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Original article

Dietary supplementation with *Lactobacillus plantarum* and β-glucan affects immune parameters in the tench (*Tinca tinca*) fry

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Abstract

The aim of the experiment was to examine the effect of a diet enriched with *Lactobacillus plantarum* and/or β-glucan on the immune parameters in the juvenile tench (*Tinca tinca*). Fish were fed for 14 days different diets (phase 1 of the experiment), a dry commercial starter feed in the control group or the same feed supplemented with: 1% β-1,3/1,6-glucan in group G, 10^8 cfu *L. plantarum* g^{-1} in group L, 1% β-1,3/1,6-glucan + 10^8 cfu *L. plantarum* g^{-1} in group G+L. During consecutive 14 days all fish were fed the commercial feed alone (phase 2). The stimulating effects of the tested preparations was evaluated twice, at the end of each experimental phase. Dietary supplementation of β-1,3/1,6-glucan considerably improved the humoral innate immune response (activity of lysozyme and total Ig) and the pinocytotic activity of phagocytes. Supplement of L. plantarum improved the ability of the head kidney phagocytes (RBA) to carry out oxygen burst in L and G+L groups. A similar effect was observed for the killing activity of phagocytes (PKA) from the head kidney after the stimulation of A. hydrophila, and the effect persisted for two weeks after the commercial feed regime was resumed. A significant increase in the proliferative activity of B lymphocytes originating from the head kidney was observed in groups L and G+L. The study has revealed that the addition of the tested G+L synbiotic to dry diet stimulates the innate immune response mechanisms in the juvenile tench.

Key words: aquaculture, synbiotic, Lactobacillus plantarum, innate immunity, tench fry

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Introduction

Aquaculture is one of the most dynamically developing branches in animal production. The growing interest in fish and fish products leads to a wider range of fish-based food available. The underlying reason is the promotion of healthy lifestyles and fish are considered to be pro-health food. Aquaculture currently plays as well an important role to provide fish for restocking, sport fishing and conservation of endangered species. Increasing market demands for live fish and fish products effect on intensification of aquaculture production. However, more intense production of fish entails a risk of more intense spread of diseases, especially bacterial ones. Administration of antibiotics is not indifferent to aquatic animals and to humans, in addition to which it leads to the emergence of antimicrobial-resistant pathogens. Moreover, some antibiotics can produce an immunosuppressive affect. Antibiotic resistance of bacteria has turned to be a genuine and increasingly widespread threat. One of the effective prophylactic methods is vaccination, which, however, can be stressful to fish as well as being costly and labour-intensive. Furthermore, due to the complex antigen structure, a single vaccine can be effective only against a specific type of pathogen (Lazado et al. 2015). Thus, it is necessary to search for other, alternative means that will successfully inhibit the growth of pathogenic bacteria, improve the health of fish and not cause a negative influence on the environment. Currently, prebiotics, probiotics and synbiotics, which meet the above expectations, draw much attention.

Tench is a commonly cultured fish from the family of Cyprinidae, highly valued for its palatability and for sport fishing. Currently, this species can be found on all continents except the Antarctic.

The objective of the research was to compare the effect of supplementation of a commercial feed with the selected probiotic (*Lactobacillus plantarum*) and/or prebiotic (β-glucan) on the selected immune parameters in tench fry.

Materials and Methods

The research was performed in compliance with the Polish animal welfare regulations and approved by the Local Ethics Committee for Animal Experimentation of The Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland.

Experimental fish

Tench larvae were pooled progeny of numerous spawners obtained from a commercial fish farm. For 10 months, the fish were raised under controlled conditions in order to prepare for the experiment. On the first day of the experiment, the fish mean (±SD) body weight (BW) and total length (TL) were 3.30±0.93 g and 6.63±0.56 cm, respectively.

Experimental conditions

The fish were stocked randomly into twelve 40 l flow-through glass aquaria. Initial stocking density was 20 fish per aquarium. Aquaria were continuously supplied with filtered, heated and aerated water from a recirculating aquaculture system (RAS) at approximately 0.4 l min^{-1}. Water temperature was 25.1±0.3 °C. The water in aquaria was continuously aerated by airstones maintaning oxygen concentration above 80% of saturation. Other water-quality parameters were monitored weekly in one aquarium per group. Total ammonia was (mean±SD) 0.20±0.06 mg l^{-1}, nitrites 0.08±0.04 mg l^{-1}, conductivity 542±28 µS cm^{-1} and pH 8.0±0.2. Aquaria were illuminated from 08:00 to 21:00 by fluorescent tubes with the intensity of about 700 lx at the water surface.

Experimental design

Fish were randomly divided into four equal groups, in three replicates (n = 3): the control group (C) and three experimental groups (G, L and G+L). The study involved two phases.

In phase 1 of the experiment, fish were fed four different diets. In the control group, fish were fed dry commercial starter feed Aller Performa 2 (Aller Aqua, Denmark). Dry feed proximate composition was (mean±SD): moisture 6.4±0.0%, ash 9.87±0.01, crude protein 54.1±0.3, total lipids 14.1±0.1. Chemical analysis were performed according to the method described by Kamiński et al. (2017). The other experimental fish groups were fed the commercial starter feed supplemented with: 1% β-1,3/1,6-glucan with a molecular mass of 100-200 kDa (Leiber® Beta-S) (G group), 10^8 cfu *L. plantarum* g^{-1} (L group), 1% β-1,3/1,6-glucan + 10^8 cfu *L. plantarum* g^{-1} (G+L group). The experiment involved five strains of *L. plantarum* obtained from the collection of strains of the Department of Molecular Biochemistry of the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw, Poland (Kazuń et al. 2018a). Probiotic-supplemented diet was prepared according to Kazuń et al. (2018b). The probiotic mixture was mixed thoroughly with 50 g of commercial feed to achieve a dose of ~10^8 cells g^{-1} of feed. Prebiotic and synbiotic supplemented diets were prepared according to Kazuń et al. (2020). The modified feed was stored in screw-top glass bottles at room temperature until required. To ensure high probiotic

level in the supplemented feed (Irianto and Austin 2002, Das et al. 2013), fresh diets were prepared on weekly basis. Initially, the daily food ration was 1.8 g per aquarium. Feed was given manually at 08:00, 14:00 and 20:00 in equal portions.

In phase 2 of the experiment, all fish were fed exclusively the commercial feed without supplementation. Due to the reduction of fish stocks, the daily feed ration was reduced to 0.9 g per aquarium.

Sample collection

At the end of phase 1 of the experiment, all fish were anaesthetized and measurements of their individual BW and TL were performed. From each experimental aquarium, 10 fish were taken randomly, euthanized by immersion with an overdose (50 mg l^{-1}) of unbuffered tricaine methanesulfonate solution (MS-222, Sigma-Aldrich) and fish blood, liver, spleen, head kidney and intestinal tract samples were taken (uptake 1). Blood was collected from the caudal vein and transferred to Eppendorf tubes. Following centrifugation (2,000 g, 10 min, 4 °C), serum was collected and stored at -20 °C until use. At the end of the experiment, all the remaining fish were euthanized as described above, individual BW and TL were determined and then the samples of tissues were taken (uptake 2).

Evaluation of non-specific humoral immunity parameters

The plasma lysozyme activity was measured in a turbidimetric assay described by Siwicki and Anderson (1993). The plasma ceruloplasmin activity was determined according to the method developed by Siwicki and Studnicka (1986) and modified for micro-methods. Total serum immunoglobulin levels were also measured using the Lowry micro-method modified by Siwicki and Anderson (1993). Total serum protein levels were determined with the specrophotometric micro-method proposed by Lowry et al. (1951) and modified by Siwicki and Anderson (1993).

Isolation of tench immune cells

Tench head kidneys and spleens were pooled within the control and experimental groups (organs from 10 individuals kept in the same aquarium). Organ immune cells were isolated using Histopaque 1077 (Sigma-Aldrich) density gradient centrifugation, suspended at a concentration of $1x10^6$ cells ml^{-1} in RPMI-1640 medium supplemented with 10% fetal calf serum and 1% antibiotic-antimycotic solution (both reagents from Sigma-Aldrich) and cultured/incubated as described before (Kazuń et al. 2020). Isolated cells were then used for assays of pinocytosis, respiratory burst activity, potential killing activity and proliferative response of lymphocytes. Samples obtained from each pool were tested in duplicate.

Pinocytosis assay – neutral red uptake (NRU) assay

The pinocytosis assay was performed using a commercially available kit from Sigma-Aldrich (TOX-4), as described earlier (Kazuń et al. 2018b). Briefly, after the removal of non-adherent immune cells (lymphocytes), the adherent cells (phagocytes) were incubated in fresh medium containing 0.033% of neutral red for 3 h at 22°C in order to allow pinocytosis. After cell washing, the solubilisation solution was added to each sample and the absorbance was measured at a wavelength of 540 nm with 690 nm as a reference wavelength using the Sunrise Absorbance Reader (Tecan, Austria). The values were compared to the optical density (OD) of baseline neutral red solution (without cells) and expressed as a percentage of ingested dye.

Respiratory burst activity (RBA) and potential killing activity (PKA) tests

The intracellular respiratory burst and potential killing activities of phagocytes were determined as described before (Kazuń et al. 2018b). In short, the adherent immune cells were incubated in fresh medium containing 0.1% NBT (nitroblue tetrazolium, Sigma-Aldrich) and PMA (phorbol myristate acetate, Sigma-Aldrich, 1 µg ml^{-1}) or *Aeromonas hydrophila* ($1x10^8$ cells ml^{-1}) for 60 min at 22 °C. Once the supernatant was removed, cells were fixed with absolute ethanol and the reduced NBT was extracted using KOH (potassium hydroxide, Chempur, Piekary Śląskie, Poland) and DMSO (dimethylsulfoxide, POCh, Gliwice, Poland). The OD of samples was measured colorimetrically at 620 nm. The results were expressed as a stimulation index (SI), which was calculated by dividing the mean OD of PMA (RBA test) or bacteria-stimulated cells (PKA test) by the OD of control, unstimulated cells.

Proliferative response of lymphocytes – MTT assay

The mitogenic response of tench lymphocytes was determined using the MTT colorimetric assay, as described before (Kazuń et al. 2018b). The head kidney or spleen immune cells were cultured in the presence of mitogens – concanavalin A (ConA) as a T-cell mitogen or lipopolysaccharide from *Escherichia coli* (LPS) as a B-cell mitogen (both mitogens purchased from Sigma-Aldrich and used at concentrations of 50 µg ml^{-1})

Table 1. The humoral-mediated immune parameters in tench fed β-glucan (G), *Lactobacillus plantarum* (L) and β-glucan+*L. plantarum* (G+L) supplemented feed or commercial diet (Control), uptake 1.

Parameter	Control	G	L	G+L
Lysozyme activity in serum (mg l^{-1})	35.8 ± 0.3^{b}	48.6 ± 0.2^{a}	36.4 ± 0.2^{b}	49.7 ± 0.3^{a}
Ceruloplasmin activity in serum (IU)	45.33 ± 0.94^{a}	47.77 ± 0.44^{a}	47.44 ± 0.27^{a}	46.53 ± 0.84^{a}
Total protein level in serum (g l^{-1})	26.69 ± 1.07^{a}	27.33 ± 1.12^{a}	27.07 ± 1.16^{a}	28.09 ± 1.05^{a}
Total γ-globulin level in serum (g l^{-1})	5.42 ± 1.16^{b}	9.82 ± 1.06^{a}	5.94 ± 1.08^{b}	10.05 ± 1.19^{a}

mean±SD, n=3, ^{a,b} - significant differences between marked values at $p<0.05$

Table 2. The humoral-mediated immune parameters in tench fed β-glucan (G), *Lactobacillus plantarum* and β-glucan+*L. plantarum* (G+L) supplemented feed or commercial diet (Control), uptake 2.

Parameter	Control	G	L	G+L
Lysozyme activity in serum (mg l^{-1})	37.01 ± 0.3^{b}	48.9 ± 0.4^{a}	36.2 ± 0.2^{b}	49.7 ± 0.4^{a}
Ceruloplasmin activity in serum (IU)	47.66 ± 0.83^{a}	47.01 ± 0.64^{a}	47.06 ± 0.54^{a}	47.77 ± 0.32^{a}
Total protein level in serum (g l^{-1})	27.15 ± 1.05^{a}	28.19 ± 0.84^{a}	27.45 ± 0.87^{a}	27.87 ± 1.76^{a}
Total γ-globulin level in serum (g l^{-1})	6.19 ± 1.02^{b}	11.13 ± 1.23^{a}	7.89 ± 0.84^{b}	11.85 ± 1.32^{a}

mean±SD, n=3, ^{a,b} - significant differences between marked values at $p<0.05$

for 72 h at 22°C. Control, unstimulated cells were maintained in a medium without mitogens. Following incubation, 10 µl of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) solution (10 mg ml^{-1}) were added to each well and the plate was incubated for the next 3 h. After the removal of the supernatant, the reduced MTT was dissolved in DMSO and the optical density was measured at a wavelength of 570 nm with 640 nm as a reference wavelength. The results were expressed as a stimulation index (SI), which was calculated by dividing the mean OD of the mitogen-stimulated cells by the OD of the control, unstimulated cells.

Statistical analysis

Data were analysed statistically by one-way analysis of variance (ANOVA). The Bonferroni's *post-hoc* test was used to determine differences between the groups ($p<0.05$). Evaluation of the results was performed with the help of a GraphPadPrism software package.

Results

Fish growth

After 14 days of feeding (experimental phase 1) fish mean BW in experimental groups ranged from 3.97 to 4.22 g and TL 6.91-7.03 cm. After consecutive 14 days (phase 2) respective values were 4.76-5.14 g BW and 7.17-7.34 cm TL. No significant difference was found between the experimental groups ($p>0.05$) for BW as well as for TL, neither on the last day of the experimental phase 1 nor on phase 2.

Evaluation of non-specific humoral immunity and biochemical parameters

The serum lysozyme activity and gammaglobulin level were significantly higher ($p<0.05$) in groups G and G+L as compared to the control group and group L in both uptake 1 and uptake 2 (Table 1 and Table 2). The serum ceruloplasmin activity and total protein level did not differ significantly among the experimental groups ($p>0.05$).

Evaluation of non-specific cellular immunity

The pinocytotic activity of the head kidney and spleen phagocytes in both uptakes were highest ($p<0.05$) in groups G and G+L (Table 3 and Table 4).

The respiratory burst activity of the head kidney phagocytes were significantly higher ($p<0.05$) in groups L and G+L in uptake 1 only (Table 3). No significant differences in the respiratory burst activity of spleen phagocytes were observed between the groups (Table 3 and Table 4). The potential killing activity of head kidney phagocytes in both uptakes was significantly higher ($p<0.05$) in groups L and G+L (Table 3 and Table 4). No significant difference was found in the potential killing activity of spleen phagocytes between the control and experimental groups ($p>0.05$).

Table 3. The cell-mediated immune parameters in tench fed β-glucan (G), *Lactobacillus plantarum* (L) and β-glucan+*L. plantarum* (G+L) supplemented feed or commercial diet (Control), uptake 1.

Parameter	Control	G	L	G+L
Pinocytic activity of the head kidney phagocytes (% of ingested NR)	34.66 ± 2.28^{b}	53.26 ± 6.06^{a}	29.37 ± 3.54^{b}	52.79 ± 2.31^{a}
Pinocytic activity of the spleen phagocytes (% of ingested NR)	9.62±1.15^{b}	21.34±4.01^{a}	10.14±2.89^{b}	21.78±1.86^{a}
Respiratory burst activity of spleen phagocytes (SI)	1.04 ± 0.07^{a}	1.21 ± 0.19^{a}	0.91 ± 0.09^{a}	1.21 ± 0.10^{a}
Respiratory burst activity of head kidney phagocytes (SI)	1.29 ± 0.21^{b}	1.11 ± 0.17^{b}	1.83 ± 0.13^{a}	1.83 ± 0.07^{a}
Potential killing activity of spleen phagocytes (SI)	1.24 ± 0.15^{b}	1.10 ± 0.16^{b}	1.18 ± 0.08^{a}	1.23 ± 0.23^{b}
Potential killing activity of head kidney phagocytes (SI)	1.27 ± 0.17^{b}	1.22 ± 0.22^{b}	1.73 ± 0.19^{a}	1.75 ± 0.07^{a}
Proliferative response of head kidney lymphocytes stimulated by ConA (SI)	1.15 ± 0.13^{a}	1.29 ± 0.15^{a}	1.21 ± 0.18^{a}	1.19 ± 0.16^{a}
Proliferative response of spleen lymphocytes stimulated by ConA (SI)	1.29 ± 0.14^{a}	1.53 ± 0.18^{a}	1.32 ± 0.11^{a}	1.29 ± 0.15^{a}
Proliferative response of head kidney lymphocytes stimulated by LPS (SI)	1.21 ± 0.15^{b}	1.35 ± 0.17^{b}	1.69 ± 0.10^{a}	1.70 ± 0.08^{a}
Proliferative response of spleen lymphocytes stimulated by LPS (SI)	1.08 ± 0.12^{a}	1.31 ± 0.19^{a}	1.34 ± 0.13^{a}	1.44 ± 0.13^{a}

mean±SD, n=3, ^{a,b} – significant differences between marked values at $p<0.05$

Proliferative response of lymphocytes – MTT reduction assay

The values of proliferative response of the head kidney and spleen lymphocytes stimulated by ConA did not differ significantly between the control and experimental groups ($p>0.05$) (Tables 3 and 4).

The proliferative response of head kidney lymphocytes stimulated by LPS in uptake 1 was significantly higher ($p<0.05$) in groups L and G+L as compared to the control group and group G (Table 3). However, in uptake 2 all differences were not significant (Table 4). No significant differences were observed in the mitogenic response of spleen lymphocytes to LPS between the groups (Table 3 and Table 4).

Discussion

When making a choice of a prebiotic to add to a well-composed synbiotic preparation, it is advisable to consider its potential use by probiotic bacteria as a source of carbon, thereby stimulating the growth and activity of thereof as well as improving their implantation in the digestive tract. In turn, the selection of a probiotic should be guided by its positive effects on the host's organism (Kolida and Gibson 2011).

Lactobacillus plantarum applied in our experiment had been previously submitted to tests in order to determine their tolerance to acid and bile as well as antagonisms against pathogens that are dangerous to fish, which is in line with the FAO/WHO (2002) guidelines for evaluation of probiotic organisms (Kazuń et al. 2018a). Five strains of the bacteria *L. plantarum* were used in the present study to achieve high antibacterial effect because feeding fish a feed supplemented with a mixture of bacterial strains has a better effect on the immunological parameters than the application of a single probiotics (Giri et al. 2014, Beck et al. 2015). Rods of the genus *Lactobacillus* have high nutritional requirements and derive metabolic energy from homofermentation or heterofermentation of carbohydrates. Studies have shown that the *L. plantarum* bacteria preferentially metabolize oligosaccharides by phosphotransferase/phospho-glycosyl hydrolase systems (Andersson et al. 2005). Therefore, we tried to design a synbiotic preparation in which selected *L. plantarum* strains would be combined with β-glucan, which is known to support the growth of probiotic bacteria. Many studies have shown that β-glucan is also able to stimulate non-specific elements of the immune system (lysozyme activity, RBA, PKA, superoxide anion production, complement activity, serum bactericidal activity, total immunoglobulin content), IL-1β, IL-6, IL-10 and TNF secretion, as well as the specific humoral resistance (antibody formation) and improve the protection against dangerous fish pathogens (Bagni et al. 2005, Selvaraj et al. 2005, Meena et al. 2013).

Table 4. The cell-mediated immune parameters in tench fed β-glucan (G), *Lactobacillus plantarum* and β-glucan+*L. plantarum* (G+L) supplemented feed or commercial diet (Control), uptake 2.

Parameter	Control	G	L	G+L
Pinocytic activity of the head kidney phagocytes (% of ingested NR)	23.75 ± 3.54^{b}	30.12 ± 1.48^{a}	20.30 ± 3.86^{b}	31.49 ± 1.13^{a}
Pinocytic activity of the spleen phagocytes (% of ingested NR)	11.35 ± 2.31^{c}	16.13 ± 1.26^{b}	13.72 ± 1.85^{c}	21.73 ± 1.21^{a}
Respiratory burst activity of spleen phagocytes (SI)	1.01 ± 0.09^{a}	0.99 ± 0.12^{a}	0.96 ± 0.07^{a}	1.06 ± 0.10^{a}
Respiratory burst activity of head kidney phagocytes (SI)	1.36 ± 0.12^{a}	1.23 ± 0.11^{a}	1.21 ± 0.10^{a}	1.38 ± 0.19^{a}
Potential killing activity of spleen phagocytes (SI)	1.41 ± 0.17^{a}	1.40 ± 0.10^{a}	1.32 ± 0.08^{a}	1.42 ± 0.11^{a}
Potential killing activity of head kidney phagocytes (SI)	1.49 ± 0.12^{b}	1.49 ± 0.12^{b}	1.86 ± 0.14^{a}	1.82 ± 0.13^{a}
Proliferative response of head kidney lymphocytes stimulated by ConA (SI)	1.30 ± 0.15^{a}	1.35 ± 0.14^{a}	1.32 ± 0.11^{a}	1.26 ± 0.10^{a}
Proliferative response of spleen lymphocytes stimulated by ConA (SI)	1.54 ± 0.11^{a}	1.66 ± 0.17^{a}	1.72 ± 0.12^{a}	1.75 ± 0.10^{a}
Proliferative response of head kidney lymphocytes stimulated by LPS (SI)	1.28 ± 0.14^{a}	1.26 ± 0.15^{a}	1.31 ± 0.11^{a}	1.25 ± 0.14^{a}
Proliferative response of spleen lymphocytes stimulated by LPS (SI)	1.32 ± 0.12^{a}	1.48 ± 0.16^{a}	1.44 ± 0.15^{a}	1.52 ± 0.12^{a}

mean±SD, n=3, ^{a,b} – significant differences between marked values at $p<0.05$

Fish serum contains numerous proteins and peptides (lysozyme, total Ig, complement, lytic components) that form part of the first-line immune defence, which prevents the adherence and colonization by microorganisms (Devi et al. 2019). In the present study that dietary supplementation of β-glucan considerably improved the innate humoral immunological parameters, i.e. activity of lysozyme and total Ig in serum, in juvenile tench. This effect persisted for two more weeks after the fish returned to being fed only a commercial feed. An increase in these parameters was also confirmed in our earlier study on roach fed a feed supplemented with β-glucan (Kazuń et al. 2020). Similar effects have been observed on other freshwater and marine fish species fed a feed with added β-glucan in different feeding strategies. Examples of tested fish species include Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), catla (*Gibelion catla*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), North African catfish (*Clarias gariepinus*), channel catfish (*Ictalurus punctatus*), Nile tilapia (*Oreochromis niloticus*), European seabass (*Dicentrarchus labrax*), silver seabream (*Pagrus auratus)*, Atlantic cod (*Gadus morhua)*, Japanese amberjack (*Seriola quinqueradiata*) (Bagni et al. 2005, Meena et al. 2013). In present study differences among the control and experimental groups for the serum ceruloplasmin activity or protein levels were not significant. The results of the ceruloplasmin activity suggested that β-glucan and *L. plantarum* had no negative influence on the hepatocytes and did not induce acute phase proteins, which are a heterogeneous group of serum proteins synthesized in the liver (Kazuń et al. 2020).

Pinocytosis is a non-specific pathway of intracellular ingestion, where cells ingest substances soluble in the extracellular fluid. In our study, we observed more intensive pinocytic activity of phagocytes of the head kidney and spleen after the fish had been fed a feed with added β-glucan for two weeks, and this effect persisted after the fish returned to being fed a commercial diet, which corresponds with the results of earlier experiments on the rainbow trout and roach (Verlhac et al. 1998, Kazuń et al. 2020).

Probiotics affect also the cellular mechanisms of an organism's innate immunity by stimulating the activity of phagocytic cells (monocytes/macrophages and neutrophils), which are the first-line cellular defence of an organism and play an extremely important role in the control of fish infections. PBA and PKA tests, which evaluate the non-specific cellular immunity in fish, are good indicators of the health and immune status (Ringo et al. 2018). Our study showed that the supplementation of a feed with *L. plantarum* improved the ability of the head kidney phagocytes to carry out oxygen burst. These research results agree with our earlier findings from a study into possible use of these strains in the nutrition of roach and carp (Kazuń et. al. 2018b, 2020) and with data reported by other researches for different fish species (Son et al. 2009, Giri et al. 2014, Beck et al. 2015, Van

Doan et al. 2016). A similar tendency has been observed for the killing activity of phagocytes. Higher PKA implicates a higher level of antibacterial protection in fish fed a feed supplemented with tested strains of bacteria, that persisted for two more weeks after the fish returned to being fed a commercial feed. Our results coincide with data reported by other researchers thus far (Newaj-Fyzul et al. 2007, Son et al. 2009, Sharifuzzaman and Austin 2010, Geng et al. 2011, Kazuń et al. 2018b).

We also investigated the effect of the administration of a prebiotic, probiotic and synbiotic on the mitogenic response of lymphocytes. A significant rise in the proliferative activity of B lymphocytes stimulated by the bacterial LPS determined in this experiment was achieved also in carp fry fed a feed enriched with *L. plantarum* (Kazuń et al. 2018b). The proliferative effect of *L. plantarum* strains on murine spleen lymphocytes has been also described by Lee et al. (2011) and by Ren et al. (2015). Although the application of *L. plantarum* in aquaculture has been studied very extensively, there are few publications concerning their effect on the adaptative immunity in fish. Most studies have focused on possibilities of producing several cytokines and chemokines such as TNF-a, interleukins (IL-6, IL-10, IL-12) and IFN-c (Banerjee and Ray 2017). B cells are a key element of the humoral acquired immunity and their main role is to produce antibodies with high affinity to the foreign antigen and acting as professional antigen presenting cell (pAPC), presenting transformed antigens to T cells in order to activate them. Hence, the effect observed in our study can be indirect evidence in favour of the stimulating influence of *L. plantarum* not only on the non-specific but also on the specific antibacterial response in tench.

The objective of the present study was to test *Lactobacillus plantarum* and β-glucan dietary supplementation in order to find an effective and inexpensive way to stimulate mechanisms of the immune system in juvenile tench. Although the results obtained thus far are promising, more research is needed to assess the application of a synbiotic diet in different feeding strategies. Moreover, it will be helpful to focus on profiling studies dedicated to possibilities of modifying the composition of a synbiotic depending on the fish rearing system, water temperature, health of fish and their age.

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