability to meet up with this standard led to so many dietary deficiency problems in man. Therefore, the need to meet the protein requirements in human diets has led to increasing demand for fish and the domestication of some animals.

Feed being a major inputs in aquaculture production, high cost of fish feed has caused a lot of problem in aquaculture sector, which has actually hindering aquaculture development in Nigeria since the fish feed cost account for at least 60% of the production cost (Gabriel, Akinrotimi, Bekibele, Onunkwo, and Anyanwu, 2007). Expensive feeds has actually reduced the profitability of fish farming thereby limiting the expansion of farms and reducing the yield in terms of quantity and quality (Adikwu, 1992). This has brought about the search for local protein feed stuffs that are cheap and high in quality as alternative protein feed for *Clarias gariepinus* quality. *Moringa olifera* leaf is said to be an excellent source of vitamins, minerals and proteins: perhaps more than any other tropical vegetable and commonly known as

"*The Miracle Tree*," "*Horseradish-tree*," or "*Ben oil tree*" is the best known and most widely distributed species of Moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world (Shuaib, Suchita, Ritesh, Anil, & Debabrata, 2012). It is used as growth hormone, green manure and for medications. Many do use *Moringa oleifera* leaves to fight against malnutrition and its associated diseases, such as the treatment of cardiovascular diseases: as the roots, leaves, gum, flowers, and infusion of seeds have nitrite, mustard oil glycosides, and thio-carbonate glycosides as their chemical constituents which are suggested to be responsible for the diuretic, cholesterol lowering, antiulcer, hepatoprotective, and cardiovascular protective property of the tree (Shuaib *et al*, 2012).

Moringa oleifera leaves contain an appreciable amount of nutrients, the nutritional composition is as following crude protein (C.P) 25.0%, crude lipids (C.L) of 10% and crude fibre (C.F) 8.4%, beyond some common fruits, milk and carrot, which contained; 25 times the Iron of Spinach, 17 times the Calcium of Milk, 15 times the Potassium of Bananas, 10 times the Vitamin A of Carrots, 9 times the Protein of Yogurt, 0.5 times the Vitamin C of Oranges (Luna Shyr, 2012). However, most plant proteins are deficient in lysine and methionine. Therefore, there is need to supplement plant diets with sources of lysine and methionine especially when used as feed for livestock.

Methionine serves as a precursor to carnitine (Tacon, 1990). It is also required for building muscles and detoxifying the liver (Health Vitamin Guide.com). Its availability in the appropriate quality is important in feeds. According to Lim and Dominy (1989) a protein with an essential amino acid composition which closely matches the essential amino acid requirements of the fish is described as being of high nutritive value. A protein that is deficient in one or more essential amino acids is of low biological value. Previous analytical work on the whole body protein of *C. gariepinus* shows that methionine makes up 2.77g/100g protein of the fish (Fagbenro, Balogun & Eyo, 2001). Apart from the inclusion of methionine in fish feed to boost the its nutrition requirement, there is need to determine optimum inclusion levels of methionine and *Moringa oleifera* in feeds to identify the best level of combination needed to grow *Clarias gariepinus.*

Fish require a well – balanced mixture of essential and non essential amino acids. The most effective, economical source of these amino acids is a proper combination of high quality natural proteins in feedstuffs. A protein that is deficient in one or more essential amino acids is of low biological value while that which closely matches that required by the fish is of high nutritive value (Lim & Dominy, 1989). The findings of the study will be of benefit to farmers of *Clarias gariepinus.* The aim of this experiment is to establish the effects of *M. oleifera* leaf and methionine on growth of African Catfish (*Clarias gariepinus*) fingerlings at different inclusion levels.

MATERIAL AND METHODS

The experiment was carried out in Agricultural Science Education Department, Federal College of Education (Technical), Asaba, Nigeria.

Fish: Two hundred and forty, post fingerling (3g) *Clarias gariepinus*, were collected from Adaeze farms, Asaba, Nigeria and transported to the school farm. The fish was acclimatized and fed the same commercial diet (Durante fish feed) for a period of two weeks, after which they were randomly distributed (n=15) into plastic aquarium tanks (60cm x 30cm x 30cm) at the school farm. The fish was raised in a flow-through system for 56 days, with adequate water supply/aeration.

Moringa: *Moringa oleifera* leaf was obtained from Delta State University Farm, Anwai. It was air dried in a clean room for about three weeks to obtain a constant weight for easy grinding. It was grounded using manual grinder to obtain right particle size for pelleted feed.

Methionine: The methionine used for the study was sourced from a reputable feed mill in Asaba, Nigeria

Experimental Diets

Feed ingredients were purchased from a reputable feed mill in Asaba. The ingredients used were maize, wheat offal, groundnut cake, fishmeal blood meal, vegetable oil, oyster shell, bone meal, salt, premix and lysine. Four experimental diets were formulated using moringa leaves; diet 1 contained 0% of *Moringa oleifera* meal and serve as control and diet 2, 3 and 4 contained 10%, 20% and 30% replacement levels of

Moringa oleifera leaf meal respectively. Also four experimental diets were formulated using methionine supplement; diet 1 contained 0% of methionine and served as control and diet 2, 3 and 4 contained 10%, 20% and 30% replacement levels.

The ingredient was weighed according to their calculated weight, mixed thoroughly and then pelletized to replaced dustiness and for proper acceptance by fingerlings. The pellets were sun dried for two days to prevent deterioration and the feeds were stored in an air tight container.

Experimental Design

The experimental design was a 4 x 4 factorial in randomized complete block design (RCBD). The two factors Moringa diet (0%, 10%, 20% and 30%) and Methionine (0%, 10%, 20% and 30%) were combined to produce 16 treatment combinations that were replicated three times.

Feeding and Management of the Fingerlings

The fingerlings were fed 3% of their total body weight daily. Feeding was done twice daily at 0900 hrs and 1500 hrs the fish regime was adjusted with respect to body weight.

Chemical Analysis of the Test Diet

The proximate composition of the test ingredient (*Moringa oleifera* leaf) was determined on dry matter basis using the method of A.O.A.C (2000).

Data Collection

Growth parameters measured were mean weight gain; percentage weight gain and specific growth rate. Nutrient utilization parameters include: feed conversion ratio, protein intake, feed intake, protein efficiency ratio.

Statistical Analysis

Data collected were analysed using Genstat (3) Discovery edition package for statistical analysis. Separation of means was carried out using Least Significant Difference (LSD) procedure as described by Obi (2002). Test of significance was done at 5% probability level

RESULTS

The proximate and nutrient composition of the moringa leaf is given in Tables 1 and 2. The result in Table 1 shows that moringa leaf was high in crude protein and energy value suggesting that moringa leaf is a good source of protein and energy for the fish. The result in Table 2 indicated that contents of the leaf were high in minerals.

Table 1: Proximate composition of the moringa leaf used for the study

Nutritional Analysis	Proximate Composition (g/100g)
Crude Protein	23.5
Moisture	3.5
Fibre	7.9
Ash	8.3
Crude fat	2.9
NFE	53.9
Energy value (Kcal/100kg)	1349.5

Table 2: Mineral composition of the moringa leaf used for the study

Minerals	Content
Calcium	1.93%
Magnesium	0.41%
Phosphorous	33.10 ppm
Manganese	80.55 ppm
Copper	6.13 ppm
Iron	109.75 ppm
Sodium	189.22 ppm
Zinc	59.12 ppm

Table 3: The effect of moringa and methionine diets on the growth parameters of *Clarias gariepinus* at 8 weeks of feeding experimental diet (WFED)

Methionine diets (%)	Moringa diets (%)				Mean
	0 (Mor 0)	10 (Mor 10)	20 (Mor 20)	30 (Mor 30)	
Mean weight gain(kg)					
0 (Met 0)	5.80	8.25	8.38	9.54	**7.99**
10 (Met 10)	6.93	8.52	8.56	9.87	**8.47**
20 (Met 20)	7.43	8.68	9.70	10.42	**9.05**
30 (Met 30)	8.55	9.07	9.92	10.79	**9.58**
Mean	**7.17**	**8.63**	**9.14**	**10.15**	8.77
Percentage weight gain					
0 (Met 0)	35.75	84.67	69.11	91.57	**70.27**
10 (Met 10)	49.16	81.12	83.50	84.10	**74.47**
20 (Met 20)	78.63	78.03	68.28	95.60	**80.13**
30 (Met 30)	80.84	66.17	81.05	95.60	**80.91**
Mean	**61.09**	**77.49**	**75.48**	**91.71**	76.44
Specific growth rate					
0 (Met 0)	1.48	1.55	2.73	2.80	**2.14**
10 (Met 10)	1.59	1.69	2.75	2.87	**2.22**
20 (Met 20)	1.60	1.75	2.83	3.88	**2.51**
30 (Met 30)	1.71	2.05	2.85	3.83	**2.61**
Mean	**1.59**	**1.76**	**2.79**	**3.34**	1.04

	MWG	PWG	SGR
$LSD_{0.05}$ for comparing 2 Meth rates:	2.34	2.59	0.18
$LSD_{0.05}$ for comparing 2 Mor rates:	2.34	2.59	0.18
$LSD_{0.05}$ for comparing 2 Meth x Mor rates:	4.68	5.18	0.36

Where: MWG= mean gain weight, PWG= percentage gain weight, SGR= specific growth rate

Result in Table 3 showed that the use of methionine, moringa and their interaction diets significantly ($P < 0.05$) influenced mean weight gain at 8 WFED. The application of 30%, 20% and 10% of moringa feeds were statistically ($P < 0.05$) the same but significantly higher than 0% of moringa diet. The use of 30%, 20% and 10% of methionine diets produced significantly ($P < 0.5$) the effect. However, 30% of

methionine diet gave the highest mean gain weight. The combination of 30% of moringa and 30% methionine diets produced the highest mean gain weight of *Clarias gariepinus*

The use of methionine, moringa and their interaction diets significantly ($P < 0.05$) influenced percentage gain weight at 8 WFED. The application of 30% of moringa diet gave significantly ($P < 0.05$) the highest percentage gain weight among moringa rates. The use of 30% of methionine produced the highest percentage gain weight among methionine treatments (Table 3). The interaction of 30% of moringa x 30% of methionine diets gave the highest percentage gain weight among the treatment combinations.

Moringa, methionine and interaction diet effects were significant ($P < 0.05$) on specific growth rate of the cat fish at 8 WFED. The use of moringa diets at 10%, 20% and 30% were statistically ($P < 0.05$) the same with respect to specific growth rate (Table 3). However, the use of 30% of moringa diet had the highest specific growth rate. The 30% of methionine feed produced the highest specific growth rate among methionine diets. The use of 30% of methionine and 30% of moringa had the highest specific growth rate and treatment combinations diets.

Nutrient utilization parameters

Table 4: The effect of moringa leaf and methionine diets on the growth parameters of *Clarias gariepinus* on feed conversion ratio, protein intake, feed intake, protein efficiency ratio

Methionine diets (%)	**Moringa diets (%)**				**Mean**
	0 (Mor 0)	10 (Mor 10)	20 (Mor 20)	30 (Mor 30)	
Feed conversion ratio (g)					
0 (Met 0)	0.05	0.15	0.40	0.59	**0.29**
10 (Met 10)	0.12	0.16	0.43	0.87	**0.39**
20 (Met 20)	0.20	0.24	0.45	0.94	**0.45**
30 (Met 30)	0.38	0.33	0.45	1.05	**0.55**
Mean	**0.18**	**0.22**	**0.43**	**0.86**	0.42
Protein Intake					
0 (Met 0)	0.13	0.20	0.28	0.35	**0.24**
10 (Met 10)	0.16	0.25	0.30	0.40	**0.27**
20 (Met 20)	0.23	0.35	0.35	0.44	**0.37**
30 (Met 30)	0.30	0.30	0.45	0.53	**0.39**
Mean	**0.20**	**0.27**	**0.34**	**0.43**	0.31
Feed Intake (kg)					
0 (Met 0)	4.94	10.07	13.00	14.58	**10.64**
10 (Met 10)	5.23	13.67	13.15	14.85	**11.72**
20 (Met 20)	7.89	13.89	14.05	15.21	**12.76**
30 (Met 30)	9.89	13.78	14.51	15.85	**13.50**
Mean	**6.98**	**12.85**	**13.67**	**15.12**	12.15
Protein efficiency ratio					
0 (Met 0)	18.50	20.56	25.13	30.09	**23.57**
10 (Met 10)	19.33	22.61	28.67	33.22	**25.95**
20 (Met 20)	23.41	25.89	31.06	36.12	**29.12**
30 (Met 30)	25.34	28.41	33.78	40.61	**32.03**
Mean	**21.64**	**24.36**	**29.66**	**35.01**	27.66

	FCR	PI	FI	PER
$LSD_{0.05}$ for comparing 2 Meth rates:	0.02	0.06	2.09	4.01
$LSD_{0.05}$ for comparing 2 Mor rates:	0.02	0.06	2.09	4.01
$LSD_{0.05}$ for comparing 2 Meth x Mor rates:	0.04	0.12	4.18	8.02

Where: FCR= Feed conversion ratio, PI= Protein Intake, FI= Feed Intake, PER= Protein efficiency ratio

The use of methionine, moringa and their interaction diets significantly ($P < 0.05$) influenced feed conversion ratio. The application of 30% of moringa diet gave significantly ($P < 0.05$) the highest feed conversion ratio among moringa rates. The use of 30% of methionine produced significantly ($P < 0.05$) the highest feed conversion ratio among methionine treatments (Table 8). The interaction of 30% of moringa x 30% of methionine diets gave the highest feed conversion ratio among the treatment combinations.

Table 4 also showed that the use of methionine, moringa and moringa x methionine interaction had significant ($P < 0.05$) on protein intake of the experimental fishes. The application of 30% of moringa had the highest protein intake of the fishes among the rates of moringa diets. The use of 30% of methionine produced the highest protiein intake among methionine treatments The combination 30% of moringa x 30% of methionine feeds produced the highest protein intake.

Moringa, methionine and interaction diet effects were significant ($P < 0.05$) on feed intake of the experimental fishes. The use of methionine diets at 10%, 20% and 30% were statistically ($P < 0.05$) the same with respect to feed intake. However, the use of 30% of moringa diet had the highest feed intake rate of the experimental fishes. The 30% of moringa feed statistically ($P < 0.05$) gave the highest feed intake of *Clarias gariepinus* among methionine diets. The use of 30% of methionine and 30% of moringa diets had the highest feed intake with respect to the fishes.

Table 4 also indicated that methionine, moringa diets and their interaction significantly ($P < 0.05$) influenced protein efficiency ratio of the fishes. The feeding of 30% of methionine diet produced the highest protein efficiency ratio among the rates of methionine diets. Highest protein efficiency ratio was achieved with the feeding of 30% of moringa diet when compared to other moringa rates. The application of 30% of methionine and 30% of moringa diets gave the highest protien efficiency ratio of *Clarias gariepinus* treatment combinations

DISCUSSION OF RESULTS

Protein is very important in fish growth and thus crucial ingredient in fish diets. A comparison between the amino acid composition of the raw and extracted moringa leaves to that of soybean revealed an almost identical composition of essential amino acids (Tagwireyi Mupangwa, Jepsen and Mwera, 2010). The crude protein (C.P) of the moringa targeted was 40%. However, the CP of the formulated diets fell within the range of 15-40% C.P (Table1) as recommended by Lovell (1997) for *Clarias gariepinus* fingerlings, contrary to minimum C.P below an average value of 35% for *Clarias gariepinus* fingerlings reported by Robinson (2001) which could be detrimental to *Clarias gariepinus* fingerlings performance. The crude fat (C.F) of 3-4% in the diet was observed, this result agreed with Robinson et al (2006) who found out that the C.F of 3-4% could be accepted by *Clarias gariepinus* fingerlings without any adverse effect. Other constituents were in right proportions to enable adequate growth and developments in fries (Table 1). The result in Table 2 showed the mineral constituent of the moringa leaves used for the study were high in values and adequate to substitute for fish feed.

Growth parameters

Mean weight, percentage weight gain and specific growth were increased with increase in the feeding rate of moringa feeds or diets, with 30% of moringa diet formulation performing better than other treatment formulation rates in the period of the experiment. This showed that the growth rate of *Clarias gariepinus* was improved with increase percentage rate of moringa diets in feeds. Ladipo, Doherty, Akinfemi and Okeme (2005) had similar observation when they used moringa leaf as constituent of fish meal. They also observed that *Moringa oleifera* leaf meal was able to maximize their feeds to produce the best final weight gain when compared with other sources of constituents to fish meals. Adewumi (2014) found out that moringa leaf substitution in fish meal of 10% and below did not significantly ($P < 0.05$) influence growth in *Clarias gariepinus.* The increase in methionine percentage rate as in the fish meal increased growth parameters of *Clarias gariepinus* throughout the experimental period. Methionine supplement in fish diet have shown to improve growth in *Clarias gariepinus*. The finding is in line with the findings of

Fauconncau (1988) who submitted that synthetic amino acid supplementation such as lysine and methionine has also been done in diets of rainbow trout for higher rate of production in fishes. Combine use of methionine and moringa leaf in fish performed better than sole use of moringa or methionine supplement. Incorporation of plant ingredients in fish feed as protein source to replace the fish meal has been a good effort of feed manufacturers. Using plant ingredients, the essential amino acid content were balanced as most of the feed stuffs of plant origin are deficient in some of the essential amino acid (NRC, 1983; Lovell, 1989; Halver, 1989; Lall, 1991; Cho and Kaushik, 1990; Li and Robinson, 1998). Higher rate of common carp production have been obtained in the intensive fish culture operations using feed with low levels of plant feed stuffs (moringa leaf meal, soya bean meal) but fortified with synthetic amino acids, lysine and methionine (Viola and Lahav, 1991).

Nutrient utilization and quality

The use of Moringa diets increased feed conversion ratio of the experimental fish as percentage rate of the moringa constituent increased. The use of methionine followed same trend. Olaniyi, Ajani and Adetomi (2013) recorded higher values of feed conversion ratio in *Clarias gariepinus* as moringa treatment rate increased in fish feed. High feed conversion ratio (less efficiency) was obtained generally in this study especially with the moringa meal-based diets. Adequate feed conversion ratio would lead to increased growth. However, poor feed conversion ratios were reported by (Olaleye, 1991 and Ipinjolu, 1997) when various plant protein based diets were fed to tilapia and carp fingerlings. This observation could partly be due to low feed intake by the fish. The interaction of methionine and moringa leaf in fish performed better than sole use of moringa or methionine supplement.

The use of Moringa diets increased protein intake of the experimental fish as treatment rate of the moringa constituent increased. The use of methionine diet rates followed the same trend. The combinations of methionine and moringa leaf in the fish feed performed better than sole use of moringa or methionine supplement with regards to protein intake. The utilization protein corresponded positively with protein intake (P.I) suggesting that the higher the protein intake the higher the utilized since protein consumed in excess of requirement would be voided in feaces (Olaniyi *et al*, 2013). This finding suggests that the rate of protein intake is equivalent to the amount of protein contained in the fish carcasses.

Moringa diets increased feed intake of the experimental fish as treatment rate of the moringa constituent increased. The use of methionine diet rates did not differ significantly in application rates in the fish feed with regards to feed intake. Treatment combination of methionine and moringa improved feed intake when compared to sole use of moringa or methionine supplement. This implies that moringa diets and interaction of moringa and methionine diets significantly ($P < 0.05$) improved feed intake while sole use of methionine diets did not improve feed intake as most levels of the supplement rate were statistically similar. This observation is an indicator of the level of acceptability and palatability of the various diets. Diet with moringa diet and treatment combination, as the protein source, was more acceptable and probably more palatable than the methionine diets. The diet acceptability was indicated by the total quantity of feed intake

The use of Moringa diets increased protein efficiency ratio of the experimental fish as treatment rate of the moringa constituent increased. The use of methionine diet rates followed the same trend. The combinations of methionine and moringa leaf in the fish feed performed better than sole use of moringa or methionine supplement with regards to protein efficiency ratio. These results seem to have direct link with palatability of the diet which causes improve feed intake (Faturoti, 1989). This could also indicate that methionine deficiency may be one of the reasons responsible for the lower growth performance and poorer diet utilization of the groups fed the diets supplemented with moringa leaves as observed by Bundit and Toshiro (2006).

CONCLUSION

The findings of the study have shown moringa leaves to be a good source of supplement to fish feeds especially in improving growth rate and protein content in fish carcasses. The results indicated that the various growth parameters measured such as mean weight gain, percentage weight gain and specific

growth rate increased with increase in methionine or moringa percentage rate. Nutrient utilization parameters like feed conversion ratio, protein intake, feed intake and protein efficiency ratio also increased with increase rate of methionine or moringa diet rates.

Combination use of moringa with methionine in fish feed performed better than sole use of moringa or methionine fish diets in the growth and nutrient utilization parameters measured. Moringa leaf supplement in fish feed rate of 30% performed better than the lower percentage rates of moringa leaf supplement while 30% of methionine supplement in fish feed performed better than other percentage rate excerpt in feed intake where it was not statistically different from other methionine rates. Combination of 30% of moringa leaf with 30% of methionine supplements produced higher than other treatment combinations and was found satisfactory to improve growth and nutrient utilization in *Clarias gariepinus* in the study.

RECOMMENDATIONS

1. Fish farmers are recommended to use treatment combination of 30% of moringa and 30% of methionine supplement rates in fish feed for *Clarias gariepinus* to improve growth and nutrient utilization (protein intake)**.** However, if restricted to moringa leaf as a source, 30% of it as feed replacement is recommended.
2. It is recommended that further research should be carried out to determine the optimum rates of treatments use for *Clarias gariepinus* growth and nutrient utilization.

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