

Growth Assessment and Microbial Flora Presence in African Catfish (*Clarias gariepinus*) Larvae Fed Live and Commercial Feeds

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Abstract: Assessment was carried on the growth and microbial flora presence in African catfish (*Clarias gariepinus*) larvae fed with live food (Artemia) and commercial feed (Aller Aqua). Two days old larvae ($n=200$) of average weight (4.8 ± 0.01 mg) and length (6.16 ± 0.03 mm) were reared in triplicates for 21 days in plastic tanks (40cm x 25cm x 25cm) dimension. The experiment was done in 12 hours static and 12 hours flow through periods each day. The physico-chemical parameters were within acceptable range for catfish culture except in the static periods where ammonia was present in values (0.30 ± 0.00) that are unacceptable. However, nitrate and nitrite were present but in low values. Both feeds showed commendable performance in growth parameters, but Artemia with lower crude protein content did better. There was presence of *Total heterotrophic bacteria*, *Vibrio*, *Total coliform* and *Salmonella/Shigella* in the microbial analysis of the fish waters in all the treatment. Apart from total heterotrophic bacteria that was significantly present in all the experimental waters *Vibrio*, *Total coliform* and *Salmonella/Shigella* were less than thirty (<30) cfu. Also, fish gets older the quantity of microbial flora present in the fish waters reduces.

Keywords: Aquaculture, Larvae, *Clarias gariepinus*, Feeds, Microbial Flora

Introduction

Aquaculture production is increasing with increase population. Between 1987 and 1997 (within 10 years) the world aquaculture production was doubled (FAO, 1999), and between 1999 to 2001 (4 years) there was 29.37% increase in the world aquaculture production (FAO, 2011). In Nigeria, the aquaculture production as at 2007 was eighty-five thousand (85,000) metric tons (FDF, 2008) and increased to two hundred thousand (200,000) metric ton as at 2012 (FAO, 2013).

Fish as an aquaculture product is a solution to one of the world greatest challenge “malnutrition” due to its high nutritive values above other protein source (Delgado *et al*, 2003; Fasakin, 2007). Omega -3 fatty acid which is one of the component of fish oil is a major source of treatment to health challenges such as cardiovascular diseases, eye disorders, cancers, neurological problems (Ukwé *et al*, 2018). The above qualities of fish have led to its high demand that keeps increasing to meet up the uncontrolled increase in human population, and this has led to serious decline in the natural catch (Delgado, 2003). Over

half a billion persons in the developing nations depends on fisheries and aquaculture for their livelihood (Future Directions International, 2013), and this had led to series of employments and job opportunities in the practice of aquaculture (Ukwé *et al*, 2018).

Despite other challenges, poor seed and feed availability are some of the major problems of the world aquaculture growth (World Bank, 2006; Future Directions International, 2013). Feed determines to a large extent the sustainability in aquaculture, since the survival and growth of the fish larvae depends on the quality of feeds used (Ukwé *et al*, 2017). The cost of feeds tends to hinder the production of aquaculture as a result of formulation, components, and management (Kaushik *et al*, 2004; Enes *et al*, 2006). Fast growth rate in fish is an expected quality in aquaculture which can be achieved by administering live and artificial feeds (Bhosale *et al*, 2010). Live food have been accepted as the best feed in aquaculture, but their cost is a bottle neck to production. Artificial feeds though cheaper have been observed to compete with live feeds and even did



well in some growth parameters (Ukwe *et al*, 2017; Ukwe, 2018).

One of the major setbacks in aquaculture production is the outbreak of diseases. It leads to economic losses, and scarcity of fish and fish products. Diseases as a result of bacteria presence are the main source of death in aquaculture especially in the hatchery (Grisez and Overvier, 1995), and two factors that determine the presence of bacteria in aquaculture are the source of water and the type of feed administered (Obianeme and Obire, 2017; Ukwe *et al*, 2018). Success in intensive aquaculture depends on the quality of fish feeds (FAO, 2011), since it determines growth of the fish and to some extent, the proliferation of bacteria in the system. The African catfish is the most farmed fish in Nigeria, because of its good market value, fast growth, good conversion of feed to flesh, and ability to resist diseases (Jamabo and Dienye, 2017).

Materials and Methods

Experimental area and design

The experiment was carried in the hatchery unit of the University of Port Harcourt fish farm, Choba, Port Harcourt, Rivers State, Nigeria. Two days old ($n=200$) *C. gariepinus* larvae of average weight (4.8 ± 0.01 mg) and length (6.16 ± 0.03 mm) were transferred in triplicate design into plastic tanks of 40cm x 25cm x 25cm dimension, containing thirty (30) litres of water. Feeding commenced when their yolk sack was completely absorbed, at 10% body weight daily; and feeding was adjusted weekly in accordance to their weight gain.

Physico-chemical parameter of the experimental water

Physico-chemical parameters (temperature, dissolved oxygen, pH, ammonia, nitrite and nitrate) were taken at 6.00am and 6.00pm daily to determine the values at the static and flow through periods. Temperature was determined by the use of mercury-in-glass thermometer calibrated in degree centigrade ($0-100^{\circ}\text{C}$). Dissolved oxygen (DO) was determined using a 9-series multi-parameter water quality meter (Bante 980 Precision D. O. Meter, Bante Instruments, Beijing China, Version Number-2009070200). The ammonia, nitrate and nitrite test was conducted using la Motte Aquaculture test Kit (Model AQ-4, Code 3635-04, Chester town, Maryland, 21620 USA).

Proximate composition of experimental feeds

The proximate composition of the test feeds were determined using the standard of analysis of the Association of Official Analytical Chemists (AOAC, 1990).

Microbiological analysis

The microbiological analysis were determined using the American Public Health Association (APHA)

1998, on waters of the static and flow through periods. Total number of coliforms in a water sample was determined by the Most Probable Number test.

Measurement of Growth Performance

The weight was determined by the use of Rohrer electric sensitive weight balance (Model: 2002N, No. 110628014, Warten Instrument Co. Ltd China), and the length was determined using a transparent millimeter calibrated ruler.

Survival: Survival rate was determined using the formula:

$$\% \text{ survival} = \frac{\text{final number of larvae}}{\text{initial number stocks}} \times 100$$

Specific growth rate (SGR): SGR was determined using the formula:

$$\text{SGR} = \frac{\ln W_t - \ln W_0}{t} \quad (\text{Arimoro, 2007})$$

Where W_t =Final body weight; W_0 = Initial body weight; t =time (days); \ln =Logarithms of numbers

Percentage Weight Gain (PWG): PWG was determined using the formula:

$$\text{PWG} = \frac{\text{Weight gain (g)}}{\text{Fish Weight (g)}} \times 100 \quad (\text{Richiur, 1979})$$

Daily Weight Gain (DWG): DWG was determined using the formula:

$$\text{DWG} = \frac{\text{Mean weight increase per day}}{\text{Fish body weight}} \quad (\text{Richiur, 1979})$$

Relative Weight Gain (RWG): RWG was determined using the formula:

$$\text{RWG} = \frac{\text{final weight} - \text{Initial weight}}{\text{Initial weight}} \quad (\text{Mbadwu and Adeniji, 1988}).$$

Absolute Growth Rate (AGR): AGR was determined using the formula:

$$\text{AGR} = \frac{\text{final Weight} - \text{Initial Weight}}{\text{Growth Period}} \quad (\text{Orisamuko, 2006})$$

Average Daily length Gain (ADLG): ADLG was determined using the formula:

$$\text{ADLG} = \frac{\text{final Length} - \text{Initial Length}}{\text{Days}} \quad (\text{Penase and Meguonhan, 2015})$$

Food Conversion Ratio (FCR): FCR was determined using the formula:

$$\text{FCR} = \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight (g)}} \quad (\text{Olusola and Olorunfeni, 2017}).$$

Protein Efficiency Ratio (PER): PER was determined using the formula:

$$PER = \frac{\text{Body weight gain (g)}}{\text{Crude protein fed}} \quad (\text{Olusola and Olorunfemi, 2017})$$

Condition Factor (K)

$$K = \frac{W}{L^3} \times 100 \quad (\text{Panase and Mengumphan, 2015})$$

W=Weight (g); Length (cm)

Statistical analysis

Statistical analysis was carried out on all data using the SPSS Version 12 for windows, 2000. Data was

pooled by treatment and presented as mean \pm standard deviation (SD) or standard error of the mean (SEM), was analyzed for treatment effect by one way analysis of variance (ANOVA). The Turkey Post hoc test was used to 95% confidence level to produce specific information on which means are significantly different from each other.

Results

The proximate composition of the two experimental diets (Aller Aqua and Artemia) are shown in Table 1. There were significant difference in all the parameters measured apart from the moisture content

Table 1: Proximate Composition of Experimental Diets (Mean \pm SD)

Parameters	Experimental Diets	
	Aller Aqua	Artemia
Moisture content (%)	10.25 \pm 0.02 ^b	10.25 \pm 0.04 ^b
Protein (%)	61.83 \pm 00.5 ^a	48.55 \pm 0.03 ^b
Fibre (%)	0.93 \pm 0.02 ^a	6.42 \pm 0.03 ^b
Fat (%)	11.35 \pm 0.05 ^a	2.95 \pm 0.04 ^b
Ash content (%)	11.50 \pm 0.02 ^a	14.74 \pm 0.03 ^b
Carbohydrate (%)	14.15 \pm 0.03 ^a	17.06 \pm 0.03 ^b
Energy (cal/100g)	366.8 \pm 0.04 ^a	298.0 \pm 0.03 ^b

Mean within the same row with different superscript are significantly different (P < 0.05).

Physico-Chemical Parameters of the Experimental Waters

The summary of the physico-chemical parameters of the experimental waters during the periods of the flow through and static water within the twenty one (21) days are shown in Tables 2. The reveals the fact that Ammonia (NH₃), nitrate (NO₃) and nitrite (NO₂) were completely absent in the flow through periods, but present in the static water period.

Growth performance and Nutrient Utilization

The results of the growth, nutrient utilization, and survival rate are shown in table 3. Through both feeds showed commendable performance in the regard, Artemia did well compared to Aller Aqua in most of parameters, except survival rate.

Microbiological Analysis

The result of the microbiological analysis is shown in table 4, with total heterotrophic count noticeably present in the experimental waters of both diets, while vibro count, total coliform count and salmonella/shigella were scanty.

Table 2: Summary of physico-chemical Parameters of Water in the Experimental tanks during flow through periods within 21 days (Mean \pm SEM)

Parameters	Experimental Diets			
	Static Period		Flow through Period	
	Aller Aqua	Artemia	Aller Aqua	Artemia
Temperature (%)	27.43 \pm 1.51 ^a	27.11 \pm 1.37 ^a	27.85 \pm 1.15 ^a	27.49 \pm 1.34 ^a
pH	6.24 \pm 0.23 ^a	6.28 \pm 0.26 ^a	6.11 \pm 0.45 ^a	6.13 \pm 0.48 ^a
Dissolve oxygen (mg/l)	6.05 \pm 0.11 ^a	6.17 \pm 0.11 ^a	6.32 \pm 0.16 ^a	6.37 \pm 0.16 ^a
NH ₃ (mg/l)	0.30 \pm 0.00 ^a	0.30 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
Nitrate (mg/l)	0.50 \pm 0.00 ^a	0.30 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
Nitrite (mg/l)	0.05 \pm 0.00 ^a	0.05 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 3: Growth/Nutrient Utilization Response in *C. gariepinus* Fry Fed Experimental Diets within 21 days (Mean± SEM)

Parameters	Experimental Diets	
	Aller Aqua	Artemia
Weight Gained (mg)	19.20± 9.89 ^a	24.64± 14.45 ^b
Length increase (mm)	5.04±3.16 ^a	7.21±4.61 ^b
Survival (%)	54.06±17.40 ^a	49.61±20.77 ^b
Specific Growth Rate (% d ⁻¹)	11.55±2.15 ^a	12.40±1.44 ^a
Condition Factor (K)	1.89± 0.46 ^a	1.42± 0.46 ^b
Percentage Weight Gained (%)	75.75±12.00 ^a	78.00 ±13.64 ^a
Absolute Growth Rate (mg)	1.33± 0.36 ^a	1.62± 0.47 ^a
Daily Weight Gain (mg)	0.09 ± 0.04 ^a	0.09 ± 0.04 ^a
Average Daily Length Gain (mm)	0.77 ± 0.08 ^a	0.78 ± 0.16 ^a
Relative Weight Gained (%)	4.00 ± 2.06 ^a	5.13 ± 3.01 ^b
Feed Conversion Ratio	1.67 ± 0.65 ^a	1.87 ± 0.55 ^a
Protein Efficiency Ratio	2.69±1.03 ^a	3.88 ± 1.16 ^b
Protein Intake	9.67± 4.2 ^a	7.97±3.69 ^b
Gross Feed Conversion Efficiency	75.02 ±45.46 ^a	75.02 ± 45.46 ^a

Mean within the same row with different superscripts are significantly different (P < 0.05).

Table 4: Microbial Analysis of Water in Experimental Tanks of *C. gariepinus* Fed with Aller Aqua and Artemia (cfu)

Feed	Duration (week)	Microbial Flora							
		Static Period				Flow through Period			
		THC	VIBC	TCC	SMS	THC	VIBC	TCC	SMS
Aller Aqua	1	5.4 x 10 ³	< 30	< 30	< 30	2.6 x 10 ²	< 30	< 30	< 30
	2	4.6 x 10 ³	< 30	< 30	< 30	2.4 x 10 ²	< 30	< 30	< 30
	3	3.9 x 10 ³	< 30	< 30	< 30	2.0 x 10 ²	< 30	< 30	< 30
Artemia	1	4.3 x 10 ³	< 30	< 30	< 30	2.2 x 10 ²	< 30	< 30	< 30
	2	3.6 x 10 ³	< 30	< 30	< 30	1.7 x 10 ²	< 30	< 30	< 30
	3	2.1 x 10 ³	< 30	< 30	< 30	1.2 x 10 ²	< 30	< 30	< 30

Key: THC-Total Heterotrophic Count; VIBC-Vibro Count; TCC -Total Coliform Count; SMS - Salmonella/Shigella.

Discussion

Proximate composition of the experimental diets

The proximate composition of the experimental diets are within the range recommended for fish growth and survival of catfish culture (Tibbets and Lall, 2003; Li *et al*, 2014), except in the energy content, which Aller Aqua had significantly higher value.

Physico-Chemical Parameters of the Experimental Waters

The values for the water quality were within the range recommended for aquaculture practice (Diyaulu, 2015; Ukwe *et al*, 2018), except in the static period that the ammonia, nitrate and nitrite were present in quantities that is threatening to fish health (Wurts and Durborow, 1992; Boyd, 1979). The presence of the ammonia, nitrate and nitrite could be as a result of leftover feed decay, and biological activities of the fish larvae and micro-flora within the period of the non-water exchange (FAO, 2015; Ukwe *et al*, 2017).

If left for a long time, the presence of ammonia, nitrate and nitrite has the capacity to negatively affect the survival, growth and causes death in fish larvae (Robiette, 2006; Ganguly *et al*, 2013), because of the negative effect on the dissolve oxygen content of the aquatic environment (Deakae, 2009; Amakiri, 2011). This was evident in this work as mortalities were recorded more at the end of every static period.

Growth performance and Nutrient Utilization

Artemia fed larvae had higher weight gain and growth rate than Aller Aqua fed larvae, despite the fact that Aller Aqua have higher protein content than Artemia. This is contrary to the report of Mondal *et al*, (2007) who reported that increase in dietary protein leads to increase in growth, but it is in agreement with report of Ukwe *et al*, (2017) were Artemia with less protein (48.55%) content did better in growth than Aqualis with protein content of (53.74%). This could be as a result of the fact that Artemia as a live feed has its protein properly utilized for growth as evident in its high protein efficiency

ratio (Madu *et al*, 2003; Ajani *et al*, 2014), it could also be as a result of the fact that the live exogenous enzymes that comes with live feeds facilitated digestion in the larvae, since their digestive system at this stage is poorly developed (Person, 1989). The higher energy content of Aller Aqua can also be a factor why Artemia with lower protein content did better than Aller Aqua, because the larvae tends to eat less quantity of the feed to be satisfied (Ukwe *et al*, 2017; Ukwe, 2018). The result of this experiment is in conformity with the report of Bukola *et al*, (2015).

The percentage survival was higher in Aller Aqua fed larvae than Artemia fed larvae, this could be as a result of the stress due to experimental procedures (Amadi and Solomon, 2011), it could also be as a result of cannibalism in the Artemia fed larvae arising from its fast growth rate. The result of survival rate in this experiment is in deviance with that of Sales (2011) who reported that larvae fed formulated diets have 2.5 times higher chance to die than larvae fed live feeds, and Person (1989) who reported that live feeds promotes survival when compared to formulated diets. But it is in agreement with Ukwe *et al*, (2017) who reported that Aqualis a commercial diet had better percentage survival when compared to Artemia as larval feed.

Microbiological Analysis

The microbiological activities of the various experimental water confirms the presence of varying quantities of the following bacteria:- *Total Heterotrophic bacteria*, *Vibro*, *Total coliform* and *Salmonila/Shigella*, and the water in Port Harcourt metropolis have been confirmed to have the presence of these micro-organisms (Obianeme and Obire, 2017). Though not all micro-flora present in fish is found in the fish environment, there is a relationship between the micro-flora in the fish environment and the fish body (Lesel, 1979; Austin, 2006). In this experiment varying quantities of the above mentioned bacteria were found in the experimental tanks of the Artemia and Aller Aqua fed fish larvae, this result is in agreement with the report of Okpokwasili and Ogbulie (1999) and Daboor (2008).

The total heterotrophic count was higher in all the experimental tanks, this could be as a result of their been more present in the water source (Borehole) (Obianeme and Obire, 2017) and the organic matter deposited as a result of uneaten feeds and metabolic activities enhanced its presence (Ukwe *et al*, 2018b). Total heterotrophic bacteria can grow to a level that may be detrimental to the fish and the fish consumer, if the ponds are not properly managed (Eze and Ogbara, 2010). The presence of *Vibro*, *Total Coliform* and *Salmonela/Shigella* count were noticed in minute qualities (< 30 cfu) this could be as a result of fish excreta, and were favored by the enabling

environment (Kay *et al*, 2008; Bhatnagah and Devil, 2013; Ukwe *et al*, 2018b). The quantity of bacteria in this experiment reduced as the fish larvae gets older this could be as a result of reduction in uneaten feed deposit that promoted the proliferation of the bacteria (Zmyslowska *et al*, 2001).

Conclusion

There were no significant difference in the physico-chemical parameters of the water in the various experimental tanks during the flow-through period of the experiment, but there were presence of ammonia, nitrate and nitrite during the static period of the experiment, which could lead to mortality. Artemia and Aller Aqua showed commendable response in growth parameter, and are recommended as good larval feed to the larvae of *Clarias gariepinus*.

There were presence of Total heterotrophic count, *Vibro*, coliform count and *Salmonela/Shigella* count in all the experimental water, but in different quantities, and level of bacterial presence depends on the type of feed used. Flow through system is recommended for larval rearing, to avoid the presence of ammonia (NH₃), Nitrate (NO₃) and Nitrite (NO₂) that can lead to mortality. High energy content in feed reduces the growth rate of the larvae, as it affects the quantity of feed eaten by the larvae and reduces its protein efficiency ratio.

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