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The Effect of Probiotics on Growth Performance and Body Composition of Common Carp (*Cyprinus carpio*)

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Abstract: This study evaluated the effects of three types of probiotics, two bacteria and one yeast on growth performance in common carp. Three diets were formulated containing the optimum protein level (40%) for common carp fry; one supplemented at 0.1% with a bacterial mixture containing *Streptococcus faecium* and *Lactobacillus acidophilus*; a 2nd supplemented at 0.1% with the yeast *Saccharomyces cerevisae* and 3rd a control diet without supplements. Two additional diets were formulated to contain 27% protein to serve as a stress factor and were supplemented at 0.1% with either the bacterial probiotic mix or the yeast. The diets were fed for 9 weeks to common carp fry stocked in 20 L tanks at two densities; a high density of 20 fry tank^{-1} as a stress factor and a low density of 10 fry tank^{-1}. Results indicate that the fry fed with diets with a probiotics supplement exhibited greater growth than those fed with the control diet. Of the four probiotic treatments, the 40% protein diet supplemented with yeast produced the best growth performance and feed efficiency, suggesting that yeast is an appropriate growth stimulating additive in common carp cultivation.

Key words: Probiotics, common carp, growth performance, supplement, fry, Iran

INTRODUCTION

The word probiotic is constructed from the Latin word pro (for) and the Greek word bios (life) (Zivkovic, 1999). The definition of a probiotic differs greatly depending on the source but the 1st generally accepted definition was proposed by Fuller (1989) as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance. Given the nature of fish farming and the fact that water harbours microbial communities it is accepted that we must have a distinctive definition for aquatic animals as opposed to that proposed by Fuller (1989) for terrestrial animals. The evolution of the definition for aquaculture throughout the 1990s is discussed by Gomez-Gil *et al.* (2000). During this period, the definition was refined and new terminology for microbial applications in aquaculture were proposed. Microbes that are antagonistic to pathogens but are not found to be present either transiently or residentially in the Gastrointestinal (GI) tract have been termed as biocontrol agents (Maeda *et al.*, 1997; Moriarty, 1998). Microbial applications that improve water quality by the breakdown of waste or pollutants have been termed bio augmentation or bio remediation (Moriarty, 1997, 1998; Gatesoupe, 1999). Thereafter, many researchers proposed definitions for probiotics in aquaculture that were inclusive of administration via rearing water but tended to restrict applications to microbes that were associated with health promoting properties (Spanggaard *et al.*, 2001; Irianto and Austin, 2002). Contrary to these definitions, some definitions do not focus only on health benefits (Moriarty, 1998; Gram *et al.*, 1999; Verschuere *et al.*, 2000; Farzanfar, 2006). Whilst many people now refer to all of these microbial applications as probiotic treatments (sensu lato), it is important to distinguish the differences between them. If we were to merge all of the proposed definitions it would appear that a probiotic application for aquaculture is a live, dead or component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving either disease resistance, health status, growth performance, feed utilisation, stress response or general vigour which is achieved at least in part via improving the hosts microbial balance or the microbial balance of the ambient environment.

The demand for animal protein for human consumption is currently on the rise and is largely supplied with terrestrial farm animals. Aquaculture, however is an increasingly important option in animal protein production. This activity requires high-quality feeds with high protein content which should contain not

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only necessary nutrients but also complementary additives to keep organisms healthy and favor growth. Some of the most utilized growth-promoting additives include hormones, antibiotics, ionophores and some salts (Fuller, 1992; Klaenhammer and Kullen, 1999). Though these do promote growth, their improper use can result in adverse effects in the animal and the final consumer as well as lead to resistance in pathogenic bacteria in the case of antibiotics. Though, probiotics are widely used in poultry and swine rearing, little has been done to incorporate them into aquaculture. Thus, this study was designed to evaluate the use of a bacteria mix containing *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as probiotic supplements in diets for common carp fry.

MATERIALS AND METHODS

The experiment was conducted for 9 weeks, using common carp (*Cyprinus carpio*, 152.3 mg average weight) fry obtained from locally farm. The experimental system was a closed recirculation system consisting of forty 20 L plastic tanks. 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 23°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 and weekly analyses were done of total ammonium, nitrite, nitrate and pH levels, using standard methods (APHA, 1992). The following values (FS.D.), appropriate for common carp cultivation were used; temperature, 23.83 F0.45°C; dissolved oxygen, 6.17 F1.64 mg L^{-1}; pH, 7.46 F0.32; ammonia, 0.07 F0.02 mg L^{-1}; nitrite, 0.07 F0.03 mg L^{-1}; nitrate, 5.93 F0.61 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 because at this growth stage, the optimum protein level for common carp is 40% (Tacon *et al*., 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). To all five diets, 0.5% chromic oxide was added 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 over population 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 $tank^{-1}$ (1 fry 2 L^{-1}) and the other 20 tanks at 20 organisms $tank^{-1}$ (1 fry L^{-1}). All fry had similar average initial weights. 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 fed with a conventional diet after which time the different treatments were randomly assigned to the tanks with four replicates per treatment.

Table 1: Formulation and proximate composition of experimental diets

	Diets				
Ingredients (%)	CON40	ALL40[a]	Y40[b]	ALL27	Y27
Anchovy fish meal	54.230	54.230	54.230	36.60	36.60
Cod liver oil	0.000	0.000	0.000	1.85	1.85
Soybean oil	3.260	3.260	3.260	6.40	6.40
Yellow corn starch	34.500	34.400	34.400	47.04	47.04
Mineral premixc	1.500	1.500	1.500	1.50	1.50
Vitamin premixd	3.000	3.000	3.000	3.00	3.00
Carboxy methyl cellulose	3.000	3.000	3.000	3.00	3.00
Chromic oxide	0.500	0.500	0.500	0.50	0.50
Probiotic	0.000	0.100	0.100	0.10	0.10
Proximate composition (% wet weight)					
Moisture	7.300	6.630	6.480	7.27	7.43
Crude protein	39.560	39.920	37.910	25.84	26.49
Ether extract	8.020	8.040	7.470	9.53	9.69
Ash	9.550	10.140	9.770	9.17	9.32
Nitrogen-free extract	34.030	33.530	36.550	44.53	43.04
Gross energy (kJ g^{-1})	19.954	19.753	20.087	19.83	19.92

[a]ALL = Bacterial mixture; Y = Yeast (Jauncey and Ross, 1982; Tacon *et al*., 1984)

Feed was manually administered *ad libitum* 4 times a day for 9 weeks. A daily record was kept of feed offered. Bulk weight was measured weekly to follow growth in weight and calculate survival and feeding ration. Briefly, the fish were taken from each tank using a net previously disinfected with a 1% benzalkonium chloride solution. This was then passed over fabric towels to eliminate excess moisture and the fish weighed on an electronic balance.

Every 3rd day, each tank was partially cleaned and the water partially changed. Once a week, the same day bulk weight measurement was done, the tanks were completely cleaned and a total change of water in the system carried out.

Beginning in the 3rd week of the experiment, feces were collected by siphoning the tank 30 min after the 2nd daily feeding to minimize leaching. Scales were removed from the collected feces, the feces was oven dried at 105°C for 24 h and then stored in hermetic containers under refrigeration until analysis.

Chemical analyses: Proximate chemical analyses were made of diet ingredients and a sample of fish at the beginning and end of the experiment according to standard methods (AOAC, 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 feces were analyzed using the acid digestion method (Furukawa and Tsukahara, 1966). Protein content was also determined for the feces to assess protein digestibility.

Statistical methods: Growth performance and feed utilization efficiency parameters were statistically compared using a one-way ANOVA ($p<0.05$) and differences among means were identified using the Duncan multiple range test. Analyses were carried out with the STATISCA Ver. 4.3, 1993 and StatGraphics Plus Ver. 2.1, 1996 computer softwares. Arcsin transformations were done when necessary.

RESULTS AND DISCUSSION

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 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 than those fed with probiotic-supplemented diets ($p<0.05$). The highest survival 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, though 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 and administered to Common carp 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 fish, Apparent Nitrogen Utilization (ANU) was significantly greater in comparison with the other treatments. The lowest apparent biological value was observed in 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 variable 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 treatments. In contrast, digestibility results for the control diets, mainly CON40/20 were lower than those for

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

	Diets										
Mean values[1]	CON40/10	CON40/20	Y40/10	Y40/20	Y27/10	Y27/20	ALL40/10	ALL40/20	ALL27/10	ALL27/20	FS.E.M.[2]
Survival (%)	75.000^{ab}	64.810^{a}	87.500^{bc}	92.590^{c}	91.670^{bc}	96.290^{c}	91.670^{bc}	85.180^{bc}	95.830^{c}	85.180^{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^{c}	0.264
SGR (% day $^{-1}$)[3]	3.330^{a}	3.570^{a}	5.800^{d}	5.430^{c}	5.460^{cd}	5.240^{c}	4.030^{b}	4.190^{b}	5.230^{c}	5.100^{c}	0.119
FCR[4]	3.113^{e}	3.260^{e}	1.430^{abc}	1.010^{a}	1.620^{bc}	1.170^{a}	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}	0.121
CND[5]	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 (%)[6]	13.580^{a}	12.440^{a}	30.350^{c}	42.700^{b}	32.950^{c}	47.300^{d}	9.620^{a}	19.270^{b}	32.950^{c}	47.510^{d}	1.843
AOMD (%)[7]	89.590^{bc}	71.300^{a}	96.180^{cd}	96.900^{cd}	90.270^{c}	92.970^{cd}	75.350^{a}	84.780^{b}	97.160^{d}	97.100^{d}	2.967
APD (%)[7]	94.280^{cd}	68.790^{a}	97.750^{cd}	98.460^{d}	91.780^{c}	96.210^{cd}	95.530^{cd}	84.780^{b}	97.160^{cd}	98.250^{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]Specific Growth Ratio (SGR) (% day^{-1}) = 100 ((log final b.w.-log initial b.w.)/time (days)). [4]FCR = IFI*/IWG*. *Individual Weight Gain, IWG (mg day^{-1}) = 1000 [(S weekly WG)/time (days)]. *Individual Feed Intake, IFI (mg day^{-1}) = 1000 [(S weekly FI)/time (days)]. [5]Carcass N Deposition (CND) (mg day^{-1}) = 1000 [(final body weight*percent final carcass protein)-(initial weight*percent initial carcass protein)]/100/time (days)/6.25. [6]Apparent N Utilization (ANU) (%) = 100 (CND/N intake). [7]Apparent organic matter and protein digestibility

Table 3: Carcass proximate composition of fish fed diets supplemented with probiotics

		Diets									
Composition (%)	Initial	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}	73.85^{a}	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 ($p>0.05$)

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 over crowding 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 the 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 the 27% protein treatments which were statistically different from the 40% 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.

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 Vazquez-Juarez *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 in cluding 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 a 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, clearly in contrast to the present results for common carp.

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. 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 common carp. This contributes to optimizing protein use for growth, a significant quality 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 (Ringa and Gatesoupe, 1998). The 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 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 (Scophthalmus maximus) feeds with the bacteria Vibrio proteolyticus. They attribute 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 shown to increase considerably with the use of a probiotic in the diet (Rychen and Nunes, 1994). The results of the present study suggest the same effect in aquatic organisms. To confirm this, more research is needed using other ingredients and protein sources because costs can be reduced by using cheaper proteins with higher digestibility.

CONCLUSION

It can be said that the addition of 0.1% probiotics in common carp 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, though the yeast produced the best results being the most viable option for optimizing growth and feed utilization in intensive common carp culture. Feed utilization was highest in common carp fry fed with the yeast-supplemented diets, meaning the nutrients were more efficiently used for growth and energy.

RECOMMENDATIONS

Based on the results, use of a 0.1% supplement of yeast in common carp fry feeds is recommended to stimulate productive performance. Further research is needed to determine the most appropriate supplement levels for optimum growth results in larger animals at a commercial scale.

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