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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effects of different levels of Immunoster as prebiotic on some haematological and immunological parameters in common carp (*Cyprinus carpio*)

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Abstract

This study was conducted to investigate the effects of different levels of prebiotic (Immunoster) on the blood parameters and immune response of the common carp (*Cyprinus carpio*). For this purpose, a total of 300 juvenile common carp (24.9±0.52g) were randomly divided into four groups in triplicates and each group was fed with diets supplemented with prebiotic (Immunoster) at the dose level of 0, 0.5, 1.5 and 2.5 g/kg for eight weeks. At the end of the experiment, the blood parameters including white blood cell Count (WBC), red blood cell count (RBC), hematocrit (PCV), hemoglobin (Hb), globular indexes (MCV; MCH and MCHC) and differential counts of WBC, as well as total protein. The immune responses included antibactericidal and lysozyme activities. The results indicated that in spite of the increase in some of the immunological indices in the fish, fed with the food including Immunoster, there were no significant differences in the blood and immunological indices. It can be concluded that the use of 0.5, 1.5 and 2.5 g/kg of Immunoster had no significant effect on the blood parameters and immune response of the common carp.

Keywords: *Cyprinus carpio*; prebiotic; Immunoster

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Introduction

Aquaculture is expanding in the world (Denev et al., 2009). Over the years, different strategies on the composition of the intestinal bacterial flora have led to improvements in growth, digestion, immune response and resistance against illness and were checked in different hosts, from animals to humans. Manipulation of the intestinal bacterial flora by enriching the ration with useful microbes is a new approach not only from the feeding point of view but also as an alternative treatment method and persistent to overcome the side effects of the anti-biotic and other medications (Nayak, 2010). Among this way, the use of prebiotics as a growth stimulus was noticed during the past few years, based on the explanation by Gibson and colleagues (1998). Prebiotics are indigestible substances stimulate growth and improve health. Based on what said, and food that reaches the intestines such as indigestible carbohydrates, some of the peptides, proteins and some lipids can be candidates for the prebiotics. The common carp (*Cyprinus carpio*) is one of the most important Warm water fish and the culture of this aquatic creature is growing along the world, therefore in the present research work, the effect of different prebiotic Immunoster levels including (19.3% Mannan Oligosaccharides stemmed from the outer wall of the yeast *Saccharomyces cerevisiae* and 22% 1-3- beta-glucan) were tested on the common carp's blood and immune response.

Materials and Methods

A total of 300 juvenile *Cyprinus carpio* (24.9±0.52g) were bought from a commercial farm (Sheyban, Ahwaz, Iran). The animals were acclimatized

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for one week to the experimental conditions and diets. At the start of the experiment, the fishes were weighed after anesthetizing them with 0.01% MS-222 (Sigma, USA). Fish were selected randomly and divided into 12 rearing cubic fibre glass tanks (capacity: 600 L) for the growth trial (40 fish/tank). The fish were hand-fed *ad libitum* three times (08:30, 12:30 and 18:00) daily for eight weeks. During the experiment, water temperature ranged from 26.8 to 29.3°C, pH 6.9–7.2, NH3–N 0.13±0.05 mg/L, dissolved oxygen content was approximately 6.5 mg/L, the photo period used was 12 h light/12 h dark cycle. The mortality was recorded during the experiment.

The prebiotic, Immunoster, used included 19.3% mannan oligosaccharides stemmed from the outer wall of the *saccharomyces cerevisiae yeast* and 22% 1-3-beta-glucan). The mentioned prebiotic was added at the rate of 0.5, 1.5 and 2.5 g/kg of the basic rations to the group 1, 2, 3 and 4 respectively.

After storing the fingerlings of common carp in the mentioned ponds, the fishes were fed with the basic diet for 10 days as an adaptation period. Afterwards, the feeding was started with specific diet. The feeding was done three times in a day. All treatments were fed for 60 days according to the plan. The water quality was kept at a preferred state. During the 60 days, the treatments were sampled randomly and six times per each cycle phlebotomy was done.

Haematological and serum analyses

At the end of 60 days nutrition trial, blood samples (five fish from each treatment) were taken from the caudal vein for haematological and immunological analyses. Blood was sampled by puncturing the caudal vein using a syringe (18 G×1 1/2). Fish were anaesthetised prior to blood sampling. Heparin was used as an anti-coagulant at a concentration of 5,000 IU–heparin sodium salt in 1 ml. Haematological examination was carried out immediately after sampling to assess indices of leukocyte profile. The number of leukocytes (WBC) was determined in blood diluted in Natt–Herrick solution using a Neubauer haemocytometer. WBC number was determined on each blood smear and calculated. The total red blood cell count of the fish was done manually using the Neubauer Haemocytometer lam and the cell count was multiplied by the dilution factor (10000). The total red blood cell per mm^3 of blood was calculated (Thrall, 2004). The cell indexes were calculated via the formula mentioned by Thrall (2004).

MCV (μm^3/cell) = (Packed cell volume as percentage/ RBC in millions cell mm^3)×10

MCH (pg /cell) = (Hb in g/100 ml/RBC in millions cell mm^3) ×10

MCHC (g 100/ml Hct) = (Hb in g100/mL/packed cell volume as percentage) ×100

The total white blood cells were done directly with haemocytometer as described by Thrall (2004) using the following formula:

200 × (10% + TWBC in 9 big squares) = TWBC in a micro liter of blood

For the differential count of white blood cell, briefly, a blood droplet was placed directly on a lam and the smear was prepared. The spreads prepared were stabilized via methyl acid and stained for 20 minutes using the GIEMSA. After staining they were observed by using an oil emersion lens. About 100 white cells were counted and the percentage of each cell was calculated. The haemoglobin (Hb) was determined using the standard Cyanomet Haemoglobin and the haemoglobin amount was calculated in units of grams per dl (Feldman et al., 2000). Packed cell volume (PCV) was done using the micro haematocrit method. The total serum protein level was estimated by the method of Lowry (1951) using the standard protein estimation kit (Zistshimi co, Iran).

Immunological analyses

Lysozyme activity was measured by the method of Ellis (1990). Serum bactericidal activity was measured according to Kajita et al. (1990) with slight modification. Sera samples from each group were diluted three times with 0.1% gelatin-veronal buffer (GVBC2) (pH 7.5, containing 0.5 mM ml^{-1} Mg^{2+} and 0.15 mM ml^{-1} Ca^{2+}). *Aeromona shydrophila* (live, washed cells) was suspended in the same buffer to make a concentration of 1 ×10^5 cfu/ml. The diluted sera and bacteria were mixed at 1:1, incubated for 90 min at 25°C and shaken. The numbers of viable bacteria was then calculated by counting the colonies from the resultant incubated mixture on TSA plates in triplicate after 24 h incubation.

Statistical analysis

All statistical analyses were performed using SPSS 18 software. The average values were compared using the ANOVA and post Hoc Duncan test that proved or disproved the significant difference on the trusted level of P=0.05.

Results and Discussion

The impact of immunoster on hematological parameters is shown in Table 1. There was no significant difference between blood parameters (P>0.05).

In the current research, the effect of prebiotic Immunoster (0.5, 1.5 and 2.5 grams per kilogram of base rations) on the common carp's blood parameters was analyzed and the results indicated that no significant effect was observed on the blood parameter (P>0.05). These results are in agreement with previous studies. Welker et al. (2007) reported no effect of the

mannan oligosaccharides in the canal catfish diet (*Ictalurus panctatus*) on the growth function, globular indexes and immune response. Yuji Sado and Almeida (2008) also found no effect of six levels of mannan oligosaccharides on the blood parameters of Nile Tilapia (*Oreochromis niloticus*). Yosefian et al. (2012) analyzed the effects of adding prebiotic on the growth effectiveness and biochemical indicators of the blood of *Rutilus frisii kutum* and found no effect. On the other hand, Zhu et al. (2012) found a positive effect of yeast polysaccharides at the amount of 0%, 0.1%, 0.2% and 0.3% for 7 weeks on the blood and morphological indicators of the canal catfish (*Ictalurus punctatus*). Akrami et al. (2012) reported that mannan oligosaccharides at the level of 1 gram per kilogram improved blood parameters in the common carp.

Currently, there are few data available on the prebiotic's effect on the blood indicators. The prebiotic type, dose, a wide spectrum of factors such as the aquatic species, size, age, physiological state, environmental factors, the amount of food in basal diet, basal diet type, vitamins and proteins are effective on the blood indicators that lead to differences in the results (Hoseinifar et al., 2011). Also Williams and Warner (1976) reported that environmental factors such as season, salinity, photoperiod, temperature, density, physiological factors in aquatic species, reproductive cycle and pubertal status, age, sex and nutritional conditions, time of sampling, how to sample preparation, measurement accuracy can affect blood's biochemical parameters and lead to a difference in results.

The results of safety factors are shown in Table 2. Although there is a slight increase in some of the fish safety factors due to feeding the food including Immunoster, there were no significant statistical differences between the immune response in analyzed treatments.

Torricelli et al. (2007) analyzed the effect of different oligosaccharides levels among the sea bass fish (*Dicentrachus labrax*) on the intestinal structure and safety factors and the results indicated that the mannan oligosaccharides had no significant effects. Cerezuela et al. (2007) analyzed the effect of prebiotic inulin on the gold head shank's (*Sparus aurata*) cell safety and its results indicated that inulin had no significant effect on the leukocyte's activity. Li et al. (2005) analyzed the effect of grubiotic-A on the growth and safety factors among the hybrid striped bass. The results indicated that the grubiotic-A improved the safety among the hybrid striped bass. Zhou et al. (2010) analyzed the effects of four types of prebiotics including inulin, glucose co-oligosaccharides, mannan oligosaccharides and yeast resulted from glucose co-mannan by the amount of 10 grams per kilogram among the red fish and it was eventually shown that the mentioned prebiotics improve the safety factors. Soleimani et al. (2012) analyzed the glucose co-oligosaccharides on growth and immune response among the Caspian red-eyed white fish and these results indicate that this prebiotic improves the safety factors. Based on studies, contradictory results from the prebiotic effects on the aquatic immune response is because of the difference in their environmental properties such as ambient temperature, fish species, environmental stress, and also, the type and amount of prebiotic (Salze et al., 2008; Ibrahem et al., 2010).

Table 1: Changes in the haematological and serum parameters in *C. carpio* fed with diet containing different levels of prebiotic Immunoster

Blood factors	G1	G2	G3	G4
WBC(10^4/mm^3)	10.00±4.38[a]	11.00±7.07[a]	9.40±4.30[a]	6.55±0.78[a]
RBC(10^6/mm^3)	1.25±0.31[a]	1.40±0.26[a]	1.20±0.28[a]	1.12±0.03[a]
PCV (%)	29.40±6.62[a]	29.60±5.13[a]	31.00±4.00[a]	32.80±3.96[a]
Hb(g/100 ml)	6.04±1.76[a]	6.00±1.43[a]	5.81±1.42[a]	6.15±1.56[a]
MCV (μm^3)*	528.19±113.53[a]	432.58±37.93[a]	548.57±129.30[a]	578.53±72.02[a]
MCH (pg)*	55.41±8.66[a]	51.51±10.74[a]	56.23±3.92[a]	54.23±18.92[a]
MCHC (g per 100 ml)*	20.73±4.45[a]	21.25±8.53[a]	18.90±4.46[a]	19.06±5.69[a]
Total protein (g/dl)	4.58±1.16[a]	3.82±1.31[a]	3.42±0.86[a]	3.44±1.06[a]

*MCV = [PCV (%)/RBC (106)] x 10 μm^3; MCH = [Hb (g)/RBC (10^6)] x 10 pg; MCHC = [Hb (g)/PCV (%)] x 100 g per 100 ml; G 1: Basic diet supplemented with 0.5 g/k Immunoster, G 2: Basic diet supplemented with 1.5 g/k Immunoster, G 3: Basic diet supplemented with 2.5 g/k Immunoster ,G 4 Control: Basic diet supplemented without any additions

Table 2: Changes in the safety factors of *C. carpio* fed with diet containing different levels of prebiotic Immunoster

Safety factors	G1	G2	G3	G4
Lymphocyte (%)	77.50±5.00[a]	78.75±8.54[a]	81.25±8.54[a]	78.00±7.58[a]
Monocyte (%)	7.50±2.89[a]	8.75±4.79[a]	8.75±4.79[a]	9.40±4.67[a]
Heterophil (%)	15.00±4.08[a]	12.50±6.45[a]	10.00±5.77[a]	12.00±4.47[a]
Bactericidal activity (%)	92.60±35.41[a]	89.00±32.71[a]	96.00±19.22[a]	82.00±20.28[a]
Lysozyme (units/ml)	125.00±17.32[a]	121.00±7.41[a]	129.00±23.29[a]	130.00±26.22[a]

G 1: Basic diet supplemented with 0.5 g/k Immunoster, G 2: Basic diet supplemented with 1.5 g/k Immunoster, G 3: Basic diet supplemented with 2.5 g/k Immunoster, G 4 Control: Basic diet supplemented without any additions

Based on overall result, it can be concluded that the current levels of Immunoster had no significant effect on the blood and immune response in common carp.

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