Effect of probiotics to the production of one-year old tench and common carp

Mirjana Miščević[1], M. Ćirković [2], R. Jovanović[3], Dragana Ljubojević[2†], Nikolina Novakov[2], Z. Mašić[2], Maja Marković[4]

1. Serbian Chamber of Commerce, Resavska 13-15, 11000 Belgrade, Serbia; 2. University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi, Serbia; 3. Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia; 4. University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia

SUMMARY

The aim of this work was to assess the effects and efficacy of probiotics on production of one-year old tench and carp fingerlings. The trial was set up as two treatments with three replications. The first group was fed standard diets without addition of probiotic, and served as control. The second group was fed the same mixture with addition of 3% probiotic. Probiotic "Digestase 1000" that was used during the experiment contains a bacterial culture of: *Bacillus suptilis*, *Lactobacillus bifidus*, *Lactobacillus acidophilus*, *Ruminococcus albus* and enzymes. Month old fingerlings of tench and carp, average body mass respectively, 2.01g and 3.51g were set on six ponds of size of one ha. 20,000 fingerlings of tench and 40,000 fingerlings of carp were set on in each pool. Weight of 60 randomly selected fingerlings from each group and both species, selected randomly, was measured at the end of growing season. Final mean weight of tench and carp was respectively, 22.9 g, 67.9g in control group and 25.78g, 74.4g in group that was fed with addition of probiotic, and difference was statistically significant ($p<0.01$). Feed conversion rate was 2.2 for tench and 1.6 for carp in control group and 1.85 for tench and 1.41 for carp in experimental group. Survival rate of tench and carp was respectively 70%, 78% in control group compared with 82% and 87% in group which received probiotic. The research results confirm the usefulness of use probiotics "Digestase 1000" as 3% addition in food for tench and carp fingerlings because it was obvious that use of probiotics reduce losses, increase growth and reduce feed consumption per unit of mass.

Keywords: body weight, common carp, feed conversion rate, survival rate, probiotic, tench

† Corresponding author e-mail: ljubojevic.ljubojevicd.dragana@gmail.com

INTRODUCTION

Aquaculture faces serious problems due to various adverse effects of antibiotics such as accumulation in the tissue and immunosuppression (Nayak et al., 2007). In the recent years, the using use of some antibiotics has been prohibited by several countries due to serious environmental hazards and also some carcinogenic effects in many teleosts (Gatesoupe, 1999; Gatesoupe, 2007). Furthermore, if antibiotics are used in the lowest possible doses for economic reasons to avoid side effects and to lessen environmental impact (Scotts, 1993), the possibility of resistance of pathogen to the action of antibiotic will increase (Suzer et al., 2008) and these pathogens are often responsible for further spreading of diseases, especially under stressful fish culture conditions Functional additive, like probiotics, is a new concept on aquaculture (Li and Gatlin III, 2004). Tannock (1997) proposed the definition "living microbial cells administered as dietary supplements with the aim of improving health". The first application of probiotics in aquaculture was relatively recent (Kozasa, 1986) and since then their use have being on the rise. Probiotics have an important role in disease control strategies for aquaculture, and may provide an alternative to the use of antimicrobial compounds (Verschuere et al., 2000). Probiotics have been widely used as dietary supplementations in aquaculture to control of disease and also to the increase of feed efficiency and husbandry parameters (Jovanović et al., 2011; Miščević et al., 2011). Effect of probiotics for common carp (*Cyprinus carpio L*.) and some other warm-water fish species based on growth performance and feed utilization (feed conversion ratio, FCR) were investigated by Wang and Xu, 2006; Wang et al., 2008; Lara-Flores et al., 2010. Common carp is an economically very important fish species in Republic of Serbia, Hungary, Croatia, Poland, Israel, Czech Republic and Russian federation (Farkas et al., 1980; Bieniarz et al., 2001; Ćirković et al., 2002). Tench (*Tinca tinca*) is a species of fish grown in aquaculture of several European countries (Steffens, 1995), mostly semi-intensive in polyculture with the cyprinid species. Besides Europe tench production increased dramatically in China since 1998. (Wang et al., 2004). The tench is likely to have a great potential for future, either as a supplementary species for pond aquaculture or for stocking open waters in Republic of Serbia (Ćirković et al., 2008). The aim of this work was to assess the effects and efficacy of probiotics, commercial named "Digestase 1000", produced by Westal Chimica Italiana, which is a mixture of saprophytic bacteria and enzymes in production of one-year old tench (*Tinca tinca*) and common carp (*Cyprinus carpio L*.) fingerlings.

MATERIAL AND METHODS

The experiment was conducted at the experimental ponds in Mošorin. The trial was set up as two treatments with three replications. Healthy tench and carp fingerlings averaging respectively, 2.01g and 3.51g in weight were procured from the ponds of Fish Hatchery and Breeding Center of Sutjeska, Republic of Serbia. The fishponds were stocked on 15th June and harvested on 15th October when the fish were transferred and kept in storage ponds until the second year of rearing. The first group was fed experimental diets without addition of probiotic, and served as control. The second group was fed the same mixture with addition of 3% probiotic. Feeding fingerlings were carried out with completed feed mixtures. The composition of mixtures is shown in Table 1.

Table 1. Complete feed mixtures for carp in the intensive system

Ingredients (%)	Control	Probiotic
Soybean	41	40
Sunflower	11	10
Yeast	5	5
Fish meal (64%)	12	12
Corn	18	18
Wheat	10.6	9.6
Probiotic "Digestase"	0	3
Lysine	0.3	0.3
Methionine	0.1	0.1
Mineral premix	1	1
Vitamin premix	1	1
Total	100%	100%

Preparing facilities for breeding fingerlings was carried out as described by Ćirković et al. (2010). The amount of natural food and its influence was not considered because the pedologic composition of all 6 ponds was the same, without significant difference, and agriculture measures for improved natural production were the same for the all ponds. Well water obtained from water well which is an excavation or structure created in the ground by digging, driving, boring or drilling to access groundwater in underground aquifers was used for filling the earthen ponds. The application of probiotics, minerals and vitamins was carried out during the pelleting with temperature which did not exceed 60°C. Probiotic "Digestase 1000" that was used during the experiment contains a bacterial culture of: *Bacillus suptilis*, *Lactobacillus bifidus*, *Lactobacillus acidophilus*, *Ruminococcus albus* and enzymes. The composition of “Digestase 1000“ probiotic is shown in Table 2. Month old fingerlings of tench and carp, average body mass respectively, 2.01g and 3.51g were set on

six ponds of size of one ha. Fish were hand-fed there time daily (8:00, 12:00 and 16:00h) Oxygen level was not lower than 3 ml/L and the water temperature was in the range from 18-26°C during the trial. The water flow was approximately 4 l/s. 20,000 fingerlings of tench and 40,000 fingerlings of carp were set on in each pool. Weight of 20 selected fingerlings from each pond (60 per group) and both species, selected randomly, was measured at the end of growing season. The survival rate was determined based on the total number of fish. Feed conversion was calculated on the basis of feed offered per pond. Health status of fish was monitored visually during the growing period and no pathological changes were found in both groups.

Table 2. Compound of probiotic „Digestase 1000“

Non-pathogenic aerobic/anaerobic bacterial culture	50.00 %
Cellulase	20.0 %
Protease	10.0 %
Amylase	2.5 %
Lipase	1.0 %
Yeast	15.0 %
Sodium carbonate	1.0 %
Sodium chloride	q.s to 100

The calculations were performed with the Statistica 10 program (StatSoft Inc.). The average results are presented as means ± SD. The differences between the mean values of the studied determinants were calculated with the Student's t-tests. The statistics was done between groups from the same species.

RESULTS AND DISCUSSION

Probiotic had significance influence on final mean weight, percentage of survival and feed conversion rate. Final mean weight in control groups was significantly lower than in treated groups ($p<0.01$) (Table 3). The common probiotics used in aquaculture, belonging to *Lactobacillus sp., Bacillus sp., Bifidobacterium sp.,Vibrio sp., Saccharomyces sp., Enterococcus sp*. (Kumar et al., 2006).

Results that were noted by Lara-Flores et al. (2010) indicated that the fry of Nile tilapia (*Oreochromis niloticus*) fed with diets containing probiotic supplements (mix *Streptococcus faecium* and *Lactobacillus acidophilus* and yeast (*Saccharomyces cerevisiae*)) exhibited greater growth rate than those fed with control diet. The positive effect of *Lactobacillus acidophilus* was confirmed in the present research.

Table 3. Production parameters of one year old common carp and tench

Parameter	Tench-control	Tench-probiotic	Carp-control	Carp-probiotic
Survival (%)	70±0.82[b]	82±1.63[a]	78±1.63[b]	87± 1.63[a]
Initial mean weight (g)	2.01±0.01[a]	2.01±0.03[a]	3.51±0.01[a]	3.51±0.01[a]
Final mean weight (g)	22.9±0.23[b]	25.78±0.29[a]	67.9±0.32[b]	74.9±0.23[a]
Feed conversion rate	2.±0.08[a]	1.85±0.02[b]	1.6±0.08[a]	1.41±0.02[a]

Values are means ± SD; Values in the same row with different letter notation differ significantly statistically at $p<0.01$. Calculation is done between groups from the same species

In the study of Kumar et al. (2006), an increased growth rate and survival rate were observed in Indian major carp (*Labeo rohita*) fed feed containing *Bacillus subtilis,* which was one of the component of probiotic in the present work, compared with control. Wang (2010) established increase the growth performance of grass carp fingerlings (*Ctenopharyngodon idella*) which were fed with feed with addition of *Bacillus coagulans*, *Rhodopseudomonas palustris* and *Lactobacillus acidophilus*. Similar observations were also made by Wang and Xu (2006) in common carp, with the difference that the common carp survival rate of all the feed treatments was very high. This result might be explained by the fine experimental condition and the bigger experimental animals used in their study compared with the present study. Previous research regarding to the effect of probiotic on production parameters of common carp fingerlings one months old (Jovanović et al., 2011) and of carp one year old (Miščević et al, 2011) showed that probiotics have beneficial effect on survival rate, body weight and feed conversion rate of common carp, which is consistent with the present results. Probiotics have an important role in disease control strategies for aquaculture, and may provide an alternative to the use of antimicrobial compounds (Verschuere et al, 2000; Irianto and Austin, 2002) reported that better survival rates were achieved with the use of probiotics, which is confirmed in the present work. Similar observations were also made by Gram et al. (1999) and by Gomez-Gil et al. (2000).

CONCLUSIONS

The research results confirm the usefulness of use probiotics "Digestase 1000" in food for tench and common carp fingerlings because it was obvious that use of probiotics reduce losses, increase growth and reduce feed consumption per unit of mass. The use of probiotic decreases the cost of growing because the price of consumed probiotics was several times lower than the value of increased production.

REFERENCES

Bieniarz, K., Koldras, M., Kaminski, J., Mejza, T., 2001. Fatty acids. fat and cholesterol in some lines of carp (Cyprinus carpio) in Poland. Archives of Polish Fisheries, 9, 5–24.

Ćirković, M., Jovanović, B., Maletin, S., 2002. Ribarstvo. Univerzitet u Novom Sadu, Poljoprivredni fakultet.

Ćirković, M., Trbović, D., Milošević, N., Đorđević, V., Janković, S., Ljubojević, D., 2010. Meat quality of two years old tench and carp grown in extensive conditions. XIV International Symposium Feed Technology, Novi Sad. Proceeding, p 400-404.

Ćirković, M., Marković, G., Maletin, S., Milošević, N., Jurakić, Ž. (2008): Reintroduction and repopulation of tench (Tinca tinca L) in Serbian warm-water ponds. Proceedings of the Vth International Workshop on Biology and Culture of Tench (Tinca tinca L.), 7, Ceresole d' Alba, Italy.

Farkas, T., Csengeri, I., Majoros, F., Olah, J. 1980. Metabolism of fatty acids in fish. Ill. Combined effect of environmental temperature and diet on formation and deposition on fatty acids in the carp, Cyprinus carpio Linnaeus 1758. Aquaculture. 20, 29-40

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture. 180, 147–165.

Gatesoupe, F.J., 2007. Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture. 267, 20–30.

Gomez-Gil, B., Roque, A., Turnbull, J.F., 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture. 191, 259–270.

Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I., Nielsen, T.F., 1999. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. Appl. Environ. Microbiol. 65, 969–973.

Irianto, A. and Austin, B., 2002. Use of probiotics to control furunculosis in rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of Fish Diseases. 25, 333-342.

Jovanović, R., Miščević, M., Ćirković, M., Milošević, N., Ljubojević, D., 2011. Effect of probiotic to the production of one month old carp. 7. Međunarodni gospodarsko – znanstveni skup o ribarstvu; Hrvatsko ribarstvo na pragu EU, Riba kao funkcionalna hrana, Proceeding summary. Vukovar, Croatia, p 7-11.

Kozasa, M., 1986. Toyocerin (Bacillus toyoi) as growth promoter for animal feeding. Microbiolial Alimentary Nutrition. 4, 121–135.

Kumar, R., Mukherjee, S. C., Prasad, K. P., Pal, A. K., 2006. Evaluation of Bacillus subtilis as a probiotic to Indian major carp Labeo rohita (Ham.) Aquaculture Research. 37, 1215-1221.

Lara-Flores, M., Olivera-Castillo, L., Olvera-Novoa, M. A., 2010. Effect of the inclusion of a bacterial mix (Streptococcus faecium and Lactobacillus acidophilus), and the yeast (Saccharomyces cerevisiae) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (Oreochromis niloticus). International Journal of Fisheries and Aquaculture. 2(4), 93-101.

Li, P., Gatlin III, D.M., 2004. Dietary brewers yeast and the prebiotic Grobiotic TM AE influence growth performance, immune responses and resistance of hybrid striped bass (Monrone chrypsops X M. saxatilis) to Streptococcus iniae infection. Aquaculture. 231, 445-456.

Miščević, M., Ćirković, M., Jovanović, R., Ljubojević, D., Mašić, Z., Novakov, N., Marković, M., 2011. Effect of probiotics to the production of one-year old carp. 22nd International Symposium Food safety production, Proceedings. Trebinje, Bosnia and Hercegovina. p 50-52.

Nayak, S.K., Swain, P., Mukherjee, S.C., 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, Labeo rohita (Ham.) Fish & Shellfish Immunology. 23, 892-896.

Scotts, P., 1993. Therapy in aquaculture. In: Aquaculture for Veterinarians (ed. by L. Brown), p 131-152. Pergamon Press, Oxford, UK.

Steffens W. (1995): The tench, Tinca tinca L., an eglected pond fish species. Polish Arch. Hydrobiol. 42: 161–180.

Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., Küçüksari, H., 2008. Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: Effects on growth performance and digestive enzyme activities. Aquaculture. 280, 140–145.

Tannock, G.W., 1997. Modification of the normal microbiota by diet, stress, antimicrobial agents, and probiotic. In: Mackie RI, With BA, Isaacson RE (Eds). Gastrointestinal Microbiology. Chapman and Hall Microbiology Series, New York, USA. 2, 1219-1228.

Turnbull, J.F., 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture. 191, 259–270.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete W., 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiolial Molelular Biology Reviews. 64, 655-671.

Wang J., Min W., Guan M. and Hu S. (2004): Tench farming in China: present status and future prospects. In: IVth. International Workshop on Biology and Culture of the Tench, Tinca tinca (L.). Wierzba, September 20–23, 2004. Programme and Abstracts, Stanislaw Sakowicz Inland Fisheries Institute in Olsztyn, Poland, 32.

Wang, Y., Tian, Z., Yao, J., Li, W., 2008. Effect of probiotics, Enteroccus faecium, on tilapia (Oreochromis niloticus) growth performance and immune response. Aquaculture. 277, 203-207.

Wang, J., 2010. Use of probiotics Bacillus coagulans, Rhodopseudomonas palustris and Lactobacillus acidophilus as growth promoters in grass carp (Ctenopharyngodon idella). Aquaculture Nutrition. onlinelibrary.wiley.com