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Utilization of corn gluten meal in the diets of C*larias gariepinus* juveniles

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Abstract
A feeding trial was conducted to investigate the effect of replacing menhaden fish meal (65%Crude Protein) with corn gluten meal (60% Crude Protein) at 0%, 25%, 50%, 75% and 100% inclusion levels in the practical diets of *Clarias gariepinus* juveniles. One hundred and fifty (150) juvenile's fish with initial mean weight of 3.54±0.05g were stocked randomly into fifteen (15) glass aquaria tanks at 10 fish/tank in five treatments with three replicates each. The experimental diets were iso-nitrogenous (45%Crude Protein) and iso-caloric (11.1kcal/kg). Using a complete randomized design, each diet was fed to the test fish for 70days. The highest weight gain was recorded in the fish fed D_3 (12.74±0.34)g containing 50% inclusion level of corn gluten meal (CGM) and the least weight gain was in the fish fed D_5(9.33±0.36) with 100% inclusion level of CGM. Specific growth rate (SGR) ranged from 1.69 to 1.84; feed conversion ratio (FCR) ranged between1.23% to 1.64%; protein efficiency ratio (PER) ranged from (0.19%) in D_3 to (0.33%) in D_5. The net protein utilization (NPU) values ranged from (14.08 ±0.06) in fish fed D_3 to (9.73 ±0.03) in D_5. The overall results indicated that 50% replacement of Fish Meal with Corn Gluten Meal in the diets of *Clarias gariepinus* juveniles gives optimal feed utilization without affecting fish growth.

Keywords: corn gluten meal, *Clarias gariepinus*, catfish, fish meal, juveniles, feed

Introduction
Fish nutrition has advanced in recent years with the development of balanced diets that promote optimal fish growth (King, 2004) [10]. The development of new diets to support the expansion of aquaculture industry cannot be overemphasized. Protein is the most expensive part of fish feed and must be adequately supplied to each species and size of cultured fish (Lim and Akiyama, 1992) [11]. Protein requirement in smaller fish is generally higher when compared with adult (Davies *et al.*, 2007) [6]. Since protein must be supplied to fish in sufficient amount, the protein content in the diet must be of good amino acid profile (Wu *et al.*, 1999) [18].

Fish meal is known to contain complete Essential Amino Acids (EEA) profile that meet the protein needs of most catfishes, but as feed ingredient, it is expensive (Webster *et al.*, 2001) [17]. Hence the need to focus on plant protein sources as an alternative to fish meal (Wu *et al.*, 1999) [18].Corn gluten meal (CGM) is considered as a plant protein source in fish diets (Cheng *et al.*, 2013) [5]. The crude protein level is between 55-60% and does not contain anti-nutritional factors like most grains and seed oils used in catfish diets (Parson, 2008). Most plants protein sources exhibit low digestibility and symptoms of deficient Essential Amino Acid (EEA) when fed exclusively to fish (FAO, 2002) [7]. Maize gluten meal is known to be deficient in lysine (Regost *et al.*, 2003) [13]. This deficiency can be corrected by using it with fish meal at a level that does not compromise optimal fish growth. The use of fish meal with corn gluten meal in this study was supplemented with L-lysine and methonine to address the imbalances in Essential Amino Acid (EEA) profile in the diet composition.

Clarias gariepinus exhibits fast growth rate which qualifies it for commercial purposes. It is an omnivore but prefers formulated feeds to flesh, unlike the hybrids and pure *Heterobranchus* species (Tacon, 1996) [16]. The objective of this study was to determine the effect on fish growth and feed utilization the graded levels of Corn gluten meal as a replacement to fish meal in the diets of *Clarias gariepinus* juveniles

Materials and Methods
Experimental Set-up
The study was conducted at the Fisheries and Aquaculture unit of the Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria. Fifteen (15) rectangular aquaria glass tanks with a dimension of (70cm × 45cm × 40cm)/tank were used for the experiment. The tanks were filled with borehole water to 70L capacity. 150 juveniles of *Clarias gariepinus* average mean weight (3.5 ± 0.16) g were purchased from Success Fish Breeding and Poultry Farm Nigeria Limited, Akure, Ondo State. The fish were buck weighed using an electronic top loading balance into the aquaria glass tanks and fed with commercial feed of size 2mm and allowed to acclimatize for a week to the experimental conditions. After which the fish were randomly stocked at 10 fish per tank in five treatments, with each treatment replicated thrice. The test diets were fed to the fish in each corresponding tank for 70 days.

Experimental Diets
Feed ingredients were purchased from Metrovet Feed Mill in Ado-Ekiti, Ekiti State, Nigeria. Five experimental diets were formulated to contain varying proportions of corn gluten meal (CGM) except the Control. The Control diet (D_1) contained 0% CGM with fishmeal as its main protein source. The remaining diets (D_2 –D_5) contained 25%, 50%, 75% and 100% CGM inclusion levels to replace fish meal respectively.

Proximate analysis of the gross composition of experimental diets was carried out after the feed formulation according to AOAC (1995) [2] to ascertain that diets were in line with the set objectives.

Table 1: Gross Composition of Experimental Diets

Ingredients	Diets				
	D_1 (control)	D_2	D_3	D_4	D_5
Corn Gluten meal	0%	25%	50%	75%	100%
Fishmeal	100%	75%	50%	25%	0%
Fish Meal (65%CP)	70.00	52.50	35.00	17.5	-
Corn Gluten Meal (60%CP)	-	17.50	35.00	52.50	70.00
Maize (8.8%CP)	25.00	22.00	22.00	22.00	22.00
L- Methonine	-	1.5	1.5	1.5	1.5
L-lysine	-	1.5	1.5	1.5	1.5
Vitamins premix	2.0	2.0	2.0	2.0	2.0
Cod Liver Oil	1.5	1.5	1.5	1.5	1.5
Starch	1.5	1.5	1.5	1.5	1.5
Total %	100	100	100	100	100

Feeding Procedure

The experimental diets were fed to the fish twice daily at 0900 and 1600 for 70 days. Accumulated dirt in the tanks was siphoned out every morning before feeding. The weights of fish were measured fortnightly to monitor their response to feed. Exchange of water in the tanks was at a flow rate of 1.5L/min in a flow through system. Data collected at the end of the feeding experiment were used to evaluate the fish performance in terms of growth and feed utilization.

Water Quality Parameters

The pH values of the water during the feeding trial were measured directly using electronic pH meter (Metler toledo 320 model) by dipping the electrode into each tank daily. Dissolved oxygen was measured using Standardized YSI Do meter (YSI Model 57) every day. The water temperature of the aquaria tanks was measured using a mercury thermometer calibrated from 0^0C to 110^0C by gently immersing it at a vertical position for 2-5 minutes, then quickly moved near the surface of the water and read.

Growth and nutrient utilization paramters

Growth performances were measured in term of mean weight gain (MWG) g, specific growth rate (SGR %), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU). These parameters were calculated as follows according to AOAC (1995) [2].

MWG = Final mean weight (W_2) - Initial mean weight (W_1)

SGR (%) = $(\ln W_t - \ln W_i)/T \times 100$

Where W_t is weight of the fish at time t, W^i is weight of the fish at time 0, and T is the culture period in days.

FCR = total dry feed fed (g)/ total wet weight gain (g).

PER = wet weight gain (g)/ amount of protein fed (g)

NPU (%) = 100 x (protein gain/ protein consumed)

Chemical Analysis

Samples of experimental diets, fish carcasses in all treatments were analyzed for proximate composition: Moisture, Ash, Crude fibre, Crude protein, Crude fat, and Carbohydrate according to the methods of AOAC, 1995 [2]. Gross energy in kcal/kg of test diets was determined using ballistic oxygen bomb calorimeter (Gallen Kamp) as described by AOAC, (1995) [2]

Statistical Analysis

Data obtained for MWG, SGR, FCR, PER and NPU were subjected to Analysis of Variance (ANOVA) using SPSS version 16 to indicate statistical significant ($P < 0.05$) (Steel *et al.*, 1997) [15]. Differences among treatment means were separated using Duncan's multiple range test at 5% level of probability.

Results

Proximate Composition of Experimental Diets

The result of the proximate analysis of experimental diets is shown in Table 2. Throughout the duration of the feeding trial, feed acceptance by fish was satisfactory. The crude protein ranged between 40.01% and 40.08% while crude fibre ranged between 5.15% and 6.78%. Similarly, lipid, ash and nitrogen free extract (NFE) varied from 9.82% to11.88%, 7.69% to 8.89% and 19.37% to 24.23% respectively. The results show that the diets were formulated in line with the experimental objective. Any change in the performance of the fish at the end of the experiment would be attributed to various inclusion levels of corn gluten meals in the diets.

Table 2: Proximate composition of experimental diets fed to *Clarias gariepinus*

Parameters (%)	Treatments				
	D_1	D_2	D_3	D_4	D_5
Moisture	14.63	13.67	13.59	13.45	11.07
Crude protein	40.08	40.05	40.07	40.02	40.01
Ash	8.89	8.73	8.63	8.53	7.69
Lipid	11.88	10.88	9.82	7.55	10.71
Crude fiber	5.15	6.78	6.73	6.22	6.34
NFE	19.37	19.89	21.16	24.23	24.18

Proximate Analysis of Experimental fish before and after the feeding trials

Table 3 shows the results of the proximate analysis of the fish before and after the feeding experiment. Significant difference ($P< 0.05$) was recorded in crude protein (CP), ether

extract (EE), nitrogen free extract (NFE) ash content of fish. The CP level ranged between 65.22% -68.08% in the body of fish fed (D_1 –D_5) with the highest value in D_3 (68.08%). Fat content was highest in fish fed D_5 (11.07%). No fibre was detected. Ash content showed increasing values from D_1 (10.50%) to D_5 (11.07%). While nitrogen free extract showed inverse relationship with the ash content by decreasing values from D_1 (2.40%) to D_5 (1.88%). It was observed that there was a slight increase in the values of some parameters examined after the experiment compared to initial values before the experiment.

Table 3: Proximate Composition of fish before and after the feeding trials

Parameters (%)	Before	Treatments After				
		D_1	D_2	D_3	D_4	D_5
Moisture	10.57±0.31[a]	10.25±0.53[a]	10.24±0.71[a]	10.20±0.53[a]	10. 15±0.67[a]	10.71±0.56[a]
Ash	10.56±0.43[b]	10.50±0.34[b]	11.25±0.65[a]	10.35±0.76[b]	11.10±0.23[a]	11.07±0.45[a]
Lipid	9.25±0.23[c]	10.76±0.32[b]	10.31±0.12[b]	10.30±0.22[b]	10.35±0.21[b]	11.12±0.17[a]
Protein	67.22±0.43[b]	65.85±1.21[b]	66.40±1.23[b]	68.08±0.54[a]	66.98±0.32[b]	65.22±0.23[b]
NFE	2.40±0.11[b]	2.64±0.56[a]	1.80±0.32[a]	1.07±0.34[a]	1.42±0.43[c]	1.88±0.21[c]

Mean values with similar superscript along the horizontal row are not significantly different (P>0.05).

Water Quality Parameters

The mean values of the physic - chemical parameters of water in the experimental tanks for the period of the experiment are presented in Table 4. Water temperature fluctuated, though not significantly within the range of 24.82 -25.15^{0}C throughout the feeding trials. While mean values obtained for Dissolve oxygen (DO), PH and nitrate ranged between 5.29mg/L, 6.82 -6.93 and 0.22 -0.23mg/L respectively. There was no significant difference (P> 0.05) in the values of the parameters analyzed among the treatments. They were within the acceptable ranges for *Clarias gariepinus.*

Table 4: Mean Value of Water Quality Parameters during the experimental period

Parameters	Treatment				
	D_1	D_2	D_3	D_4	D_5
DO (mg/L)	5.32±0.02	5.30±0.00	5.34±0.01	5.29±0.03	5.35±0.02
pH	6.83±0.02	6.91±0.01	6.82±0.02	6.97±0.21	6.93±0.01
Temp (ºC)	25.15±0.17	24.86±0.23	2486±0.21	25.17±0.22	24.82±0.21
NO_3 (mg/L)	0.22±0.02	0.23±0.01	0.23±0.02	0.22±0.00	0.22±0.01

Growth performance and nutrient utilization of experimental fish

The growth parameters of the fish fed the various experimental diets are presented in Table 5. There was a significant difference (P<0.05) in growth performance of *Clarias gariepinus* juveniles fed with different test diets. Mean weight gain showed similar pattern to that of SGR with the best values in fish fed D_3 (12.74g, 1.69) respectively. Feed utilization expressed as feed conversion ratio (FCR), was not significantly (P>0.05) different in fish fed diets D_4 (1.57) and D_5 (1.64). Diet D_3 had the lowest FCR (1.23) with the best growth performance. The dietary protein utilization expressed as the protein efficiency ratio (PER) and as net protein utilization (NPU) were significantly highest (P<0.05) in fish fed D_3 (0.33%, 14.08%) respectively

Table 5: Growth Performance and Nutrient Utilization of *Clarias gariepinus* fed with experimental diets.

Growth Parameters	Diets				
	D_1	D_2	D_3	D_4	D_5
Initial Mean weight (g)	3.54±0.08[a]	3.56±0.07[a]	3.55±0.00[a]	3.54±0.05[a]	3.54±0.05[a]
Final Mean weight (g)	15.71±0.30[c]	16.28±0.08[b]	16.35±0.34[a]	13.05±0.25[d]	12.87±0.41[e]
Mean weight gain (g)	11.17±0.22[b]	12.72±0.01[a]	12.80±0.34[a]	9.51±0.20[c]	9.33±0.36[d]
SGR	1.54 ±0.03[b]	1. 56 ±0.05[b]	1.69 ±0.06[a]	1.47 ±0.01[c]	1.46 ±0.02[c]
PER (%)	0.24 ±0.05[c]	0.29 ±0.00[b]	0.33 ±0.08[a]	0.24 ±0. 05[c]	0.24 ±0.09[c]
FCR	1. 39 ±0.03[c]	1.35 ±0.02[b]	1.23 ±0.01[a]	1.57 ±0.08[d]	1.64 ±0.05[d]
NPU (%)	13.80 ±0.07[b]	13.89±0.04[b]	14.08 ±0.06[a]	11.19 ±0.06[c]	9.73 ±0.03[d]

Mean values with similar superscript along the horizontal row are not significantly different (P>0.05).

Discussion

The excellent nutritional composition, coupled with the rising demand and limited supply of fish meal makes it an expensive protein source (FAO, 2007) [8]. However, different inclusion levels of protein feed materials from plant have been tested by many researchers to replace fish meal in the diets of fish (Regost *et al.*,1999; Fournier *et al.* 2004; Bonaldo *et al.* 2015 and Albert *et al.*,2016) [12, 9, 4, 1]. The suitability of corn gluten meal (CGM) as alternative protein source in the diets of *Clarias gariepinus* juveniles in this study was evaluated using growth performance indicators such as final body weight, mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU). In the present study, 50% replacement of fish meal with Corn Gluten Meal did not affect the growth of fish negatively. Corn gluten meal can substitute up to 30% of fish meal without negative effects on growth of turbot (Sevgili *et al.*, 2015. Regost *et al.* 1999) [12,

[14] observed unaffected growth performance of turbot fed diets containing 33% replacement of fish meal with corn gluten meal. According to Albert *et al.* (2016) [1], since the protein level in most plant materials is lower than in fish meal, there should be reduction in their uses as substitute to fish meal.

This result in this study shows that source of protein affected the growth of *C. gariepinus* juvenile. The trends in growth performance especially at high inclusion level of CGM (75 - 100%) indicate poor growth. According to Bonaldo *et al.* (2015) [4], when substituting for different components in fish diets, the balance of nutrients must satisfy the nutritional requirements of the fish. The amino acids profile in most plant protein materials such as CGM (60% Crude Protein), though very high lacks the most essential amino acids (EAAs). This decrease in dietary essential amino acids leads to poor growth in fish (Bonoldo *et al.* 2011) [3].

Conclusion

Since fish meal has become a critical ingredient in aquafeed production, the search for alternative protein sources from plant protein is continuous. Substituting fish meal with other ingredients from plant origin, especially at high inclusion level may comprise growth as seen in this study. Also, there is need for further investigation on the utilization of Corn gluten meal by adult *Clarias gariepinus*, since data on it are currently lacking. Conclusively, Corn gluten meal can serve as potential alternative protein source to partially replace fish meal in the diet of *Clarias gariepinus* juvenile, with a reasonable balance of essential amino acids by supplementing with most limiting amino acids.

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