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Original Paper

Some Physiological Responses of *Clarias gariepinus* Fed Graded Levels of *Cirina forda* Larvae Based Diets

Adewumi, A.A.[1*], Idowu, E.O.[1], Edward, J.B.[1], Iwalaye, O.A.[1], Fadiya, O.O.[2] & Obafemi, B.D.[2]

[1] Department of Zoology and Environ. Biology, Ekiti State University, Ado-Ekiti, Nigeria

[2] Department of Zoology, University of Ibadan, Nigeria

* Adewumi A.A., Department of Zoology and Environ. Biology, Ekiti State University, Ado-Ekiti, Nigeria

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Abstract

The study examined the growth performance, hematological and digestive enzymes of Clarias gariepinus juveniles fed Cirina forda meal (CFM) based diets in the laboratory for a period of 10weeks. Five iso-nitrogenous (30%) experimental diets were formulated at various levels of CFM inclusion levels of 0% (control), 10%, 20%, 30%, 40% and 50%, designated as diets Q, A10, B20, C30, D40 and E50 respectively. Fish fed the CFM based diets showed mean weight gain (MWG), specific growth rate (SGR) and protein efficiency ratio (PER) comparable to the control diet. There was significant differences in the digestive enzyme activities of the fish as the CFM level in the experimental diets increased. Protease and maltase activities significantly increased, with diet C30 recording the highest maltase activity (4.37) while the cellulase and glucanase activities of the fish significantly ($P<0.05$) reduced. There was no significant difference ($P>0.05$) between the PCV and RBC of the blood of the fish fed the various diets. Highest RBC (2.75 $x10^6/\mu L$) was obtained in fish fed the control diet while the lowest (2.55 $x10^6/\mu L$) was recorded in the fish fed diet E50. The White Blood Cell count and the Neutrophils of the fish fed the trial diets were not significantly higher ($P>0.05$) than those of the fish fed the control diets. From the results of this experiment, it is concluded that up to 50% inclusion level of Cirina forda meal in the diet of Clarias gariepinus was tolerable for good growth and physiological well-being of the fish.

Keywords

Clarias, growth, haematology, digestive enzymes

1. Introduction

Fish culture is one of the fastest growing sectors of the world's animal production with an annual increase of about 10% (FAO, 2010). The African catfish; *Clarias gariepinus*, is the most sought after species among fish farmers and consumers because it commands good commercial value, not only in Nigeria, but all over Africa. The growth of aquaculture in Nigeria is now largely being boosted by a steady rise in catfish culture. To sustain such high rates of increase in production, a matching increase in fish feed production is imperative. The high cost and fluctuating quality as well as the uncertain availability of fish meal have led to the need to identify alternative protein sources for fish feed formulation. Therefore, in order to attain more economically, sustainable, environmentally friendly and viable production, research interest has been directed towards the evaluation and use of non-conventional sources of protein.

The edible larvae of *Cirina forda* insect (Figure 1) has a wide acceptability as a food source, and also serves as an important item of commerce in such Nigerian states like Oyo, Kwara, Kogi, Niger and Kaduna, where it has become the most important and marketable insect (Ande, 1991; Fasoranti & Ajiboye, 1993). Osasona and Olaofe (2010) analyzed the insect's larvae as composed of digestible protein (45.10%) (Table 1), fats (18.03%) and small, but significant amount of carbohydrate, minerals, vitamins and polyunsaturated fatty acids. Table 2 contains the reports of Akinnawo and Ketiku (2000) and Omotoso on the mineral composition of *C. forda*.

Apart from being a widely acceptable food source, a number of factors are known to enhance the availability of the larvae of the insect. Such factors include its capability for artificial rearing, the short-lived larval stage, and the high conversion rate (Ande, 1991). These factors make it a resourceful replacer for fish meal in animal diets (Oyegoke et al., 2006). Omotoso (2006), Ifie and Emeruwa (2011) reported that *Cirina forda* contains less than 0.005% oxalate. Omotoso observed that the anti-nutrient composition (mg/100 g) of *C. forda* larva to include phytic acid (1.02 ± 0.00) and oxalate (4.11 ± 0.05). He however commented that these values are lower than those reported in some proteinous foods. This amount is under the tolerance limit because much higher amounts have been observed in various plant food materials (Kalita et al., 2007). Vijayakumari et al. (1997) observed that 513 mg of phytic acid was present in 100 g of *P. chilensis*, a consumable legume, hitherto reported by de Lumen et al. (1986), to be very rich in methionine and cysteine.

Enzymes are protein in nature and comprise biological molecules which are involved in metabolic processes in living organisms. The digestion and absorption of nutrients are mostly dependent on enzyme activities involved in breakdown and assimilation of food (Klein et al., 2006). Therefore, analysis of enzyme activities is a convenient and reliable technique that can provide comprehensive information relating to digestive physiology and nutritional conditions in the fish (Bolasina et al., 2006). Digestive enzyme activities in fishes are associated with feeding ecology and composition of diet (Fernandez et al., 2011). Digestive enzymes activity influence feed utilization by fish, and its understanding is important to optimize diet formulation. Feed nutrients must be digested for their

utilization, and pancreatic digestive enzymes have essential roles for the digestion; trypsin and chymotrypsin are the main pancreatic proteases, lipase is the major pancreatic lipolytic enzyme, and amylase is known as the major pancreatic digestive enzyme for carbohydrates (Murashita et al., 2015). In general, herbivorous fish species possess greater carbohydrate enzyme activity, while carnivorous fish species exhibit higher proteolytic enzyme activity (Hidalgo et al., 1999). The digestion and absorption of nutrients are mostly dependent on enzyme activities involved in breakdown and assimilation of food (Klein et al., 2006). The digestion and metabolism of carbohydrates (and other feed ingredients) is dependent on fish species and on the source, inclusion level and treatment of the ingredient (Krogdahl et al., 2005; Stone, 2003). Knowledge of the capacity of a fish to utilize the nutrients in the diet is an essential pre-requisite for appropriate formulation of fish feed (Wilson, 1994).

Blood analysis is a valuable means of evaluating the physiological condition of cultured fish with respect to the effect of diets and other stress factors on fish health. Changes in haematology of fish in response to stressing agents are indicators of the stressful stage of fish, producing useful information to curb any unfavourable condition that may affect the fish health (Fagbenro et al., 2010).

This research is being conducted to evaluate the effects of *Cirina forda* pupae replacement for fish meal in the diet, on the growth, digestive enzymes and haematological parameters of *Clarias gariepinus.*

Figure 1. Dried Whole *Cirna Forda* Pupae

Table 1. Proximate Composition (% DM^{-1}) and Energy Value of *Cirina Forda* Pupae

Components	Percentage (%)				
	This work	Akinnawo and Ketiku (2000)	Oso and Ola-Oladimeji (2016)	Adepoju and Daboh (2013)	Omotoso (2006)
Moisture	6.02	-	5.25	-	10.85
Fibre	-	9.40	7.69	-	-
Ash content	6.62	7.12	6.49	2.6	10.26
Protein	52.34	33.12	45.10	52.6	55.50
Fat	17.56	12.24	18.03	16.8	4.68
Carbohydrates (by difference)	17.36	38.12	17.44	-	18.70
Energy (Kcal)	-	359.00	-	458.4	-

Table 2. Mineral Composition (mg/100g) *C. Forda* Pupae

Minerals	Omotoso (2006)	Akinnawo and Ketiku (2000)
Calcium	33.16	7.0
Potassium	64.02	2130
Magnesium	62.31	32.4
Phosphorus	215.54	1090
Sodium	45.26	210
Iron	5.34	64
Zinc	3.81	8.6
Manganese	1.14	7.0
Copper; Cobalt; Lead; Chromium; Nickel	Not available	-

Source: Akinnawo O. and Ketiku, A.O. (2000) and Omotoso (2006).

2. Materials and Method

2.1 Experimental Site

The experiment was carried out at the Aquaculture Centre of the Department of Zoology and Environmental Biology, Ekiti State University, Ado-Ekiti.

2.2 Procurement of the Experimental Fish and Feedstuffs

One hundred and eighty juveniles (31.01 ± 0.23 g) of *Clarias gariepinus* were purchased at Adebayo fish hatchery in Ado-Ekiti, Ekiti State. The fish were acclimatized for two weeks being fed with a commercial feed (Coppen's feed). After acclimatization, the fish were randomly distributed into well labeled aquarium tanks in triplicates per treatment.

2.3 The Preparation of Experimental Feed

Dry *Cirina forda* pupae was purchased from Oja-Oba (king's market) in Ado-Ekiti, Ekiti State. The

Cirina forda was sundried and milled into fine powder meal, tagged CFM. Other feed ingredients used for the diet formulation include; fish meal, maize, lysine, methionine, wheat bran, vitamin premix, groundnut oil and salt purchased from Afe Babalola University, Ado-Ekiti (ABUAD), Ekiti State, while the groundnut oil and salt were obtained from Oja-Oba. Calculated amounts of each feed ingredient were weighed separately using Pearson's square method of feed formulation. Fishmeal was replaced at 0% (control diet), 20%, 40%, 60%, 80% and 100% respectively and the treatments were tagged diets Q, A20, B40, C60, D80, and E100 respectively. The various ingredients were properly mixed together and pelleted to a particulate size using a 2mm pellet disc. Mixing and pelleting was done at the Federal University of Technology, Akure (FUTA), in Ondo State. The pelleted feeds were sundried for a week and kept inside labeled polythene bags till required. The feed formulation is as shown in Table 3.

Table 3. Proximate Composition (g 100^{-1} DM) of the Experimental Diets

Ingredient	Q	A20	B40	C60	D80	E100
Fish meal	43.10	38.79	34.48	30.15	25.86	21.55
Cirina forda		4.31	8.62	12.93	17.24	21.55
Maize	51.40	51.40	51.40	51.40	51.40	51.40
Wheat bran	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	0.50	0.50	0.50	0.50	0.50	0.50
Methonine	0.50	0.50	0.50	0.50	0.50	0.50
Groundnut oil	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix*	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100

*Contains Vit. A 4000000 IU, Vit. D: 800000 IU, Vit. E: 40000 mg, Vit. K: 3800 mg, Vit. B1: 1000 mg, Vit. B2: 6000 mg, Vit. B6: 5000 m, Vit. B12: 25 mg, Niacin: 6000 mg, Patothenic acid: 20000 mg, Folic acid: 200 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese: 300000 mg, Iron: 80000 mg, Zinc: 20000 mg, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg and Choline: 800000 mg.

2.4 Fish Sampling

Three fish samples were taken randomly from each bowl. The initial individual weight and length of these were measured using the weighing scale and calibrated ruler, respectively. Triplicate samples of the fish from each bowl were weighed bi-weekly until the experiment was completed. The bi-weekly weighing allowed the adjustment of feeding levels for the subsequent weeks. Unconsumed feeds and faecal matters were siphoned off every other day.

2.5 Growth Performance and Nutrient Utilization Parameters

Using the weight data and the quantity of feed fed, the growth response and nutrient utilization parameters were determined. Mean weight gain (MWG), relative weight and specific growth rate

(SGR), percentage weight gain (PWG), feed conversion ratio (FCR), specific growth rate (SGR) were calculated, using the following formulae;

$$SGR = \frac{InW_2 - InW_1 \times 100}{T-t}$$

Where,

W_1=Initial weight (gram) at time t

W_2= final weight (gram) at time T

$$FCR = \frac{\text{Weight of food consumed per fortnight}}{\text{Weight gained by fish per fortnight}}$$

MWG= mean final body weight (g)-mean initial body weight (g)

$$\text{Survival Rate (SR)} = \frac{\text{Total fish number harvested}}{\text{Total fish number stocked}}$$

Relative Weight gained (RWG)

$$RWG = \frac{W_2 - W_1}{W_1} \times 100$$

$$PWG = Wt - W0 \times 100$$

Where,

W0 = Weight at initial

Wt = Weight at time t.

2.6 Biochemical Analysis

The proximate compositions of the various diets (Table 4) and the carcass of the flesh of the fish were carried out in the laboratory using the methods of AOAC (2006).

Table 4. The Proximate Composition (% DM^{-1}) of the Various Diets

Parameters	Q	[a]A20	B40	C60	D80	E100
Ash	9.51[a]	9.36[a]	9.91[a]	9.21[a]	8.29[ab]	7.45[ab]
Moisture	12.26[a]	12.64[a]	12.03[a]	11.82[a]	12.35[a]	12.65[a]
Carbohydrate	35.07[a]	43.16[b]	39.81[b]	37.75[b]	37.26[b]	42.55[b]
Crude fiber	0.90[a]	1.20[a]	0.20[b]	0.21[b]	0.47[b]	0.31[b]
Crude Protein	40.06[a]	39.63[a]	38.77[a]	41.81[a]	39.69[a]	39.75[a]
Fat	0.20[a]	0.20[a]	1.20[ab]	2.10[b]	4.01[b]	2.30[b]

Table 5. The Proximate Composition (% DM^{-1}) of the Carcass of *C. Gariepinus* Fed with the Various Diets

Parameters	Q	A20	B40	C60	D80	E100
Ash	12.77^{ab}	15.67^{a}	13.55^{a}	14.06^{a}	13.77^{a}	13.91^{a}
Moisture	9.45^{b}	11.98^{a}	12.03^{a}	12.34^{a}	12.73^{a}	11.69^{a}
Crude Protein	73.44^{a}	75.10^{a}	71.78^{a}	72.33^{a}	69.23^{ab}	68.21^{ab}
Fat	8.26^{a}	7.78^{a}	7.39^{a}	7.55^{a}	6.97^{a}	6.89^{a}

2.7 Enzyme Analysis

2.7.1 Amylase Assay

Amylase activity was assayed by the method of Negi and Banerjee (2010). 0.5 mL of properly diluted enzyme was added into a tube containing 1.5 mL of 2 % (w/v) of potato starch solution and 1 mL of 0.05 M acetate buffer, pH 5.0. The reaction mixture was incubated at 40 ℃ for 15 min. Then, 1 mL of the mixture was transferred to a new tube containing 1 mL of 3,5-dinitrosalicylic acid and kept in boiled water for 10 min. The color density was determined spectrophotometrically at 520 nm. One unit was defined as 1 μmol of glucose released per minute by 1 mL of enzyme.

2.7.2 Sucrase Assay

0.5 mL of properly diluted enzyme was added into a tube containing 1.5 mL of 2 % (w/v) of Sucrose solution and 1 mL of 0.05 M acetate buffer, pH 5.0. The reaction mixture was incubated at 40 ℃ for 15 min. Then, 1 mL of the mixture was transferred to a new tube containing 1 mL of 3,5-dinitrosalicylic acid and kept in boiled water for 10 min. The color density was determined spectrophotometrically at 520 nm.

2.7.3 Maltase Assay

0.5 mL of properly diluted enzyme was added into a tube containing 1.5 mL of 2 % (w/v) of maltose solution and 1 mL of 0.05 M acetate buffer, pH 5.0. The reaction mixture was incubated at 40 ℃ for 15 min. Then, 1 mL of the mixture was transferred to a new tube containing 1 mL of 3,5-dinitrosalicylic acid and kept in boiled water for 10 min. The color density was determined spectrophotometrically at 520 nm.

2.7.4 Glucanase Assay

Glucanase was assayed by incubating 500 uL of 5.0% Laminarin in 50 Mm acetate buffer pH 4.8 with 200 uL enzyme solution at 45 ℃ for 30 min and determination of reducing sugars with DNSA (Danielson et al., 2010).

The amount of reducing sugars was calculated as mmol of glucose per min per ml.

2.7.5 Cellulase Assay

Cellulase was measured according to Ghose (1987). A 900 uL of 1% CMC solution was added to 100 uL enzyme solution in a test tube. 1.5 mL DNS reagent was added and incubated at 50 ℃ in water bath

for 30 min. The absorbance was measured at 540 nm. Glucose standard graph was prepared from 0-500ug glucose. One unit of cellulase activity was defined as the amount of enzyme that liberates 1 micromole of reducing sugars equivalent to glucose per minute under the assay conditions.

2.8 Haematological Analysis

Blood samples of a set of three fish were collected, at the beginning of the feeding trial (week 0) and at the end of trial (week 10), following the procedure described by Stockopf (1993) and Joshi et al. (2000a). Two ml of the blood sample from each fish was collected by cardiac puncture with 2 ml syringe and needle and put in ethylene-diamine tetra-acetic acid (EDTA) treated Bijou bottles. The blood was stored at -40℃ prior to analysis. Analysis includes the direct measurement of erythrocyte values: Haemoglobin (Hb), estimated by cyanomethemglobin method, red blood cells (RBC) and white blood cell (WBC) counted by Neubauer's improved haemocytometer using Hyem's and Turks solution as a diluting fluid respectively.

2.9 Statistical Analysis

Growth performance, nutrient utilization parameters, haematological parameters and proximate composition data were analyzed using One-way Analysis of Variance (ANOVA). Significant differences among means were determined using Duncan's Multiple Range Test (DMRT) on SPSS 15.0.

3. Results

3.1 Growth Performance

Table 5 shows the growth performance and feed utilization of *Clarias gariepinus* fingerlings fed the varying diets. Even though the initial weight of the fish used for this trial were not significantly different from one another ($P>0.05$), the final weight gain of the fish fed diets B20,C30,D40,E50 were significantly lower ($P>0.05$) than that of the control. The MWG of the fish fed diets A10 and B20 were high, comparable to the control diet, but not significantly higher ($P>0.05$) than the other experimental diets. The fish fed A10 had the highest MWG (21.50), while the fish fed E50 had the lowest (21.28). Fish fed diet C30 had the highest percentage weight gain while the lowest was recorded for the fish fed diet E50. The SGR of fish fed the experimental diets were not significantly different ($P>0.05$) from one another nor from the control, but the highest value (0.75) was recorded for the fish fed control diet, while the least (0.47) was recorded for the fish fed diets B20 and D40. The FCR of the fish fed the experimental diets were not significantly different ($P>0.05$) from one another, but significantly lower ($P<0.05$) than that of fish fed the control diet.

Table 6. Growth Performance of *Clarias Gariepinus* Fed with the Experimental Diets

Parameters	Q	A10	B20	C30	D40	E50
Initial weight (g)	30.49^{a}	30.42^{a}	30.42^{a}	30.47^{a}	30.50^{a}	30.49^{a}
Final weight(g)	52.55^{b}	51.92^{ab}	51.89^{a}	51.82^{a}	51.79^{a}	51.77^{a}
Mean weight gain(g)	21.46^{ab}	21.50^{ab}	21.47^{ab}	21.35^{a}	21.29^{a}	21.28^{a}
% Weight gain(g)	72.35^{a}	70.68^{a}	70.58^{a}	70.07^{a}	69.80^{a}	69.79^{a}
SGR	0.75^{a}	0.50^{a}	0.47^{a}	0.49^{a}	0.47^{a}	0.48^{a}
FCR	1.39^{a}	2.34^{b}	2.21^{b}	2.59^{b}	2.52^{b}	2.48^{b}
PER	1.45^{a}	1.43^{a}	1.39^{ab}	1.42^{a}	1.39^{ab}	1.38^{ab}

Note. Suffixes of different letters indicates statistical significant difference among means and same letters in column indicate no significant difference (P>0.05). PER = Protein Efficiency Ratio, FCR = Feed conversion ratio, SGR = Specific Growth Rate.

3.2 Enzyme Activity

Table 4 shows the enzyme activity in the gut of the experimental fish before and after the experiment. There was no significant difference (P>0.05) between the initial amylase enzyme activity and that of the control. However, the amylase activity of the fish fed the trial diets were significantly (P<0.05) higher than the initial and those of the fish fed the control diet. For cellulase, there was significant difference (p<0.05) between the initial and the control. There was significant reduction (P<0.05) in the cellulase activity of the fish as the CFM level in the experimental diets increased. Maltase activity significantly (P<0.05) increased as the fish were fed high level of CFM based diets, with diet C30 recording the highest maltase activity (4.37). The glucanase activity of the fish significantly (P<0.05) reduced in the fish fed the experimental diets when compared with the initial, but when compared with the control, there was significantly higher (P<0.05) glucanase activity of the fish fed the trial diets. When compared with the initial, protease activity of the fish fed the trial diets increased as the fish was fed both the control diet and CFM based diets. The highest protease activity (4.34) was recorded in diet C30.

Table 7. Digestive Gut Enzyme Activities of the Experimental Fish

Enzyme	Initial	Q	A10	B20	C30	D40	E50
Amylase	4.18^{a}	3.78^{a}	3.25^{a}	3.72^{a}	4.25^{b}	4.26^{b}	4.28^{b}
Cellulase	3.17^{a}	0.73^{b}	0.62^{bc}	0.73^{ab}	0.58^{c}	0.69^{ab}	0.65^{b}
Glucanase	1.79^{a}	0.77^{bc}	1.00^{b}	0.67^{c}	0.52^{c}	0.93^{b}	0.90^{b}
Maltase	1.22^{a}	1.88^{ab}	3.26^{b}	3.33^{b}	4.37^{b}	2.94^{bc}	3.95^{b}
Protease	0.59^{a}	2.56^{b}	3.77^{c}	4.02^{c}	4.34^{c}	3.67^{c}	4.17^{c}

Note. Means with the same letters in rows are not significantly different (P>0.05).

3.3 Haematological Parameters

The haematological parameters of *C. gariepinus* fed the control diet and the various *C. forda* fortified diets are presented in Table 5. The PCV of fish fed the control diet was not significantly higher (P>0.05) than the fish fed the *C. forda* diets, but diet E50 recorded the least PCV level. There was no definite pattern in the Hb of the blood of the variously fed fish but the Hb of the fish fed the control diet was significantly higher (P<0.05) than those of the fish fed the experimental diets.

There was no significant difference (P>0.05) between the RBC of the blood of the fish fed the control and the experimental diets. However, the highest RBC (2.75 $x10^6$/µL) was obtained in fish fed the control diet and the lowest (2.55 $x10^6$/µL) in the fish fed diet E50. The White Blood Cell (WBC) count and neutrophils of the fish fed the experimental diets were not significantly different (P>0.05) from those of the fish fed control diets. The lowest WBC (7.81 $x10^6$/µL) was recorded in fish fed the control diet and highest (9.03 $x10^6$/µL) in fish fed diet E50. Neutrophils recorded was highest (70.21 $x10^6$/µL) in fish fed diet A50 and the least (56.17 $x10^6$/µL) obtained in diet D10.

Table 8. Haematological Parameters of the Fish Fed the Various Diets

Parameters	**Experimental Diets**					
	Q	**A10**	**B20**	**C30**	**D40**	**E50**
PCV (%)	26.64^a	25.33^a	24.33^a	24.51^a	24.44^a	24.00^b
Hb (g/100ml)	8.51^a	7.37^{ab}	8.01^a	8.33^a	7.31^{ab}	7.55^{ab}
RBC ($x10^6$/µL)	2.75^a	2.65^a	2.61^a	2.65^a	2.61^a	2.55^{ab}
WBC ($x10^6$/µL)	7.81^a	7.69^a	8.40^{ab}	8.74^{ab}	8.82^{ab}	9.03^{ab}
Neutrophil ($x10^6$/µL)	56.33^a	56.17^a	59.00^a	60.33^{ab}	63.13^{ab}	63.27^{ab}

Mean ± S.E with different superscript are significantly different from each other (p<0.05)

Hb = Haemoglobin; PCV = Pack Cell Volume; WBC = White Blood Cell; RB = Red Blood Cell.

4. Discussion

In the present study, fish fed the experimental diets showed increase in weight without an external sign of nutritional deficiency because growth performances of fish fed *Cirina forda* diet, at various levels up to 50% replacement, improved in terms of weight gain, percentage weight gain and specific growth rate. The fish showed good appetite to all the diets as attested to by the increase in body weight. This shows that *Cirina forda* contained some of the necessary growth factors required by *Clarias gariepinus*. This might also be due to good digestibility of the diet. The experimental fish showed great increases in weight, which indicates that the fish was able to convert the feed protein to extra muscles. Weight gain and species growth rate are usually considered as the most important measurement of productivity of diets (Adesina et al., 2013). Fish fed *Cirina forda* meal showed high specific growth rates comparable to the fishmeal based diet. This result agreed with the findings of Oyegoke et al. (2006), who reported

that there were no significant differences between the growth performances of broiler chicks fed the compounded *Cirina forda* larvae and those fed the conventional fishmeal.

The compounded larval diets contained a crude protein level which is comparable and even higher in quality to that present in the conventional fishmeal. *Cirina forda* larvae, in dried form, had been confirmed to contain 57.96% crude protein (Ande, 1991). Kodondi et al. (1987) have also analyzed three species of saturniid caterpillars (larvae) prepared by the traditional techniques of smoking and drying and found them to be high in riboflavin and niacin. *Cirina forda* was able to cause higher growth rate compared to that observed in the fishmeal probably because of its high protein content and presence of essential minerals and vitamins like sodium, potassium, zinc and manganese as reported by Ande (1991). Keshavanath et al. (2002) reported better utilization of protein from low protein-high carbohydrate diets by common carp grown in manured tanks. Adepoju and Daboh (2013) further reported that the trypsin inhibitor level of *C. forda* was very low and cannot cause protein malabsorption. The positive growth response of Clarias to *Cirna forda* diet could also be attributed to its high gross energy (458.40), crude protein (45.10-55.50), lipid (16.82-18.80) and acceptability by the fish. Adepoju and Daboh (2013) observed that addition of *C. forda* at 5, 10, and 15% levels to fermented sorghum and maize flours significantly increased both micro- and macronutrients of the complementary foods and the nutrient density increased with the inclusion level.

High digestive enzyme activity in fish receiving the test diets would have resulted in better utilization of diets, leading to higher growth. High protease activity in the fish fed the control diet and test diets when compared to the initial value, indicates efficient utilization of protein from all the diets. This is also reflected by the PER value. However, different researchers have shown different results for protease activity. Lopez-Lopez et al. (2005) reported that there is no strong correlation between protease activity and dietary crude protein. According to Le Moullac et al. (1994) and Krogdahl et al. (1999) the quantity of protease and amylase enzyme fluctuate with variation in concentration of carbohydrate and protein in fed diets. However, if concentration of these components increases beyond limits, concentration of amylase and protease start to decrease (Cara et al., 2003). In this experiment, as also reported by Haider et al. (2018), all treatment diets were iso-nitrogenous so there was no significant difference in protease activity.

Kikuchi (1999) reported that fish usually use less carbohydrate, demanding higher protein levels in the feeds. Fish species differ greatly in their ability to digest carbohydrates. De Almeida et al. (2006) reported that digestive functions capable of hydrolyzing a greater variety of carbohydrate-containing feedstuffs have been developed in herbivorous and omnivorous fish, in contrast to carnivorous fish. Lower amylase levels may be indicative of the limited potential of fish to exploit diets containing high carbohydrate levels.

Measuring the activity of digestive enzymes is not enough to determine the value of a specific fish feed, as enzymes act in combination with feed composition, thus when associated with metabolic parameters they are a more reliable indicator of the fish nutritional status (Lundstedt et al., 2004). Digestive enzyme

responses can also be influenced by the feeding period, as changes in protein synthesis and enzyme activity in fishes can be observed after a long feeding period (Krogdahl et al., 1994; López et al., 1999). An increase in White Blood Cells (WBC) and lymphocyte count (lymph) is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Bello, 2013). A measurable increase in white blood cells and neutrophil counts of fish or any animal is a function of immunity and animals' resistance to some vulnerable illness or disease (Akinwande et al., 2004). This increase might indicate that the fish under study had high immunity or resistance to diseases.

From the results of this feeding trial, it can be asserted that *C. forda* can serve as a good source of nutrients in formulating nutrient-rich feed for fish. Fish farmers can utilize the advantages of the insect's availability and nutrient potentials in enhancing the productivity of *Clarias gariepinus* at reduced cost of production.

5. Conclusion

This feeding trial revealed that up to 50% of *Cirina forda* meal inclusion level in the diet of *Clarias gariepinus* was utilized efficiently for good growth and physiological performances. This indicates, that *Cirina forda* meal if thermo-treated to reduce anti-nutrition factor such tannin, as in the dried form, could replace fishmeal up to 50% in the fish feed composition without physiological distress. This level of inclusion would be significant replacement for the expensive fishmeal in feed formulation, since *Cirina forda* meal is an animal resource with less competition for it's use.

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