Original Article

Supplementation of probiotics in Nile tilapia fingerling cultivation subjected to microbial challenge

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Abstract

The intensification of aquaculture has brought about the need to find alternative feed supplement sources to reduce production costs. One of the alternative sources of feed in fish farming is animal manure. However, the use of animal manure may cause health problems in fish cultures. The objective of this study was to determine the effects of probiotics on *Nile tilapia* fingerlings subjected to microbial challenge using swine manure. Three hundred (300) fingerlings weighing 2±0.05g and measuring 5±0.06 cm, were distributed among four treatments with three replications each. Swine manure was inoculated in water in the proportion of 10 % of fingerlings live weight. Probiotics *Saccharomyces cerevisiae* and *Lactobacillus plantarum* were used as ration supplements in the proportions of 0.5 % per 100 g of ration. The results showed no significant differences (P>0.05) in terms of growth between treatments. The inclusion of *S. cerevisiae* reduced the number of pathogenic bacteria in the intestine of fingerlings and resulted in better production performance. Feed supplementation with *L. plantarum* had no effect, neither on production, nor on intestinal microbial population. The fingerlings under study showed typical signs of yersiniosis, edwardiellosis and mycobacteriosis. From the experiment it was concluded that the supplementation of the probiotic *S. cerevisiae* on the Nile tilapia fingerlings treated with swine manure showed a positive effect in the sense that this probiotic avoided the intestinal colonization of fingerlings by pathogenic bacteria. Additionally, the presence of the probiotic seems to promote better grow performance and hence reinforce the results of previous studies on the role of probiotics in aquaculture. More experimental studies are required, particularly *in situ* experiments, with the addition of other performance analyses linked to the immune response of the fingerlings.

Keywords: aquaculture, fish farming diseases, *Oreochromis niloticus*, probiotics

Introduction

Aquaculture is an agricultural system for the production of aquatic organisms, which has been expanding globally with the increase of human populations and urbanization. It is estimated that aquaculture production will double by 2030 in order to meet the demand for fish (Subasinghe, 2015). In Mozambique fish farming began in the 1950s, with the goal of producing food rich in animal protein to help improve diets and the quality of life for rural communities (INAQUA, 2012). Nationally, fish farming has developed at an industrial and small scale. Aquaculture production of 5,517 tons was expected in 2019, of which 3,770 were reached, against 3,245 tons produced in 2018 (Ministério do Mar, Águas Interiores e Pesca, 2020).

The expansion of fish farming resulted in increasing demands for fish ration and its main ingredient, fish meal, which is seldom available and expensive. Fish meal limitations in turn leads to the need for alternative sources of good nutritional quality feed, which would enable fish farmers to improve production, and maintain the integrity of the ecosystems (Boscolo *et al*., 2005; Castellani and Abimorad, 2012).

Integrated fish farming encompasses a combination of fish culture with agricultural systems (Mishra, 2010), an excellent alternative for overcoming ration deficits in fish farming (Castellani and Abimorad, 2012). In such integrated systems, swine manure is used as fertilizer in fish tanks to promote the growth of photosynthetic microorganisms, or it can be fed directly to fish (Dang *et al*., 2011). The use of swine manure as fertilizer involves some risks due to the accumulation of organic matter in fish tanks, which stimulates the growth of pathogenic bacteria which can cause illnesses in cultivated organisms (Hilbrands and Yzerman, 2004).

A viable alternative for preventing the occurrence of illnesses in fish farming could be the use of probiotics, which when administered in the proper amounts, provide health benefits for the host organisms by improving the balance of their intestinal flora (Azevedo and Braga, 2012). Although evidence already exists that probiotics play an important role in aquaculture, the present study sought to further explore the subject to obtain a better understanding, using Nile tilapia (*Oreochromis niloticus*) fingerlings grown in closed systems in Mozambique. Specifically, the objective of the study was to assess the effects of probiotic supplements on Nile Tilapia fingerlings subjected to microbial challenge using swine manure.

Material and methods

Nile tilapia fingerlings

Sex reversed Nile tilapia fingerlings were obtained from Aquacultura Chibaha Ltd, a company located in the district of Vilankulo, Inhambane Province, Mozambique, weighing an average 2 ±0.05 g and measuring 5 ±0.06 cm. The fingerlings were transported in 50-liter transparent plastic bags with approximately 5 L of water and 15 L of dissolved oxygen, at a density of 250 g of fish per 5 L of water. Once in the laboratory, the fingerlings were placed inside aquaria for 15 minutes, while still in plastic bags, to equilibrate the temperatures before their release.

Analysis of the microbial composition in swine manure

Swine manure was collected weekly at the Agrarian Institute of Umbeluzi, located in the district of Boane, Maputo Province, Mozambique. A sterilized spatula was used for collection, and the collected manure was placed in a tightly closed container and transported in 4 °C temperature. The manure was kept in the laboratory for one week, after which the remaining manure was discarded and substituted with fresh manure to avoid reduction of quality during storage (Hilbrands and Yzerman, 2004).

The presence of pathogenic bacteria in swine manure was determined from 10^{-1} to 10^{-6} dilutions of 1 g swine manure in 0.1 % peptone water. From each of the dilutions, 1 ml was drawn and double inoculated in Agar Lowenstein-Jensen and Agar MacConkey. One-half of each dilution was incubated at 37°C by 48 hours in the Agar Lowenstein-Jensen culture and the other half for 24 hours in Agar MacConkey culture. Following the incubation period, the presence of pathogenic enterobacteria in samples of swine manure was determined, using the Gram method. In order to determine the presence of Mycobacteria in the manure, 12 samples were sent to the microbiology laboratory at the Central Hospital of Maputo for analysis, based on the Ziehl-Neelsen test.

Experimental design

The experiment was carried out for a period of 90 days, from May to July, at the Ecology Laboratory in the Department of Biological Sciences, Faculty of Sciences of the Eduardo Mondlane University in Maputo. The climate in Maputo is humid subtropical, with a cool dry (winter) season from April to October, and a warm wet (summer) season for the rest of the year (Gomes *et al.*, 2014; Macamo *et al.*, 2015). During summer the average temperature is around 30-31 °C in January and in the winter season the average temperature is about 25-26 °C during the months of July and August (Gomes *et al.*, 2014). Agriculture and fisheries are two of the most important economic activities in Maputo and its satellite cities (Macamo *et al.*, 2015).

The experimental design was a complete randomized design with four treatments with three replicates. The fingerlings were distributed among 12 aquaria of 50 litres each at 25 fingerlings per aquarium. In aquaria designated for the control treatments (C), fingerlings were fed commercial fish rations without probiotics and swine manure. Treatment (T1) consisted of treating the fingerlings with swine manure and feed ration without probiotic supplements. For treatment (T2) the fingerlings were treated with swine manure and a ration supplemented with the probiotic *Saccharomyces cerevisiae,* while treatment (T3) consisted of treating fingerlings with a combination of swine manure and a ration supplemented with the probiotic *Lactobacillus plantarum.*

The aquaria were equipped with an aeration system composed of micropores linked by silicone hoses connected to an air pump. Water replacement was carried

out daily at a rate of 50 % in the mornings by means of a siphon to remove faeces and the remainder of rations. Water quality was assessed through daily measurements of temperature and the dissolved oxygen in the mornings (8:00 hrs) and in the afternoons (16:00 hrs), with a PCE-PHD 1 measurement device. The pH was measured once a day (at 16:00 hours) using a pH meter (HI 9025 Microcomputer, HANNA Instruments) and the electric conductivity was determined weekly, in the mornings before siphoning, with a conductivity measuring device (524 S/N 4289 CRISON). Sodium thiosulfate was applied to the aquaria in the proportion of one drop for every litre of water, immediately after water was replaced (Meurer *et al*., 2006) to neutralize the effect of chlorine present in the water.

The application of swine manure to treatments 1, 2 and 3 was done in the morning soon after syphoning in the proportion of 10 % of the live weight of the fingerlings adjusted biweekly in accordance with the determined weights of the fingerlings. Swine manure was diluted in 100 ml of distilled water and then sterilized to inoculate the aquaria (Meurer *et al*., 2006).

The probiotic *S. cerevisiae* containing about $3.2*10^{10}$ live microorganisms for each gram of the product was obtained from the Mozambican brewery (2M), located in the city of Maputo, Mozambique. This probiotic was added to treatment 2 at the proportion of 0.5 g per 100 g of ration. The number of yeasts per gram of the product as well as of ration were analysed by counting in plates with 1 ml of the dilutions from 10^{-1} to 10^{-3}. The selective culture medium *Yeast Growth Cloramphenicol* (YGC) was used for yeast.

The probiotic *L. plantarum*, containing about $1.8*10^{10}$ live microorganisms per gram of the product was obtained from the ProLab laboratories. This probiotic was added in the proportion of 0.5 g per 100 g of ration. Dilutions 10^{-1} to 10^{-3} in peptone water 0.1 % solution were made for the quantification of acid-lactic bacterium per gram of the product and of the ration. One (1) ml of each dilution was incubated in anaerobic conditions in *Man Rugoso and Sharp* (MRS) culture medium in the microbiology laboratory of the Central Hospital in Maputo.

Determination of the microbial composition in fingerlings supplemented with probiotics following microbial challenge

In order to determine the presence of pathogenic bacteria in the water of the experimental aquaria, three (3) ml of aquarium water was sampled in the morning, at the beginning of the experiment before siphoning and inoculated in duplicate samples of Agar MacConkey, Agar Lowenstein-Jensen and YGC e MRS medium. To determine the extent of intestinal colonization by the swine manure bacteria as well as the ration supplemented with probiotics, six fingerlings were collected from each treatment after a 24-hour fast and sacrificed by decapitation. After local disinfection with a sterile gauze soaked in 70 % alcohol, the intestine was removed and transferred to sterilized Petri dishes. The intestine was then triturated while still fresh and placed in previously sterilized test tubes, diluted in 2 ml of distilled water and then homogenized in a vortex for 1 min. Following the homogenization, decimal dilutions were made from 10^{-1} to 10^{-3} in sterile distilled water. One (1) ml of each dilution was drawn and inoculated in duplicate, in plates containing the above-mentioned culture media for counting of the colony-building units.

Determination of growth and survival

Growth was determined by measuring the length of 300 fingerlings under study at the beginning and at the end of the experiment, using the formulae of Pooramini *et al.*, (2009, 2014):

Weight gain = average final fish weight – average initial fish weight (1)

% Weight gain = [(average final weight – average initial weight)]/average initial weight (2)

Specific growth rate = [(lnWf – lnWi)]/(t2-t) (3)
Where: lnWf = ln for the average final fish weight;
lnWi= ln for the average initial fish weight;
(t2-t1) = duration of the experiment in days.

Condition Factor (CF) = [(W/L)*100] (4)
Where W = fresh fish weight;
L = length of fish (cm).

Apparent Feed Conversion Rate (AFC) = [F/ (Wf–Wi) (5)
Where F= Feed provided (g);
Wf = average final fish weight;
Wi = average initial fish weight.

Furthermore, the determination of survival was carried out using the formula of Koch *et al*., (2015):

Survival = [(Nf/Ni)]*100 (6)
Where: Nf – number of fish at the end of the experiment;
Ni = number of fish at the start of the experiment.

Observation of clinical signs of the *O. niloticus* fingerlings

In order to determine the clinical signs in fish, 15 fingerlings from each treatment were captured every 15 days and observed for possible signs of illnesses (Ostrensky and Boeger, 1998). Essentially, the fingerlings were assessed for the presence or absence of typical signs of yersinioses, edwardsioloses and of micobacterioses, including mutilated fins, absence of scales, dark pigmentation of the skin, reddish-coloured gills, puffy and opaque eyes, with signs of haemorrhages around the eye and/or on the body skin. At the end of the experiment six fingerlings were sacrificed in each treatment for observation of internal signs including dark gall bladders and empty intestine (Kubitza, 2005).

Data analysis

Collected data were subjected to the Shapiro-Wilk test for normality, and the Leven test for the homogeneity of variances. Mean comparisons were based on one-way ANOVA, for the variables with normal distribution and homogeneous variances (weight gain, % weight gain, specific growth rate, and apparent feed conversion rate). For the variables which did not show normal distribution (condition factor and survival), the Kruskal-Wallis test was used. As for data on colony-building units from which significant differences were observed, the Tukey and Newman-Keuls tests were used for variables with normal distribution and those without normal distribution, respectively.

Results and discussion

Water quality

The results of the analyses of water quality parameters observed in the aquaria used in this experiment were as follows: water temperature in the morning and in the afternoon 21.5 ±0.01 °C, and 22.3 ±0.01 °C respectively; dissolved oxygen in the morning and in the afternoon 7.1 ±0.05 mg/l, and 6.8 ± 0.04 mg/l respectively; pH 8 ± 0.01; and conductivity 99.9 ±0.23µS/cm. Statistical analyses showed no significant differences among the treatments (P>0.05). The results from this study were in line with the observations of Meurer *et al*. (2006) and Hortmam *et al*. (2014). The experiments were carried out within the established guidelines for good species performance, except for the variable temperature, which was a few degrees below the recommended level. Previous studies with *O. niloticus* culture reported ideal parameters to range between 6.5 to 9 for pH; 25 to 35 °C for water temperature; dissolved oxygen concentration above 5.0 mg/L and water conductivity of 96µS/cm (Hilbrands and Yzerman, 2004; Meurer *et al.*, 2008; Dias *et al*., 2011). The low temperatures observed in the present study are reflective of seasonal weather patterns in the city of Maputo in May to July which corresponds to the cold season with temperatures averaging 20.0±1.0 °C.

Microbial population in swine manure

Non-lactose fermented enterobacteria to the value of $7.1*10^6$ UFC and *Mycobacteria* spp to the value of $1.2*10^5$ UFC were identified as microbial challenges from the swine manure utilised for the present study. The values obtained correspond to those by Bona *et al*. (2013) when they isolated the microbial flora of anaerobic treatment systems of swine manure inoculants and found a $8.0*10^6$ UFC enterobacteria composition. These values confirm the findings of Ziemer *et al*. (2008), which from his studies with swine manure observed that the fresh swine manure usually contains 10^{10} bacterial cells per gram. The pathogenic bacterium found in swine manure in the present study were also reported by Ziemer *et al*. (2008), from a study which showed that swine manure contains pathogenic bacterium which include *Mycobacterium spp, Yersinia spp, Eschericia coli, Brucella spp, Listeria monocytogenes, Bacillus anthracis, Leptospira spp, Clamydia spp and Campylobacter spp.*

Amount of probiotics

The microorganisms used as probiotics in this study accounted for 100 g of ration, $1.6*10^5$ UFC of *S. cerevisiae* and $9*10^4$ UFC of *L. plantarum*. These values were lower compared to those recommended by Martins *et al*. (2006). However, the values for *S. cerevisiae* were similar to the values of 10^5 live cells per gram of ration obtained by Meurer *et al*. (2008). The values for the *L. plantarum* probiotic found in this research contradicted the values observed by Leandro *et al*. (2010) in studies where they found that feed supplemented with *L. plantarum* contained about 10^6 UFC/g of the food.

Microbial composition of pathogenic bacteria in water and in the Nile tilapia fingerlings

Enterobacteriasis non-lactose fermentors and mycobacteria were isolated in aquarium water and in the intestine of fingerlings used in this experiment. The latter were not observed in the intestine of the fingerlings subjected to Treatment (T2). Similar results were obtained by Pupo (2006) in studies where he isolated several species belonging to the Enterobacteriaceae and which have been known for their

Table 1. Microorganism colony-building units in aquarium water and in the intestine of *O. niloticus* fingerlings, by treatment (with or without probiotics).

		Treatments			
		C(1)	T1(2)	T2(3)	T3(4)
Non-lactose fermenting Enterobacteria	In the Water	$5*10^{3}$ (a)	$7.7*10^{4}$(b)	$2.3*10^{4}$(a)	$4.7*10^{4}$(a)
	In the Intestine	$6.7*10^{4}$(b)	$1.5*10^{5}$(c)	$8*10^{3}$(a)	$1.7*10^{5}$(c)
Mycobacteria	In the Water	$5*10^{4}$	$6.2*10^{4}$	$3.3*10^{4}$	$4.5*10^{4}$
	In the Intestine	$5.7*10^{4}$(f)	$6.6*10^{4}$(df)	0(e)	$1*10^{5}$(f)
Yeast	In the Water	$5*10^{3}$(c)	$2.8*10^{4}$(d)	$7*10^{3}$(c)	$2.7*10^{4}$(d)
	In the Intestine	$1.5*10^{5}$	$2.5*10^{4}$	$3*10^{4}$	$1.4*10^{5}$
Acid-Lactic Bacteria	In the Water	$2*10^{4}$(c)	$4.8*10^{4}$(c)	$1*10^{4}$(c)	$8.2*10^{4}$(c)
	In the Intestine	$1.5*10^{5}$(b)	$1.7*10^{5}$(b)	$4.5*10^{4}$(b)	$2.1*10^{5}$(b)

Mean comparisons showed significant differences among treatments (P<0.05). Different letters (a,b), (c,d) in the data for microbes in water indicate significant differences based on Tukey's and Newman Keuls tests, respectively. Different letters (a,b,c), (e,f) in the intestinal microbiology data also indicate significant differences based on Tukey's and Newman Keuls tests, respectively.

pathogenic potential. Eissa *et al*. (2008) isolated the pathogenic bacterium *Yersinia ruckeri* in 100 cultivated fish. Ahmed and Refaey (2013) isolated 5 % of enterobacteriacea non-lactose fermentors in *Rhamdia quelen* (*Edwardsiella delays and Y. ruckery*) in the kidneys and external lesions of the same species.

In relation to mycobacteria, the results of this study are similar to the results of work carried out with ornamental fish and other freshwater species (*Cichlasoma bimaculatum, Carassis auratus* and *Cichlasoma meeki*), where five mycobacteria species were isolated including *Mycobacterium fortuitum, Mycobacterium marinum*, among others (Ishikawa *et al*., 2001). In a study with *O. niloticus*, the inclusion of 10^{9} UFC of *M. marinum* did not induce fish death during the experiment, but indicative signs of the presence of Micobacteriasis were observed in fish (Ishikawa *et al*., 2001). However, in *Carassius auratus*, mycobacterial inoculations at the concentrations of 10^{9} and 10^{8} UFC per animal provoked diseases followed by the death of organisms after 17 days of its inoculation.

Colony-building units (CBU)

The average number of colony-forming units in aquarium water and in the intestines of fingerlings are presented in Table 1. Significant differences were observed among treatments (P <0.05). Supplementation of *S. cerevisiae* under treatment T2 resulted in a significantly lower number of CBU of pathogenic enterobacteriaceae and average number of colony-forming units of acid-lactic bacteria, compared with other experimental treatments. Results published by Meurer *et al*. (2006) showed that the number of CBU of pathogenic bacteria were significantly less in fish fed a ration containing these probiotics, as compared with the control treatment, which is corroborated by the present study.

Supplementation of *L. plantarum* in treatment T3 resulted in a higher number of CBU of acid-lactic bacteria in the intestines of fingerlings. However, it did not reduce the intestinal colonies of pathogenic bacteria in fingerlings, when compared with the control treatment. Similar results were observed by Jotobá

Table 2. Growth parameters of *O. niloticus* fingerlings evaluated in treatments with and without probiotics.

Growth Parameters	Treatments			
	C(1)	T1(2)	T2(3)	T3(4)
Final Weight	3.3	3.2	3.5	3.2
Final Length	5.8	5.6	5.6	5.6
Weight Gain	1.0	0.9	1.3	0.9
% Weight Gain	30.3	28.1	36.1	28.1
Specific Growth Rate	1.2	1.2	1.3	1.2
Condition Factor	1.7	1.8	2.0	1.8
Apparent Feed Conversion Rate	0.45	0.5	0.3	0.5
Survival	100	96	100	100

(1) Control, (2) Treatment with swine manure without probiotics, (3) Treatment with swine manure with *S. cerevisiae*, (4) Treatment with swine manure and *L. plantarum*. There were no significant differences amongst treatments. (P> 0.05).

and Mouriño (2015), whereby supplementation with acid-lactic bacteria resulted in a large population of these bacteria (about $5*10^4$ CBU per gram of the intestine) in fish. Hortmam *et al.* (2014) also reported similar results which showed that the intestines of tilapia fingerlings fed with probiotics had a larger number of lactic acid and a lower number of pathogenic bacteria, compared with fish fed with the control diet.

Effects of probiotics on the growth and survival of the *O. niloticus* fingerlings

Results of the fingerling growth parameters and survival under each treatment are presented in Table 2. Fish weight changes during the experiment are illustrated in Figure 1. Statistical analyses showed no significant differences in terms of growth parameters and survival among the treatments ($P>0.05$). However, the death of one fish was recorded in Treatment 1 (T1) during the experiment and the fish in question had lost the scales on its skin.

Treatment 2, together with *S. cerevisiae* resulted in better performance in the Nile tilapia fingerlings for all of the growth parameters, except for the fish length which showed higher average values in the control treatment. Similar results were observed by Pooramin *et al.* (2009; 2014) and Jotobá and Mouriño (2015) who worked with probiotics, using fish as the indicator organism. The longest final length observed in the control treatment without probiotic supplements corroborates with the findings of Pooramini *et al.* (2009; 2014) who also found longer final lengths for fish artificially fed control diets without the inclusion of probiotics.

Supplementation of *L. plantarum* in Treatment 3 did not have any effect on the performance of fingerlings as compared with the control treatment, but resulted in better performance than Treatment 1 (T1). Similar results were found by Suzer *et al.* (2008) from studies where the use of *Lactobacillus sp* as a probiotic in *Sparus aurata* had no effect on weight gain. The main disadvantage of the use of some probiotics resides in the absence of spores, which hinders their inclusion and durability in commercial diets (Leandro *et al.*, 2010; Vieira, 2010).

Effect of swine manure on *O. niloticus* fingerlings

Internal and external clinical signs were observed to determine the effects of swine manure on *O. niloticus* fingerlings. Results from the observations revealed the following signs: dark gall bladders and empty intestine, an indication that the fingerlings did not feed for several days; bleeding around the eye and the skin; absence of scales; opaque eyes; mutilated fins; reddish-coloured gills (sign of anaemia); lack of appetite, and the death of one fingerling. The clinical signs illustrated in Figure 2 were observed under Treatment 1 which included swine manure without probiotic supplements. However, they were not observed either in the control or in Treatments 2 and 3, which included *S. cerevisiae* and *L. plantarum* supplements, respectively.

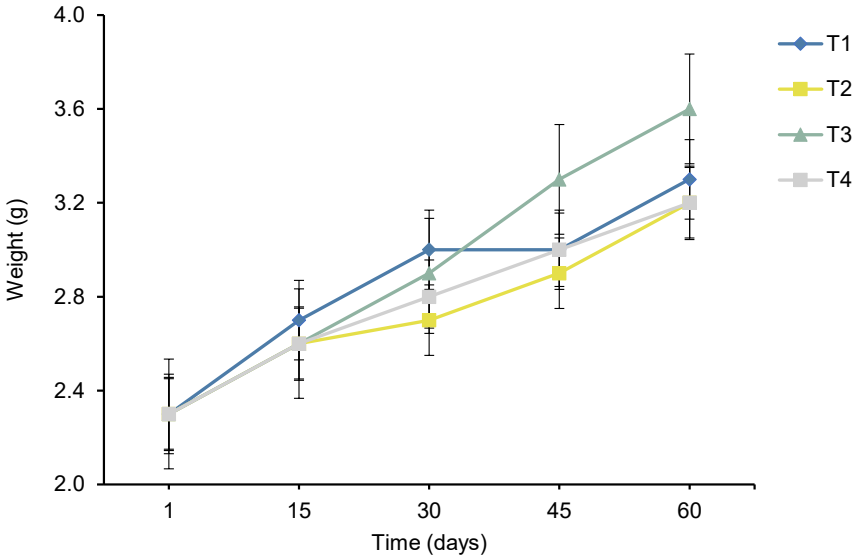

Figure 1. Differences in weight during the experimental period (starting from date of swine manure inoculation in aquariums) in treatments with and without probiotics. (T1) Control treatment, (T2) Treatment with swine manure without probiotics supplements, (T3) Treatment with swine manure with the probiotic *S. cerevisiae*, (T4) Treatment with swine manure with the probiotic *L. plantarum*.

Figure 2. Signs observed on fish under Treatment 1 (Swine manure without probiotics supplement). A. Dark-green gall bladder; B. Empty stomach; C. Haemorrhage (bleeding) around the eye; D. Dead tilapia fingerling showing no scales, opaque eyes and mutilated fins; E. Mutilated fins; F. Haemorrhages on the skin; G. Pale-red to rose coloured gills.

The presence of these signs suggest that the microbial challenge had an effect on *O. niloticus* fingerlings, from contact with pathogenic microorganisms contained in the swine manure. These are typical signs of yersiniosis, edwardsiellosis and mycobacteriosis in fish (Ostrensky and Boeger, 1998; Carson and Wilson, 2009).

The signs described above were also observed by Meurer *et al*. (2009). According to Kubitza (2005) the emergence of these signs is associated with high concentrations of faecal material in the cultivation tanks. These signs where fish were infected with enterobacteriacea *Y. ruckery*, showed clinical signs including lack of appetite, dark pigmentation of the skin, haemorrhages around of the mouth, the eye and the fins and swollen abdomen were associated with faecal material in cultivation tanks (Carson and Wilson, 2009; Ahmed and Refaey, 2013).

Swine manure inoculated in the experimental aquariums contained bacteria that are pathogenic for fish. Pathogenic microorganisms present in cultured fish have the potential to colonize the intestine of fingerlings. Pathogenic bacteria contained in swine manure had no effect on growth, but affect survival of fingerlings due to the inclusion of probiotics in the commercial ration used.

The supplementation of the probiotic *S. cerevisiae* in the feed of *O. niloticus* fingerlings treated with swine manure showed a positive effect in the sense that the probiotic prevented the intestinal colonization of fingerlings by pathogenic bacteria. The application of manure in fish farming to increase food production seems to be a good alternative, but supplementation with probiotics to reduce contamination by pathogenic bacteria as observed in the present experiment provides an added advantage.

The circular economy approach, which focuses on "reducing, reusing and recycling" resources including animal waste such as swine manure, can be used due to the presence of proteins and other valuable compounds for cultivated organisms in the manure. In the present experiment, swine manure was added directly to the fish cultivation system similarly to the practice used by farmers. Instead of applying manure directly to the fishponds that can lead to contamination from pathogenic bacteria, an alternative approach could be to add manure to separate food production systems to stimulate the growth of protei-rich phytoplankton and zooplankton that are the basis of the food web and can then be fed to omnivorous fish.

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