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# Diet-Influenced Performance of Juvenile Common Carp (*Cyprinus carpio* L.) after Experimental Aeromonas Infection

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**Abstract**

The effects of two diets, SGP 493 (Aller Aqua, Denmark; SGP) and chironomid larvae (Katrinex, Poland; CHI), on juvenile carp survival and hematological values after subcutaneous infection with *Aeromonas veronii* bt. *sobria* (strain K144) were studied. All infected fish developed skin ulcers and both groups developed anemia, i.e., a severe reduction in hemoglobin content without a decrease in red blood cell count and a minor decrease in hematocrit. There were higher levels of hematocrit, hemoglobin, and mean cell hemoglobin in the CHI group at the beginning of the infection, together with a slightly higher erythropoietic potential. At the end of the experiment, partial recovery of hemoglobin levels took place. The SGP diet induced considerable leukocytosis in healthy fish, which did not enhance their resistance to the infection. Leukopenia, lymphopenia, and reduced phagocyte activity took place in all infected fish. Survival 15 days after injection was 50% in SGP and 63% in CHI, suggesting that fish fed natural food were slightly less susceptible to *Aeromonas*-induced anemia even though feeding natural food or SGP did not significantly affect the performance of infected fish.

## Introduction

Much attention has been paid to study of the effects of diets on juvenile cyprinid fish. Dry commercial diets are generally inferior to natural ones as far as growth, survival, and biological quality are concerned (Myszkowski et al., 2002; Kaminski et al., 2005; Kamler et al., 2006; Wolnicki et al., 2006). Little is known about how diets and feeding influence fish blood parameters as indicators of physiological status or the resistance of fish against dis-

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ease. Fish immune response may be enhanced by dietary probiotic additives such as bacteria (Irianto and Austin, 2002; Salinas et al., 2005), antioxidants such as vitamin C (Verlhac et al., 1998; Sahoo and Mukherjee, 2003) and vitamin E (Clerton et al., 2002; Sahoo and Mukherjee, 2002), natural and synthetic immunomodulators (Sahoo and Mukherjee, 1999; Siwicki et al., 2000; Sakai et al., 2001; Peddie et al., 2002; Bagni et al., 2005; Cuesta et al., 2005; Siwicki et al., 2005), natural carotenoids (Amar et al., 2004) and hormones (triiodothyronine; Sahoo, 2003), and dietary chromium (Gatta et al., 2001).

On the other hand, immunocompetence and disease resistance may be impaired by nutrient deficiencies especially of certain vitamins and minerals (Sealey and Gatlin, 1999) or essential fatty acids (Montero et al., 2004). A dietary protein deficiency may reduce the red blood cell count in fish (Daniels and Gallagher, 2000) while leucocrit values and plasma protein tend to increase and phagocyte activity to decrease as the feed ration increases in *Oncorhynchus tshawytscha* (Alcorn et al., 2003).

Bacteria belonging to the mesophilic *Aeromonas* group commonly occur in the water environment and are responsible for a wide spectrum of diseases among poikilothermic and homoiothermic organisms including humans (Khardori and Fainstein 1988; Austin et al., 1996). Such bacteria are also important pathogens of many fish species. They cause motile aeromonad infection (MAI) and aeromonad septicaemia (MAS) which occur in adverse environmental conditions. Mesophilic aeromonads are frequently isolated from asymptomatic fish and often occur in damaged fish tissues as a secondary pathogen. *Aeromonas veronii* bt. *sobria, A. bestiarum*, and *A. salmonicida* are most often associated with disease in common carp (*Cyprinus carpio* L.) cultured in Poland (Kozinska et al., 2002). The risk of fish disease associated with mesophilic aeromonads is increasing in Poland because of stress and environmental contamination resulting from production intensity.

It seems possible that juvenile cyprinids reared on dry commercial diets may perform considerably worse than those fed natural foods, after release into outdoor ponds. *Aeromonas* causes skin lesions in fish after subcutaneous administration (Stosik, 1989; Rehulka, 2002; Harikrishnan et al., 2003), facilitating observation of disease development and recovery.

Therefore, the aim of the present study was to evaluate the effect of two commercial diets, one artificial and one natural, on the performance of juvenile *C. carpio*, measured by survival and hematological values, after experimental infection with *Aeromonas veronii* bt. *sobria* (strain K144).

## Materials and Methods

*Fish preparation. Cyprinus carpio* progeny of four female and four male spawners were pooled. Juveniles began to be prepared for the experiment 120 days after hatching, when their mean wet body weight and total length reached 3.31±0.38 g and 6.03±0.34 cm. The fish were stocked into 40-l glass flow-through aquaria at the density of 1.25 individuals per l (50 per aquarium) and fed a dry commercial starter feed SGP 493 (Aller Aqua, Denmark) or frozen commercial chironomidae larvae (Table 1). Feed was given manually at 08:00, 11:00, 14:00, 17:00, and 20:00 at daily feeding rates noticeably below satiation.

Aquaria were continuously supplied with filtered and aerated water from the recirculation system at approximately 1.5/min and heated to 22.0±0.5°C. Aquaria were artificially illuminated from 08:00 to 21:00 by fluorescent tubes producing a light intensity of 700 lux at the water surface. Aeration was provided by airstones to maintain the oxygen concentration in the water at about 60% saturation.

The preparatory period lasted 70 days. The final mean wet body weight and total length were 24.36±3.57 g and 11.60±0.49 cm for fish fed SGP 493 and 16.70±3.44 g and 10.40±0.70 cm for those fed chironomids.

*Experimental procedures*. The experiment begun on the day after completion of the preparatory period and lasted 15 days. The two experimental groups included 40 juveniles fed SGP 493 (SGP) and 35 juveniles fed chi-

Table 1. Composition of artificial (SGP 493) and natural (CHI; chironomidae larvae) diets.

| *Component (%)* | *SGP* | *CHI** |
|---|---|---|
| Moisture | - | 86.69 |
| Protein | 56.0 | 9.34 |
| Lipid | 11.0 | 1.57 |
| Carbohydrate | 13.0 | 1.05 |
| Ash | 11.0 | 1.35 |

* Wolnicki et al. (2006)

ronomids (CHI). Assuming that natural food is an optimum source of nutrients for fish, the CHI group served as the control.

On day 1 of the experiment, all the fish were subjected to thermal shock by rapidly transferring them from water of 22.4±0.5°C to water of 10.5±0.2°C for one hour, then exposing them to cold atmospheric air (11.0-12.0°C) for 2 min. Next, they were hypodermically injected on the left side of the body (1 cm below the dorsal fin) with 0.05 ml of 0.85% NaCl solution containing 4 x $10^7$ cfu/ml of the Polish strain K144 *Aeromonas veronii* bt. *sobria* which is very virulent for carp (unpubl. data). The experimental concentration of bacteria was established in preliminary tests after it induced distinct symptoms in the fish without killing them.

Since fish in good condition may resist bacterial infection (Jara and Chodyniecki, 1999), the fish were weakened with a second thermal shock to assure infection by rapidly putting them into water of 21.4°C. The optimum thermal shock procedure was determined in preliminary tests where there was no fish mortality during a 10-day observation period. The fish were transferred in this water to 20-l experimental aquaria filled with stagnant water of 22.5±0.5°C that was continuously aerated. The SGP fish were stocked in four aquaria at 10 fish per aquarium while the CHI fish were stocked in one aquarium of five fish and three aquaria of 10 fish. Water in the aquaria was changed manually once a day and fish were not fed throughout the 15-day experiment.

Fish mortality and changes on the skin were monitored continuously. Dead fish were counted and removed immediately after being noticed. Irreversible loss of balance and complete cessation of all movement indicated death.

*Blood analysis*. Blood was sampled by heart puncture. Needles and plastic Eppendorf tubes were heparinized and chilled. Blood (200-300 µl) was first sampled six days before the experiment, i.e., six days before infection, and on days 1, 8, and 15. Each sampled fish was sampled only once and then excluded from further observations and survival calculations. Therefore, data from only three replicates (10 fish each) per experimental group were used to determine survival.

The blood was transported (about 2 h) at 4.0ºC to the laboratory of the Department of Animal Physiology of the University of Podlasie in Siedlce and immediately subjected to analysis. Hematological parameters were used as indicators of physiological status in fish. Red blood parameters are related to oxygen transport capacity and therefore indicate the energetic status of the organism (they usually increase under stress and decrease during infection or intoxication). White blood parameters are related to immune functions, in fish represented primarily by non-specific mechanisms, mainly phagocytosis. These parameters are sensitive to environmental and intrinsic factors. Thus monitoring of hematological parameters provides reliable information on disease development and recovery.

The following blood parameters were evaluated: hematocrit (Ht) – after centrifugation of blood in heparinized capillary tubes for 5 min at 12,000 rpm; hemoglobin concentration (Hb) – using the spectrophotometric cyanmethaemoglobin method (with Drabkin reagent), extinction read at 540 nm; erythrocyte count (RBC) – blood was diluted 100 times with Hayem solution and the cells were counted in a Burker chamber under 12.5 x 40 magnification; mean cell volume (MCV) – calculated according to the formula MCV = 10(Ht

x RBC); mean cell hemoglobin (MCH) – calculated according to the formula MCH = Hb x RBC; mean cell hemoglobin concentration (MCHC) – calculated according to the formula MCHC = 100(Hb x Ht); leukocyte count (WBC) – blood was diluted 100 times with Hayem solution and cells were counted in a Burker chamber under 12.5 x 40 magnification at the same time that red blood cells were counted; and activity of reactive oxygen radical production by phagocytes (NBT) – by spectrophotometric measurement of formasan concentration (at 546 nm), a product of intracellular reduction of nitotetrazolium blue by phagocytes, according to Sychlowy and Lukas (1978), modified for fish blood by Sopinska (1985). Blood smears were prepared and stained according to Pappenheim to calculate differential erythrocyte and leukocyte counts.

*Statistical analysis.* Survival percentages were normalized using angular transformation (Sokal and Rohlf, 1969) and differences between and within experimental groups were considered significant at $p \leq 0.05$. Results of blood analyses were subjected to the non-parametric Mann-Whitney U-test and differences were assumed significant at $p \leq 0.05$.

## Results

All fish developed skin ulcers and red spots around the place of infection within 16 h after injection. Large amounts of mucus were secreted on the skin and swimming ability decreased. Ulcers first began to burst three days after injection in group CHI and four days after injection in SGP. By day 6, all ulcers had burst. Injuries caused by bacteria began to heal on day 8. There were external deformities (spinal curvatures) in some motionless weak specimens. Mortality occurred on days 4-9 in group SGP and 3-7 in group CHI. By the end of the experiment, CHI had a lower average mortality rate – 37% (30%, 40%, and 40% in the three replicates) than SGP – 50% (in all replicates), but the differences within and between groups were not significant (Fig. 1).

Hematocrit did not significantly differ between groups before injection (Table 2) and was within the normal range for *C. carpio* (Witeska, 2003). On day 1, hematocrit decreased only in SGP and was significantly lower than in CHI while on days 8 and 15, hematocrit was significantly lower than before injection in both groups.

Hemoglobin gradually decreased after infection in both groups and on day 1 was significantly lower in SGP than in CHI. On day 8, when symptoms were fully developed, it was extremely low in both groups (Witeska, 2003). On day 15, it had slightly recovered but was still lower than before injection.

Red blood cell counts changed little throughout the experiment and were typical for the species (Witeska, 2003). It was slightly lower in CHI than in SGR and significantly increased on day 15 in CHI.

Mean cell volume was higher in CHI but the difference was not significant until day 15. Mean cell hemoglobin gradually decreased after infection and began to recover on day 15 but was still significantly lower than before infection. The decrease and recovery of mean cell hemoglobin concentration was similar but there were no differences between the groups.

The percent of immature erythrocytes (polychromatophilous erythroblasts) on day 1 was significantly higher and on day 8 slightly higher in CHI than in SGP, possibly indicating

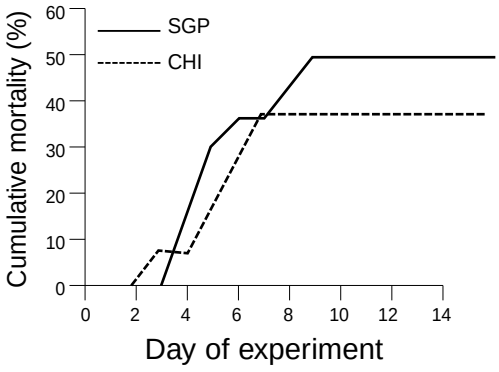


Fig. 1. Cumulative mortality of carp juveniles fed an artificial (SGP) or natural (chironomid larvae; CHI) diet after infection with *Aeromonas veronii* bt. *sobria*.

Table 2. The effects on hematological parameters of feeding an artificial (SGP) or a natural (chironomid larvae; CHI) diet to carp juveniles infected with *Aeromonas sp*.

| | *Before infection* | | *Days after infection* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *1* | | *8* | | *15* | |
| | *SGP* | *CHI* | *SGP* | *CHI* | *SGP* | *CHI* | *SGP* | *CHI* |
| n | 10 | 10 | 10 | 5 | 7 | 7 | 10 | 12 |
| Ht (%) | 27.9±1.6 | 28.6±2.2 | 25.7±2.2$^{a\#}$ | 29.2±2.2$^{b}$ | 24.7±1.5$^{\#}$ | 22.0±2.8$^{\#}$ | 21.5±3.2$^{\#}$ | 22.6±3.5$^{\#}$ |
| Hb (g/l) | 88.9±12.0 | 95.3±11.6 | 64.8±6.3$^{a\#}$ | 74.3±5.9$^{b\#}$ | 28.6±9.4$^{\#}$ | 32.0±8.3$^{\#}$ | 60.6±19.4$^{\#}$ | 59.5±22.5$^{\#}$ |
| RBC ($10^6$/µl) | 1.96±.66 | 1.53±0.48 | 2.05±0.44 | 1.98±.41 | 2.26±0.53 | 1.73±0.73 | 2.29±0.53 | 2.01±0.45 $^{\#}$ |
| MCV (fl) | 167±66 | 197±55 | 132±35 | 152±31 | 97±10 | 212±23 | 92±15 $^{a\#}$ | 113±17$^{b\#}$ |
| MCH (pg) | 50.0±17.6 | 66.6±18.5 | 32.8±6.9$^{\#}$ | 38.3±5.4$^{\#}$ | 12.2±2.6$^{a\ \#}$ | 20.1±4.2$^{b\#}$ | 26.5±5.6$^{\#}$ | 31.2±10.2$^{\#}$ |
| MCHC (g/l) | 321±32 | 343±42 | 254±38$^{\#}$ | 254±19$^{\#}$ | 138±17$^{\#}$ | 115±2$^{\#}$ | 301±66 | 276±65$^{\#}$ |
| Erythroblasts (%) | 4.7±3.6 | 3.4±4.1 | 2.1±0.8$^{a}$ | 5.4±1.7$^{b}$ | 2.2±1.4 | 3.8±2.8 | 2.0±1.7 | 1.2±1.1 |
| WBC ($10^3$/µl) | 178.0±93.3 | 92.7±23.1 | 80.3±20.9$^{a\ \#}$ | 40.4±15.8$^{b\ \#}$ | 59.5±29.0$^{\#}$ | 40.6±22.0$^{\#}$ | 93.3±34.2 | 89.0±50.6 |
| Lymphocytes (%) | 97.5±3.3 | 98.5±1.3 | 86.3±7.3$^{\#}$ | 91.6±5.9$^{\#}$ | 77.6±26.4 | 89.2±23.9 | 98.8±2.1 | 98.6±2.3 |
| Neutrophils (%) | 2.1±3.4 | 0.8±0.9 | 13.7±7.3$^{\#}$ | 8.0±5.7$^{\#}$ | 6.8±9.9 | 3.6±7.4 | 0.3±0.5$^{\#}$ | 1.4±2.3 |
| Monocytes (%) | 0.1±0.3 | 0.5±0.8 | 0.0±0.0 | 0.4±0.5 | 14.4±17.4 $^{\#}$ | 6.6±15.9 | 0.0±0.0 | 0.0±0.0 |
| NBT (g/l) | 1.08±0.49 | 0.89±0.22 | 0.62±0.22$^{\#}$ | 0.67±0.12 | 0.63±0.11$^{\#}$ | 0.61±0.11$^{\#}$ | 0.45±0.07$^{\#}$ | 0.42±0.09$^{\#}$ |

Values in a row with different superscripts significantly differ between days ($p$<0.05). Values marked with # significantly differ from before infection.

Ht = hematocrit, Hb = hemoglobin concentration, RBC = red blood cell count, MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, WBC = white blood cell count, NBT = phagocyte activity in reduction of NBT dye

greater erythropoietic potential in fish fed natural food. However, the percent of erythroblasts observed in smears was rather low as, in healthy cyprinids, it may reach 5-9% (Wlasow et al., 1990) or even 15-20% (Murad and Houston, 1992).

## Discussion

The red blood parameters show that both groups of fish began to develop anemia immediately after infection and severe anemic conditions occurred within one week after injection. Signs of partial recovery were seen on day 15.

The higher levels in CHI of hematocrit and hemoglobin on day 1 and mean cell hemoglobin on day 8, the slightly greater erythropoietic potential, and the increase in red blood cells on day 15 suggest that fish fed natural food are less susceptible to *Aeromonas*-induced anemia than those fed artificial food. The anemic response to *Aeromonas*-induced ulcerative dermatitis was also observed in rainbow trout (Rehulka, 1998, 2002) while reduced hemoglobin was observed in brook trout infected by *A. salmonicida* (Shieh and Maclean, 1976). There was a similar reduction in hematocrit and hemoglobin, increase in red blood cells, and subsequent erythroblastosis leading to complete recovery of these three values thirty days after infection in common carp intraperitoneally injected with *A. hydrophila* (Harikrishnan et al., 2003).

The reduction of the red blood values cannot be attributed to starvation. Hematocrit and red blood cells in *Hoplias malabaricus* (a neotropical warmwater fish that is more susceptible to starvation-induced changes than carp) remained unchanged after 30, 60, and 90 days of starvation, and low as late as 150 days after starvation (Rios et al., 2002). Likewise, 17 days of starvation did not affect red blood parameters in common carp juveniles (Witeska, unpubl.).

The average white blood cell count was extremely high in SGP, exceeding values typical for this species (Witeska, 2003), but normal in CHI, although the difference was not significant due to very high variability within SGP. The white blood cell count sharply dropped in both groups on day 1, was significantly lower in CHI, and remained low until day 8. Leukopenia probably resulted mainly from the *Aeromonas* infection, but since the leucocrit value in *Coho salmon* is positively related to the food ration (Alcorn et al., 2003), the leukocyte count may have been affected by starvation of the fish during our experiment. Nevertheless, 17 days of starvation did not reduce the white blood count in carp juveniles (Witeska, unpubl.). The white blood cell count recovered on day 15, but did not return to the high pre-injection level in SGP. Leukopenia and a progressive decrease of immunoglobulin was also observed in common carp infected with ulcerative *A. salmonicida*, interpreted by the authors as a state of immune suppression (Evenberg et al., 1986). A similar reduction was reported in carps suffering from *Aeromonas*-induced erythrodermatitis (Stosik, 1989). However, an increase in white blood cells may occur in *Aeromonas*-infected fish (Shariff et al., 2001; Harikrishnan et al., 2003).

Before injection, lymphocytes comprised 97.5-98.5% of the leukocytes in both groups, while on day 1 the percentage significantly decreased, accompanied by the decrease in white blood cell count. Thus, a drop in absolute lymphocyte count took place. *Aeromonas hydrophila* and its exotoxins trigger apoptosis in lymphocytes as demonstrated *in vitro* for isolated crucian carp cells (Shao et al., 2004). It is possible that these cells migrated and concentrated in the ulcers. After infection, the percentage of phagocytes began to increase. On day 1, the proportion of neutrophils significantly increased, and, on day 8, the levels of monocytes were high (the differences were not significant because of very high individual variability; some fish did not develop monocytosis). Leukocyte counts did not deviate from normal values for common carp (Witeska, 2003). Almost all the neutrophils were in juvenile stages (myelocytes and metamyelocytes) and their percentage increased with time. Similar changes were observed in blood and spleen leukocyte populations of *Oncorhynchus mykiss* infected with *A. salmonicida*; the percent of granulocytes rose throughout the period of infection while

the percent of monocytes rose from day 2 to 7 after infection (Kollner and Kotterba, 2002). The number of polymorphonuclear leucocytes increased in *Aeromonas*-infected carps (Stosik, 1989).

Phagocyte activity did not significantly differ between SGP and CHI. After infection, phagocyte activity gradually decreased to very low values on day 1 in SGP and on day 8 in CHI. This drop may have been related to rejuvenation of the blood phagocyte population. On the other hand, the later decrease of phagocyte activity in CHI may indicate that fish fed natural food are less susceptible to the immunosuppressive action of *Aeromonas*, supported by their slightly higher survival and faster recovery. In *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, leukocytes immunized *ex vivo* with *A. hydrophila* increased production of reactive oxygen species, indicating phagocyte activation (Basheera et al., 2002), although there was no significant change in phagocyte activity of *Aeromonas*-infected brook trout (*Salvelinus fontinalis*; Dautrempuits et al., 2006).

The changes in the white blood cell system show that SGP induced considerable leukocytosis in healthy fish which, however, did not enhance their immune response when infected with strain K144 *Aeromonas veronii* bt. *sobria*. Despite slightly better performance of the fish fed natural food (CHI), results show that diet did not significantly affect the ability of fish to overcome the disease, indicating that SGP 493 meets the dietary requirements of common carp juveniles reared under hatchery conditions.

## References

**Alcorn S.W., Pascho R.J., Murray A.L. and K.D. Shearer**, 2003. Effects of ration level on immune functions in chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, 217: 529-545.

**Amar E.C., Kiron V., Satoh S. and T. Watanabe,** 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. *Fish Shellfish Immunol*., 16:527-537.

**Austin B., Altwegg M., Gosling P.J. and S. Joseph,** 1996. *The Genus Aeromonas*. John Wiley & Sons, Chichester, U.K.

**Bagni M., Romano N., Finoia M.G., Abelli L., Scapigliati G., Tiscar P.G. and G. Marino,** 2005. Short- and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). *Fish Shellfish Immunol.*, 18:311-325.

**Basheera J.M., Chandran M.R., Aruna B.V. and K. Anbarasu,** 2002. Production of superoxide anion by head-kidney leukocytes of Indian major carps immunised with bacterins of *Aeromonas hydrophila*. *Fish Shellfish Immunol.*, 12:201-207.

**Clerton P., Trotaud D., Verlhac V., Gabaudan J. and P. Deschaux,** 2002. Dietary vitamin E and rainbow trout (*Oncorhynchus mykiss*) phagocyte functions: effect on gut and on head kidney leukocytes. *Fish Shellfish Immunol*., 11:1-13.

**Cuesta A., Rodriguez A., Esteban M.A. and J. Meseguer,** 2005. *In vivo* effects of propolis, a honeybee product, on gilthead seabream innate immune responses. *Fish Shellfish Immunol*., 18:71-80.

**Daniels H.V. and M.L. Gallagher,** 2000. Effect of dietary protein level on growth and blood parameters in summer flounder, *Paralichthys dentatus*. *J. Appl. Aquac*., 10:45-52.

**Dautrempuits C., Fortier M., Croisetiere S., Belhumeur P. and M. Fournier,** 2006. Modulation of juvenile brook trout (*Salvelinus fontinalis*) cellular immune system after *Aeromonas salmonicida* challenge. *Vet. Immunol. Immunopathol*., 110:27-36.

**Evenberg D., de Graaff P., Fleuren W. and W.B. van Muiswinkel,** 1986. Blood changes in carp (*Cyprinus carpio*) induced by ulcerative *Aeromonas salmonicida* infections. *Vet. Immunol. Immunopathol*., 12:321-330.

**Gatta P.P., Thompson K.D., Smullen R., Piva A., Testi S. and A. Adams,** 2001. Dietary organic chromium supplementation and its effect on the immune response of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol*., 11:371-382.

**Harikrishnan R., Nisha Rani M. and C. Balasundaram,** 2003. Hematological and

biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture*, 221:41-50.

**Irianto A. and B. Austin,** 2002. Use of probiotics to control furunculosis in rainbow trout *Oncorhynchus mykiss* Walbaum. *J. Fish Dis*., 25:333-342.

**Jara Z. and A. Chodyniecki,** 1999. *Ichthyopathology.* Agric. Univ. Wroclaw, Poland. 478 pp.

**Kaminski R., Korwin-Kossakowski M., Kusznierz J., Myszkowski L. and J. Wolnicki,** 2005. Response of a juvenile cyprinid, lake minnow *Eupallasella perenurus* (Pallas), to different diets. *Aquac. Int*., 13:479-486.

**Kamler E., Myszkowski L., Kaminski R., Korwin-Kossakowski M. and J. Wolnicki,** 2006. Does overfeeding affect juvenile tench *Tinca tinca* (L.)? *Aquac. Int*., 14:99-111.

**Khardori, N. and V. Fainstein,** 1988. *Aeromonas* and *Plesiomonas* as etiological agent. *Annu. Rev. Microbiol.*, 42:395-419.

**Kollner B. and G. Kotterba,** 2002. Temperature-dependent activation of leukocyte populations of rainbow trout, *Oncorhynchus mykiss*, after intraperitoneal immunisation with *Aeromonas salmonicida. Fish Shellfish Immunol*., 12:35-48.

**Kozinska A., Figueras M.J., Chacon M.R. and L. Soler,** 2002. Phenotypic characteristics and pathogenicity of *Aeromonas* genomospecies isolated from common carp (*Cyprinus carpio* L.). *J. Appl. Microbiol.*, 93:1034-1041.

**Montero D., Socorro J., Tort L., Caballero M.J., Robaina L.E., Vergara J.M. and M.S. Izquierdo**, 2004. Glomerulonephritis and immunosuppression associated with dietary essential fatty acid deficiency in gilthead seabream, *Sparus aurata* L., juveniles. *J. Fish Dis*., 27:297-306.

**Murad A. and A.H. Houston**, 1992. Maturation of the goldfish (*Carassius auratus*) erythrocyte. *Comp. Biochem. Physiol*., 102A: 107-110.

**Myszkowski L., Kaminski R., Quiros M., Stanny L.A. and J. Wolnicki,** 2002. Dry diet-influenced growth, size distribution, condition coefficient and body deformities in juvenile crucian carp *Carassius carassius* L. reared under controlled conditions. *Arch. Pol. Fish*., 10:51-61.

**Peddie S., Zou J. and C. Secombes,** 2002. Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. *Vet. Immunol. Immunopathol*., 86:101-113.

**Rehulka J.,** 1998. The blood indices of the rainbow trout, *Oncorhynchus mykiss* (Walbaum) in *Aeromonas*-induced ulcerous dermatitis. *Acta Vet. Brno*, 67:317-322.

**Rehulka J.,** 2002. Aeromonas causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology and biochemistry. *Acta Vet. Brno*, 71:351-360.

**Rios F.S., Kalinin A.L. and F.T. Rantin,** 2002. The effects of long-term food deprivation on respiration and haematology of the neotropical fish *Hoplias malabaricus*. *J. Fish Biol*., 61:85-95.

**Sahoo P.K.,** 2003. Immunostimulating effect of triiodothyronine: dietary administration of triiodothyronine in rohu (*Labeo rohita*) enhances immunity and resistance to *Aeromonas hydrophila* infection. *J. Appl. Ichthyol*., 19:118-122.

**Sahoo P.K. and S.C. Mukherjee,** 1999. Influence of the immunostimulant, chitosan, on immune responses of healthy and cortisol-treated rohu (*Labeo rohita*). *J. Aqua. Trop*., 14:209-215.

**Sahoo P.K. and S.C. Mukherjee,** 2002. Influence of high dietary tocopherol intakes on specific immune response, nonspecific resistance factors and disease resistance of healthy and aflatoxin B-induced immunocompromised Indian major carp, *Labeo rohita* (Hamilton). *Aquac. Nutr.*, 8:159-167.

**Sahoo P.K. and S.C. Mukherjee,** 2003. Immunomodulation by dietary vitamin C in healthy and aflatoxin B-induced immunocompromised rohu (*Labeo rohita*). *Comp. Immunol. Microbiol. Infect. Dis*., 26:65-76.

**Sakai M., Taniguchi K., Mamoto K., Ogawa H. and M. Tabata,** 2001. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio* L. *J. Fish Dis*., 24: 433-438.

**Salinas I., Cuesta A., Esteban M.A. and J. Meseguer,** 2005. Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. *Fish Shellfish Immunol*., 19:67-77.

**Sealey W.M. and D.M. Gatlin,** 1999. Overview of nutritional strategies affecting health of marine fish. *J. Appl. Aquac*., 9:11-26.

**Shao L., Liu J. and L. Xiang,** 2004. *Aeromonas hydrophila* induces apoptosis in *Carassius auratus* lymphocytes *in vitro*. *Aquaculture*, 229:11-23.

**Shariff M., Jayawardena P.A.H.L., Yusoff F.M. and R. Subasinghe,** 2001. Immunological parameters of Javanese carp *Puntius gonionotus* (Bleeker) exposed to copper and challenged with *Aeromonas hydrophila*. *Fish Shellfish Immunol*., 11:281-291.

**Shieh H.S. and J.R. Maclean,** 1976. Blood changes in brook trout induced by infection with *Aeromonas salmonicida*. *Wildl. Dis*., 12: 77-82.

**Siwicki A. K., Fuller J.C., Nissen S., Ostaszewski P. and M. Studnicka,** 2000. *In vitro* effects of beta-hydroxy-beta-methylbutyrate (HMB) on cell-mediated immunity in fish. *Vet. Immunol. Immunopathol.*, 76:191-197.

**Siwicki A.K., Zakes Z., Fuller J.C., Nissen S., Trapkowska S., Glabski E., Kazun K. and E. Terech-Majewska,** 2005. The effect of feeding the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) on cell-mediated immunity and protection against *Yersinia ruckeri* in pike-perch (*Sander lucioperca*). *Aquac. Res*., 36:16-21.

**Sokal R.R. and J.F. Rohlf,** 1969. *Biometry. The Principles and Practice of Statistics in Biological Research.* H.F. Freeman and Co., San Francisco, CA, USA. 776 pp.

**Sopinska A.,** 1985. Przydatnosc testu NBT do badan aktywnosci metabolicznej granulocytow krwi obwodowej karpi. *Med. Wet.*, 41:738-740 (in Polish).

**Stosik M.,** 1989. Aktywnosc metaboliczna neutrofili u karpi zdrowych i chorych na erythrodermatitis. *Rocz. Nauk Rol*., 102 H:77-81 (in Polish).

**Sychlowy A. and A. Lukas,** 1978. Ocena mikroilosciowej metody redukcji NBT przez granulocyty krwi obwodowej. *Pol. Tyg. Lek*., 33:45-47 (in Polish).

**Verlhac V., Obach A., Gabaudan J., Schuep W. and R. Hole**, 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol*., 8:409-424.

**Witeska M.,** 2003. Wplyw metali (Pb, Cu, Cd i Zn) na parametry hematologiczne i morfologie komrek krwi karpia. [The effects of heavy metals (Pb, Cu, Cd and Zn) on haematological parameters and blood cell morphology of common carp.] Rozprawa naukowa nr 72, Wydawnictwo Akademii Podlaskiej, Siedlce. 113 pp. (in Polish).

**Wlasow T., Dabrowska H. and E. Ziomek,** 1990. Hematology of carp in prolonged sublethal ammonia intoxication. *Pol. Arch. Hydrobiol*., 37:429-438.

**Wolnicki J., Myszkowski L., Korwin-Kossakowski M., Kaminski R. and L.A. Stanny,** 2006. Effects of different diets on juvenile tench, *Tinca tinca* (L.), reared under controlled conditions. *Aquac. Int*., 14:89-98.