

Growth and Haematological Studies of African Catfish (*Clarias gariepinus*) Fingerlings Fed with *Drosophila melanogaster* Pupa as Feed Supplement

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Abstract

A three months study was carried out to determine the growth rate and haematological performance of *Clarias gariepinus* fingerlings fed with *Drosophila melanogaster* pupa as feed supplement. The fingerlings were subjected to different insect supplement inclusions to commercial feed in the following rations, 0:100, 20:80, 30:70, and 45:55 respectively, with the 0:100 (no insect inclusion) ration as control. Each of the treatments had 15 fingerlings per 30litres plastic tank at 24.5-26.5°C. At the commencement of the experiment, the fingerlings had initial mean length and weight of 3.0 ± 2.0 cm and 1.8 ± 3.0 g respectively. The fishes were fed twice a day and data were taken at the end of every two weeks to determine the growth and haematological performance of the fishes. The mean weight gains (MWG) recorded at the end of the period of study were 11.5g, 16.8g, 32.7g, 44.1g for the Control and treatments 1, 2 and 3 while the Specific Growth Rate (SGR) were 4.08, 6.17, 7.41, 7.53 respectively. The Feed Conversion Ratio (FCR) and Percentage survival rate values were 30.43, 31.07, 35.70, 46.60, and 86.6%, 93.3%, 86.6%, 86.6%. The greatest performance was recorded in the treatment 3 with insect supplement and fish meal ratio of 45:55. The same trend was recorded for the haematological parameters, with the control having the least values in all the measured parameters. The highest value in the haematological parameters was from tank 3 with Haemoglobin count (HB 8.0 ± 0.80^a), Pack cell volume(PCV 24.0 ± 2.0^a), Total red blood cell (TRBC 3.3 ± 0.50^a) Total white blood cell(TWBC 5.1 ± 0.80^a), Mean corpuscular volume(MCV 7.4 ± 0.50^a), Mean corpuscular haemoglobin (MCH 4.0 ± 2.83^a) and Mean corpuscular haemoglobin concentration (MCHC 3.4 ± 0.06^a) as compared to the control with the following; (HB, 3.02 ± 0.3^c), PCV(9.02 ± 1.04^d), TRBC(1.13 ± 0.27^b), TWBC(1.51 ± 0.06^c), MCV(8.30 ± 1.04^a), MCH($2.72 \pm .40^a$), (MCHC, 3.30 ± 0.37^a). Significant differences were recorded for the HB, PVC, TWBC, TRBC values of the treatment tank 3 juveniles. The result obtained from this study shows that *Drosophila melanogaster* pupa can be administered as supplementary feed for *Clarias gariepinus*.

Keywords: growth, haematological studies, performance, African catfish, *Drosophilla melanogaster* pupa,

Introduction

Fish is a vital source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO, 2012). It is particularly an important protein source in regions where livestock is relatively scarce. FAO (2012) estimates that about one billion people world-wide rely on fish as their primary source of animal protein.

With this increasing demand for intensive aquaculture, demand for more efficient diets for fish is rising (Agbo *et al.*, 2011). Nigeria's aquaculture industry is currently faced with the problem of inadequate supply and prohibitive cost of quality fish feeds (Omitoyin, 2006). The principal

operating cost in the production of fish is feed and the main protein source has been the commercial fish meal (Glencross *et al.*, 2007), which is however scarce and expensive (Agbo *et al.*, 2011). Currently, insects are being considered as a new protein source for animal feed (Premalatha *et al.*, 2011). This is due to the discovery of the nutritional value of insect diets (Banjo *et al.*, 2006). There are approximately one million known species of insects, although it has been estimated that their global diversity is as high as 80 million (Erwin, 2004). Some insect species can be grown on organic side streams, reducing environmental contamination and transforming waste into high protein feed that can replace increasingly more expensive compound feed ingredients, such as fish meal. Then, from an environmental point of view, insect cultures are sustainable; culturing insects is usually performed in warehouses, with no need for large areas or much water, particularly when compared with crops. Culturing insects also contributes to the recycling of waste. In addition, insects are efficient food converters because they do not use energy to maintain a high body temperature (Nijdam *et al.*, 2012).

The African catfish (*Clarias gariepinus*) is generally considered to be one of the most tropical catfish species for aquaculture and since the 1970's it has been a fish of great promise for fish farming in Africa. It has high growth rate, and is a very strong fish appreciated by a wide number of African consumers. Insects have been shown to constitute a part of the natural diet of catfish *Clarias gariepinus*, (Bailey and Harrison, 1945) reported that its insects portion of natural diet consist 2.16% of their organic food material.

Drosophila melanogaster is a species of fly (Class Insecta; order Diptera) in the family *Drosophilidae*. The species is known generally as the common fruit fly or vinegar fly (James *et al.*, 2001). *D. melanogaster* continues to be widely used for biological research in studies of genetics, physiology, microbial pathogenesis, and life history evolution, because it is easy to care for, has four pairs of chromosomes, breeds quickly, and lays many eggs (James *et al.*, 2001).

Haematological (Blood) analysis is a valuable means of evaluating the physiological condition of cultured fish with respect to determining 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 (Bello-Olusoji *et al.* 2006). Adeparusi and Ajayi (2004) reported that analysis of blood is an important factor that could be considered in fish feed assessment. The use of haematological values as indices of diagnosing diseases and stress induced condition as well as for feed assessment is well documented by (George *et al.*, 2007; Yue and Zhou, 2008; Akintayo *et al.*, 2008).

Since the increasing human population of the nation is challenged with measures to meet the protein demand of its populace. There is need for aquaculture to replace the actual depletion of fish stock in the wild coupled with over-exploitation of marine and fresh water resources. Therefore, in order to attain more economically, sustainable, environmentally friendly and viable aqua-cultural production, research interest has been directed towards the evaluation and use of non-conventional sources of insect protein, which could enhance the growth and haematological performance of fishes. Hence this study aims at rearing pupa of *D. melanogaster* for use as feed supplement for *Clarias gariepinus*; Determination of the growth performance of *Clarias gariepinus* fed with pupa of *D. melanogaster*; and the Analysis of the effect of the feed supplement on the haematology of *Clarias gariepinus*.

Materials and methods

Study area

The study was carried out in the Biology laboratory of the Michael Okpara University of Agriculture, Umudike Abia state, Nigeria. Umudike is situated along longitude 7° 32' east and latitude 5° 29' north, 129m² above sea level, within the rainforest zone of south-eastern Nigeria with a mean altitude of 123m. The annual rate of rainfall ranges from 1800-2100mm. It has warm bound climate and temperature that ranges from above 29°C in the wet to slightly over 25°C in the hot season.

Procurement of Materials and Experimental Design

70 pieces of fingerlings of the *Clarias gariepinus* species were purchased at the Oriental Integrated Farms Limited located k/m 4 Ovom Street by Ikot-Ekpene, Aba Abia State. They were carried in a 10litre gallon filled with water to the biology laboratory of Michael Okpara University of Agriculture, Umudike.

The experiment was carried out with four rectangular mini plastic tanks with 30 litres water capacity, fused with a tap to ease discharge of water. The fingerlings were allowed to acclimatize for one week in dichlorinated municipal water. The fishes adapted to the laboratory condition when less than 4% death was recorded in a period of 14 days. Water supply was from the university water system and the water quality was maintained by changing the water in the tanks every three days. After one week of this acclimatization, they were divided into 15 pieces per rectangular treatment tanks labelled tank 1, tank 2, tank 3 and control tank. They were covered with nets so as to help the fishes stay within and not to jump out. The initial length and weight of the fishes were measured and recorded.

Production of *D. Melanogaster* pupa

50 appropriate sized containers were filled with tomatoes fruit and used for the culture of the *Drosophila* pupa. The containers were left open for the introduction of insects (*Drosophila*), and constantly the containers were always made wet with water to make it moist and hence attract flies to lay eggs in it. Pupae were generated from the third to fourth day and were then harvested and stored in the oven to enable drying.

Feeding of the fishes

The fingerlings were fed morning and evening with 1mm of commercial feed (coupens) containing 35% crude protein. They were fed 3% of their body weight and care was taken to avoid over feeding, The *D. melanogaster* pupa was introduced to the fish feed with the following percentages; 0% (control), 20% (T1- treatment 1), 30% (T2- treatment 2), 45% (T3- treatment 3), making for ratios 0:100, 20:80, 30:70 and 45:55 respectively.

Data was collected once in every two weeks throughout the twelve weeks of experimental study to ascertain the weight of the fingerlings (five fingerlings were randomly collected from each tank using a hand net sieve), and subsequently the quantity of feed given was adjusted.

Temperature was taken with thermometer; dissolved oxygen and pH were also measured for water quality using Winkler's solution and pH meter (EIL Richmond Model) respectively.

Growth parameter measurement

The initial, total and standard lengths of the fingerlings were measured at commencement using a meter rule. Similarly, their weights were taken, using a weighing scale (the Ohaus Model Cs Capacity 5000X2g).

From the experimental data obtained, growth rate was determined using the following parameters; Specific Growth Rate (SGR), Mean weight gain (MWG) and Daily weight gain (DWG). Other parameters calculated were Feed Conversion Ratio (FCR) and Survival rate (SR). They were calculated as follows,

Specific growth rate (SGR%/DAY)

$$\text{SGR\% / Day} = \frac{(\ln wf - \ln Wi)}{t} \times \frac{(100)}{1} \text{ (Kolkovski et al., 1995)}$$

Where $\ln wf$ = natural log of final average weight at the end of the experiment.

$\ln wi$ = natural log of initial average weight at the beginning of the experiment.

t = culture period in days

Feed conversion ratio (FCR)

FCR = Amount of Feed/Weight gain(g) (Kolkovski et al., 1995)

$$\text{FCR} = \frac{\text{Dry weight of the diet fed (g)}}{\text{fish weight gain (g)}}$$

Survival rate (SR %)

$$\text{SR\%} = \frac{N_1}{N_0} \times \frac{(100)}{1} \text{ (Kolkovski et al., 1995)}$$

Where N_1 = total no of fish alive at the end of the experiment

N_0 = Total no of fish stocked at the beginning of the experiment

Daily weight gain (DWG)

$$\text{DWG} = \frac{wf - wi}{t} \text{ (Kolkovski et al., 1995)}$$

Where W_f = final average weight at the end of the experiment

W_i = initial average weight at the beginning of the experiment

t = culture period in days

Mean Weight Gain (MWG)

$Mw_2 - Mw_1$ (Kolkovski et al., 1995)

Where Mw_2 = final mean weight after experiment

Mw_1 = initial mean weight at beginning of experiment.

Haematological Analysis

Three weeks to the end of the 12 weeks experiment, three fishes were randomly taken from each tank. Blood (1-2ml, depending on fish size) was collected from the vertebral blood vessel towards the caudal peduncle of each fish using a 2ml string. The blood samples were collected from cardiac puncture using 2mL disposable heparinised syringe treated with EDTA as anti-coagulant. The blood produced after centrifugation were analyzed using the Haematocrit for the (PCV), whole blood hemoglobin (HB), Total red blood cell (RBC) and white blood cell (WBC). The blood analyses was done according to the method described by Svobodova et al. (1991).

Blood cell count: Haemocytometer was used in blood cell count through the Neubauer chamber. The blood diluting fluid was prepared as described by Svobodova et al., (1991). The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope:

- **RBC** = No of cells counted $\times 3 \times 10 \times 200$ (10^6 mm³)
- **WBC** = No of cells counted $\times 0 \times 25 \times 10 \times 20$ (10^4 mm³)

- **Haemoglobin estimation:** Haemoglobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI):
- **Packed cell volume (PCV):** The packed cell volume was measured after placing sealed micro-haematocrit tube in a centrifuge at 10,500 rpm using micro-haematocrit reader and expressed as percentage (Svobodova *et al.*, 1991).
- **Mean corpuscular volume (MCV);** this is the mean volume of the erythrocyte counted in the sample and it was calculated using the standard formula
$$MCV = \frac{\text{hematocrit} \times 1000}{\text{red blood cell count}}$$
 (Svobodova *et al.*, 1991).
- **Mean corpuscular haemoglobin (MCH);** this represents the mean mass of haemoglobin in the red blood cell (RBC) and it is calculated thus
$$MCH = \frac{\text{hemaglobin} \times 10}{\text{red blood cell count}}$$
 (Svobodova *et al.*, 1991).
- **Mean corpuscular haemoglobin concentration (MCHC);** this is the mean concentration of haemoglobin in the red blood cell, it is calculated thus,
$$MCHC = \frac{\text{hemaglobin}}{\text{hematocrit}}$$
 (Svobodova *et al.*, 1991).

Data Analysis

Analysis of variance (ANOVA) was used to analyse the variation in growth rate and hematological results obtained for *Clarias gariepinus*. This was based on the mean monthly weight and length gained through the period of this research.

The results were analyzed using one way ANOVA, fisher's least significant differences 0.05 was used in separating possible treatment mean differences. SPSS V₂₀ was used to determine the statistical analysis.

RESULTS

Growth performance

Weight

The three treatments with different inclusion level of *Drosophila melanogaster* pupa as feed supplement together with the fish meal and the control (Fish meal alone) recorded different mean weight gains. The highest was from treatment 3 with 44.1g which indicate that the diet was acceptable to the fish. The next to treatment 3 was treatment 2 (32.7g), followed by T1 (16.8g) and finally the control (11.5g) which had no inclusion of the insect supplement.

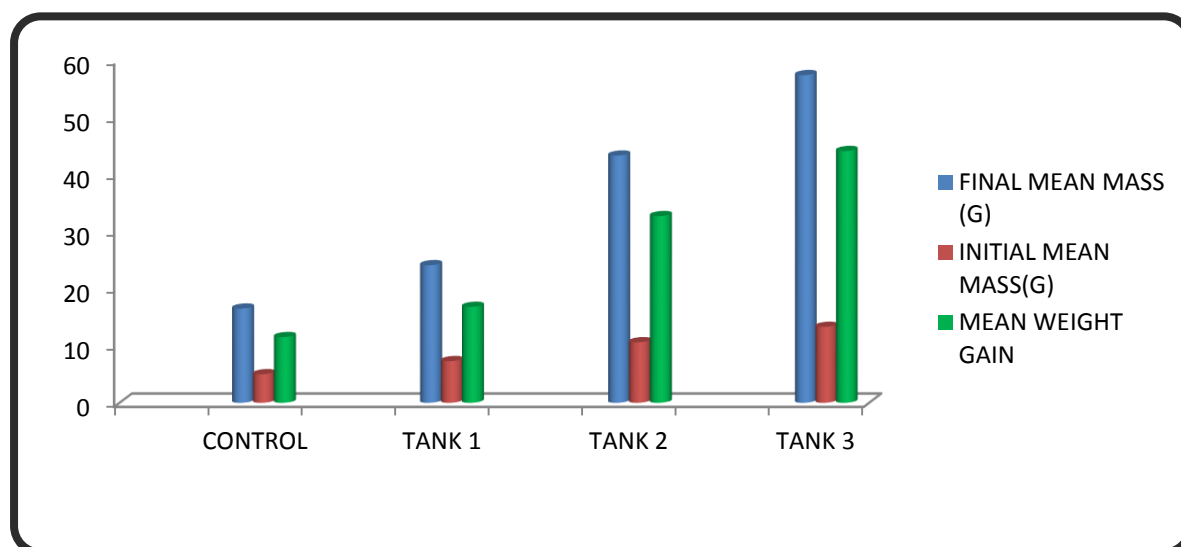


Figure 1 showing the mean weight gain of the fishes.

Length Gain

Table 1 shows the increase in the length of the fishes. Tank 3 had the greatest increase in length, with an average length gain of 6.14cm, followed by tank 2 which had 4.02cm, then tank 1 having 3.74cm. The control tank recorded the lowest increase in length.

Table 1 showing the average length gain

Treatments	Initial mean length (cm)	Final mean length (cm)	Average length gain (cm)
Control	3.78	7.12	1.67
Tank 1	3.94	11.42	3.74
Tank 2	4.42	12.46	4.02
Tank 3	5.20	17.48	6.14

Mean Growth Rate

Figure 2. below shows the result for the mean growth rate of the fishes in the 3 months of this research. The mean growth rate values of the tanks were 1.530, 1.396, 1.381 and 1.32 for Tanks 3, 2, 1 and the control respectively.

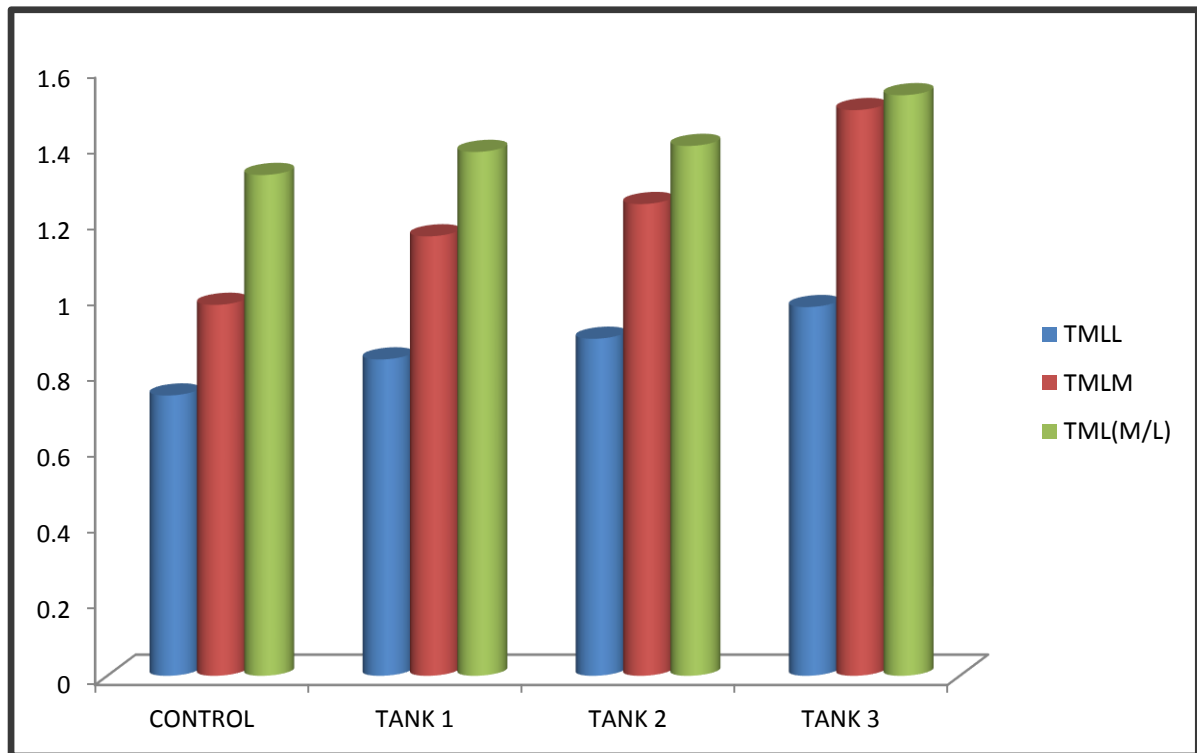


Figure 2 showing the mean growth rate

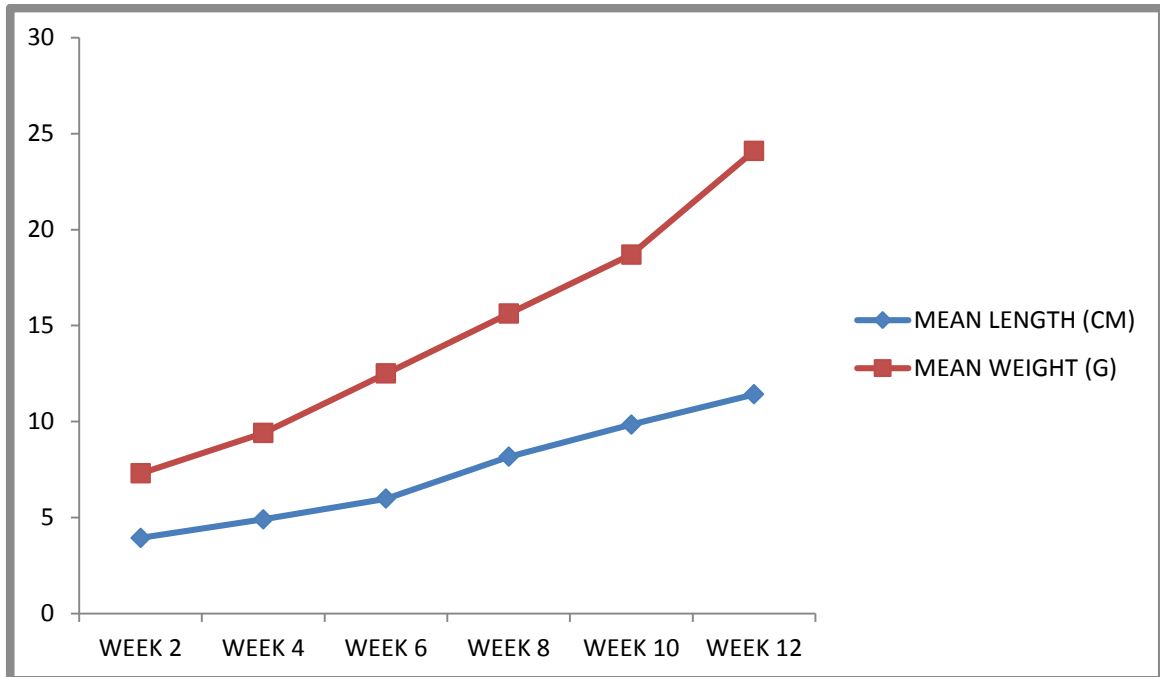


Figure 2. Showing the Growth Rate in control tank for 12 Weeks

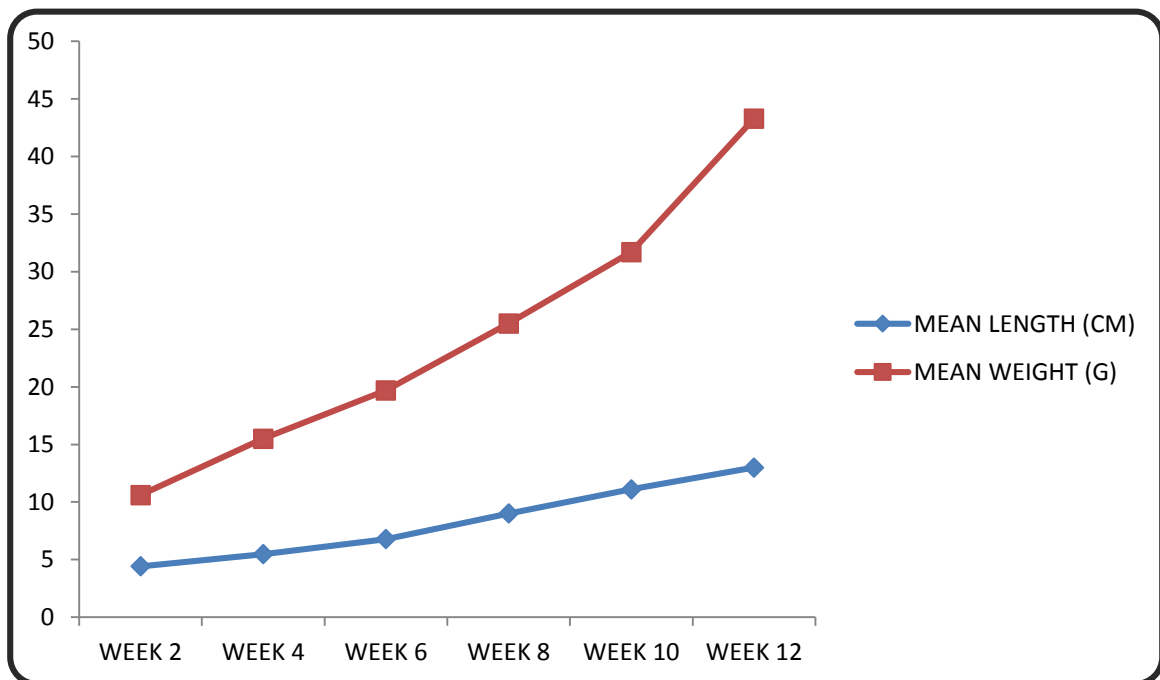


Figure 3 Showing the Growth Rate in Tank 1 for 12 Weeks

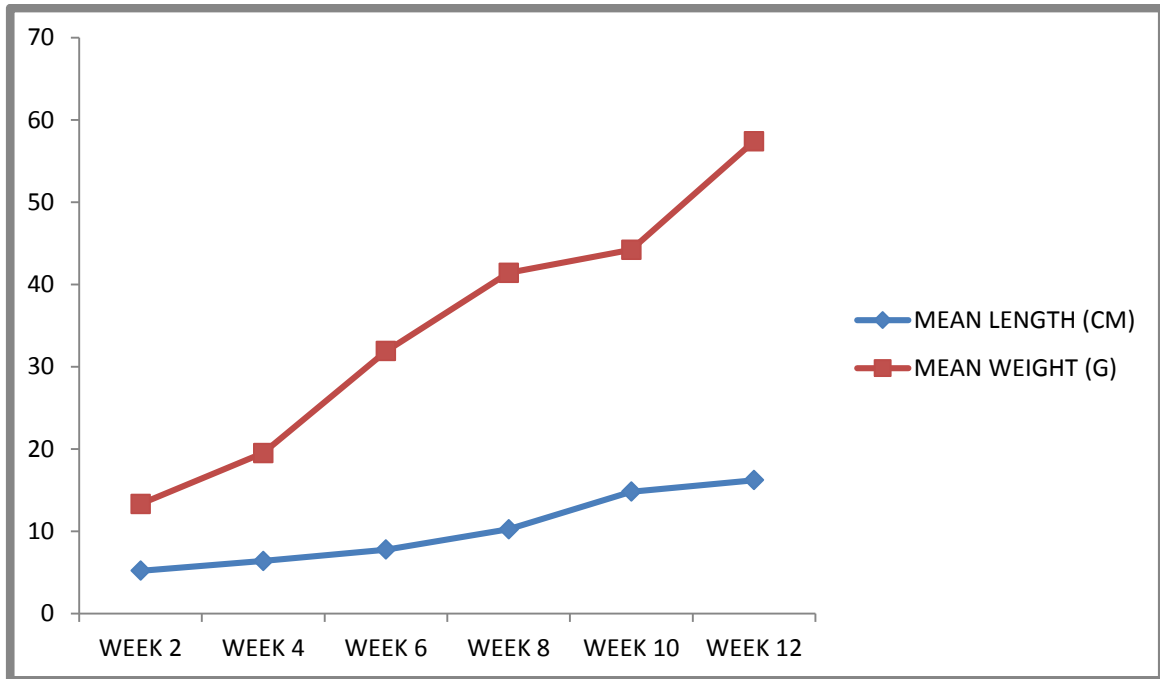


Figure 4. Showing the Growth Rate in Tank 2 for 12 Weeks

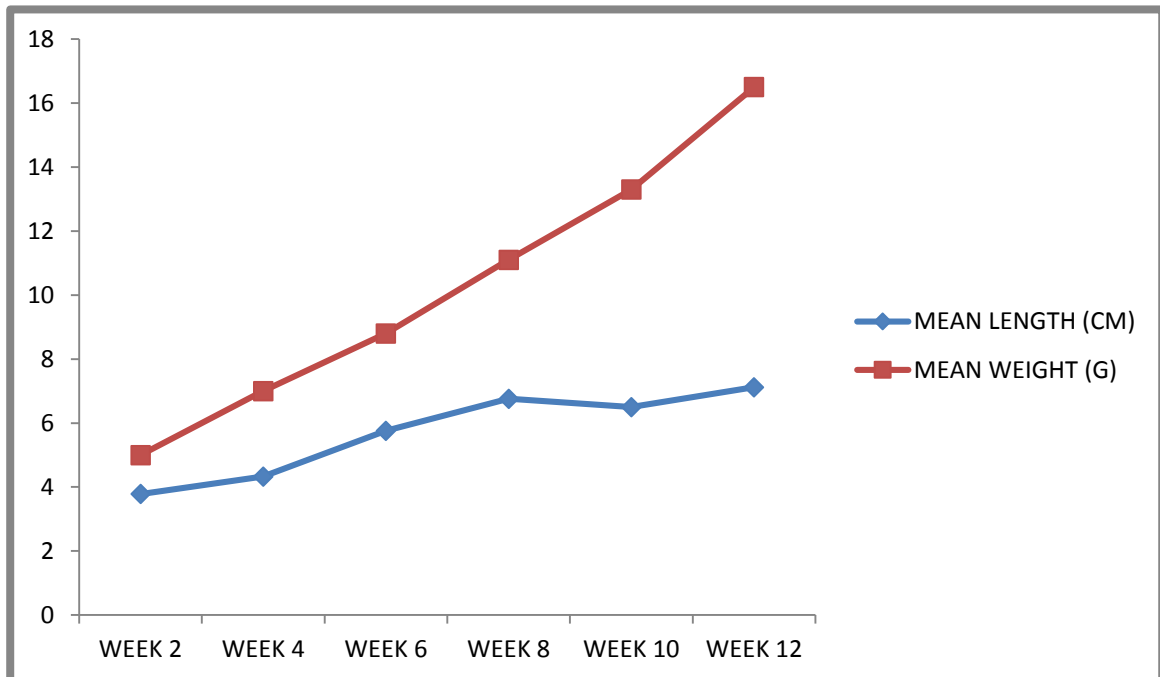


Figure 5. Showing the Growth Rate in Tank 3 for 12 Weeks

Specific Growth Rate;

The specific growth rate of the various tanks were 7.5321g, 7.4071g, 6.1690g and 4.0845g for tanks 3, 2, 1 and the control respectively (Table 2)

Table 2. Showing the specific growth rate in the various tanks.

TREATMENTS	FINAL MEAN LOG OF MASS (G)	INITIAL MEAN LOG OF MASS(G)	SGR
CONTROL	1.2167	0.6985	4.0845
TANK 1	1.3818	1.0387	6.1690
TANK 2	1.6461	1.0239	7.4071
TANK 3	1.7589	1.1262	7.5321

3.1.5 Feed Conversion Ratio

Table 3 shows that conversion ratio. The results for the various tanks were 46.60g, 35.70g, 31.07g and 30.43g for control, tanks, 1, 2, and 3 respectively.

Table 3 showing the feed conversion ratios of the fishes

TREATMENTS	FCR
CONTROL	46.6
TANK 1	35.7
TANK 2	31.07
TANK 3	30.43

Percentage Survival Rate

Figure 3 shows the percentage survival rate. The highest was in tank 1 with 93.3% survival rate followed by other tanks which had the same percentage survival rate of 86.6%.

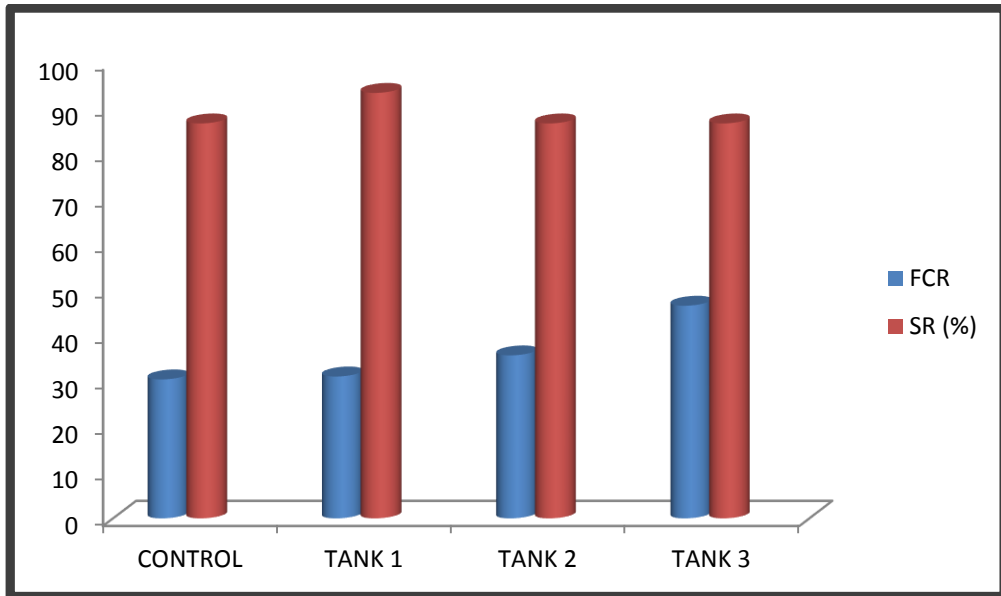
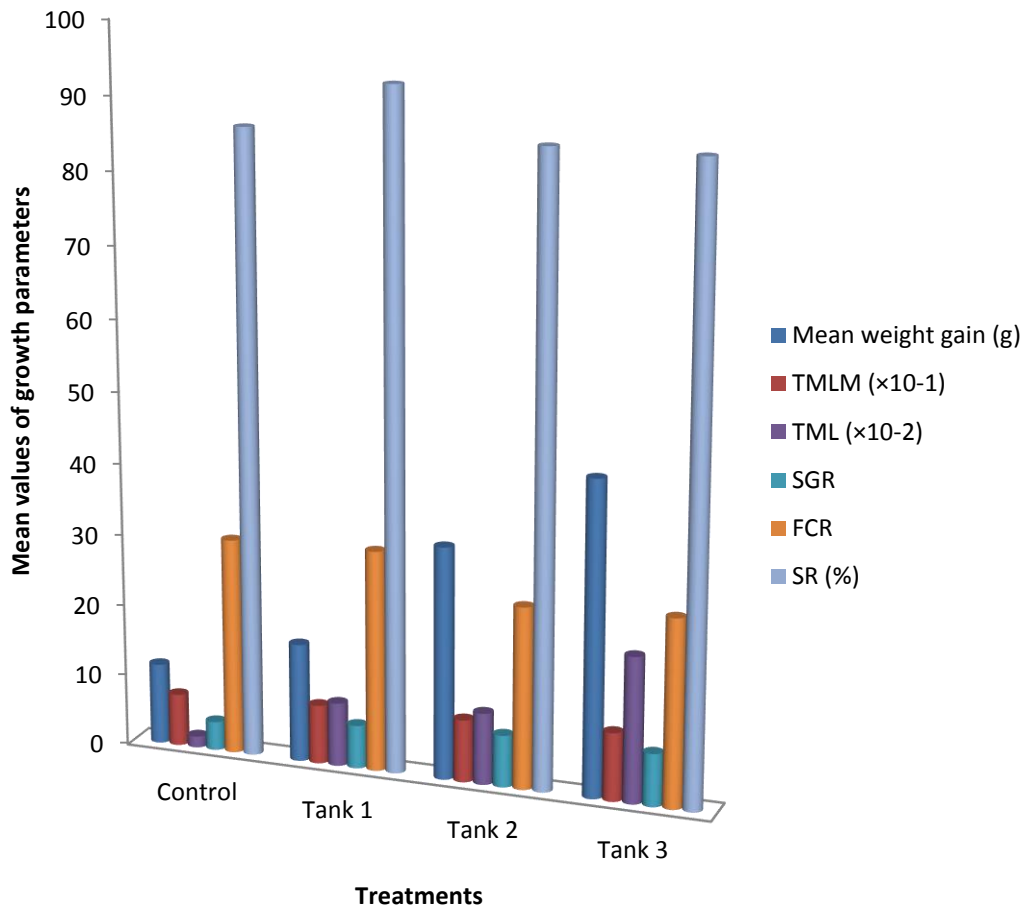


Figure 3; showing the feed conversion ratio and the percentage survival.

A Bar Chart Showing All the Growth Parameters Together



Heamatological Results

Table 4 shows the hematological changes in the fingerlings fed with the different levels of the insect and the control. The fingerlings fed with the insect supplement showed a significantly remarkable increase ($P < 0.05$) in hematological parameter values compared to the fish fed with fish meal diet only. Table 5 shows the level of significance in the hematological parameters.

Table 4; Showing the result for the hematological analysis.

TREATMENTS	HB	PCV	TRBC	TWBC	MCV	MCH	MCHC
CONTROL	3.02 $\pm 0.3^c$	9.02 $\pm 1.04^d$	1.13 $\pm 0.27^b$	1.51 $\pm 0.06^c$	8.30 $\pm 1.04^a$	2.72 $\pm 0.40^a$	3.30 $\pm 0.37^a$
TANK 1	4.12 $\pm 0.86^c$	12.3 $\pm 2.51^c$	1.62 $\pm 0.47^b$	3.2 $\pm 0.55^b$	7.71 $\pm 1.21^a$	2.91 $\pm 0.66^a$	3.3 $\pm 0.17^a$
TANK 2	6.5 $\pm 0.46^b$	19.01 $\pm 1.02^b$	2.6 $\pm 0.59^a$	4.30 $\pm 0.64^{ab}$	7.3 $\pm 1.47^a$	2.5 $\pm 0.46^a$	3.4 $\pm 0.07^a$
TANK 3	8.0 $\pm 0.80^a$	24.0 $\pm 2.0^a$	3.3 $\pm 0.50^a$	5.1 $\pm 0.80^a$	7.4 $\pm 0.50^a$	4.0 $\pm 2.83^a$	3.4 $\pm 0.06^a$

HB - hemoglobin count, PCV - pack cell volume, TRBC - total red blood cell count, TWBC, - total white blood cell count, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, and MCHC - mean corpuscular hemoglobin concentration. Superscripts a, b, c, d is showing the level of significance.

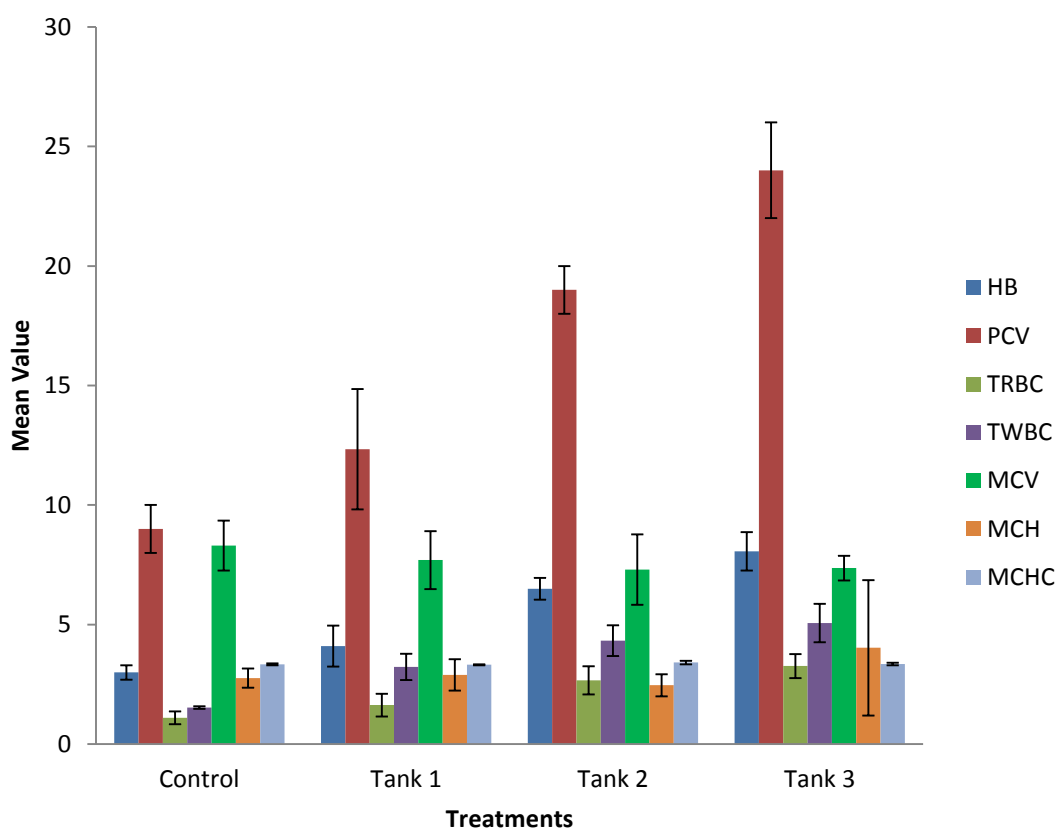


Figure 5; showing a graphical representation of all the haematological parameters

Discussion

The result of this experiment indicating increase in the growth of the fishes fed with the pupa is in consonance with the findings of Steffens (1989) who reported that insect pupa used as feed supplement contributed to the increase in growth of *Clarias* species. There was a drastic increase in the treatment 3 when compared to other tanks with lower inclusion of the insect pupa while the control which has no insect supplement was recorded as having the lowest growth rate.

The increase in tank 3 as well as the other treatments when compared to the control tank is in line with the work of Glencross *et al.*, (2007) who reported that the growth of fish was dependent on the percentage ratio of insect meal to fish feed. This is also in agreement with comparative feed trials of Henken *et al* (1986) who recorded best growth results for feed containing 40% and 58% insect supplement respectively. The result also concurs with that of Giri *et al.*, (2003) who reported an increase in body weight gain and SGR in post larvae of *Clarias* hybrid fed increased level of protein.

The feed conversion ratio (FCR) was highly efficient in tank3 with 30.43g which indicate more efficient utilization of the diet. Treatment 2 (31.07g) followed and finally treatment one (35.7g) and then the control (46.6g). The increase in the treatment 3 is supported by Fasakin *et al.* (2003) who attributed the reduction in growth performance of experimental maggot to low protein digestibility of the feed stuff among other reasons.

Haemoglobin had the highest increase (from tank3) (8.0 ± 0.80) followed by T2 (6.5 ± 0.46^b), T1 (4.12 ± 0.86^c) and Control tank (3.02 ± 0.3^c) in which there was no significant differences at ($p < 0.05$). The increase recorded on the haematological parameter is supported by Akintayo *et al.* (2008) who reported a measurable increase in the haematological parameter in experimental fish fed with toasted sunflower seed meal. Also, similar results were recorded when cotton seed meal was fed to juvenile hybrid Tilapia (El-Saidy *et al.*, 2004). There was also an increase in the PCV level in T3 (24.0 ± 2.0^a), followed by T2 (19.01 ± 1.02^b), T1 (12.3 ± 2.51^c), CT (9.02 ± 1.04^d). The reported increase in the TRBC of the fishes as compared with the control 1.13 ± 0.27^b and tank 3 (3.3 ± 0.50^a) recorded no significance at ($p > 0.05$). Also, there was an increase on the TWBC of the fishes compared with the control tanks which recorded no significance. But there was significance at ($p > 0.05$) in the control diet and fish fed test diets with respect to MCV, MCH, MCHC. This is in line with Joshi *et al.* (2002), which reported that the survival of any fish can be correlated with increase in antibody production which helps in their survival and recovery.

Conclusion

Based on the results of the research, using *Drosophila melanogaster* as a supplement to the diet of African catfish *Clarias gariepinus* is a good alternative to commercial fish feed. This will help reduce the cost of fish (*Clarias gariepinus*) farming, as the conventional fish meal which has high demand and thus, not always readily available and costly will to a great extent be supplemented with *drosophila melanogaster* pupa. The cheap cost of *Drosophila melanogaster* (fruit fly) pupa with high nutritional values as feed supplement to fishes therefore is an attraction to aquaculture.

Recommendation

It is therefore recommended that *Drosophila melanogaster* pupa be used in the formulation of fish meal to obtain optimal growth since the conventional feed is very expensive. Emphasis should hence be made on the culture and availability of the pupa of *Drosophila melanogaster*

supplement which holds great promises for the future of aquaculture in Nigeria and the world at large. Further researches are therefore advocated for, in order to fortify and improve on this diet for better performances.

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