

UTILIZATION OF *NYMPHAEA LOTUS* AS PARTIAL REPLACEMENT FOR MAIZE IN THE DIET OF *CLARIAS GARIEPINUS* FINGERLINGS

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ABSTRACT

The use of Nymphaea lotus as energy replacement in the diet of Clarias gariepinus fingerlings was investigated in a 12-week experiment. Five isonitrogenous (40% crude protein) diet were formulated using dried Nymphaea lotus leaves at 0% (T1), 12.5% (T2), 25% (T3), 37.5% (T4) and 50% (T5) inclusion levels as partial substitutes for maize meal, with three replicates each. Growth and nutrient utilization, Haematological and serum biochemistry parameters were determined and the data were subjected to one-way analysis of variance. The results showed that fish fed with T1 recorded the highest mean weight gain (26.58±3.00g), which was not significantly ($p>0.05$) different from those of the other dietary treatments. The least growth indices were recorded in fish fed T5 (50%). There was no significant difference ($p>0.05$) among the values of Heterophil (HET), lymphocyte (LYM), and mean corpuscular haemoglobin concentration (MCHC) among the treatments. The serum biochemistry analysis showed that the highest value (14.10±1.83) of Total protein (TP) was observed in T5 which was not significantly different ($p>0.05$) from other treatment while the lowest value (9.70±0.60) of TP was observed in T2. The highest value (63.70±3.60) of Aspartate aminotransferase (AST), was recorded in T5 while the least value (30.30±1.50) was observed in T1. It can be concluded that dried N. lotus can only replace maize at 25% inclusion level in the diet of C. gariepinus.

Keywords: *Clarias gariepinus, growth indices, Nymphaea lotus*

INTRODUCTION

Aquaculture has grown geometrically in response to an increasing demand for fish as a source of animal protein globally (Akinrotimi *et al.*, 2007). The reason is that production of capture fisheries has attained its maximum potential possible, as the catch keeps declining every day (Gabriel *et al.*, 2007). Fish is nutritionally accepted as the cheapest and direct protein source and micro nutrients in diets of several millions of

Africans (Agbabiaka, 2010; Bene and Heck, 2005).

The major fish species cultured in the Nigerian aquaculture sector are Tilapia and Clarias. Inadequate and outrageous cost of quality fish feeds is a major hindrance to development of this sector. Feed accounts for a minimum of 60% of the total cost of fish production (Jamu and Ayinla, 2003) and it is a major factor that determines the viability and profitability of fish farming enterprise. Therefore, efforts to develop practical diets of

high quality at affordable and reduced cost for farmed fish using locally available feedstuffs is indispensable (Ayoola, 2010; Bamidele *et al.*, 2015; Fagbenro *et al.*, 2003; Omitoyin, 2005). The cost of maize, especially in developing countries like Nigeria, is quite high compared to other sources of energy used in livestock feed as a result of human competitive uses. This has made it essential to assess other non-conventional ingredients to substitute maize which is the major source of energy with cheaper carbohydrates (Olurin *et al.*, 2006).

Nymphaea lotus is one of the most abundant among aquatic flora in water bodies. The plant has wide-round leaves that float on the water surface, greenish in colour and the matured flower produces bulbs (Ezeonu *et al.*, 2017). It is an indigenous wild crop plant and belongs to herbaceous aquatic plant widely distributed in the stream, rivers, lakes, and ponds (Fulckar, 2005). It is a plant with perennial rhizomes or stock anchored with mud, floating or submerged leaves and solitary showy flower (Conard, 1905; Wasagu *et al.*, 2015). Though *N. lotus* is in abundant in our water bodies, Mohammed and Awodoyin (2008) stated that there is dearth of information on this plant. Mohammed *et al.*, (2012) studied *N. lotus* from Tatabu flood plain and revealed that they have some nutritional values that can be of immense benefit to livestock feed. The objective of this study was to evaluate the

effect of dried *Nymphaea lotus* as partial replacement for maize in the diets of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

A total of two hundred fingerlings of *Clarias gariepinus* with average body weight of 3.89 ± 0.13 g were obtained from a reputable hatchery and acclimatized to experimental condition for seven days prior to the feeding trial and were fed commercial feed. The fishes were starved for 24 hours in order to empty their stomach and prepare their appetite for the new feed. Fifteen (15) experimental tanks, each with water holding capacity of 50 liters ($49 \times 33.5 \times 33.5$ cm), were randomly allocated to five treatment diets, each treatment was replicated thrice in a completely randomized design at a stocking density of ten fish per replicate. The fishes were fed *ad libitum* for twelve weeks. The fishes were weighed every two weeks with an electronic sensitive scale (Model No-JLV, type Di).

Nymphaea lotus plants were harvested from a water reservoir in Ogun State, Nigeria. The whole parts of the plant were sun-dried for two weeks, and milled to powder to form *Nymphaea lotus* meal (NLM)

Five iso-nitrogenous and iso-caloric diets were formulated with NLM replacing maize at 0%, 12.5%, 25%, 37.5% and 50% inclusion levels. The gross composition of the experimental diets is shown in Table 1.

TABLE 1: GROSS COMPOSITION OF THE EXPERIMENTAL DIETS.

| Feed Ingredients | Diet 1 (control) | Diet 2 (12.5%) | Diet 3 (25%) | Diet 4 (37.5%) | Diet 5 (50%) |
|---------------------------|---------------------|-------------------|-----------------|-------------------|-----------------|
| Fishmeal | 18.61 | 18.52 | 18.42 | 18.33 | 18.22 |
| Soybean meal | 37.21 | 37.03 | 36.84 | 36.66 | 36.44 |
| GNC | 18.61 | 18.52 | 18.42 | 18.33 | 18.22 |
| Maize | 18.58 | 16.57 | 14.49 | 12.31 | 10.07 |
| <i>Nymphaea lotus</i> | - | 2.37 | 4.83 | 7.38 | 10.07 |
| Dicalciumphosphate | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Methionine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Lysine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Fish premix | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vegetable oil | 4.75 | 4.75 | 4.75 | 4.75 | 4.75 |
| Total | 100 | 100 | 100 | 100 | 100 |

Growth and nutrient utilization of *Clarias gariepinus* fed diets containing NLM was determined by calculating the following parameters as described in Bamidele *et al.*, (2015).

$$\text{Weight gain (w)} = \text{Final weight (W2)} - \text{Initial weight of fish (W1)};$$

Feed Conversion Ratio (FCR)

$$= \frac{\text{Total feed consumed by fish (g)}}{\text{Weight gain by fish (g)}}$$

Survival %

$$= \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish stocked}} \times 100$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{weight gain(g)}}{\text{feed intake (g)}}$$

Specific growth rate (SGR, % per day)

$$= \frac{(\text{Loge W2} - \text{Loge W1})}{(T2 - T1)} \times 100$$

Blood was drawn from the caudal vein through the lateral line of fish into ethylenediamine tetra-acetic acid (EDTA) bottles by the use of needle and syringe (1 ml). Packed Cell Volume (PCV) and Red

Blood Cell (RBC) were determined using the method described by Kelly, (1979). The method described by Schalm *et al.*, (1975) was used to determine White Blood Cell (WBC) and haemoglobin. The Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin were estimated using the formula described by Blaxhall and Daisley, (1973).

Data resulting from the experiment were subjected to one-way analyses of variance (ANOVA) using the IBM SPSS (version 20). Duncan's Multiple Range Test was used to compare differences among the means (Duncan 1955).

RESULTS

The proximate composition of dried *Nymphaea lotus* leaves and experimental diets are presented in Table 2. The result showed that *Nymphaea lotus* leaves contained 16.30 ± 0.01 % crude protein and fat content of 5.61 ± 0.13 %. The values of ash, crude fibre, moisture content and nitrogen free extract are 15.63 ± 0.01 %, 18.46 ± 0.01 %,

$8.17 \pm 0.01\%$ and $35.82 \pm 0.01\%$ respectively. There was no significant difference ($p > 0.05$) in the crude protein and crude lipid content of the diets.

The proximate composition of the experimental fishes in Table 3 showed that crude protein level in treatment 1 has the highest value of $21.84 \pm 0.90\%$ compared to other treatment, however there was no

significant difference ($p > 0.05$) between T1 and T2 but T1 was significantly different ($p < 0.05$) from other treatments. No significant difference ($p > 0.05$) was observed in the fat and ash contents of the fish. The highest value of moisture content (MC) of $76.67 \pm 1.65\%$ was recorded in treatment 4 while the least value of $70.30 \pm 1.35\%$ was recorded in treatment 1.

TABLE 2: THE PROXIMATE COMPOSITION OF *NYMPHAEA LOTUS* MEAL (NLM) AND EXPERIMENTAL DIETS FED TO *CLARIAS GARIEPINUS*

| Parameters (%) | NLM | T1 (0%) | T2(12.5%) | T3 (25%) | T4 (37.5%) | T5 (50%) |
|------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Crude protein | 16.30 ± 0.01 | 39.39 ± 0.07 | 40.15 ± 0.35 | 39.37 ± 0.53 | 39.57 ± 0.27 | 39.87 ± 0.15 |
| Moisture content | 8.17 ± 0.01 | 10.58 ± 0.01 | 10.70 ± 0.01 | 10.81 ± 0.01 | 10.35 ± 0.01 | 10.47 ± 0.02 |
| Fat content | 5.61 ± 0.13 | 7.19 ± 0.01 | 7.28 ± 0.02 | 6.12 ± 0.01 | 6.41 ± 0.02 | 6.66 ± 0.02 |
| Ash | 15.63 ± 0.01 | 13.89 ± 0.01 | 14.33 ± 0.01 | 15.89 ± 0.01 | 15.99 ± 0.01 | 16.21 ± 0.01 |
| Crude fibre | 18.46 ± 0.01 | 5.72 ± 0.01 | 5.99 ± 0.01 | 5.87 ± 0.15 | 6.13 ± 0.01 | 6.20 ± 0.01 |
| Nitrogen free extract | 35.82 ± 0.01 | 22.71 ± 0.01 | 21.54 ± 0.01 | 21.65 ± 0.01 | 21.56 ± 0.01 | 20.51 ± 0.01 |

TABLE 3: CARCASS COMPOSITIONS OF *CLARIAS GARIEPINUS* FED DIETS CONTAINING *NYMPHAEA LOTUS* MEAL

| | T1(control) | T2 (12.5%) | T3 (25%) | T4 (37.5%) | T5(50%) | Initial |
|--------------------------|--------------------|-----------------------|-----------------------|--------------------|-----------------------|-----------------------|
| Moisture (%) | 70.30 ± 1.35^b | 71.36 ± 1.76^{ab} | 73.85 ± 1.55^{ab} | 76.67 ± 1.65^a | 74.20 ± 1.83^{ab} | 75.89 ± 1.99^{ab} |
| Ash (%) | 6.04 ± 0.14 | 5.92 ± 0.31 | 5.94 ± 0.18 | 5.74 ± 0.47 | 5.65 ± 0.19 | 6.06 ± 0.28 |
| Fat Content | 5.82 ± 0.38 | 5.73 ± 0.13 | 5.64 ± 0.35 | 5.55 ± 0.32 | 5.09 ± 0.41 | 5.16 ± 0.23 |
| Crude Protein (%) | 21.84 ± 0.90^a | 19.44 ± 1.07^{ab} | 16.53 ± 1.16^{bc} | 14.77 ± 1.22^c | 14.43 ± 1.14^c | 14.31 ± 1.08^c |

Mean \pm S.E with the same superscripts along the rows were not significantly different ($p > 0.05$)

Table 4 showed the growth and nutrient utilization of *Clarias gariepinus* fed diets containing *N. lotus* meal at different level of inclusion. Initial weight (IW) has no significant difference among the treatments. The final weight (FW) showed that treatment 1 (control) has the highest final weight which was not significantly different ($p > 0.05$) from other treatments. Similar trend was observed in mean weight gain (MWG). Feed conversion ratio (FCR) ranged from

1.29 ± 0.02 in treatment 1 to 1.55 ± 0.23 in treatment 2. Feed Efficiency Ratio (FER) values were not significantly different ($p > 0.05$) among the treatments. The highest significant ($p < 0.05$) feed intake (FI) value of 38.49 ± 0.18 was observed in T2 while the least value of 30.28 ± 0.16 was observed in treatment 4.

Result of hematological analysis showed that there was no significant difference ($p > 0.05$) among the values of HET, lymphocyte

(LYM) and mean corpuscular haemoglobin concentration (MCHC) among the treatments. The highest value (28.20 ± 0.58) of packed cell volume (PVC) was recorded in fishes fed T1 while the lowest value (18.00 ± 0.31) of PCV was recorded in fishes fed diet T5. However, there were significant differences ($p < 0.05$) among the treatment. The highest significant ($p < 0.05$) value of haemoglobin (HB) of 9.60 ± 0.37 was observed in fishes fed diet T2 while the least value of 6.10 ± 0.19 was recorded in T5. The white blood cell (WBC) values decreases with increase in the inclusion of the test ingredient in the diet. However, there were no significant difference in the values of WBC recorded in T1, T2 and T3.

The serum biochemistry results presented in Table 6 showed that the highest value

(14.10 ± 1.83) of Total Protein (TP) was observed in fish fed diet T5 while the lowest value (9.70 ± 0.60) was observed in fish fed diet T2. However, there were no significant difference ($p > 0.05$) among the treatments. The same trend was observed for Alanine aminotransferase (ALT). The value observed in globulin (GLO) showed that fishes fed diet T5 has the highest significant ($p < 0.05$) value of 9.00 ± 0.57 while fishes fed diet T1 and T3 had the lowest value and were not significantly different ($p > 0.05$) from T2. The fishes fed diet T3 recorded the highest value of Albumin (ALB) while the lowest value was recorded in fishes fed diet T1. The highest value of Aspartate aminotransferase (AST), 63.70 ± 3.60 was recorded in fishes fed diet T5 while the least value of 30.30 ± 1.50 was recorded in T1.

TABLE 4: GROWTH AND NUTRIENT UTILIZATION OF *CLARIAS GARIEPINUS* FED DIETS CONTAINING *NYMPHAEA LOTUS*

| | T1(control) | T2 (12.5%) | T3 (25%) | T4 (37.5%) | T5(50%) |
|------------|--------------------|-----------------------|----------------------|--------------------|----------------------|
| IW | 3.93 ± 0.06 | 3.86 ± 0.14 | 3.75 ± 0.33 | 3.94 ± 0.23 | 3.92 ± 0.38 |
| FW | 30.51 ± 2.94 | 28.64 ± 3.43 | 28.35 ± 2.21 | 24.58 ± 2.95 | 24.45 ± 2.75 |
| MWG | 26.58 ± 3.00 | 24.78 ± 3.43 | 24.60 ± 2.03 | 20.64 ± 2.96 | 20.53 ± 2.78 |
| FCR | 1.29 ± 0.02 | 1.55 ± 0.23 | 1.50 ± 0.27 | 1.47 ± 0.38 | 1.53 ± 0.08 |
| SGR | 3.64 ± 0.15^a | 3.55 ± 0.15^{abc} | 3.62 ± 0.02^{ab} | 3.25 ± 0.11^c | 3.26 ± 0.03^{bc} |
| SR | 50.00 ± 3.73^c | 76.67 ± 3.05^a | 80.00 ± 0.00^a | 66.67 ± 3.05^b | 66.67 ± 2.05^b |
| FI | 34.17 ± 0.85^c | 38.49 ± 0.18^a | 36.83 ± 0.09^b | 30.28 ± 0.16^d | 31.49 ± 0.25^d |
| FER | 0.78 ± 0.21 | 0.64 ± 0.24 | 0.67 ± 0.02 | 0.68 ± 0.09 | 0.65 ± 0.22 |

Mean \pm S.E with the same superscripts along the rows were not significantly different ($p > 0.05$)

IW=Initial Weight; FW=Final Weight; MWG=Mean Weight Gain; FCR=Feed Conversion Ratio;

SGR = Specific growth rate; SR=Survival Rate; FI=Feed Intake; FER=Feed Efficiency Ratio.

TABLE 5: HAEMATOLOGICAL PARAMETERS OF *CLARIAS GARIEPINUS* FINGERLINGS FED DIFFERENT LEVELS OF *NYMPHAEA LOTUS* DIET.

| | T1(control) | T2 (12.5%) | T3 (25%) | T4 (37.5%) | T5 (50%) |
|---|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| PCV (%) | 28.20±0.58 ^a | 27.70±1.27 ^a | 25.30±0.46 ^b | 21.00±0.69 ^c | 18.00±0.31 ^e |
| HB (g/100ml) | 9.00±0.23 ^a | 9.60±0.37 ^a | 8.70±0.47 ^a | 7.13±0.36 ^b | 6.10±0.19 ^b |
| RBC (10 ³ mm ⁻³) | 3.60±0.02 ^a | 3.40±0.01 ^b | 2.80±0.02 ^c | 2.31±0.05 ^d | 1.82±0.05 ^e |
| WBC (10 ³ mm ⁻³) | 7.30±0.29 ^a | 7.10±0.17 ^a | 6.70±0.13 ^a | 5.30±0.26 ^b | 4.10±0.38 ^c |
| HET (%) | 36.00±0.00 | 33.00±0.00 | 32.00±0.00 | 32.00±0.00 | 37.00±0.00 |
| LYM (%) | 60.00±3.06 | 63.00±2.60 | 67.00±2.25 | 65.00±3.52 | 60.00±2.77 |
| MCV (fl) | 78.33±1.44 ^c | 81.47±2.66 ^b | 90.36±3.12 ^{ab} | 90.91±3.71 ^{ab} | 98.90±3.07 ^a |
| MCH (pg) | 25.00±0.87 ^c | 28.24±0.84 ^b | 31.07±1.24 ^{ab} | 30.87±1.05 ^{ab} | 33.52±0.96 ^a |
| MCHC (%) | 31.91±1.33 | 34.66±1.06 | 34.39±0.72 | 33.95±0.95 | 33.89±1.21 |

Mean ± S.E with the same superscripts along the rows were not significantly different (p>0.05)

PCV=Packed cell volume; WBC=white blood cell; RBC=red blood cell; Hb=haemoglobin; LYM=lymphocyte; MCHC=mean corpuscular haemoglobin concentration; MCH=mean corpuscular haemoglobin; MCV=mean corpuscular volume, HET = Heterophil

TABLE 6: MEASUREMENTS OF SERUM BIOCHEMISTRY PARAMETERS OF *CLARIAS GARIEPINUS* FINGERLINGS FED DIFFERENT LEVELS OF *NYMPHAEA LOTUS* DIET.

| | T1(control) | T2 (12.5%) | T3 (25%) | T4(37.5%) | T5(50%) |
|-----------|--------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| TP(g/dL) | 10.30±1.28 | 9.70±0.60 | 12.10±1.54 | 13.70±1.22 | 14.10±1.83 |
| GLO(g/dL) | 5.00±0.15 ^c | 5.40±0.11 ^c | 5.00±0.10 ^c | 7.40±0.12 ^b | 9.00±0.57 ^a |
| ALB(g/dL) | 5.23±0.12 ^b | 4.30±0.09 ^c | 7.10±0.51 ^a | 6.30±0.18 ^a | 5.10±0.19 ^{bc} |
| AST | 30.30±1.50 ^c | 31.40±1.21 ^c | 43.70±1.99 ^b | 50.40±3.27 ^b | 63.70±3.60 ^a |
| ALT | 17.30±1.33 ^{cd} | 14.00±0.84 ^d | 21.70±1.85 ^{bc} | 23.80±2.30 ^b | 37.40±2.82 ^a |
| CREAT | 0.50±0.01 ^b | 0.40±0.01 ^c | 0.80±0.01 ^a | 0.80±0.02 ^a | 0.50±0.01 ^b |

Mean ± S.E with the same superscripts along the rows were not significantly different (p>0.05)

TP=Total Protein; GLO=Globulin; ALB, Albumin; AST=Aspartate aminotransferase ALT=Alanine aminotransferase; CREAT=Creatinine.

DISCUSSION

The crude protein of *Nymphaea lotus* recorded in this study was a little lower than 19.54±0.78 reported by Mohammed *et al.* (2012) for *N. lotus* obtained from Tatabu flood plain, North-central, Nigeria. The reason might be due to the difference in nutrients availability in different locations. The crude protein content was high when compared to some other plant materials used

as non-conventional feed ingredients like water lettuce leaf meal (Adedokun *et al.*, 2017), *Chrysophyllum albidum* seeds (Jimoh *et al.*, 2014) and Tigernut meal (Agbabiaka *et al.*, 2012) but lower than that recorded for Moringa (*Moringa oleifera*) seed meal reported by Bamidele *et al.*, (2015), African star apple kernel meal (Agbabiaka *et al.*, 2016) and *Albizia lebbeck* leaf meal (Oyelere, 2015)

The best growth performance from the values of FW, MWG and FCR were recorded in fishes fed diet T1 (control) followed by T2 (12.5%). Similar results were also reported by Agbabiaka *et al.*, (2012) when maize was replaced with tiger-nut meal in which the control group has significantly higher value than the other dietary treatments in terms of growth performance. In addition, Jimoh *et al.*, (2014) also observed higher significant difference in the control when maize was replaced with *Chrysophyllum albidum* seed meal. The value of final mean weight recorded in fishes fed *N. lotus*-based diets were lower than that of fishes fed control diet. This may be attributed to the inherent anti-nutritional factors in plant-based feedstuffs (Agbabiaka *et al.*, 2016)

The least tissue protein was observed in the fishes fed with diet 5 (50% inclusion level). This has been previously observed by Alegbeleye, (2005), who stated that the presence of anti-nutritional stress factors essentially tannin were known to decrease protein retention in the body. The least survival rate was observed in the fishes fed with diet 1 (control) while the highest survival rate was observed in the fishes fed diet 3. The mortality was due to cannibalism which was primarily caused by uneven fish growths in the population and high growth variations between individuals that consumed less of the experimental feeds (Marimuthu *et al.*, 2011, Musa *et al.*, 2012). The quantity and frequency of feeding were not factors of cannibalism (Tarekegn, 2015).

There was a reduction in the red blood cells as the inclusion level of *N. lotus* increased. Das *et al.* (2004) reported that the concentration of blood plasma protein is an

indicator to general health condition of fish. Although, Abdali *et al.* (2011) reported that a reduction in plasma protein is an indicator of the effect of toxins in the kidney, spleen and liver. The increase in protein level with higher levels of the plant protein-based diets may be due to mobilization of protein to meet energy requirements and to sustain increased physiological activity (Martinez *et al.*, 2004). The increase in blood glucose level recorded in this study agrees with the findings of Kumar *et al.* (2010) who observed higher blood glucose concentration in fish exposed to plant protein-based diets. Other researchers with similar observations are Kikuchi *et al.*, (1994) and Kikuchi, (1999). Increase in the blood glucose concentration might result from an increase in plasma catecholamine and corticosteroid hormones (Pickering, 1981).

CONCLUSION

The result of the growth performance showed that *Nymphaea lotus* can be used up 25% inclusion level, because it competes favourably with the control (0% inclusion), with positive impact on growth performance, nutrient utilization and health status. The result obtained from this study revealed the fact that dried *Nymphaea lotus* based diet is a profitable alternative energy source for partial replacement of maize in feeding *C. gariepinus* fingerlings as the plant stuff is readily available in various water bodies and reduces cost of production.

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