



Growth Performance of *Clarias Gariepinus* Fed Soaked *Moringa Oleifera* Leaf Meal Ayegba, E. O., * Ayuba, V. O. and Annune P. A.

Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Benue state, Nigeria

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Email: ayegbaemmanuel45@gmail.com

ABSTRACT

The present study evaluates the nutritional potential of soaked-dried *Moringa oleifera* leaf meal in the diet of *Clarias gariepinus*. Four isonitrogenous (35% crude protein) diets were formulated with Moringa leaf replacing soybean meal at 0%, 10%, 20% and 30%. Result obtained revealed declined in weight gain, specific growth rate, feed conversion efficiency, protein efficiency ratio and apparent net protein utilization as dietary replacement of Moringa leaf meal increased beyond 10%. It is concluded that *Moringa oleifera* leaf meal can replace soybeans meal up to 10% without affecting the growth performance of African catfish.

INTRODUCTION

Locally produced feed reduces the cost of production and hence, cheaper means of meeting the protein requirement improve food security and reduce the level of poverty in developing countries. The search for alternative protein sources which are not suitable for direct human consumption has been the focused of research for decades now (Hoffman et al., 1997). Fishmeal and soybean has been used as the main source of animal and plant protein in fish feeds. However, the periodically occurring low availability, competition and continuously fluctuating prices of these conventional protein sources are affecting aquaculture feed production and consequently the profitability of Aquaculture venture. As a result, a lot of effort has been focussed on feed alternatives of plant and animal origin to help replace the conventional protein sources (El-saidy and Gaber, 2004; Rinchar et al., 2002; Hossain et al., 2002). These alternatives should hold the potential to supplement, replace or partly replace the conventional feedstuffs.

Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Hasting, 1976; Sotolu., 2012). The inclusion of plant protein sources in the ration of fish requires investigation on proper processing for effective utilization (Pillay, 1990; Francis et al., 2001). Presence of certain factors in plant ingredients such as high crude fibre content and anti-nutritional factors (Alegbeleye et al., 2001; Sotolu., 2012) have been demonstrated as the reason for limiting their use in fish culture. Excessive consumption of plant protein sources by fish could also cause slower growth rates and poor performance which may result in mortalities if conditions become critical. (Cho et al., 1974; Francis et al., 2001; Bello et al., 2013).

Moringa oleifera Lam, is a multipurpose tree that thrives in both tropical and sub-tropical conditions. It is native to the sub-Himalayan regions of north-west India and now indigenous to many countries in Africa, Arabia, South East Asia, the Pacific and Caribbean Islands and South America (Wikipedia, 2014). The leaves have high levels of macronutrient and very rich in vitamins and minerals (Bau et al., 1994), Moringa leaves are free from anti-nutrients except for saponins and phenols. The concentration of phenol is much below the toxic threshold levels for animals (Makkar and Becker, 1997) and saponins were inactive as far as haemolytic properties are concerned. This property of Moringa has necessitated the investigation of this study which seeks to determine the performance of African catfish fed diet containing *Moringa olifera* meal.

MATERIALS AND METHODS

Study location

The study was conducted at the Fisheries and aquaculture Department earthen pond of the University of Agriculture, Makurdi, Benue state, Nigeria.

Sources of ingredients and diets preparation

The feed ingredients used in the feed formulation which includes Fish meal, Soybean meal, Maize meal, Vitamin and Mineral premixes were purchased from the Makurdi Modern market, Benue state, Nigeria. They were then processed and grinded into meal for storage. Moringa leaves were freshly plucked, and the leaves were thoroughly rinsed with water to remove dirt, soaked for three days, drained and properly dried. Thereafter, the leaves were grinded into fine powder and stored for feed formulation. 35% crude protein control diet (DT1) was formulated using Pearson square method without Moringa olifera leaf meal, the other experimental diet were however formulated by simply substituting soybeans meal for *Moringa olifera* leaf meal at 10% (DT2), 20% (DT3) and 30% (DT4) substitution levels (Table i). The diets so formed were pelletized using a pelleting machine after weighing appropriately and thorough mixing of the ingredients. The diet formulated is represented in table 1.

Experimental procedure

The fingerlings of *Clarias gariepinus* for this study were obtained from the research farm of the Fisheries Department, University of Agriculture Makurdi. The experiment lasted for 56 days. Hapas made from nets measuring 1m x 1m x 1m were mounted on a kuralon rope and set across the pond surface and properly staked to the dyke of the pond using bamboo sticks. Stones were attached to the four bottom corners of the hapas to serve as sinkers. This enables the bottom surface of the hapas to spread uniformly and to extend properly. This extension made easy inflow and outflow of water through each hapa immersed in the pond water half way to enable ease of access. Fifteen *Clarias gariepinus* fingerlings were evenly distributed in each of the hapa.

Fish feeding and culture

The daily feeding was done by hand at 5% of the cumulative body weight of fish of each hapa. Daily ration was divided into two feedings per day (08:00 and 16:00) and the fingerlings were weighed weekly so as to adjust the feed by virtue of weight gained. Water quality parameters: Temperature, Dissolved Oxygen, and pH were determined using Thermometer, Dissolved Oxygen meter and pH meter respectively.

Data collection and analysis

A Tefal electronic digital scale was used to measure weights of fingerlings per week till the end of the experiment (8 weeks), growth performance were estimated as stated below.

(a) Mean Weight Gain (MWG) = Mean final weight – Mean initial weight

(b) Feed conversion ratio (FCR) = $\frac{\text{dry feed intake}}{\text{wet weight gain}}$

(c) Specific Growth Rate (%/day) = $\frac{\log_e(wt_2) - \log_e(wt_1)}{t_2 - t_1}$

Where Wt1= Initial weight gain
 Wt2= Final weight gain
 T2-T1= Duration (in days) considered between Wt2 and Wt1

$$(d) \text{ Protein efficiency ratio} = \frac{\text{wet weight gain}}{\text{protein fed}}$$

$$\text{Where Protein fed} = \frac{\% \text{protein in diet} \times \text{total diet consumed}}{100}$$

$$(e) \% \text{ survival rate} = \frac{\text{total number of fish} - \text{mortality}}{\text{total number of fish}} \times 100$$

Proximate compositions of *Moringa olifera*, diets formulated, initial and final carcass of fish were determined according to standard methods by AOAC (2000). However Nitrogen free extracts in samples were determined by difference. The analyses were conducted in triplicate and all reagents were of analytical grade.

Statistical analysis

The data obtained from the study were analyzed using Gen stat® discovery edition 4 and Minitab® 14, descriptive statistics were done and mean gotten were subjected to analysis of variance, where significant differences were obtained (P<0.05), means were separated using Duncan's least significant difference (LSD).

RESULTS AND DISCUSSION

The potentials of feedstuffs such as leaf meal in fish diets can be evaluated on the basis of its proximate chemical composition (Adewolu 2008). However, the presence of anti-nutrients reduced the nutritional quality of the fish feeds (Francis et al; 2001). There was observed reduction in crude protein content after soaking (33.19% lower than the raw sample with 36.05% CP table 2), this shows that soaking reduced the protein content of the leaf. This is in agreement with the work of Nwaoguikpe et al (2011) who reported a reduction in crude protein content from 28.23% CP to 22.17%CP of *Mucuna pruriens* seeds (velvet Beans) after soaking.

Table 1 : Percentage inclusion of diets I, II, III and IV

	Diet I	Diet II	Diet III	Diet IV
Ingredient	(%)	(%)	(%)	(%)
Soybean meal	25.87	23.23	20.70	18.11
Moringa meal	0.0	2.64	5.17	7.76
Fish meal	25.87	25.87	25.87	25.87
Maize meal	23.13	23.13	23.13	23.13
Rice bran	23.13	23.13	23.13	23.13
Mineral premix	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50
Oil	0.50	0.50	0.50	0.50
Binder	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100

Table 2 : Proximate analysis of unprocessed and processed *M. oleifera* leaves

Parameters	Unprocessed Moringa leaves	Processed moringa leaves	P-Value
Crude protein	36.05 ± 0.01 ^a	33.19 ± 0.02 ^b	0.001
Moisture	5.63 ± 0.23 ^a	3.46 ± 0.11 ^b	0.001
Crude fat	3.84 ± 0.48 ^a	2.61 ± 0.00 ^b	0.001
Crude fibre	8.53 ± 0.02 ^b	11.66 ± 0.10 ^a	0.001
Ash	12.95 ± 0.28 ^a	7.59 ± 0.19 ^b	0.001
NFE	33.00 ± 0.11 ^b	41.49 ± 0.12 ^a	0.001

Mean in the same row with different superscripts differ significantly (P<0.05)

Table 2 : Anti-nutritional content of unprocessed and processed *M. oleifera* leaves

Parameters (mg/100g)	Unprocessed Moringa leaves	Processed Moringa leaves	P-Value
Oxalate	879.50 ± 0.50 ^a	296.00 ± 1.00 ^b	0.001
Tanin	62.23 ± 0.12 ^a	40.35 ± 0.18 ^b	0.001
Phytic acid	16.49 ± 0.18 ^a	11.11 ± 0.11 ^b	0.001
Cyanogenic	10.53 ± 0.18 ^a	0.13 ± 0.15 ^b	0.001

Mean in the same row with different superscripts differ significantly (P<0.05)

Table 4 : Proximate composition (%) of the experimental diets

Parameters (%)	Diet 1	Diet 2	Diet 3	Diet 4	P-Value
Crude Protein	34.56 ± 0.18	34.62 ± 0.06	34.23 ± 0.35	34.95 ± 0.07	0.100
Crude fat	5.19 ± 0.14 ^a	4.99 ± 0.03 ^{ab}	4.69 ± 0.06 ^b	4.24 ± 0.08 ^c	0.005
Crude fibre	4.15 ± 0.15 ^d	5.37 ± 0.18 ^c	6.91 ± 0.09 ^b	7.89 ± 0.09 ^a	0.001
Ash	5.39 ± 0.08 ^d	7.99 ± 0.02 ^c	8.81 ± 0.35 ^b	9.79 ± 0.07 ^a	0.001
Moisture	1.51 ± 0.02 ^b	1.59 ± 0.02 ^a	1.32 ± 0.03 ^c	1.58 ± 0.01 ^{ab}	0.002
NFE	49.22 ± 0.29 ^a	45.45 ± 0.25 ^b	44.06 ± 0.100 ^c	41.51 ± 0.05 ^d	0.002

Mean in the same row with different superscripts differ significantly (P<0.05)

Table 5 : Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* fingerling fed different levels of Moringa leaf meal diet

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	P-Value
Mean Initial Weight(g)	4.42 ± 0.08	4.34 ± 0.06	4.28 ± 0.03	4.37 ± 0.09	0.589
mean Final Weight(g)	12.27 ± 0.34 ^a	9.88 ± 0.77 ^b	8.86 ± 0.29 ^b	8.80 ± 0.29 ^b	0.002
Mean Weight gain(g)	7.85 ± 0.29 ^a	5.54 ± 0.83 ^b	4.57 ± 0.29 ^b	4.44 ± 0.20 ^b	0.003
Growth rate	0.14 ± 0.001 ^a	0.099 ± 0.015 ^b	0.082 ± 0.001 ^b	0.079 ± 0.004 ^b	0.003
Specific growth rate	1.82 ± 0.04 ^a	1.46 ± 0.16 ^b	1.29 ± 0.06 ^b	1.25 ± 0.02 ^b	0.007
Feed Fed	28.27 ± 0.34 ^a	23.26 ± 0.94 ^b	21.05 ± 0.42 ^{bc}	21.60 ± 0.17 ^c	0.001
FCR	3.61 ± 0.11	4.33 ± 0.46	4.64 ± 0.32	4.87 ± 0.21	0.076
FCE	27.76 ± 0.85 ^a	23.63 ± 2.55 ^{ab}	21.76 ± 1.54 ^b	20.53 ± 0.85 ^b	0.050
PER	0.224 ± 0.008 ^a	0.158 ± 0.023 ^b	0.131 ± 0.008 ^b	0.127 ± 0.006 ^b	0.003
ANPU	27.84 ± 0.53 ^a	21.26 ± 0.49 ^b	13.60 ± 0.43 ^c	11.26 ± 0.77 ^d	0.003
% Survival	96.67 ± 3.33	86.67 ± 3.33	93.33 ± 6.67	96.67 ± 3.33	0.389

Table 6: Proximate composition of *Clarias gariepinus* carcass before and after feeding trial

Parameters (%)	Initial	Diet 1	Diet 2	Diet 3	Diet 4	P-Value
Crude Protein	41.17 ± 0.94 ^d	49.98 ± 0.19 ^a	47.67 ± 0.17 ^b	44.99 ± 0.15 ^c	44.17 ± 0.27 ^c	0.001
Crude fat	11.67 ± 0.14 ^d	24.46 ± 0.56 ^a	17.26 ± 0.23 ^b	16.24 ± 0.16 ^b	15.09 ± 0.11 ^c	0.001
Crude fibre	1.38 ± 0.06 ^{bc}	1.28 ± 0.06 ^c	1.41 ± 0.06 ^{bc}	1.53 ± 0.02 ^{ab}	1.62 ± 0.03 ^a	0.026
Ash	15.33 ± 0.14 ^a	12.26 ± 0.09 ^c	13.67 ± 0.07 ^b	13.31 ± 0.11 ^b	15.21 ± 0.25 ^a	0.001
Moisture	7.85 ± 0.03 ^a	6.38 ± 0.03 ^a	6.99 ± 0.04 ^d	7.26 ± 0.05 ^c	7.56 ± 0.01 ^b	0.001
NFE	22.61 ± 0.58 ^a	5.65 ± 0.74 ^d	13.00 ± 0.15 ^c	16.68 ± 0.23 ^b	16.35 ± 0.58 ^b	0.001

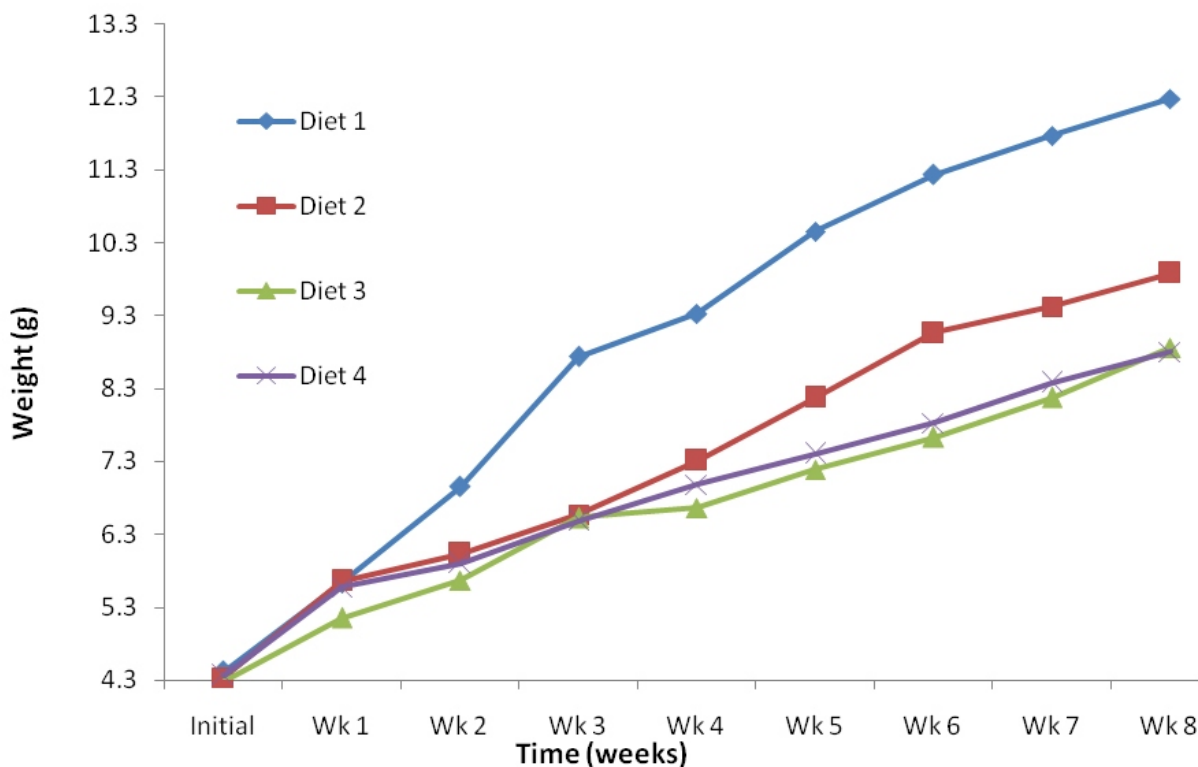


Figure 1 : Weekly growth of *Clarias gariepinus* fed experimental diet

Table 7: Water quality parameters

Parameter	treatment 1	treatment 2	treatment 3	treatment 4
Temperature (°C)	27.0	26.5	26.0	26.5
Dissolved oxygen (mg/l)	6.37	6.35	6.20	6.01
pH	6.21	6.30	6.17	6.11

Table 8 :Cost benefit analysis of the feed fed

Feed ingredient	Price of feed ingredient/kg (₦)	Cost of each feed ingredient in the Diet (₦)			
		Diet 1	Diet 2	Diet 3	Diet 4
Soyabean	150	38.805	34.845	31.05	27.165
Moringa Meal	0	0	0	0	0
Fish meal	700	181.09	181.09	181.09	181.09
Maize meal	100	23.13	23.13	23.13	23.13
Rice bran	0	0	0	0	0
Vitamin Premix	300	1.5	1.5	1.5	1.5
Mineral premix	300	1.5	1.5	1.5	1.5
Oil	150	0.75	0.75	0.75	0.75
Starch	150	0.375	0.375	0.375	0.375
Salt	100	0.25	0.25	0.25	0.25
Price of formulated diet per Kg (₦)		247.4	243.44	239.645	235.76
FCR (Amount of feed in kg to produce 1kg fish)		3.61	4.33	4.64	4.87
Cost of feed to produce 1Kg fish (₦)		893.114	1054.095	1111.953	1148.151

This may be due to solubilisation and leaching of some nitrogenous compounds during soaking. The result of anti-nutritional factors after soaking also shows that the Oxalate, Cyanide, Tannin and Phytic levels were substantially reduced ($P < 0.05$) while acids show a fairly significant ($P < 0.05$) reduction. However, result shows that soaking was more effective in the reduction of Oxalate and Cyanide than in removing Tannin and Phytic acids. The result of this study is in accord with the findings of Tamburawa (2010) which indicated that processing reduced the levels of oxalate from raw locust bean seed meal and this reduction increased with increase in duration of soaking time. Balogun (2013) also reported that oxalate level of Bauhinia seed was significantly reduced from 12.08mg/100g in raw to 1.94mg/100g in the soaked seed sample. The reduced level of undesirable anti-nutritional components is essential in order to improve the nutritional quality of the leaf meals and to effectively utilize their full potentials as feed (Balogun et al., 2005).

Growth and nutrient utilization by fish decreased as level of moringa leaf meal inclusion increases in the diets. Despite reduction of anti nutrient by soaking the persistent increase in Moringa leaf meals consumption due to replacement with soyabeans must have presented levels anti-nutrient levels that are not tolerated in the diet of the fish hence leading to reduce growth, Phenol, tannins, phytates and saponins have been reported to retard fish growth rate by Bello et al., (2013). Dienye and Olumuji (2014) and Richter et al., (2003) further buttressed this fact as they recorded progressive depressed growth rate in *Clarias gariepinus* fed increasing graded levels of moringa leaf meal based-diet. This present study also agrees with the work of Sotolu and Faturoti, (2009) who recorded progressive decrease in growth rate in catfish fed increasing graded levels of leucaena seed meal based-diet. Phytic acid level in fish feed above the concentration of 0.5% has been shown to be detrimental to the growth of fish by Francis et al., (2001) and in the present study the level exceed the concentration of 0.5% after processing. Berrker and Marker (1999) also reported that tannins interfere with digestive processes by inhibiting protease and also forming indigestible complexes with dietary protein at considerable inclusion rate. These anti-nutrients might be responsible for the decrease in the growth rate as level of inclusion of moringa leaf meals increase in the diet. The highest weight gain was recorded in diet 1 (0% *M. oleifera*) however, diet ii (10% *M. oleifera*) showed the highest weight gain compared to all the other diets with inclusion of moringa leaf meals although there was no statistical variation ($p > 0.05$) among them. This observation is in conformity with the findings of Madalla et al., 2013 and Tagwireyi et al; 2008, where aqueous extracted moringa leaf meal and heat treated moringa leaf meal at 10% inclusion in the diets of *Oreochromis niloticus* recorded higher weight gain than all other diets with inclusion of Moringa except for the control diet (0% moringa). This is contrary to the findings of Richter et al; 2003 and Bello et al; 2013 where moringa inclusion at 10% in the diets of *O. Niloticus* and *C. gariepinus* give better performance than 0% moringa inclusion. This variation might be due to differences in the processing methods. The decrease in the values of specific growth rate (SGR) could be due to differences in the moringa leaf meals level which decreased at increasing level of moringa leaf meal in the diet. The decrease trends have been reported in the diets containing black gram seed meal (Ramachandran and Ray; 2007), Moringa oleifera leaf meal diets (Bello et al; 2013) and Aqueous extracted moringa leaf meal diets (madalla et al; 2013). The decrease in the SGR could be due to reduction of protein and amino acids in the diets having higher substitution level (Russel et al; 1983) or to anti-nutritional factors contained in the moringa

leaf meal based diets (Dienye and Olumuji; 2014). There was no statistical significant difference in the feed conversion ratio (FCR). However, FCR values decreases across the treatment diets. This agrees with the report of Bundit and Toshiro (2012) where growth parameters namely, weight gain, FCR, and survival were similar in the partial replacement of soybean meal with Moringa leaf meal. This also has a direct link with the result of effects of dietary supplement of methanol extracted leaf meal found on the growth of Nile tilapia (*Oreochromis niloticus*) (Afuang et al, 2003). This might be as a result of lower level of methionine in diets supplemented with moringa leaf meal more limited in the amino acid than in the soybean meal in the control diet. It has been demonstrated that low dietary levels of methionine suppress growth and feed utilization (Gaber; 2006 and Bundit and Toshiro; 2012). The decrease trend in the FCR across the treatments observed might be due to high fibre content of the moringa leaf meal (Ramachandran and Ray; 2007). This trend was also reported by Ramachandran and Ray (2007) in black gram seed meal in the diets of *labeo rohita* (Hamilton) fingerlings and in grass pea seed meal (Ramachandran et al; 2005). Bello et al (2013) and Dienye and Olumuji (2014) reported same trend in varying levels of moringa leaf meal in the diets of *C. gariepinus*. Protein efficiency ratio (PER) was highest in fish fed 0% moringa leaf meal which differ statistically ($p > 0.05$) from values of 10%, 20% and 30% moringa leaf meal inclusions but also displayed the same decreasing trend as other growth parameters. These results seem to have direct link with feed intake. It could also be attributed to high fibre content and presence of protease inhibitor in the leaf meal (Adewolu; 2008). The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized (dienye and olumuji; 2014, Preston and Leng, 1987). This result is similar to the report of diets containing black gram seed meal which showed a decreased in PER across the treatments as the level of inclusion increased in the diets of *O. Niloticus* (Ramachandran and Ray; 2007) and diets of *C. gariepinus* fed different plant leaf meal sources (Bairagi et al; 2004). The high survival in the present study indicates that moringa leaf meal can be used for a very long periods. This contrast with the work of Wee and Wang (1987), who reported the appearance of ocular cataracts in *O. Niloticus* fed diets with leucaena leaf meal after 4-10 weeks of feeding.

All diets produced higher values of fish carcass protein and lipid than initial values, yet there existed marginal differences among them indicating different utilization levels of the diets. These relatively high values of crude protein and lipid could be viewed alongside the work of Alegbeleye et al (2001) who reported that effective utilization of bambara groundnut at varying rates was responsible for variation in *Heteroclaris carcass* protein and lipid. This could also be linked to the report of Sotolu and Faturoti (2009) who reported higher values of carcass protein and lipid than initial values with some variations among them indicating different utilization levels of leucaena seed meal based diet in *C. gariepinus*. The final protein, lipid and ash contents were similar to the levels in previous reports (Anderson et al., 1984; Wee and Wang 1987). These authors demonstrated that protein, and lipid levels declined as plant protein replacement levels increased, mainly due to lower digestibility and consequent lower nutrient availability in diets with high plant protein levels. The low level lipid of contents might be attributed to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. Presence of saponin might have also been contributed to inhibited pancreatic lipases activity and hence delayed intestinal

absorption of dietary fat (Han et al; 2000). The observed moisture content of 6.38% to 7.56% was similar to the value obtained in the work of Garduno and Olvera, (2008) in *O. niloticus* fed diet with groundnut leaf meal which recorded the highest value of 5.60% moisture content. But this was lower than the value reported by Sotolu and Faturaoi (2009) who recorded 11.14% in *C. Gariepinus* fed leucaena leaf meal.

CONCLUSION

The result of the utilization of moringa leaf meal by *C. gariepinus* processed by soaking in water and drying was better at 10% inclusion level than at any higher inclusion rates. Since weight gain of fish is what would translate into income for farmers at the end of the production cycle. However, other processing methods should be employed for better utilization of Moringa leaf meal in fish diet as soaking fairly denatured the Crude protein in this investigation. Therefore, this work recommends inclusion of Moringa leave meal in *C. gariepinus* diets in order to minimize the cost of fish production among small scale fish farmers.

Disclosure statement

No potential conflict of interest was reported by the author.

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