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The effect of oyster mushroom β-1.3/1.6-D-glucan and oxytetracycline antibiotic on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in common carp (*Cyprinus carpio* L.)

Radka Dobšíková [a, *], Jana Blahová [a], Ivana Mikulíková [a], Helena Modrá [a], Eva Prášková [a], Zdeňka Svobodová [a], Mišo Škorič [b], Jiří Jarkovský [c], Andrzej-Krzysztof Siwicki [d]

[a] *Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, 612 42 Brno, Czech Republic*
[b] *Department of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, 612 42 Brno, Czech Republic*
[c] *Institute of Biostatistics and Analyses, Research Centre for Toxic Compounds in the Environment (RECETOX), Kamenice 126/3, 625 00 Brno, Czech Republic*
[d] *Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego St. 13, 10-719 Olsztyn, Poland*

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ABSTRACT

The aim of the study was to evaluate the effect of micronized β-1.3/1.6-D-glucan (BG) derived from the oyster mushroom *Pleurotus ostreatus* Hiratake and tetracycline antibiotic oxytetracycline (OTC) on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in tissues of one- to two-year-old common carp (*Cyprinus carpio* L.). The fish tested were divided into five experimental groups and one control. Carp in the control group were fed commercial carp feed pellets. Fish in the five experimental groups were fed the same pellets supplemented with either OTC, a combination of OTC and BG, or BG as follows: 75 mg oxytetracycline kg^{-1} bw (OTC group), 75 mg oxytetracycline kg^{-1} bw and 0.5% β-glucan (OTC + 0.5% BG group), 75 mg oxytetracycline kg^{-1} bw and 2.0% β-glucan (OTC + 2.0% BG group), 0.5% β-glucan (0.5% BG group), and 2.0% β-glucan (2.0% BG group). OTC- and BG-supplemented diets and the control diet were administered to experimental and control carp for 50 days (i.e. samplings 1–3, the exposure period); for the following 14 days, fish were fed only control feed pellets with no OTC or BG supplementation (i.e. sampling 4, the recovery period). Blood and tissue samples were collected both during, and at the end of the study. No significant changes in biometrical indices (i.e. total length, standard length, total weight, hepatosomatic and spleen somatic index, and Fulton's condition factor) were found in experimental carp compared to control in any sampling. In haematological indices, significant changes were found only in sampling 2, in which shifts in PCV ($P < 0.01$), Hb ($P < 0.01$), and WBC ($P < 0.01$), and in the counts of lymphocytes ($P < 0.01$), monocytes ($P < 0.01$), and neutrophil granulocytes-segments ($P < 0.05$) were revealed. As for biochemical profiling, plasma concentrations of glucose, albumins, cholesterol, natrium, and chlorides (all $P < 0.01$), and total proteins, lactate, phosphorus, and potassium (all $P < 0.05$) as well as the catalytic activity of ALP ($P < 0.05$) were altered in common carp. A significant change in induced (opsonizedzymosan particles, OZP) chemiluminescence ($P < 0.05$) in sampling 3 and no shifts in serum immunoglobulins concentration were found in the immunological analysis. Histopathological examination of skin, gills, liver, spleen, and cranial and caudal kidneys revealed no obvious specific changes in any tissue analysed. The use of β-glucans in clinically healthy aquaculture remains an issue. Nevertheless, their use in breeding endangered by stress stimuli, infectious disease, or adverse environmental factors is defensible.

1. Introduction

Immunostimulants have been used as feed additives for many years in farm animal husbandry as well as in aquaculture. They

* Corresponding author. Tel.: +420 541 562 784; fax: +420 541 562 790.
E-mail address: dobsikovar@vfu.cz (R. Dobšíková).

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augment innate and adaptive immune responses in fish and are regarded as a promising supplement to vaccination and selective breeding, which remain the key strategies for the prevention of diseases in fish aquaculture [1]. Immunostimulants have been replacing antibiotics in aquatic animal farming practices to control pathogenic organisms which develop antibiotic resistance [2].

β-glucan is one of the most important compounds used in fish culture for strengthening the defence of fish against pathogens [3,4]. β-glucans is the most commonly used term for a heterogeneous group of glucose polysaccharides consisting of a backbone of β-(1.3)-linked β-D-glucopyranosyl units with β-(1.6)-linked side chains of varying length and distribution [5]. They are a major structural component of fungi cell walls and are also found in some bacteria, plants, algae, yeast, and mushrooms. One of the most common sources of β-glucans is derived from the cell wall of baker's yeast *Saccharomyces cerevisiae*. β-glucans are also extracted from the bran of oat, barley, rye, and wheat grains, and some species of seaweed [6].

β-glucans bind to specific cell surface receptors of macrophages and neutrophilic granulocytes that promote the enhancement of an organism's protective activity against infection and sepsis through the activation of leukocytes, phagocytic activity, and the production of inflammatory cytokines, chemokines, and reactive oxygen free radicals. The stimulation of receptors also increases the activity of antioxidant enzymes and the elimination and killing of microorganisms, and initiates the development of adaptive immunity, all of which contribute to the anti-infective and anti-tumorigenic properties of β-glucans [7].

In fish, β-glucans enhance resistance to bacterial and viral infections by means of the effective stimulation of non-specific cellular and humoural immune functions such as lysozyme and complement activities. β-glucans have a proven protective effect against bacterial fish pathogens including *Aeromonas salmonicida*, *Edwardsiella tarda* [8], and *Vibrio salmonicida* or *Vibrio ruckeri* [9]. The administration of glucans in fish diets has been shown to enhance respiratory burst activity [10,11], phagocytosis [11], and lysozyme levels [12]. Growth promotion induced by the administration of β-glucan is also reported in fish [3].

In addition to individual administration, β-glucans have also been used with bacterial vaccine as an adjuvant [13] or with lipopolysaccharide as synergients [14], so as to increase immune response and protect fish against pathogens.

Tetracycline antibiotics are among the most frequently used drugs in aquaculture. Oxytetracycline (OTC) is a broad-spectrum antibiotic produced by *Streptomyces rimosus*. It is widely used for the treatment of systemic bacterial infections in fish [15]. OTC is cost effective and legally available. This bacteriostatic agent acts as an inhibitor of bacterial proteosynthesis. OTC is the first choice antibiotic for the treatment of all bacterial fish diseases, such as vibriosis, flavobacteriosis, furunculosis, and columnaris [16], as well as erythrodermatitis in carp [17]. It can be administered orally, intravenously, and in the form of a bath.

OTC has various effects on the fish immune system, depending on the species and size of fish, water temperature and the route of drug administration. Negative side effects (e.g. the presence of antibiotic residues in fish, water, and sediments; growth retardation; the selection of resistant bacterial strains; as well as immunosuppression) have been identified [18,19]. Despite these side effects, OTC remains one of the most frequently used antibiotics in aquaculture [20]. The aim of the study was to evaluate the effect of the long-term oral administration of micronized β-1.3/1.6-D-glucan derived from the oyster mushroom (*Pleurotus ostreatus* Hiratake) and the antibiotic oxytetracycline, administered separately and in combination, on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in one- to two-year-old common carp (*Cyprinus carpio* L.).

2. Materials and methods

2.1. Chemicals

Micronized β-1.3/1.6-D-glucan (BG, dry matter 94.4%, nitrogen 1.5%, ash 1.76%, fat 0%, proteins 0%) derived from the oyster mushroom *P. ostreatus* Hiratake and oxytetracycline antibiotic (OTC, oxytetracycline HCl, sulphated ash up to 0.5%, water up to 2.0%) were obtained from Natures (Trnava, Slovakia) and Univit (Uničov, Czech Republic), respectively. The micronization of BG involves grinding it down to 5 μm particles (to allow better absorption from the gastrointestinal tract, as stated by the producer).

2.2. Fish

Common carp (185.5 ± 35.1 g) were obtained from a local fish farm in Pohořelice (Rybníkařství Pohořelice, Czech Republic). Prior to the test, fish were adapted to laboratory conditions in 12 stock tanks (each with a capacity of 200 L) for four weeks. During this period, the fish were fed commercial carp feed pellets in the amount of 1% (first two weeks) and 2% (second two weeks) body weight daily. A commercial carp diet (Carpco Prime EF 6.0 mm, Coppens International bv, Helmond, Holland) consisted of protein (30%), fat (5%), fibre (6.3%), ash (6.3%), phosphorus (0.9%), calcium (0.5%), vitamins A (10.000 IU kg^{-1}), D3 (1.000 IU kg^{-1}), E (200 mg kg^{-1}), and C (stabilized, 150 mg kg^{-1}) and trace elements: Fe ($FeSO_4 \cdot H_2O$, 75 mg kg^{-1}), I ($Ca(IO_3)_2$, 5 mg kg^{-1}), Co ($CoCO_3 \cdot H_2O$, 1 mg kg^{-1}), Cu ($CuSO_4 \cdot 5H_2O$, 5 mg kg^{-1}), Mn (MnO, 20 mg kg^{-1}), Zn ($ZnSO_4 \cdot H_2O$, 80 mg kg^{-1}), and Se (Na_2SeO_3, 0.3 mg kg^{-1}). The feed was made from soya extractable scrap, wheat meal, wheat, sunflower scrap, cod-liver oil, and fish meal.

Average water quality parameters during the acclimatization period were as follows: dissolved oxygen 8.86 ± 0.73 mg L^{-1}, pH 7.72 ± 0.09, and temperature 21.9 ± 1.1 °C.

2.3. Feed preparation

For experimental groups of carp, commercial carp basal diet pellets were crushed and divided into 5 portions. The first portion was supplemented with OTC in the amount of 3.75 g per kg feed (i.e. 75 mg OTC per kg bw per day, which is a standard daily therapeutic dose, OTC group); the second portion was supplemented with OTC and BG at concentrations of 3.75 g and 5.0 g per kg feed, respectively (OTC + 0.5% BG group); the third portion was supplemented with OTC and BG at concentrations of 3.75 g and 20.0 g

Table 1
Carp medicated feed preparation for control and experimental carp.

	Control	OTC	OTC + 0.5% BG	OTC + 2.0% BG	0.5% BG	2.0% BG
OTC (g)	0	26.25	26.25	26.25	0	0
β-glucan (g)	0	0	35.00	140.00	35.00	140.00
Feed (g)	7000.00	6973.75	6938.75	6833.75	6965.00	6860.00
Total (g)	7000.00	7000.00	7000.00	7000.00	7000.00	7000.00