

3 **The effect of diet with different inclusion levels**
4 **of tigernut (*Cyperus esculentus* Lativum) on**
5 **the growth performance of *Clarias gariepinus***
6 **fingerlings**

7
8 **ABSTRACT**

9 The growth performance of the African catfish, *Clarias gariepinus* fed with different inclusion levels of
10 processed tiger nut was studied for 168 days (24 weeks). A total of 144 catfish fingerlings with
11 0.66 ± 0.12 g mean weight and 3.45 ± 0.24 cm mean total length were collected from the University of
12 Calabar Fish Farm, Calabar, Cross River State, Nigeria. Eighteen (18) rectangular plastic tanks were
13 used for this study. Tigernut seeds (*Cyperus esculentus*) were purchased in Bogobiri, Calabar, Cross
14 River State. The tiger nut seeds were sundried for fourteen (14) days prior to milling to produce
15 tigernut meal. The drying process was done to reduce excess moisture so as to prevent unpleasant
16 smell, stale taste and growth of fungus as well as to minimize the presence of cyanogenic glycosides.
17 The dried nuts were ground into fine powder and analysed for proximate composition in the
18 Department of Biochemistry, University of Calabar. Complete Randomized Block Design (CRBD)
19 consisting of 18 treatments was adopted for the study. Triplicate culture tanks in treated (L25-L100),
20 and control groups (L0 and Ac) were stocked with eight fingerlings per square meter, respectively.
21 Weight gain, specific growth rate (SGR), feed efficiency (FE) and feed conversion ratio (FCR) of
22 catfish in different treatments were estimated. Five isonitrogenous diets were formulated to contain 0,
23 25, 50, 75 and 100% tiger nuts to replace the equal weight of fish meal. Commercial fish feed
24 (Coppens) was used as a control feed. All the diets were fed to the catfish fingerlings in triplicate for
25 24 weeks. The results revealed that optimum requirement of tiger nut level in the formulation of
26 practical diets for improved growth of *C. gariepinus* was 25% having the highest mean weight gain
27 (42.3 ± 0.68) and feed efficiency (23.50 ± 0.18). Therefore, it is concluded that tigernut meal could be
28 incorporated in *C. gariepinus* diet at 25% level without any negative effect on growth.

29 **INTRODUCTION**

30 The tigernut (*Cyperus esculentus* Lativum) is called "Isip Accra" in Efik. It was first discovered about
31 4000 years ago and comes in several sizes. Tigernuts have long been recognized for their health
32 benefits, as they are high in fibre, proteins, and natural sugars. The tiger nut is not actually a nut, but
33 a small tuber that has been cultivated for centuries in some parts of Africa with favourable climate.
34 The tigernut is known to be rich in energy and protein while its lipid content (about 25%) is resistant to
35 peroxidation [1]. It is fermented and processed as beverages, eaten raw and also has a medicinal
36 quality of preventing colon cancer, heart failure and diabetes [2]. In recent years, its use in
37 aquaculture as an ingredient for fish meal has not been extensively documented. Proximate analysis
38 of tigernut qualifies it as a good fish feed ingredient being rich in energy, protein and lipid. Agbabiaka
39 [3] recommended the use of tigernut alongside maize and other ingredients in catfish fish feed
40 formulation.

41 *Clarias gariepinus*, the African catfish, is an omnivorous freshwater fish which is popular delicacy in
42 Nigeria. It is a prominent culture species because of its fast growth rate and resistance to diseases
43 and stress factors like over-stocking and poor water quality [4]. It is distributed mainly in fresh waters
44 of Africa hence the name African catfish, although it is also available in Asia. They are nocturnal
45 omnivorous animals feeding on living as well as dead organic matter. They are capable of swallowing
46 large prey because of large mouth. However, there is not much information on the use of tigernut as

catfish feedstuff in Nigeria[8]. This study is to evaluate the effect of diet with different inclusion levels of tigernut on the growth of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Procurement of experimental fish

A total of one hundred and forty-four experimental catfish with 0.81 ± 0.22 g mean weight and 4.22 ± 0.34 cm mean total length were collected from University of Calabar Fish Farm, Calabar, Cross River State, Nigeria. Thereafter, the fish specimens were transported to the Fisheries and Aquaculture Laboratory, Institute of Oceanography, University of Calabar, Calabar and allowed to acclimatize for 14 days (2 weeks) with rectangular plastic tanks.

Preparation of culture tanks

Eighteen (18) rectangular plastic tanks were used for fish culture for a period of 168 days (24 weeks). Although each tank had a water-holding capacity of 95 L, water level was maintained at 60 L covered with mosquito net to prevent escape of fish, as well as entry of predators, and other extraneous bodies. All tanks were continuously aerated using air pump to maintain optimum dissolved oxygen level.

Collection and Processing of the tigernut

The tigernut seeds used for the study were purchased in Bogobiri, Calabar. The tigernut seeds were sundried for fourteen (14) days prior to milling to produce tigernut meal. The drying process is to reduce excess moisture so as to prevent unpleasant smell, stale taste and growth of fungus. The tigernut meal produced was subjected to proximate and elemental analysis in the Department of Biochemistry, University of Calabar following the methods of [5]. The proximate composition of the nutrient contents is shown in Table 1.

Formulation of fish feed with tigernut

Proximate analysis of dietary ingredients was carried out. Then, five iso-nitrogenous diets were prepared using Pearson Square Method. They contained 0, 25, 50, 75 and 100% processed tigernut, which were labelled T0, T25, T50, T75, and T100 respectively, to replace the equal weight of fish meal. Prior to diets formulation, feedstuffs other than the oil were pulverised into powdered form and mixed thoroughly. The oil was added and then pelletized (2mm). After which the pellets were oven dried at 50°C for 3 h (Odo *et al.*, 2016). The pelleted diets were packed in water impermeable bags (Nylon bags), labelled accordingly and kept at room temperature. The proximate analysis of the diet was carried out according to [5] as shown in Table 2.

Table 1. Proximate and mineral composition of tigernut

Parameters	Concentration
Moisture content (%)	7.76
Crude protein (%)	8.59
Ether extracts (%)	28.21
Crude fibre (%)	11.90
Ash (%)	3.57
Nitrogen Free Extract (%)	41.78
Sodium (mg/100g)	535.55
Potassium (mg/100g)	551.95
Calcium (mg/100g)	37.82
Magnesium (mg/100g)	27.87
Zinc (mg/100g)	46.01

Iron (mg/100g)	5.24
Manganese (mg/100g)	0.02
Phosphorus (mg/100g)	12.00

79

80 **Table 2. Percentage composition of experimental diets in different treatments with tigernut**

Ingredients	Treatments				
	T0 Control (0% tigernut inclusion)	T25 (25% tigernut inclusion)	T50 (50% tigernut inclusion)	T75 (75% tigernut inclusion)	T100 (100% tigernut inclusion)
Fish meal	40	30	20	10	0
Tigernut	0	10	20	30	40
Maize meal	15	15	15	15	15
Soybean	30	30	30	30	30
Wheat middling	7	7	7	7	7
Vitamin premix	1	1	1	1	1
Mineral premix	1	1	1	1	1
Vegetable oil	2	2	2	2	2
Bone meal	2	2	2	2	2
Salts	0.5	0.5	0.5	0.5	0.5
Binder	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100

81

82 **Experimental design**

83 The experimental design adopted for this study was Complete Randomized Block Design (CRBD)
84 involving 18 treatments in triplicates. The culture tanks labelled T25 to T100, and control groups
85 labelled T0 and CT were stocked with eight (8) fingerlings each [4].

86 The control group CT was fed with Coppens feed while control group T0 was fed with 0% dietary level
87 tigernut. Fish were acclimatized for two weeks prior to commencement of feeding trial during which
88 they were fed twice a day with a commercial diet at 5% body weight as recommended by [6]. Before
89 feeding trials, the fish in each tank were starved for 2 days to allow utter digestion of any food in their
90 stomach. At the end of acclimatization, fish in each tank were batch weighed with mettler top loading
91 balance to the nearest 0.01g to determine their initial mean weight. The fish were fed daily in two
92 rations (at 8:00am and 3:00pm) and adjusted fortnightly according to weight gain at 5% of the total
93 body weight of the fish in each tank [6].

94 **Growth Performance Indices**

95 **Weight gain:** Weight gain was calculated as: $W_2 - W_1$

96 Where W_1 = live weight of fish at the beginning of the test period

97 W_2 = live weight of fish at the end of the test period

98 **Specific growth rate (%/day):** Specific growth rate was calculated according to [7] as:

$$\frac{\log W_2 - \log W_1}{T_2 - T_1} \times \frac{100}{1}$$

100 Where W_2 = weight at time T_2 (days); W_1 =weight at time T_1 (days)

102 **Feed Utilization**

103 **Feed efficiency (FE):** Feed efficiency for catfish in different treatments was calculated using the
104 formula:

105 $FE = \frac{\text{Weight gain}}{\text{Feed intake}}$

Feed intake
Feed intake was estimated as weight of feed fed – weight of dry feed left
Feed conversion ratio (FCR): Feed conversion ratio was calculated as: $F/(W_2 - W_1)$
 Where F= weight of food supplied to the fish during test period
 W_1 = live weight of fish at the beginning of the test period
 W_2 = live weight of fish at the end of the test period

Statistical analysis

Data collected from the experiment were subjected to One Way Analysis of variance (ANOVA) to test for significance in fish fed different inclusion levels of tigernut using PASW windows software (predictive analytical software) program (version 19.0). Effects with a probability of $p < 0.05$ were considered significant whereas probability of $p > 0.05$ were not considered significant. Means were separated using Duncan Multiple Range Test of difference at significant level of 0.05.

RESULTS

Growth performance

Results showing indices of growth performance (weight gain, Feed efficiency ratio, specific growth rate, and feed conversion ratio) of *C. gariepinus* fingerlings fed different inclusion level of tigernut are presented in Table 3.

Table 3. Growth performance and feed utilization of *Clarias gariepinus* fed different dietary levels of tigernut meal for 24 weeks.

Group	Initial weight (g)	Final weight (g)	Feed intake	Weight gain (g)	Feed Efficiency Ratio	Specific Growth Rate (%/day)	Feed Conversion Ratio
To	11.62±0.14 ^a	51.68±0.71 ^a	1.88±0.08 ^a	40.06±0.13 ^a	21.31±0.16 ^a	0.39±0.04 ^a	0.13±0.03 ^a
T25	12.16±0.28 ^a	54.46±0.68 ^a	1.8±0.11 ^a	42.3±0.68 ^a	23.50±0.18 ^a	0.39±0.04 ^a	0.13±0.01 ^a
T50	13.58±0.19 ^a	43.31±0.45 ^b	1.4±0.13 ^b	29.73±0.23 ^b	21.24±0.99 ^a	0.3±0.01 ^a	0.14±0.02 ^a
T75	12.96±0.26 ^a	37.25±0.23 ^b	1.17±0.09 ^b	24.29±0.28 ^b	20.76±0.22 ^a	0.27±0.08 ^a	0.14±0.04 ^a
T100	11.17±0.71 ^a	23.42±0.27 ^c	0.65±0.14 ^c	12.25±0.21 ^c	18.85±0.62 ^a	0.19±0.04 ^a	0.17±0.04 ^a
CT	10.31±0.44 ^a	61.26±0.81 ^a	2.16±0.06 ^a	50.95±0.92 ^a	23.59±0.84 ^a	0.46±0.01 ^a	0.13±0.05 ^a

Values are means of three replicates ±SE. Column means followed by different letters are different at 0.05 significant level

Weight gain

The mean weight gain (12.25±0.21) of catfish fed diet T100 (100% tigernut meal) was very low compared to the mean weights of those fed diets T0, T25, T50, T75 and CT (40.06±0.13, 42.3±0.68, 29.73±0.23, 24.29±0.28 and 50.95±0.92 respectively). Comparing the results of weight gain between weight gain with CT control group and other treatments, CT had the highest weight gain followed by T25 and T0. Analysis of variance showed significant difference ($p=0.00$) in weight gain between *C. gariepinus* fingerlings fed different dietary levels of tigernut. However, Duncan multiple range test of difference showed no difference ($p=0.27$) between T0, T25 and CT (Fig. 1).

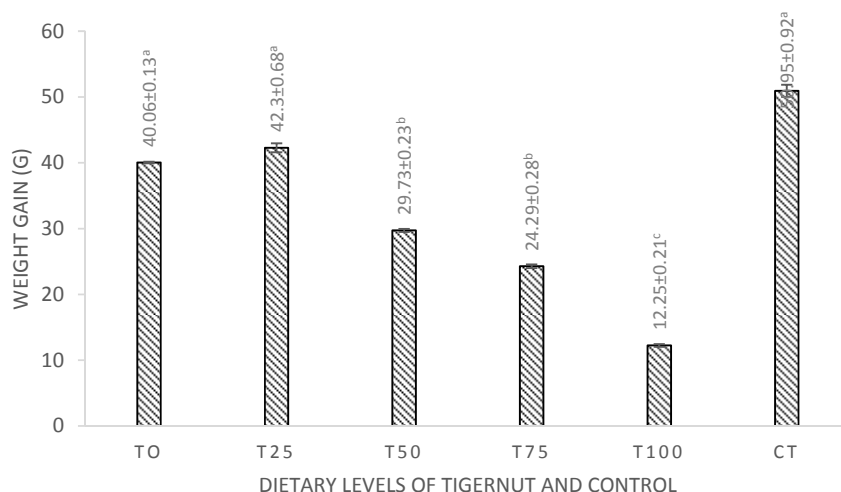


Fig. 1. Weight gain of *Clarias gariepinus* fed different dietary levels of tigernut meal for 24 weeks.

Values are means of three replicates ±SE. Column means followed by different letters are different at 0.05 significant level

Specific growth rate

Specific growth rate was highest (0.46 ± 0.01 %/day) in CT control group followed by T0 and T25 (with respective SGR of 0.39 ± 0.04 %/day and 0.39 ± 0.04 %/day), T50 (0.30 ± 0.01 %/day), T75 (0.27 ± 0.08 %/day) and least (0.19 ± 0.04 %/day) in T100 (Fig. 2). Analysis of variance showed significant difference ($p=0.00$) in specific growth rate between *C. gariepinus* fingerlings fed different dietary levels of tigernut. However, Duncan multiple tests of difference showed no difference ($p=0.34$) between T0, T25 and CT (Fig. 2).

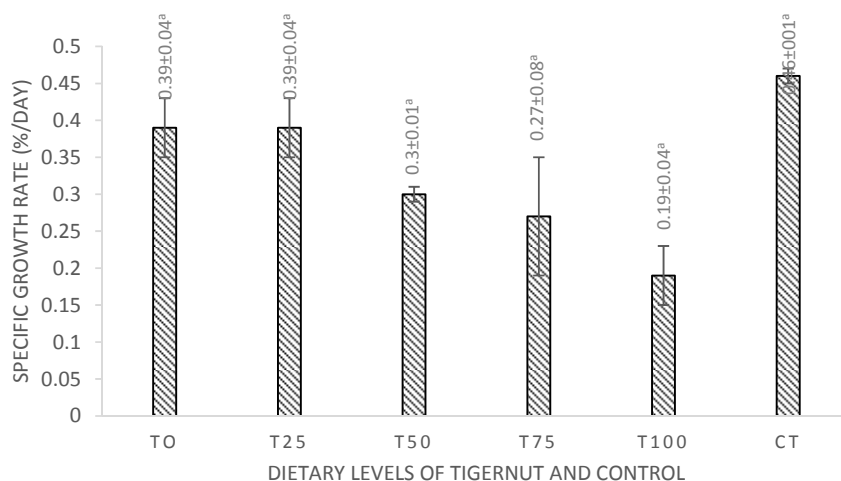


Fig. 2. Specific growth rate (%/day) of *Clarias gariepinus* fed different dietary levels of tigernut meal for 24 weeks.

Values are means of three replicates ±SE. Column means followed by different letters are different at 0.05 significant level

Feed Utilization

Feed efficiency

Feed efficiency was highest (23.59 ± 0.84) in CT control group followed by T25 (23.50 ± 0.18), T0 (21.31 ± 0.16), T50 (21.24 ± 0.99), T75 (20.76 ± 0.22) and least (18.85 ± 0.62 %) in T100 (Fig. 3). There was no significant difference ($p=0.25$) in weight gain between *C. gariepinus* fingerlings fed different dietary levels of tigernut (Fig. 3).

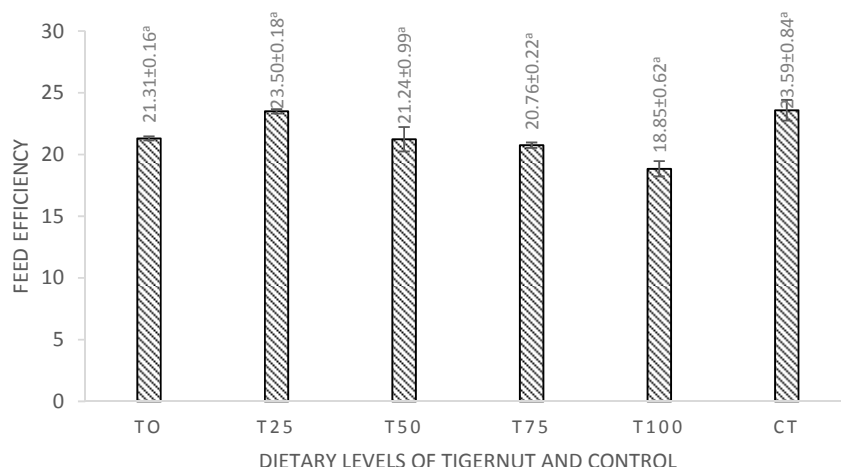


Fig. 3. Feed efficiency of *Clarias gariepinus* fed different dietary levels of tigernut meal for 24 weeks.

Values are means of three replicates ± SE. Column means followed by different letters are different at 0.05 significant level

Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was highest (0.17 ± 0.04) in T100 followed by T75 and T50 (with respective FCR of 0.14 ± 0.04 and 0.14 ± 0.02) and least in T25, T0 and CT groups (0.13 ± 0.01 , 0.13 ± 0.03 , 0.13 ± 0.05 respectively) There was no significant difference ($p=0.33$) in feed conversion ratio between *C. gariepinus* fingerlings fed different dietary levels of tigernut (Fig. 4).

(Fig. 4).

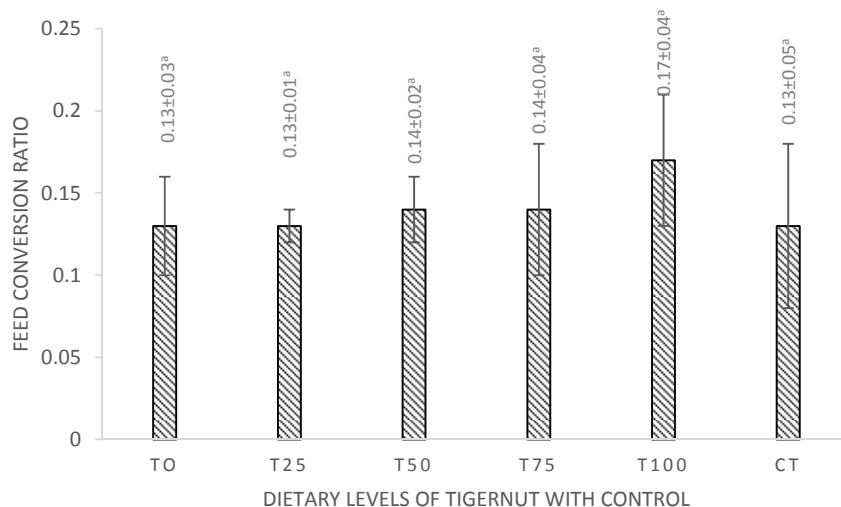


Fig. 4. Feed conversion ratio of *Clarias gariepinus* fed different dietary levels of tigernut meal for 24 weeks.

Values are means of three replicates ± SE. Column means followed by different letters are different at 0.05 significant level

DISCUSSION

Results obtained for the growth parameters showed that fish fed all experimental diets responded positively to the experimental diets although fish in some treatment performed better than some. This indicates that all the diets were of good quality providing all the nutrients required by fish for good health and optimal growth. This fact could be confirmed in the results obtained for proximate composition of all the experimental diets which showed that crude protein level, crude fibre, ash,

moisture, crude fat and nitrogen free extract were within the range recommended by [8] and [9] for optimal growth of *C. gariepinus*.

The ability of the test organisms to convert food materials to usable forms positively influences its growth performance. This was justified by the growth performance in 25% tigernut inclusion level in diet T25. According to [4], lower feed conversion ratio indicates better utilization of the feed by the fish. The least mean feed conversion ratio was observed in diets CT, T0 and T25, indicating that fish in these diets had the best utilization of the feed. However, the mean feed conversion ratio was highest in diet T100 (100% tigernut inclusion), indicating that fish in this diet had the worst utilization of the feed.

Feed efficiency (FE) is a vital tool used in evaluating the efficiency and quality of a given feed [10]. Results obtained for FE in the present study showed that there was no significant difference ($P>0.05$) in FE of fish fed all the experimental diets. This implies that there was not much difference in all the experimental diets with regards to quality as the experimental fishes were able to convert them to body mass. The best FE value was obtained in fish fed CT (control/Coppens) and T25 (25% tigernut meal) compared to fish fed other experimental diets containing tigernut meal at varying levels.

This finding could be attributed to the presence higher fiber content in other groups compared to the control and diets with low tigernut inclusion level. According to Ekanem *et al.*, [11] high fiber content is a major problem when plant meals are used in fish diets as this can impair fish growth through poor food utilization. Positive growth performance has been reported by Ochang *et al.*, [12] on the African catfish (*Clarias gariepinus*) fed with *Moringa oleifera* leaf meal (MLM); Agbabiaka *et al.*, [3] on *C. gariepinus* fed with tigernut based diets; Ekanem *et al.*, [11] for *Heteroclinus* fed Cassava leaf meal diets. Highest values of weight gain and total feed intake were significantly highest ($P<0.05$) in fish fed control and T25 and least in fish fed T50-100. FCR was not significantly different ($P>0.05$) from fish fed other experimental diets. Highest values of growth parameters including weight gain, SGR and total feed intake obtained in T0 and T25 could be attributed to low level of tigernut meal in these diets.

Comparatively, there was no significant difference ($P>0.05$) in for growth parameters of fish fed Diet 1 (T0) and Diet 2 (T25). This implies that tigernut meal could be incorporated at 25% level without any negative influence on the growth of *C. gariepinus*. According to [2], tigernut like other plants has been reported to contain some phytotoxins such as alkaloids, trypsin inhibitors, tannins, phytase and saponins that are deleterious to animals including fish when fed in high dosage. Nevertheless, some of these toxins can be curtailed through processing such as fermentation, toasting among others [1]. Similarly, [11] reported that antinutrients in plant-based feed ingredients could be reduced through processing techniques which will lead to increased palatability, acceptability and growth in fish. However, in the present study, tigernut meal was processed based on standard methods to reduce antinutrients, although it is not possible to reduce the anti-nutrients to 0 % level.

Findings of this study disagrees with findings of [1] who reported the greatest body weight gain of fish at 50% dietary inclusion of tigernut. According to [1], there was a synergetic effect on the utilization of polysaccharides in maize and tigernut tubers by the catfish, enabling its conversion to muscle for growth.

SUMMARY AND CONCLUSION

Findings of the present study revealed that tigernut is a good plant-based ingredient for aquaculture feed due to its rich nutrient and elemental composition which is needed by fish for good health and fast growth. However, feeding of high dietary levels of tigernut meal to *C. gariepinus* may result in adverse effect on the intestinal mucosal such as mucous degradation and poor growth due to high fibre content and presence of anti-nutrients which could not be completely eliminated. In conclusion, inclusion of tigernut meal at 25% level in the diet of *C. gariepinus* will not impose any adverse effect on growth.

Ethical Disclaimer:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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