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Effect of a strain of *Lactobacillus* used as probiotic on the biological parameters of tilapia (*Oreochromis niloticus*)

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Aquaculture is among the fastest growing sectors in agriculture. Freshwater and marine aquaculture production has increased remarkably recently as has the growing demand for seafood. A study was conducted for 15 days on *Oreochromis niloticus* fry aiming to assess the effect on animal performance of a *Lactobacillus* strain used as probiotic incorporated in a diet formulated for tilapia fry. The parameters measured were daily live weight gain, standard length, total length and survival rate. Our results showed that the overall individual average weight gain as well as the survival rate were not significantly higher for animals supplemented with probiotic when compared to control animals. Longer lasting experiments are required to confirm these results.

Introduction

Due to an increased demand for animal protein over the previous twenty years, animal production has become more industrialized. However, intensive modern farming practices provides animals with unfavorable artificial conditions including high population density, industrial food and stress that result in numerous concerns and therefore major threats to the sustainability of the industry (Li & Gatlin 2005). Sensitivity towards infectious diseases is one of the major issues facing intensive fish farming. Traditionally, antibiotics and chemicals are used to control aquatic animal diseases (Li & Gatlin 2005). The worldwide indiscriminate use of antibiotics in aquaculture since 1960 has led to the development of drug-resistant bacteria that are increasingly difficult to control and eradicate (Watanabe *et al*. 1992). Further, the use of antibiotics in animal production, including aquaculture, has been met with public opposition in most developed countries. Thus, we conducted research into substitute substances or beneficial microorganisms that have positive effects similar to antibiotics with moderate impact (Irianto & Austin 2002).

The implementation of European regulations on the use of additives in animal feed has led to a progressive reduction in the antibiotic molecules used as additives in animal feed (FAO 2002). However, disease prevention is a better way to control infectious diseases than treating sick fish. Probiotics are bio-friendly agents that can be either administered as a feeding supplement or added to drinking water to control pathogens and enhance feed utilization, growth rate, and microbial balance by different modes of action

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(Kuebutornye *et al*. 2019). Hence, there has been considerable interest in recent years to evaluate the possibility of using non-nutritive food additives, especially probiotics, to improve the growth, stimulate the immune system and/or improve the resistance of fish towards infectious diseases.

The application of probiotics originally targeted humans and terrestrial farm animals. It was extended to aquatic animals only at the beginning of the 1980s. Yasuda and Taga (1980) were the first to suggest the beneficial effects of probiotics on fish. However, the first studies were only published by the end of the 1980s (Kosaza 1986, Gatesoupe *et al*. 1989). Since then, many studies focused on dietary supplementation with probiotics to improve farming water quality (Abdel-Aziz *et al*. 2020). A variety of probiotics have been widely used in aquaculture, including Gram-negative or Gram-positive bacteria, fungi, and algae (Soccol et al. 2010, Austin & Austin 2012). Commonly used probiotics of bacterial origin include Gram positive bacteria of *Bacillus* species, such as *B. subtilis*, followed by various species of *Lactobacillus* (*L. plantarum*, *L. rhamnosus*) (Sharifuzzaman and Austin, 2017). The aim of this study was to determine the effects of a *Lactobacillus* strain on tilapia performance.

Methods

Animals

Sixty unsexed specimens of tilapia (*Oreochromis niloticus*), 60 days old and 1.64 g average weight were obtained from the National Center of Research and Development of Fishing and Aquaculture of Bousmail (CNRDPA, Tipaza district) to be used in this experiment. The fish were divided into two groups (Control, C and Experimental, E) with three replicates per group.

Probiotics

A strain of *Lactobacillus sp*. bacteria was isolated in a selective culture medium (MRS), from an aquatic Algerian environment (Probiont AH 78). These bacteria were identified based on morphological, physiological and biochemical characteristics. The strain is Gram positive, non-sporulating and catalase negative rods that ferment various carbohydrates mainly to lactate and acetate and shows capacity to inhibit highly pathogenic bacteria in vitro, by its ability to produce bacteriocin and lactic acid. It is resistant to the gastro-intestinal environment and adheres to intestinal epithelial cells (Abdullah *et al*. 2011).

The putative probiont was prepared following the method described by Abd El-Rhman *et al*. (2009). Briefly, 1000-ml flasks of MRS broth were inoculated with 1% of an overnight culture of AH 78 cells and incubated for 48 h at 37 °C. Bacterial pellets were collected by centrifugation at 3000 rpm for 10 min at 4 °C, and washed three times with a sterile saline solution (0.9% NaCl). Cells were adjusted by McFarland standards to approximately 10^{10} CFU ml^{-1}.

Feed formulation

Feed based on corn, soybean meal, and olive pomace was used. The fish diet (crude protein 32%, crude lipid 8%, crude fiber 10% and ash 12%) was formulated as previously reported, with minor modifications (FAO 2002). The experimental diet was sprayed with the selected *Lactobacillus* strain previously grown in MRS at a concentration of 1×10^{11} CFU ml^{-1} and a rate of 100 ml kg^{-1} feed. The sprayed feed was incubated for 24 h at 35 °C in a hermetically sealed container. Next, the feed was dried in an oven for 24 h at 35 °C. The control feed was sprayed with a sterile MRS culture medium (Jatobá *et al*. 2008), then left to dry at ambient temperature for 4–5 hours, stirring feed manually. During this period, the probiotic attaches to the formulated diet (Apun-Molina *et al*. 2009).

Infrastructural breeding setup

The animals were placed in an experimentation room at the experimental station of Blida Institute of Veterinary Sciences. Healthy juvenile tilapia were selected and acclimated in cylindrical plastic tanks for 2 weeks (before starting the feeding trial) at 28 ± 0.3 °C, pH 7.6 ± 0.2, with a

12 h light/dark photoperiod and continuous aeration under laboratory conditions and were fed a basal diet.

The fish were then randomly distributed among six 57-l tanks (20 × 20 × 20 cm) at low density (37 fish m^{-3}) (n = 10 fish per tank). Tank was considered the experimental unit. During the study, an intensive breeding system was used where physicochemical conditions were controlled, such as water temperature, oxygen concentration, and light brightness on a daily basis throughout the duration of the experiment. Water temperature was checked on a daily basis (27 ± 1 °C) due to immersion heaters. The dissolved oxygen concentration (O_2) was kept to a minimum of 44 ppm using oxygen pumps inside the aquaria.

The experiment lasted for 15 days following Jatobá *et al*. (2018), Wanguyun *et al*. (2019) with the time-span of the experiment affected by COVID-19 restrictions. Aquarium water was renewed at a rate of 20% daily, and waste evacuated manually with a hose without handling the fry. Weekly maintenance consisted of rigorous cleaning of the walls and a complete cleaning of the aquaria. Water was changed every week to maintain good water quality.

Fish feeding

Animals were fed twice (08:00, 14:00) per day. Initially, the fish were fed at a rate of 1% biomass per day. Feeding frequency was the same for the two groups. No medication or antibiotic was administered during the experiment. The only treatment was the presence or absence of the probiotic strain in the feed.

Biological parameters

Individuals were weighed on a daily basis. Mean animal weight per aquarium was obtained from the total values divided by the number of animals. Mean individual average daily gain (ADG) was calculated accordingly. Weight biometrics was performed using an electronic balance (OHAUS-PIONEER PX 224). Measurements of fish sizes were carried out individually at the same time as weights, using a simple geometric ruler. Total length (TL) was measured from the tip of the snout to the tip of the caudal peduncle. Standard length (SL) was measured from the tip of the snout to the base of the caudal fin (FAO 2018).

Survival rate was calculated from the total number of fish at the end of the experiment compared to the initial number at the start.

Statistical analysis

Data were analyzed using a general linear mixed model (SAS 2009) including the fixed effect of group (control vs. experimental), and the random effect of aquarium within group. For time series analysis, a type 1 covariance structure was used, allowing taking into account autocorrelation between successive data measured on the same experimental unit. In this case, time and the interaction group × time were considered. Student's t-test was used to determine whether differences between means were significant. The results were considered significant at $P < 0.05$. Mortality rates were compared using Fischer's Exact test.

Results and discussion

The initial and final weight and length parameters were not significantly different between the two groups (control, experimental) (× 1). Overall, ADG and Relative ADG did not differ between groups, although they were twice as high in the experimental group compared to the control group. The final SL and TL values did not differ significantly in tilapia fed with probiotic compared to the control fishes (Table 1).

The survival rate obtained during this study was not significantly different ($P = 0.679$) between groups (2 cases in the experimental tanks *vs*. 4 in the control tanks). In our experiment, the observed survival rate was achieved during a life stage sensitive to diseases (hatchery). This survival rate indicated good adaptation of animals to the feed formulated. Mortality recorded during the experiment did not seem to be related to infectious or parasitic diseases.

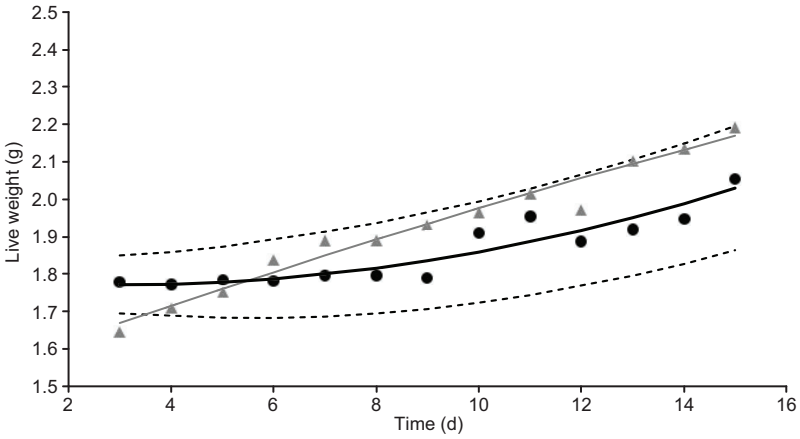

Fig. 1. Means, quadratic regression curves of the means and confidence intervals of the growth curves of the control (black circles, black lines) and experimental (grey triangles, grey lines) groups. Means were obtained following mixed model for repeated measures and autoregressive covariance structure. Confidence intervals were weighted according to experimental day^{-1}.

Muangkeow *et al*. (2007) obtained a higher survival rate ranging from 84.7% to 90.8%, while growth rate was between 0.21 and 0.24 g day^{-1} but during a longer period of seven weeks. Our results are comparable to those obtained by Huang *et al*. (2004) who recorded a survival rate of 90% after 70 days, but lower than that reported by Ayoola *et al*. (2009) on the juveniles of *Clarias gariepinues* fed with food containing bacteria with probiotic properties (*Lactobacillus sp*. and *Bifidobacterium sp*.) for 90 days. Ramos *et al*. (2017) reported that no effect of probiotic feeding was observed after a long period of probiotic supplementation.

Our results demonstrated a statistically insignificant, but higher growth rate in the experimental group (Fig. 1), yet it became close to statistically significant at the end of the experimental period. This suggests that a longer experimental period could have shown significant differences.

Currently, several types of beneficial feed additives such as probiotics, prebiotics, and synbiotics are used in aquaculture to improve growth performance (Hoseinifar *et al*. 2018). The use of probiotics as growth promoters of edible fishes has been reported by Lara-Flores *et al*. (2003) who found significant improvements in growth performance and the feed conversion ratio of Nile tilapia fry (0.16 g) fed for 63 days with commercial probiotic intended for terrestrial animals and consisting of a mixture of *Streptococcus faecium* and *Lactobacillus acidophilus*. El-Haroun *et al*. (2006) reported a 33%, 43% and 30% increase in the daily growth rate, feed conversion ratio and

Table 1. The performance of *Oreochromis niloticus* fed a diet with or without a *Lactobacillus* strain as probiotic.

Parameters		Groups		SEM[1]	$P > t$[2]
		control (30)	experimental (30)		
Weight (g)					
	Initial	1.75	1.64	0.084	0.27
	Final	2.06	2.13	0.117	0.67
ADG (g/d)		0.026	0.041	0.007	0.22
Relative ADG (%, d^{-1})		1.3	2.1	0.37	0.20
SL (cm)	Initial	3.78	3.72	0.067	0.52
	Final	4.02	4.08	0.079	0.63
TL (cm)	Initial	4.77	4.74	0.085	0.77
	Final	5.05	5.22	0.097	0.23

[1] SEM = Standard error of the difference between means. [2] Probability of a difference higher than that observed, according to Student's *t*-test.

profit, respectively in 25 g Nile tilapia fed for 84 days with Biogen® (commercial probiotic) consisting of a mixture of *Bacillus licheniformis* and *Bacillus subtilis*.

The diet of the Nile tilapia was amended with a probiotic *Streptococcus* strain increasing significantly the content of crude protein and crude lipid in the fish, also weight increased from 0.154 g to 6.164 g in 9 weeks of culture (Ghosh *et al*. 2008). Examples of growth improvement of ornamental fishes were reported, including swordtail (*Xiphophorus helleri*, *X. maculatus*) and guppy (*Poecilia reticulate*, *P. sphenops*). Their feed was supplemented with *Bacillus subtilis* and *Streptomyces* sp., which significantly increased in growth and survival of *Xiphophorus* spp. and *Poecilia* spp. after 90 and 50 days of administration, respectively (Ghosh *et al*. 2008).

Our results disagreed with those of Lara-Flores *et al*. (2003) and Wang *et al*. (2008). These authors reported that after 40 days, tilapia supplemented with *Enterococcus faecium* probiotic showed significantly higher final weights and daily weight gain than those fed a control diet ($P < 0.05$). In addition, a study on clownfish *Amphiprion sebae* showed a considerable weight gain when the fish received a diet supplemented with *Lactobacillus sp*. and yeast. Weight gain was 20% higher for the *Lactobacillus sp*. treatment and 25% for the yeast treatment (Pushparaj *et al*. 2012). El-Haroun *et al*. (2006) reported that the growth performance and nutrient utilization of Nile tilapia, including weight gain, specific growth rate, protein efficiency, protein production and fluid retention were significantly ($P \leq 0.01$) higher in the probiotic than in the control group. In addition, Li and Gatlin (2004) reported that dietary supplementation with the probiotic Grobiotic® significantly improved the growth and disease resistance of a *Moronechrysops* × *Saxatilis* hybrid. A similar observation has been made with other species, notably the red drum *Sciaenopsocellatus* and the Nile tilapia (Shelby *et al*. 2006). Furthermore, *Haliotis* sp. supplemented with probiotics had a survival rate of 62% against the pathogenic bacterium *Vibrio anguillarum* compared to 25% survival of untreated animals (Cruz *et al*. 2012).

Gatesoupe (1997) reported an improvement in the growth rate of turbot larvae *Scopthalmus maximus* in hatcheries when treated with probiotics. The same observations were made in the work of Paulmony (1996). The latter indicated that a diet enriched with yeast significantly influenced growth, food conversion rate and specific growth rate of *Cyprinus carpio*. A large percentage increase in growth was obtained for fish fed a diet supplemented with 6% yeast. A similar result was reported by Singh *et al*. (1980) for *Labeo rohita*.

The application of bacteria with high probiotic potential in aquaculture is booming. Gatesoupe (2007) and Aly *et al*. (2008) evaluated the probiotic potential of a *Bacillus pumilus* strain in culture of tilapia and showed that a low dose of this strain led to a significant increase in weight gain for 2 months. A similar result was obtained by Taoka *et al*. (2006), who studied the effect of commercial probiotics (*B. subtilis, L. acidophilus, Clostridium butyricum* and *S. cerevisiae*) on the growth of *Paralichthys olivaceus*. Maurilio (2011) also reported that yeast (*S. cerevisae*) stimulated growth in tilapia as do *Streptococcus faecium* and *L. acidophilus*, which also improved specific growth of tilapia. In addition, Hoseinifar *et al*. (2018) reported that probiotics enhanced growth performance and feed utilization in aquatic animals through increasing digestive enzyme activity. Overall, there was a similarity in these results in terms of growth improvement (Gnikpo *et al*. 2014). However, our results agree with the study of Gunther and Montealegre (2004) and Shelby *et al*. (2006) who reported no positive effect on the weight and length of tilapia fish. Moreover, Wang *et al*. (2008) indicated that supplementation with *E. facium* in the diet of tilapia had no significant effect on weight gain during the first 40 days.

In addition, *Lactobacillus* sp. has been reported to improve survival by 72% and performance of the pearl oyster, *Pinctada mazatlanica* (Aguilar-Macias *et al*. 2010). Furthermore, in a study with juvenile tiger shrimp (*Penaeus monodon*), *Lactobacillus acidophilus* (10^5 CFU g^{-1}) was administered for 1 month and an increase in resistance (80% survival) was observed following exposure with pathogenic *Vibrio alginolyticus* (Sivakumar *et al*. 2012). Interestingly, Dash *et al*. (2015) administered a heat-killed form of *Lactobacillus plantarum* at rate of 10^8 CFU g^{-1}

in *Macrobrachium rosenbergii* diet for 90 days. While no significant effects were observed on growth performance, feeding on probiotic supplemented diet noticeably enhanced immune responses and disease resistance.

Finally, Hansen and Olafsen (1988) noted that the incorporation of probiotics has proven advantageous in domestic animal production, and the search for effective probiotics may have great potential in aquaculture of marine organisms.

Conclusions

The probiotic strain used in this experiment did not improve growth parameters or morphological traits in tilapia. The treated group also had a similar mortality rate compared to the control group. Despite the fact that the current study did not produce positive results in the growth of tilapia, growth rates between the two groups requires further research on the effects of the probiotic on tilapia and other culture fish. Longer experiments could be carried out to highlight the potential effects of the probiotic strain on tilapia performance.

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