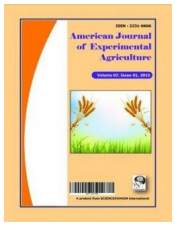

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# Effect of *Gliricidia sepium* (Jacq) Leaf Meal Supplemented with Enzymes (Roxazyme® G2 and Maxigrain®) on Growth Performance of *Clarias gariepinus* Burchell, 1822

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***Authors' contributions***

*This work was carried out by all the three authors. Authors OAO and MOO designed the study and wrote the first draft of the manuscript. Authors OAO and AAL reviewed the experimental design and all drafts of the manuscript. Authors MOO and AAL managed the analyses of the study. Author MOO identified the plants. Authors OAO, MOO and AAL performed the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

The growth performance of *Clarias gariepinus* Burchell, 1822 fed *Gliricidia sepium* leaf meal and supplement with enzymes (Roxazyme®) and Maxigrain® were examined. The fingerlings having weight of about 6.34±0.43 g were stocked in12 plastic aquaria with 50 liters capacity at the rate of 25 juvenile per aquarium and fed on four different dietary treatments for 42 days. The experimental diets varied with increasing levels of *G. sepium* leaf meal, 0%, 10% without enzyme supplementation, 10% supplemented with enzyme (Roxazyme G2), and 10% supplemented with enzyme (Maxigrain). The growth performance, Feed Conversion Ratio (FCE) and water quality were evaluated during and by the end of trial. The results showed that all experimental treatments promoted positive growth and no mortality was encountered during the experimental period.

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However, theFinal Body Weight (FBW) of *C. gariepinus* was found to differ significantly among fed experimental treatments. *C. gariepinus* fed with T4 had the highest significant (P< 0.05) FBW (14.15 g), followed by T3 (12.58 g) and T1 (12.10 g) whereas least FBW was recorded in *C. gariepinus* fed T2 (10.49 g). *C. gariepinus* fed with T4 had higher WG which was found to be significantly different from other treatments while no significant difference (P> 0.05) in WG was found amongT1 and T3 and the least WG was recorded in *C. gariepinus* fed T2. *C. gariepinus* fed T2 had the highest significant (P<0.05) value of FCR followed by T1 and T3 while the least significant (P<0.05) value FCR was recorded in *C. gariepinus* fed T4. Mean temperature, pH and dissolved oxygen (DO) ranges from 26.1°C to 28.10C, 6.89 to 8.13 and 1.9 mg/l to 8.13 respectively while the ammonia value varied from 0.21 mg/l to 0.67 mg/l. Therefore, it can be concluded that *G. sepium* can be used up to 10% supplement with enzyme in the diets of *C. gariepinus* at fingerlings stage with good performance with better quality of water.

*Keywords: Gliricidia sepium; Clarias gariepinus; growth; water quality; Maxigrain® and Roxazyme®.*

## 1. INTRODUCTION

The major problem being faced by developing countries especially Nigeria is the ever increasing population without a corresponding increase in animal protein production. Animal products contribute only 15 to 20% of the total protein intake of an average Nigerian contrary to 33% recommended by [1]. This wide margin between the recommended protein intake and the average consumption rate has resulted in an ever-increasing demand for animal protein by the Nigerian populace. The increasing demand for required protein production can be satisfied through fish culture.

Fish has a great potential of ameliorating the protein deficiency in developing world especially Nigeria, but feed and feeding has been the major problem militating against the effective and efficient production in this venture. Shortage and high cost of some conventional animal based protein feed like fish meal and plant protein sources such as cowpea, soybean and groundnut cake due to ever increasing demand as staple food for man, raw material in industries and as feed ingredients in farm animals has called for alternative plant (legumegrains) which are rich in protein and of less industrial use and human preference [2]. The drive to find alternative feed ingredients that do not compete with man's own food needs has stimulated interest in the possible use of various leaf meals [3]. *Gliricidia sepium* is a leguminous browse plant with relatively high protein content which has been used solely or combined with other feed ingredients to feed livestock and poultry at different proportions and forms with satisfactory results [4]. *G. Sepium* leaves have a high feeding value, with crude protein comprising 20-30% of the dry matter, a crude fibre content of only about 15% and in vitro dry matter digestibility of 60-65% [5,6].

Roxazyme® G2 and Maxigrain® are commercial multi-enzymes complex. Roxazyme G2contains â-glucanase and â-xylanaseand Maxigraim® contains 10,000 IU cellulose, 200 beta glucanase, 10,000 UI xylanase and 2500 FTU phytase. Addition of any of these enzymes to poultry diets usually result in numerous beneficial effects, such as increased utilization of nutrients, improved apparent metabolizable energy values and decreased viscosity of intestinal digesta [7].

In lieu of the challenges of high cost of production associated with the production of fish due to high cost of feed ingredients, there is need to exploit the great potential of forage materials that has the potentials of replacing other conventional feed ingredients for fish production. The study intends to incorporate *Gliricidia sepium* with enzymes to produce *Clarias gariepinus.*

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted college of Agricultural sciences, Olabisi Onabanjo University, Ayetoro, Ogun State, situated within longitude 2º 45' E and 3º 5'E, latitude 7º 15' N and 6º 55' N in deciduous/derived savannah zone of Ogun state with annual rainfall of 1,900.3 mm, maximum temperature 29-340C and relative humidity of 81% [8].

## 2.2 Collection and Preparation of Gliricidia Leave Meal

Fresh leaves of *Gliricidia sepium* were collected from the campus college of Agricultural sciences, Olabisi Olabanjo University, Ayetoro, Ogun State, Nigeria. The collected samples were washed thoroughly with tap water to move dirt and debris, drained properly and sunned for 7 days. The dried leaves were milled using a laboratory miller, packed in the freezer at -2°C until use.

## 2.3 Experimental Diets

Feeds were formulated from *G. sepium* leaf mealalong with other ingredients and supplemented with enzymes to test their efficiency in the growth performance of cat fish. Four experimental diets (T1, T2, T3, and T4) were formulated as shown in Table 1.

The enzymes used were Roxazyme® and Maxigrain®. Roxazyme® (Hoffman la Roche, Mississauga, ON L5N 6L7, Canada) which contains 18,000 units' cellulose activity and B glucanase and 8,000 units cellulose activity and 26,000 unit xylanase per gram. Maxigrain® contains 10,000 IU cellulose, 200 beta glucanase, 10,000 UI xylanase and 2500 FTU phytase.

Diet 1 Containing 20% FSM, 0% GLM without enzyme supplementation.
Diet 2 Containing 10% FSM, 10% GLM without enzyme supplementation.
Diet 3 Containing 10% FSM, 10% GLM supplemented with enzyme (Roxazyme G2).
Diet 4 Containing 10% FSM, 10% GLM supplemented with enzyme (Maxigrain). FSM Fish meal, GLM Gliricidia leaf meal.

Dietary ingredients (Table 1) were weighed according to the gross composition. The ingredients were thoroughly mixed together and pelleted with locally fabricated pelletizer and oven dried to constant weight and kept in air tight containers.

## 2.4 Experimental Design and Feeding Trials

Three hundred (300) *C. gariepinus* fingerlings mean weight 6.34±0.43 g were purchased from a reputable fish farm in Lagos State and transported to fishery laboratory, College of Agricultural Science, Olabisi Onabanjo University, Yewa campus, Ogun state. The *C. gariepinus* fingerlings were acclimatized for 14 days and feed on commercial feed prior to the start of the experiment, records of fish weights were taken with aid of a top loading electronic meter balance (model PT 600).

The fish were stocked in flow through system having 12 plastic aquaria with 50 litres capacity at the rate of 25 juvenile per aquarium with provision of diet at the rate of 5% body weight throughout the experiment except on weighting days when they were fed after weighing. Control weighting was carried out every 14 days and feed was administered in accordance with body weight of fish after every 14 days. The experiment lasted for 42 days (6weeks).

## 2.5 Water Analysis

The water quality was routinely monitored following standard procedures [9,10] to ensure adequate and appropriate water quality for fish growth. Temperature (°C) of the culture system was monitored using mercury thermometer, dissolved oxygen (DO) was measured using Jenway DO meter model 9071 and pH was measured using glass electrode pH meter (E 520) metrolin model.

## 2.6 Data Collection and Analysis

Fish weight gain, feed conversion ratio and mortality were determined as follows

i. Weight gain = Final weight of fish – Initial weight of fish
ii. Percentage weight gain (PWG)= Mean weight gain/Mean initial weight X 100/1
iii. Specific growth rate (SGR)= $\text{Loge}W_2 - \text{Log}W_1 / T_2 - T_1$ X 100/1 where $W_2$ = Weight of fish at time $T_2$ in days $W_1$ = Weight of fish at time $T_1$ in days Log = Natural log to base
iv. Feed Conversion Ratio (FCR) = Weight of feed fed/ Live weight gain in fish X 100/1
v. Mortality (M) was calculate as

$$M = \frac{(No - Nt) \times 100}{NO}$$

Where

No = Number of fishes at the start of the experiment.
Nt = Number of fishes at the end of the experiment

## 2.7 Chemical Analysis

Samples of *Gliricidia sepium* and experimental diets were analysed for their proximate composition according to the methods of [11].

## 2.8 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) and means were analysed for significance difference using the Multiple Range Test [12].

## 3. RESULTS

The proximate composition, Ca and P of GLM used for the experiment is indicated in Table 2. The DM content is 86.26; CP, EE, CF, OM, Ash and gross energy were 16.88, 1.14, 16.97, 89.63, 10.37 (%DM) and 3.01(kcal/g) respectively. Calcium and P content was 0.20 and 0.40%. Table 3 shows the anti-nutritional factors of the GML. The Phytate is 1.74 mg/g, oxalate tannin and Saponin were 0.74, 0.47 and 0.42 (%DM) respectively.

The growth performance of *C. gariepinus* fed with *G. sepium* leaf meal supplemented with enzymes is depicted in Table 4. The initial body weight of the fish were not significantly different, suggesting that difference in their final body weights can be attributed the dietary effects on the fish. There was a significant difference ($P< 0.05$) in the FBW of *C. gariepinus* fed with experimental treatments. *C. gariepinus* fed with T4 had the highest significant ($P< 0.05$) FBW (14.15 g), followed by fish on T3 (12.58 g) and T1 (12.10 g) while the least FBW was recorded in *C. gariepinus* fed T2 (10.49 g). WG was also found to be significantly different ($P< 0.05$) among the treatments. *C. gariepinus* fed with T4 had higher significant ($P< 0.05$) WG (5.16g) while there was no significant difference ($P> 0.05$) in WG of T1 and T3 and the least WG (1.14 g) was recorded in *C. gariepinus* fed T2. There was no significant difference ($P> 0.05$) in the MOR of the *C. gariepinus* fed with the experimental treatments. There was a significant difference in the FCR of the fish across the experimental treatments. *C. gariepinus* fed T2 had the highest significant ($P<0.05$) value of FCR (0.19) followed by T1 (0.15) and T3 (0.14) while the least significant ($P<0.05$) value FCR was recorded in *C. gariepinus* fed T4.

Table 5 show the water quality analysis in the experimental plastic tanks used in the study. Mean temperature ranges from 26.1ºC to 28.10ºC and pH value ranges from 6.89 to 8.13.Dissolved oxygen (DO) ranged from 1.9 mg/l to 8.13.and the ammonia value varied from 0.21 mg/l to 0.67 mg/l.

**Table 1. Percentage composition of experimental diets**

| | Experimental treatments | | | |
|---|---|---|---|---|
| Feed ingredients | T1 | T2 | T3 | T4 |
| Maize | 25 | 25 | 25 | 25 |
| Soya bean meal | 32 | 32 | 32 | 32 |
| Glicidia leaf meal | - | 10 | 10 | 10 |
| Fish meal | 20 | 10 | 10 | 10 |
| Wheat offal | 10 | 10 | 10 | 10 |
| Blood meal | 10 | 10 | 10 | 10 |
| Bone meal | 1.5 | 1.5 | 1.5 | 1.5 |
| Premix | 0.5 | 0.5 | 0.5 | 0.5 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.20 | 0.20 | 0.20 | 0.20 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin C | 0.05 | 0.05 | 0.05 | 0.05 |
| Enzyme | - | - | 0.1 | 0.1 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| CP (%DM) | 41 | 39.7 | 39.7 | 39.7 |
| Energy (Kcal/kg) | 2863.5 | 2731.5 | 2731.5 | 2731.5 |
| Crude fibre | 3.341 | 4.511 | 4.511 | 4.511 |
| Ether extract | 4.363 | 3.538 | 3.538 | 3.538 |
| Ash | 6.285 | 4.366 | 4.366 | 4.366 |

**Table 2. Proximate composition, calcium and phosphorus of *Gliricidia sepium* leaf meal (%DM)**

| | Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **DM** | **CP** | **EE** | **CF** | **OM** | **ASH** | **GE** | **Ca** | **P** |
| GLM | 86.26 | 16.88 | 1.14 | 16.97 | 89.63 | 10.37 | 3.01 | 0.20 | 0.40 |

*GLM=Gliricidia sepium leaf meal; DM= Dry matter; CP= Crude protein; EE= Ether extract; OM= Organic matter; GE= gross energy (Kcal/g)*

**Table 3. Anti-nutritional factors of *Gliricidia sepium* leaf meal**

| | Parameters | | | |
|---|---|---|---|---|
| | **Phytate (mg/g)** | **Oxalate (%DM)** | **Tannin (%DM)** | **Saponin (%DM)** |
| GLM | 1.74 | 0.74 | 0.47 | 0.42 |

**Table 4. Growth performance of *Clarias gariepinus* fed with *Gliricidia sepium* leaf meal**

| **Parameters** | **Experimental treatments** | | | | **SEM** | **LOS** |
|---|---|---|---|---|---|---|
| | **T1** | **T2** | **T3** | **T4** | | |
| IBW (g) | 7.08 | 8.29 | 8.52 | 8.66 | 0.85 | NS |
| FBW ( g) | $12.10^{c}$ | $10.49^{d}$ | $12.58^{b}$ | $14.15^{a}$ | 0.56 | * |
| WG (g) | $4.05^{b}$ | $1.14^{c}$ | $4.05^{b}$ | $5.16^{a}$ | 0.04 | * |
| MOR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | NS |
| FCR | $0.15^{b}$ | $0.19^{a}$ | $0.14^{c}$ | $0.13^{d}$ | 0.005 | * |

*a, b, c, d means with different superscript on thesame row are significantly different (P < 0.05); SEM= Standard error of mean; LOS= Level of significant; IBW= Initial body weight; FBW= Final body weight; WG= Weight gain; FCR= Feed conversion ratio; MOR= Mortality*

**Table 5. Water quality in experiment tanks containing the *C. gariepinus* fed with *G. sepium* leaf meal**

| **Treatments** | **Temperature (ºC)** | **DO (mg/l)** | **pH** | **$NH_3$-N (mg/l)** |
|---|---|---|---|---|
| T1 (0%GLM) | 26.5 -28.1 | 2.4 | 7.15 – 7.63 | 0.35 |
| T2 (10%GLM) | 26.4 – 27.9 | 3.9 | 6.89 - 8.05 | 0.21 |
| T3 (10%GLM + Roxazyme® | 26.9 – 28.1 | 1.9 | 6.95 – 8.13 | 0.67 |
| T4 (GLM+ Maxigrain® | 26.1 – 28.0 | 2.1 | 6.90 – 8.10 | 0.59 |

## 4. DISCUSSION

The nutrients values for *G. sepium* used in this study were similar to those reported by [13] except the CP with slight difference (19.72 vs. 16.88). The 16.88%CP recorded in the present study is close to 15.60% CP reported by [14] for dried *G. sepium*, also higher than CP (10.94 reported by [15]. Ash content was in line with the findings of [16] who reported higher values of ash for forages. The high level of ash content is an indication of high mineral content inherent in the forages, which are essential in the formation and function of blood and bones [16]. The Ca (0.22%) and P (0.44) content of GLM also varied from what (Ca 0.44% and P 0.60%) [17] reported. The variation observed might be due to the age, soil and time as factors affecting the nutrients and minerals contents of forages [18,19]. The anti-nutritional factors reported for GML favorably compared with ANF reported in some Nigerian browse plants and required uses of enzyme to improve the digestibility and utilization of nutrients.

There was a significant difference (P< 0.05) in the FBW of *C. gariepinus* fed experimental treatments. *C. gariepinus* fed with T4 had the highest significant (P< 0.05) FBW compare to other treatments, followed by fish on T3 and T1 least FBW was recorded in *C. gariepinus* fed T2. Improvement in growth rate of *C. gariepinus* fed with T4 and T3 may be attributed to presence of enzymes in the two diets, because enzyme is expected to improve the digestibility of the carbohydrate portion of leaf meals, and so energy level. The digestibility of all nutrients, including protein, carbohydrates and minerals are affected [20,21]. Feed intake and growth rate in Carp fed diets containing multi-enzyme premix

at 5 or 10 gkg-1 had 12.3 and 27.5% faster growth rate than the control [22]. However, WG was significant difference (P< 0.05) among the treatments. *C. gariepinus* fed with T4 had higher significant (P< 0.05) WG, there was no significant difference (P> 0.05) in WG of T1 and T3, the least WG was recorded in *C. gariepinus* fed T2. This major difference in weight gain may be associated with increased feed intake.

The highest feed conversion ratio was noticed in T2. The higher the value of the FCR the less desirable is the diet as animals consumes more feed to produce a unit of weight gain [19]. The best FCR (0.13) recorded in T4 implies that the fish utilized the supplied feed with highest efficiency. This simply suggests that the nutrients are more readily released for utilization than others. The slight variations in water temperature; pH, Dissolve oxygen and ammonia during the experience were within the acceptable range for fish growth and health [10].

## 5. CONCLUSION

The *G. Sepium* has potential to make considerable contribution to growth of the African catfish. This Study has demonstrated that, *G. Sepium* leaf meal could be included up to 10% level in *C. gariepinus* diet without any negative effect on the growth and feed efficiency. However, the results revealed that diet supplemented with enzymes can significantly improve growth performance and feed utilization in *C. garipinus*. Maxigrain seems more advantageous for *C. gariepinus* fed with *G. Sepium* leaf meal. It is, therefore more economical to partially replace fish meal with Gliricida leaf meal at 10%.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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