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The Effect of Partial Replacement of Fishmeal with *Citrullus lanatus* and *Moringa oleifera* Seed Meals on Growth Performance of *Clarias gariepinus* (Burchell, 1822) Juveniles

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Authors' contributions

This work was carried out in collaboration between both authors. Author FEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FE managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Four experimental diets were formulated to contain *Citrullus lanatus, Moringa oleifera* seed meal, *Citrullus lanatus* and *Moringa oleifera* seed meals as partial replacement for fishmeal (Treatment 2, 3 and 4 respectively) and Treatment 1 as control, which included fishmeal and soy bean meal as the protein sources. The fed were to one hundred and twenty (120) African catfish (*Clarias gariepinus*) of mean weight of 25±5.0 g and mean length of 14±2.0 cm in replicates for 10 weeks growth period. The results obtained showed that fish fed treatment 3 had highest mean weigh gain. More so, treatment 1 had the highest percentage weight gain and specific growth rate. Treatment 4 had the highest food conversion ratio and treatment 1 had the highest length increase. The result of the economic analysis of the four treatments showed the treatment 4 and 3 are the cheapest while treatment 1 is the most expensive. All the treatment diets showed that growth due to the

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different protein sources were not significant (p>0.05). The result of this study therefore revealed that partial replacement of fishmeal with *Moringa oleifera* seed meal can be used in the diet of *Clarias gariepinus* juveniles.

Keywords: Citrullus lanatus; Moringa oleifera; Clarias gariepinus; diets.

1. INTRODUCTION

Aquaculture production is a major industry in many countries, and it will continue to grow as the demand for fisheries products increases and the supply from natural sources decreases. Nutrition plays a critical role in intensive aquaculture because it influences not only production costs but also fish growth, health and waste production [1].

Aquaculture production is becoming more and more intensive because production from capture fisheries has reached its maximum possible potential, as the catch is dwindling with each passing day [2]. Jamiu and Ayinla [3] had stated that feed management determines the viability of aquaculture as it accounts for at least 60 percent of the cost of fish production [4], therefore the establishment of economically viable fish culture ventures requires the incorporation of agricultural wastes or by-products as feed ingredients or direct feed [5] to replace conventional feed stuffs whose dwindling supply has resulted into arbitrary hike in prices. Conventional ingredients used in fish feed are in high demand for human consumption and their yield are currently being affected by climate change, hence out of concern for and the implications for food security as well as water and land use, there is urgent need to get local materials especially agricultural by-products of lower price to replace these costly feed materials.

The importance of artificial feeding in aquaculture cannot be over-emphasized. It promotes faster growth, allows higher stocking density and shorter cultivation periods. However, the unavailability and affordability of adequate fish feed has significantly affected the development of aquaculture in Nigeria. A well prepared and carefully formulated fish feed plays a significant role in fish culture [6]. The higher cost and competition imposed on some feed ingredients, such as soybean, groundnut cake, maize and sorghum used by human population as food, have necessitated the use of unconventional material for fish feed formulation. The price of finished feed in our part of the world continues to be on the rise, thereby removing the margin of profit accruing to the fish producers [7]. Thus, overcoming the burden of feed ingredients and reducing the cost of fish feed and fish products have been the burden of numerous researchers [8]. In fish farming, nutrition is critical because feed represents 40-50% of the production cost [9].

With the ever increasing cost of fishmeal in the tropics, [10] noted that several researchers have attempted to incorporate plant protein into formulated fish diet in order to reduce the quantity and cost of fishmeal without reducing the quality of the feed. According to [11] fishmeal contains some amino acids and fatty acids that are not present in tissues from terrestrial plants. The most limiting amino acids in plant protein are lysine and methionine [12,13].

Citrullus lanatus seed meal is one of such agricultural by-product whose nutritive potential has not been effectively tapped in animal nutrition [14]. *Citrullus lanatus* seed is rich in minerals, protein, vitamins, carbohydrate and fibre [15,16]. Watermelon seeds are rich in oil and protein [17,18]. *Citrullus lanatus* seed oil proved to be good source of high quality edible oil characterized by low free fatty acid content [17]. The experience with *Citrullus lanatus* seed cake or meal in rations for animals showed that it is a good source of digestible protein comparable to other oil seed cakes like cottonseed, linseed etc [19] hence can be safely incorporated in animal feeds [20].

Moringa oleifera ,which is one of the most widely cultivated species of the genius *Moringa* serves an example of nutritious vegetable tree with a variety of potential uses [21]. The immature green pods called “Drumstick” are probably the most valued and widely used part of the tree. The seeds are sometimes removed from more mature pods and eaten like peas or roasted like nuts. The flowers are edible when cooked and are said to taste like mushrooms. The roots are shredded and used as condiment in the same way as horseradish.

In view of the increasing demand for fish and high cost of conventional feed ingredients, it is

therefore necessary to investigate the effect of partial replacement of fish meal with *Citrullus lanatus* and *Moringa oleifera* seeds meals in the diets of *Clarias gariepinus* juveniles.

2. MATERIALS AND METHODS

The juveniles of *Clarias gariepinus* for this research were obtained from Arazu's fish farm, Awka, Anambra State. The experiment which lasted for 70days was carried out also in 75 liters plastic tanks at Arazu's fish farm.

2.1 Procurement and Preparation of Seed Plants and Conventional Feed Ingredients for Feed Formulation

Fishmeal, soybean, corn meal, wheat bran, vitamin and mineral premix, vegetable oil, salt and starch binder were procured from "Afor-Nnobi" market in Nnobi, Anambra state.

2.2 Preparation of *Citrullus lanatus* Seeds

Citrullus lanatus seeds were procured from large quantities of rotten or spoilt matured watermelon fruits, and were identified by a botanist. The seeds were soaked for twelve (12) hours to separate the seed nut from the fruit flesh [22]. The seed were sun dried, packaged in polythene bags and stored for processing. The dried seed were grounded into powder using a melon mill (Henry West GX 200). The milled watermelon seeds were then sieved with a hand sieved to separate the fine powder from the remaining fibre. The processed seeds were analyzed for proximate composition using the method described by the [23], to determine the crude protein, carbohydrate, fibre, fat, ash, dry matter, moisture content and Nitrogen free extract.

2.3 Preparation of *Moringa oleifera* Seed for Feed Formulation

Moringa oleifera seeds were collected from Polycarp farm in Onitsha, Anambra state where it is commonly grown. The seeds were identified by a botanist. The seeds were shelled manually to obtain the cream coloured kernels. *Moringa* kernels were properly sorted out to remove stones and other dirt's. The clean kernels were grounded into powder using a melon mill (Henry West GX 200). The milled *Moringa* kernel seeds were also analyzed for proximate composition using the method described by the [23], to determine the crude protein, carbohydrate, fibre, fat, ash, dry matter, moisture content and Nitrogen free extract.

2.4 Formulation of Experimental Diets

The Experimental diets were formulated at Ezelagbo farms, Nise, Anambra State. Four diets were formulated. The percentage composition for each of the four diets is shown in Table 1. The experimental diets with crude protein levels of 40% were formulated using the Pearson square method. The individual ingredients were weighed using an electronic weighing balance (Citizen 5000MP) and then thoroughly mixed together in a bowl by hand. Water was added to the premixed ingredients and homogenized to a dough-like paste. The diets were then pelletised using a 3-mm electronic pelletizer (Model KL120B). The diets were sun-dried and stored in airtight plastic containers throughout the experimental period [2,24].

2.5 Experimental Design

The experimental design used, was Completely Randomized Design (CRD). In this experiment, a total of 120 juvenile fish were used. After the acclimatization period which lasted for one week, fish were weighed using an electronic weighing balance (Citizen 5000MP) and homogenous sizes were randomly distributed in twelve (12) experimental plastic tanks with each tank containing ten juveniles of *Clarias gariepinus*. The fish were randomly assigned to four treatment diets of T_1 – Fishmeal (control), T_2 - Fishmeal and *Citrullus lanatus* seed meal, T_3 - Fishmeal and *Moringa oleifera* seed meal and T_4 - Fishmeal, *Citrullus lanatus* and *Moringa oleifera* seed meal. Each treatment had three replicates. The experimental fish were fed twice daily (8.00AM and 4.00PM) at 5% body weight for ten weeks. The experimental tanks were thoroughly washed and water changed regularly at interval of three days throughout the experimental period. Water samples from the experimental tanks were collected and some physico-chemical parameters of the water analysed. These include; temperature, pH and dissolved oxygen (DO) using water sample kit (mercury in glass thermometer, pHep pocket-sized digital pH meter and Eutech Dissolve oxygen meter (model MW600) respectively). The juveniles were weighed every week to determine weight gain, and feed quantity given was adjusted accordingly.

Table 1. Percentage composition of the experimental diets

Ingredients	Treatment 1 (Control)	Treatment 2 (F+C)	Treatment 3 (F+M)	Treatment 4 (F+C+M)
Fishmeal	40	30	30	20
Soybean	15	15	15	15
Citrullus lanatus	0	10	0	10
Moringa oleifera seed meal	0	0	10	10
Corn meal	20	20	20	20
Wheat flour	10	10	10	10
Wheat bran	10	10	10	10
Vit|Min Premix	2	2	2	2
Vegetable oil	2	2	2	2
Salt	1	1	1	1
Total (%)	100	100	100	100

**Keys: % (percentage), F (Fishmeal), C (Citrullus lanatus) and M (Moringa oleifera seed meal)*

2.6 The Proximate Analysis of the Ingredients and Diets

The feed used were analyzed for proximate composition at SMO Laboratory consult, Ibadan. Nigeria, using the methods of the Association of Official Analytical Chemists (AOAC, 2000) for crude protein (CP), moisture content, crude fibre, fat, ash and Carbohydrate.

2.7 Indices of Growth and Feed Utilization Measured

A Citizen 5000 MP electronic weighing balance was used to measure weights of juveniles per week till the end of the experiment (10 weeks), growth performance were estimated.

2.8 Statistical Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using Computer SPSS Version 22. Differences in means were evaluated at 5 percent (0.05) level of significance and direction of interaction between treatments was analyzed using Duncan HSD Post Hoc Test.

3. RESULTS AND DISCUSSION

The results of the growth indices (Mean Weight Gain, Percentage Weight Gain, Specific Growth Rate, Food Conversion Ratio, and Mean Length Increase) of *Clarias gariepinus* juveniles fed on four dietary treatments showed that there was no significant difference *(P>0.05)* in the fish growth.

The mean weight gain of the fish in the four treatments (T_1-18.36±1.86, T_2-17.21±3.3.4, T_3-21.32±1.96 and T_4-18.39±0.37) revealed that T_3 (21.32±1.96) had highest mean weigh gain however not significantly different *(P>0.05)* from others. The increased mean weight gain in T_3 (Fishmeal and *Moringa* seed) could be attributed to the high crude protein content of the feed type which is higher than T_1 (control diet) containing fishmeal. This agrees with the report of [25] that T_2 (Fishmeal and *Citrullus lanatus* seeds) had the lowest mean weight gain (17.21±3.34). [26], concluded that the inclusion level of 10% raw *Citrullus lanatus* seed meal in the diet of common carp is suggested to enhance growth performance and nutrient utilization. Although, [27] reported that *Citrullus lanatus* seed meal can be used as partial replacement for soybean in the practical diets of *Clarias gariepinus* up to 60%. He however suggested that the presence of anti-nutritional factors in plant ingredients could also be pointed at as a factor causing reduced growth rate and nutrient utilization by fish. Hence, the poor growth recorded in fish fed with *Citrullus lanatus* seed meal may be caused by the presence of these anti-nutritional factors [28].

Table 2. Water quality parameters

Parameters	Mean values
Temperature (°C)	30.18±0.21[a]
pH	6.03±0.13[a]
Dissolve oxygen (mg/l)	4.42±0.10[a]

Mean values with the same superscript in the same column are not significantly different at (P>0.05)

This result was in line with the findings of [29] who stated that growth is influenced through better food conversion and not just through stimulated feed intake. This indicates that fish which consumed more food, utilizes it efficiently

unlike the fish that consumed less food. Therefore, food consumption and not food conversion efficiency is a growth-limiting factor [30].

The mean Length Increase of the fish exposed to the four treatments (T_1- 4.63±0.12, T_2- 3.70±0.42, T_3- 3.67±0.63 and T_4- 3.30±0.23) revealed that T_2 (3.70±0.42) had highest mean Length Increase while the least was recorded by those fed on T_2 (T_4- 3.30±0.23). The mean Length Increase was not significantly different *(P>0.05)* among the four feed types. They all portray good length increase.

The mean percentage mean weight gain of the fish in the four treatments (T_1-89.99±2.5, T_2-97.99±3.0, T_3-76.43±2.3 and T_4-67.21±2.2) revealed that T_2 (97.99±3.0) had highest mean percentage weigh gain while the least was recorded by those fed on T_4 (67.21±2.2). The mean percentage weight gain was not significantly different *(P>0.05)* among the four feed types. They all portray good weight increase.

The result of the specific growth rate (weekly) revealed that there was no significant difference *(P>0.05)* in the specific growth rate.

Table 3. Proximate composition of *Citrullus lanatus* and *Moringa oleifera* seeds used in formulation of experimental diets

Nutrients	*Citrullus lanatus* (%)	*Moringa oleifera* (%)
Crude protein	19.98	20.39
Crude fat	3.75	3.68
Crude fibre	3.89	3.82
Ash	6.63	6.65
Moisture	9.61	9.59
Dry matter	90.39	90.41
N.F.E	56.14	55.97

**Key: N.F.E = Nitrogen Free Extract, % = percentage*

Table 4. The Proximate compositions of the experimental diets of *Citrullus lanatus* and *Moringa oleifera* dry seeds in percentage (%)

Parameters	T_1 (Control)	T_2 (F+C)	T_3 (F+M)	T_4 (F+C+M)
Crude protein	20.18	19.89	20.26	20.58
Crude fat	3.59	3.65	3.61	3.57
Crude fibre	3.76	3.84	3.79	3.86
Ash	6.48	6.59	6.45	6.67
Moisture	9.46	9.55	9.41	9.63
Dry matter	90.54	90.45	90.59	90.37
N.F.E	56.53	56.48	56.47	55.69

**Key: N.F.E = Nitrogen Free Extract, T_1 = (Fishmeal as control), T_2 = F+C (Fishmeal and Citrullus lanatus seed meal), T_3 = F+M (Fishmeal and Moringa oleifera seed meal) and T_4 = F+C+M (Fishmeal, Citrullus lanatus and Moringa oleifera seed meal)*

Table 5. Growth performance and feed utilization indices of *Clarias gariepinus* fed *Citrullus lanatus* and *Moringa oleifera* seed meal as partial replacement for fishmeal

	T_1	T_2	T_3	T_4
MIW	$20.40±2.0^a$	$17.57±2.44^b$	$27.90±1.86^a$	$27.37±3.27^a$
MFW	$38.76±2.8^b$	$34.78±2.0^c$	$49.22±2.28^a$	$45.76±3.0^a$
MWG	$18.36±1.86^a$	$17.21±3.44^a$	$21.32±1.96^a$	$18.39±0.37^a$
PWG	$89.99±2.5^a$	$97.99±3.0^a$	$76.43±2.3^a$	$67.21±2.2^a$
SGR	$0.68±0.8^a$	$0.81±0.1^a$	$0.41±0.4^a$	$0.43±0.2^a$
FCR	$0.107±0.08^a$	$0.106±0.12^a$	$0.116±0.01^a$	$0.125±0.03^a$
MLI	$4.63±0.12^a$	$3.70±0.42^a$	$3.67±0.63^a$	$3.30±0.23^a$
SURVIVAL (%)	100±0.00	100±0.00	100±0.00	100±0.00

Mean values with the same superscript in the same column are not significantly different at (P>0.05).
Keys: MIW: Mean Initial Weight. MFW: Mean Final Weight. MWG: Mean Weight Gain.
PWG: Percentage Weight Gain. SGR: Specific Growth Rate. FCR: Food Conversion Ratio
M LI: Mean Length Increase. %: Percentage

The mean food conversion ratio of the fish exposed to the four treatments (T_1-0.107±0.8, T_2-0.106±0.1, T_3-0.116±0.1 and T_4-0.125±0.03) revealed that T_4-0.125±0.03 had highest mean food conversion ratio while the least was recorded by those fed on T_2 (0.106±0.1). The mean food conversion ratio was not significantly different *(P>0.05)* among the four feed types. These showed they were able to convert their feed well into tissues and muscles which gave them good growth.

The results obtained from the water quality analysis showed that Temperature and Dissolve oxygen met the standard values for tropical fish culture as postulated by [31]. However, the mean value of pH was slightly lower than the standard value. No significant difference was recorded in the various parameters, suggesting that the fish can be reared in these water conditions comfortably.

The results obtained from the proximate analysis of *Citrullus lanatus* and *Moringa oleifera* seeds showed that the values of crude protein (CP) were 19.98% and 20.39% respectively. The crude protein value of *Citrullus lanatus* in this study was observed to be relatively lower when compared with the findings of [32] 24.4%, [33] 23.37%, [34] 21.46% and [35] 23.4% respectively. The differences could be in the environmental factors such as season, geographical location and stage of maturity which play a minor role in determining nutritive value of *Citrullus lanatus* seeds meal [35]. The result obtained on crude protein value of *Citrullus lanatus* seed obtained from the study disagreed with the work of [36] who reported that *Citrullus lanatus* seeds, decorticated contained up to 34.5% crude protein content.

The result on crude protein value of *Moringa oleifera* seeds obtained from the study (20.39%) agrees with the findings of [37] who reported a range of 15.8 – 20.7% for protein content of Moringa (*Moringa oleifera*), pods. The value obtained was higher than the crude protein of raw *Moringa oleifera* seed flour (18.86%) and defatted *Moringa oleifera* seeds (17.13%) as reported by [38] and [39]. The value was however lower than raw *Moringa oleifera* seeds (35.97%), germinating and fermented *Moringa oleifera* seed flour (23.69% and 21.15%) as reported by [39]. The increased protein value of germinating and fermenting seed flour could be attributed to the biochemical activities of the germinating seeds and the activities of the microorganisms during fermentation [38]. Foyd et al. [11] reported that Moringa seeds are good sources of fats, proteins, and crude fibers.

The results of the crude fibre contents in *Citrullus lanatus* and *Moringa oleifera* seeds conform to the range of 3-6% necessary for the growth of African catfish growth [40].

The results obtained from the proximate analysis of the four experimental diets types (T, T_2, T_3 and T_4) compared favourably with the findings of [25] who stated that usually fish growth will be directly proportional to the level of nutrient composition of its diet and if the level of crude protein ranges approximately within 20 to 40%. The results of the crude protein content of the four experimental diets were slightly lower than the recommendation of [41] who suggested that herbivorous and omnivorous fish require a diet with 25-35% crude protein. According to the report of [42], the nutrient requirement for growth, reproduction and normal physiological function of fish must be met, like other animals; but fish have much higher requirement for protein, so feed mixture with 25-45% of crude protein are mainly used.

4. CONCLUSION

This research provides more insight in enhancing better growth performance of *Clarias gariepinus* juvenile fed with *Citrullus lanatus* and *Moringa oleifera* seeds as partial replacement for fishmeal. The results showed that their inclusion in the diet of *Clarias gariepinus* did not significantly increase the growth performance. However, partial replacement of fishmeal with *Moringa oleifera* seed meal gave a better growth performance and it's also cost effective when compared to fishmeal which is usually very expensive. Therefore, it can be included in the diet of *Clarias gariepinus* as this will boost the protein content and other nutrients present in the diet.

ETHICAL DISCLAIMER

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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