

INFLUENCE OF PROBIOTIC SUPPLEMENTED FEED ON GROWTH RATE OF COMMON CARP (*Cyprinus carpio* L.) IN AN INTENSIVE CAGE-CULTURED SYSTEM

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Abstract: As a new technology solution, probiotic fish feed supplementation was the initial approach for this experiment to assess the influence of probiotic-supplemented food on the growth rate of common carp (*Cyprinus carpio* L.). The experimental design was set in a registered cage farm in a reservoir. For the trial, three cages were dimensioned. Two experimental groups were observed (A – with lower probiotic concentration, and B – with higher probiotic concentration) and a control group C, without probiotic feed addition. An equal number of 323 carp individuals were stocked in each group. The experiment was conducted over 180 days. The growth rate was evaluated during the experimental trial. The carp fed with probiotic-supplemented feed with lower concentration had a positive impact on growth rate (GR) (total, specific, and individual GR), i.e. carp individuals in group A during the first and the second phase, compared with control group C.

Key words: aquaculture; carp; feed; probiotic; growth rate

ВЛИЈАНИЕ НА ДОПОЛНИТЕЛНА ПРОБИОТИЧНА ХРАНА ВРЗ РАСТЕЖОТ НА ОБИЧНИОТ КРАП (*Cyprinus carpio* L.) ВО СИСТЕМ НА ИНТЕНЗИВНО ОДГЛЕДУВАЊЕ ВО КАФЕЗНИ

Апстракт: Новото технолошко решение за аквакултурата – храна збогатена со пробиотици, претставуваше иницијален поттик за изведување на овој експеримент, со цел да се утврди влијанието на пробиотикот додаден во храната врз прирастот на крапот (*Cyprinus carpio* L.). Експериментот се изведуваше во кафезна фарма во резервоар. За експериментот беа димензионирали три кафези. Беа следени две експериментални групи – група А, каде што рибите беа хранети со храна збогатена со пониска концентрација на пробиотик, група В со повисока концентрација на пробиотик во храната, и група С – контролна група во која единките крап беа хранети со храна без пробиотик. Во секоја група беа насадени еднаков број единки. Експериментот траеше 180 дена. Во текот на целиот експериментален период беше следен прирастот на рибите. Кај единките крап од групата А, кои беа хранети со храна со пробиотик додаден со пониска концентрација, беше констатирано позитивно влијание на пробиотикот врз прирастот (вкупен, специфичен и индивидуален) во споредба со контролната група С во текот на првата и втората фаза од експериментот.

Клучни зборови: аквакултура; крап; храна; пробиотик; прираст

INTRODUCTION

Producers are focused on intensive cage fish cultivation to increase production worldwide. It is a

modern method of intensive rearing of freshwater fish species, and it is popular due to several advantages, in contrast to conventional methods of cultivation of freshwater fish species (De Silva,

1991). Fish farming is conducted in cages closed from all sides, with various materials that keep the fish inside while simultaneously freely changing and moving the water and removing feces. Intensive cage system fish rearing implies high stocking density, controlled feeding, relatively easy manipulation of fish and simple implementation of production procedures (Yang, 1982; Graneli, 1987; Beveridge, 1996; Starling, 1997).

Common carp (*Cyprinus carpio* L.) is the most suitable and adequate freshwater species, characterized by relatively fast growth and development, good meat taste and quality and high market demand.

Setting up the cages is not always that easy and straightforward. The cages where carp is reared are placed in natural lakes or reservoirs. To set up a cage system, it is necessary to meet certain prerequisites and technological/technical requirements (depth under the cages, water quality, possibility of fixing in a certain place, etc.). The risk of stratification disturbance with consequent increase in hypolimnetic water, which can cause mortality in caged fish, according to Soemarwoto et al. (1990) and Zoran et al. (1994), should also be considered. The intensive cultivation of carp, as well as other fish in a cage system, in addition to all the advantages, also leads to increased incidence of diseases, and thus a significantly increased need for the application of medicines, especially antibiotics, as well as regular disinfection and the use of disinfectants (Chen et al., 2014).

Experience over the years has confirmed that the application of antibiotics causes a number of negative, unwanted consequences, such as nephrotoxicity (Hentschel et al., 2005), immunosuppression, and immunomodulation (Nayak, 2010). Antibiotic residues accumulation in fish tissues causing carcinogenic effects in many teleosts was reported by Gatesoupe (1999), and their environmental hazards was also reported (Allameh et al., 2015).

As a result of the current knowledge about the negative effects of the use of antibiotics in aquaculture, in recent years, there has been a pronounced trend and effort among fish producers to reduce their use in fish farming. The scientific community is intensively working on finding a new alternative approach, the main goal of which is to increase the resistance of fish to diseases and to improve the immune system without antibiotics.

Food additives provide bacteriostatic and bactericidal effects or stimulate populations of bacteria living in the digestive tract of fish, e.g., acids, plant

extracts, prebiotics, probiotics, synbiotics, etc. (Piva, 1998; Freitag et al., 1999; Verschuere et al., 2000; Balcazar et al., 2006; Marzouk et al., 2008; Ćirković et al., 2012, and Bandyopadhyay et al., 2015).

The modern generation of probiotics is being developed in response to the demands of the development of sustainable aquaculture (intensive carp rearing) through two key factors: growth performance and disease resistance (Dawood and Koshio, 2016).

The nutritional and health benefits of high quality fish meat in aquaculture are among the most important factors for the continuous increase of fish demand in the worldwide fish meat market (Burger and Gochfeld, 2009). Several scientists have conducted studies on the effect of different strains of bacteria on farmed fish, which have been shown to have a positive effect on both the health status of the fish and their growth. Some researchers are dedicated to the effect of probiotics on juveniles of different fish species (Metailler et al., 1991; Bogut et al., 1998, and Przybyl et al., 2006), while other researchers were focused on the effect of probiotics on larger size fish (Cahill, 1990; Bogut et al., 1998; Wang & Xu, 2006; Nayak, 2010).

For the prevention of fish diseases in our state, there is a common practice, as well as for treating fish when a certain disease appears, that carp producers implement the classical and already established treatment methods. It is about using medicines (primarily antibiotics) and disinfectants (blue stone, methylene blue, malachite green, formalin, etc.). We should emphasize that some of the mentioned substances are already prohibited from being used in aquaculture. The results of previous studies indicate that precisely the probiotic from a *Paenibacillus* strain had a positive effect in the treatment of fish infected by *Aeromonas hydrophila* (Nielsen et al., 2001; Chen et al., 2019), providing an alternative approach to treating diseased fish without using antibiotics. Considering that it is a disease to which even the carp cultivated in our state is not immune, the challenge of applying the probiotic from the *Paenibacillus* strain in carp production was to be carried out within this experiment.

All research results indicate that probiotics stimulate the appetite and thus improve the nutritional regimen through the production of vitamins, detoxification of food components, and the breakdown of indigestible ingredients (Lee et al., 1999; Li and Gatlin, 2004).

Considering the increased trend of freshwater fish intensive production and the fact that certain

laws in many countries worldwide, including our neighbor states, regulate the use of antibiotics in aquaculture fish farming, which, of course, will have to be implemented in our country in the future, the need to find new alternative technological solutions to improve the welfare of fish is imposed.

In this experiment, an attempt was made to produce carp in cages without using antibiotics and chemical treatments with probiotic-supplemented feed. The main aim of the experiment was to assess the probiotic influence on production parameters as crucial factors for successful aquaculture production. The fish growth rate was estimated among the evaluated production parameters.

MATERIALS AND METHODS

The experiment was performed on a registered production facility cage farm in the "Kozjak" reservoir. To carry out the research, three partitions/cages with dimensions 5×5×5 m, that is, 3×125 m³ volume (A, B and C) were selected. Before starting the experiment, a preparatory phase was conducted.

At the beginning of the experiment, the food was controlled in the chemical laboratory of the UKIM Institute of Animal Science and Fishery and its chemical composition was examined.

The carp's diet in the experiment was based on commercial pelleted food from a manufacturer "Aqua" from Austria, with a pellet size of 4 – 6 mm.

According to standard chemical methods, the following parameters were analyzed:

- moisture by drying in an oven at a temperature of 105 °C to constant weight;
- raw fiber;
- crude proteins according to the Kjeldahl method (Nx6.25);
- crude fats through extraction with diethyl ether according to the Soxhlet method, and
- crude ash through combustion in a furnace for 8 hours at a temperature of 600 °C.

The feed composition declared values were the following: protein 30%, fat 10%, crude fiber was used 4.5%, crude ash 6.5%, calcium (Ca) 0.90%, sodium (Na) 0.25%, and phosphorus (P) 1.10%.

Bacterial culture – probiotic *Paenibacillus alvei* DZ-3, in strictly defined quantities, was added to the basic food as pellets, with a special procedure.

The production and preparation of probiotics was conducted in the Department of Microbiology

and Microbial Biotechnology Laboratory at the Faculty of Natural Science and Mathematics – University Ss. "Cyril and Methodius" in Skopje.

The probiotic preparation was carried out within the following procedure: *Paenibacillus alvei* was used, i.e., its 24h culture, at 37°C. Furthermore, culture multiplication was performed in NB (nutrient medium) at 37°C/24h/180 rpm. The biomass was collected at 4000 rpm/15 min., then washed with 5 ml of PBS (phosphate buffer) (pH = 7.2) twice consecutively. It was then diluted in PBS to 1.5×10⁸ CFU/ml (= Mcfarland 0.5). The probiotic was applied in two concentrations: 1 ml/kg and 2 ml/kg of food.

The food with added probiotics was prepared using the following procedure: The probiotic in liquid form was sprayed onto the pelleted food in a mixer for 3 minutes. Then, it was mixed in a mixer for 5 minutes. The mixed food with the added probiotic was applied evenly, distributed in a layer of 2 cm, and left to dry in a dry, ventilated place for 2 hours.

Food with probiotics for experimental groups A and B was prepared every two weeks, and daily rations were determined according to the table prescribed by the food manufacturer, depending on the water temperature and body weight of the fish.

In the food for the first experimental group (A), the probiotic was added at 1 ml/kg of food at a concentration of 1.5×10⁸ CFU/ml.

In the food for the second experimental group (B), the probiotic had the same concentration but was added at 2 ml/kg of food.

The third group (C) was the control group and the fish were fed with commercial food without the probiotic.

The fish were fed with automatic feeders throughout 24 hours.

The experiment was set up during one rearing technological season (180 days). Before the start of the experiment, the sorting and selection of the biological material necessary for the formation of the three experimental groups was carried out. The sorted and separated carp individuals had an average body mass of 170 g. The exact number of carp individuals (323) were stocked in each cage. After stocking the fish into separate groups, the total ichthyomass in each group was determined, which represented the initial weight of the groups.

The experiment was conducted in two phases: the first phase (juvenile rearing) and the second phase (commercial phase).

In the first phase five consecutive control measurements of fish were carried out at 14 to 16 days. In the second phase, four control measurements of the total ichthyomas in each cage of the groups were performed. The second phase (commercial rearing) followed the completion of the first phase of the technological process. In each cage, groups A, B, and C were stocked with sorted individuals of carp in equal quantities (kilograms of fish), i.e., total mass of 121 kg in each cage. Growth rate (GR), specific growth rate, and individual growth rate, during control measurements were calculated by following formulas:

- total weight gain to the formula:

$$(WG) = Wf - Wi,$$

- specific growth – the growth of the unit from stocking to the last measurement is calculated according to the formula:

$$(SGR) = (\ln Wf - \ln Wi) \times 100/t,$$

- individual (daily) growth rate – average increase of an individual per day from planting to the last measurement, calculated according to the formula:

$$IP = kg$$

of fish/number of individuals: 1000,

where: (WG) represents the growth, (SGR) is the specific growth, Wi and Wf (g) represent the initial and final weights, t is the duration of experimental days and \ln is the natural logarithm.

RESULTS AND DISCUSSION

In Table 1 are presented the results of carp food composition analysis. Determination was performed on commercial carp food without probiotic addition and both probiotic concentrations added in carp commercial food, 1 ml/kg food and 2 ml/kg food, respectively.

Fodd containing probiotic in concentration of 1 ml/kg, had higher values of moisture content and lower values of dry matter content. Raw protein, fiber and fat content presented quite stable values regarding probiotic feed addition. Nitrogen free extracts percentage was decreased in food with probiotic addition of 1 ml/kg (Table 1).

After determining the body weight of the experimental fish for all subsequent control measurements, the growth rate was calculated from the groups A, B, and C, whose values are presented in Table 2.

Table 1

Results of chemical analysis of commercial food without probiotic addition and food with probiotic concentration 1 and concentration 2

Parameter	Food without probiotic additon	Probiotic food 1	Probiotic food 2
Moisture	7.27	11.35	10.36
Dry matter	92.73	88.65	89.64
Raw protein	31.13	31.00	30.97
Raw fiber	4.94	4.85	4.86
Raw ash	6.13	5.85	5.91
Raw fat	8.74	8.60	8.60
Nitrogen free extracts	41.79	38.35	39.76

Table 2

Total growth rate (g) of 30 carp individuals in groups A, B, and C during the experimental period

Group	Total growth rate (g)	
	1st phase	2nd phase
A	10512	262912
B	9052	241800
C	7165	207600

Table 2 presents the values for the total growth rate of each group, for the entire experimental period (first and second phase).

Probiotic treatment represents the highest growth rate of 10512 g and 9052 g, respectively, for groups A and B. The experimental groups presented superior results compared to control group C, fed without probiotic supplement, with a total growth rate of 7165 g. The lowest total growth rate for the second phase was also estimated in the control group C. The highest growth rate values were observed in fish of group A, treated with a probiotic with a lower concentration (1 ml/kg food). The application of a commercial *Streptococcus faecium* probiotic in Israeli carp diets, as shown by the research of Bogut et al. (1998), improved growth and feed conversion rate. The authors highlights the possibility of a difference between the types of probiotics, as well as finding the approach to combining them and the optimal concentration assessment because they determined a positive influence on the daily and specific growth in the experimental

group treated with photosynthetic bacteria and *Bacillus* sp.

During the research of Khalil et al. (2012) an increased total growth rate was observed, increased food intake and increased use of nutrients, as well as in the parameters of the chemical composition of the fish meat. Feed supplementation with probiotics significantly increased growth rate, feed utilization and decreased mortality rate, i.e., the survival rate (Munir et al., 2016).

Table 3 presents the specific growth rate by groups from the first and the second phase of the experimental period. During the first phase of the experiment, the experimental groups A and B note the specific growth rate increase through the values of 245.6 g and 218.2 g, respectively, finally as increased values compared to the control group C, estimated by 162.0 g. At the end of the experiment, experimental group A presented an increased trend-line than groups B and C, with values of 435.7 g. In contrast, group C had the lowest total growth rate values during all measurements and, at the end of the experiment, estimated 259.9 g.

Table 3

Specific growth rate (g) of 30 carp individuals in groups A, B, and C during the experimental period

Group	Specific growth rate (g)	
	1 st phase	2 nd phase
A	245.6	435.7
B	218.2	411.0
C	162.0	259.9

This experiment's results were in accordance with Xu, Wang and Lin (2014), in which research has been determined the impact of the probiotic *B. coagulans* in the carp diet and presented a positive impact on the final body weight of the fish and the growth rate (specific, individual, and daily), as well as on the feed conversion ratio. Similar results were reported by Lara-Flores et al. (2003); El-Haroun et al. (2006), and Ramakrishnan et al. (2008).

The calculated daily (individual) growth rate per fish/individual (g) during the two phases of the experiment is presented in Table 3.

From Table 4 it can be concluded that the fish treated with a lower concentration of probiotic, i.e., experimental group A, in contrast to experimental

group B (treated with a higher concentration of probiotic), presented a difference in the values of increased individual growth rate.

Table 4

Individual daily growth rate (g) of 30 carp individuals in groups A, B, and C during the experimental period

Group	Individual growth rate (g)	
	1st phase	2nd phase
A	8.2	15.6
B	7.3	14.7
C	5.4	9.3

In relation to the control group C, where the fish were presented with the lowest individual growth rate, in both experimental groups A and B, this parameter presented higher values.

Considering individual growth rate, experimental group A maintained the trend of increasing the value of specific and individual growth rate, compared to groups B and C. The results obtained from the conducted experiment were in accordance with the results of Swain et al. (1996) and Ghosh and Ray (2003), who performed experiments with Indian carp.

Faramarzi et al. (2011), during research on the influence of probiotic feed addition, observed increased growth rate values when feeding juvenile carp with food containing 0.1% probiotic (*Bacillus subtilis*) compared to the control group.

Probiotic addition influence on the growth rate of carp reared in intensive cage system, as one of the objectives of this experiment, presented positive effects about this surveyed production parameter. In terms of growth rate (total, specific, individual/daily), the obtained results presented that group A, in which the fish were treated with a lower probiotic concentration, stands out not only in relation to group C, but also in relation to group B.

As a conclusion we highlight that probiotic concentration of 1 ml/kg had positive influence on carp total, specific and individual growth rate, during the both experimental phases.

Probiotic addition in aquaculture, as an alternative to antibiotics and chemical agents, results in significant benefits such as improving the health status, fish growth, and survival rate, and the most

important technological impact on fish production is the increase in production performance and economic profitability.

REFERENCES

- Allameh, S. K., Yusoff, F. M., Ringo, E., Daud, H. M., Saad, C. R., Ideris, A. (2015): Effects of dietary mono- and multi-probiotic strains on growth performance, gut bacteria and body composition of Javanese carp (*Puntius gonionotus*, Bleeker 1850). *Aquaculture Nutrition*.
- Balcazar, J. L., De Blais, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., Muzquiz, J. L. (2006): The role of probiotics in aquaculture, *Veterinary Microbiology* **114**, 173–186.
- Bandyopadhyay, P., Mishra, S., Sarkar, B., Swain, S. K., Pal, A., Tripathy, P. P., Ojha, S. K. (2015): Dietary *Saccharomyces cerevisiae* boosts growth and immunity of IMC *Labeo rohita* (Ham.) juveniles. *Indian Journal of Microbiology* **55**, 81–87.
- Beveridge, M. C. M. (1996): *Cage Aquaculture*. 2nd edition. Fishing News Books Ltd., Oxford. 346 pp.
- Bogut, I., Milaković, Z., Bukvić, Z., Brkić, S., Zimmer R. (1998): Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). *Czech Journal of Animal Science* **439**, 231–5.
- Burger, J., Gochfeld, M. (2009): Perceptions of the risks and benefit of fish consumption: Individual choices to reduce risk and increase health benefits. *Environmental Research* **109**, 343–349.
- Cahill, M. M. (1990): Bacterial flora of fishes: A review. *Microb. Ecol.* **19**, 21–41. <https://doi.org/10.1007/BF02015051>
- Chen, Y., Zhu, X., Yang, Y., Han, D., Jin, J., Xie, S. (2014): Effects of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance of gibel carp, *Carassius auratus gibelio*. *Aquatic Nutrition* **20**, 532–546.
- Chen, S.-W., Liu, C.-H., Hu, S.-Y. (2019): Dietary administration of probiotic *Paenibacillus ehimensis* NPUST1 with bacteriocin-like activity improves growth performance and immunity against *Aeromonas hydrophila* and *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology* **84**, 695–703. <https://doi.org/10.1016/j.fsi.2018.10.059>
- Ćirković, M., Ljubojević, D., Đorđević, V., Novakov, N., Petronijević, R., Matekalo-Sverak, V., Trbović, D. (2012): The breed effect on productivity and meat nutrient composition of fish. *Kafkas Univ Vet Fak Derg*; **18** (5), 775–780. DOI:10.9775/kvfd.2012.6383
- Dawood, M., Koshio, S. (2016): Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture* **454**. DOI:10.1016/j.aquaculture.2015.12.033
- De Silva, S. S., Zhitang, Y., Lin-Hu, X. (1991): A brief review of the status and practices of the reservoir fishery in mainland China. *Aquaculture and Fishery Management* **22**, (1), 73–84. <https://doi.org/10.1111/j.1365-2109.1991.tb00496.x>
- El-Haroun, E. R., Goda, A. M. A.-S., Kabir Chowdhury, M. A. (2006): Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture Research* **37**, (14), 1473–1480. <https://doi.org/10.1111/j.1365-2109.2006.01584.x>
- Faramarzi, M., Kiaalvandi, S., & Iranshahi, F. (2011). The effect of probiotics on growth performance and body composition of common carp (*Cyprinus carpio*). *Journal of Animal and Veterinary Advances* **10** (18), 2408–2413. DOI:10.3923/javaa.2011.2408.2413
- Freitag, M., Hensche, H. U., Schulte-Sienbeck, H., Reichelt B. (1999): Biological effects of conventional and alternative performance enhancers. *Feed Magazine* **2**, 50–57.
- Gatesoupe, F. J. (1999): The use of probiotics in aquaculture. *Aquaculture* **180**. 147–165.
- Ghosh, K., Sen, S. K., Ray, A. K. (2003): Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. *Israeli Journal of Aquaculture* **55**, 13–21.
- Graneli, W. (1987): Restoration and management of lakes in tropical and sub-tropical areas – a Swedish perspective. *Archiv für Hydrobiologie. Beihefte Ergebnisse der Limnologie* **28**, 563–571.
- Hentschel, D. M., Park, K. M., Cilenti, L., Zervos, A. S., Drummond I. (2005): Acute renal failure in zebrafish: a novel system to study a complex disease. *American Journal of Physiology – Renal Physiology* **288**, 923–929.
- Khalil, F. F., Mehrim, A. I., Montaha, E. M. (2012): Hassan Effect of Hydroyeast Aquaculture® as growth promoter for adult Nile tilapia (*Oreochromis niloticus*). *J. Animal and Poultry Production*, Mansoura University, Vol. **3** (6), 305–317.
- Lara-Flores, M., Olvera-Novoa, M. A., Guzman-Mendez, E. B., Lopez-Madrid, W. (2003): Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **216**, 193–201.
- Lee, Y. K., Nomoto, K., Salminen, S., Gorbash, S. L. (1999): *Handbook of Probiotics*. New York, Wiley.
- Li, P., Gatlin III D. M. (2004): Strategies of dietary supplementation for health management of hybrid striped bass *Morone chrysops* M. *Saxatilis*: A review of research at Texas A & M University. In: Cruz Suarez, L. E., Ricque Marie, D., Nierto Lopez, M. G., Villarreal, D., Scholz, U., Gonzalez, M., *Avances en Nutrición Acuicola*, VII. *Memorias del VII Simposium International de Nutrición Acuicola*. Hermosillo, Sonora, Mexico.
- Marzouk, M. S., Moustafa, M. M., Mohamed, N. M. (2008): Evaluation of immunomodulatory effects of some probiotics on cultured *Oreochromis niloticus*. *Proceedings of 8th International Symposium on Tilapia in Aquaculture*, Cairo, Egypt, pp. 1043–1058.
- Metailler, R., Hollocou, Y. (1991): Feeding of European sea bass (*Dicentrarchus labrax*) juveniles on diets containing probiotics. *Fish Nutrition in Practice*. Institut national de la recherche agronomique, Paris, 429–432.
- Munir, M. B., Roshada, H., Yam, H. C., Terence, M. L., Siti, A. M. N. (2016): Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings. *Aquaculture* **460**, 59–68.
- Nayak, S. K. (2010): Probiotics and immunity: A fish perspective. *Fish Shellfish Immunology* **29**, 2–14.

- Nielsen, M. E., Høi, L., Schmidt, A. S., Qian, D., Shimada, T., Shen, J. Y., Larsen, J. L. (2001): Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China. *Diseases of Aquatic Organisms* **46** (1), 23–29.
- Piva, A. (1998): Non-conventional feed additives. *Journal of Animal Feed Science* **7**, 143–154.
- Przybyl, A., Mazurkiewicz, J., Sip, A., Grajek, W. (2006): Probiotic effect on lactic bacteria *Carnobacterium* sp. in the feed on growth and survival of common carp (*Cyprinus carpio*). In press.
- Ramakrishnan, C. M., Haniffa, M. A., Manohar, M., Dhanaraj, M., Arockiaraj, A. J., Seetharman, S. (2008): Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). *Israeli Journal of Aquaculture – Bamidgeh* **60**, 128–133.
- Soemarwoto, O., Roem, C. M., Herawati, T., Costa-Pierce B. A. (1990): Water quality suitability of Saguling and Cirata reservoirs for development of floating net cage aquaculture. In: *Reservoir Fisheries and Aquaculture Development for Resettlement in Indonesia* (Costa-Pierce, B.A. and O. Soemarwoto, eds.), 18–111. ICLARM Tech. Rep. **23**. ICLARM, Manila.
- Starling, F., Beveridge, M., Lazzaro, X., Baird, D. (1997): Testing two biomanipulation approaches in eutrophic paranoá reservoir (Brasília – Brazil): Control of tilapia overpopulation vs. introduction of herbivorous silver carp. (In press.)
- Swain, S. K., Rangacharyulu, P. V., Sarkar, S., Das, K. M. (1996): Effect of a probiotic supplement on growth, nutrient utilisation and carcass composition in Mrigal fry. *Journal of Aquaculture* **4**, 29–35.
- Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W. (2000): Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, **64**, 655–671.
- Wang, Y. B., Xu, Z. (2006): Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Animal Feed Science. Tech.* **127**, 283–292.
- Xu, Y., Wang, Y., Lin, J. (2014): Use of *Bacillus coagulans* as a dietary probiotic for the common carp (*Cyprinus carpio*). *Journal of the World Aquaculture Society*, Vol. **45**. N°4.
- Yang, S. L. (1982): Fish culture and reservoir management in the Republic of Singapore. *Proc. Biotrop.*, Bogor, Indonesia. 8 p.
- Zoran, M., Milstein, M., Krambeck, H. J. (1994): Limnological aspects of dual purpose reservoirs for irrigation and fish culture in the coastal area of the Jordan valley. *Israeli Journal of Aquaculture* **46**, 64–75.

