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Effect of *Moringa* Leaves and Methionine on Growth of *Clarias gariepinus* Fingerlings at Different Inclusion Levels

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ABSTRACT

An experiment was conducted in the Department of Agricultural 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 higher than sole application. The combination of 30% Moringa with 30% Methionine had higher effect than other treatment combinations during the experimental period and was found satisfactory for achieving growth of *Clarias gariepinus* fingerlings. It is therefore recommended that fish farmers 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).

Keywords: *Clarias gariepinus*, *Moringa oleifera* leaf, methionine,

INTRODUCTION

Early catfish (*Clarias gariepinus*) producers depend primarily on natural pond productivity to provide nutrients for fish growth. Fish production was enhanced by the addition of fertilizers to pond water to stimulate the growth of natural food organism (Robinson, 1991) but as aquaculture developed and expanse, supplementary feeding became an important part for higher productivity, and now accounts for at least 60% of the total cost of fish production in Africa, hence, it determines the viability and profitability of fish farming enterprise (Jamu and Ayinla, 2003).

Feed ingredients that are consumed by humans are competed for by the livestock sector (Obun, 2008). The high cost, scarcity and insufficient supply of soybean meal have necessitated the need for a search for alternative plants protein ingredients in livestock feeds (Daniel, 1992). Atteh (1995) suggested that the alternative plant protein source to replace conventional once may have comparative nutritive value or preferably be cheaper than the conventional protein sources, hence locally available and cheap feedstuff are the interest of practitioners in the feed industry. *Moringa oleifera* (family Moringaceae) has been identified to hold the potential to make contributions to fish culture.

Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It is a non-leguminous tree with a high crude protein in the leaves (251g/kg DM) and negligible content of tannins and other anti-nutritional factors (Makkar and Becker, 1985). It has an impressive range of

medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, -carotene, amino acids and various phenolics. Every part of moringa tree is said to have beneficial properties that can serve humanity. Nutritional analysis indicates that Moringa leaves contain a wealth of essential, disease preventing nutrients. They even contain all of the essential amino acids, such as methionine, cystine, tryptophan (Makkar and Becker, 1985), as required by aquatic animals (WHO, 1985). This is unusual for a plant source. Based on a number of reports on the nutritional/medicinal values of moringa, it is being promoted as a "healthful" food, used traditionally to combat a number of common ailments. Since the dried leaves are concentrated, they contain higher amounts of many of the nutrients, except Vitamin C. Leaves and pods of Moringa therefore, offers an alternative source of protein to fish. However, studies have shown that high levels of plant protein in fish diets or complete substitution of animal proteins has resulted in poor growth and feed efficiency in feed (Dabrowski, Poczyczynski, Kock and Berger 1989; Lim, 1992).

Poor growth in fishes have been attributed to anti nutritional factors or toxic substances; improper balance of essential nutrients such as amino acids especially methionine, energy and minerals, high amount of fibre and carbohydrate, decrease in palatability of the feed; and reduction of pellet quality especially water stability (Lim and Dominy, 1989). Thus, 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 and provisional source of essential amino-acid such as methionine in feedstuffs.

Methionine serves as a precursor to carnitine (Tacon, 1990). It is also required for building muscles and detoxifying the liver (Health Vitamin Guide.com, 2015). 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 and Eyo, 2001). Apart from the inclusion of methionine in fish feed to boost 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.* 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. Specifically, the study seeks to:

1. determine the effect of *M. oleifera* leaves as supplement in fish feed on the growth of *Clarias gariepinus;*
2. examine the effect of methionine as supplement in fish feed on the growth of *Clarias gariepinus;* and
3. determine the effect of combined *M. oleifera* and methionine as feed supplement on utilization and quality of *Clarias gariepinus*

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 Department of Crop Science, University of Nigeria, Farm, Nsukka. 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.

Feed ingredients were formulated. 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 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 respectively

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

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 (Table 1) that were replicated three times.

Table 1: Treatment combinations used for the study

	Moringa leaves (%)			
Methionine supplement (%)	0 (Mor 0)	10 (Mor 10)	20 (Mor 20)	30 (Mor 30)
0 (Met 0)	Mor 0/met 0	Mor 10/met 0	Mor 20/met 0	Mor 30/met 0
10 (Met 10)	Mor 0/met 10	Mor 10/met 10	Mor 20/met 10	Mor 30/met 10
20 (Met 20)	Mor 0/met 20	Mor 10/met 20	Mor 20/met 20	Mor 30/met 20
30 (Met 30)	Mor 0/met 30	Mor 10/met 30	Mor 20/met 30	Mor 30/met 30

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

The parameters measured were Weight Gain; Average Daily Weight Gain and Feed Intake. Each of these parameters were measured at 2 weeks interval. Performance characteristics were evaluated according to the method of (Olvera-Novoa, Campros, Sabido and Martinez-Palacious, 1990) as follows:

Mean Weight Gain (MWG) = Final mean weight (kg) - Initial mean weight (kg);

Percentage Weight Gain (PWG) = Mean weight gain (kg) / Initial mean weight; x 100

Specific growth rate (SGR % /day) = 100[(LogeW2 – LogeW1)/NO of days

Feed conversion ratio (FCR) = total feed fed (g) / net weight gain (g)

Protein Intake (PI) = total feed consumed X % Crude protein in feed

Feed intake (FI) = Amount of feed consumed throughout the period of the experiment;

Protein Efficiency Ratio (PER) = Net weight gain (g) / Amount of protein fed (g)

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 AND DISCUSSION

The proximate and nutrient composition of the moringa leaf is given in Tables 2 and 3. The result in Table 2 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 3 indicated that contents of the leaf were high in minerals.

Table 2: 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
Energyvalue (Kcal/100kg)	1349.5

Table 3: 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

Growth parameters

Table 4: The effect of moringa leaf and methionine diets on the growth parameters of *Clarias gariepinus* at 2 weeks of feeding experimental diet (WFED)

Methionine diets (%)	Moringa diets (%)				Mean
	0 (Mor 0)	10 (Mor10)	20 (Mor20)	30 (Mor 30)	
Mean weight gain					
0 (Met 0)	0.12	0.19	0.21	0.25	**0.19**
10 (Met 10)	0.15	0.28	0.22	0.30	**0.24**
20 (Met 20)	0.18	0.32	0.38	0.42	**0.32**
30 (Met 30)	0.20	0.43	0.40	0.49	**0.38**
Mean	**0.16**	**0.30**	**0.30**	**0.36**	0.28
Percentage weight gain					
0 (Met 0)	67.75	74.67	79.11	81.57	**75.77**
10 (Met 10)	68.16	81.12	83.50	84.10	**79.22**
20 (Met 20)	82.63	87.03	88.28	89.60	**86.88**
30 (Met 30)	83.84	87.17	91.97	95.60	**89.64**
Mean	**75.59**	**82.49**	**85.71**	**87.71**	82.87
Specific growth rate					
0 (Met 0)	0.11	0.29	0.43	0.46	**0.32**
10 (Met 10)	0.24	0.45	0.55	0.58	**0.45**
20 (Met 20)	0.35	0.68	0.73	0.77	**0.63**
30 (Met 30)	0.43	0.45	0.71	0.81	**0.60**
Mean	**0.28**	**0.46**	**0.60**	**0.65**	0.50

	MWG	PWG	SGR
$LSD_{0.05}$ for comparing 2 Meth rates:	0.04	3.21	0.12
$LSD_{0.05}$ for comparing 2 Mor rates:	0.04	3.21	0.12
$LSD_{0.05}$ for comparing 2 Meth x Mor rates:	0.08	6.42	0.24

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

The feeding of Methionine, Moringa and their interaction diets had high ($P < 0.05$) effect on mean weight gain of *Clarias gariepinus* at 2 weeks of feeding experimental diet (Table 4). The feeding of methionine diet at 30% was statistically ($P < 0.05$) higher than other levels of methionine diet as regards mean weight gain. The feeding of moringa diet at 30% was ($P < 0.05$) higher than other percentages of moringa diets with respect to mean weight gain. The interaction of 30% of methionine and 30% of Moringa ($P < 0.05$) gave the highest mean gain weight of the *Clarias gariepinus* at 2 weeks of feeding.

The result in Table 4 showed that the feeding of methionine, moringa and interaction diets produced higher ($P < 0.05$) percentage weight gain of *Clarias gariepinus* at 2 weeks of feeding regime. The effect of the use of methionine diets at 30% and 20% were statistically higher ($P < 0.05$) on percentage mean weight. However, both diet levels were ($P < 0.05$) higher than 10% and 0% of methionine. The feeding of *Clarias gariepinus* with 30% and 20% of moringa were statistically ($P < 0.05$) the same but higher than the use of moringa diet at 10% and 0% with respect to percentage mean weight. The combination of methionine diet at 30% and moringa at 30% gave the highest percentage mean weight.

At 2 weeks on the use of the experimental diets, the feeding of methionine, moringa and their interaction diets highly ($P < 0.05$) influenced specific growth rate. The use of moringa diet at 30% was ($P < 0.05$) higher that other treatments of moringa diets with regards to specific growth rate. Methionine diet at 20% gave the highest specific growth rate among methinine diets. The interaction of 30% of moringa and 30% of methionine diets resulted to the highest specific growth rate of *Clarias gariepinus.*

Table 5: The effect of moringa and methionine diets on the growth parameters of *Clarias gariepinus* at 4 weeks of feeding experimental diet (WFED)

Methionine diets (%)	Moringa diets (%)				Mean
	0 (Mor 0)	10 (Mor10)	20(Mor 20)	30(Mor 30)	
Mean weight gain					
0 (Met 0)	2.18	2.22	2.28	4.25	**2.73**
10 (Met 10)	2.23	2.32	3.56	4.57	**3.17**
20 (Met 20)	2.25	2.38	3.70	4.42	**3.18**
30 (Met 30)	2.32	2.47	3.92	4.49	**3.30**
Mean	**2.25**	**2.34**	**3.36**	**4.43**	3.09
Percentage weight gain					
0 (Met 0)	77.75	84.67	69.11	81.57	**78.27**
10 (Met 10)	68.16	51.12	73.50	54.10	**61.72**
20 (Met 20)	82.63	87.03	58.28	79.60	**76.88**
30 (Met 30)	73.84	67.17	61.97	85.60	**72.14**
Mean	**75.59**	**82.49**	**85.71**	**87.71**	82.87
Specific growth rate					
0 (Met 0)	0.36	0.49	0.43	0.46	**0.43**
10 (Met 10)	0.54	0.65	0.55	0.58	**0.58**
20 (Met 20)	0.55	0.78	0.73	0.77	**0.70**
30 (Met 30)	0.53	0.45	0.71	0.81	**0.62**
Mean	**0.49**	**0.59**	**0.60**	**0.65**	0.58

	MWG	PWG	SGR
$LSD_{0.05}$ for comparing 2 Meth rates:	0.21	1.89	0.10
$LSD_{0.05}$ for comparing 2 Mor rates:	0.21	1.89	0.10
$LSD_{0.05}$ for comparing 2 Meth x Mor rates:	0.42	3.78	0.20

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

The result on presented in Table 5 showed that methionine, moringa diets and their interaction ($P < 0.05$) influenced growth parameters of the cat fish at 4 weeks of feeding experimental diets. The feeding of 30% of methionine diet produced the highest mean weight gain among the rates of methionine diets. Highest mean weight gain was achieved with the feeding of 30% of moringa diet when compared to other moringa rates. The application of 10% of methionine and 30% of moringa diets gave the highest mean gain weight of mean gain weight of *Clarias gariepinus*

At 4 WFED, the use of moringa, methionine and their interaction diets ($P < 0.05$) influenced percentage weight gain. The use of 20% of methionine diet ($P < 0.05$) produced the highest percentage gain weight among the rates of methionine diets. The application of 30% of moringa feed had the highest percentage weight gain when compared with the other moringa feed rates. The interaction of 30% of moringa and 30% of methionine diets significantly ($P < 0.05$) produced the highest percentage weight gain on the cat fish.

Moringa, methionine and interaction diet effects were significant ($P < 0.05$) on specific growth rate of the cat fish at 4 WFED. The use of moringa diets at 10%, 20% and 30% were statistically ($P < 0.05$) the same with respect to specific growth rate. However, the use of 30% of moringa diet had the highest specific growth rate. The 20% 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.

Table 6: The effect of moringa and methionine diets on the growth parameters of *Clarias gariepinus* at 6 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					
0 (Met 0)	5.34	6.22	7.28	8.25	**6.77**
10 (Met 10)	5.93	6.52	7.56	8.57	**7.14**
20 (Met 20)	6.25	6.68	7.70	8.42	**7.26**
30 (Met 30)	6.52	7.07	7.92	9.09	**7.65**
Mean	**6.01**	**6.62**	**7.61**	**8.58**	7.20
Percentage weight gain					
0 (Met 0)	79.75	64.67	89.11	71.57	**76.27**
10 (Met 10)	69.16	71.12	53.50	74.10	**66.97**
20 (Met 20)	88.63	77.03	88.28	79.60	**83.38**
30 (Met 30)	80.84	67.17	61.00	95.60	**76.15**
Mean	**79.59**	**69.99**	**72.97**	**80.21**	75.69
Specific growth rate					
0 (Met 0)	0.48	0.55	0.73	1.80	**0.89**
10 (Met 10)	0.59	0.69	0.75	1.85	**0.97**
20 (Met 20)	0.64	0.75	0.83	1.88	**1.02**
30 (Met 30)	0.71	0.75	1.85	1.93	**1.31**
Mean	**0.60**	**0.68**	**1.04**	**1.86**	1.04

	MWG	PWG	SGR
$LSD_{0.05}$ for comparing 2 Meth rates:	2.21	3.89	0.15
$LSD_{0.05}$ for comparing 2 Mor rates:	2.21	3.89	0.15
$LSD_{0.05}$ for comparing 2 Meth x Mor rates:	4.42	7.78	0.30

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

The result presented in Table 6 showed that the use of methionine diets did not have high ($P < 0.05$) effect on mean gain weight. However, the use of moringa and moringa x methionine interaction had ($P < 0.05$) effect on mean gain weight at 6 weeks of feeding experimental diets. The application of 30% of moringa gave the highest mean gain weight among the rates of moringa diets. The combination 30% of moringa x 30% of methionine feeds produced the highest mean gain weight.

The use of moringa, methionine and their interaction diets gave significant ($P < 0.05$) effect on percentage mean weight at 8 weeks of feeding experimental diet on African cat fish (Table 6). The application of 30% of methionine diet had ($P < 0.05$) the highest percentages mean weight among methionine rates. Highest percentage mean weight was obtained when 30% of moringa diet was used. The application of 30% of moringa diet and 30% ($P < 0.05$) gave the highest percentage mean weight.

It was observed that the use of methionine, moringa and their interaction produced significantly ($P < 0.05$) effect on specific growth rate at 6 weeks of feeding experimental diets (Table 6). The highest specific growth rate was obtained when 30% of methionine x 30% moringa diets were fed to the fishes

Table 7: 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 (Mor10)	20 (Mor20)	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 7 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 7). 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 7). 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 8: 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 (Mor10)	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 the highest feed conversion ratio among moringa rates. The use of 30% of methionine produced 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 8 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 protein 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 8 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 protein efficiency ratio of *Clarias gariepinus* treatment combinations

DISCUSSION

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 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 and Li (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 2). The result in Table 3 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 have significant ($P < 0.05$) influence on 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 (Cho and Kaushik, 1990; Robinson and Li, 2006). 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, 2001).

Nutrient utilization

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) 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 utilized 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 intraction 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, Pezzato and Barros, 2004). 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. 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.

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