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Research Article

Growth and Microbial Indices in African Catfish (*Clarias Gariepinus*) Larvae Fed Formulated and Commercial Diets

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Abstract: This study investigated the effects of formulated and commercial diets as starter feeds on the performance of *Clarias gariepinus* larvae in the hatchery. Two hundred larvae were randomly distributed in experimental tanks and were fed with a commercial diet Aqualis and Special formulated feed (poultry feed + fishmeal + vitamin C+premix) for 21 days. The result obtained revealed that there was no significant difference (P>0.05) in the physico-chemical parameters in all treatments. Total heterotrophic bacteria, *Vibro*, Total coliform and *Salmonela/ Shigella* were identified in the microbial analysis of the fish waters in all treatments. The total heterotrophic, and *Vibro* counts in the tank waters were significantly higher in specially formulated diet than Aqualis starter feed. Total coliform count and *Salmonela/Shigella* were less than thirty (<30) in all the water samples. Fish fed Special feed had a higher survival rate than Aqualis. Special feed was also higher in length increase of larvae (8.96 ± 7.11 mm), than the larvae fed with Aqualis. There was steady reduction of the microbial flora as the fish became older. The weekly results showed that condition factors were less than one, in all the treatments, at the end of the third week.

Keywords: Fish feed; Aquaculture; Microbial flora; Catfish; *Clarias gariepinus*; Nutrients

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Introduction

In aquaculture practices, successful larval rearing at early stage of the cultured fish depends majorly on the availability of appropriate diets that are readily consumed, efficiently digested and that provide the required nutrients to support growth, health and good rearing conditions (Adewunmi, 2015). Fish hatchlings generally depend on live foods at first feeding for survival and development (Adewolu *et al.,* 2009). Nutrients which are found in reasonable proportion in both natural and formulated fish feeds are proteins, lipids and carbohydrates. Also, some nutrients such as vitamins and minerals are required in some measurable quantity. When the fish obtain sufficient supplies of certain essential nutrients, body functions and growth process will be effective and commensurate (Ajah, 2007; Gabriel *et al.,* 2007). The African catfish, *Clarias gariepinus* (Burchell, 1822), evolves through different developmental stages in its life time. The larval stage, which is the first developmental stage, depends on some environmental and management factors which include temperature and nutrition, and takes 14-42 days to complete (Arimoro and Ofojekwu, 2003). The fry stage, which is the second stage, is characterized by the some movement of the fish to the surface of the water. This movement also signifies that the fish can be stocked into ponds. The fingerling stage is reached when the fins are fully developed and most organs have been formed (Bengston, 2003; Akbary *et al.,* 2010).

In fish hatchery management, food and feeding parts are obvious key factors for a major achievement in larval rearing. Diets of fish larvae may be selected according to different sets of conditions depending upon the perspective of the fish farmer and some physiological requirements set by the developing larva (Adikwu and Haruna, 1999; Gabriel *et al.,* 2009). Recently, there is growing advocacy to reduce costs involved with feed preparation in the hatchery. Survival rate is given more importance than growth rate as fingerlings are sold based on numbers rather than weight (Adekunle and Joyce, 2013). Constant availability of the diet is also emphasized upon. Palatability and digestibility are most important criteria for the choice of feed. These factors are in turn affected by size of the diet in relation to the size of the fish (Craig *et al.,* 1994). In general, the food size should be around 2-3% of the larval length (Ukwe *et al.,* 2016).

Formulated dry feed have been used as a first feed in *C. gariepinus* larval rearing with the aim of avoiding the dependence on expensive and time consuming live feed (Ataguba *et al.,* 2009). In developing countries of the world, starter feeds which include live feeds such as capsulated artemia and commercial starter feeds are imported, therefore expensive and in most cases, the prices are beyond the reach of the farmers. Many of the hatchery operators therefore resort to formulated feeds for feeding of fry in the hatchery. This study therefore focused on growth performance and microbial indices in *C. gariepinus* larvae fed with specially formulated feed in the hatchery.

Materials and Methods

Experimental location

The project work was carried out in the hatchery unit of the University of Port Harcourt, Choba Campus, Rivers State, Nigeria.

Experimental feeds

The commercial starter feed (Aqualis) used was produced by Aqualis and imported into Nigeria by Crown Flour Mills Nigeria Limited. It was obtained from Agro-Services Center at Rumudomaya, Port Harcourt, and Rivers State. The special feed was prepared by mixing Vital broilers starter feeds (poultry), Dana fish meal, vitamin C, and premix (containing vitamins) they were procured from Agro-services centre at Rumudomaya, in Port Harcourt, Rivers State. This Special feed contains 1.18 kg of fish meal, 0.78kg of broilers starter feeds, 0.02 kg of vitamin C and 0.02 kg of premix, given a total of 2 kg of starter fish feed.

Proximate nutrient composition of experimental diets

The four experimental feed samples were analyzed using the standard analysis method of the Association of Official Analytical Chemist (AOAC, 1990). They were analyzed as follows: The moisture content of the sample was determined by air oven method at 150°C, crude protein was obtained by using micro-kjeldahi method. Crude lipid was determined by soxhlet extraction method using petroleum ether as extracting solvent. The ash content was by muffle furnace at 550°C for four (4) hours until constant weight of ash was obtained. Crude fibre was determined by exhaustive extraction of soluble substance in sample using 1.25% H_2S0_4 and 1.25% Na0H solution after the residue was ashed and loss in weight was recorded as crude fiber. Carbohydrate content was determined by difference 100 – (% moisture + % protein+ % fat + % crude fiber). All analysis was carried out in triplicates.

Stocking density

A total of two hundred (200) larvae of 4.8 ± 0.16 mg weight and 6.16 ± 0.30 mm lengths were transferred to each of the experimental tanks (40 × 25 × 25 cm^3) that were properly labeled. The weight was obtained by the use of a Rohr sensitive electric weighing balance (Model no. 3002N, by Want Instrument Co Ltd, Shanghai, China). A wet filter paper was placed in the balance and adjusted to zero, twenty five larvae were collected randomly from the hatchery at the end of endogenous feeding(after 48 hours from hatching time) and placed at the zero weight filter paper, and the weight was taken, and its average determined. This was repeated five times, and the average of the results of the five sets was taken as the weight of the individual larvae in the hatchery. Five (5) set of five larvae each were measured using a transparent millimeter calibrated ruler and a magnifying hand lens. The average lengths of each of the sets were taken and the mean of the various sets was taken as length of each larva in the hatchery. Feeding commenced twelve (12) hours after stocking.

Physico-chemical parameters

Temperature, Dissolved Oxygen, pH, were monitored twice daily, but other parameters; Ammonia, Nitrate and Nitrite were measured twice a week due to the constant quality of the water source and water retention time in the tank (Noori *et al.,* 2001). The temperature was taken by the use of mercury in glass thermometer calibrated in degree centigrade (0-100˚C). The pH value of the water was determined by the use of a pH meter, pocket pen pH meter model 700, made in Japan. The dissolved oxygen (D.O) was determined using a 9-series multi-parameter water quality meter (Bante 980 Precision DO. Meter) Version Number: 2009070200. The ammonia, nitrite and nitrate test were conducted using La Motte Aquaculture test kit MODEL AQ-4, CODE 3635-04, chester town, Maryland, 21620. USA.

Feeding of fish larvae: The larvae were fed at 10% of their body weight of feeds per day. They were twice daily early in the morning and late in the evening.

Growth parameters: During the experiment the growth parameters were measured as follows.

Length: The length was measured by the use of a transmitted millimeter calibrated ruler and a magnifying hand lens. The initial larva length was 6.16 ± 0.30 mm and measurements were done at days.

Weight: The weight was determined by the use of an electric sensitive weighing balance (model: 3002N, No.110628014, made in Shangai, China by Wart Instrument Co. Ltd). The initial larva weight before stocking was 4.8 ± 0.16 mg.

Survival: The survival rate was determined using the formula

$$\% \text{ survival rate} = \frac{final\ number\ of\ larva}{initial\ numberoflarvae\ stocked} x100$$

Specific growth rate (SGR): This was calculated as

$$SGR = \frac{InW_t - InW_o}{t}$$ (Arimoro, 2007)

Where: W_1=Final body weight; W_o=Initial body weight; t=Time (days); Ln=Logarithms of numbers

Fulton's condition factor (K): This was calculated as

$$K = \frac{W}{L^3} \times 100\%$$ (Peanase and Mengumhan, 2015)

W=Weight (g); L=Length (cm)

Feed conversion ratio (FCR): This was calculated as

$$FCR = \frac{Live Weight\ gain\ (g)}{Dry\ feed\ fed\ (g)}$$ (Tacon, 1990)

Feed intake (g): Total weight of food consumed by fish within the experimental period.

Protein efficiency ratio (PER): This was calculated using the formula

$$PER = \frac{Gain\ in\ Fish\ Weight\ (g)}{\text{Protein intake}\ (g)}$$ (EIFAC, 1980)

Percentage weight gain

$$PWG = \frac{Weight\ gain\ (g)}{Fish\ weight\ (g)} x\ 100$$ (Mbagwu and Adeniji, 1988)

Absolute growth rate

$$AGR = \frac{Final\ weight\ -\ Initial\ weight}{Growth\ Period}$$ (Orisamuko, 2006)

Daily weight gain

$$DWG = \frac{Mean\ wt\ increase\ per\ day}{Body\ weight\ of\ fish}$$

(Mbagwu and Adeniji, 1988)

Average daily length gain

$$ADLG = \frac{Final\ Length - Initial\ Length}{Days}$$

(Peanase and Mengumhan, 2015).

Relative weight gain (RWG)

$$\frac{Final\ weight - Initial\ weight}{Initial\ weight}$$ (Orisamuko, 2006)

Gross feed conversion efficiency (GFCE)

$$GFCE = \frac{I}{FCR} x100$$ (Mbagwu and Adeniji, 1988)

Microbiological analysis

The water samples were analyzed using the methods of American Public Health Association (APHA 1998). The total number of coliforms in a water sample was determined by the most probable number (MPN) test.

Statistical Analysis of Data

Statistical analysis was carried out on all data using the SPSS VERSION 22 for windows, 2000. Data was pooled by treatment and presented as mean ± standard deviation (SD) and standard error (SE), Data 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

Proximate composition of experimental diets

The proximate composition of experimental diets used in feeding *C. gariepinus* larvae is shown in **Table 1**. Significant differences were recorded in all the parameters between Aqualis

starter feed and special formulated feed. The values of moisture, fibre, ash and carbohydrates content were significantly higher in special feeds than that of Aqualis.

Physiochemical parameter of water in experimental tanks

The results of the physic-chemical parameters of water in experimental tanks in the *C. gariepinus* larvae fed with special feed and aqualis is shown in **Table 2**. There were no significant difference (P>0.05) in the values of temperature, pH and dissolved oxygen of the water in all the experimental tanks. While ammonia, nitrate and nitrite were 0.00 (zero) in all the experimental tanks.

Growth response and nutrient utilization in *C. gariepinus* larvae fed experimental diets

The growth responses of larvae to the experimental diets are shown in **Table 2**. The final length, final weight, weight gained and length increase significantly (P>0.05) higher in larvae fed with special feeds than that of aqualis within the experimental period. However, percentage weight gained was higher in fish fed with aqualis than the special formulated diet. While other parameters were within the same range **(Table 3)**. The nutrient utilization values is shown in **Table 4**, includes protein intake, protein efficiency ratio, feed conversion ratio and gross feed conversion efficiency were within the same range with no significant different (P>0.05).

Comparatively, changes in weight and length gained in *C. gariepinus* fed with special feed and aqualis indicated that their values increased steadily in the experimental weeks, also, across the experimental period the values of weight gained and length increase in fish fed with special feed were consistently higher than

Table 1: Proximate composition of experimental feeds (Mean ± S D).

Parameters	Aqualis	Special Feeds
Moisture Content (%)	8.95 ± 0.25[a]	11.76 ± 0.04 [b]
Protein (%)	53.74 ± 0.04 [b]	42.72 ± 0.03[a]
Fibre (%)	2.53 ± 0.03 [a]	7.22 ± 0.02[b]
Fat (%)	7.94 ± 0.02 [a]	10.64 ± 0.02[b]
Ash Content (%)	12.25 ± 0.03 [a]	12.11 ± 0.10 [a]
Carbohydrate (%)	14.65 ± 0.04[a]	15.64 ± 0.03[a]
Energy (cal/100g)	345.12 ± 0.08 [b]	329.25 ± 0.03[a]

Table 2: Physico-chemical parameters of water in the experimental tanks during 21 days (Mean ± SE).

Parameters	Aqualis	Special Feeds
Temperature (°C)	27.85 ± 1.15[a]	27.96 ± 1.34[a]
pH	6.11 ± 0.45[a]	6.26 ± 0.19[a]
Dissolved Oxygen (mg/l)	6.32 ± 0.16[a]	6.09 ± 0.11[a]
NH_3(mg/l)	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Nitrate (mg/l)	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Nitrite(mg/l)	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Note: EPU- Endopentosanase units.

Table 3: Growth response in C.gariepinus fry fed Experimental diets within 21 days (Mean ± SE).

Parameters	Aqualis	Special Feed
Final Length (mm)	11.85 ± 3.56 [a]	15.19 ± 7.30[b]
Final Weight (mg)	20.44 ± 8.72 [a]	22.11 ± 11.30[b]
Weight Gained (mg)	15.76 ± 8.83 [a]	16.29 ± 11.30[b]
Length Increase (mm)	5.73 ± 3.53 [a]	8.96 ± 7.11[b]
Survival (%)	58.78 ± 11.90[b]	65.53 ± 11.33[b]
Specific Growth Rate (% d^{-1})	9.92 ± 1.83 [a]	9.72 ± 1.85 [a]
Condition Factor	1.42 ± 0.37[b]	0.94 ± 0.31 [a]
Feed Intake (mg)	13.23 ± 10.08 [a]	13.16 ± 10.50 [a]
Percentage Weight Gained (%)	71.11 ± 16.51[b]	67.45 ± 20.00[a]
Absolute Growth Rate (mg)	1.05 ± 0.30 [a]	1.03 ± 0.43 [a]
Daily Weight Gained (mg)	0.09 ± 0.04 [a]	0.09 ± 0.04 [a]
Average Daily Length Gain (mm)	0.71 ± 0.18[a]	0.78 ± 0.16[a]
Relative Weight Gained (%)	3.28 ± 1.84 [a]	3.95 ± 2.37 [a]

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

Table 4: Nutrient utilization in C. gariepinus fry fed experimental diets within 21 days (Mean ± E).

Parameters	Aqualis	Special Feed
Protein Intake	7.11 ± 3.59[b]	5.61 ± 3.24 [a]
Protein Efficiency Ratio	2.60 ± 1.00 [a]	2.03 ± 1.45 [a]
Feed Conversion Ratio	1.40 ± 0.54 [a]	1.33 ± 0.45 [a]
Gross Feed Conversion Efficiency	79.75 ± 25.87[b]	80.27 ± 30.00[a]

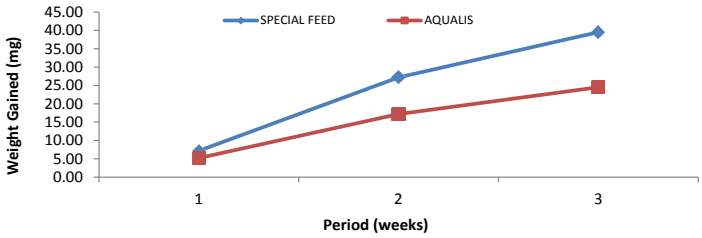

Figure 1: Changes in weight gained of C.gariepinius fed with special feed and aqualis.

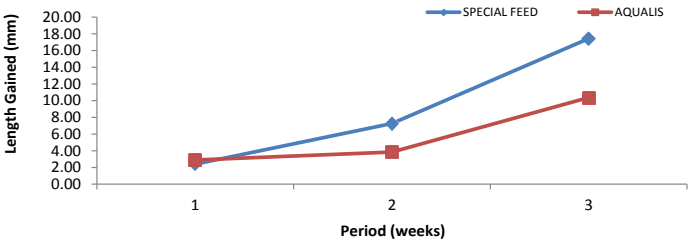

Figure 2: Changes in length increase of C.gariepinius fed with special feed and aqualis.

the fish fed with Aqualis **(Figures 1 and 2)**. Whereas, parameters such as survival, condition factor and food conversion ratio reduces in both feeds under consideration as the experimental

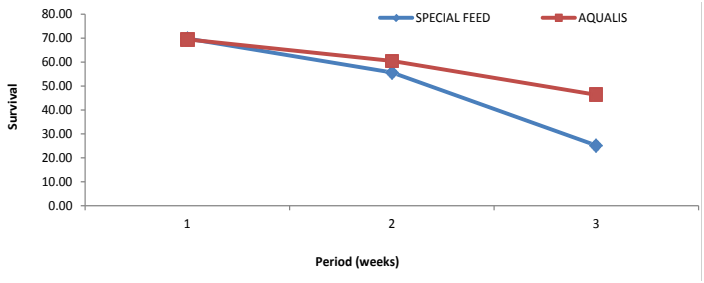

Figure 3: Changes in survival of *C.gariepinius* fed with special feed and aqualis.

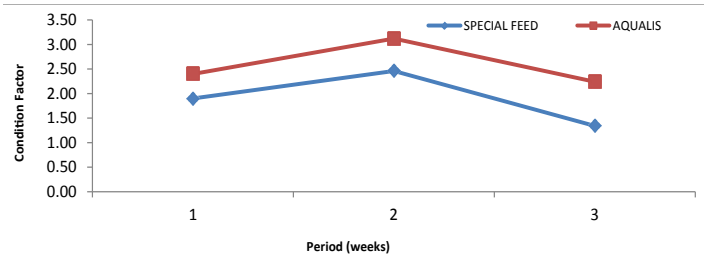

Figure 4: Changes in condition factor of *C.gariepinius* fed with special feed and aqualis.

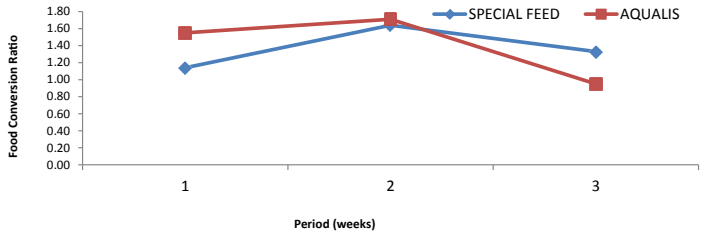

Figure 5: Changes in food conversion ratio of *C.gariepinius* fed with artemia and aqualis.

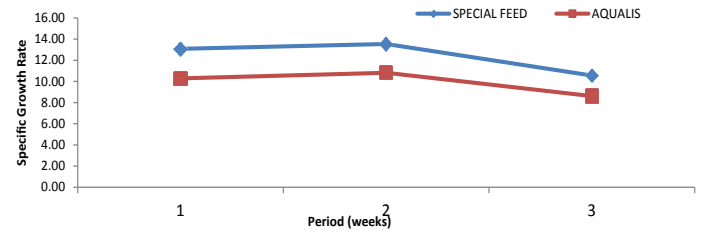

Figure 6: Changes in specific growth rate of *C. gariepinius* fed with special feed and aqualis.

Table 5: Microbial Analysis of Water in Experimental Tanks of *C. gariepinus* fed with Special Feeds and Aqualis (Mean±SE).

Parameters	Aqualis	Special Feeds
THC	5.2 x 10^3	7.1 ± 1.34[a]
VIBC	<30	5.2 × 10^2
TCC	<30	<30
SMS	<30	<30

Note: THC- Total Heterophic Count ; VIBC - Vibro Count ; TCC -Total Coliform Count; SMS- Salmonela/ Shigella.

period increases (**Figures 3-5**). While, the specific growth rate values were within the same range in all the experimental period **(Figure 6)**.

Microbial analysis of water in experimental containers

The experimental waters in the larvae fed with Aqualis and Special feed during the periods of the experiment was analyzed for the four microbial floras **(Table 5)**. Higher values of Total heterotrophic count and Vibro Count were recorded in experimental waters of fish fed with special feed when compared to Aqualis. While the other parameters such as Total Coliform Count and *Salmonela/Shigella* were less than thirty (<30) in both experimental waters.

Discussion

Water quality in relation with growth of larvae

In the present study, fish feeding does not leads to alterations in water quality parameters like dissolved oxygen, pH and ammonia. These parameters were not affected by different feed type application during the three weeks feeding trial. However, dissolved oxygen levels vary slightly with different feed type. However, the parameters were within the appropriate ranges for the catfish culture. Other studies also found and recommend that every growth rate is related to the water quality of the rearing containers (Adeniji and Ovie, 1990; Owhonda *et al.,* 2007; Abu *et al.,* 2010). This study result shows the larvae during first feeding days have good condition, and applying different type of feeds doesn't influence water quality.

Temperature and pH plays a vital role in determining the effectiveness of digestive enzymes in the fish larvae as a whole (Makinde *et al.,* 2015). A study by Keremah *et al.* (2010), shows temperature influences fish growth, specifically on the sensitive early stage. According to present study, the physico-chemical parameter range, pH (6.12-6.92) and temperature of (27.80-28.0˚C) were considered suitable according to Keremah *et al.* (2010). A fair amount of studies do not concern conductivity and salinity, usually if the water source is from bore hole which have more consistent water quality compared to surface water, and is less likely to be contaminated by pathogens and other pollutants (Boyd, 2005).

Growth performance of *Clarias gariepinus* larvae

Ajah (2010) observed that, at the onset of exogenous feeding, larvae of the African catfish are able to eat, digest, absorb, and metabolize nutrients with their sizeable mouth and digestive system. In this study *Clarias gariepinus* larvae readily consumed both feeds after yolk absorption. During the experimental period the larvae reached an overall average growth of 11.85 and 15.99 mm in Total Length in Aqualis and special feed treatment respectively. Also, at the end of 21 days trial, average total weight of 20.44 mg was recorded for the larvae fed with Aqualis, while an average weight of 22.11 mg was in the larvae fed with special formulated diet. This result is within the standard average

recommended by Gross *et al*. (2006) for catfish larvae fed with artificial diets in limited period of time.

Our findings in this study are based on the fact that locally formulated diets resulted in higher growth when used as starter feed. Diets that are readily consumed and efficiently digested to provide the required nutrition's for fish growth and development must be rich in protein. As protein quality and quantity determine the growths of fish larvae (Giri *et al.,* 2002). If there is a big difference of crude protein content between feeds, the larvae also shows different growth performances as the study examples indicate (Ovie, 2010; Olurin *et al.,* 2012). In This study higher growth of *C. gariepinus* larvae was observed in fish fed with special formulated feed, when compared to Aqualis starter diets. Whereas, the crude protein content in special was lower than that of Aqualis. This observation may be due to palatability of the feeds. Low palatability and digestibility are considered possible causes for poor growth performance (Amadi and Solomon*,* 2001).

Microbial analysis of experimental waters

The microbial analysis of the water in the various experimental tanks confirmed the presence of the following microbial flora, in different quantities:-*Total Heterotrophic bacteria, Vibrio, Total Coliform and Salmonela/Shigella.* Microflora especially total heterotrophic bacteria counts were found in borehole waters within Port Harcourt metropolis, but in different quantities (Obianeme and Obire, 2017). There is a link between the microflora present in fish and its water, Lesel (1979) and Austin (2006), but not all microfloras present in fish is in the fish water. Esteve and Garay (1991) also reported that the quantity of bacteria found in the tank water varies with the quantity found in the fish. Fish injects bacteria from the environment through the mouth, Olafsen (2001). In this experiment, some of microfloras were found in the various tanks though in different quantities. This is in agreement with report of Okpokwasili and Ogbulie (1999), and Daboor (2008).

The total heterotrophic count was high in all the tanks in this experiment, this could be as result of its presence in the borehole water before the administration of the fish feeds, and the organic matter deposited as a result of uneaten feed and metabolic activities enhanced the presence of the heterotrophic bacteria, Obianeme and Obire, (2017). At a conducive environment for bacterial growth, heterotrophic bacteria can grow to the level that may be detrimental to fish and fish consumers (Eze and Ogbaran, 2010). The *Vibro* count in all the experimental tanks except Special feed were less than (<30). The presence of the *Vibro* could be as a result of fish excreta (Kay *et al.,* 2008), and the presence of the organic matter from the feed could have encouraged their presence. The presence of *Vibro* noticeable in the tank fed Special feed could be due to the fact that organic matter in the Special feed deposited favored the proliferation of *Vibro*, and the temperature of the water may have contributed to this (Bhatnagah and Devi, 2013).

The total Coliform count and the Salmonela/Shigella count were less than thirty (<30) in all the experimental tanks. According to Kay *et al.,* (2008) and Obianeme and Obire (2017) this could be as a result of the presence of the fish excreta. The presence of this microflora as seen in this report could lead to the transmission of diseases (Piet, 2009). There was a noticeable reduction in quantity of microflora from the first to the third week, this could be as a result of the reduction in the quantity of leftover of uneaten feeds as the fish gets older, this led to reduction in organic matter presence that favoured their proliferation. This result is in agreement with Zmyslowska *et al.* (2001), who reported that, the presence of bacteria decreased as the fish gets older.

Conclusion

There was no significant difference in the physico-chemical parameters of the water in all the experimental tanks, as they were all within the range of fish survival. The results obtained from this experiment indicated that the two feeds showed commendable response to growth parameters. The larvae of *C. gariepinus* fed Special feed (a combination of broiler starter feed, fish meal, vitamin C and premix) did better than Aqualis in terms of length increase, weight gained and survival rate of the fish. The observed microbial flora: Total heterotrophic count*, Vibro,* Coliform count *and Salmonela/Shigella count* were all present in the various experimental waters in different quantities. Vibro count was only present in considerable quantity in Special feed tanks. There is need for constant monitoring of the physico-chemical parameters and microbial flora presence of the fish water, as different feeds alter the physico-chemical parameters and microbial flora presence of the water.

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