Available online at https://asric.africa/natural-sciences
ASRIC Journal on Natural Sciences Vol 3(2) (2023) 83-90

Dietary Evaluation and Partial Budget Analysis of Earthworm Meal Based Diet on the Performance Characteristics of Catfish (*Clarias gariepinus*)

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Abstract
Fishmeal is considered the best protein source in fish feeding but increasing cost and high demand of it in animal and aquaculture industries have necessitated the need for good alternatives without compromising quality. The thrust of this study was to evaluate the potential of earthworm meal as a replacement of fishmeal in *Clarias gariepinus* diets. In a completely randomized design, 120 *Clarias gariepinus* (25±0.36g) were randomly distributed into 12 tanks (35L), each containing 10 fish in triplicate. Experimental diets were formulated with earthworm meal (EWM) replacing fishmeal at 0% (EWM_0; control), 25% (EWM_{25}), 50% (EWM_{50}) and 100% (EWM_{100}) and fed to experimental fish twice daily at 5% body weight for eight weeks. Growth performance parameters, nutrient utilization, profitability index and survival were measured and calculated using standard procedures. Result showed that protein intake ranged from 6.71g/d to 10.85g/d while protein efficiency ratio was 1.05 to1.59. Gross feed conversion efficiency was between 0.33 and 0.47. Weight gain varied significantly across the treatments with the highest in fish fed EWM_{50} and EWM_0 and least in those fed EWM_{100}. Specific Growth Rate, Feed Conversion Ratio and Profitability index were highest in fish fed EWM_{50}. Survival rate were higher in fish fed EWM (85 – 90%) compared to those fed control diet, EWM_0 (80%). Results from this study concluded that, fishmeal can be replaced with up to 50% earthworm meal in fish feed for better growth performance and economic viability of *Clarias gariepinus* culture.
Keywords: Earthworm meal, African mudfish, Growth performance, Nutrient utilization, partial budget analysis

Introduction
Fish nutrition is critical in fish farming because feed represents over 70% of production cost (Ploegmakers, 2022 and Fregene *et al.*, 2020). Growth performance and nutrient utilization of fish is determined by gross composition of the feed ingredients, processing and storage of the feed products. To culture catfish on an intensive level, it is important to feed fish feeds that provide nutrients and energy required for normal metabolic functions such as reproduction and growth like every other animal (Ajidhaslin *et al.*, 2022).
Due to declining catch from capture fisheries, intense competition between human food and animal feed industries, the rise in energy prices worldwide, and an unpredictable year-round supply, fishmeal has become increasingly rare and expensive (Nairuti *et al.*, 2022 and FAO, 2022). The constant use of fishmeal in aquaculture has consequently challenged the long-term viability of fisheries resources and increased fish demand, which has impacted fish farmers' profit margins (Tidd *et al.*, 2022). Although there is an obvious demand for less expensive fish feed, it is crucial that catfish feeds be formulated to be cost-effective rather than just less expensive (Ajidhaslin *et al.*, 2022). It is also important that the nutritional and physical quality of the feed, fish development, processed production, and product quality are not compromised by using less expensive substitute feedstuffs (Robinson and Li, 2015).

There are still some questionable possibilities for making inexpensive, dependable, and eco-friendly substitute fish feeds. Lack of certain amino acids, such as methionine, lysine and isoleucine in meat, bone meal and blood meal, limits the utilization of animal-based protein sources for fish diets, (Musyoka et al., 2019). Moreover, microbial contamination and the possibility for disease transmission provide difficulties for the sustainability of the usage of animal-based protein sources (Ogello *et al.,* 2014).

Earthworms are almost always present throughout the year and are a good candidate for use as non-conventional fish and poultry feed ingredients due to their high reproductive rate and biomass output (Hasanuzzaman *et al*., 2022). Fish feeds incorporated with non-conventional feedstuff such as earthworm meal are not often found in the market and are not the conventional ingredients in the preparation of commercial fish feed (Pandey, 2013). Hence, the thrust of this study was to evaluate the effect of earthworm meal on the performance characteristics of catfish as well as assessing the cost effectiveness of the experimental diets.

Materials and methods

Study Location

This study was conducted at the University of Ilorin, University of Ilorin is located in the North Central, Nigeria with a mean temperature of 25^0C -30^0C and mean annual rainfall of 1,200mm, humidity of 89% and pressure 1014mb (BBC weather Ilorin), while the University has a coordinate of 8.4795^0N and 4.6712^0E.

Earthworm Culturing, Collection and Processing

Earthworms used for the experiment were primarily cultured through a process known as Vermiculture. This is an artificial cultivating or rearing of worms and the technology is the scientific process of using them for the benefit of human and industrial goals. The process was done by collecting and using waste animal dung, paper and cardboard at a ratio of 1:1:1 as culturing media for the growth of the earthworm (Manohar *et al.*, 2016) however, the temperature, humidity and pH were taken into consideration for optimum growth. The process will help in converting garbage into compost by the earthworm with increasing number in their growth. During harvesting, the worms were collected and washed in clean water to remove the rubble and soil and was later processed into Earthworm meal.

The cultured earthworms were firstly sun dried for about two days, later subjected to oven drying at about 40 ^{0}C for 6 hours to prevent the decomposition of the earthworms. The earthworms were then milled to produce earthworm meal (EWM) and stored in plastic containers in preparation for its addition in the experimental diet

Preparation of the Experimental Diets

The prepared earthworm meal was included in the experimental diets as follows; experimental diet 1, EWM_0 (Control), contained 0% EWM while experimental diets 2, 3, and 4 (EWM_{25} EWM_{50} and EWM_{100}) contained 3.5% EWM, 7% EWM and 14% EWM respectively to replace fishmeal while other ingredients were of fixed proportions (table 1).

Table 1: Composition of the Experimental Diets

Ingredients (%)	Experimental Diets			
	EWM_0	EWM_{25}	EWM_{50}	EWM_{100}
Fishmeal	14.00	10.50	7.00	0.00
Blood meal	5.00	5.00	5.00	5.00
Soybean meal	32.00	32.00	32.00	32.00
Groundnut cake	22.00	22.00	22.00	22.00
Earthworm meal	00.00	3.50	7.00	14.00
Maize	25.00	25.00	25.00	25.00
Fish Pre-mix	0.50	0.50	0.50	0.50
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Vitamin C	0.10	0.10	0.10	0.10
Common Salt	0.20	0.20	0.20	0.20
DCP*	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

*Dicalcuim Phosphate

Experimental fish

A total of One hundred and twenty (120) *Clarias gariepinus* (average weight of 25g) for this study were acquired from a reputable fish farm in Ilorin metropolis and transported early in the morning in a 50L fish transporting tank to the study area. The fish samples were divided into four plastic tanks for acclimatization under aerated condition and fed with commercial feed for 7 days before the commencement of the experiment.

Experimental design and procedure

The experimental design was a Completely Randomized Design, where experimental fish were randomized against the experimental diets. The fish were fed with 5% of their body weight per day (twice daily between 8 – 9am and 4 – 6pm) for 8 weeks. Using a sensitive scale, fish in individual tanks were batch-weighed on a weekly basis during the feeding period, and the meal was adjusted as necessary.

Total changing of water was done every other day and replaced with aerated de-chlorinated water. Water quality parameters were monitored at interval throughout the study period, these were; temperature, pH, dissolved oxygen and conductivity. Hanna instruments pH/EC/TDS/Temperature meter were used to measure temperature and pH while dissolved oxygen was determined using Winkler titration method, conductivity was measured with digital conductivity meter.

Chemical analysis

All diets were analyzed for their proximate composition using the method of AOAC (2011) while metabolizable energy (ME) was calculated using the method of Capentar and Clegg (1956).

Growth Performance Characteristics

Growth performance characteristics such as Weight gain (WG), Mean Weight Gain (MWG), Specific Growth Rate (SGR); nutrient utilizations parameters such as Feed Intake (FI), Feed Conversion Ratio (FCR), Feed Efficiency (FE), Gross Feed Conversion Ratio (GFCR) Protein Intake (PI), and Protein Efficiency Ratio (PER) were determined using the following formulas according to Ojewole *et al.* (2022) and Olaniyi *et al.* (2013);

Weight gain (g) $= W_2 - W_1$

Mean Weight gain (g) $= \frac{W_2 - W_1}{N}$

SGR $= \frac{lnW_2 - lnW_1}{T_2 - T_1} \times 100$

Feed intake $= Total\ feed\ consumed\ through\ out\ duration\ of\ experiment$

FCR $= \frac{Weight\ of\ feed\ fed}{weight\ gain}$

Feed Efficiency $= \frac{Average\ weight\ gain}{Average\ feed\ consumed}$

GFCR $= \frac{1}{FCR}$

Protein intake $= total\ feed\ consumed\ x\ percentage\ protein\ in\ feed$

Protein Efficiency Ratio (PER) $= \frac{Weight\ gain\ (g)}{Protein\ intake}$

Survival Rate (%) $= \frac{\text{Number of fish that survived}}{\text{Total number of fish stocked}} x\ 100$

Where;

W_1 = initial weight of fish,

W_2 = final weight of fish and

N = Number of fish

T_1 and T_2 = First and last day of experiment (days).

Ln = Natural logarithm

Partial Budget Analysis

The profitability of feeding EWM based diets to *C. gariepinus* was determined using the partial budget analysis and marginal rate of return. Utilizing market pricing, the cost of feed was computed while accounting for transportation and processing expenses.

i. Profitability index being a measure of a project or investment attractiveness was calculated as:

$$\text{Profitability index} = \frac{Present\ value\ of\ future\ cash\ flow}{Initial\ investment}$$

ii. Rate of return on capital was calculated using the formula:

$$\text{Rate of return on capital} = \frac{Net\ Return\ (NR)}{Total\ Cost\ (TC)}$$

iii. Percentage marketing margin was determined by using the formula:

$$\text{Marketing margin (\%)} = \frac{Selling\ Price - Cost\ Price}{Selling\ Price}\ x\ 100$$

Statistical analysis

All data collected were subjected to Analysis of variance and means were separated using Duncan Multiple Range Test at P<0.05 level.

Results

Proximate composition of the experimental diets and the earthworm meal are shown in Table 2. The earthworm meal reported herein contains 50.97% Crude Protein, 6.79% ether extract, 1.44% Crude fiber and 6.96% Ash. Crude protein of experimental diets ranged from 29.53% to 37.41% while the other nutrient parameters showed a direct proportional relationship with EWM levels in the diet.

Table 2: Proximate Composition of the Experimental Diets and Earthworm meal (EWM)

Parameters (%)	Experimental Diets				EWM
	EWM_0	EWM_{25}	EWM_{50}	EWM_{100}	
Dry matter	92.74	90.77	94.62	95.12	95.86
Crude Protein	29.53	32.81	37.41	31.94	50.97
Ether extract	11.12	10.08	11.97	12.01	6.79
Crude fiber	4.20	4.65	5.12	5.20	1.44
Ash	6.72	8.39	9.72	8.10	6.96

The growth characteristics and nutrient utilization of *Clarias gariepinus* fed diets with varying inclusion levels of Earthworm meal are shown in Table 3. Average weight gain varied significantly (P<0.05) from 400g and 670g in fish fed EWM_0 to EWM_{100}. Fish fed EWM_{50} had the highest weight gain and least weight gain was in fish fed EWM_{100}. Feed intake per percentage body weight (%BW) increased as the inclusion levels of earthworm meal increased in the diets but this increase was not statistically significant. Feed Conversion Ratio (FCR) varied significantly (P<0.05) in the different diets with the lowest FCR recorded in fish fed EWM_0 and the highest in fish fed EWM_{100}. Highest Gross Feed Conversion Ratio (GFCR) was recorded in EWM_0 with 0.47 and least in EWM_{100} with 0.33, though not significantly different. Fish given EWM_0 had the highest specific growth rate (1.16) in this study while those fed EWM_{100} had the least (0.71), an observation that was statistically significant. The highest Protein intake was observed in fish fed EWM_{50} and least in fish fed EWM_{100}, this varied significantly across the treatments. Protein efficiency ratio ranged significantly from 2.67 (EWM_{50}) to 3.37 (EWM_0). Survival rate was least (80%) in fish fed EWM_0 and highest in those fed EWM_{50} and EWM_{100} (90%).

Table 3: Growth Performance and Nutrient Utilization of *C. gariepinus* fed EWM Based Diets

Parameters	EWM_0	EWM_{25}	EWM_{50}	EWM_{100}
Weight gain (g)	650^a	610^b	670^a	400^c
Feed Intake (%BW)	3.79	4.03	4.34	5.36
Specific Growth Rate	1.16^a	1.09^b	1.20^a	0.71^c
Feed efficiency	2. 71	2.48	2.31	1.82
FCR	2.13^d	2.27^c	2.43^b	3.03^a
GFCR	0.47	0.44	0.41	0.33
Protein Efficiency Ratio	3.37^a	3.05^{ab}	2.67^b	3.13^{ab}
Protein intake (g/day)	7.29^c	8.07^b	10.85^a	6.71^d
Survival rate (%)	80	85	90	90

Means along the rows with the same superscript are not significant different (P>0.05)

Partial budget analysis of feeding *C. gariepinus* earthworm meal based diets is presented in Table 4. The profitability index in this study was 2.74 (EWM_{50}) > 2.62 (EWM_{25}) > 2.04 (EWM_0) > 1.79 (EWM_{100}) while marketing margins were highest in EWM_0 and EWM_{50} with 6.25% and 6.30% respectively. Rate of return of capital was highest in fish fed EWM_{50} and least in fish given EWM_{100}.

Table 4: Partial Budget analysis of feeding *C. gariepinus* EWM based diets

Parameters	EWM_0	EWM_{25}	EWM_{50}	EWM_{100}
Profitability index	2.04^c	2.62^b	2.74^a	1.79^d
Rate of Return on Capital	1.02	1.01	1.02	0.54
Marketing margin	6.25	4.69	6.30	2.50

Means along the rows with the same superscript are not significant different (P>0.05)

Results of the water quality parameters had no significant differences (P>0.05) in all the parameters measured except conductivity and the values were within the normal standard for rearing *Clarias gariepinus* (table 5).

Table 5: Water quality parameters

Parameters	EWM_0	EWM_{25}	EWM_{50}	EWM_{100}
pH	6.70	6.75	6.80	6.76
Temperature (^{0}C)	26.00	27.00	27.00	26.00
Dissolved Oxygen (Mg/L)	5.30	5.80	5.70	5.45
Conductivity (µs/cm)	768.00^c	826.00^b	898.00^a	908.00^a

Means along the rows with the same superscript are not significant different (P>0.05).

Summary

Nutrient composition of the experimental diets in the present study showed a direct relationship with increasing EWM levels in the diet. The study examined the growth characteristics and nutrient utilization of *Clarias gariepinus* fed diets with varying levels of Earthworm meal. The average weight gain varied significantly between 400g and 670g, with EWM_{50} having the highest weight gain and EWM_{100} having the least. The profitability index was 2.74, with highest marketing margin in EWM_{50} at 6.30%. The rate of return of capital was highest in EWM_{50} fish. The water quality parameters measured for *Clarias gariepinus* during this study were within the normal standard.

Discussion

The Crude protein content of Earthworm meal in the present study were similar to the values reported by Kirens *et al*. (2019); Sun *et al*. (2019); Davis and Raja (2019) and Deepika *et al.* (2018). Additionally, the ether extract (6.79%) reported in this study were higher than 1.25% reported by Deepika *et al*. (2018) but lower than 11.0% reported by Onura *et al*. (2022) while the crude fiber (1.44%) was higher than 1.25% and lower than 3.75% reported by Onura *et al.* (2022) and Deepika *et al.* (2018) respectively. The Ash content reported herein (6.96%) was similar to the result (6.71%) obtained by Onura *et al.* (2022) but was lower than 10.31% and 45.66% noted by Davis and Raja (2019) and Deepika *et al.* (2018) respectively. The proximate composition of the experimental diets showed variation in composition among treatment diets due probably to the various inclusion levels of earthworm meal (EWM) in the diets. The range of Crude protein noted for the experimental diets reported herein were within the values of 25 and 50 % reported by Omoike *et al*. (2020) as the range for catfish requirements. Hence, the diets in this study were nutritionally adequate to feed *C. gariepinus*.

Good weight gain was recorded across fish fed different earthworm meal-based diets except those fed with the highest earthworm level. This indicated that the experimental diets are nutritionally balanced and adequate for *C. gariepinus*. The lower weight gain of fish fed diets 4 can be attributed to the total replacement of fishmeal by earthworm meal in the diet. Similar results have been reported by numerous authors (Omoike *et al.*, 2020) for *Clarias gariepinus.* According to several studies, earthworms meet the nutritional requirements of many fish species and have acceptable levels of protein, essential amino acids, and lipids similar to those found in fishmeal (Hasanuzzaman *et al.*, 2022; Parolini *et al.*, 2020; Musyoka *et al.*, 2019; Gunya *et al.*, 2016; de Chaves *et al.*, 2015). The result of Feed Conversion Ratio (FCR) varied significantly due probably to the inclusion of EWM in the experimental diet. EWM_0 had the lowest FCR (2.13) followed by EWM_{25} (2.27) and EWM_{50} (2.43) while EWM_{100} (3.03) indicated poor utilization which may have led to depressed growth shown in the result. Some studies in the past showed that the total replacement of fishmeal by maggot or earthworm meal solely resulted in reducing growth performance of *Heteroclarias* fingerlings (Olele, 2011 and Monebi and Ugwumba, 2013); and *Macrobrachioum rosenbergii* (Habashy, 2012) *Oreochromis nioticus* (Ezewudo et al., 2015) and *Common carp* (Pucher *et al*., 2014) which was evidenced in the present study. However, other studies have shown contrary results, indicating better growth performance in *Oreochromis niloticus* (Gbai *et al.*, 2018); *Clarias gariepinus* (Djissou *et al.*, 2016a) and *Oreochromis niloticus* (Djissou *et al.*, 2016b) and *Penaeus merguiensis* (Rachmawati, and Nurhayati, 2022).

The specific growth rate (SGR) was similar to that of Omoike *et al.* (2020) for *Clarias gariepinus.* It is interesting to note that fish sample fed on diet 3 gave the best and significant ($P<0.05$) specific growth rate. This supported the work of Olele and Okonkwo (2012) who fed heteroclarias fingerlings partial and total replacement of fishmeal with earthworm meal. The SGR of *Clarias garienpinus* fed with diets containing up to 7% earthworm meal in the current study was similar to the reports of Olele and Okonkwo (2012) where the authors asserted that inclusion of EWM improved the performance characteristics of fish. The value of PER noted in this study was higher than the value reported by Omoike *et al.* (2020). The variation may be due to different sources of protein used in the studies as well as the feed composition. The PER was best for EWM_{50} (7% EWM inclusion) and poorest from EWM_0. The Gross Feed Conversion Efficiency which is a reciprocal of FCR reported was 0.47 (EWM_0); 0.44 (EWM_{25}); 0.41 (EWM_{50}) and 0.33 (EWM_{100}). All fish samples in the present study on EWM based diets had high survival rate compared to the Control, EWM_0, and these results were in tandem with the values obtained by Omoike *et al.* (2020).

The highest profitability index noted for EWM_{50}, and EWM_{25} showed that these diets were more attractive and better compared to EWM_0 (Control) while EWM_{100} had poor Profitability index. The higher values of 6.30% and 6.25% Marketing margins recorded for EWM_0 (6.25) and EWM_{50} (6.30) are very encouraging for farmers to include 50% Earthworm meal in the diet of Catfish for more gain. Additionally, the EWM_{50} had a higher Rate of Return on Capital with the potential to produce more profit per N1 employed, which should encourage farmers to use earthworm meal at a 50% replacement for fishmeal in order to increase profits. The poor performance of fish samples on EWM_{100} could be due to higher inclusion level of earthworm meal in this diet.

Our study showed that the water temperatures of 26 ^{0}C and 27 ^{0}C were within the tolerable range for the culturing of Catfish and were similar to results (26.74 ^{0}C – 26.95 ^{0}C) obtained by Onura *et al.* (2022). The values in our study also agreed with the reported values of between 25 and 30 ^{0}C by Kasihmuddin *et al.* (2021); Basita and Rajis (2021) and Omike *et al*. (2020). The dissolved oxygen reported in the current study agreed with the assertion of Ott (2023) and Basuki *et al.* (2018) that dissolved oxygen values should be greater than 3mg/l in water used for Catfish culture and Basita and Rajis (2021) who recommended a value not less than 3mg/l. The pH value noted in the current study was in conformity with the value reported by Omoike *et al*. (2020) and was within the range of 6.5 – 8.5 recommended by Basita and Rajis (2021). Since, conductivity is a measure of the quality of water, the results of the conductivity of water reported here in our study was higher than the value (150 - 500µs/cm) noted by Russel *et al.* (2011) but was within the range of values (100-2000µs/cm) reported by Stone *et al.* (2013). This indicates that the water was of good and adequate quality for the fish samples.

Conclusion and Recommendation

In the present study, earthworm meal was used partially in place of fishmeal in the diet of *Clarias gariepinus*. The results showed that, when compared to other diets, the inclusion of earthworm meal at a rate of 50% produced the highest growth performance and rate of return on capital. Backed by evidences of other reported studies with similar results, farmers can be encouraged to explore the utilization of earthworm meal inclusion in the diets of *Clarias gariepinus* up to 50% as it holds a potential to reduce cost of production with better growth performance of fish.

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