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Full Length Research Paper

Effect of the use of commercial probiotic feed as a growth enhancer in the enzymatic activity of the intestinal tract of Nile tilapia

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This study evaluated the effects of two types of probiotics, a mix of two bacteria and one yeast, on growth performance and intestinal enzyme activity in Nile tilapia. Three diets were formulated containing the optimum protein level (40%) for tilapia fry: one was supplemented at 0.1% with a bacterial mixture containing *Streptococcus faecium* and *Lactobacillus acidophilus*; a second was supplemented at 0.1% with the yeast *Saccharomyces cerevisiae*; and a third, was complemented with a control diet without supplements. Two additional diets were formulated to contain 27% protein to serve as a stress factor. They were supplemented at 0.1% with either the bacterial probiotic mix or the yeast. The diets were fed for 9 weeks to tilapia fry housed in 20-L tanks at two densities: a high density of 20 fry per tank as a stress factor; and a low density of 10 fry per tank. Every week an organism was selected from each tank for the enzymatic analyses of unicellular protein, alkaline phosphatase, disaccharidases and peptidase. Results indicate that the fry fed with diets containing probiotic supplement exhibited greater growth rate than those fed with control diet. Of the four probiotic treatments, the 40% protein diet supplemented with yeast produced the best growth performance and feeding efficiency. This was attributed to an increase in the alkaline phosphatase activity, suggesting that yeast is an appropriate growth-stimulating additive in tilapia cultivation.

Key words: Probiotic, Nile tilapia, enzymatic activity, streptococcus, *Lactobacillus*, *Saccharomyces*.

INTRODUCTION

The human requirement for the consumption of aquatics products and aquarist activity is high and the demand is not satisfactory met by the world fishery production. This throws open an extensive range of opportunities to aquaculture industry (FAO, 2004). Aquaculture has become an important economic activity in many countries (Balcazar et al., 2006). However, its development is fraught with many problems such as widespread epizootics, feed efficiency and growth performance (Subasinghe, 1997; Fegan, 2001; Gaiotto, 2005). This is principally caused by large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to disease, inadequate balance of nutrient in the artificial diets and deterioration of environmental conditions. It has been noted that physiological stress is one of the primary contributing factors of aquatic organisms' disease, poor growth and mortality in aquaculture (Balcazar et al., 2004; El-Haroun et al., 2006; Rollo et al., 2006).

Presently, the principal aims of the aquaculture industry

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are to increase the growth or survival performance, feed efficiency, and resistance of aquatic organisms. This in turn will show a positive effect on production costs (Gatlin III, 2002). Hormones, antibiotics, ionophers and some salts compounds have been used as growth promoters and to some extent to prevent disease. However, their inadequate applications show a negative effect on aquaculture production and environment (Gongora, 1998).

Functional additive, like probiotics, is a new concept on aquaculture (Li and Gatlin III, 2004) where the additions of microorganisms on diets show a positive effect on growth caused by the best use of carbohydrates, protein, and energy (Moriarty, 1998; Skejermo and Vadstein, 1999; Chang and Liu, 2002; Irianto and Austin, 2002a, b). It further diminishes mortality by disease, antagonism to pathogen, and better microbial intestinal balance in the environment (Subasinghe, 1997; Moriarty, 1998; Holmström et al., 2003).

The concept of probiotics was originally used by Lilley and Stillwell (1965) to mean a substance (s) that stimulates the growth of other microorganisms (Chunkeatirote, 2002). Parker in 1974 modified the definition to “organisms and substances which contribute to intestinal balance”. Fuller (1992) revised the definitions as “A live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance”. This revised definition has put forward the importance of live cells as the essential component of a potential probiotic .This clears the confusion created by the use of the term “substances”. However, an effect on intestinal microbial balance has been defined and demonstrated using only few cases. This was noted by Tannock (1997), and he proposed the definition “living microbial cells administered as dietary supplements with the aim of improving health”.

According to the accepted definition of probiotic, this product is a live microorganism food supplement, which improves the microbial balance of the host intestinal flora (Fuller, 1992; Vine et al., 2006; Ziaei-Nejad et al., 2006). Other definitions in aquaculture show that probiotic is a live microbial food supplements that are consumed with the aim of providing health benefit to the host by contributing to an improved microbial balance within the intestinal microbiota (Gram et al., 1999; Crittenden et al., 2005). They are biologically active components of single or mixed cultures of microorganisms capable of improving the health of the host (Salminen et al., 1999; Ochoa-Solano and Olmos-Soto, 2006), live micro-organisms and/or are disease resistant (Tacon, 2002). Live microorganisms administered in adequate amounts confer a health effect on the host (Gomez et al., 2007). The components of these definitions reflect the use of microorganism or their products (microbial cells element o cell free supernatant factors) on tanks and ponds in which animals live. They were used as biological control or for their capacity to modified the bacterial composition of aquatic animal’s intestine, water and sediment, or used with feed as health supplement and/or biological control.

The present work assessed the effect of the use of a commercial probiotic feed as a growth enhancer in the enzymatic activity of the intestinal tract of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

The experiment was conducted for 9 weeks, using 3-week old Nile tilapia (*O niloticus*, with 152.3 mg average weight) fry obtained from the Aquaculture Laboratory of CINVESTAV in Mérida, Yucatán, México. The experimental system was a closed recirculation system consisting of 40 self- cleaning 20^{-l} plastic tanks, sedimentation tanks, a biological filter and UV filter to prevent cross-contamination of microorganisms in-between treatments.

The system was installed in an environment-controlled laboratory maintained at 22°C, with a photoperiod of 12 h light and 12 h darkness. Water in the system was maintained at a temperature of 28 °C with two 2000-W bayonet -titanium heaters, and the dissolved oxygen level was controlled by adjusting water flow into each tank to 1 l min^{-1}. For water quality control, temperature and dissolved oxygen were measured daily. Weekly analyses were done on total ammonium, nitrite, nitrate and pH levels, using standard methods (APHA, 1989). The following appropriate values (±S.D.), were used for tilapia cultivation: temperature, 28.83 ± 0.45°C; dissolved oxygen, 6.17 ± 1.64 mg l^{-1}; pH, 8.46 ± 0.32; ammonia, 0.07 ± 0.02 mg l^{-1}; nitrite, 0.07 ± 0.03 mg l^{-1}; nitrate, 5.93 ± 0.61 mg l^{-1}.

Experimental diets

Five isocaloric diets were formulated: three containing 40% protein, and two with 27% protein. The lower protein in the latter diets was used as a stress factor (Wilson, 2000) because at its present growth stage, the optimum protein level for tilapia was 40% (Tacon, 1984). A commercial probiotic for terrestrial vertebrates based on *S. faecium* and *L. acidophilus* was added to one of the 40% protein diets (ALL40) and one of the 27% protein diets (ALL27) . The yeast (*S. cerevisiae*) was added to separate 40% (Y40) and 27% (Y27) protein diets. Finally, a control diet was formulated with 40% protein and no supplements (CON40). 0.5% chromic oxide was added to all five diets as a marker for determining digestibility. Table 1 shows diet formulation and proximate composition.

Population density in the tanks was also used as a stress factor, under the assumption that overpopulation is one of the main growth-inhibiting factors in intensive aquaculture systems. To this end, 20 tanks were stocked at a density of 10 organisms per tank (one fry per 2 l) and the other 20 tanks at 20 organisms per tank (one fry per liter). All the fries had similar average initial weight. The different diet formulations were assigned within the tanks in each density set so that for each protein level there were four diets within each density category. The animals were allowed to adapt to the experimental system for a week, and were fed with a conventional diet, after which different treatments were randomly assigned to the tanks, with four replicates per treatment.

Feed was manually administered ad libitum four times a day, for 9 weeks. A daily record was kept of the feed given. Bulk weight was measured weekly to follow growth in weight and to calculate survival and feeding ration. The fish were taken from each tank, briefly, using a net previously disinfected with a 1% benzalconium chloride solution. This was then passed over fabric towels to eliminate excess moisture and the fish weighed on an electronic balance. Every third day, each tank was partially cleaned and the water partially changed. Once a week, bulk weight measurement was done on the same day. The tanks were completely cleaned and a total change of water in the system was carried out.

Table 1. Formulation and proximate composition of experimental diets.

Ingredients (%)	Diets				
	CON40	ALL40[a]	Y40[b]	ALL27	Y27
Anchovy fish meal	54.23	54.23	54.23	36.60	36.60
Cod liver oil	0.00	0.00	0.00	1.85	1.85
Soybean oil	3.26	3.26	3.26	6.40	6.40
Yellow corn starch	34.50	34.40	34.40	47.04	47.04
Mineral premix[c]	1.50	1.50	1.50	1.50	1.50
Vitamin premix[d]	3.00	3.00	3.00	3.00	3.00
Carboxyl methyl cellulose	3.00	3.00	3.00	3.00	3.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Probiotic	0.00	$1x10^6$ CFU/g	$1x10^6$ CFU/g	$1x10^6$ CFU/g	$1x10^6$ CFU/g
Proximate composition (% wet weight)					
Moisture	7.30	6.63	6.48	7.27	7.43
Crude protein	39.56	39.92	37.91	25.84	26.49
Ether extract	8.02	8.04	7.47	9.53	9.69
Ash	9.55	10.14	9.77	9.17	9.32
Nitrogen-free extract	34.03	33.53	36.55	44.53	43.04
Gross energy (kJ g^{-1})	19.954	19.753	20.087	19.830	19.920

[a] ALL = Bacterial mixture; [b] Y = Yeast; [c] Jauncey and Ross, 1982; [d] Tacon, 1984.

Beginning from the third week of the experiment, faeces were collected by siphoning the tank 30 min after the second daily feeding to minimize leaching. Scales were removed from the collected faeces, the faeces were oven dried at 105°C for 24 h, and then stored in hermetic containers under refrigeration until analysis.

Weight increase and feed consumption were used to calculated different parameters; Individual weight gain (IWG (mg day^{-1}) = 1000[(weekly WG)/time (days)]; Individual Feed intake (IFI (mg day-1) = 1000[(weekly FI)/time (days)]); Specific growth ratio (SGR (%day-1) = 100((log. Final body weight – log initial body weight)/time (days))); Feed conversion rate (FCR=IFI/IWG); Protein efficiency ratio (PER=IWG/protein intake); Carcass N deposition (CND (mg day-1) = 1000[(final body weight x percent final carcass protein) – (initial weight x percent initial carcass protein)]/100/ time (days)/6.25); Apparent N utilization (ANU (%) = 100 CND/N intake).

Chemical analyses

Proximate chemical analyses were made using diet ingredients and a sample of fish at the beginning and end of the experiment according to standard methods (A.O.A.C., 1992). Gross energy in the feed was determined by combustion in a Parr adiabatic calorimeter. To evaluate digestibility, the chromic oxide content of each diet and the collected faeces were analyzed using the acid digestion method (Furukawa and Tsukahara, 1966) . Protein content was also determined by using the faeces to assess protein digestibility.

Intestinal enzymatic analysis

Every week an organism from each tank was selected to make the enzymatic analyses. The fish were sacrificed with benzocaine solution at 400 ppm, disinfected with benzalconium chloride at 1% and washed three times with sterile saline solution (NaCl 0.85%). Before this the intestine was extracted in aseptic conditions. The intestines were weighted and then homogenized in a phosphates buffer solution (pH 9.5). From this suspension 1.5 ml were placed in tubes and then were storage at –10°C (Markwell et al., 1978).

Unicellular protein was determined using the method of Folin-Lowry modified by Markwell et al. (1978) that allows the use of samples that contain membrane or lipoprotein. For the determination of alkaline, Weeb and Morrow's (1959) method of phosphatase to measures the activity of the variations of pH in the intestine. The method of Dahlqvist (1964) was also used to determine the activity of disaccharidases on the intestinal mucus. The intestinal peptide hydrolase activity was determined by the one-step L-amino-acid oxides assay (Nicholson and Kim, 1975)

Statistical methods

Growth performance, feed utilization efficiency parameters and intestinal enzymatic activity were statistically compared using a one-way ANOVA ($P<0.05$), and differences among the mean scores were identified using the Duncan Multiple Range Test. Analyses were carried out with the STATISTICA ver 4.3 (1993) and StatGraphics Plus ver. 2.1 (1996) computer software. Arcsin transformations were done when necessary.

RESULTS

Growth and feeding performance

Of the five experimental diets and the two density categories, the 40% protein diet supplemented with yeast administered to the 10-fry density treatment (Y40/10) produced the best growth rate (Table 2). All the diets supplemented with yeast showed better results than those with the microbial mixture and control diets, though

Table 2. Growth and feeding performance of fish fed diets supplemented with probiotics.

Mean values[1]	Diets										
	CON40/10	CON40/20	Y40/10	Y40/20	Y27/10	Y27/20	ALL40/10	ALL40/20	ALL27/10	ALL27/20	± S.E.M.[2]
Survival (%)	75.00^{ab}	64.81^{a}	87.50^{bc}	92.59^{c}	91.67^{bc}	96.29^{c}	91.67^{bc}	85.18^{bc}	95.83^{c}	85.18^{bc}	5.163
Initial mean weight (g)	0.156^{a}	0.152^{a}	0.154^{a}	0.150^{a}	0.156^{a}	0.149^{a}	0.151^{a}	0.148^{a}	0.153^{a}	0.156^{a}	
Final mean weight (g)	1.285^{a}	1.439^{a}	6.164^{d}	4.570^{bc}	4.831^{c}	4.026^{bc}	1.910^{a}	2.081^{a}	4.145^{bc}	3.826^{b}	0.264
SGR (% day^{-1})	3.33^{a}	3.57^{a}	5.80^{d}	5.43^{c}	5.46^{cd}	5.24^{c}	4.03^{b}	4.19^{b}	5.23^{c}	5.10^{c}	0.119
FCR	3.113^{e}	3.260^{e}	1.430^{abc}	1.010^{a}	1.620^{bc}	1.170^{ab}	4.130^{f}	2.220^{d}	1.620^{bc}	1.170^{ab}	0.153
PER	0.830^{ab}	0.780^{ab}	1.890^{c}	2.640^{d}	2.260^{c}	3.170^{e}	0.610^{a}	1.140^{b}	2.230^{c}	3.380^{e}	0.1.21
CND	0.442^{a}	0.482^{a}	1.902^{c}	2.402^{d}	2.227^{cd}	3.150^{e}	0.565^{a}	1.035^{b}	2.422^{d}	3.472^{e}	0.136
ANU (%)	13.58^{a}	12.44^{a}	30.35^{c}	42.70^{d}	32.95^{c}	47.30^{d}	9.62^{a}	19.27^{b}	32.95^{c}	47.51^{d}	1.843
AOMD (%)[3]	89.59^{bc}	71.30^{a}	96.18^{cd}	96.90^{cd}	90.27^{c}	92.97^{cd}	75.35^{a}	84.78^{b}	97.16^{d}	97.10^{d}	2.967
APD (%)[4]	94.28^{cd}	68.79^{a}	97.75^{cd}	98.46^{d}	91.78^{c}	96.21^{cd}	95.35^{cd}	84.78^{b}	97.16^{cd}	98.25^{d}	2.686

[1]Values with the same superscript in the same row are not statistically different (P < 0.05); [2]Standard error, calculated from the mean-square error of the ANOVA; [3]Apparent organic matter digestibility; [4]Apparent protein digestibility.

ALL27/10 and ALL27/20 showed similar responses to Y27/20. The four diets ALL40/10, ALL40/20, CON40/10 and CON40/20 had the lowest growth performance.

Table 2 shows growth and feed utilization data. Fish fed with the CON40/10 and CON40/20 diets had statistically lower survival rate than those fed with probiotic-supplemented diets ($p < 0.05$). The highest survival rate was recorded with the probiotic treatments.

The addition of yeast to the diets with optimum protein content (40%) administered in the low organism density tanks (Y40/10) produced the best growth (individual weight gain, IWG; specific growth rate, SGR), with values statistically higher than the other treatments ($p < 0.05$). The diets supplemented with probiotics produced an IWG and SGR significantly higher than the control diets ($p < 0.05$).

The ALL40/10 treatment had the statistically highest feed conversion ratio of the probiotic-supplemented diets, although all the other probiotic-supplemented diets had feed conversion ratios significantly lower than those for the control diets ($P<0.05$). The best conversion ratio was recorded for the Y40/20, Y40/10, Y27/20 and ALL27/20 treatments. In general, fish fed with the diets supplemented with yeast showed better feeding efficiency than those fed with diets containing the bacterial mixture.

The Protein Efficiency Ratio (PER) was significantly higher in the treatments containing 27% protein supplemented with probiotics that were administered to tilapia at high densities (Y27/20 and ALL27/20) than in other treatments (Table 2). The lowest PER was recorded for the ALL40/10 and the control treatments. For these same fishes, Apparent Nitrogen Utilization (ANU) was significantly greater in comparison with the other treatments. The lowest apparent biological value was observed on the control diets, with results significantly lower than those obtained with the diets including probiotics.

In general, Apparent Organic Matter Digestibility (AOMD) and Apparent Protein Digestibility (APD) values were variables among the treatments. The maximum value was seen in the ALL27/10 treatment, though this was not statistically different from the ALL27/20, Y40/10 and Y40/20 treatment. In contrast, digestibility results for control diets, mainly CON40/20, were lower than those for the probiotic-supplemented diets, but APD for the low population control treatment (CON40/10) showed better results than those for CON40/20, suggesting an adverse effect of overcrowding on digestibility performance.

No statistical differences were observed in carcass moisture content between treatments (Table 3). Differences were observed in carcass protein content, with the highest value recorded in fish fed with the ALL40/20 and Y40/20 diets, which were statistically different from values produced in the ALL27/20, Y27/20 and Y27/10 treatments. Carcass protein was clearly related to dietary protein, with lowest values in fish fed with the 27% protein diets. Carcass lipid content was also affected by dietary protein content, with the highest values in the27% protein treatments, which were statistically different from the 40%

Table 3. Carcass proximate compositions of fish fed diets supplemented with probiotics.

Composition (%)	Initial	Diets									
		CON40/10	CON40/20	Y40/10	Y40/20	Y27/10	Y27/20	ALL40/10	ALL40/20	ALL27/10	ALL27/20
Moisture	81.09	73.36^{a}	74.26^{a}	73.40^{a}	73.09^{a}	73.46^{a}	74.10^{a}	73.85a	73.19^{a}	73.70^{a}	73.90^{a}
Crude protein	11.80	15.61^{bc}	15.72^{bc}	15.83^{bc}	16.22^{c}	14.72^{ab}	14.55^{ab}	15.39^{bc}	16.39^{c}	15.50^{bc}	13.77^{a}
Crude lipid	3.06	5.64^{a}	7.76^{bc}	8.37^{c}	7.03^{b}	10.25^{d}	9.86^{d}	7.84^{bc}	7.32^{bc}	9.50^{d}	10.21^{d}
Ash	2.46	3.49^{a}	3.76^{cd}	3.56^{b}	3.54^{a}	3.72^{c}	3.81^{d}	3.73^{c}	3.55^{ab}	3.52^{a}	3.85^{d}

Values with the same superscript in the same row are not statistically different (P>0.05).

protein treatments. The lowest overall lipid content was recorded for the CON40/10 treatment, which was statistically different from all other treatments. Statistical differences were observed also in the carcass ash content among the fish fed with the different diets, but it was not possible to establish a relation of this parameter with the protein level, fish density or with the type of probiotic used in each treatment.

Enzymatic activity

Figure 1 shows the unicellular protein content. All the treatments presented an increase in the content during the time of experimentation. They appeared at the highest values in treatment Y40/20 with statistical difference with the other treatments $p < 0.05$). The lowest values were observed in the animals fed with Y27 diet without significant differences between both densities ($p > 0.05$).

In the diets supplemented with the yeast, the content of Alkaline-phosphatase increased during the experiment with statistical difference with the other treatments ($p < 0.05$). The highest value was observed in the Y40-10 treatment. The other treatments presented an exponential decrease in the content of this enzyme (Figure 2).

All the treatment supplemented with a microorganism presented higher values of disaccharides than the controls (Figure 3). The highest values were observed in the diets with yeast ($p < 0.05$). The peptidase activity presented an exponential decrease in all the diets without statistical differences between the treatments ($p > 0.05$) (Figure 4).

DISCUSSION

All the probiotic -supplemented diets resulted in growth higher than that of the control diets, suggesting that the addition of probiotics mitigated the effects of the stress factors. This resulted in better fish performance, with better growth results in the diets supplemented with the yeast. Similar results were observed by Vázquez-Juárez et al. (1993) when yeast isolated from the intestines of wild rainbow trout was introduced into the digestive tracts of domestic rainbow trout, producing a significant increase in the growth of the cultured trout. In contrast, the use of the bacterial mixture in the optimum protein diets at either density caused no significant growth increases when compared to the control and yeast treatments.

These results may be explained by the greater adaptive capacity of yeasts in aquatic environments in contrast to bacteria such as Lactobacillus and Streptococcus. It is also necessary, however, to consider the possibility of interspecies differences, as suggested by Noh et al. (1994), who studied the effect of supplementing common carp feeds with different additives, including antibiotics, yeast (*S. cerevisiae*) and bacteria (*S. faecium*). They observed better growth response with probiotic-supplemented diets, but obtained the best growth with a bacterium, not yeast. Similar results were reported by Bogut et al. (1998), who fed common carp diets supplemented with *S. faecium*, reporting that the bacterium has a better probiotic additive for carp than yeast. This was clearly in contrast to the present results for tilapia.The best FCR values observed with probiotic-supplemented diets suggest that addition of probiotics improved feed utilization even under stress conditions, with yeast being the most effective of the supplements tested in the present study. Similar results have been reported for probiotics use in diets for piglets (Gil, 1998a). In practical terms, this means that probiotic use can decrease the amount of feed necessary for animal growth which could result in production cost reductions.

The PER and ANU results indicate that supplementing diets with probiotics significantly improves protein utilization in tilapia. This contributes to optimizing the use of protein for growth, given that protein is the most expensive feed nutrient. The improvement in the biological value of the supplemented diets in those treatments with high population and low dietary protein demonstrated that the probiotics supplements performed more efficiently in stress situations (Ringo and Gatesoupe, 1998).

Better digestibility obtained with the supplemented diets suggests that the addition of probiotics improved diet and protein digestibility, which may in turn explain the better

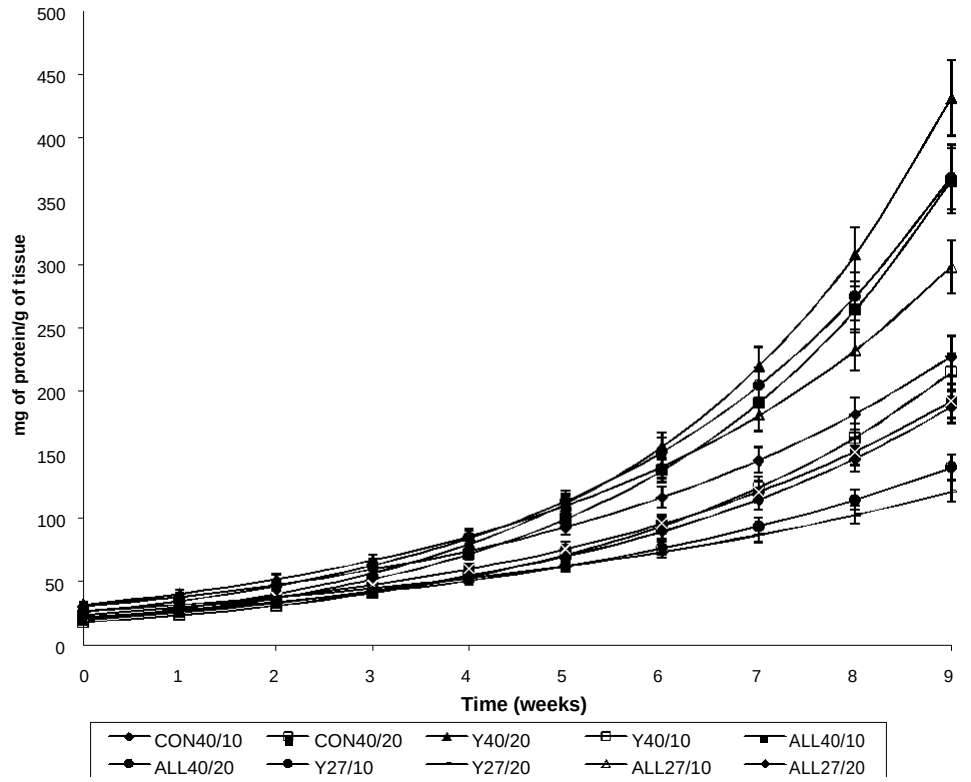

Figure 1. Unicellular protein content through time of experimentation.

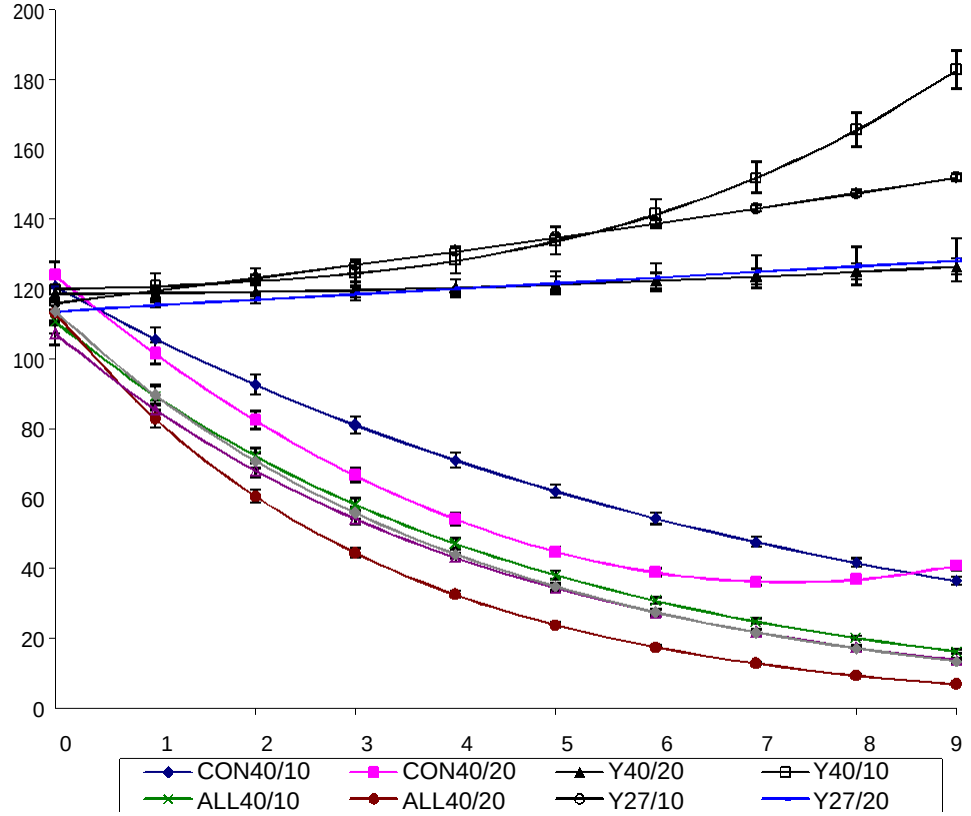

Figure 2. Alkaline phosphatase activity through time of experimentation.

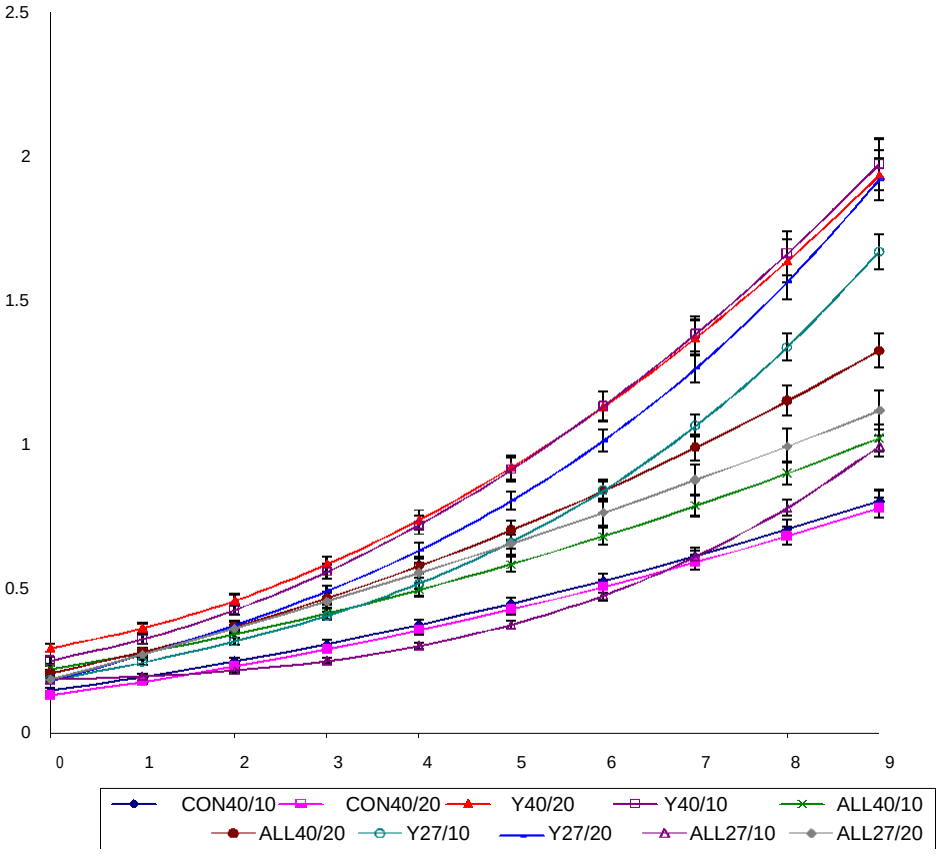

Figure 3. Disaccharidases activity through time of experimentation.

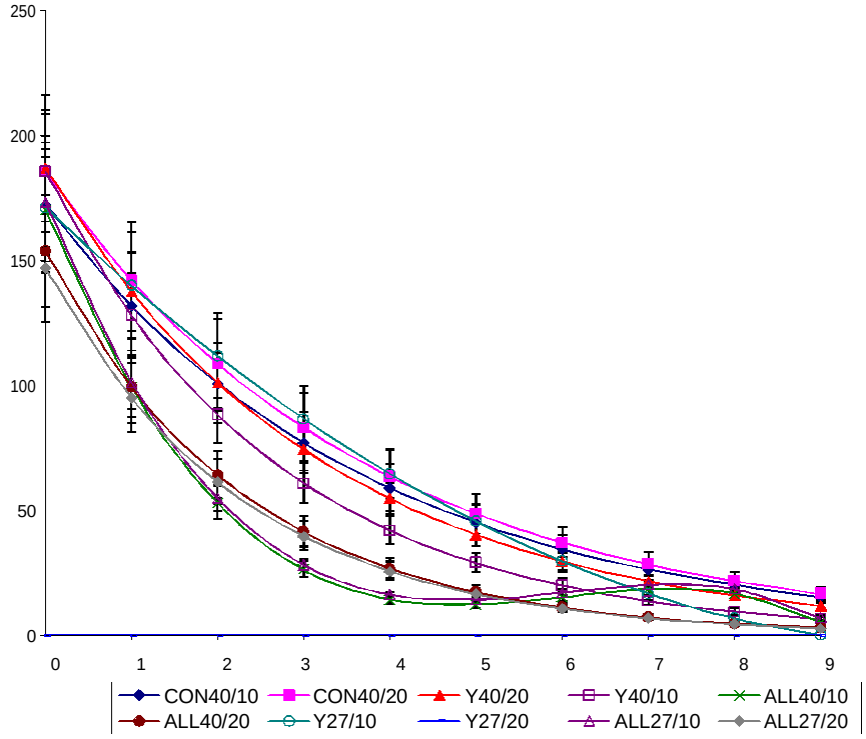

Figure 4. Peptidase activity through time of experimentation.

growth and feed efficiency seen with the supplemented diets. Similar results were obtained by De Schrijver and Ollevier (2000), who reported a positive effect on apparent protein digestion when supplementing turbot (Scophtalmus maximus) feeds with the bacteria Vibrio proteolyticus. They attributed this effect to the proteolytic activity of bacteria. The lower digestibility values for the CON40/20 treatment support this supposition. It may also indicate that high density had adverse effects on digestibility in the control diets, resulting in lower growth in the fish receiving these diets. Similar effects have been reported for terrestrial animals in which digestibility is showed to increase considerably with the use of probiotic in the diet (Bougon et al., 1998; Rychen and Nunes, 1994; Gil, 1998b). The results of the present study suggest the same effect in aquatic organisms. To confirm this, research which makes use of other ingredients and protein sources is needed because costs can be reduced by using cheaper proteins with higher digestibility.

In the present experiment, the content of unicellular protein was not affected by the inclusion of the probiotics. In contrast Bougon (1988) observed that the administration of *S. cerevisiae* in poultry diets increased the content of unicellular protein. It is necessary to consider that there are no studies of the influence of the probiotic on the content of unicellular protein in aquatic animals and this result can be different from those obtained in terrestrial animals. The content of peptidase and disaccharides were not significantly affected by the inclusion of the probiotics. The observed variations could be attributed to the normal physiological development of the fish and to the ingredient of the diets, and these variations did not affect the growth and feed conversions (Sunde et al., 2001; Bélanger et al., 2002; German et al., 2004).

The alkaline phosphatase showed the highest activity when administered to the yeast in the diet. The strong increase in the activity of alkaline phosphatase reflected a possible development of brush border membranes of enterocytes that can be stimulated by the yeast (Cuvier-Péres and Kestemont, 2002). Activities of this brush border enzyme have been reported to be indicators of the intensity of nutrient absorption in the enterocytes of fish (Gawlicka et al., 2000) . The high alkaline phosphatase activity also has been reported to be an indicator of carbohydrate and lipid absorption (Calhau et al., 2000; German et al., 2004) . This can be explained by the higher weight gain and the best feed conversion of the fish fed with diets supplemented with the yeast. Saenz de Rodrigañez et al. (2008) observed an increment in the alkaline phosphatase activity when two bacteria of genus Shewanella were administered in diets for Solea sengalensis; this author notes that this is probably the cause of significant improvement in nutritive utilization of feed. In this study similar results were observed.

It can be concluded that the addition of 0.1% (1x106 CFU/g of diet) probiotics in tilapia fry diets improves animal growth, and mitigates the effects of stress factors. The two bacterial strains used in the present study were effective in stimulating fish performance. The yeast produced the best results, being the most viable option for optimizing growth and feed utilization in intensive tilapia culture. Feed utilization was highest in tilapia fry fed with the yeast-supplemented diets, meaning that the nutrients were more efficiently used for growth and energy. This can be attributed to the high activity of alkaline phosphatase in the intestinal tract by the development of the brush probably by the yeast stimulation. Based on these results, use of 0.1% (1x106 CFU/g of diet) supplement yeast in tilapia fry feeds is recommended to stimulate productive performance.

Further research is needed to identify the mode of action in the intestinal tract of the microorganisms at enzymatic level and to determine the most appropriate supplement concentration for growth results in larger animals at a commercial scale.

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