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EFFECTS OF PROBIOTIC AND STOCKING DENSITY ON NILE TILAPIA FINGERLINGS (*Oreochromis niloticus*) PRODUCTIVE PERFORMANCE

Efectos de un probiótico y de la densidad sobre el comportamiento productivo de alevines de tilapia del nilo (*Oreochromis niloticus*)

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ABSTRACT

A study was conducted with the purpose to assess the effect of a commercial probiotic and stocking density on productive performance of Nile tilapia (*Orechromis niloticus*) fingerlings. Fingerlings were stocked in four 16.56-m^3 aerated concrete tanks at two initial densities, low (50 fish/m^3) and high (100 fish/m^3) and fed four times a day with a commercial food sprayed with 2.6 or 6.6 g/kg food of a commercial probiotic containing strains of *Lactobacillus acidophilus* and *Pediococcus acidilacticii,* and inactivated *Saccharomyces cerevisiae.* Water temperature ranged between 25.9 to 26.1 °C, dissolved oxygen level was 4.95 mg/L and pH value was about 6.9. Daily weight gain, final weight and body length, height and width were greater (P<0.05) in fish reared at low density and added with 2.6 g/kg of probiotic. Specific growth rate was greater (P<0.05) in fish reared at low density without probiotic and in fish reared at high density and fed 6.6 g/kg of probiotic. Inclusion of probiotic improved (P<0.05) feed conversion regardless of stocking density but the cost of feed was significantly increased (P<0.05) by the addition of 6.6 g/kg of probiotic. It can be conclude that the addition of 2.6 g/kg of probiotic improved productive performance of tilapia fingerlings and juveniles rearing at low densities without a significantly increasing the cost of feeding. Productive performance of fish reared at high stocking density could be successfully improved with the addition of 6.6 g/kg of probiotic.

Key words: *Lactobacillus acidophilus*, stocking density, growth, feed conversion.

RESUMEN

Se realizó un estudio con el objetivo de evaluar el efecto de la adición de un probiótico comercial y de la densidad de cultivo sobre el comportamiento productivo de alevines de tilapia (*Orechromis niloticus*). Los alevines fueron sembrados en cuatro tanques de concreto de 16,56-m^3 provistos con sistema de aireación a dos densidades iniciales, baja (50 peces/m^3) y alta (100 peces/m^3). Fueron alimentados cuatro veces al día con un alimento comercial asperjado con 2,6 o 6,6 g/kg de alimento de un probiótico comercial que contenía cepas de *Lactobacillus acidophilus* y de *Pediococcus acidilacticii,* así como células inactivas de *Saccharomyces cerevisiae.* Durante el estudio la temperatura del agua varió de 25,9 a 26,1°C, con un nivel de oxígeno disuelto de 4,95 mg/L y un pH de 6,9. La ganancia diaria de peso, el peso final así como la longitud, altura y grosor del cuerpo fueron mayores (P<0,05) en los peces cultivados a baja densidad y adicionados con 2,6 g/kg de probiótico. El crecimiento específico fue mayor (P<0,05) en los peces cultivados a baja densidad sin la inclusión de probiótico y en aquellos cultivados en alta densidad alimentados con 6,6 g/kg de probiótico. La inclusión del probiótico mejoró (P<0,05) la conversión alimenticia en ambas densidades pero el costo de alimentación se incrementó significativamente (P<0,05) al adicionar 6,6 g/kg del probiótico. Se concluye que la adición de 2,6 g/kg de probiótico mejoró el comportamiento productivo de los alevines de tilapia cultivados a baja densidad sin un incremento significativo en el costo de alimentación. El desempeño productivo de los peces cultivados a alta densidad puede ser mejorado con la adición de 6,6 g/kg de probiótico.

Palabras clave: *Lactobacillus acidophilus*, densidad de cultivo, crecimiento, conversión alimenticia.

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INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world at an average rate of 8.9% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period [27]. Currently, the purpose of the aquaculture industry is to increase growth and or survival performance, feed efficiency, and resistance of aquatic organisms, while reducing production costs [4].

Tilapia (*Orechromis niloticus*) culture has been growing at an outstanding rate during the past two decades. As a result, the production of farmed tilapia has witness a 6-fold increase during the past 15 years, jumping from 383,654 metric tons in 1990 to 2,348,656 metric tons in 2006 [11, 12]. In addition, there has been a gradual shift in tilapia culture from traditional semi-intensive to more intensive farming systems, leading to rear fish at high stocking densities [6].

In aquaculture, stocking density is considered to be one of the important factors that affect fish growth, feed utilization and gross fish yield [18]. Even though the full utilization of space for maximum fish production through intensive culture can improve the profitability of the fish farm, several studies have indicated an inverse relationship between the stocking density and growth rate of tilapia fingerlings, caused by voluntary appetite suppression, more expenditure of energy because of intense antagonistic behavioral interaction, competition for food and living space [8] and increased stress [22]. Also, the relationship between feed conversion ratios and survival rates of the fish and stocking density is not found consistent [10, 23].

On the other hand, probiotics can be an alternative to reduce the use of chemical agents. In aquaculture probiotics have been defined as ‘‘microbial cells that are administered in such a way to enter the gastrointestinal tract and to be kept alive, with the aim of improving health’’ [13]. Beyond their action in improving the immune system, probiotics improved the growth rate of Nile Tilapia under stress factors such as stocking density [17].

The use of probiotics in aquaculture is now widely accepted with an increasing demand for environment friendly aquaculture. Nowadays, a number of preparations of probiotics are commercially available and have been introduced to tilapia farming as feed additives, or are incorporated in pond water [2, 14, 29]. The aim of this study was to assess the effect of a commercial probiotic on growth performance of Nile tilapia fingerlings and juveniles rearing at different stocking densities.

MATERIALS AND METHODS

A 120 days (d) experiment was conducted from October 2011 to February 2012 in Jala Aquaculture Center (19°04’35? N; 103°53’24? W), located in the State of Colima, Mexico. Male Nile tilapia var. Stirling (*Oreochromis niloticus*) fingerlings with an initial average weight of 0.95 ± 0.04 g were stocked in four 16.56-m^3 aerated concrete tanks supplied with a water flow-through system, at two initial densities: low (50 fish/m^3) and high (100 fish/m^3). Stocking densities were reduced to 25 and 50 fish/m^3, respectively, after the first eight weeks of the test.

Two commercial feed (crude protein 53%, crude fat 15%, flour, semi-floating, particle size <0.35 mm) and (crude protein 44%, crude fat 15%, extruded, floating pellet size 1.5 mm) were offered the first six weeks and the last nine week of the trial, respectively. Both was sprayed with 2.6 or 6.6 g/kg food of the commercial probiotic FloraMax-B11® (Pacific Vet Group, Arkansas, USA), intended to use in poultry and contained strains of *Lactobacillus acidophilus* and *Pediococcus acidilacticii,* skim milk, fructo-oligosaccharides and inactivated *Saccharomyces cerevisiae.* In order to avoid probiotic dilution, food was stored for five d prior to administration.

Four treatments were designed (T_1) group fed a commercial diet reared at low stocking density; (T_2) group fed a commercial diet added with 2.6 g/kg of probiotic reared at low stocking density; (T_3) group fed a commercial diet reared at high stocking density, and (T_4) group fed a commercial diet added with 6.6 g/kg of probiotic reared at high stocking density. Tilapia groups were fed four times a d (10:00, 12:00, 14:00, and 16:00 h). Initial daily feeding rate was about 10% of total biomass, and was progressively reduced to 5.0, 3.5 and 2.0% of total biomass per d in subsequent feeding times.

Water temperature during the experiment ranged between 25.9 to 26.1 °C with photoperiod of 11.48 h light and 12.12 h darkness. The dissolved oxygen level was 4.95 mg/L and the pH value was about 6.9. Water samples were daily analyzed for total ammonia nitrite, nitrate and pH levels using a photometer (AquaManager, YSI 5200A, Greece) to adjust the appropriate water quality parameters for tilapia cultivation.

Thirty-five fish were fortnightly sampled from each treatment to determine body weight, length, height and width. Additionally, specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR) and feed cost per kilogram gain (FCK) were calculated as El-Sayed *et al* [11]:

SGR = (ln final weight – ln initial weigth) * 100 / Time

DWG = Weight gain / Time

FCR = Feed given / Weight gain

FCK = FCR * Feed cost

Initially, Levene’s test was used to evaluate equality of variances. Subsequently, assuming unequal variances (P<0.05, Levene´s test), data were analyzed using a one-way analysis of variance followed by Dunnett´s T3 post hoc test, setting the alpha level at 0.05. The statistical computer software SPSS was used in data analysis [26].

RESULTS AND DISCUSSION

Final weight and body length, height and width were greater (P<0.05) when the fish were reared at low stocking density and fed 2.6 g of probiotic. Addition of 6.6 g of probiotic allowed fish stocking at high densities overcome body width and also, equaled final weight and body length and height of fish reared at low density without probiotic (TABLE I).

Specific growth rate was greater (P<0.05) in fish reared at low density without probiotic and in fish stocking at high density and fed 6.6 g of probiotic, whereas daily weight gain was greater (P<0.05) in fish reared at low density and added with 2.6 g of probiotic. Addition of 6.6 g of probiotic allowed fish reared at high densities equaled daily weight gain of fish stocking at low density without probiotic. Inclusion of probiotic improved (P<0.05) feed conversion regardless of stocking density, while in no probiotic supplemented groups, feed conversion was not affected (P>0.05) by stocking density. Cost of feed was significantly increased (P<0.05) by the addition of 6.6 g of probiotic (TABLE II).

According to Jatobá *et al.* [16] Nile tilapia juveniles fed a probiotic (*Lactobacillus plantarum*) supplemented diet showed significantly higher values for final weight and feed efficiency. Likewise, tilapia juveniles fed probiotic bacteria (*Lactobacillus rhamnosus*) improved growth performance and feeding efficiency ratio compared to animals fed with normal diet [14]. On the contrary, Meurer *et al*. [20] did not find an increase in weight gain for tilapia fed a live cells *Saccharomyces cerevisiae* probiotic.

The administration of a probiotic bacterial diet to tilapia is often reported as being responsible for increasing growth rates, feed conversion efficiency, and nutrient absorption. In some cases this beneficial effect was attributed to the capacity of the probiotic to stimulate and or produce some enzymes on the intestinal tract. Lara *et al*. [17] observed a high activity of alkaline phosphatase when probiotics were administered in the diet; and that this high activity reflected a possible development of brush border membranes of enterocytes, stimulated by the probiotic, which can be an indicator of carbohydrate and lipid absorption and explain the higher weight gain and the better feed conversion. Other studies indicate that lactic acid bacteria produce lactic acid in the intestinal lumen of the host and may acidify the digestive tract. This acidification of the digestive tract might contribute to dietary acidification and improve the solubility and uptake of minerals and accordingly improving feed efficiency [24, 25]. Further, a probiotic may produce a positive effect in water quality when this generate a better organism digestion, which reduces the nutrient excretion (protein principally) to pond water and while generating a better growth of the cultured aquatic organisms [2].

TABLE I

EFFECTS OF PROBIOTIC ADDITION AND STOCKING DENSITY ON FINAL WEIGHT AND BODY MEASUREMENTS OF NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS

Parameter	T_1	T_2	T_3	T_4
Final weight (g)	55.49 ± 3.73^{bc}	93.66 ± 5.05^{a}	40.25 ± 2.31^{c}	61.12 ± 2.10^{b}
Body length (cm)	14.10 ± 0.16^{bc}	17.50 ± 0.47^{a}	13.29 ± 0.24^{c}	15.18 ± 0.17^{b}
Body height (cm)	4.47 ± 0.07^{bc}	6.00 ± 0.17^{a}	4.28 ± 0.10^{c}	4.88 ± 0.08^{b}
Body width (cm)	2.20 ± 0.03^{c}	2.97 ± 0.10^{a}	2.11 ± 0.05^{c}	2.51 ± 0.04^{b}

T1= Low stocking density-without probiotic, T_2= Low stocking density-2.6 g probiotic, T_3= high stoking density-without probiotic, T_4= high stocking density-6.6 g probiotic.
^{abc} Means in the same row with different superscripts are significantly different (P<0.05).
Mean ± standard error.

TABLE II

EFFECTS OF PROBIOTIC ADDITION AND STOCKING DENSITY ON SPECIFIC GROWTH RATE, DAILY WEIGHT GAIN, FEED CONVERSION RATIO AND FEED COST PER KILOGRAM (FCK) OF NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS

Parameter	T_1	T_2	T_3	T_4
SGR (% /day)	3.07 ± 0.06^{a}	2.47 ± 0.09^{b}	2.42 ± 0.06^{b}	2.87 ± 0.04^{a}
DWG (g/day)	0.44 ± 0.03^{bc}	0.77 ± 0.04^{a}	0.32 ± 0.02^{c}	0.50 ± 0.02^{b}
FCR	1.70 ± 0.11^{a}	0.78 ± 0.25^{bc}	1.10 ± 0.^{17ab}	0.67 ± 0.03^{c}
FCK (US Dollars)	2.40 ± 0.33^{b}	2.62 ± 0.38^{b}	1.70 ± 0.12^{b}	4.29 ± 0.18^{a}

T1= Low stocking density-without probiotic, T_2= Low stocking density-2.6 g probiotic, T_3= high stoking density-without probiotic, T_4= high stocking density-6.6 g probiotic.
SGR= Specific growth rate, DWG= Daily weight gain, FCR= Feed conversion ratio, FCK= Feed cost per kilogram.
^{abc} Means in the same row with different superscripts are significantly different (P<0.05).
Mean ± standard error.

As has been previously described, increasing stocking density resulted in a significant reduction in tilapia specific growth rate [14, 21]. This is consistent with the findings of this study among fish reared with no addition of probiotic. However probiotic addition allowed that fish reared at high densities overcome feed conversion and equaled final weight, daily weight gain of fish reared at low density without probiotic. A previous studies showed that among both crowd stressed and unstressed fish, probiotic administration improved final weight, daily weight gain, and specific growth rate but did not improve feed conversion in fish rearing at high stocking density, as happened in the present study [14]. Another study [19] indicated that probiotic addition increased growth performance and feed conversion of tilapia fingerlings reared in a intermediate stocking density (30 fish/m^3) compared with no supplemented fingerlings stocked at low density (10 fish/m^3), with no effect on fish stocking at high densities (50 and 60 fish/m^3).

Lower growth performance and food utilization of tilapia at higher stocking density could have been caused by social interactions through competition for food and/or space. Higher stocking densities lead to intensified stress that result in increased energy requirements causing a reduction in growth rates. Metabolic energy is shifted toward activities closely bound to homeostatic recovery in detriment of investment activities, such as growth or reproduction [3, 5, 9]. Moreover, Yi *et al*. [28] and Huang and Chiu [15] argued that tilapia is a territorial and aggressive fish so that the density effect on growth might be explainable by their competition for territories, as well as the permanent stress caused by crowding. Also, increase in stocking density may cause deterioration in water quality, resulting in stressful conditions [9].

On the other hand, as happened in this study at low stocking densities, lack of competition for food and/or social hierarchy can lead to decreased feed utilization efficiency. In this case, the difficulty of tracing food particles may have led to the reduction of feed consumption, and to the flushing of uneaten food with the drainage water, causing the deterioration of feed utilization efficiency [7].

Stocking density did not affect body length of fish reared without probiotic supplementation, which is contrary to that observed by Abdel-Hakim and Moustafa [1]. These authors showed that there were slight but insignificant decreases in body length with increasing stocking density from 80 to 100 or 120 fish/m^3. However, they found a significant decrease in body length of fish stocked at 140 fish/m^3.

CONCLUSION

Addition of 2.6 g of probiotic improved growth performance of tilapia fingerlings and juveniles reared at low densities without increasing significantly the cost of feeding. Productive performance of fish reared at high stocking density could be successfully improved with the addition of 6.6 g of probiotic.

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