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Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia[1]

Avaliação econômica de prebiótico, probiótico e simbiótico para juvenis de tilápia do Nilo

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ABSTRACT - This study aimed to evaluate economically the inclusion of prebiotics, probiotics and symbiotics in diets of Nile tilapia (4.07 ± 0.30 g), at two stocking densities (0.6 and 1.2 kg m^{-3}). A total of 288 fish were distributed over 32 tanks (40 L) in a completely randomised design, in a 2 x 4 factorial (stocking density x additives), with four replications, over six weeks. The following were evaluated: total feed consumption (FC), final biomass (FB), relative gain in biomass (RGB), apparent feed conversion (AFC), survival rate (SUR), average cost of feed per kg of live weight gain (ACF), total cost of feed (TCF), total cost of production (TCP), gross income (GI), operating profit (OP) and economic efficiency index (EEI). No effect was seen on the evaluated parameters from the interaction ($p>0.05$) between stocking density and inclusion of the feed additives. No influence was observed ($p>0.05$) on SUR from the stocking density, although this significantly influenced the parameters FB, FC, RGB, AFC, ACF, TCF, TCP, GI and OP. There was no significant influence from the inclusion of prebiotics, probiotics and symbiotics on FC, SUR and TCF, however there was an influence ($p<0.05$) on the parameters FB, RGB, AFC, ACF, TCP, GI and OP. The control diet at the higher density displayed the worst EEI. The best EEI was obtained by fish at the lower density which received feed with added probiotics and symbiotics. The best indices of economic and zootechnical performance obtained demonstrate the economic viability of including prebiotics, probiotics and symbiotics in the diets of Nile tilapia.

Key words: *Bacillus subtilis*. Diets. Fish-feeding. Mannan oligosaccharides. *Oreochromis niloticus*.

RESUMO - Objetivou-se avaliar economicamente a inclusão de prebiótico, probiótico e simbiótico em rações para tilápia do Nilo (4,07 ± 0,30 g) em duas densidades de estocagem (0,6 e 1,2 kg m^{-3}). Um total de 288 peixes foram distribuídos em 32 aquários (de 40 L), em delineamento inteiramente casualizado, em esquema fatorial 2 x 4 (densidade de estocagem x aditivos), com quatro repetições, durante seis semanas. Foram avaliados: consumo total de ração (CR), biomassa final (BIOF), ganho relativo em biomassa (GBIO), conversão alimentar aparente (CAA), taxa de sobrevivência (SOB), custo médio da ração por quilograma de peso vivo ganho (CMR), custo total com ração (CTR), custo total de produção (CTP), receita bruta (RB), lucro operacional (LO) e índice de eficiência econômica (IEE). Não foi observado efeito da interação ($p > 0,05$) entre densidade de estocagem e inclusão dos aditivos à ração sobre os parâmetros avaliados. Não foi observado influência ($p > 0,05$) da densidade de estocagem sobre a SOB, embora tenha influenciado significativamente os parâmetros BIOF, CR, GBIO, CAA, CMR, CTR, CTP, RB e LO. Não houve influência significativa da inclusão de prebiótico, probiótico e simbiótico sobre CR, SOB e CTR, mas houve influência ($p < 0,05$) sobre os parâmetros BIOF, GBIO, CAA, CMR, CTP, RB e LO. A ração controle, na maior densidade, apresentou o pior IEE. Os melhores IEE foram obtidos pelos peixes que, na menor densidade, receberam as rações acrescidas de probiótico e simbiótico. Os melhores índices econômicos e desempenho zootécnico apresentados mostram a viabilidade econômica da inclusão de prebiótico, probiótico e simbiótico em rações para tilápia do Nilo.

Palavras-chave: *Bacillus subtilis*. Mananoligossacarídeo. *Oreochromis niloticus*. Peixes-alimentação. Rações.

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INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) occupies a prominent position in Brazilian fish farming, representing around 70% of production in 2010 (BRASIL, 2012). Among other features, desirable in fish farming, this species stands out for its rapid growth, excellent performance in intensive production systems, omnivorous eating habits and easy acceptance of feed, from the post-larva period until the termination phase (BOSCOLO *et al*., 2001; FURUYA *et al*., 2008).

The rapid expansion and intensification of fish farming, combined with the increase in ever more intensive production strategies at higher stocking densities, has resulted in the emergence of diseases that cause considerable economic losses, and hinder the sustainable development of the industry (GÓMEZ *et al*., 2007).

Antibiotics have been used on a large-scale as strategies for the prevention and treatment of diseases in fish farming, and in sub-therapeutic dosages have often been used for promoting growth. These actions are considered to be of high risk, since they result in the development of resistant bacteria, the presence of antibiotic residues in the flesh and the destruction of the microbial population in the aquatic environment (MARQUES *et al.,* 2005; VINE; LEUKES; KAISER, 2004). As a result, various alternative strategies for the use of antibiotics have been proposed (GÓMEZ *et al*., 2007). One that has generated great interest among researchers is the introduction of prebiotics and probiotics in diets. Prebiotics are compounds which are not digestible by the enzymes, salts and acids produced by the organism, but which are fermented selectively by microorganisms in the gastrointestinal tract, present in ingredients of the diet or added to it from concentrated exogenous sources (SILVA; NÖRNBERG, 2003). Probiotics are live microorganisms, which when administered in suitable amounts, confer benefits to the health of the host by improving the balance of the microbiota in the intestine (VERSCHUERE *et al*., 2000). Symbiotics are a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival rate and modulating the microbial community in the gastrointestinal tract, selectively stimulating the growth, or activating the metabolism, of one or a limited number of beneficial bacteria (lactic acids), thus improving the welfare of the host (LI; TAN; MAI, 2009).

Several studies have demonstrated the beneficial effects of prebiotics and probiotics for fish, such as improvements in the usage of food, modulation of intestinal microflora, increases in the immune response and an antagonism to pathogens, resulting in greater survival of the fish (RINGO *et al.,* 2010; VERSCHUERE *et al.,* 2000). Probiotics and prebiotics are generally studied separately, with information on the use of symbiotics in fish farming being scarce.

The aim was to evaluate economically the addition of prebiotics, probiotics and symbiotics to the diets of juvenile Nile tilapia reared at two stocking densities.

MATERIAL AND METHODS

The experiment was carried out for six weeks in the Sector for Aquaculture of the Laboratory of Animal Husbandry and Nutrition, the Centre for Agricultural Sciences and Technology at the Darcy Ribeiro North Fluminense State University (LZNA / UENF), in Campos dos Goytacazes, in the state of Rio de Janeiro, Brazil (RJ).

For the prebiotic, a mannan-oligosaccharide, derived from the cell wall of the yeast *Saccharomyces cerevisiae* was used. As the probiotic microorganism, *Bacillus subtilis* was used (a commercial probiotic, containing 1 x 10^{10} colony-forming units of probiotic per gram of product). The symbiotic was formed from a mixture of the above prebiotic and probiotic.

In the experiment, 288 juvenile, sexually-reversed male Nile tilapia (*Oreochromis niloticus*) were used, having an initial weight of 4.07 ± 0.30 g, distributed in a completely randomised design and a 2 x 4 factorial (stocking density x additives), giving a total of eight treatments, each with four replications (Table 1).

The fish were randomly distributed into 32 tanks with a working capacity of 40 L and a closed-circulation water supply system, using a biological filter (one for each group: control, prebiotic, probiotic and symbiotic) and thermostats to control the water temperature. The photoperiod was kept at 12 hours and controlled by timer.

Throughout the experiment the parameters of temperature, pH and dissolved oxygen were measured daily every morning, using digital multiparameter analysers.

An experimental control diet was prepared in accordance with the requirements of the species as per Furuya *et al.* (2010), using the apparent digestibility coefficients as obtained by Boscolo, Hayashi and Meurer (2002) (Table 2).

To prepare the diets, the food was processed individually in a knife mill with a 0.5 mm sieve. The feeds were then mixed according to their formula, and moistened with water to be pelletized and dried in an oven at 55 °C for 48 hours. The prebiotics, probiotics and symbiotics were included in place of the wheat bran (AI *et al*., 2011). The feed for each replication was weighed and kept in plastic containers. When feeding the tilapia, the diets were crumbled to a suitable size

Table 1 - Distribution of treatments by additive and stocking density

Treatment	Type of treatment	Mannan oligosaccharide (g kg^{-1})	*Bacillus subtilis* (g kg^{-1})	Density (kg m^{-3})
1	Control	-	-	0.6
2	Control	-	-	1.2
3	Prebiotic	0.2	-	0.6
4	Prebiotic	0.2	-	1.2
5	Probiotic	-	0.2	0.6
6	Probiotic	-	0.2	1.2
7	Symbiotic	0.1	0.1	0.6
8	Symbiotic	0.1	0.1	1.2

Table 2 - Percentage composition of the control diet used for feeding juvenile Nile tilapia (*Oreochromis niloticus*)

Ingredient	(g kg^{-1})
Soybean meal	410.0
Wheat bran	250.0
Corn meal	200.0
Fish meal	79.7
Corn flour	34.0
Soybean oil	16.1
Supplement (mineral and vitaminic)[1]	10.0
Antioxidant BHT	0.20
Item	**Calculated value**
Digestible protein (g kg^{-1})	254.8
Digestible energy (kcal kg^{-1})	3078
Raw fiber (g kg^{-1})	51.2
Fat (g kg^{-1})	43.0
Total Lysine (g kg^{-1})	16.5

[1]Composition Kg^{-1}: Mg - 2,600 mg; Zn - 14,000 mg; Fe - 10,000 mg; Cu - 1,400 mg; Co - 20 mg; I - 60 mg; Se - 60 mg; Vit. A - 1,000,000 UI; Vit. D3 - 400,00 UI; Vit. E - 10,000 mg; Vit. K3 - 500 mg; Vit. B1 - 2,500 mg; Vit. B2 - 2,500 mg; Vit. B6 - 2,500 mg; Vit. B12 - 3,000 mcg; Vit. C - 35,000 mg; Folic Acid - 500 mg; Pantothenic Acid - 5,000 mg; Niacin - 10,000 mg; Biotin - 80,000 mcg; Choline - 200,000 mg; Methionine - 130 g; Inositol - 5,000 mg; Ethoxyquin - 15,000 mg

for the mouths of the fish, and offered twice a day to apparent satiation.

To evaluate zootechnical performance, the following were determined: total feed intake, final biomass, relative gain in biomass [(final biomass / initial biomass) x 100], apparent feed conversion (feed intake / weight gain) and survival rate [(dead individuals / living individuals) x 100].

To analyse the economic viability of the use of experimental feeds, information on the price of ingredients was obtained from suppliers. The cost of the feeds was calculated based on retail prices, with the values being converted to US dollars (at US$ 2.21, the exchange rate for the month of November, 2013). The cost per kilogram of the control feed, and the prebiotic, probiotic and symbiotic feeds were respectively US$ 0.460, US$ 0.467, US$ 0.473 and US$ 0.470. The sales price of one kilogram of fish was taken to be US$ 2.04.

The average cost of feed per kilogram of live weight gain was calculated according to Bellaver, Fialho and Protas (1985). The estimated total cost of production, using only the parameters of feeding costs and animal performance, was obtained according to Matsunaga *et al.* (1976). Gross income and operating profit were obtained as per Martin *et al.* (1998). To evaluate the financial impact of the treatments in relation to the addition or not of prebiotics, probiotics and symbiotics, the economic efficiency index was calculated according to Barbosa, Fialho and Ferreira (1992).

The data were subjected to bifactorial variance analysis at 5% probability, and when there were significant differences, the F-test was applied for stocking density, and the Tukey test for the use or not of prebiotics, probiotics and symbiotics. The data, expressed as percentages, were transformed using the formula y = arcsine √x for later evaluation. For the analyses, the Statistical Analysis System 9.1 software (SAS INSTITUTE, 2006) was used.

RESULTS AND DISCUSSION

The values for the physical and chemical parameters of the water were on average 28.5 ± 1.1 °C, 6.6 ± 0.5 and 3.8 ± 0.4 mg L^{-1} respectively for temperature, pH and dissolved oxygen, which were within the suitable range for fish farming (MOREIRA *et al.*, 2001).

No effect was seen (p>0.05) from the interaction between stocking density and the addition or not of prebiotics, probiotics and symbiotics to the feed, on zootechnical performance and the indices of economic evaluation (Tables 3 and 4).

No significant influence was seen from stocking density on survival, obtaining on average 95.5%. Similar results were obtained by Ayroza *et al.* (2011) with juveniles of Nile tilapia (31.3 ± 0.1 g), when evaluating different stocking densities in net fencing, and Silva *et al.* (2002) for the same species (20.0 g) in raceways. In this study, the experimental conditions favoured satisfactory survival rates.

The stocking density had an influence (p<0.05) on the final biomass and total feed intake. The higher number of individuals at the greater density and the similarity in survival rates between treatments influenced these results. Similar results were obtained by Marengoni *et al.* (2008) with juvenile Nile tilapia (0.65g) grown in ponds, and by Maeda *et al.* (2006) with fingerlings (8.04 ± 1.81 g) reared in raceways.

The stocking density influenced (p<0.05) the relative gain in biomass and apparent feed conversion. Despite final biomass at the higher stocking density

Table 3 - Zootechnical performance of juvenile Nile tilapia (*Oreochromis niloticus*) by stocking density and the addition or not of prebiotics, probiotics or symbiotics[1]

Treatment	Variable[2]					
	IB (kg m^{-3})	FB (kg m^{-3})	RGB (%)	TFC (kg m^{-3})	AFC (g g^{-1})	SUR (%)
Density						
0.6 kg m^{-3}	0.62 b	2.44 b	399.00 a	2.33 b	1.30 b	94.79
1.2 kg m^{-3}	1.21 a	4.07 a	336.68 b	4.62 a	1.63 a	95.31
Additive						
Control	0.90	2.94 b	335.11 b	3.45	1.65 a	90.63
Prebiotic	0.91	3.34 a	372.49 a	3.45	1.40 b	95.83
Probiotic	0.94	3.34 a	372.46 a	3.49	1.42.b	96.88
Symbiotic	0.90	3.39 a	391.31 a	3.51	1.39 b	96.88
CV (%)	6.51	6.93	11.04	4.65	9.51	5.73
Value of F						
Density	40.35	39.02	40.69	37.34	12.03	0.98
Additive	1.25	7.83	4.23	0.10	19.52	2.12
Density x Additive	0.42	1.10	0.89	0.71	0.51	0.54
Value of P						
Density	0.0001	0.0001	0.0002	0.0001	0.0001	0.7891
Additive	0.6131	0.0018	0.0469	0.8689	0.0027	0.0915
Density x Additive	0.8768	0.1546	0.4443	0.3090	0.2899	0.8476

[1]Means followed by different letters in a column differ at 5% probability by Tukey test. CV, coefficient of variation; [2]IB, initial biomass; FB, final biomass; RGB, relative gain in biomass; TFC, total feed consumption; AFC, apparent feed conversion; SUR survival rate

Table 4 - Economic evaluation of feeds for juvenile Nile tilapia (*Oreochromis niloticus*) by stocking density and the addition or not of prebiotics, probiotics or symbiotics[1]

Treatment	Variable[2]				
	ACF (US$ kg^{-1})	TCF (US$ m^{-3})	TCP (US$ kg^{-1})	GI (US$ m^{-3})	OP (US$ m^{-3})
Density					
0.6 kg m^{-3}	0.61 b	1.09 b	0.45 b	4.98 b	3.56 b
1.2 kg m^{-3}	0.76 a	2.16 a	0.53 a	8.30 a	4.79 a
Additive					
Control	0.76 a	1.59	0.53 b	6.01 b	3.27 b
Prebiotic	0.65 b	1.61	0.48 a	6.82 a	4.51 a
Probiotic	0.67 b	1.65	0.48 a	6.80 a	4.36 a
Symbiotic	0.65 b	1.65	0.48 a	6.92 a	4.55 a
CV (%)	9.44	4.64	7.21	6.93	14.45
Value of F					
Density	31.94	29.20	22.35	25.34	18.35
Additive	16.74	0.54	8.45	12.12	16.21
Density x Additive	0.26	0.45	0.33	1.25	2.23
Value of P					
Density	0.0001	0.0001	0.0001	0.0001	0.0001
Additive	0.0086	0.3090	0.0281	0.0018	0.0007
Density x Additive	0.2692	0.4047	0.3314	0.1546	0.0718

[1]Means followed by different letters in a column differ at 5% probability by Tukey test. CV, coefficient of variation; [2]ACF, average cost of feed per kilogram of live weight gain; TCF, total cost of feed; TCP, total cost of production; GI, gross income; OP, operating profit

being about 65% higher than at the lower, the relative gain in biomass in the fish at the lower stocking density was 18.51% higher than for the fish at the higher density. Proportionally therefore, the higher the storage density, the lower the performance of the fish. The same was observed by Silva *et al.* (2002) for Nile tilapia.

Similar results to those obtained in this study for apparent feed conversion were obtained by Marengoni *et al.* (2008), where an increase in stocking density (1 Fish m^{-3} to 4 fish m^{-3}) impaired the apparent feed conversion (0.98:1 to 1.84:1).

Factors that act by modifying metabolic stress, social interaction, changes in hormones, enzymes and growth factors (BARTON; IWAMA, 1991) may result in a reduction in growth. Therefore fish subjected to higher storage densities tend to reduce their growth, since energy consumed in the diet and in body reserves is mobilised for the physiological changes due to that stress (ELLIS *et al.*, 2002). Poorer apparent feed conversion may be a physiological response to conditions of higher storage density, which would explain the lower growth rate for that treatment.

There was no significant influence from the addition of prebiotics, probiotics and symbiotics on total feed consumption or survival rate. A common difficulty, observed when new additives or alternative food sources are used in fish diets, is acceptability, which is related to palatability (AZEVEDO; TONINI; BRAGA, 2013; CARVALHO *et al.*, 2012; RODRIGUEZ; OLVERA; CARMONA, 1996; SENA *et al.*, 2012). The similarity in values for total feed consumption suggests that including the additives under evaluation did not alter the palatability of the feed.

The addition of prebiotics, probiotics and symbiotics to the feed increased ($p<0.05$) final biomass and relative gain in biomass, and significantly improved apparent feed conversion, when compared to the results obtained with the animals fed the control diet.

Some studies have reported improvements in the zootechnical performance of those tilapia that received additives in the feed (ESSA *et al.*, 2010; EL-RHMAN; KHATTAB; SHALABY, 2009; GHAZALAH *et al.*, 2010; LARA-FLORES *et al.*, 2003; LARA-FLORES; OLIVERA-CASTILLO; OLVERA-NOVOA, 2010), however other researchers did not observe this effect on zootechnical performance (FERGUSON *et al.*, 2010; SCHWARZ *et al.*, 2011; SHELBY *et al.*, 2006). According to Welker and Lim (2011), it is difficult to arrive at and provide specific recommendations on the effects of these additives on the performance of tilapia, since studies vary widely with respect to the age and size of fish, stocking density, composition of feed, concentration of the additive used, its time of administration, type and source.

Intestinal microbial flora is important in fish nutrition, as it may increase the production of digestive enzymes, amino acids, short-chain fatty acids and vitamins, improving the use of nutrients (NAIAK, 2010). An increase in the production of amylase, lipase and protease was seen in tilapia fed diets containing *Bacillus subtilis* (ESSA *et al.*, 2010), and an increase in the height of the intestinal villi was observed when the fish were fed a diet supplemented with a prebiotic, which can favour the absorption of nutrients (SCHWARZ *et al.*, 2010). These factors may have been responsible for the better apparent feed conversion in fish receiving diets containing prebiotics, probiotics and symbiotics, compared to those which received the control diet, and which in this study resulted in greater weight gain.

In the economic evaluation, stocking density influenced ($p<0.05$) the average cost of feed per kilogram of live weight gain, total cost of feed, total cost of production, gross income and operating profit.

On average, 15% more was spent on feed to produce one kilogram of fish at the higher storage density in relation to the lower, confirming the results obtained by Ayroza *et al.* (2011). The total cost of production was, on average 17.78% lower at the lower stocking density compared to the higher density, similar to results obtained by Marengoni *et al.* (2008). However, even with the higher cost of production, the gross income and operating profit were on average, respectively 66.67 and 34.55% greater at the higher stocking density compared to the lower, which may be related to the high survival rates obtained with the treatments at the higher stocking density.

Greater densities generally result in greater production, but individual growth tends to be smaller. As a consequence, the fish present worse ratios of apparent feed conversion, due to their being subjected to limited space, making access to food difficult. However, the increase in productivity obtained in this experiment with the increase in stocking density can offset the larger growth and better apparent feed conversion shown by the fish at the lower density, resulting in higher gross income and operating profit (Tables 3 and 4).

The inclusion of prebiotics, probiotics and symbiotics did not affect ($p>0.05$) the total cost of feed, although significantly altering the other parameters. The cost of feed ranged from US$ 0.460 (control) to US$ 0.473 (probiotics), making the economic analyses dependent on the zootechnical performance of the animals. The worst economic indices were seen therefore for the treatment where the fish received the control diet. Similar results were obtained by Ghazalah *et al.* (2010), when evaluating the addition of a probiotic in Nile tilapia (1 g), and by Dias *et al.* (2012), evaluating the addition of a probiotic in *Brycon amazonicus*.

The control diet at the higher density had the worst economic efficiency index of all the treatments. The highest rates were obtained by fish at the lower density which were fed diets with added probiotics and symbiotics (Figure 1).

Analysing the indices of economic efficiency within the same stocking density, it can be seen that at the lower density the best indices were obtained with the feeds with the added probiotics and symbiotics. However, at the higher density, the best index was obtained with the feed with an added prebiotic. At the two densities, the worst indices were obtained with the control feed (Figure 2).

At the lower stocking density, the inclusion of additives in the experimental feeds resulted in indices of economic efficiency on average 10.48% higher compared to the control feed, while at the higher density these rates were on average 14.92% higher. These results confirm those of Grisdale-Helland, Helland and Gatlin III (2008), who reported that under conditions of stress (in this study,

Figure 1 - Economic efficiency index of diets for juvenile Nile tilapia (*Oreochromis niloticus*) by stocking density and the addition or not of prebiotics, probiotics and symbiotics

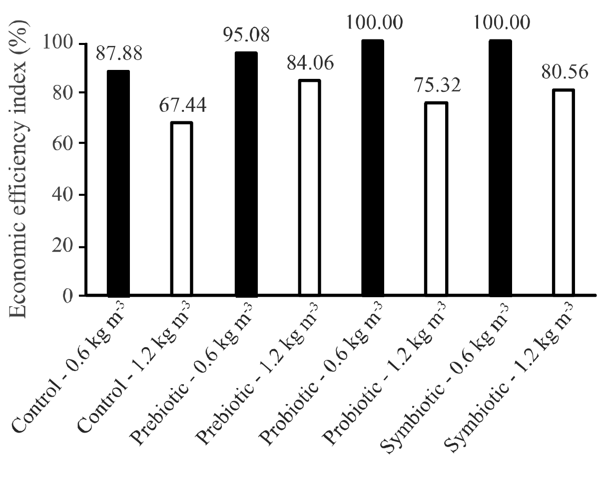
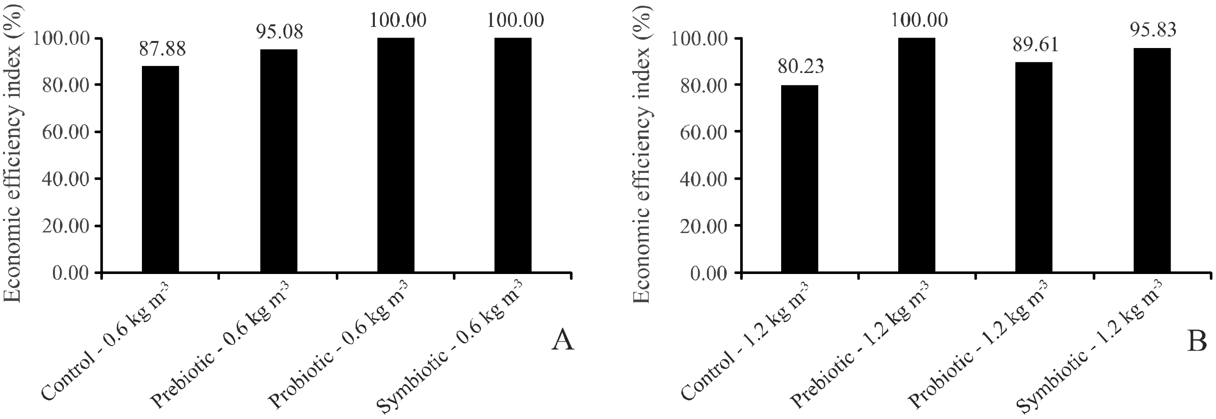


Figure 2 - Economic efficiency index of diets for juvenile Nile tilapia (*Oreochromis niloticus*). A - Stocking density 0.6 kg m^{-3}. B - Stocking density - 1.2 kg m^{-3}



the high stocking density), the beneficial effect of including immunostimulant additives in the diet can be seen.

CONCLUSION

The highest indices of zootechnical performance and economic efficiency presented in this study show the viability of including prebiotics, probiotics and symbiotics in the feed for Nile tilapia; the costs and availability of each of these additives should be watched when using them as components in diets for this species.

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