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Nutrient Digestibility of Non-conventional Protein Sources Blend in African Catfish, *Clarias gariepinus* (Burchell, 1822) Post Juveniles Using Novel Aquafeed Formulation Software

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, the growth performance, carcass composition, and apparent digestibility coefficient of African catfish, Clarias gariepinus, were evaluated in relation to different non-conventional protein sources blends diets that were formulated using a novel feed formulation software (FUTA AQUAFEED). Diet 1 served as the control diet, and the other four experimental diets (Diets 2 through 5) included non-conventional protein sources such blood meal, water hyacinth, palm kernel cake, palm beetle meal, blackfly meal, water fern, and Moringa. The gross composition of the

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experimental feeds exhibited notable variations in nutrient content. The crude protein content in the diets were iso-nitrogenous. Analysis of the composition of the carcass showed that diets varied significantly. Diet 5 had the highest fat content (10.66%), while Diet 3 had the highest crude protein amount (61.57%). Growth performance parameters such as initial and final weights, weight increase, feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) exhibited diverse patterns. Diet 5 produced the greatest final weight (324.00 ± 122.94 g) and weight gain (173.32 ± 96.06 g), suggesting that the varied protein blend may have advantages. Dietary differences were seen in the apparent digestibility coefficients of crude protein and lipids. The crude protein digestibility coefficient (90.68%) was best in Diet 3, while the crude lipid digestibility coefficient (92.00%) was highest in Diet 1. In conclusion, the inclusion of unconventional protein sources in the diets of C. gariepinus affects both their growth performance and nutritional makeup. Through the incorporation of alternate protein sources into fish diets, the findings offer insights for improving aquaculture techniques and advancing sustainability.

Keywords: Aquafeed; blends; non- conventional; formulation; nutrients.

1. INTRODUCTION

Aquaculture growth has often occurred at the expense of the environment. Sustainable aquaculture development remains critical to supply the growing demand for aquatic foods. Rising incomes and urbanization, improvements in post-harvest practices and changes in dietary trends are projected to drive a 15 percent increase in aquatic food consumption. Consumption of fish provides important nutrients to a large number of people worldwide and thus makes a very significant contribution to nutrition (Fasakin and Aberejo, 2002). The rearing of African catfish started in the early 70s in central and western African countries. It received wide acceptance when it was realized to be a very suitable species for aquaculture and of high economic value. It has since been the most widely cultured fish in Nigeria and even in Africa. It matures quickly and has a wide range of tolerance to climatic conditions.

Aquaculture depends on common input ingredients such as, fishmeal and soybean, for which it competes in the marketplace with the animal husbandry sector, as well as with direct human consumption. Furthermore, many of the key ingredients traditionally used in formulating feed for commercial or on-farm aquaculture feeds are internationally traded commodities. Reduction in inclusion level of these conventional protein sources, especially fishmeal, will therefore be important to reduce feed costs and avoid competition with other users. Numerous investigations on substitute components in fish diets have been done over the years due to the shortage of fishmeal and the rising production of aqua-feeds [1]. Due to its balanced amino acid (AA) profile, great digestibility, and delectable flavour, the vast majority of these investigations have confirmed the validity of fishmeal as the most acceptable protein source for fish (Hardy, 2010).

Investigating the digestibility values of these ingredients is an essential step in formulating balanced practical diets. The quality of a feed ingredient depends on its digestible amino acid profile, protein, and energy when it comes to feed composition (Fagbenro, 1996, Fagbenro, [1].

Reliable information on the digestible AA content of these various components for each species is believed to be an essential requirement because the majority of feed formulation is focused on the protein content. Compared to other farmed fish species like rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), and Atlantic salmon (*Salmo salar*), information on the AA digestibility of feed components for African catfish (*Clarias gariepinus*), is particularly scarce [2].

2. MATERIALS AND METHODS

2.1 Experimental Design

Completely randomized design was used for this study. This experiment lasted for twelve weeks (3 months). A total number of 225 African catfish, *Clarias gariepinus* [3] post juveniles were used and evenly distributed, forty-five (45) per treatment. The test organisms were not fed for 24 hours before the commencement of the feeding trial. The initial mean weight and length were measured with the aid of a digital weighing balance and metre rule respectively. At the end of the experiment, individual weights of all

surviving fish from all the treatments were measured to obtain their final mean weight after evacuation of feed by starving the fish for 24 hours. The composition of ingredients is shown in Table 1.

2.2 Moisture Content

This is the removal of water from the fish and diet samples and it is measured a loss of weight or the amount of water removed. It was determined by using a moisture extraction oven (Gallenkamp). The dishes with their content was removed from the oven, cooled in the desiccators for 30minutes, weighed and recorded as W3.

$$\%\ Moisture\ content = \frac{W2 - W3}{W2 - W1}\ X\ 100$$

2.3 Ash Content

The ash content was determined by bashing the samples. Crucibles were prepared, oven-dried for 30minutes, cooled in the desiccator for 30 minutes and weighed as weight one (W1). One gram (1g) of the sample was put into clean dried pre-weighed crucibles with a lid (W2).. Ashing of the samples continued until a light grey white ash is obtained. The crucibles were then cooled in the desiccator and weighed (W3).

$$\%\ Ash\ content = \frac{W3 - W1}{W2 - W1}\ X\ 100$$

2.4 Ether Extract

The lipid extract was done by soxhlet extraction. About 150ml of anhydrous diethyl ether at a boiling point of 40-60°c was placed in a flask. Fifteen (15) filter papers were placed in the oven and cooled in the desiccator for 30 minutes each. The samples were then transferred into the oven for thirty minutes (30 min) after which they were transferred into the desiccator to cool for another thirty minutes (30 min) and then weighed as weight three (W3).

$$\%\ \text{Lipid content} = \frac{W2 - W3}{W2 - W1}\ X\ 100$$

2.5 Crude Fibre

The crude fibre content of the samples was determined by putting ether extract residue into one litre (1litre) conical flask. A portion (1.5g) of the sample was weighed into the flask and denoted as W1 followed by the addition of 200ml of boiling 1.25% of H²SO4. The solution was boiled gently for 30 minutes maintained at a constant volume. Percentage fibre was then calculated using the formula below:

$$\%\ Fibre = \frac{W2 - W3}{W1}\ X\ 100$$

2.6 Crude Protein

Micro Kjeldahl apparatus (AOAC, 1990 and 2019) was used to determine the crude protein content of the fish and the diet samples in a three (3) stage process namely: digestion, distillation and titration. The protein content was calculated using this formula:

$$\%\ Nitrogen = \frac{Titre\ value\ x\ 0.1HCl\ x\ 0.014\ x\ 100\ 10}{weight\ of\ sample\ (g)}$$

% crude protein = % nitrogen × 6.25

2.7 Nitrogen Free Extract (NFE)

This is an approximate carbohydrate available in the diet and fish respectively. It was determined by the subtraction method as shown below:

%NFE = 100 – moisture + crude protein + crude lipid + crude fibre + ash

2.8 Growth Performance and Nutrient Utilization Parameters

Growth is often reported in fish nutrition as absolute (gain per day), relative (percentage increase in size) or specific growth rate (percentage increase in size per day). The following parameters are widely used in fish nutrition research to monitor growth performance and nutrient utilization in fish.

Feed Intake (g/day): Total amount of food consumed by fish in grams X Number of days.

Weight Gain: Final weight - Initial weight.

Specific Growth Rate

$$\text{SGR}(\%\text{days}) = \frac{(FW_{\log e} - IW_{\log e})}{T}$$ ...Brown (1957)

Table 1. Gross composition of the experimental diet (g/100g) for culturing *C. gariepinus*

Feed Stuffs	Diet 1 (CONTROL)	Diet 2	Diet 3	Diet 4	Diet 5
Maize	11	17	10	10	10
Soyabean meal	10.9	-	-	-	-
Groundnut cake	13	14.5	-	-	-
Fishmeal	55.1	-	-	-	-
soyabean oil	5	6	6	6	6
Alginate	2	2	2	2	2
Vitamin premix	2	2	2	2	2
Water fern	-	-	24	-	-
Water hyacinth	-	-	23	10	-
Blood meal	-	40.5	32	-	-
Palm kernel cake	-	17	-	-	-
Palm beetle meal	-	-	-	53	61.5
Blackfly meal	-	-	-	-	9
Moringa	-	-	-	-	8.5
Poultry intestines	-	-	-	16	-
Methionine	-	0.5	0.5	0.5	0.5
Lysine	-	0.5	0.5	0.5	0.5

**Vitamins supplied mg/100 g diet: thiamine (B_1), 2.5 mg; riboflavin (B_2), 2.5 mg; pyridoxine (B_6), 2.0 mg; pantothenic acid, 5.0 mg; inositol, 3 mg; biotin, 0.3 mg; folic acid, 0.75 mg; para-amino benzoic acid, 2.5 mg; choline, 200 mg; niacin, 10.0 mg; cyanocobalamin (B_2), 10.0 mg; ascorbic acid, 50.0 mg; menadione (K), 2.0 mg. Minerals: $CaHPO_4$, 727.8 mg; $MgSO_4$. 127.5 mg; NaCl, 60.0 mg; KCl, 50.0 mg; $FeSO_4$, 250 mg, $ZnSO_4$, 5.5 mg; Mn_4SO_4, 2.5 mg; $CuSO_4$, 0.79 mg; $CoSO_4$, 0.48 mg; $CaClO_3$, 0.3 mg; $CrCl_3$, 0.123 mg.*
Key: Diet 1 - 5 = Treatment 1 - 5, Treatment 1 = Control

Feed Conversion Ratio

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Feed Efficiency Ratio or Feed Conversion Efficiency

$$\text{FER} = \frac{\text{Weight gain}}{\text{Feed intake}}$$

2.9 Statistical Analysis

Statistical analyses were performed using SPSS (version 21) software. The data obtained from the study was analysed statistically using one-way analysis of variance (ANOVA) and the significant differences (P<0.05) among means was determined using a follow up procedure. Data was presented as mean ± SD. All the data were tested for normality (Kolmogorov- Smirnov test).

3. RESULTS

3.1 Proximate Composition of African catfish (*Clarias gariepinus*) Juveniles

Table 2. Shows the initial and final proximate analysis of fishes in each tank with different experimental feed, the analysis covered the moisture content (MC), the crude protein (CP), the Lipids, Ash and the NFE. There was no significant difference (P> 0.05) in the Crude protein (CP), Lipids and Moisture across the treatment groups. The highest ash content was recorded in T1 (18.27%) while the least was recorded in T3 (14.19%) which were significantly different (P>0.05). The NFE show the highest value recorded at T5 (5.49%) while the least value at T1 (4%).

3.2 Growth Performance of African catfish (*Clarias gariepinus)* Juveniles

Table 3. shows the result for the growth performance and nutrient utilization of *C. gariepinus* fed with experimental feed formulated with different non-conventional protein sources blend (water hyacinth, blood meal, water fern, poultry intestine, palm beetle, black fly meal, Moringa) for 30 days. The growth performance of *C. gariepinus* fed experimental feed formulated with non-conventional feed stuffs showed no significant differences (P>0.05) in the mean initial weight (MIW). At the end of the experiment the fish in the treatment 5 has the highest mean final weight (MFW) with the value of 324.0g while the treatment 2 has the lowest mean final weight (MFW) with value 248g. The Specific growth rate, ranges from 1.86 ± 0.40 to 3.08 ± 0.68 and was significantly different with the highest value recorded in T 4 and the lowest value was

recorded in T1 respectively. There were significant differences (P<0.05) in the FCR and the PER across the treatment groups. The lowest and best FCR was observed in the T1 (1.23 ± 0.21) while the highest was in T5 (2.41 ± 1.09). Finally, the highest value for the PER was recorded in T1 (3.43 ± 0.20) and the lowest value was in (2.78 ± 0.28).

3.3 Apparent Digestibility Coefficient of African catfish (*Clarias gariepinus)* Juveniles

Table 4. shows the result for the apparent digestibility coefficient of *C. gariepinus* fed with experimental feed formulated with different non-conventional protein sources blend (water hyacinth, blood meal, water fern, poultry intestine, palm beetle, black fly meal, Moringa) for 30 days. The apparent digestibility coefficient of *C. gariepinus* fed experimental feed formulated with non-conventional feed stuffs were significantly different (P>0.05) in the crude protein with the highest value recorded in T3 (90.68 ± 0.82) and the lowest value observed was in T5 (76.40 ± 1.88) [4-7]. There were significant difference (P>0.05) in the Crude Lipids across the treatment group. The highest value was recorded in T3 (93.05 ± 0.13) while the lowest value was recorded in T2 (92.00 ± 0.60).

4. DISCUSSION

4.1 Proximate Compositions of Experimental Diets in % Dry Matter

Protein content is a key nutritional parameter, essential for growth and development. The diet 2 stands out with the highest protein content at 45.37%, aligning with the observations of Robinson *et al*. (2020) on the importance of protein-rich diets in supporting optimal animal performance. Conversely, treatment 1 (control) (44.84%), with the lowest protein content among the diets, may necessitate supplementation to meet the protein requirements of the intended recipients [8,9].

Table 2. Carcass composition of *C. gariepinus* fed non-conventional protein sources blend

Parameter	Initial	T1(CONTROL)	T2	T3	T4	T5
CRUDE PROTEIN	58.73	60.71 ± 1.50^{a}	61.46 ± 2.14^{a}	61.57 ± 2.06^{a}	59.97 ± 0.90^{a}	61.02 ± 1.51^{a}
LIPIDS	12.65	9.63 ± 0.82^{a}	9.98 ± 0.97^{a}	10.20 ±0.33^{a}	10.09 ± 0.18^{a}	10.66 ± 1.12^{a}
MOISTURE	6.03	7.38 ± 0.13^{a}	7.72 ± 2.30^{a}	8.22 ± 0.54^{a}	8.06 ± 1.04^{a}	7.62 ± 1.29^{a}
ASH	16.24	18.27 ± 0.70^{b}	16.97 ± 0.96^{ab}	14.19 ± 1.97^{a}	16.08 ± 1.83^{ab}	15.22 ± 1.56^{a}
NFE	9.052	4.00 ± 0.76^{a}	3.87 ± 0.28^{a}	5.82 ±0.17^{b}	5.81 ± 0.63^{b}	5.49 ± 0.35^{b}

*Values with different superscript in the same row indicate significant difference at P<0.05. **Data presented are means and standard deviation (mean ± SD) for fifteen fish from three replicates (n = 15)*

Table 3. Growth performance of *C. gariepinus* fed non-conventional protein sources blend

Parameter	T1	T2	T3	T4	T5
MIW	126.20 ±8.44 ^{a}	130.29 ± 16.66 ^{a}	144.48 ± 23.88 ^{a}	106.50 ± 39.44 ^{a}	150.69 ± 35.86 ^{a}
MFW	220.45 ± 12.00 ^{a}	248.02 ± 43.77 ^{b}	301.99 ± 48.67^{c}	259.82 ± 43.80 ^{d}	324.00 ± 122.94 ^{e}
FI	76.21 ± 4.37 ^{a}	61.75 ± 6.15 ^{a}	82.96 ± 4.80 ^{a}	72.14 ± 19.64 ^{a}	69.08 ± 14.81 ^{a}
MWG	94.25 ± 19.81 ^{a}	117.72 ± 36.26 ^{b}	157.52 ± 62.41 ^{c}	153.31 ± 17.15 ^{d}	173.32 ± 96.06 e
FCR	1.23 ± 0.21 ^{a}	1.89 ± 0.42 ^{b}	1.92 ± 0.83 ^{b}	2.25 ± 0.78 ^{c}	2.41 ± 1.09 ^{c}
SGR	1.86 ± 0.40 ^{a}	2.13 ± 0.45 ^{b}	2.46 ± 0.97 ^{c}	3.08 ± 0.68 ^{d}	2.42 ± 0.86 ^{c}
PER	3.43 ± 0.20 ^{c}	2.78 ± 0.28 ^{a}	3.73 ± 0.22 ^{d}	3.25 ± 0.88 ^{c}	3.11 ± 0.67 ^{b}

*Values with different superscript in the same row indicate significant difference at P<0.05. **Data presented are means and standard deviation (mean ± SD) for fifteen fish from three replicates (n = 15)*
Key: MIW=Mean Initial Weight; MFW=Mean Final Weight; MWG=Mean weight Gain; SGR=Specific Growth Rate; MFI=Mean Feed Intake; FCR=Feed Conversion Ratio; PER=Protein Efficiency Ratio

Table 4. Apparent digestibility coefficient

Parameter	T1	T2	T3	T4	T5
Crude protein	88.84 ± 0.89 ^{d}	84.81 ± 0.54 ^{c}	90.68 ± 0.82 ^{d}	81.20 ± 2.24 ^{b}	76.40 ± 1.88 ^{a}
Crude lipids	92.00 ± 0.60 ^{a}	91.90 ± 0.45 ^{a}	93.05 ± 0.13 ^{b}	91.87 ± 0.47 ^{a}	92.08 ± 0.79 ^{ab}

*Values with different superscript in the same row indicate significant difference at P<0.05. **Data presented are means and standard deviation (mean ± SD) for fifteen fish from three replicates (n = 15)*

Fiber, a critical component influencing digestive health, varies significantly among the diets. Treatments 4 and 5 exhibit higher fibre content of 6.11% and 5.93%, as supported by the work of Anderson and Smith (2017), who highlight the positive impact of dietary fiber on digestive processes and overall gut health in animals. This aligns with the findings of Patel and Williams (2018), who underscore the significance of lipid-rich diets in meeting the energy demands of livestock. The inclusion of poultry intestine in treatment 4 may also contribute to a higher lipid content, aligning with the established role of lipids in energy provision and essential fatty acid intake for fish (Tocher, 2003).

4.2 Growth Performance

The growth performance analysis evaluates the effectiveness of unconventional protein sources in promoting fish growth. For final weight, diets 3 (144.48) and 5 (150.69) demonstrate the highest final weights. Diet 5 shows the highest weight gain (173.32). The non-significant differences (P>0.05) of the evaluated growth and nutrient utilization indices among the five treatments imply that African palm weevil larvae meal, black fly meal, Moringa can successfully replace the entire fishmeal portion of the fish diet. This study corroborates with the finding of Ogunji *et al.* (2006), who observed a better growth performance and nutrient utilization of *Clarias gariepinus* fed experimental diets containing Palm beetle meal over those solely fed on fish meal diets. Feed Conversion Ratio (FCR) Diets 3, 4, and 5 exhibit higher FCR values than the control diet with values (1.92 ± 0.83), (2.25 ± 0.78), (2.41 ± 1.09) respectively.

PER evaluates how efficiently dietary protein is converted into fish biomass. Higher PER values indicate better protein utilization. In this case, T3 has the highest PER at 3.73±0.22, suggesting that the blend of non-conventional protein sources in T3 led to the most efficient conversion of dietary protein into fish biomass. T1 and T5 also show relatively high PER values, indicating good protein efficiency. T4 has the lowest PER value, suggesting that the protein sources in T4 may be less efficient in promoting fish biomass production.

4.3 Apparent Digestibility Coefficient

The table shows the apparent digestibility coefficients for crude protein and crude lipids for 5 different treatments (T1-T5). Higher values indicate greater digestibility. For crude protein, digestibility was highest in T3 (90.68%) and lowest in T5 (76.40%). T1, T2 and T4 had intermediate digestibility values. Treatment 1 contains only conventional protein sources as the control diet. In treatment 2, blood meal was the only non-conventional protein source. In treatment 3, water hyacinth, water fern and blood meal were the non- conventional protein sources. Treatment 4 contains poultry intestine, palm beetle and water hyacinth. Treatment 5 contains black fly meal, palm beetle and Moringa.

The highest APDC for crude protein among all the ingredients was observed in water hyacinth, water fern and blood meal (90.68± 0.82) that showed significant difference (P<0.05) with all others ingredients except the control diet (88.84 ± 0.89). Black fly meal, Palm beetle and Moringa exhibited significantly (P<0.05) lowest APDC among all the feed and ingredients.

The highest APDC for crude lipids among all the ingredients was observed in water hyacinth, water fern and blood meal (93.05 ± 0.13) that showed significant difference (P<0.05) with all others ingredients except the treatment 5 (92.08 ± 0.79) fed the blend of black fly meal, palm beetle and Moringa. Blood meal in Treatment 2 exhibited significantly (P<0.05) lowest APDC among all the feed and ingredients [10,11].

5. CONCLUSION

Aquaculture professionals can optimize feed formulas, save production costs, and improve economic viability by assessing the nutritional digestibility of different substitute protein sources in African catfish. Conventional protein sources, like fishmeal, frequently come from stocks of fish that have been harvested in the wild. The balance of aquatic food chains can be disrupted and marine ecosystems damaged if these resources are overused. The findings suggest that the inclusion of specific unconventional protein sources in catfish diets can influence growth performance, feed efficiency, and nutrient utilization. Diets rich in water fern, water hyacinth, blood meal, Palm beetle meal, blackfly meal, and Moringa show promise in promoting favourable growth outcomes. Further investigations into the long-term effects, economic feasibility, and environmental sustainability of these diets are warranted. Additionally, the data underscores the importance of understanding the impact of unconventional feed ingredients on

catfish farming for optimizing production practices.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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