Effects of Vitamin C and E Administration on Leukocyte Counts in Rainbow Trout (Oncorhynchus mykiss)

Abstract

White blood cells (WBC) or Leukocytes are defense mechanism indicators in fishes. Vitamins C and E are among the most important nutrients, providing protection to the leukocytes and subsequently influence the fish health and immune system. This study was conducted to investigate the effects of different levels of vitamins C and E (0, 100, 200 and 1000 mg/kg diet) on leukocyte counts of rainbow trout fingerlings during 60 days of feeding trial. The results showed that vitamin E administration increases significantly (P<0.05) the percent of the lymphocytes at 200 and 1000 mg/kg treatments. In addition, a significant increase (P<0.05) was found in lymphocytes of fish fed with 1000 mg/kg vitamin C administration. No significant effects of different levels of Vitamins C and E on neutrophils, eosinophils and monocytes were found during the 60 days experiment (P>0.05). The results revealed that administration of Vitamins C and E can be led to a significant increase in lymphocytes that may influence immune response and resistance against disease.

Keywords: WBC; Fish; Immune system; Lymphocytes

Introduction

Nutrition influences the immune system of fishes and plays a crucial role in their health and ability to withstand against diseases [1-3]. In fishes, essential nutrients, including fatty acids, proteins, polysaccharides, vitamins, and some minerals have pivotal importance to reinforce their immune functions [4]. Vitamins C and E are among the most important nutrients influencing specific and non-specific immune responses of fishes [5-8]. Vitamin C is an essential micronutrient and water-soluble antioxidant in biological fluids, which is associated to biochemical reactions in cells and tissues. Its deficiency can increase disease susceptibility in fishes affecting their immune system [9,10]. In addition, this vitamin has been suggested having a positive role in stress amelioration [11]. Vitamin E is related with immune system function, which acts as an antioxidant in biological membranes. This vitamin can enhance both humoral and cellular defenses, whereas its deficiency can reduce immune responses [12].

Both vitamins may prevent the immunosuppression [13-15], help the inflammatory responses [14,16,17], improve the respiratory burst on the phagocytes [6,13], and enhance the phagocytic activity [6,8,18] and antibody production in fishes [10,19]. Furthermore, they are important antioxidants offering protection against oxidative damages [20], enhancing the resistance of red blood cell membranes [18,21-24], protecting leukocyte functions [5,18,20,25,26], and influencing blood cell indices such as leukocytes profile [24,27].

The major functions of the leukocytes are to fight infection, defend the body against foreign organisms in the immune response. It has been shown that natural and exogenous stressors in the environment elicit a stress response in fish leukocyte counts, suggesting leukocyte counts can be considered as a novel marker of immunotoxicity [28-30]. Therefore, hematologic evaluation, especially leukocyte counts can be useful in monitoring of fish health and its immune response, due to the influence of intrinsic and extrinsic factors on the appearance of cells and its quantitative values [31]. Hence, this study was conducted to investigate the effects of different levels of vitamin C and E (i.e. each of 0, 100, 200 and 1000 mg/kg diet) on leukocyte counts of rainbow trout (Oncorhynchus mykiss) during 60 days of experiments.

Materials and Methods

Experimental design, procedures and diets

The experiment was conducted for a period of 60 days in Tehran Ghezel Ala Co. (Firoozkooh city, northern Iran). Eight experimental diets with similar energy and protein levels containing different levels of the vitamin E (i.e. 0.0, 100, 200, 1000 mg/kg diet) and Vitamin C (i.e. 0.0, 100, 200, 1000 mg/kg diet) was selected as treatments and evaluated. The rainbow trout fingerlings (as three replicates of eight treatments) with mean initial weight of 2.1 ± 0.15 g were introduced into twenty-four 100 L tanks, each containing 50 fish. During the experiment, the water flow rate, pH, average dissolved oxygen and water temperature were 0.5 L/S, 7.8, 8.5 ppm and 10-13 °C, respectively, with a natural photoperiod (10 L: 14 D).

Formulations and proximate composition of the experimental diets are presented in Table1. Briefly, the dietary ingredients were thoroughly mixed, made into pellets and air-dried at room temperature. The pellets were ground and sieved into small size and
Table 1: Composition of the experimental diet (dry weight).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>58</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>14</td>
</tr>
<tr>
<td>Meat flour</td>
<td>12</td>
</tr>
<tr>
<td>Dextrin</td>
<td>5</td>
</tr>
<tr>
<td>Fish oil</td>
<td>6</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>2.2</td>
</tr>
<tr>
<td>Filler (sawdust)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mineral mixture ¹</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin mixture (vitamin E or C free)²</td>
<td>1</td>
</tr>
</tbody>
</table>

Proximate composition (%)

- Moisture: 10 ± 0.8
- Crude protein: 49 ± 1
- Crude lipid: 15.1 ± 0.8
- Ash: 11.5 ± 0.92
- Energy (Kcal/g): 380

¹ One kilogram mineral premix contained 130.6 g calcium phosphate dibasic, 327 g calcium lactate, 29.7 g ferric citrate, 137 g magnesium sulfate, 239.8 g potassium phosphate dibasic, 87.2 g sodium phosphate dibasic, 43.5 g sodium chloride, 0.15 g aluminum chloride hexahydrate, 0.15 g potassium iodide, 0.1 g cupric chloride, 0.8 g manganese sulfate monohydrate, 1 g cobalt chloride hexahydrate, and 3 g zinc sulfate heptahydrate.

² Vitamin mixture was manually provided according to feed requirements of the fish and ingredients were obtained from Science Laboratories (Ghazvin, Iran), which provides 6600 IU vitamin A (retinol palmitate), 2400 IU vitamin D3, 28 mg vitamin K (menadione sodium bisulfate), 47 mg thiamin, 53 mg riboflavin, 38 mg pyridoxine, 115 mg pantothenate, 220 mg niacin, 0.6 mg biotin, 12.7 mg folic acid, 0.06 mg vitamin B12, and 300 mg inositol per kg feed. The diets of vitamin E and C controls supplemented with 100 mg/kg diet, vitamin C, E, and respectively. Vitamin E and C used in the diets were Dl-al-rac-o-tocopherol and L (+)-Ascorbic acid calcium salt dihydrate, (Sigma, USA).

Discussion

Aquaculture is one of the fastest growing animal production sectors in the world, which has constraint due to emerging of disease outbreaks [34]. There is a rapidly increasing literature pointing to the success of probiotics, vitamins, plant products and immunostimulants, in immune modulation, namely stimulation of the adaptive and innate immune response, and the control of fish diseases [35,36]. The dietary supplements of vitamins have promised reducing the severity of disease, and enhancing of immune response [35,36]. Leukocytes are defense mechanism indicators in fishes, and it has shown that vitamins E and C are potent antioxidants that can provide the protection to the leukocyte function [5,18,25,26]. The vitamin E and/or C supplementation in the diet of fishes has shown a greater efficiency in the production of antibodies, lysozyme levels, phagocyte activity and stress attenuation [6,14].

In the present study, the administration of high levels of Vitamin C and E showed a significant increase in lymphocytes. Although, there are some literatures regarding the application of the dietary Vitamin C and/or E on fishes with immunological assay, but there are no enough results regarding their effects on leukocyte counts. Safarpour-Amlashi et al. reported higher lymphocytes in Beluga (Hucho huso) fed with the high levels of vitamin E (400 mg/kg diet) for 8 weeks [37]. It has also shown a significant increase of lymphocytes in fish fed vitamin C at an elevated dose for longer periods [20,38]. In contrast to our findings, Menezes et al. pointed out that vitamin E supplemented in fish diet reduces the number of total thrombocytes, lymphocytes and neutrophils, and increases eosinophils [39]. They also reported that vitamin C+E and C increase total leukocytes, thrombocytes and eosinophils in pirarucu (Arapaima gigas). It can be suggested that differences in fish species and environmental factors are related to different results.

Lymphocytes are responsible for the production of antibodies in fishes, and may be affected by an alteration in their membrane stability, which can be influenced by these vitamins. It has been found that feeding with vitamin E deficient diets decreases the function of T and B-lymphocytes in rainbow trout sensitized against Yersinia.
It has also shown that vitamin C enhances antibody production against *Vibrio anguillarum* in rainbow trout [41] and against *Edwarsiella ictaluri* in channel catfish [9]. Therefore, antibody production may be influenced as a result of observed change in lymphocytes value; however, it needs to be evaluated.

Vitamins C and E provide cellular defense against the uncontrolled generation of reactive oxygen species (ROS) from normal aerobic metabolism, and by oxidative challenges such as pollution, infection, tissue damage and oxidative drug, which can damage biological membranes and DNA [42]. Furthermore, It has been confirmed that oxidation of leukocytes membrane lipids can change the synthesis of some complement proteins and consequently their functions [43]. Hence, alterations in leukocyte membrane due to different intakes of vitamin E and C, and also function of these vitamins through ROS pathway maybe modulate the complement activity and enhance immunity in fish.

In conclusion, our findings suggest the effects of diets with

![Figure 1: Leukocyte differential count ((a) neutrophils, (b) lymphocytes, (c) monocytes and (d) eosinophils) in rainbow trout fingerlings fed with different levels of vitamin E. Data is shown as means ± SEM (n=15). Different letters indicate significant differences between the treatment and control groups at *P*<0.05.](image)

![Figure 2: Leukocyte differential count ((a) neutrophils, (b) lymphocytes, (c) monocytes and (d) eosinophils) in rainbow trout fingerlings fed with different levels of vitamin C. Data is shown as means ± SEM (n=15). Different letters indicate significant differences between the treatment and control groups at *P*<0.05.](image)
sufficient Vitamin E and C on leukocytes in rainbow trout fingerlings, which may influence disease resistance and immune response. Further research is necessary to evaluate the antibody production and complement activity. Furthermore, the mechanisms by which the high dose of vitamin E and C influences lymphocytes is unclear, which require further investigation as well.

References

ISSN: 2469-4185


