

## RESEARCH ARTICLE

# Could the Vitacel R200 improve the Immune Response and Hemato-Biochemical Parameters of Rainbow Trout (*Oncorhynchus mykiss*) challenged with *Aeromonas hydrophila*?

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## ABSTRACT

The fermentable fiber, Vitacel R200 is industrial compound cellulose exerting many beneficial health effects at the hosts. In this study, rainbow trout (*Oncorhynchus mykiss*) fingerling ( $120 \pm 4.5$  grams) were fed dietary 1% (10 g/kg feed) Vitacel R200 for 7 weeks, then challenged with *Aeromonas hydrophila*. Two weeks after the challenge, hemato-biochemical and immune parameters in the challenged fish were studied. The lysozyme and complement activity, as well as, white blood cells (WBCs), hemoglobin (Hb%), percentage of neutrophil and eosinophil in the fish fed Vitacel were significantly increased compared with the control group. Furthermore, the serum total protein, albumin,  $\text{Na}^+$ ,  $\text{K}^+$ , P, and  $\text{Ca}^{++}$  concentrations in the fish fed diet containing 1% Vitacel were significantly increased, while alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST), as well as, cortisol, cholesterol, and glucose concentration were significantly decreased in comparison to the control group. These results concluded that dietary Vitacel could improve the hemato-biochemical and immune parameters, as well as, the stress response in *O. mykiss* infected with *A. hydrophila*.

**Keywords:** *Aeromonas hydrophila*, Hematology, Trout, Vitacel.

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**Conflict of interest:** None

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## INTRODUCTION

The rainbow trout (*O. mykiss*) is one of the most important farmed fish species with increasing interest to intensive culture, which induces stress and negatively affects the physiological response and immune system of fish,<sup>1</sup> consequently resulting in the emergence of different infectious pathogens.<sup>2-4</sup> The *A. hydrophila* is an opportunistic gram-negative and motile pathogen causing *Aeromonas* septicemia with various symptoms, such as, hemorrhagic septicemia, infectious abdominal dropsy, and fin and tail rot in different fish species.<sup>5</sup> Despite the great concerns regarding the resistant pathogen and environmental issues, the control of fish diseases has mainly focused on the antibiotics, and more recently on the vaccination.<sup>6-8</sup> Thus, there is increasing interest in using dietary immune-stimulants (such as, prebiotic, probiotic, and plant extracts) as a potential and promising alternative to

improve the health status and disease control in aquaculture.<sup>9,10</sup> The prebiotics in fish have revealed many beneficial effects on growth performance and feed utilization,<sup>11</sup> intestine microbiota, and morphology,<sup>12</sup> and resistance against pathogenic bacteria.<sup>13</sup> Vitacel is industry compound cellulose in the category of non-starch polysaccharides (NSP) with at least 70% of crude fiber. Previous studies in fish have shown the positive effects of dietary Vitacel on hematological parameters and immune response in beluga sturgeon *Huso huso*,<sup>14</sup> as well as, humoral immune parameters.<sup>15</sup> There is limited information about the protective effect of dietary Vitacel on physiological and stress response, hematological, and immune parameters of infected fish. Therefore, this study was aimed to investigate the effects of Vitacel on innate immune response, hemato-biochemical parameters, stress resistance, and enzyme activities of rainbow trout challenged with *A. hydrophila*.

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**MATERIALS AND METHODS**

**Fish and Experimental Diets**

Rainbow trout (*O. mykiss*), with the mean weight of  $120 \pm 4.5$  grams, were obtained from local fish culture (Karaj, Iran), and transferred to the indoor laboratory condition adapted for 14 days. During the experiment, fed on the basal diet (Behparvar Co, Iran). The tap water was used, and the water temperature, dissolved oxygen, and pH levels were  $17 \pm 1.5^\circ\text{C}$ , 6.5 ppm, and  $7.8 \pm 0.2$ , respectively. After acclimation, 80 fish were distributed into two triplicate groups (each of 20 fish per tank). The treatment group fed a diet containing 10 g/kg feed of Vitacel. For the formulation experimental diet, 1% of the dietary supplement Vitacel were added into commercial diet rainbow trout (Behparvar Co, Iran). A daily feeding rate of 3% of the biomass was performed for 7 weeks. The approximate composition of the experimental diets has been shown in Table 1.

**Bacterial Challenge (*A. hydrophila*)**

After 7 weeks feeding with experimental diets, fishes anesthetized with clove powder (200 mg/L), and were injected intraperitoneally with 0.2 mL ( $5 \times 10^8$  CFU/mL) bacterial suspension of *A. hydrophila* (RTCC1032) cultured in Brain Heart Infusion (BHI, Merck, Germany) at  $37^\circ\text{C}$ .

**Serum Lysozyme and Alternative Complement Activity ( $\text{ACH}_{50}$ )**

Lysozyme activity was measured according to Parry *et al.*<sup>16</sup> Alternative complement activity assays were also carried out using rabbit red blood cells (RaRBCs) according to the method of Van Doan *et al.*<sup>17</sup>

**Blood Collection and Hematological Assay**

Two weeks post-challenges, five fish from each tank were sampled and anesthetized with clove powder (200 mg/L), and then, blood collection was taken from the caudal peduncle and transferred in two heparinized tube and non-heparinized tubes. Hematological parameters, including WBCs, differential leukocyte count (DLC), red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb), mean erythrocyte hemoglobin (MCH), mean erythrocyte cell volumes (MCV), and mean erythrocyte hemoglobin concentration (MCHC) were measured, as described by Svobodova Z *et al.*<sup>18</sup>

**Serum Biochemical Analysis**

Serum enzymes lactate dehydrogenase (LDH), ALP, ALT, AST activities, total protein, cortisol, cholesterol, triglyceride, glucose, as well as, serum  $\text{Na}^+$ ,  $\text{K}^+$ , P, and  $\text{Ca}^{++}$

**Table 1:** Approximate composition of the experimental diets

Item	Proximate analysis (%)
Dry matter	14
Crude protein	45.3
Crude lipid	37
Ash	10
Phosphorous	> 0.7

levels were determined by an auto-analyzer (Eppendorf, EPOS, Germany), using commercial kits (Pars Azmoon, Iran), according to the manufacturer protocols.

**Statistical Analysis**

All data were analyzed and expressed as means and standard error ( $M \pm SE$ ). The data were subjected to an independent sample t-test, using SPSS 23 package at 0.05 significance level.

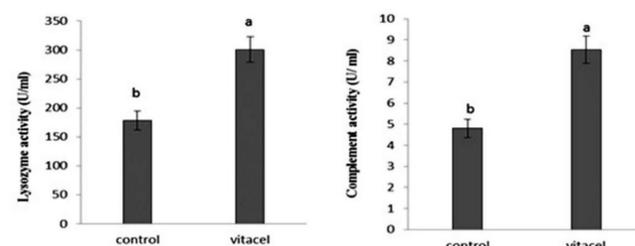
**RESULTS**

**Hematological Parameters**

The results of hematological parameters of rainbow trout fed Vitacel, and challenged with *A. hydrophila* has been shown in Table 2. The hemoglobin value, the total number of white blood cells (WBCs), and the percentage of monocyte and eosinophil were significantly increased in the fish fed Vitacel compared with the control ( $p < 0.05$ ). However, no significant difference was found in RBCs, lymphocyte, monocyte hematocrit (HCT), as well as, MCV, MCH, MCHC values ( $p > 0.05$ ).

**Serum Lysozyme and Alternative Complement Activity ( $\text{ACH}_{50}$ )**

The results of serum lysozyme and complement activity in fish fed Vitacel, and infected with *A. hydrophila* are presented in Figure 1. The serum lysozyme and complement activity in infected fish fed with 10g/kg feed Vitacel were significantly higher in comparison to the group that fed the control diet ( $p < 0.05$ ).



**Figure 1:** Lysozyme and complement activity of *O. mykiss* fed Vitacel R200, and infected with *A. hydrophila*; different letters indicate significant differences between the control and experiment groups ( $p < 0.05$ )

**Table 2:** Hematological parameters of *O. mykiss* fed Vitacel R200, and infected with *A. hydrophila*

Parameters	Control	Vitacel
RBC ( $10^4/\mu\text{L}$ )	$1.43 \pm 0.1^a$	$1.38 \pm 0.1^a$
WBC ( $10^4/\mu\text{L}$ )	$5.95 \pm 0.47^a$	$4.03 \pm 0.42^b$
Hct (%)	$41 \pm 1^a$	$38 \pm 2^a$
Hb (g/dL)	$12.86 \pm 0.35^a$	$11.5 \pm 0.43^b$
MCH (pg cell <sup>-1</sup> )	$89.8 \pm 7.62^a$	$83.7 \pm 8.24^a$
MCHC (%)	$31.38 \pm 0.88^a$	$30.31 \pm 1.91^a$
Lymphocyte (%)	$82 \pm 2^a$	$85.66 \pm 1.15^a$
Monocyte (%)	$1.5 \pm 0.5^a$	$1.5 \pm 0.5^a$
Neutrophil (%)	$10 \pm 1^a$	$7.33 \pm 0.57^b$
Eosinophil (%)	$6.5 \pm 1.32^a$	$5.5 \pm 1^b$

Different letters indicate significant differences between the control and experiment groups ( $p < 0.05$ )

### Serum Enzyme Activity

The effect of dietary Vitacel on some serum enzymes activity in infected rainbow trout with *A. hydrophila* is shown in Figure 2.

The serum level of ALP, AST, and ALT were significantly lower in infected fish fed with Vitacel ( $p < 0.05$ ). However, there was no significant difference in serum LDH activity ( $p < 0.05$ ).

### Serum Ions Concentration

In this experiment, some of the ions to assess stress levels were measured (Figure 3). It was found that the amount of  $\text{Na}^+$ ,  $\text{K}^+$ , and P in the fish fed dietary supplementation of Vitacel R200, and challenge with *A. hydrophila* were significantly higher than the control group ( $p < 0.05$ ). However, no significant difference was observed in the serum  $\text{Ca}^{++}$  concentration.

### Serum Biochemical Parameters

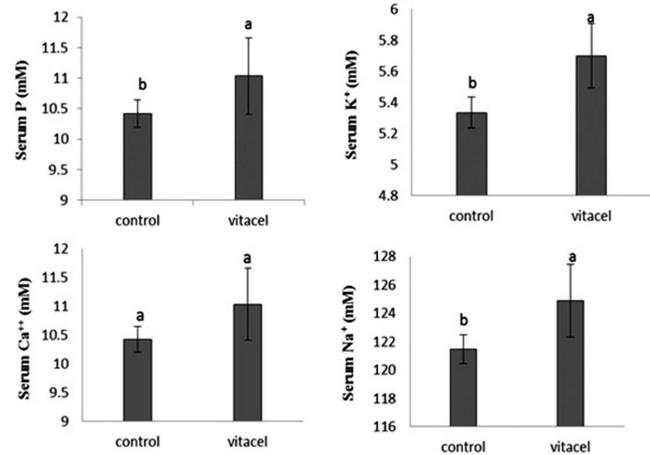
The results of serum biochemical analysis are shown in Figure 4. The serum total protein and albumin concentration in the fish fed diet containing 1% Vitacel were significantly increased, while cortisol, cholesterol, and glucose concentration were significantly decreased in comparison to the control group ( $p < 0.05$ ). However, the serum triglyceride level was not affected by dietary Vitacel.

### DISCUSSION

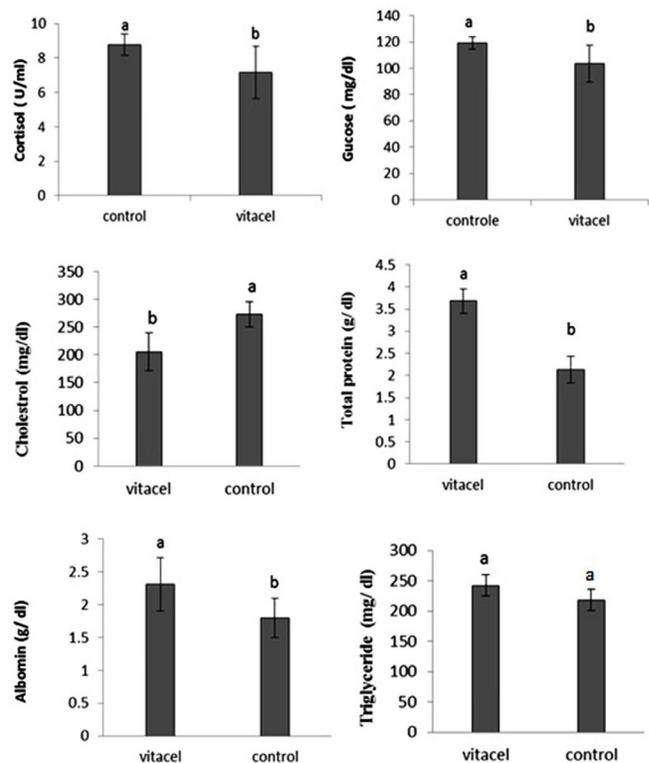
Consistent with these studies, our study revealed that Vitacel dietary can improve innate immune response and hemato-biochemical parameters of rainbow trout (*O. mykiss*) challenged with *A. hydrophila*.

Since the blood parameters can be influenced during stressful conditions induced by a variety of factors, such as, infection, dietary imbalance, and environmental pollution, have considered useful tools to evaluate the physiological and health status of fish.<sup>19-21</sup> It is well documented that

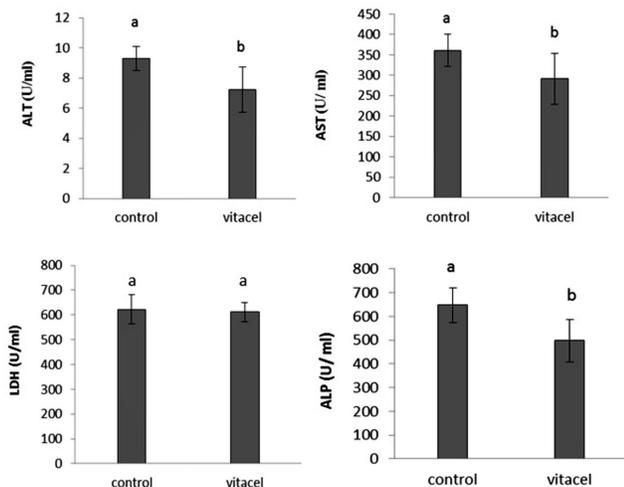
hemorrhagic septicemia caused by *A. hydrophila* negatively affects hematological and other physiological aspects of different fish species, which in this hematological parameters study have been positively influenced by dietary Vitacel. In agreement with our results, Heidarieh and his colleagues<sup>14</sup> reported that dietary administration of Vitacel increased WBC and eosinophil percentage in beluga. Likewise, dietary fermentable fiber positively increased the level of immune-



**Figure 3:** Serum concentration of  $\text{Ca}^{++}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and P in rainbow trout fed with 1% Vitacel in diet, and challenged with *A. hydrophila*; different letters indicate significant differences between the control and experiment groups ( $p < 0.05$ )



**Figure 4:** Serum biochemical parameters of rainbow trout fed with 1% Vitacel, and challenged with *A. hydrophila*; different letters indicate significant differences between the control and experiment groups ( $p < 0.05$ )



**Figure 2:** Serum enzyme levels in rainbow trout fed with 1% Vitacel in diet after challenge with *A. hydrophila*; different letters indicate significant differences between the control and experiment groups ( $p < 0.05$ )

related genes, humoral immune factors, and WBC level in health rainbow trout after 45 days.<sup>15</sup> However, there was no data regarding the effect of dietary fermentable fiber, and other prebiotics on the hematological and immune system of infected fish. Besides the lysozyme and complement activity, considering the elevated level of total protein and albumin, as well as, WBC, especially neutrophils and eosinophils having high phagocytic capacity, and an important role in lysozyme and immunoglobulin production,<sup>22</sup> our results further confirm the immunostimulants properties of Vitacel.

Dietary fermentable in the intestine produce metabolite, such as, short-chain fatty acids and lactate, and transfer them from the blood to the liver that can affect the health of the liver.<sup>22</sup> Thus, it can be suggested that decreased serum levels of these enzymes in the group fed with Vitacel R200 may be due to the protective effects of Vitacel and the lower damage in the liver of the experiment fish.<sup>23</sup> In addition, these results may be associated with a lower synthesis rate of glycogen and less energy demand, as fishes fed dietary Vitacel.

Consistent with our study, Liu co-authors<sup>19</sup> reported that infection with *A. hydrophila* significantly increased AST, ALP, and ALT level in *M. amblycephala*, but in group fed 0.1% anthraquinone were significantly lower in comparison to the control group. Likewise, ALT, ALP, and AST levels were elevated in *Labeorohita* infected with *A. hydrophila*, but in infected fish treated with *Achyranthes*, the levels were similar to the uninfected control group.<sup>24</sup>

Moreover, our study indicated that fish fed Vitacel compared to the control had significantly decreased glucose and cholesterol level, which may associate with reduced activity of liver enzymes, such as, ALP, AST, and ALT, involved in gluconeogenesis and energy metabolism. Changes in serum corticosteroids, such as, cortisol as an initial response and serum glucose level in fish as a secondary response can be used as indicators of stress in fish.<sup>25</sup> Different stressors, such as, an infectious disease can change the serum cortisol, glucose, and serum osmolality, especially with disease progression, and reduction of the disease resistance to expend more energy, greater stress response can occur.<sup>26</sup> Therefore, the lower concentration of glucose and cortisol in the treatment group, suggesting the lower level of stress in this group under *A. hydrophila* infection. Fish ionic regulation is influenced by a group of endocrine hormones, such as, cortisol that can be influenced under stress conditions, consequently increases the excretion of ions.<sup>27</sup> Therefore, the low levels of ions in fish fed Vitacel may be associated with the levels of cortisol influenced by the dietary, providing more energy for dealing with stress.

## CONCLUSION

Vitacel R200 can improve hemato-biochemical and immune parameters, as well as, serum enzyme activity and stress response in *O. mykiss* infected with *A. hydrophila*. Further studies are needed to evaluate the immune response and disease resistance, as well as, the mode of action of Vitacel R200 in fish.

## SOURCE OF FUNDING

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## ETHICAL CLEARANCE

In the present study, the experimental design was read and approved via the Institutional Animal Care and Use Committee (9130/2019).

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