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ORIGINAL ARTICLE

Determination on Growth indices, Blood parameters, Immune responses and Intestine bacterial flora of Bester juvenile fish (*Huso huso×Acipenser ruthenus*) Fed by Biomin Nutritional supplements

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ABSTRACT
This study is evaluated the effects of Biomin P.E.P supplement on growth indices, blood and immune factors, and intestine bacterial flora of Bester juvenile fish. The survey is conducted on Bester juvenile sturgeons in fisheries laboratory located in Islamic Azad University Science and Research of Guilan Branch in the fall of 1392. The completely random scheme of the research includes 4 treatments each of which has 3 repetitions. The supplement was added to the basal diet on 4 levels of 0, 1, 1/5, and 2 g / kg.72 of 45-g Bester fish were fed for 60 days based on 5 percent of the body weight in 12 tanks (100-liter) that 6 fish in each tank. At the end of the farming period some of the growth indices, blood and immune factors, and intestine bacterial flora were compared. So that in treatment 2 and in the level of 1/5 g/kg in some growth and nutrition factors such as average initial weight, total weight, total length, FCR, SGR, PBWI, BWI, ADG, at the end of the period, a significant difference was observed between treatment and control (P<0.05). But in the factors like Average initial length and Condition Factor(K), there was no statistically significant differences between treatment and control at the end of the period (P>0.05). Evaluation of blood factors indicated that the rate of red blood cells (RBC), white blood cell(WBC), hemoglobin (Hb), hematocrit (HCT), neutrophils, has decreased by increasing amounts of Biomin P.E.P compared to the control, and showed significant statistically difference (P<0.05). In evaluation of biochemical and immune factors, the amount of total immunoglobulin, albumin, total protein with different amounts of Biomin P.E.P had significant statistically difference (P<0.05).Therefore, this supplement affects the blood biochemical factors and can influence on their recovery. The total count of bacterial flora in the dilution TSA 10^{-1} showed that the levels of 1 and 1/5 g/kg has less amount compared to the control, and in MRS10^{-1} at the level of 1 g/kg LAB bacteria were higher compared to control, and it can be said with reducing bacteria on TSA, LAB bacteria have been replaced. it is therefore suggested of 1/5 g Biomin Nutritional Supplement in the 1 kg diet.

Keywords: *Bester Sturgeon (Huso huso×Acipenser ruthenus), Biomin P.E.P, Intestine bacterial flora, Growth, Blood and Immune responses.*

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INTRODUCTION

In recent years with reducing Sturgeons fishing in the Caspian Sea, their fattening cultivation has achieved strong motivation in the world, including Iran. As today in many parts of the world containing Iran, meat production and the possibility getting larvae from natural spawners and thus Forming sturgeon breeding herds in soil pools, fiberglass tubs and concrete tanks is provided [1].Today scientists believe that the only way to save valuable resources of Sturgeon is to fulfill research and executive activities with the aim of maintaining and restocking resources and development the technology of breeding sturgeon in breeding environments, preventing illegal fishing, and cooperation with international organizations to protect biodiversity and endangered species. Scientific studies show that the efficiency of feeding, feeding rate, water temperature and fish size are the economic factors that

determine the commercial Production competency of fish [2].So in order to increase production efficiency and provide greater profitability, economic evaluation of nutrition and determining the nutritional requirements of fish are essential [3]. For commercial and effective production of Acipenseri management, good conditions for breeding, feeding with proper diet including less expensive, yet effective ingredients that provide optimum growth with lowest FCR are seem necessary [4]. Currently the major problem in commercial aquaculture is improving formulated diets in order to growth acceleration and enhancing the health of the fish. Researchers believe that increasing of nutrition efficiency in aquaculture production depends on the food ingredients such as proteins, fats, vitamins, minerals and also factors like price, and availability [5]. Growth rate and disease resistance in farmed fish are two important issues [6]. Bacterial diseases are much lower intensive aquaculture production and the need to improve the safety and survival of fish and economic gains control of this place. In many marine fish, microbial diseases in particular during the production of young fish caused mortality of fish, and limited fish production to a sustainable level on an industrial scale [7]. Traditionally antibiotics have been added in aquatic foods to prevent and treat bacterial diseases. It is reported that antibiotics can increase growth rate and fish nutrition efficiency by killing the bowel pathogenic bacteria, but use of antibiotics may leads to the state of pretending to cure, that is, more resistant bacteria species don't respond to antibiotic treatment and cause environmental risks [8].Given the dangers of antibiotics and their prohibition in many developed countries, these changes affected in government policy on aquaculture, and thus development of other methods have been considered for Disease Control very quickly, such as; the use of dietary supplements including probiotics, prebiotics and immune stimuli which are very popular [6].
In the present study the Biomin PEP dietary supplement contains two sets of combination that combined in an isotonic method including essential oils (oregano essential oil, which contains caracrol material that has potent anti-microbial and anti oxidative effects.

MATERIALS AND METHODS

Requirement materials: Biomin dietary supplement, 12 fiberglass tanks of 100-liter for storage farmed juvenile Bester, pumps and aerators stone, bioassay boards to measure the length of the fish, WTW device for measuring water parameters like temperature, oxygen, pH. Digital weight, hardness test kit, ethanol, 2^{cc} syringe, Heparin, distilled water, Saline, cotton, test tubes, Scalper razor No. 23, Yuri bottle sampling containers, 8 cm plates, capillary pipettes, tissue paper, freezer bags, TSA & MRS culture medium, meat grinder, small pan, oven.

Experiment methods: Survey procedures are included field assay and laboratory experiment. This study was conducted in the fall of 2013 at Azad University Science and Research, Guilan Branch of located in Rasht. The survey was conducted with 1control group and 3 treatment groups in three replicates, so that the treatment 1,2,3 is composed of 1,1/5,2 g Biomin per kg of food respectively and treatment 4(control) considered no Biomin in the diet. Mean while, 6 Bester juvenile Sturgeons with average weight of 48.22 ± 1.89 and average total length of 24.5± 1.18 are placed in each treatment. Feeding is carried out at 5percent of the average weight at 3 times per day, every 8 hours for 2 months. Average water temperature during breeding period was 22.8 ° ^{C} and average of dissolved oxygen was 10 ^{ppm}, and water hardness was about 307^{ppm}, CaCO3. Bioassay operation was conducted during the third shift (Initial, middle and end of the period).

Dietary making: For food preparation, the values of 1, 1/5 and 2 g of Biomin should be milled and mixed well into 1 kg of ration. In order to have a homogeneous mixture, some water should beaded to this material, after mixing, we changed the shape of the material into cylindrical plates by meat grinder to make them proportional to the size of the fish's mouth. Then we put the rations into trays that was covered with aluminum sheet and placed them in oven at 30 ° C for 24 hours to dry. After that the plates were chopped in proportion to the size of the fish's mouth, and prepared rations were weighed and packed to use in each 24 hours, additional food for each treatment group was packed within 2 plastic bag (two layer), in order to avoid increasing the humidity.

Basal diet ingredient: Basal diet is Biomar (EFICO Sigma840 No. 6.5) which includes the following ingredients: protein brute %43, crude fats %18, cellulose brute %3.7, crude Ash %7.1, phosphor %1.15, calcium % 1.40, and Sodium %0.50.

Specifics Growth: (Average Daily Growth) ADG%, Body Weight Increase (BWI), Percent Body Weight Increase (PBWI), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Condition Factor (K). [9,10].

Hematological and intestine bacterial studies: After 8 weeks of farming trial and 24 hours of feeding cut off and full ensuring of completely defecation, the fish were caught and blood samples were taken from the tail vein or artery located behind the Anal fin of bed fish. For hematology studies 2^{cc} syringes were used. After taking the blood by syringe from caudal vein of the fish, 0.5^{cc} of the blood was poured

into a numbered vial smeared with blood anticoagulant (heparin) for blood factors examination, and the remaining 1.5cc was poured into a numbered, non heparin vial for blood serum biochemical and immune factors studies. For serological studies the blood in eppendorf tubes without blood anticoagulant(heparin) was centrifuged (Labofuge model by Heraeus sepatch) with 4,000 rpm for 10 minutes, the serum was separated and poured into new eppendorfs by sampler, then the samples away from vigorous shaking were sent to hematology laboratory while packed into a freeze-dried ice box. It should be noted that because of the potential impact on the levels of blood indices no anesthetic substances were used during blood sampling [11]. Immediately after blood sampling we cut the stomach of the fish, removed intestine and put it into a sterile Yuri bottle containing 10 ml of saline, and transferred it to Viro Med bacteriological laboratory located in Rasht, Guilan for the study of intestinal flora. Measurement of blood serum biochemical factors, total immunoglobulin, lysozyme, IgM, glucose, triglycerides, total protein and albumin done by [12].White blood cell differential diagnosis, measurement of hematocrit done (PCV) [13], Measured hemoglobin [14], Bacterial culture method [15], Method of preparing 10^{-1} and 10^{-7} dilutions, Primary culture method [16]. And Colonies counting method and their classification done by [17].

Statistical analysis: this research was carried out in a completely random pattern. Initially, data normality and homogenity with the Shapiro-Wilk and Levene tests respectively was performed, in the case of normal distribution of data, for the comparison between the dietary treatments One-way ANOVA test and for separation of homogeneous groups Tukey test at %5 probability levels were used. For abnormal data with non-parametric Kruskal-Wallis test was used and meaningfulness of examined groups was determined using the Mann-Whitney. Statistical software SPSS Version 17 for data analysis and Excel 2007 for drawing graphs was carried out.

RESULTS

Growth indices: Adding the levels of 1, 1.5 and 2 g/kg Biomin PEP supplement containing fructo oligo saccharide prebiotic to each 1kg of food ration or diet for 60 days caused the fed fish to show better growth in the last biometry. So that in treatment 2 at 1.5 g per kg some of growth and nutritional factors such as: Average initial weight, average total weight, Average total length, average daily growth (ADG), body weight increase (BWI), percentage of body weight increase (PBWI), specific growth rate (SGR) and feed conversion ratio (FCR) the statistical significant difference was observed between the treatment and control at the end of the course ($P<0.05$)، however, in the factors such as: average initial length and obesity coefficient (K), there was no a significant difference between the control and treatments at the end of the course ($P>0.05$), Table 1.

Table 1: Mean changes (mean ± SD) some of the fish growth indices in different treatments compared to the control.

Control	P.E.P 2g	P.E.P 1.5g	P.E.P 1g	Treatment ---------- factor
1.89[b]± 48.33	2.29 [a]±47.27	0.58[b] ±48.75	1.08 [a] ±47.71	Average initial weight (g)
1.83 [a] ± 69.15	1.59 [b] ±71.34	2.57 [c]± 112.76	1.17 [a] ±68.12	Average total weight(g)
1.09 ± 24.5	1.18 ± 24	1.18 ± 24.5	1.26 ±24	Average initial length (cm)
1.18 [a] ± 28	1.18 [a] ± 28.5	1.18 [b] ±35.5	1.26 [a] ±28	Average total length (cm)
0.04 [a] ±0.72	0.075 [b] ±0.85	0.035 [c] ± 2.18	0.015 [a]±0.69	ADG (%)
[a] 3.04 ± 124.94	[b] 4.69 ± 144.4	10.13[c]±384.1	2.76 [a] ±122.4	BWI (%)
2.31[a] ± 43,17	4.27 [b] ± 51	2.02 [c]±131.17	[a] 1.26 ±42.83	PBWI (%)
[a] 0.026 ± 0.59	[b] 0.051 ±0.69	[c] 0.012 ± 1.39	[a] 0.012±0.59	SGR (%)
[c] 0.29 ± 6.17	[b] 0.29 ± 5.17	[a] 0 ± 2	[c] 0.29 ± 6.33	FCR (%)
0.25 ± 3.13	0.4 ±3.07	0.25 ±2.53	0.45 ± 3.07	K (%)

Non-similar abbreviation signs in each row indicate statistically significant difference between treatments. ($P < 0.05$).

Blood factors: The results showed that the amount of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Hct), and neutrophils, decreased with increasing percentages of Biomin PEP compared to control and showed statistically significant differences ($P<0.05$),in other blood factors, no statistically significant differences were observed ($P>0.05$), Table 2.

Table 2: Comparison of blood factors of Bester juvenile fish (Mean ± SD) in different treatments compared to the control.

Control	P.E.P 2 g	P.E.P 1.5 g	P.E.P 1g	Blood parameters
650.6 [b] ±9766.7	665.8 [ab]±8666.7	1014.9 [ab]±8400	[a] 850.5± 6833.3	WBC No×10^3
18036.9[b]±628333.3	38552.9[ab]±595333.3	38742.7[ab]±567000	22810.8 [a]±522666.7	RBC No×10^6
[b] 0.15±6.33	[ab] 0.4 ±6.07	[ab] 0.42±5.73	[a] 0.25±5.27	Hb g/dl
1.53 [b]±31.3	[ab] 2.08±29.7	[ab] 2.08±27.7	1[a]±26	PCV٪
10.2 ±498.3	3.1 ±497.7	4.2 ±487.7	4.5 ±497.3	MCV FI
0.58±100.3	0.58 ±101.7	0.58 ±100.7	0.58 ±100.3	MCH pg
0.58±20.3	0.58±20.3	0±21	0±20	MCHC٪
[b] 0.58±31.3	[ab] 1.16±29.3	1.53[b]±30.7	[a] 1.53±26.3	Neutrophils٪
1.16±66.3	1.16 ±66.7	4.16±67.3	1±71	Lymphocytes٪
0.58±2.33	0.58±3.33	1±3	0.58 ±2.33	Monocytes٪
0.58±0.31	0.58 ± 0.67	0.58±0.33	0.58±0.33	Eosinophils٪

Non-similar abbreviation signs in each row indicate statistically significant difference between treatments. (P < 0.05).

Blood serum biochemical parameters: The results have shown that between the studied treatments in terms of total protein amount and albumin of blood, there is a statistically significant difference (P<0.05) but there is no statistically significant difference in glucose and triglycerides. However, due to the amount of total protein and albumin in the treatment of 1.5 g per kg were higher compared to control, so we can say that the Biomin P.E.P food supplement affected blood biochemical factors and improve their recovery. Table 3.

Table 3: Comparison of blood biochemical factors (mean ± SD) in different treatments compared to the control.

Control	P.E.P 2 g	P.E.P 1.5 g	P.E.P 1g	Blood parameters
3 ± 31	12.5±47.7	7 ± 35	1.16 ±32.3	Glucose (mg/dl)
79.5±312.7	112.9±327.3	33.4 ±337	100.1 ±174.7	Triglycerides (mg/dl)
[b] 0.1±1.4	[b] 0.12 ±1.47	0.15 [b] ±1.43	0.1[a] ± 1.1	Total protein (g/dl)
[b] 0.064 ± 0.483	[a] 0.015 ± 0.307	0.095[b]±0.617	[b] 0.08±0.503	Albumin (g/dl)

Non-similar abbreviation signs in each row indicate statistically significant difference between treatments. (P < 0.05).

Blood immune factors: The results showed that between the treatments compared to the control, in terms of total immunoglobulin levels in blood, statistically significant difference is observed (P<0.05), However, no statistically significant differences observed in lysozyme and IgM (P> 0.05),while the amount of lysozyme and IgM compared to control, respectively, in treatment 2 and 1/5 and 1 g per kg have increased. So we can say that the Biomin food supplement affects blood immune factors and can play a role in improving the immune system Table 4.

Table 4: Comparison of blood immune factors (Mean ± SD) in different treatments compared to the control.

Control	**P.E.P 2 g**	**P.E.P 1.5 g**	**P.E.P 1g**	**Blood parameters**
1.53[b]**±**12.67	[b] 1.53 **±**12.33	[b] 1.16 **±** 11.67	[a] 0.58 **±** 9.33	Total immunoglobulin mg/dl
1.53 **±** 12.67	4.16 **±**19.67	4.93 **±** 18.33	2.08 **±** 13.67	Lysozyme (µg/dl)
1.53 **±**9.67	5.03 **±** 13.67	2.08 **±** 12.67	2.08 **±** 12.67	IgM (mg/dl)

Non-similar abbreviation signs in each row indicate statistically significant difference between treatments. (P< 0.05).

Intestine bacterial flora at MRS (10^{-1}, 10^{-7}) and TSA (10^{-1}, 10^{-7}) media: The results showed that between the treatments in terms of amount of intestinal bacterial flora in vitro TSA (10^{-1}) statistically significant difference is observed (P<0.05),so we can say that respectively the levels of 1, 1/5 g per kg decreased compared to the control. Also in TSA (10^{-7}) medium in spite of absence of statistically significant difference (P>0/05) a reduction in amount of intestinal bacterial flora in the levels of 1, 1/5 g

per kg can be observed compared to control. In terms of intestinal bacterial flora amount in MRS (10^{-1}) medium statistically significant difference is observed (P<0.05),as at 1 g per kg compared to control increasing of LAB bacterial obvious. But in MRS(10^{-7}) medium no statistically significant differences observed (P>0/05), So in general we can say that by reduction of bacteria in the TSA culture medium LAB bacterial were replaced. Table 5.

Table 5: Comparison of the intestinal bacterial flora (Mean ± SD) in different treatments compared to the control.

Control	P.E.P 2 g	P.E.P 1.5 g	P.E.P 1g	bacterial medium culture
^{c} 0 ± 1000	^{c} 0 ±1000	55.77^{b}±66.67	143.11^{a}±141.33	**TSA10^{-1}**
4.62 ± 2.67	2.31 ±1.33	0± 0	0.58 ± 0.33	**TSA10^{-7}**
^{a} 0 ± 0	16.15^{ab}±10	24.18^{ab}±18	107.24^{b}±109	**MRS 10^{-1}**
0 ± 0	0 ± 0	0 ± 0	0 ± 0	**MRS 10^{-7}**

Non-similar abbreviation signs in each row indicate statistically significant difference between treatments. (P < 0.05).

DISCUSSION

Economic aquaculture has grown considerably in recent years [18]. In recent studies have been conducted to improve the quality of food used in rearing. Fish, especially when grown in high-density, need high-quality and balanced diet full of nutrients to grow faster. Recently, the use of prebiotic in aquaculture as a strategy has been proposed. These combinations are parts of non-digestible food ingredients, and it is believed that they can reduce the deleterious effects of infectious agents and increase survival in the face of pathogens, by improving the intestinal bacterial flora [19].

Growth indices: The use of F.O.S prebiotic at levels 1 and 2 percent in the experimental diets of juvenile *Acipenser stellatus*, and its impact on some of the growth and nutrition factors indicated that F.O.S had a significant effect on the growth and nutrition factors. As a significant difference is observed in the two treatments containing 1 or 2% compared to the control (P<0.05). Also the amount of FCR in 1% treatments was less than other treatments [20].The use of inulin prebiotic in the levels 0,1,2,3 percent in the diet of juvenile *Huso huso* showed that different levels of inulin prebiotic don't have high potential to impact on growth performance and feed efficiency in farmed *Huso huso* and this prebiotic cannot be a suitable supplement for *Huso huso* [21]. Compared with a control population, increase in final weight, feed efficiency ratio and Survival percentage were observed in *Ictalurus punctatus* which were fed for 6 weeks with diets containing 2g/kg Bio-MOS [22].Adding immunogen prebiotics at 1% level to the diet of juvenile *Acipenser persicus* showed the best percentage of weight gain, specific growth rate and the rate of obesity compared with other treatments which had statistically significant difference [23].Adding1% level immunogen prebiotics to the diet of juvenile *Acipenser baerii* has resulted in better growth than the control group at 75 days in the last biometry. As final weight, final length and biomass of the fish in the last biometry have statistically significant difference compared to the control [24]. Adding 1% fructo oligo saccharide prebiotic to the diet of juvenile *Acipenser stellatus* had statistically significant effect on the factors of weight increase, specific growth rate, feed conversion ratio, feed efficiency and protein efficiency ratio (P<0.05).As final conclusion, fructo-oligosaccharide prebiotic has good capability to influence growth performance increasing and feed efficiency in *Acipenser stellatus* [25]. In the present study, adding 1.5 g per kg Biomin PEP in the diet of bed juvenile *Huso huso* × *Acipenser ruthenus* has resulted in better growth than the control group at 60 days in the last biometry. As in some of the growth and nutritional factors such as: Average initial weight, average total weight, total length, average daily growth (ADG), body weight increase (BWI), percentage of body weight increase (PBWI), specific growth rate (SGR) and feed conversion ratio (FCR) the statistical significant difference was observed between the treatment and control at the end of the course (P<0.05).

Blood and Immune parameters: Innate or non-specific immune system in fish is an essential defense mechanism against pathogens, strengthen this system is very valuable for farmed fish because they are vulnerable against many bacterial agents [26].Adding 1 and 3 percent levels of immunoster and immunoval immune stimulants to the diet of *Huso huso* with an average weight of 95 g over a period of 8 weeks, has resulted increase in safety factors such as lysozyme and IgM compared to the control group. However, this increase was not significant [27]; While in this study despite the increase in the amount of lysozyme and IgM respectively in 2, 1.5, 1 g treatments compared to the control, no statistically significant difference in lysozyme and IgM was observed (P>0.05). Therefore, we can say that the Biomin PEP food supplement affects blood immune factors and can play important role in improving the immune system. Adding 1, 2, 3 percent levels of inulin prebiotic in the diet of *Huso huso* showed that the control group had a better condition in terms of number of red and white blood cells, hematocrit and hemoglobin, MCHC,

MCV, MCH, total protein, than the groups that have fed of inulin [28]; While also in this study the control group had a better condition than the groups that have fed of Biomin PEP. Adding 0, 2, 4 percent levels of immunoster prebiotic to the diet of juvenile *Rutilus kutum* with an initial weight of 0.02±0.35 g over a period of 8 weeks, showed statistically significant difference in differential diagnosis leukocytes (P<0.05).Thus, by increasing levels of immunoster in the diet, respectively, the percentage of lymphocytes increased and monocytes, neutrophil and eosinophil significantly decreased. As the level of 4% immunoster had the highest amount of lymphocytes and lowest amount of monocytes, neutrophils and eosinophil.The results revealed that the studied levels didn't have any significant effect on growth performance. While as an immune stimulus is capable of positively affect blood leukocytes and increasing the efficiency of lymphocytes as one of the important indicators of specific immune cell [29]. While in the present study by adding different amounts of Biomin PEP to the diet of juvenile *Huso huso* × *Acipenser ruthenus* with an initial weight of 48/33 ± 1/89g over a period of 8 weeks, statistically significant difference was observed(P<0.05), but in the other leukocytes no statistically significant difference was observed (P>0.05).But in the differential diagnosis of leukocytes it can be seen that in lymphocytes by decreasing the amount of Biomin PEP in 1g level diet compared to the control has increased, and in 1g level neutrophils and monocytes has decreased compared to the control, and also in eosinophils control had the least amount. The results showed that the different studied levels of Biomin PEP in diets in addition to have significant effect on growth performance can positively affect blood leukocytes and increasing the efficiency of lymphocytes as one of the important indicators of specific immune cell. In study of effect of F.O.S prebiotic on some blood biochemical indices of blood serum and liver enzymes of *Huso huso*, the results showed that Oligo-fructose can be immune stimulator and improve metabolism of fat [30]. In the present study also the amount of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Hct), and neutrophils, decreased with increasing percentages of Biomin PEP compared to control and showed statistically significant differences (P<0.05),),in other blood factors, no statistically significant differences were observed (P>0.05).But in amount of total protein and albumin statistically significant difference was observed (P<0.05), however, no significant difference was observed in glucose and triglycerides. So due to the higher amount of total protein and albumin in the 1.5g treatment compared to the control, we can say that Biomin PEP can affect the blood biochemical and immune factors and can play important role in improving them.

Intestine Bacterial Flora: Microbial flora existing in the digestive tract of human beings and the land creatures is almost constant. While, because aquatic are cold-blooded and their body temperature comply with temperature of water microbial flora is constantly changing [31]. After entering the gastrointestinal tract, bacteria either settle in the digestive tract and become a part of the normal flora of the colon or will be destroyed by antimicrobial agents, digestive secretions and unsuitable conditions of the gastrointestinal tractor directly excreted with feces. Bacteria may also after settling in the outer surfaces of the gastrointestinal tract act as a primary pathogen and causing disease. Records published in 1993 by Austin indicate the existence of a wide variety of moving *Aeromonas* and *Pseudomonas* genera in fish ponds [32]. In the digestive tract of freshwater fish *Enterobacter*, *Aeromonas* and *Acinetobacter* are the dominant species. On the other hand, in the digestive tract of saltwater fish *Vibrio*, *Pseudomonas*, *Achromobacter*, *Corynebacterium*, *Micrococcus* and *Flavorbacterium* are dominant [33]. Adding 1.5 percent *Saccharomyces cerevisiae* cell wall prebiotic in the diet of *Huso huso* improves their intestinal micro-flora [34]. The results of study of the composition and antimicrobial properties of *Zataria multiflora* essential oil showed its anti-microbial power [35]. However, in this study statistically significant difference is observed in the amount of Intestinal bacterial flora in TSA (10^{-1}) medium between the studied treatments (P<0.05). So we can say that respectively 1 and 1/5, g levels decreased compared to the control. Despite the absence of statistically significant difference in TSA (10^{-7}) medium (P>0/05) ‹the reduction of bacterial flora in the 1.5 and 1 g per kg levels compared to control is obvious. Statistically significant difference is observed in the amount of intestinal bacterial flora in MRS (10^{-1}) (P<0.05). As in 1 g per kg level the increasing of LAB bacteria compared to the control is obvious. But no statistically significant difference observed in MRS (10^{-7}) medium (P>0/05). So we can conclude that by reduction of bacteria in TSA medium LAB bacteria were replaced. It is therefore suggested of 1/5 g Biomin Nutritional Supplement in the 1 kg diet.

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