



Effect of Dietary Prebiotic Safmannan and Bio-antibiotic Fluconazole on some Growth and Haemato-immunological Parameters of Common Carp *Cyprinus carpio* Linnaeus

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Abstract: This study was conducted in the laboratories of the College of Agriculture, University of Basrah for 75 days, in order to study the effect of using prebiotic (Safmannan) and bio-antibiotic (Fluconazole) on some growth and haemato-immunological parameters of the common carp *Cyprinus carpio* L. The fishes were divided into four categories of treatments; the first treatment was the control (basal diet), the second and third treatments (T2 and T3) contained 500 and 1000 mg.kg⁻¹ diet of Safmannan, respectively while the fourth treatment consisted of adding 150 mg.100 g⁻¹ diet of bio-antibiotic Fluconazole. The second and third treatments were significantly higher (P<0.05) than control and antibiotic treatments in the growth parameters such as the average of daily weight gain, the average of specific growth and the feed conversion of the second and third treatments. Blood proteins (albumin, globulin and total protein) showed significantly higher (p <0.05) values in the second and third treatments than control and antibiotic treatments. No significant differences were noted in both packed cells volume (PCV) and haemoglobin (Hb) among different treatments. The value of alanine aminotransferase (ALT) showed no significant differences among all treatments, while alkaline phosphatase (ALP) was significantly higher and aspartate transaminase (AST) was significantly lower in the fourth treatment. Hepatosomatic index (HSI) was significantly higher in all treatments compared with the control, while no significant differences in spleen somatic index (SSI) were noted among all treatments. Differential white blood count exhibited no significant differences among the treatments for eosinophils, basophils, lymphocytes and monocytes, while neutrophil count was significantly decreased in all treatments in comparison with the control.

Keywords: Fish, Dietary supplement, Safmannan, Fluconazole, Health, Haematology.

Introduction

Fish resources are considered as one of the main pillars for the productive base in livestock, so the importance of fish aquaculture has grown over the past years due to the great advantages enjoyed by this activity. Common

carp (*Cyprinus carpio* L.) remains the most important group among aquatic organisms, which contributes to 72% of the freshwater production, because it has the ability to withstand the environmental conditions of

temperature and oxygen concentration reduction below 4.5 mg. l⁻¹ (Baldry, 2000; Kühlwein *et al.*, 2014). The expanding of aquaculture, the spread of intensive and semi-intensive culture, and the competition to get higher production in growth averages have led to problems that have accompanied these activities, e.g. the emergence of diseases, often due to increased production, in the limited unit area (Chen *et al.*, 2014). The use of antibiotics and chemotherapy to overcome these problems and diseases have led to the spread of drug-resistant strains of bacteria and fungi and the weakening of the immune system of fishes (Broden *et al.*, 2014; Allameh *et al.*, 2016).

The accumulation of antibiotics in both the environment and fishes possess a potential risk to consumers and the environment (Carrias *et al.*, 2012). Furthermore, due to the various fungal infections, drugs and medicines were used in treating these infections, but there was a significant evolution in resistance of the fungi to antibiotics and the failure of some of these antibiotics in treating most disease cases (Wisplinghof *et al.*, 2014). As a result, many researchers have recently turned their attention to the use of dietary supplements in addition to remedies and vaccines, where an alternative method was found to enhance the resistance of pathogens, immune responses and other health benefits, namely the addition of probiotic and prebiotic, which have a variety of traits to enhance the health that improve fish immunity and increase the nutritional value of the diet. It stimulates growth, immune system development, improves feed digestion as well as other health and productivity benefits (Tuan, 2013; Bandyopadhyay *et al.*, 2015). This study aims to test the effect of adding prebiotic (Safmannan) and bio-antibiotic (Fluconazole),

in fish diets, on some growth and hematoimmunological criteria of the common carp.

Materials & Methods

Fishes of the experiment and water quality

A total of 130 common carp fries (10.8-11.5 g in weight) were used. The fishes were sterilized with a saturated salt solution (NaCl) for approximately 35-40 seconds (Herwig, 1979) to get rid of any external disease agents. The fishes were randomly distributed into 13 glass aquariums, acclimatized for 14 days in pre-prepared glass aquariums for research measures (30 × 40 × 60 cm), and filled by 50-litres of aged tap water. The fishes were fed the control diet during the period of acclimatization, which was manufactured in the laboratory (table 1). The temperature was measured by a mercury thermometer with a gradient of 0-100 °C. The dissolved oxygen concentration was measured by using the modified Winkler's method and pH by using pH-009-1-A Meter while a WTW electrical conductivity device was used to measure water salinity.

Fish-diet formulation

Table (1) explained the percentages of different diet ingredients used in control diet. Each of feed item was grinded alone by laboratory mill (Hinduston-MFG-Co, England) and after mixing all of the ingredients for homogeneity, the diet put in Rapid Content Analyser to determine the chemical composition of the diet as shown in table (2). The contents of French commercial prebiotic (Safmannan) was shown in table (3). Antifungal fluconazole (=Forcan-150, Indian origin) was added to the control diet to form the fourth treatment (T4). Four experimental treatments were formed including

basal (control) treatment. The control (T1) with two replicates, antibiotic with three and the rest (T2 and T3 of safmannan) with four replicates each. In each replicate, ten fishes were used. The homogeneity of the components for each diet was taken into account when the diet was prepared (Lovell, 1989). Hot water was gradually added up to 600 ml.kg diet⁻¹, then manually mixed and the mixture temperature

was raised to 75°C. The mixture was then left to cool down and then the additives from the fluconazole or safmannan were added and then mixed well with ingredients until the formation of a dried paste, which was entered into a 50 ml plastic syringe to form yarns with a size of 5 mm. The yarns of the four treatments were spread in the laboratory and exposed to air with constant flux until dry.

Table (1): The proportions of the calculated diet components as a dry matter.

Diet item	Percentage
Flour	25
Soybean meal	25
Fish meal	25
Yellow corn	10
Black barley	10
Fine flour bran	5
Total	100

Table (2): Chemical contents of the basal diet by Rapid Content Analyser.

Component	Percentage
Humidity	7.95
Crud protein	31.67
Ether extract	3.09
Ash	7.60
Crud fibre	1.64
Nitrogen free extract (NFE)	48.05

Table (3): The composition of prebiotic Safmannan.

Component	Percentage
Humidity	6
Crud protein	17.5
Ether extract	20
β-Glucan	20
Mannan oligosaccharide	20

Each diet was placed after drying in a labelled nylon bag and kept in refrigerator at a temperature of -4 °C until use. The fishes were starved for two days and then fed on the control diet by 3% of diet per body weight for two weeks. The experiment lasted for 75 days exclusive of the acclimation period (14 days). Water was ventilated throughout the experiment by using air pumps, and 20-25% of the water of aquarium were exchanged daily with new amount that was left in a stock tank for a period of 24 hours. Clips wire was used to cover the aquarium to prevent fishes from escaping outside. After finishing the acclimation period, fishes were fed experimental diet two time a day at 8 am and 2 pm. Feeding was adjusted after measuring the weight of the fishes every 15 days to monitor growth using a Metler PE600 sensitive balance.

Growth parameters

Specific Growth Rate (S.G.R), Feed Conversion Ratio (FCR) and Daily Weight Gain (DWG) were calculated according to Hephher (1988).

Blood proteins

Serum albumin, total protein and cholesterol were calculated by using special kit from German company "Human" and measured at a wavelength of 570 nm, 580 nm and 500 nm, respectively by using the Polish

Haemato-immunological analyses

Fishes were fasted for 24 hours prior to blood sampling. The blood was withdrawn from different groups of fishes after cutting the caudal peduncle. Each fishes was caught in a way where the head was to the top which helped to flow the blood by fine massage on the caudal peduncle and filled the capillary tubes which contained the anticoagulant, where one of the ends was closed with artificial clay (Plastine), then the capillary tubes were placed in the microhaematocrit centrifuge to separate the serum from the plasma at 3000 rpm for two minutes (Yang & Chen, 2003). The serum was then kept in glass tube and placed in a freezer until the laboratory tests, where it was measured.

Packed cell volume (PCV)

It was read by a microhaematocrit reader and represents the packed cell volume per 100 ml of blood.

Haemoglobin (Hb)

Haemoglobin was directly calculated by the packed cell volume, using the equation mentioned by Campbell (1995) which is:

$$\text{Haemoglobin (Hb)} = \frac{\text{Packed cell volume} - 1}{3}$$

spectrophotometer "Philips"(model SP-3000 plus) according to the attached method of work by the concerned company.

The level of globulin in serum was estimated according to Wolf & Darlington (1971) through the following equation

:

Globulin (mg.100 ml⁻¹ serum) = Total protein- Albumin.

Albumin/globulin ratio (A/G) was estimated by dividing the albumin to globulin (Richards & Pickering, 1979).

Liver enzymes

The concentration of alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzymes in the serum were estimated by using special kit from a German company (Human) and read at a wavelength of 400 nm, 340 nm and 550 nm, respectively against distilled water by using Polish spectrophotometer "Philips"(model SP-3000 plus) according to the attached method attached by the company.

Differential white blood cell count

A thin blood film was prepared, fixed in methyl alcohol solution and then stained with Giemsa

stain. White blood cells were calculated on a 400x as a general view of the slide, then the cells were differentiated by 1000x. Randomly count 100 of different types of WBCs e.g. monocytes, lymphocyte, neutrophil, eosinophils and basophils and identified according to Mumford *et al.* (2007) and Palić *et al.* (2011).

Some biological measurements

Samples of fishes were randomly taken from all treatment groups and the internal organs were carefully isolated. The liver and spleen were weighed by using a Chinese sensitive balance (type XY-8006) and the following equations were applied to determine the hepatosomatic index (HSI) and spleen somatic index (SSI) according to Schreck & Moyle (1990).

$$\text{HSI \%} = \frac{\text{Liver weight(g)}}{\text{weight of fish}} \times 100.$$

$$\text{SSI \%} = \frac{\text{Spleen weight (g)}}{\text{Fish weight}} \times 100$$

Statistical analysis

The statistical analysis system (SAS, 2012) was used in the analysis of data to study the effect of different treatments in the studied traits according to the completely randomized design (CRD). The significant differences among the means were compared with Duncan's multiple range test (Duncan, 1955).

Results

Water quality

The temperature of water in the aquariums ranged between 26-27 °C, the dissolved oxygen concentrations 6-7 mg. l⁻¹, the pH 8-8.4 and the salinity between 2.8 - 3.9 PSU.

Growth parameters

No mortality of fishes happened during the experiment. The daily weight gain (DWG)

exhibited a significantly higher ($P < 0.05$) values for the second (500 mg safmannan.kg diet⁻¹) and third treatments (1000 mg safmannan.kg diet⁻¹) which were 1.44 and 1.49 g fish.day⁻¹, respectively compared with 0.935 and 1.06 g.fish.day⁻¹ for control and fourth treatments (Fluconazole), respectively. Feed conversion ratio (FCR) was significantly excelled ($P < 0.05$) for the second and third treatments which amounted 2.72 and 2.56, respectively compared with the first and fourth treatments which amounted 3.89 and 3.34, respectively. The averages values of the SGR% harmony with the average values of the daily weight gain, where the values of the second and third treatments were significantly higher with 0.377 g.day⁻¹ and 0.422 g.day⁻¹, respectively in

comparison with 0.270 and 0.303 % g.day⁻¹, for first and fourth treatments (Table 4).

Haemato-immunological analyses

Table (5) explained the results of blood proteins, serum cholesterol and liver enzymes. The albumin was significantly different ($P < 0.01$) among the treatments. The second and third treatments recorded significant lower (2.15 and 2.23 mg.100 ml⁻¹) compared with control and fourth treatments (2.88 and 2.63 mg.100 ml⁻¹, respectively). In the case of total protein, the third treatment was significantly ($p < 0.01$) excelled with 5.47 mg.100 ml⁻¹ compared with 4.78, 4.96 and 4.84 mg.100 ml⁻¹, for the first, second and fourth treatments, respectively.

Table (4): Effect of dietary Safmannan or Fluconazole on the averages of the daily weight gain, feed conversion ratio and the Daily growth rate for common carp (Mean±SE).

Treatment	Initial weight (g)	Final weight (g)	Daily weight gain (g.day ⁻¹)	Feed conversion ratio	Daily growth rate (% g.day ⁻¹)
Control	115.80 ± 5.93a	± 186.25 10.69 b	0.93 ± 0.03 b	± 3.89 0.21 a	± 0.270 0.01 b
Safmannan 500 mg.kg diet ⁻¹	115.30 ± 7.47 a	± 223.31 10.96 a	± 1.44 0.08 a	± 2.72 0.12 b	± 0.377 0.02 a
Safmannan 1000 mg.kg diet ⁻¹	± 108.55 5.62 a	220.80 ± 10.96 a	± 1.49 0.15 a	± 2.56 0.18 b	0.422 ± 0.02 a
Fluconazole 150mg.100 g diet ⁻¹	114.90 ± 5.7 a	± 195.40 12.9 b	± 1.06 0.06 b	± 3.43 0.22 a	0.303 ± 0.01 b
Significant level	NS	*	*	**	**

Means with different letters within the same column differ significantly among them * ($P < 0.05$) ** ($P < 0.01$).

As for the globulin, it was observed a significant excelling ($P < 0.01$) for the second and third treatments (2.80 and 3.24 mg.100 ml⁻¹) compared with 1.90 and 2.21 mg.100 ml⁻¹ for first and fourth treatments, respectively. The results of albumin/globulin ratio showed that the second and third treatments were signific-

antly ($P < 0.01$) lower (0.775 and 0.719, respectively), compared to the control and fourth treatments (1.63 and 1.72, respectively). Serum cholesterol showed a significant decrease ($P < 0.01$) in the second treatment (173.81 mg.100 ml⁻¹) followed by the third treatment (184.43 mg.100 ml⁻¹), while the first

treatment (control) and the fourth treatment recorded the highest concentration for cholesterol (204.08 and 201.70 mg.100 ml⁻¹, respectively) as shown in table (5). The results showed that there was no significant difference among all treatments in the concentration of the Alanine transaminase (ALT) enzyme (1.20, 1.23, 1.19 and 1.25 IU.l⁻¹, respectively).

Significant (P <0.05) higher value was observed for the fourth treatment (54.30 IU.l⁻¹) in Alkaline phosphatase (ALP) and significant decrease (P <0.01) Aspartate transaminase (AST) enzymes (24.57 IU.l⁻¹), compared with other treatments (30.85, 29.30 and 30.34 IU.l⁻¹, respectively).

Table (5): Effect of dietary Safmannan or Fluconazole on the concentration of blood proteins, serum cholesterol and liver enzymes of common carp (Mean±SE).

Treatment	Albumin (mg.100 ml ⁻¹)	Total protein (mg.100ml ⁻¹)	Globulin (mg.100 ⁻¹ ml)	A/G	Cholesterol (mg.100 ⁻¹ ml)	ALT (IU.l ⁻¹)	ALP (IU.l ⁻¹)	AST (IU.l ⁻¹)
Control	± 2.88 0.11 a	± 4.78 0.24 b	1.90 ± 0.21 b	1.63 ± 0.23 a	204.08 ± 2.66 a	1.20 ± 0.03 a	36.42 ± 5.13 b	30.85 ± 1.26 a
Safmannan 500 mg.kg diet ⁻¹	2.15 ± 0.08 b	± 4.96 0.11 b	2.80 ± 0.09 a	0.775 ± 0.04 b	173.81 ± 9.12 b	1.23 ± 0.03 a	36.62 ± 1.83 b	29.30 ± 0.90 a
Safmannan 1000 mg.kg diet ⁻¹	2.23 ± 0.13 b	5.47 ± 0.16 a	3.24 ± 0.18 a	0.719 ± 0.07 b	184.43 ± 6.21 ab	1.19 ± 0.02 a	36.47 ± 5.16 b	30.34 ± 0.84 a
Fluconazole 150mg.100 g diet ⁻¹	± 2.63 0.12 a	± 4.84 0.69 b	2.12 ± 0.64 b	1.72 ± 0.29 a	201.70 ± 3.98 a	1.25 ± 0.06 a	54.30 ± 4.62 a	24.57 ± 1.96 b
Significant level	**	**	**	**	*	NS	*	**

Means with different letters within the same column differ significantly among them, *=(P <0.05) ** =(P <0.01).

Table (6) exhibited no significant differences among all the treatments in the packed cell volume (PCV) and haemoglobin (Hb) with 40.01, 43.00, 39.06 and 41.75% for PCV and 13.33, 14.32, 13.02 and 13.91, respectively for haemoglobin. Eosinophils and basophils did not recorded significant differences among the treatments with 1.23, 1.26, 1.31 and 1.33% for Eosinophils and 1.23, 1.26, 1.19 and 1.22% for basophils. Neutrophil did not showed significant differences among the second, third and fourth treatments, and all were significantly decreased (p <0.05) from the control with 22.57 compared with 20.24, 19.65 and 20.72, respectively. A granulocytes lymphocytes and monocytes did not revealed significant

differences among the treatments with 73.85, 76.04, 76.59 and 75.24% and 1.12, 1.20, 1.26 and 1.49, respectively (Table 6).

Hepatosomatic index (HSI) and spleen somatic index (SSI)

Table (7) indicated a significant increase (p <0.05) in the hepatosomatic index for the last three treatments (2.482, 2.47 and 2.60) compared with the control (2.032). On other hand, these three treatments were not significantly different among them. The values of the spleen somatic index did not show significant differences among them with 0.021, 0.029, 0.046 and 0.033, respectively.

Table (6): Effect of dietary Safmannan or Fluconazole on packed cell volume (PCV) and haemoglobin (Hb) and the differential white blood cell count of common carp (Mean±SE).

Treatment	PCV	Hb	Eosinophil	Basophil	Neutrophil	Lymphocyte	Monocyte
Control	40.01 ± 1.30a	13.33 ± 0.43a	1.23 ± 0.07 a	1.23 ± 0.02 a	22.57 ± 0.55 a	73.85 ± 0.50 a	1.12 ± 0.07 a
Safmannan 500 mg.kg diet ⁻¹	43.00 ± 2.03a	14.32 ± 0.67a	1.26 ± 0.09 a	1.26 ± 0.04 a	20.24 ± 0.28 b	76.04 ± 0.88 a	1.20 ± 0.03 a
Safmannan 1000 mg.kg diet ⁻¹	39.06 ± 0.61a	13.02 ± 0.20a	1.31 ± 0.02 a	1.19 ± 0.04 a	19.65 ± 0.43 b	76.59 ± 0.85 a	1.26 ± 0.04 a
Fluconazole 150mg.100 g diet ⁻¹	41.75 ± 1.03a	13.91 ± 0.34a	1.33 ± 0.02 a	1.22 ± 0.02 a	20.72 ± 0.71 b	75.24 ± 0.35 a	1.49 ± 0.06 a
Significant level	NS	NS	NS	NS	*	NS	NS

Means with different letters within the same column differ significantly among them * (P <0.05) ** (P <0.01).

Table (7): Effect of dietary Safmannan or Fluconazole on the on the hepatosomatic Index (HSI) and spleen somatic index (SSI) of the common carp (Mean±SE).

Treatment	HSI	SSI
Control	± 2.0320.12b	0.021±0.003a
Safmannan 500 mg.kg diet ⁻¹	2.482±0.12a	0.029±0.01a
Safmannan 1000 mg.kg diet ⁻¹	2.47 ±0.13a	0.046±0.01a
Fluconazole 150mg.100 g diet ⁻¹	2.60±0.13a	0.033±0.012a
Significant level	*	N.S

Means with different letters within the same column differ significantly among them * (P <0.05) ** (P <0.01).

Growth parameters

In aquaculture, the conditions which result from stress, water quality and nutrition are interacted factors, complementing each other and any damage can affect the physiological and immunological status of the affected animal and making it susceptible to the disease (Staykov *et al.*, 2007). The positive results obtained in the growth traits (daily weight gain, feed conversion ratio and daily growth rate) when using the prebiotic (Safmannan) compared with the control group can be attributed to stimulating the targeted microorganisms for growth and reproduction,

leading to an increase in the conversion of complex contents to simple materials. This, in turn, ends in the production of some digestive enzymes for starch, proteins and fatty substances (El-Haroun *et al.*, 2006; Azevedo *et al.*, 2015). Thus, it leads to activate the role of the trypsin and amylase enzymes in the intestine, the increase in the activity of digestive enzymes and the developing of the microvillus structure on the inner surface of intestinal cells which in turn increases the surface area of nutrient absorption, which in turn bring about to increasing efficiency of

food utilization compared with the control group (Zhou *et al.*, 2010; Anguiano *et al.*, 2013).

The results of the study coincided with those of Staykov *et al.* (2005), which revealed significant surpassing for the growth rates of rainbow trout fed on a diet supported with prebiotic MOS compared with unsupported diet (control). This may be attributed to stimulating the growth, reproduction and colonization of beneficial microorganisms at the expense of other non-beneficial pathological microorganisms, and the consequent improving digestion of consumed feed and absorbing it in intestine. Similar results were obtained by Mazurkiewicz *et al.* (2008) when feeding common carp on prebiotic (Fermacto) at concentrations of 1, 2 and 3 g.kg⁻¹ diet, where it showed significant superiority compared with the control diet in both the specific growth rates and the feed conversion ratio which attributed to stimulating the voluntary growth for endemic organisms in gut. Also, the present results are coincided with Kühlwein *et al.* (2014) when adding 5 and 10 g.kg⁻¹ diet from the prebiotic (Beta-glucan) in the diet of the mirror carp for eight weeks and obtained high values for weight indicators. The results of the present study also are comparable with the study of Eleraky *et al.* (2014) when adding 1.5 and 2.5 g.kg⁻¹ diet from the prebiotic containing MOS and Beta-glucans (1: 3 and 1: 6) in the feeding of common carp fries, when they showed a significant superiority compared with the control diet in terms of the final weight of the body and the average of specific growth and weight gain. They are also agreed with the results of Amani Denji *et al.* (2015), when adding 1, 2.5 and 4.0 g.kg⁻¹ diet from the prebiotic (mannan oligosaccharide) in the feeding of rainbow trout (*Oncorhynchus mykiss*), where it showed a significant

superiority compared with the control diet in terms of the final weight of the body and the average of specific growth and weight gain. However, the results did not agree with the results mentioned by Genc *et al.* (2007), which did not show significant differences between the *Oreochromis niloticus* × *O. aureus* fed on different levels of MOS compared with the control diet in both growth performance and final weight and growth rate, in addition to the feed conversion ratio and protein efficiency ratio for the duration of the experiment. The results of the current study did not agree with Dobšíková *et al.* (2013) when feeding common carp on beta-glucans, where a reduction in the weight gain was observed.

Antibiotics have been used to feed fish as growth promoters and immune stimuli by many researchers (Ahmad *et al.*, 2008; Rodgers & Furones, 2009), so they has been used as a treatment compared with the prebiotic, which many researches has illustrated their importance in increasing the beneficial bacteria and reduce the number of pathogenic ones by preventing the adhesion of pathogenic bacteria mediated by the villi in the lining of the digestive tract, which prevents their association with epithelial cells, which means that these bacteria are out of the body, thus cause no injury (Wysong, 2003). Therefore, a free from dietary additives diet, was used as a negative control treatment and antibiotics as a positive control treatment to make comparison on the effect of these additives (antibiotic and safmannan) on the growth and traits of blood and biochemical parameters and some fish organs. The results of the present study are in agreement with those of El-Houseiny *et al.* (2015) when compared by using the prebiotic (Cinnamon) and bio-antibiotic (oxytetracycline) in the diet of Nile tilapia which exhibited significant

superiority in prebiotic compared with bio-antibiotic in terms of final weight, the weight gain and daily growth rate, along with resistance of fishes fed on the prebiotic for pathogenic bacteria (*Clostridium perfringens*). These results did not coincide with the results of Reda *et al.* (2013) when adding 100 mg.kg⁻¹ diet from prebiotic (oxytetracycline) and 5 mg.kg⁻¹ diet from Florfenicol for cultured Nile tilapia where the addition led to an increase in final weight and a significant increase in the weight gain and feed conversion ratio compared with basal diet.

Haemato-immunological parameters

Blood is considered as one of the important tissue for determining the health of fishes that are given dietary supplement where they determine the suitability of the added feed. However, biochemical criteria are considered as one of the methods for determining fish health and their breeding conditions in terms of stress and disease (Peres *et al.*, 2013; Gharaei *et al.*, 2016).

The treatments with dietary safmannan of the current study revealed significantly higher values in immunological parameters compared with control, and the increase of protein in the serum was associated with high immune response in the body (Wiegertjes *et al.*, 1996; Ta'ati *et al.*, 2011). Similar results could see in the study of Ebrahimi *et al.* (2011) when using the prebiotic (immunogen) at concentrations of 0.5, 1, 1.5 and 2.5 g.kg⁻¹ diet in the nutrition of young common carp in value of the serum protein, but did not agree with the level of globulin and albumin and the albumin/globulin ratio and hence may be resulted from the type of prebiotic and the ecological criteria between the studies. It was worth to mention that the low cholesterol in safmannan treatments (second and third

treatments) is advantage result in the current study and this significant difference compared with the control is due to the prebiotic which led to increase growing the beneficial bacteria in the intestine, which increase secretion of organic acids, thus inhibits the activity of enzymes that prevent the building of fatty acids (Chen *et al.*, 2014).

When using bio-antibiotic (fluconazole), it did not show significant differences in the level of albumin, globulin, total protein and cholesterol compared with the control. These results agreed with Saha *et al.* (2017) when giving dietary fluconazole for *Labeo rohita* at concentrations of 100, 200 and 300 mg.100 g⁻¹ diet, where it did not record significant differences in the level of total protein in the concentration of 100 and 300 compared with the control, while it recorded a decrease in the level of total protein at the concentration of 200 mg.100 g⁻¹ diet. The same study recorded no significant differences in albumin at the 30th and 45th days and no significant differences in globulin during 15 and 30 days from experiment and hence agreed with the present results.

Liver enzymes

The activity of enzymes varies depending on the quality of the fishes (Shalaby, 2005). The activity of ALP, ALT and AST enzymes in the serum can be used to monitor of fish health due to the increased permeability of damaged hepatocytes which cause a liberation of these enzymes into the blood (Suárez *et al.*, 2015). The results of the ALT and AST in the second and third treatments (500 and 1000 safmannan, respectively) revealed no significant differences compared with the control, which means that the concentrations of enzymes are within the normal ranges of the prebiotic treatments.

Akrami *et al.* (2013) found similar results when they recorded no significant differences in the treatments of MOS with two levels (2 and 4 g.kg⁻¹ diet) when fed great sturgeon (*Huso huso*). In addition, similar upshots were demonstrated by Amani Denji *et al.* (2015) when adding 1, 2.5 and 4.0 g.kg⁻¹ diet of prebiotic MOS and by Adel *et al.* (2017) when feeding juvenile *H. huso* on the prebiotic GroBiotic with different concentrations. In the case of bio-antibiotic (fluconazole), there were significant differences in the enzymes of AST and ALP, while there were no differences in ALT compared with the control. This rise in the level of liver enzymes was due to the stress and toxicity of which the fishes were exposed due to the continuous using of antibiotics (Ovie *et al.*, 2010). Related results were demonstrated by Reda *et al.* (2013) when adding bio-antibiotic oxytetracycline and Florfenicol by 5 and 100 mg.kg⁻¹ feed, respectively to the Nile tilapia where AST values showed significant differences compared with the control.

Hemoglobin (Hb) and packed cell volume (PCV)

Knowing blood criteria is considered as one of the main indicators in diagnosing the health status of fishes especially when some additives are used in feeding (Abu-Elala *et al.*, 2013). Plosker & Figgitt (2003) indicated that the PCV is considered as an indicator of environmental influences and sometimes as a pathological case. The current results presented no significant differences in hemoglobin (Hb) and PCV. Welker *et al.* (2007) noted same results in both parameters when fed Channel catfish (*Ictalurus punctatus*) on the MOS. Also, Akrami *et al.* (2013) when feeding great sturgeon (*Huso huso*) on two levels of MOS (2 and 4 g.kg⁻¹

feed) and Amani Denji *et al.* (2015) when adding MOS with 1, 2.5, 4.0 g.kg⁻¹ feed on rainbow trout (*Oncorhynchus mykiss*) and with Gelibolu *et al.* (2018) when adding different concentrations of the prebiotic MOS by 1, 2, 3 and 4 g.kg⁻¹ diet to Sea bream (*Sparus aurata*), while Akrami *et al.* (2012) used prebiotic MOS (1, 2 and 3 kg⁻¹ diet) in feeding common carp and found significant differences in PCV with treatment of 1 kg⁻¹ diet.

Regarding fluconazole, there were no significant differences compared with the control in the PCV and hemoglobin (Hb), which agreed with Saha *et al.* (2017) in the PCV when used dietary fluconazole (100, 200 and 300 mg.g⁻¹diet) to *Labeo rohita* in the 15th and 45th days of the experiment. For Hb, in the same experiment, it was applied with the results of the present investigation on the 45th day, where no significant differences were recorded, while there were significant differences in the 15th and 30th days of the experiment.

HSI and SSI

The hepato-somatic index (HSI) revealed significant differences compared with the control, while the spleen somatic index (SSI) did not display significant differences among the treatments. There were differences among the treatments compared with the control, excelled by the treatment (T2), where these results supported the theory that the prebiotic improves the health of a particular animal and provides a beneficial physiological effect (Hutkins *et al.*, 2016). Ashourpour *et al.* (2012) noted the same findings when fed *Acipenser nudiventris* on different concentrations of the prebiotic (0.5, 1 and 1.5 g.kg⁻¹diet), where significant differences were found in HSI of all treatments compared with the control. Ahmed (2014) investigated the

comparative effect of a prebiotic Fructooligosaccharide (FOS) and a probiotic (*Saccharomyces cerevisiae*) and their combination in different levels as a symbiotic on young common carp. The study did not recorded significant differences in hepatosomatic index in all eight treatments except in probiotic with dietary yeast of 2.5g.kg⁻¹ diet.

Differential WBC count

White blood cells are the most important immune defences in vertebrates (Tierney & Farrell, 2004) where number of white blood cells may increase in response to disease, season, environmental conditions and physiological status of fishes. Number of WBC in fishes cannot be determined because they are few compared with red blood cells. Therefore, their use as an index for pathological infections is limited (Hoole *et al.*, 2001).

The present results did not displayed significant differences in the differential WBC count but demonstrated significant superiority in the number of neutrophils in fishes fed on the control diet, which may be due to the stress of fishes fed on the diets free from the health-promoting prebiotic. In lymphocyte cells, although there was no significant differences, but they exhibited statistical excelling in prebiotic treatments compared with the control. Similar findings were illustrated by Amani Denji *et al.* (2015) when rainbow trouts were fed with 1, 2.5 and 4.0 g. kg⁻¹ diet of the prebiotic MOS, where it did not showed significant differences compared with the control diet in terms of lymphocytes and eosinophils. In contrast, Akrami *et al.* (2012) revealed that feeding of common carp with 1, 2 and 3 g.kg⁻¹ diet prebiotic MOS have a significant superiority at a concentration of 1 g.kg⁻¹ diet compared

with the control in terms of lymphocytes. The reason for this rise is due to the effect of prebiotic in immunostimulation of fishes. Ahmed (2014) recorded significant increasing in granulocytes count in FOS 5g.kg⁻¹diet and significant increasing in monocytes count in symbiotic treatment FOS 2.5 g.kg⁻¹diet with yeast (2.5 g.kg⁻¹diet).

Conclusion

Adding Safmannan to the diet exhibited positive results in growth parameters, healthy aspects and the liver enzymes. The present adding of Safmannan in the diet is still in the normal concentrations. The biochemical criteria exhibited better in safmannan treatment compared with the antibiotic and the control treatments. It is recommended to use dietary safmannan in heavy aquaculture system especially in cage culture to immuno stimulate fishes toward various pathogens.

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