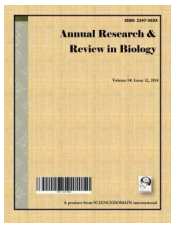

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Use of Probiotics as First Feed of Larval African Catfish *Clarias gariepinus* (Burchell 1822)

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Authors' contributions

This work was carried out in collaboration between both authors. Author EUD designed the study, wrote the protocol and interpreted the data. Authors OJU and EUD anchored the field study, gathered the initial data and performed preliminary data analysis. While author EUD managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

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ABSTRACT

African catfish larvae do not have fully developed digestive system at onset of first exogenous feeding. Live feed like artemia have been used more than dry diets but is also costly. The gut microbiota may be influential in larval diet utilization. We therefore made three larval feed from two commercial probiotics and tested their growth and nutritional effects on first feeding hatchlings (larvae) of African catfish. The three diets were labeled as feed 1 (F1) made up of mixtures of mixtures of *Lactobacillus acidophilus, Bacillus subtilis* and *Lactobacillus bugaricus*, feed 2 (F2) was made up of 100% *Saccharomyces cerevisiae* and feed 3 (F3) was made up of 50% Lactobacillus mixtures and 50% *Saccharomyces cerevisiae*. The control diet F4 was decapsulated artemia. African catfish hatchlings were produced by artificial dry fertilization of brooders maintained in our lab. First feeding hatchlings 48 hours post fertilization (48 h pf) were stocked in well aerated 15l plastic aquariums at density of 100 larvae aquarium^{-1}. There were three replicate aquariums per treatment feed. The photoperiod of the larval rearing system was 12 h D: 12 h L and the light intensity were maintained at 8 lux using black nylon coverings. The hatchlings were fed to satiation

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5 times daily for the whole larval period of 20 days. Survival of larvae were similar (P>0.05), for those fed with feed 1 (100% mixed lactobacillus sp), (LAB) 62.59%, feed 2 (100% *Saccharomyces cerevisiae*) (SAC) 57.89% and feed 4 (decapsulated artemia) 57.89%. The lowest surviving larvae were those fed with F3, 50% LAB: 50% SAC 54.67%.Weight gain was however better for larvae fed with artemia AWG 0.15 g than the rest of the treatments (P<0.05). The larvae fed with 50% LAB: 50% SAC (F3) had higher AWG 0.11 g than those fed with LAB (F1) 0.07 g and SAC (F2) 0.09 g (P<0.05). The result indicates that probiotics can be used in enhancing growth and survival of larval of African catfish. Nutritional value of probiotics is similar to live feed. Prolonged use of artemia reduces survival.

Keywords: Lactobacillus; Saccharomyces; larval feed; African catfish; larviculture; probiotics.

1. INTRODUCTION

Freshly hatched African catfish larvae do not have fully developed digestive system [1]. First feeding catfish have little or no gut microbiota community as other larvae [2]. Fish larvae are exposed to microbes in their water [3,4], and feed [5]. The presence of microbes in the system would influence general well being of the fish, the digestive tract and body [6]. Inclusion of exogenous microbes like the probiotics could control or enhance the microbial communities of the fish [7,8]. Larval fish ability to utilize live feed may be associated with presence of microbiota within the feed. Larval fish may increase its intestinal microbial community by ingesting particle in ambient water and egg remains [9]. The role of intestinal microbiota is associated with feed. The feeding of sea bass (*Sparus aurata*) and sea bream (*Dicentrarchus labrax*) larvae with rotifers resulted in recovering of *V. anguillarum*, *V. tubiashii*, and nonvibrio groups from the gut of the fish. However when same fish were fed with artemia more species of vibrio sp were recovered *V. alginolyticus, V. proteolyticus*, *V. harveyi*, and *V. natriegens* [10], suggesting that feed sources of the fish are focal contact points of microbial introduction into fish gut. Intestinal microfauna and biota positively contributes to the health, nutritional performances and zootechnical attributes of cultured fish [11,12]. Consequently there is increased research in fish nutrition with regards to gut microbiota and health to develop dietary supplementation using probiotics strategies to promote health and growth [13-17]. The use of probiotics in aquaculture has been in various forms like application in culture water [3,6,18], to inclusion in feeds [19-22], and therapeutic applications [23-25]. Beneficial effects of probiotics have been attributed to several factors like modulation of the intestinal microbiota and immune system, enhanced growth survival and development, nutrition and disease resistance [21]. *Lactobacillus acidophilus* and yeast *Saccharomyces cerevisiae* are probiotics that modulate gastrointestinal tract, leading to improved nutritional performances and immunity in fishes [26]. Incorporation of *L. acidophilus* as probiotic in diet of African catfish resulted in higher growth rate and better nutrient utilization [27]. The incorporation of sesame seed meal fermented with *L. acidophilus* into diets of *Labeo rohita* improved their growth and nutritional performances [28]. In a previous research, noted that there were improved growth rate of *O. mossambicus* when fed with diets like *Lactobacillus*, *Vibrio* sp, *Aeromonas*, and *E. coli* [29]. The addition of probiotics (lactic acid bacteria) to larval starter diets seemed to enhance soybean meal (SBM) utilization in first feeding rainbow trout [30]. The incorporation of yeast *S. cerevisiae* in the diets of Nile tilapia produced better growth [31]. Similarly, improved growth performances have been noted when *S. cerevisiae* was used in diets of sea bass [20], hybrid striped [32] and Japanese flounder [33]. The beneficial effects of yeast could be associated with its beneficial compounds like nucleic acid, β-glucans, mannan oligosaccharides and proteins [21,32-34]. Yeast naturally occurs in the gastrointestinal tract of healthy fish and constitutes an important part of the gut microbiota [35]. Yeast is able to stand pelletizing and retains its quality after pelleting. It has been reported that yeast supplemented diets had effects of stimulating growth, feed efficiency, blood biochemistry, survival rate, and non- specific immune responses in olive flounder (*Paralichthys olivaceus*) challenged with *Uronema marinum* infection [36]. Mixing of probiotic can be beneficial than using single probiotic. In the diets of rainbow trout juveniles challenged with *Yersinia ruckeri* administration of *S. cerevisiae* treated with beta-mercaptoethanol was better than whole cell yeast and n-3 highly unsaturated fatty acids (HUFA)-enriched yeast, in enhancing immune system and growth stimulation [37].

This research will examine the effects of novel diets made up of mixtures of LAB species, whole diets made of Saccharomyces, and a third diet made from mixing 50:50 inclusion levels of both LAB and SAC, on the first feeding hatchlings of African catfish *Clarias gariepinus* throughout the larval stages of the fish.

2. MATERIALS AND METHODS

2.1 Experimental Fish

Larval African catfish used for the experiment were produced by artificial dry fertilization of catfish brooders maintained in our wet laboratory. Female catfish brooder of weight 1.85 kg was injected with a GnRHa hormone (Ovopel) injection. Ovopel contains metoclopramide, a blocker of dopamine receptors, and mammalian hormone GnRH analogue (D-Ala6, Pro9NEt-mGnRH) [38]. The injected female was placed alone in well aerated plastic aquarium of 20 liter capacity. Ovulation, vitellogenesis and stripping were completed after 8 hours. Mature males catfish (*C. gariepinus*) of weight 2 kg was sacrificed and the testes was extracted and used in dry fertilization of stripped eggs. The eggs were incubated in 15 liter flow-through plastic aquaria at 28.5℃ and the eggs hatched after 22 hours post fertilization. During incubation, water parameters were as follows; (mean ± SD, n=3), pH 6.7±0.02 measured with pH meter. Dissolved oxygen was 5.65±0.08 mg^{-1} liter, measured with dissolved oxygen meter. Water temperature was 28±0.06℃, measured with mercury in glass thermometer. Water turbidity was 2.4±0.05 NTU, measured with calibrated Nephelometer. The general turbidity values obtained for the treatment feed aquaria range from 1.1 – 6.9 NTU.

There were three replicate aquariums per treatment feed and first feeding larvae were stocked at 100 fish $aquarium^{-1}$. Aquarium dimensions were length, width, depth sizes (30, 25, 20 cm). Experimental fish were electronically weighed together per replicate treatment feed. Water flow rate was adjusted to 100 mL min^{-1}, water temperature ranged from 25℃-28℃, light intensity at the tank surface was c. 8lux maintained by covering with black nylon sheet. Photoperiod was 12 h D: 12 h L.

2.2 Diet Formulation and Feeding

Three novel diets were made from commercial probiotics available from Canada. The diets were labeled feed 1 (F1) to feed F3. Feed 1 (LAB) was made up of mixtures of 10^{8-10} lactic bacteria, *L. acidophilus, L. bulgaricus, S. thermophilus,* embedded g^{-1} in dry white edible starch. Feed 2 (SAC) was made of dried *Saccharomyces cerevisiae* and feed 3 was made of a mixture 50:50 of 10^{8-10} LAB: SAC g^{-1} dry white edible starch. The feed were all dry and used as dry feed. The control diet feed 4 (F4) was decapsulated artemia. The catfish hatchlings were hand fed to satiation four times daily. Feeding was done in the early morning hours 08 h, 12 h, 16 h and 20 h. Care was taken to avoid over feeding. The aquariums were always cleaned every morning and the uneaten food and fecal maters were removed. Amount of feed was adjusted as the larvae grew bigger.

2.3 Chemical Analysis

The feed samples were dried and ground to powder and stored under -4℃ for chemical analysis. Protein content of the treatment feeds were determined by Kjeldahl method from dried feed samples and crude protein was expressed as Kjeldahl -nitrogen N x 6.25. Crude fat was analyzed gravimetrically after chloroform: methanol (2:1) extraction (after methods in [39]). Ash was measured after burning samples at 550℃ in a furnace for 18 h. % Ash (dry basis) = Weight of ash X 100/Weight of original feed = W_3 - W_1 X 100/ W_2 – W_1, where W_1 = weight of empty crucible, W_2 = weight of crucible + feed before drying/or ashing, and W_3 = weight of crucible + ash.

Fibre was measured according to methods in [40]. Phytate was measured using spectrophotometer. The absorbance was measured and phytate was calculated as,

$$Y = 0.512^* X$$

Where

X = concentration of phytate (mg/100 g unit), 0.512 = constant, Y = absorbance.

2.4 Sampling and Weighing of Fish

Fish larvae were weighed together per replicate tank using electronic balance sensitive to 0.0001 g. Larvae were carefully netted together out of water dried in a filter paper and placed in glass petri dishes and weighed. Weighing was done early in the morning to avoid stress and three times in the experimental period.

Larvae were not fed for four hours before weighing.

2.5 Calculations and Statistics

The following growth parameters were calculated for each aquarium: Average weight gain (AWG) = Initial average weight (g) - final average weight (g). Larval survival was calculated as Survival =100 x Final number of fish / Initial number of fish. Specific growth rate (SGR, % / day) of the larvae was calculated as 100 (Ln W_2 – Ln W_1) /t, where W_1 and W_2 were average weights in g at the start and the end of the experiment and t was the length of the experiment in days (i.e. 20). The larval protein efficiency ratio was calculated as; Protein efficiency ratio (PER) = weight gain (g)* protein intake-1 (g). Results were analyzed using oneway ANOVA and least significant difference (LSD) 0.05 was used in separating possible differences of treatment means. SPSS version 14.0 statistical package was used for analyses of the results.

3. RESULTS

Experimental diets were easily accepted by the *Clarias gariepinus* larval fish. There was differential growth of the experimental larvae based on diets. The highest larval specific growth rate was from those fed control diet (artemia) F4 with SGR of 18.484±0.02% day^{-1}. The catfish larvae fed with 50:50% mixture of LAC:SAC and 100% mixtures of LAB had similar SGR of 16.566±0.01% day^{-1} and 16.165±0.03% day^{-1} respectively (P>0.05). The lowest growth rate 14.867±0.02% day^{-1} was from the catfish fed with SAC F2 this was significantly different from SGR of F4, F3 and F1 (P>0.05). There were no significant differences in the initial weight of the larvae (P>0.05). After the 20 d feeding experiment larvae fed with artemia F4 had the highest final weight of 0.16±0.01 g. The larvae fed with F3 had the next higher final weight of 12±0.03 g but was significantly lower than those of F4 (P>0.05). There was however no significant differences between the final weight of catfish fed with F1 0.113±0.02 g and F3 (P>0.05). The lowest final weight was recorded for the larvae fed with 100 SAC feed F2, weighing 0.093±0.03 g (Table 2). The weight gain of the larvae follows similar trend with the final weight. Larvae fed with F4 artemia had the highest weight gain of 0.15±0.03 g. The weight gain of larvae fed with 50:50, mixture of LAB and SAC had the next best weight gain of 0.11±0.04 but was not significantly different from the larvae fed with 100% mixed LAB diet F1 0.103±0.02 g (P<0.05) (Table 2). The lowest weight gain was from catfish larvae fed with 100% SAC F2, 0.083±0.04 g.

Table 1. Composition of experimental diets and proximate composition of experimental diets used in the larviculture of African catfish *Clarias gariepinus* for 20 d. F4 is control (decapsulated artemia)

	Feeds			
Ingredients	**F1**	**F2**	**F3**	**F4**
L. acidophilus	35	-	50	-
L. bulgaricus	35	-	-	-
S. thermophilus	30	-	-	-
S. cerevisiae	-	100	50	-
Artemia	-	-	-	100
Total	100	100	100	100
Proximate analyses				
Moisture	6.78	10.87	6.73	
Protein	5.44	3.18	5.39	
Ash	3.6	4.1	4.1	
Fibre	0.23	13.5	5.9	
Starch	80.8	65.08	74.42	
Phytate	3.15	3.27	3.46	

Table 2. Growth and nutritional parameters of larval African catfish *C. gariepinus* fed with probiotic and mixed diets for 20 d larval period

Parameters	**Feeds**			
	F1	**F2**	**F3**	**F4**
Ini. Weight (g)	0.01±0.02^{a}	0.01±0.03^{a}	0.01±0.02^{a}	0.01±0.01^{a}
Fin. Weight (g)	0.113±0.02^{b}	0.093±0.03^{c}	0.12±0.03^{b}	0.16±0.01^{a}
Wt. gain (g)	0.103±0.02^{bc}	0.083±0.04^{c}	0.11±0.04^{b}	0.15±0.03^{a}
SGR% day^{-1}	16.165±0.03^{b}	14.867±0.02^{c}	16.566±0.01^{b}	18.484±0.02^{a}
PER	0.019±0.03^{a}	0.026±0.01^{b}	0.020±0.04^{b}	0.003±0.01^{c}
Survival (%)	62.56±0.04^{a}	59.89±0.03^{ab}	54.67±0.04^{b}	57.89±0.03^{b}

Means not followed by same superscript are statistically significantly different P<0.05

Protein efficiency ratio (PER) was best for the larvae fed with F1 and this was significantly ($P<0.05$). There was no difference in the PER of larvae fed F2 and F3 ($P>0.05$). However the control diet F4 had the lowest PER. The survival of catfish larvae fed with F1 (mixture of LAC) 62.56±0.04% was highest and significantly different ($P<0.05$) from the rest of the treatment feed. There was however no significant differences ($P>0.05$) between the survival off larvae fed with F1 and F2 (SAC). The lowest larval survival percentage was 54.67±0.04% recorded for catfish larvae fed with F3, 50:50 LAB:SAC. The survivability of larvae fed with F4 artemia was however similar to those fed with F3 ($P>0.05$). The water parameters of the aquariums were affected by treatment feed. The application of SAC increased the turbidity of the larviculture water in the aquarium. Consequently the aquariums receiving experimental feeds 2 and F3 had the highest turbidity. The tanks receiving LAB F1 had the lowest turbidity. This could be due to further replication of the yeast cells in the culture environment.

Table 3. Water parameters of aquaria used in culturing larval African catfish *C. gariepinus* For the larval period of 20 d with probiotics and mixed probiotic diets

Parameters	F1	F2	F3
pH	6.5	6.8	6.7
Temperature (℃)	25	28	28
Dissolved Oxygen (ppm)	0.42	0.5	0.44
Turbidity (NTU)	1.1	6.7	6.9

NTU=Nephelometric Turbidity Unit, ppm=Parts per million

4. DISCUSSION

Single cell proteins diets had been noted as alternatives to fish meal [41,1]. The specific growth rates of the larvae fed with probiotics diets were high as compared with those fed artemia. The experimental diet F3 and F1 were close to the control diet artemia. Essentially these two diets were composed of mixed ingredients. It seems that combination of probiotics is better than single source. This highlights the advantages of probiotic mixture in aqua diets. This suggests that the larvae utilized the probiotic experimental diets like they did the live feed. Comparison of observed optimal SGR day^{-1} of artemia fed larvae (F4) SGR, 18.48±0.02% day^{-1}, F3, 50:50% (LAB:SAC) fed larvae SGR 16.566±0.01% day^{-1} and 100% LAB (F1) fed larvae SGR 16.165±0.03% day^{-1} suggests that probiotic diets produced high larval SGR that is comparable to that of the non probiotic but live feeds. The SGR of the probiotic fed larvae in our experiment was in line with previous experiment, where larval catfish were fed with tubifex and had SGR of 15%, those fed with artemia had 14.5% and 14.4% for moina sp [42]. However larval catfish in our experiment grew better than same age larvae of *Clarias macrocephalus* fed live feed and artemia, larval SGR was 15.2% day^{-1} [42]. Similarly, in a related experiment larval African catfish fed commercial diets, daphnia and artemia had lower SGR (SGR 0.228% day^{-1}) [43], than we had in this experiment. The growth enhancement of the probiotic larvae fed could also be as a result of secondary effects of the probiotics as, nutritious food and as intestinal modulators. *S. cereviciae* fermentation of cereals leads to higher production of all essential amino acids [44]. The probiotics in this research were embedded on starch matrix and it seems the microbial fauna acted to release nutrients beneficial to the fish. Moreso continual feeding of the probiotic could have lead to colonization of the gut thereby leading to intestinal modulation and better utilization of the diets and growth performances. This had been suggested in the nutrition of the following fishes fed with probiotics; brown trout [45], salmonids [15] and *Oreochromis niloticus* fed with prebiotics [16]. In our experiment, the effects of feed 1 (F1) made up of mixed lactic bacteria on the larvae were similar to that of F3, which was composed of 50:50% mixtures of LAB: SAC, suggesting that growth effects could be as a result of some probiotic actions on the fish and the feed ingredient such as proteins and starch. Mixtures of *L. acidophilus* and *B. subtilis* had been shown to improve growth in *Tilapia nilotica* [25]. Enhanced growth has been observed in channel catfish subjected to *B. subtilis* probiotics feed [46]. The performance of fish larvae on artemia has been noted to be due to its essential amino acid profile [47]. The growth performances of the larvae fed F3 could be due to specific action of yeast in removing ANF from starch based feed. *S. cerevisiae* removes monosaccharide, disaccharides and trisaccharides by fermentation [48]. The breaking down of the sugars by the probiotic enhances utilization by the larvae. Based on the results the combination of LAB and SAC in feed production provided feed better utilized by the larvae. The reasons for this are not very clear but seem among other things as a result of the combined amino acid value of the diet. Moreover it had been noted that yeast is good probiotic and is

been used either as live feed or processed as a feed ingredient and then colonize host intestine [49]. The quality of artemia had been noted to be different depending on whether it decapsulated or not [47]. Decapsulated artemia had lower protein values and reduced amino acids than the artemia [47]. This could be reason for closer performances of our experimental diets when compared with the decapsulated artemia. Consequently the weight gain of our catfish was highest for those fed F4 (artemia) but closely followed by the probiotic fed larvae. The higher weight gain of the control could be related to its higher fatty acid profile compared to the experimental diets. However the application of mixed LAB diet F1 and its mixture with SAC (50:50), produced larvae of high weight gain. Lactobacillus sp. has been known to secret exoenzymes that aid nutrient digestibility [50,51]. It could be possible that such enzymes would help larval *C. gariepinus* growth. Increased growth performances has been noted in fishes fed with *S. cerevisiae*, Israeli carp [52], hybrid striped bass [32] and Nile tilapia [26]. In our research we noted high growth with SAC but a higher growth rate with (F1) LAB, this is in line with previous experiment with African catfish *C. gariepinus* that had better growth rate when fed LAB probiotic supplemented diets [27]. We also noted higher growth of the larvae when fed diets with combination of 50:50% SAC and LAB (F3). Better fish growth performances was also noted by for *Oreochromis niloticus* fed diets supplemented with bacteria *Streptococcus faecium*, *Lactobacillus acidophilus* and *S. cereviciae* [31]. Combination of probiotics as feed supplements seems more beneficial than a use of a single strain. In a similar experiment it was shown that rabbit fish (*Siganus rivulatus*) cultured only through commercial probiotic cocktail biogen®(mixture of probiotic and prebiotic) supplemented diet reduced feed cost by 73% – 78% [53]. The use of feed 3 (F3) in this research could be cost reducing in practical fish farming terms.

The protein efficiency ratios of the feeds were higher for the probiotic diets than the artemia. This could be because of the regular enhanced utilization of the probiotic feed by the microbes. Higher survival of the probiotic fed larvae than artemia could be due to nutrients requirement of the larvae. Artemia has been noted to be useful as first feed, but prolonged usage will reduce growth and survival. This seems to be the case in this research. The survival reduction seems to be because the larvae could still continue using the nutrients processed and released by the probiotics long after that of artemia diet was insufficient. The survivals of catfish recorded in this research were similar to those of Asian catfish [42]. The catfish survivals were also similar to that of Japanese flounder *Paralichthys olivaceus* that significantly had higher survival when fed commercial probiotics made from mixed cultures of bacteria and yeast [33]. The probiotics-treated flounder showed significantly higher survival rate than control in a 50 days culture.

The costs of the probiotic diets were much cheaper than artemia. In our present research, the commercial preparation of mixed LAB costs 12.56 USD^{-1} kg diet while commercial dried SAC costs 1.51 USD^{-1} kg of diet. Meanwhile a tin decapsulated artemia costs approximately 50USD (current prices of feed ingredient in Nigeria market September 2015).

5. CONCLUSIONS

This research shows that growth of larval African catfish *C. gariepinus* can be enhanced by use of *L. acidophilus, L. bulgaricus, S. thermophilus* and *S. cereviciae.* The high growth performances and higher survival of catfish larvae fed with probiotics compared to artemia are indicators of the advantages of probiotic diets over ordinary live feed like artemia or dry diets, in larviculture of African catfish *Clarias gariepinus.* Combination of probiotics like equal proportions of LAB: SAC improved growth but at similar rate to combined LAB of F1. Prolonged usage of artemia reduces growth and survival of the catfish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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