Effects of methionine supplemented groundnut cake on the Growth Performance and Haematology of *Clarias gariepinus* fingerlings

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ABSTRACT
This study sought to evaluate effects of graded levels of methionine supplementation of groundnut cake on the growth and haematological parameters of Clarias gariepinus fingerlings. Clarias gariepinus fingerlings of 6 g were allocated to 6 treatments having different levels of methionine and fed 5% of their body weight twice daily for 56 days. Treatments were GFM (fish meal-based diet) GM0 (control, 0 kg methionine) while GM1, GM2, GM3 and GM4 contained 0.25 kg, 0.5 kg, 0.75 kg and 1 kg methionine for every 100 kg feed, respectively. Fishes fed GM0 (control diet) had significantly (P<0.05) higher mean weight gain (MWG) than fishes fed methionine supplemented diets. The SGR and PER also followed the same trend. The packed cell volume (PCV) values were significantly higher (P<0.05) in the fish fed diet GM1, GM3 and GM4 than those of fish fed GM0 diets. The Haemoglobin concentration followed the same pattern. The total white blood cell was significantly higher (P<0.05) in fishes fed methionine supplemented diets than fish fed GM0 (control diet). The results of the study showed that methionine supplemented diets might have been affected by leaching, hence, (control diet) G0 not supplemented with crystalline methionine performed better in terms of MWG. However, supplementation of the diets with methionine increased the immunity of Clarias gariepinus fingerlings as shown in the haematological parameters.

Keywords: Methionine, Groundnut cake, Growth Performance, Haematology, *Clarias gariepinus*

INTRODUCTION

Feed is very important in aquaculture representing the single largest input constituting between 50-70% of the operating costs (Olapade and George, 2019). High-quality, cost-effective feeds improve fish performance, survival, health and wellbeing of the fish. Conventional protein feed ingredients are expensive due to competition for their use by man (Oyedokun et al., 2021). Therefore, there is the need to explore other less competitive unconventional and locally available protein feed ingredients that are cheaper especially plant proteins. Groundnut cake is a plant-based protein with a crude protein content of 40-45% and a by-product of groundnut oil extraction (Davies and Ezenwa, 2010). It is relatively cheap, abundant and available. Groundnut cake is reported to be palatable with better binding properties than soyabean meal (Olapade and George, 2019). However, it is deficient in some essential amino acids such as lysine and methionine (Oyedokun et al., 2021). Supplementation of groundnut cake with amino acids such as methionine has been reported to enhance its utilization (Olapade and George, 2019). Methionine is an indispensable supplement in aquafeed, that plays an important role in initializing protein synthesis (Elesho et al., 2021). Haematological profile has been used as a tool to evaluate fish welfare and disturbance on fish metabolism (Oyedokun et al., 2021). Some work has been done on utilization of groundnut cake in *Clarias gariepinus* diets (Davies and Ezenwa, 2010; Olapade and George, 2019). However, there are gaps on supplementation of the groundnut cake with methionine in the diet of *Clarias gariepinus* fingerlings. Hence, this study sought to evaluate effects of graded levels of methionine supplementation of groundnut cake on the growth and haematological parameters of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Study area

The experiment was carried out at the Fish farm of the National Open University of Nigeria, Kaduna, situated in Igabi local government area, between latitude 9°34'N and longitude 8°17'E. It falls within the Guinea Savannah ecological zone in the North-western part of Nigeria.

Experimental Diets

Fishmeal, vitamin premix, groundnut cake maize, soyabean cake and bonemeal were purchased from a feed mill in Kaduna. The feed ingredients were ground into fine powder using a hammer mill. The appropriate proportion of the ingredients based on Pearson's square method to produce 35% crude protein diet were weighed and dry mixed. Treatments were GFM (fishmeal-based diet) GM0 (control, 0kg methionine) while GM1, GM2, GM3 and GM4 contain 0.25 kg, 0.5 kg, 0.75 kg and 1 kg methionine for every 100 kg feed, respectively. The proximate composition of the experimental feed was determined as outlined in AOAC (1990). Table 1 shows the ingredients and proximate composition of the experimental diets.

Experimental Fish

One hundred (120) fingerling of *Clarias gariepinus* fingerlings (6 g) weight were purchased from a reputable farm in Kaduna and transported in 50 litres jerry cans. The fish were stocked in 60-liter capacity plastic tank filled with 50 litres of water at 15 fish/tank.

Experimental Design

The fishes were deprived of food for 24 hours before commencing the experiment. The fishes were allocated to 6 experimental diets in triplicates in a completely randomized design. The water was replaced twice weekly. Left over feed was flushed out daily while any dead fish found was removed and recorded. Fishes were fed 5% body weight twice daily for 56 days. The ration was recalculated after every two weeks according to the weight increase recorded when the fishes were sampled. Water quality parameters were taken every two weeks before sampling.

Table 1: Composition of Experimental Diet

	Treatments						
Ingredients (g/kg)	GFM	GM0	GM1	GM2	GM3	GM4	
Maize	19.00	7.00	7.20	7.00	7.00	7.00	
G/Cake	61.00	85.00	85.00	85.00	85.00	85.00	
Soya cake	5.00	4.50	4.00	4.00	3.70	3.50	
Fish meal	12.00	0.00	0.00	0.00	0.00	0.00	
Palm oil	1.00	1.00	1.00	1.00	1.00	1.00	
Starch	0.50	1.00	1.00	1.00	1.00	1.00	
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	
Salt	0.25	0.25	0.25	0.25	0.25	0.25	
Vit Premix	0.25	0.25	0.25	0.25	0.25	0.25	
Dl-Methionine			0.25	0.50	0.75	1.00	
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	
Proximate composition							SEM
Moisture	11.23^{c}	10.94^{d}	11.70^{b}	10.67^{e}	12.51^{a}	9.76^{f}	0.38
Crude protein	38.21^{a}	36.81^{b}	34.00^{c}	34.05^{c}	33.60^{d}	34.05^{c}	0.78
Crude fibre	2.00^{d}	2.11^{c}	2.45^{b}	2.48^{ab}	2.51^{a}	2.55^{a}	0.10
Ether extract	7.08^{c}	6.77^{d}	6.98^{d}	7.01^{c}	7.75^{b}	8.02^{a}	0.20
Ash	10.52^{b}	10.16^{c}	9.96^{d}	10.02^{c}	11.31^{a}	11.55^{a}	0.28
Energy (kcal/kg)	2497.80^{b}	2456.10^{c}	2417.80^{e}	2442.50^{d}	2500.20^{a}	2502.70^{a}	14.64

Means in the same row with different superscripts are significantly different (P<0.05); SEM= Standard Error of Mean

Growth Parameters

The following parameters were calculated for each treatment to determine the growth response of *clarias gariepinus*.

i. $\text{Mean Weight Gain (MWG)} = \text{Mean final weight} - \text{Mean initial weight}$
ii. $\text{Feed Conversion Ratio (FCR)} = \text{Total feed consumed}/\text{Weight gain by fish}$
iii. $\text{Survival rate} = \text{Total number of fish harvested}(100/\text{initial number of fish stocked})$
iv. $\text{Specific Growth Rate(SGR)} = \ln \text{W1} - \ln \text{W2} \times {}^{100}/_{\text{T2} - \text{T1}}$
v. $\text{Protein efficiency ratio (PER)} = \text{weightgain by fish}/\text{protein intake by fish}$

Determination of the Haematological parameters:

At the end of the feeding trial, 3 fishes were randomly taken per treatment. Blood samples were collected for haematological analysis according to methods described by Wedemeyer and Yasutake (1977). The blood samples were collected and preserved for analysis according to standard haematological procedures described by Blaxhall and Daisley (1973).

Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA). The level of significance set for treatments was P<0.05. SAS software (SAS, 2005) was employed for the analysis.

RESULTS AND DISCUSSION

From Table 2, fishes fed GM0 (control diet) had significantly (P<0.05) higher mean weight gain (MWG) than fishes fed methionine supplemented diets. Fishes fed GM4 showed the lowest mean weight gain (MWG). The

SGR and PER also followed the same trend being significantly (P<0.05) higher in fishes fed GM0 than those fed methionine supplemented diets.

Table 2 Growth parameters of *Clarias gariepinus* fed methionine supplemented diets.

Treatments	GFM	GM0	GM1	GM2	GM3	GM4	SEM
Parameters							
Initial weight (g)	6.29^{a}	6.58^{a}	6.01^{a}	6.54^{a}	5.74^{a}	4.96^{a}	0.25
Final weight (g)	11.87^{a}	10.87^{b}	8.65^{c}	8.40^{c}	8.99^{c}	6.45^{d}	0.78
Mean weight gain	5.57^{a}	4.29^{b}	2.64^{d}	1.87^{e}	3.25^{c}	1.49^{e}	0.63
SGR (%)	0.30^{b}	0.35^{a}	0.18^{d}	0.12^{e}	0.22^{c}	0.14^{e}	0.04
FCR	5.84^{e}	7.28^{d}	11.30^{bc}	12.02^{b}	8.71^{c}	14.95^{a}	1.37
FI	33.05^{a}	28.33^{bc}	29.10^{b}	22.32^{c}	27.85^{bc}	20.83^{d}	1.86
PER	0.46^{a}	0.43^{a}	0.26^{c}	0.24^{c}	0.34^{b}	0.29^{c}	0.04
Survival rate	55.56^{b}	48.89^{cd}	73.33^{a}	46.66^{d}	51.11^{c}	48.89^{cd}	4.04

Means in the same row with different superscripts are significantly different (P<0.05); Treatments were GFM (fishmeal-based diet) GM0 (control, 0kg methionine) while GM1, GM2, GM3 and GM4 contain 0.25kg, 0.5kg, 0.75kg and 1kg methionine for every 100kg feed respectively.SEM= Standard Error of Mean

Table 3 shows the haematological parameters. The packed cell volume (PCV) values were significantly higher (P<0.05) in the fish fed diet GM1, GM3 and GM4 than those of fish fed GM0 (control) diets. The Haemoglobin (HB) followed the same pattern. The total white blood cell (TWBC) was significantly higher (P<0.05) in fishes fed methionine supplemented diets than fish fed GM0 (control diet). The total red blood cell (TRBC) was highest in the fishes fed diet GM1, followed by GM3, while GM0 and GM2 had the lowest. The TRBC did not follow any definite pattern.

Table 3. Haematological parameters of *Clarias gariepinus* fed methionine supplemented diets

INDICES	GFM	GM0	GM1	GM2	GM3	GM4	SEM
PCV (%)	28.00^{a}	24.00^{c}	27.00^{ab}	18.00^{d}	27.00^{ab}	26.00^{b}	1.51
HB (g/dl)	9.30^{a}	8.30^{c}	9.00^{b}	6.00^{d}	9.00^{b}	8.60^{bc}	0.49
TP (g/dl)	6.00^{b}	8.40^{a}	8.00^{a}	6.70^{b}	8.00^{a}	5.80^{c}	0.46
TWBC (10^{9}/L)	11.80^{d}	8.80^{e}	15.00^{b}	19.00^{a}	13.80^{c}	12.00^{d}	1.41
TRBC (10^{6}/L)	3.40^{a}	2.80^{c}	3.10^{b}	2.60^{d}	3.00^{b}	2.70^{cd}	0.12

Means in the same row with different superscripts are significantly different (P<0.05); Treatments were GFM (fishmeal-based diet) GM0 (control, 0kg methionine) while GM1, GM2, GM3 and GM4 contain 0.25kg, 0.5kg, 0.75kg and 1kg methionine for every 100kg feed respectively. SEM= Standard Error of Mean

The higher MWG of the fishes fed control diet observed in this study was similar to the work of Olapade and George (2019) who reported that despite the fact that groundnut cake diets were supplemented with lysine and methionine, the MWG of the experimental diets were still lower than fishes fed the control diet. Machado et al. (2021) also reported that there was no significant difference in MWG of rainbow trout (*Oncorhynchus mykiss*) juveniles fed methionine supplemented diets. The reason for this observation could possibly be due to leaching of the methionine added to the diets. Gu et al. (2013) reported that there was high potential for leaching of crystalline amino acids (CCA). Moreover, Ambardekar et al. (2009) stated that there was difference between uptake of CCA and amino acids in intact protein. However, Davies and Ezenwa (2010) reported an increase in MWG when *Clarias gariepinus* fry were fed different lysine and methionine supplemented groundnut cake diets. The variations in different works on the effectiveness of crystalline amino acids (CCA) supplementation could probably be dependent on fish species and sizes, different feed ingredients in the feed, feed formulation techniques and differences in experimental conditions (Billah et al., 2022). Haematological parameters help to evaluate fish welfare and disturbance on fish metabolism. The increased PCV, HB and TWBC observed in this study was similar to the works of Machado et al. (2021) on rainbow trout juveniles and Billah et al. (2022) on *Heteropneustes fossilis* fry on methionine supplemented diets. Machado et al. (2021) suggested that methionine promotes immunity by improving stress and pathogen resistance of fish.

CONCLUSION

The results of the study suggest that methionine supplemented diets might have been affected by leaching; hence, control diet not supplemented with crystalline methionine, performed better in terms of mean weight gain. However, supplementation of the diets with methionine increased the immunity of *Clarias gariepinus* fingerlings as shown in the haematological parameters. It is recommended that the challenges of methionine

supplementation be addressed through the adoption of encapsulation techniques to minimize leaching and enhance nutrient retention. Additionally, species-specific research is crucial to optimize amino acid balance and develop tailored supplementation strategies that meet the unique needs of different aquaculture species.

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