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Effects of diet supplementation with different level of Celmanax® in rainbow trout *(Oncorhyncus mykiss)*

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ABSTRACT. The aim of this study was to evaluate the effect of a prebiotic (Celmanax®) containing yeast cell wall with mannan oligosaccharides on the haematological and serum biochemical parameters in rainbow trout. Three levels of prebiotic (0, 0.1, 0.5 and 1 %) were mixed into pellets. Fish (19.08±1.45 g) were fed a supplemented commercial diet for 60 days. Blood samples were colected from the onset and on days 30 and 60 of the trial to measure the haematological and serum biochemical parameters in rainbow trout. The results showed significant differences in haemoglobin, mean corpuscular volume,mean corpuscular haemoglobin concentration, white blood cells and neutrophil count between control and all test groups ($p<0.05$). The highest and the lowest white blood cells and neutrophil count (on day 60) were observed in the 0.1 %, prebiotic-received and control groups, respectively. Also, the result showed significant differences in Alkaline phosphatase enzymes, serum glutamic oxaloacetic transaminase, Serum glutamic-pyruvic transaminase, between the test and control groups($p<0.05$) while non-significant elevation of blood urea nitrogen, creatinine and total protein levels was found in the Celmanax®-received groups ($p>0.05$). These results suggest that the Celmanax® supplementation enhances white blood cells and neutrophil count, and changes some biochemical parameters in rainbow trout.

Keywords: Celmanax®; Rainbow trout (*Oncorhynchus mykiss*); Haematological; Biochemical parameters; Mannan-oligosaccharides

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INTRODUCTION

Worldwide interest in prebiotics has increased in animals as well as in rainbow trout. Various sectors of the aquaculture industry would benefit if cultured organisms were conferred with improved growth performance, feed efficiency, disease resistance, modulation of the gut micro biota and enhanced immune responses (Ringø et al., 2010). Since then the most common prebiotics used in fish are inulin, fructooligosaccharides (FOS), oligofructose, mannanoligosaccharides (MOS), trans-galactooligosaccharides (TOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO) and various commercial products containing multiple prebiotic combinations. Although the potential of prebiotics may have interesting applications in aquaculture in order to improve growth performance, survival, feed conversion, digestibility, gastrointestinal (GI) enzyme activities, immune functions and the presence of beneficial gut bacteria as well as the suppression of potentially pathogenic bacteria. In addition, several papers have investigated the effect of prebiotics on GI morphology (Pryor et al. 2003; Genç et al. 2007; Dimitroglou et al. 2009; 2010; Ringø et al. 2010; Sweetman et al. 2010; Dimitroglou et al. 2011a; 2011b; 2011c). Salmonids such as Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are among the most well documented fish species in respect to prebiotic applications. Indeed, MOS, GOS, FOS, inulin, and commercial products containing multiple prebiotic combinations have been investigated in studies on Atlantic salmon, brook trout (*Salvelinus fontinalis*), rainbow trout and arctic charr (*Salvelinus alpinus*) (Merrifield et al., 2010 and Ringø et al., 2010). In a recent study, Rehulka et al., (2011) reported results from a 105-day experiment, where the effect of dietary scFOS on rainbow trout (initial weight 240±34.9 g) growth and plasma biochemical parameters was determined. Inclusion of scFOS at 1 g kg−1 did not significantly affect Special Grow Rate (SGR), Feed Conversion Ratio (FCR) and survival. The results of the biochemical parameters indicated significant differences in creatinine, Na+ and alkaline phosphatase (ALP, involved in intestinal mucosal defence).

The prebiotic, Celmanax™ (Vi-COR®, Mason City, IA, USA), consists of a non-living formulation of yeast cell walls, mannan oligosaccharide (MOS) and yeast metabolites (*Saccharomyces cerevisiae*). Haematological parameters changes would be sign of fish physiological responses against environmental stresses e.g. such as pH alteration, salinity of water pollution or bacterial infections (Zorriehzahra, 2010). Thus important internal organs such as kidney, spleen, liver and pancreas that have important duty in fish physiology must be affected by infectious pathogens to change the haematological items in response to the invading pathogens. In many cases of fish infectious diseases diagnosis could be assisted by haematological study. Several studies have demonstrated that prebiotic of MOS can improve the growth parameters, survival, haematological and biochemical parameters, gut morphology and modulate the intestinal microbiota in various aquatic species, including sea bass (*Dicentrarchus labrax*) (Torrecillas et al., 2007), rainbow trout (Staykov et al., 2007; Dimitroglou et al., 2009), atlantic salmon (Grisdale-Helland et al., 2008); (Andrews et al., 2009), sea bream (*Sparus aurata*) (Gultepe et al., 2011), Japanese flounder (*Paralichthys olivaceus)* (Ye et al., 2011). Haematological parameters are repeatedly used as diagnostic and effective tools to assess the health status of fish (Thrall et al., 2012). Knowledge of haematological parameters of Salmonidae family are frequently used as an essential diagnostic tool to assess the growth and health condition. Haematological parameters are closely response of the animal to the environment and indication that the effect of diet on the haematological characteristics.

Rainbow trout (*Oncorhynchus mykiss*) is a fish belonging to the family of Salmonidae and native to the cold water rivers and lakes of the Pacific coasts of North America and Asia; the habitat and food of rainbow trout determine both their actual colour and shape (Roozbahani, et al., 2009). Since 1874 *O. mykiss* has been introduced to all continents except Antarctica, for recreational angling and aquaculture purposes, because it is a resistant fish, easy to spawn, fast growing and capable of occupying many different habitats because it can tolerate a wide range of environmental and production conditions better than other trout species (Parisi et al., 2014).

Today, nearly all rainbow trout on the European Union market come from aquaculture. Most of the EU supply of trout is locally produced. Currently the largest producers worldwide are Chile, Norway, Iran and Turkey. In 2016, a total of one million tons of seafood was grown for human consumption in Iran. The amount was 82000 tons more than in 2015. However, out of this, about 140,000 tons were rainbow trout. The rest consisted of sturgeon and caviar, shrimp and other fishes.

Rainbow trout is one of the most commercially important species grown in Iran and all around the world. Hence, the objective of the experiments was to determine the effects of Celmanax® prebiotic on haematological and some biochemical parameters of cultured rainbow trout.

MATERIALS AND METHODS

Experimental design

Rainbow trout (19.08±1 g) were purchased from a commercial fish farm in Urmia, west Azerbaijan province, Iran. Acclimatization to the laboratory condition was performed for 10 days in 1000 L tanks using aerated free-flowing well water with the following characteristics: temperature (13.5 ± 1ºC); pH (7.5); dissolved oxygen (8 ± 0.2 mg/L); natural photoperiod (10 h light/14 h dark); flow rate (1.25 l/s). Fish were fed three times daily with commercial fish feed (40% protein), 3% of average initial body weight per day.

Diet preparation and feeding trial

Commercial basal diet (21 Baiza, Shiraz, Iran) was used in this study; three experimental diets, commercial diet supplemented with 0.1% (T1), 0.5% (T2) and 1% (T3) Prebiotic. After spraying the different level of Celmanax® on commercial feed, pellets were dried at room temperature for 2 h and then the diets were stored at 4°C until use. Fish were randomly divided into 4 groups (in triplicate) of 50 animals per tank and were fed for a 60-day period. On days 0, 30, 60 a sample of three individuals per tank (nine per treatment) was taken to measure haematological and biochemical parameters.

Haematological parameters

Fish were anesthetized with 200 mg/L clove oil, then blood was collected from cardinal vein using heparin coated syringe and transferred into sterile tubes. The blood was allowed to clot at room temperature for 1 h and stored in a refrigerator overnight. The clot was then centrifuged at 1500 × g for 5 min. Then the serum was collected and stored in sterile eppendorf tubes at -20ºC until use for biochemical assays. Also, blood collected by caudal vein puncture in heparinized syringes. Haematocrit values (Ht) were determined by centrifuging fresh blood in heparinised glass capillary tubes for 5 min. Haemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethaemoglobin using a commercial kit (Pars Azmoon, Tehran, Iran). Red blood cells (RBCs) and white blood cells (WBCs) were counted under a light microscope using a Neubauer haemocytometer after dilution with phosphate buffered-saline (PBS). Deferential leukocyte counts (neutrophil, lymphocyte, eosinophil and monocyte) were determined using blood smears under a light microscope. Cells were identified on the basis of morphology and cell ultra-structure as documented in previous fish leukocyte studies (Jalali et al., 2009).

Biochemical parameters

In this study alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic-pyruvic transaminase (SGPT), Blood urea nitrogen (BUN), Creatinine and total protean were assayed in collected serum samples. An automatic blood enzyme analyser (Hitachi 704) was used for the following determinations: Alkaline phosphatase, Serum glutamic oxaloacetic transaminase, Serum glutamic-pyruvic transaminase, Blood Urea Nitrogen, Creatinine and total protean serum. The apparatus is based upon dry chemical technology and colorimetric reaction. Kits obtained from PrasAzmoon®, Iran, were used for the determination of all parameters.

Statistical analysis

The data (Mean ±SD) were analysed by one-way analysis of variance (ANOVA) followed by Tukey's test to compare the means between individual treatments with SPSS (Version 21; SPSS Inc.,) at $p < 0.05$ level.

Table 1. Haematological parameters in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment at start of the study.

Hematological parameters	Control	T1	T2	T3
Haematocrit (%)	32.15± 1.2 [a]	32.17±1.2 [a]	32.8±1.1 [a]	33.1±1.3 [a]
Hemoglobin (g/dl)	6.24±0.1 [a]	5.11±0.1 [a]	5.86±0.1 [a]	6.60±0.1 [a]
RBC (10^6 cell/mm3)	1.06± 0.1 [a]	1.00±0.18 [a]	1.009±0.12 [a]	1.00±0.18 [a]
MCV (Fl)	303±5 [a]	301±5 [a]	305±5 [a]	305±5 [a]
MCH (pg)	58.67±0.5 [a]	58.77± 0.5 [a]	58.64 ± 0.5[a]	58.55± 0.5 [a]
MCHC (g/dl)	17.20±0.5 [a]	16.90±0.5 [a]	18.15±0.5 [a]	18.50±0.5 [a]
WBC (10^3 cell/mm3)	13.72±0.2 [a]	14.02±0.1 [a]	13.85±0.2 [a]	14.10±0.2 [a]
Lymphocyte (%)	89.30±1.01 [a]	90.32±1.21 [a]	89.8±1.2 [a]	89.16±1.4 [a]
Neutrophil	6.78±1.1 [a]	6.2±1.7 [a]	7.1±1.1 [a]	7.08±1.0 [a]
Eosinophil	3.21±0.1 [a]	2.89±0.2 [a]	3.2±0.6 [a]	3.4±0.3 [a]
Monocyte	0.58±0.1 [a]	0.5±0.1 [a]	0.54±0.1 [a]	0.49±0.1 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

RESULTS

The effect of dietary Celmanax® on rainbow trout blood profiles is presented in Tables 1-3. Red blood cell count and haematological parameters such as haematocrit, Eosinophil and Neutrophil count did not significantly differ between treatments and control (p>0.05). The highest haematological parameters value at day 60 of study were recorded HB (7.23±0.1 g/dL), MCHE (21.61±0.9 g/dL), WBC (14.80±0.3 cell/mm3), Lymphocyte (92.41±0.1%), Neutrophil (7.24±1.7%), Monocyte (1.7±0.1%) in treatment 0.1% Celmanax® diet and RBC (1.47±0.08×106

Table 2. Haematological parameters in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment at day's 30 of the study.

Hematological parameters	Control	T1	T2	T3
Haematocrit (%)	31.81±2 [a]	30.53±2 [a]	29.36±2 [a]	29.18±1 [a]
Hemoglobin (g/dl)	5.34±0.1 [a]	5.51±0.1 [a]	5.47±0.1 [b]	5.38±0.1 [c]
RBC (10^6 cell/mm3)	0.895±0.1 [a]	0.862±0.01 [a]	0.847±0.02 [a]	0.88±0.01 [a]
MCV (Fl)	332.65±3 [a]	329.42±5 [a]	328.05±5 [a]	316.32±5 [b]
MCH (pg)	61.89±0.5 [a]	59.86±0.5 [d]	60.53 ± 0.5 [c]	61.32± 0.5 [b]
MCHC (g/dl)	23.46±0.5 [a]	17.13±0.5 [c]	16.21±0.5 [d]	18.37±0.5 [b]
WBC (10^3 cell/mm3)	14.07±0.01[c]	14.82±0.01 [a]	14.32±0.01 [b]	14.41±0.2 [b]
Lymphocyte (%)	91.50±0.1[a]	89.54±0.1 [a]	89.97±0.1 [a]	90.12±0.3 [a]
Neutrophil	6.33±1.0 [c]	9.93±1.7 [a]	9.55±0.5 [b]	9.37±0.1 [b]
Eosinophil	0.5±0.1 [a]	0.6±0.2 [a]	0.4±0.1[a]	0.4±0.1 [a]
Monocyte	0.6±0.1 [a]	0.45±0.1 [a]	0.48±0.1 [a]	0.51±0.1 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

Table 3. Haematological parameters in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment at day's 60 of the study.

Haematocrit (%)	32.18±1.3 [a]	32.52±1.2 [a]	33.56±2.1 [a]	33.28±2.3 [a]
Hemoglobin (g/dl)	6.27±0.1 [d]	7.23±0.1 [b]	6.74±0.1 [a]	7.07±0.1 [c]
RBC (10^6 cell/mm3)	1.21±0.1 [a]	1.29±0.11 [a]	1.36±0.01 [a]	1.47±0.08 [a]
MCV (Fl)	228.24±5 [d]	238.28±5 [b]	236.33±5 [c]	241.05±5 [a]
MCH (pg)	51.86±0.5 [a]	50.11± 0.5 [c]	49.59 ± 0.5 [d]	51.08±0.5 [b]
MCHC (g/dl)	19.13±0.5 [d]	21.61±0.9 [a]	20.11±0.5 [b]	19.45±0.5 [c]
WBC (10^3 cell/mm3)	11.70±0.2 [c]	14.80±0.3 [a]	13.02±0.2 [b]	12.90±0.2 [b]
Hematological parameters	Control	T1	T2	T3
Lymphocyte (%)	91.24±0.1 [a]	92.41±0.1 [a]	91.73±1.2 [a]	91.86±2.4 [a]
Neutrophil	6.26±1 [c]	7.24±1.7 [a]	7.1±1.1 [b]	6.38±1.0 [b]
Eosinophil	0.64±0.1 [a]	0.59±0.2 [a]	0.52±0.23 [a]	0.40±0.1 [a]
Monocyte	1.1±0.1 [a]	1.7±0.1 [a]	1.2±0.1 [a]	1.5±0.1 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet)

cell/mm3), PCV (2.3±33.28%), MCV (241.05±5 Fl) were in treatment 1% Celmanax® diet. However, the lowest value of MCH (49.59±0.5pg) which were in treatment 0.5% Celmanax® diet lower the respective control in all the treatment groups. In day 60 of study, Total white blood cell (WBC) was significantly different (P<0.05) between the prebiotic-received groups compared to control. Similar trends was recorded in the value of neutrophil. The values of Monocyte in all the treatment up than control and were raised above the control with highest value recorded at in treatment revised 0.1% Celmanax® diet. Eosinophil value in the fish varied widely with both increase and decrease relative to the control value. The results in day 60 showed significant differences in Haemoglobin, MCV, MCH, MCHC, white blood cells and neutrophil count between the control and treatment groups (P<0.05). Evaluation of alkaline phosphatase enzyme showed significantly (p<0.05) decreased between control (1002±1) and all treatment groups (T1:664± 10, T2: 645± 5 and T3: 928±3). In day 60 of study, the highest decrease of alkaline phosphatase was seen in treatment 0.5% Celmanax® diet (T2: 645± 5) (table 4). The results also showed that addition of Celmanax® on to the diet in day 60, significantly (p<0.05) increased the SGOT enzyme in two treatments (T2:399±10 and

Table 4. Alkaline Phosphatase level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	1255±1 [a]	1221±5 [a]	1296±1 [a]	1252±3 [a]
30	1658±5 [a]	1608± 10 [b]	1306±5 [c]	711±10 [d]
60	1002±1 [a]	664± 10 [c]	645± 5 [d]	928±3 [b]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

Table 5. SGOT level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	807±5 [a]	795±5 [a]	818±1 [a]	790±3 [a]
30	597±5 [d]	724± 5 [c]	751±5 [b]	892±5 [a]
60	464±1 [b]	399± 10 [a]	635± 5 [d]	484±3 [c]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

T3:484±3) and SGPT enzyme significant increase in two treatments (T1: 21±5 and T3:15±4), however, there was significant difference among the prebiotic-received groups in different days. Also in day 60, SGOT level was significantly ($p<0.05$) decreased between 0.1% Celmanax® diet treatments (399±10) and control (464±1) (tables 5-6). However, a non-significant ($p>0.05$) change among the Blood urea nitrogen (BUN), Creatinine and total protean levels was found in groups prebiotic-received compared to control ($p>0.05$) (table 7-9).

Table 6. SGPT level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	22±5 [a]	20±3 [a]	18±1 [a]	19±2 [a]
30	13±1 [b]	21±4 [a]	13±1 [b]	12±1 [b]
60	7±3 [c]	21±5 [a]	10±1 [c]	15±4 [b]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

Table 7. Blood urea nitrogen level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	3±1 [a]	4±1 [a]	4±1 [a]	3±1 [a]
30	4±1 [a]	3±1 [a]	2±1 [a]	3±1 [a]
60	3±1 [a]	3±1 [a]	3±2 [a]	2±1 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

Table 8. Creatinine level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	0.75±0.05 [a]	0.45±0.05 [a]	0.30±0.05 [a]	0.35±0.05 [a]
30	0.34±0.02 [a]	0.30±0.01 [a]	0.31±0.01 [a]	0.41±0.01 [a]
60	0.20±0.01 [a]	0.30±0.01 [a]	0.24±0.02 [a]	0.25±0.03 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

Table 9. The serum Total protein level in rainbow trout fed diets with Celmanax® prebiotic. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Day	Control	T1	T2	T3
0	3.32±0.05 [a]	3.85±0.05 [a]	3.21±0.1 [a]	3.45±0.1 [a]
30	3.35±0.05 [a]	3.98±0.15 [a]	3.56±0.1 [a]	3.44±0.1 [a]
60	3.19±0.2 [a]	3.25±0.3 [a]	3.66±0.5 [a]	3.29±0.1 [a]

*The same superscript alphabets in the same row indicate a non-significant different at P<0.05. (T1: 0.1, T2: 0.5 and T3: 1 percent of prebiotic in commercial pellet).

DISCUSSION

Hematologic evaluation of fish is not routinely used in establishing the diagnosis of fish diseases, but it can be useful in the detection of diseases and different materials in the food affecting the cellular components of blood. Certain diseases of fish result in anemia, leukopenia, leukocytosis, thrombocytopenia, and other abnormal changes of the blood cells. Evaluation of the hemogram also may be useful in following the progress of the disease or the response to therapy.

Routine assay methods for the biochemical evaluation of mammalian blood appear to be useful for fish blood; however, interpretation of the results can be difficult. Many endogenous (species, age, nutritional status, gender, reproductive status) and exogenous factors (environmental conditions, population density and method of capture) influence the plasma biochemistry results of fish (Thrall et al., 2012).

In the present study, SGPT, SGOT and ALP levels were affected by Celmanax® Commercial product ($p<0.05$). The liver tissue of teleosts appears to be rich in aspartate aminotransferase (AST) and possible alanine aminotransferase (ALT). Therefore, plasma activity of these enzymes may elevate with severe hepatocellular disease in some piscine species. High activities of AST and creatine kinase (CK) also occur in muscle of fish; therefore, elevated plasma activities of these enzymes will increase following muscle injury or strenuous muscle activity associated with capture and restraint (Thrall et al., 2012).

In recent years, less studies have been done on the efficacy of probiotics on blood parameters and enzymes in aquaculture. In 2007, Welker et al. reported that 0.2% dietary MOS had no effect on

the WBC, RBC, Hct, Hb and total serum protein of channel catfish *(Ictalurus punctatus*). Sado et al. (2008) also reported that 0.2-1% MOS had no effect on tilapia (*Oreochromis niloticus*) RBC, WBC, Hb, Hct, MCV, MCH, MCH, MCHC and plasma total protein. In the prebiotics research, Ahmdifar et al. (2011) and Hoseinifar et al. (2011) who reported that prebiotic inulin and oligofructose had no effects on SGPT, SGOT and ALP enzymes in beluga *(Huso huso)* serum, also Amani Denji et al., (2015) report no significant differences in serum enzymes activity (SGPT, SGOT and ALP), cholesterol, triglyceride and total protein among treatment use 1, 2.5 and 4 gr /kg MOS prebiotic in rainbow trout.

Some studies on effect of prebiotics, in contrast to the above results and showed the effect of prebiotic on blood parameters and enzymes. In this study, the diet supplementation with Celmanax® in all treatments too changed Alkaline Phosphatase level in rainbow trout (table 4). Alkaline phosphatase activity occurs in multiple tissues including bone and intestine, and increased plasma AP activity results not from leakage of the enzyme but from increased cellular production. Metabolism of minerals in the liver and bone have a direct effect on Alkaline Phosphatase level in serum. The increase in the Alkaline Phosphatase could be due to the rapid growth phase and the production of collagen in the cartilage before bone formation. Ninety percent of the secretion of the alkaline phosphatase enzyme is related to the liver and bones, and small amount to the intestines. The Alkaline phosphatase plays a role in the metabolism of minerals, distribution of carrier proteins and the activity of DNA and RNA polymerases. In hepatic damage, the activity of alkaline phosphatase and gamma-glutamyl transferase are increased in blood.Isoenzymes of alkaline phosphatase (L-ALP, CL-ALP) increase in bone growth and various diseases, too (Bargerand MacNeill, 2015). In this study, probably bone growth could be the reason of alkaline phosphatase increase in treatment Fed with Celmanax®.

Bone mass of an adult is dependent on supply as well as bioavailability of calcium. Even though, a lot of studies have been carried out on calcium metabolism using rats, results depicted that prebiotics play a role in escalating the bioavailability of calcium (Wong et al., 1988).

On day 60 of the study, there was a significant difference in secretion of SGPT enzyme between the groups fed with the 0.1% of Celmanax® between control (P=0.001) and the group fed with 1% of Celmanax® between control (P=0.01). Considering the significant increase in the SGPT in fish need for additional histopathological observations is required. Blood urea nitrogen, Creatinine and total protean of the control and experimental groups were not significantly affected by addition of Celmanax® to the diet (P>0.05) in all treatment groups and the prebiotics were not affected to these enzymes.

In other study Anguiano et al, (2012) showed effects of four prebiotics (fructo-oligosaccharide, Bio-MOS, transgalacto-oligosaccharide and GroBiotic-A) on digestive enzymes and intestinal morphology in juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) and red drum (*Sciaenops ocellatus*). The results of this study showed no significant changes in the enzyme activities were detected at week 8 in both species (Anguiano et al. 2012). In 2015, Amani Denji et al. reported the effect of dietary MOS (Active MOS®; Biorigin, LencoisPaulista, Sa¨o Paulo, Brazil) supplementation on rainbow trout (1, 2.5 and 4 gr /kg MOS). Result of this study showed ALT, AST and ALP activities did not significantly (p>0.05) differ between treatments. In 2015, Gültepe et al., showed effects of prebiotic MOS on the histology and biochemical blood parameters of the gilthead sea bream (*Sparus aurata*). MOS additives to feed did not have a significant effect on AST, ALT and ALP. Adel et al., (2016), reported Activities of lysozyme and alkaline phosphatase in *Huso huso* skin mucus were significantly enhanced in Grobiotic®-A fed group, particularly at an inclusion level of 1% and higher (2% group compared to the control).

The results of the present study showed that dietary containing Celmanax® had effects on Hb, MCV, MCH, MCHC and WBC and Neutrophil counts (neutrophilia). Neutrophils are a critical component in the first line of defense against invading pathogens. Multi-receptor recognition of PAMPs and DAMPs define intruding pathogens, resulting in the activation of cellular antimicrobial responses designed to kill infiltrating pathogens. Antimicrobial responses are tailored to the type and

location of the pathogen, and can be divided into two main categories: intracellular and extracellular. Intracellular defense mechanisms are designed to provide protection against pathogens found within membrane-enclosed structures. These defenses are not limited to killing pathogens that have been internalized through phagocytosis, but also to provide protection against pathogens actively hiding from humoral immune defenses. Neutrophils are armed with an extensive antimicrobial arsenal designed to limit the dissemination of a broad range of pathogens. Interestingly, many of the antimicrobial mechanisms present in teleost neutrophils are utilized both as intracellular and extracellular defences (Havixbeck et al, 2015).

According to the results of Marzouket al. (2008), a positive effect represented by significant increase in differential leukocytes count, these could be attributed to the fact that, the probiotics used increased the blood parameter values because of haematopoietic stimulation. However, WBC levels, particularly neutrophil, were elevated in fish fed dietary containing Celmanax® (Tables 2,3). This was similar to the results of Andrews et al., (2009), who observed a significant improvement in WBC, RBC and Hb in rohu *(Labeo rohita)* fed MOS supplemented diet in comparison with those fed on the control diet.

In other study, Talpur et al., (2014) showed effects of commercially available probiotic and prebiotics on growth performances, haematological and immune response and disease resistance in *Channa striata* fingerlings against the pathogenic bacteria *Aeromonas hydrophila*. Dietary probiotic and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *A.hydrophila* in Snakehead (*Channa striata*) fingerlings. Fish were fed six different diets up to 12 weeks containing single dosage of *Lacto acidophilus* at 1 g kg-1 feed (1010CFU), yeast 1%, β-glucan 0.1%, Galactooligosacharide (GOS) 1% and Mannan oligosaccharide (MOS) 0.2 % and control without any supplement. Dietary probiotic and prebiotics improved haematology parameters. There was a significant improvement in red blood corpuscles (RBCs), white blood corpuscles (WBCs), pack cell volume (PCV), haemoglobin (Hb%) concentration, erythrocyte sedimentation rate (ESR) and serum protein content in treated groups over the control (Talpur et al., 2014).

A similar study was conducted on the effect of Mannan oligosaccharide supplementation in carps by Momeni-Moghaddam et al., in 2015. Momeni-Moghaddam et al., results showed administration of MOS at 0.05-0.20% improves FCR, modulates intestinal microbiota and at 0.20% elevates the humoral immune response of common carp by increasing the alternative complement activity, lysozyme activity and serum total immunoglobulin (Momeni-Moghaddam et al., 2015).

CONCLUSIONS

The administration of probiotics varies from oral/water routine to feed additives, of which the latter is commonly used in aquaculture. Probiotic applications can be either mono or multiple strains, or even in combination with prebiotic, immune stimulants such as symbiotic and symbiotic, and in live or dead forms. Accordingly, data in this study showed that addition of Celmanax® (0.1% diet) to the rainbow trout diet could order better Hb, MCV, MCH, MCHC, white blood cell and neutrophil rate with values statistically higher than those from the control. In conclusion, the results of this study show that the addition of Celmanax® in died could increase the immunity performances, betterment alkaline phosphatase level and supposition improve phagocytosis (neutrophilia) and health management in aquaculture.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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