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RESEARCH PAPER

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Using of Black Cumin Seed Powder (*Nigella sativa*) as Immunostimulant and Growth Promoter in Rainbow Trout, *Oncorhynchus mykiss* (Walbaum)

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Abstract

In this study the effect of black cumin seed (*Nigella sativa*) on nonspecific defense mechanisms of rainbow trout (*Oncorhynchus mykiss*) was investigated. The percentage of black cumin seed in diet were 0.0; 0.5; 1.0; 2.5; 5.0; 10.0 and 20.0 g/kg respectively. Rainbow trout (*O. mykiss*) of initial weights 108.7±17g were used for experiment. The biometric measurements and the blood parameters were determined in each group after feeding 7, 15, 25, 40 and 60 days respectively. In 7 day experiment, plasma protein, MCH, MCHC, RDW_SD and RDW_CV, MPV values at 1.0 and 2.5 g/kg dose groups differed favorably from the control group. In 15 day feeding experiment, there is a positive mathematical difference in almost all parameters in the 2.5 g/kg dose group compared to control group. In 25 day experiment, there is a positive mathematical difference according to control group in almost all parameters and statistically significant difference between MCH and MPV values in the 2.5 g/kg dose group ($P<0.05$). In 40 day feeding experiment, plasma protein, MCV, RDW_CV, PLT values differed positively from the control group at the 1.0 and 2.5 g/kg dose groups and mathematical differences were observed in other parameters. In 60 day feeding experiment, at the doses of 1.0 and 2.5 g/kg, many parameters differ from the control group and from the other groups.

Introduction

In the world, very important investments have been made in cultural fisheries to meet the protein needs. In parallel with the development of this industry, diseases have also increased (FAO, 2016). For a healthy and efficient production, the main cause of these problems must be identified and solved. Because the fish diseases are the most important factor limiting fish production and affect the profitability of fish breeding and incubation enterprises. It is also necessary to pay attention to the fight against diseases in order to develop this sector in a sustainable way (Subasinghe, D. Soto, & Jia, 2009; Bondad-Reantaso *et al.*, 2005; Hanol Bektas, Ucar, & Savaser, 2017). Correct and effective protection efforts will prevent incidence of fish diseases and increase the productivity of the industry. Due to the European Union has been decided prevent using of antibiotic as feed additive in 2006, scientists have turned to research on natural medicines. These alternative products are enzymes, organic acids, probiotics, prebiotics, immunostimulating products, polysaccharides, bacterial particles, spices, herbal juices and powders of animal or vegetable origin. The widespread use of chemotherapeutic agents is known to cause increased antimicrobial resistance in pathogens, but also pose a risk on living organisms in the recipient environment. Especially in the last 10 years, there have

been considerable in vitro and in vivo studies of the use of such products chitosan, glucagan, polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, muramyl dipeptide in the aquatic products (Mastan, 2015). The use of immunostimulants has been tested in a very limited number of trout farm in Turkey. Most of farm are unaware of these products. The work carried out in this regard is not at the level where clear recommendations can be made to the manufacturers. In this respect, our work sheds light on manufacturers.

Throughout history, people have benefited from plants for therapeutic purposes (Petrovska, 2012). According to many researcher, it have been reported that medicinal plant having many properties such as growth promotion, appetite stimulation, antimicrobial, immunostimulant, antinflammatory, antistress, anticancer and healing (Bulfon, Volpatti & Galeotti, 2013). Immunostimulants are used as adjuvant in fish vaccines and as additives in aquafeeds. Especially use of immunostimulants as a prophylactic agent to hinder prevalence of epidemics is began to popular, so the interest in this issue continues to grow in scientific circles in the aquaculture sector (Tafallaa, Bøgwald, & Dalmo, 2013). From these *N. sativa*, which belongs to the Ranunculacea family is a medicinal herb has many therapeutic characteristics (Ziaee, Moharreri, & Hosseinzadeh, 2012). *N. sativa* has been used for treatment for more than 2000 years. *N. sativa* is commonly known as black cumin seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Turkey, Saudi Arabia (Ahmad *et al.*, 2013). *N. sativa* seeds and oils have been commonly used as treatment for a variety of health conditions pertaining to the respiratory system, digestive tract, kidney and liver functions, cardiovascular system, and immune system support, as well as for general well-being (Ahmad *et al.*, 2013). *N. sativa* also has a wide range of applications in aquaculture such as enhancement of immunity (Khatun *et al.*, 2015; Dorucu, Colak, Ispir, Altınterim, &Celayir, 2009; Khondoker *et al.*, 2016; Awad, Austin & Lyndon, 2013) and shelf life of fish (Ozpolat & Duman, 2016). Dorucu *et al.* (2009) used *N. sativa* at a rate of 1, 2.5 and 5% for 21 days and determined serum protein and total immunoglobulin level, hematocrit (Hct), leukocyte levels, glass-adherent NBT positive cell activation of *O. mykiss*. Khatun *et al.*, (2015) studied effect of Black cumin seed oil (*Nigella sativa*) on enhancement of immunity in the climbing perch, *Anabas testudineus* and investigated immunological parameters such as bactericidal activity and phagocytic activity. Also Khondoker *et al.*, (2016) used *Nigella sativa* in 2%, 4% and 6% doses and investigated immunological parameters such as bactericidal activity and phagocytic activity. Awad *et al.* (2013) studied Effect of black cumin seed oil (*Nigella sativa*) in a rate of 1%, 2% and 3% and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) and investigated Humoral immune parameters including lysozyme, antiprotease, total protein, myeloperoxidase, bactericidal activity, and IgM titers. So the distinctive things that makes our studies different from others can be list as follows. We used black cumin seed in a wider range and Awad &Awad, (2017) have recorded the importance of determining the ideal dose of medicinal plant to improve the immune system of fish and avoid the risk of immunosuppression. Also we regarded the duration of time with dosages to determine the optimal dose levels and we believe that this is important in terms of suggestion to producers beyond being a study for the research paper. Furhermore we studied the effect of black cumin seed extract on *O. mykiss* both in a sense of growth and broad range of non specific immunological parameters and specific immunological parameters. In summary we can say that our study is a detailed updates of existing work.

Actually, it is clear that the most appropriate method for controlling diseases is protection from diseases and increased immunity. Therefore, in this study we used immune-enhancing herbs *N. sativa* in rainbow trout in terms of growth, blood levels and immune-indicative parameters in vivo condition and *Yersinia ruckeri* infection were occured that is the most common disease in trout in Turkey generated in the group fed with experimental diet supplemented with *N. sativa* powder.

Materials and Methods

Fish and Experimental Design

A total of 1260 (315 of for infection and 1575 for feeding experiment) rainbow trout of average weight 108.7±17.0 g were obtained from commercial fish farms in Turkey and acclimatized in aerated freeflowing freshwater for 20 days. 21 tanks with 3 replications were used for feeding trial; 21 tanks with 3 replicates for challenge experiment were used. Regular and continuous water flow was provided to the tanks. The experiment were carried out in the temperature of 16,0°C, 7.50 pH, total hardness of 46.19 (°F), 0,032mg/L nitrite (NO_2^{-1}), 1.3mg/L nitrate (NO_3^{-1}) and 0.04mg/L ammonium (NH_4^{+1}). Black cumin powder was added to the experimental diets at a 0.5 g/kg, 1.0 g/kg, 2.5 g/kg, 5.0 g/kg, 10.0 g/kg and 20.0 g/kg ratio. The control diet did not include any supplementation. The fish were fed with the experimental diets twice daily for 60 days. 10 fish from each experimental unit were anaesthetized using 0,5 ml/L quinaldine at the 7th, 15th, 25th, 40th days of the study. The blood samples of the fish were obtained from the caudal vein and transferred to blood collection tubes containing K3-EDTA for determination of non-specific immune activities and centrifuged at 3000 rpm for 25 min at 4°C. Serum was collected, and stored at −20 °C until use.

Preparation of Black Cumin Seed Powder

Black cumin (*N. sativa*) was purchased from an herbalist in Isparta province. The commercial trout diet and *N. sativa* seeds were pulverized and than pelleted (Pakravan, Hajimoradloo, & Ghorbani, 2012). Then, it was mixed with the pellet diet at a concentration of 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 g/kg. The pellets were prepared freshly at every turn. Composition of feed and additives used in the experiment are shown in Table 1; and the essential oil contents of cumin seed are shown in Table 2. It is thought that the high cymene is due to the grass mixing during harvesting.

Table 1. Composition of Feed and Additives Used in the Experiment

	Feed	N
Moisture (%)	8,21	4,97
Ash (%)	8,96	4,09
Protein (%)	40,71	20,96
Oil (%)	20,56	35,11
Cellulose (%)	2,61	6,81

Table 2. Essential oil content ratio (%) of cumin seed used in the experiment

p – cymene	35,9
α - tuyen	9,8
Palmitic acide	8,6
Trans-4-metoksituyen	6,3
Thymoquinone	5,1
Other	34,3

Challenge Test

$1x10^7$ cfu/ml^{-1} of *Yersinia ruckeri* mixed in 100 µl PBS was injected to all fish intraperitoneally at the end of dietary feeding trial (after 15 days) and survival of groups were observed during 20 days post injection. Survival rate was determined using this formulae: SR (%)= (number of fish survived /number of fish injected) × 100 (Aydın, Çıltaş, & Akyurt, 1997; Bricknell, Bowden, & Bruno, 1999).

Determination of Hemogram Parameters

The immune system is a series processes that protects organisms from pathogens. So any repression of this system cause deterioration of cells and ultimately leads to death. The immune system in fish consists of the innate (non-specific) and the adaptive (specific) immune system. In fact, the immune responses in fish are mediated by a diversity of cells and secreted soluble mediators which act in synergistic form for complete protection. Leucocytes are considered as back-bone of all immune responses. They comprise lymphocytes (T cells, B cells), phagocytes (monocytes and neutrophils) (Secombes, &Wang, 2012; Awad, &Awaad, 2017). The major components of the innate immune system are macrophages, monocytes, granulocytes (neutrophils, eosinophils and basophils) and humoral elements, such as lysozymes or the complement system (Secombes and Fletcher 1992; Magnado´ttir 2006: Nakanishi, Aoyagi, Xia, Dijkstra, Ototake, 1999).

In fish, White blood cells (WBCs) play an important role in the cellular immunity and resistance to infectious diseases (Whyte, 2007). The significant increases in WBCs counts in fish fed with the nigella or combination diets may be due to the activation of the hemopoietic tissues either by the black cumin (Nair, Salomi, & Panikkar, 1991). Prokan PE 6800 Blood Counter vehicle was used for the determination of hemogram parameters.

Total Protein Content

Total serum protein is one of the most important indicators of general health of fish. (Yang & Chen, 2003). Ahmed and Ali (2013) reported that the total protein concentration in fish serum differ, depending on a series of factors such as food diet, species, season, degree of sexual maturity, water temperature. Biuret protein assay method was applied for total protein measured by using bovine serum albumin (BSA) as the standard (Tietz, 1999).

Lysozyme Activity

In fish, innate immunity is very important in terms of defence mechanism. There are many components of the immune system and from these lysozyme lever or activity is an important indicator of innate immunity of fish and is found everywhere in distribution among living organisms (Saurabh, S., & Sahoo, P.K, 2008). It is known that lysosyzme can activate complement system and phagocytes (Grinde, 1989).

Lysozyme activity were carried out according to Ellis (1996). Hen egg white lizozim (HEWL) were used as standard (Grinde 1989). For this purpose, 1% agar and 0,60 mg/ml lyophilized *Micrococcus lysodeicticus* (SIGMA M3770, ATCC 4698) was added to PBS (Phosphate Buffer Saline). 5mm diameter holes opened in the petri dishes after solidifying. 25µl of fish serum was added to holes and after incubation at 25°C for 20 h diameters of zones were measured.

Biometric Measurements

One of the most important goals of successful breeding is the increased growth performance. So various study have been conducted on the subject. Most of the studies carried out included the addition of medicinal plant extracts to fish feed. The addition of herb extract into feeds given to aquatic animals,

especially fish, increases the growth performance of the species being cultivated (Bell *et al.*, 2000; Montero *et al.*, 2005). At the beginning of the experiment, groups with normal distribution were formed in the trout with a mean weight of 108.7 ± 17.0 g without any difference between the groups.

Morphometric measurements in fish carried out during each period of blood intake in experimental and control groups. Total length and weight measurements were made during each period of the study. Feed conversion rate calculated as follows.

Feed conversion ratio (FCR) =feed intake (g)/weight gain (g)

Statistical Analysis

Data were analyzed by SPSS v20 package software. Kolmogrov Smirnov test were used for normal distribution of data. ANOVA test were used to comparing parametric data in normal distribution and non parametric data with no normal distribution were analyzed by Kruskal–Wallis. In case of difference between the groups, Duncan or Tukey HSD multiple comparison tests were applied according to the normal distribution result (Özdamar, 2011). The results were expressed as mean ± standard deviation ($\bar{X}$ ± SD). In all statistical analysis significant level were assumed as α=0,05. All statistical analyses were performed using the statistical software package IBM SPSS v.23 for Windows 2013 program.

Results

Biometric Findings

The weight measurements determined in relation to the feeding experiment are given in Table 3 and the total length measurements are given in Table 4. Weight measurements in 7th, 15th, 25th, 40th and 60th days are given in Table 5,6,7,8 and 9 respectively. There was no statistical difference in weight increases in feeding experiments carried up to 40 days. It is noteworthy that the feed group at the 2.5 g/kg dose mathematically has a higher weight gain than the other groups, and this difference becomes statistically significant after 60 days of feeding (Table 3). When we look at the total length measurements of black cumin seed powder additive, there was no difference between groups in length increase (Table 4).

Table 3. Weight measurements of feeding with black cumin seed powder additive

Dose (g/kg)	Feding period (day)				
	7	15	25	40	60
0	116,5 ± 14,9	124,5 ± 22,6	150,9 ± 27,9	181,5 ± 33,1	211,4 ± 39,4^{a}
0,5	115,3 ± 18,1	125,8 ± 17,5	146,5 ± 24,1	178,9 ± 28,0	213,8 ± 38,8^{a}
1	119,4 ± 15,9	130,1 ± 24,0	152,3 ± 27,3	188,1 ± 27,3	220,4 ± 34,3^{ab}
2,5	123,3 ± 20,5	134,1 ± 25,7	159,2 ± 30,3	193,3 ± 32,6	232,9 ± 44,9^{b}
5	115,4 ± 17,3	123,4 ± 19,4	148,0 ± 30,6	176,8 ± 39,8	209,5 ± 38,8^{a}
10	114,3 ± 22,8	122,4 ± 20,4	141,6 ± 27,4	175,3 ± 27,9	208,5 ± 40,8^{a}
20	122,7 ± 21,5	130,6 ± 24,7	153,0 ± 30,8	188,3 ± 34,8	217,5 ± 49,1^{ab}

There was no statistical difference in weight increases in feeding experiments carried up to 40 days. It is noteworthy that the feed group at the 2.5 g/kg dose mathematically has a higher weight gain than the other groups, and this difference becomes statistically significant after 60 days of feeding.

Table 4. Total length measurements of black cumin seed powder additive

Dose (g/kg)	Feding period (day)				
	8	15	25	40	60
0	22,5 ± 1,1	22,8 ± 1,4	23,5 ± 1,6	24,7 ± 1,6	26,4 ± 1,6
0,5	22,5 ± 1,3	23,1 ± 1,3	23,7 ± 1,2	24,7 ± 1,3	25,8 ± 1,8
1	22,7 ± 1,2	22,9 ± 1,6	23,5 ± 1,3	24,7 ± 1,2	26,0 ± 1,4
2,5	22,8 ± 1,2	23,2 ± 1,2	23,7 ± 1,7	25,2 ± 1,6	26,3 ± 1,4
5	22,8 ± 1,2	22,9 ± 0,9	23,3 ± 1,7	24,0 ± 2,0	25,9 ± 1,7
10	22,3 ± 1,5	22,8 ± 1,3	22,9 ± 1,5	24,3 ± 1,5	25,6 ± 1,6
20	22,7 ± 1,6	23,1 ± 1,4	23,6 ± 1,6	24,8 ± 1,4	26,4 ± 2,2

There was no difference between groups in length increase.

Blood Parameters

Differences in blood parameters determined after each feeding period according to dose groups were statistically determined. Blood parameters results in 7th, 15th, 25th, 40th and 60th day are given in Table 5,6,7,8 and 9 respectively. Non specific parameters such as WBC (leucocyte), LYM (lymphocyte), MID (monocyte), GRAN(granulocytes), RBC (erythrocyte), HGB(hemoglobin), HCT (hematocrit), MCV (mean cell vloume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), RDW-SD (red cell distribution width), RDW-CV, PLT (platelet), MPV (mean platelet volume), PDW (platelet distribution width) in rainbow trout in the 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 g/kg doses were analysed.

According to the 7-day feeding experiment plasma protein, MCH, MCHC, RDW_SD and RDW_CV, MPV values at 1.0 and 2.5 g/kg dose groups differed favorably from the control group (Table 5). Blood parameters determined in 15-day feeding experiment are shown in Table 6. Although the statistical difference is not in any group, there is a positive mathematical difference in almost all parameters in the 2.5 g/kg dose group according to the control group.

Blood parameters determined in 25 day feeding experiment are shown in Table 1. In the 2.5 g/kg dose group, there is a positive mathematical difference according to control group in almost all parameters and statistically significant difference between MCH and MPV values. Blood parameters determined in 40-day feeding experiment are shown in Table 8. According to the results; plasma protein, MCV, RDW_CV, PLT values differed positively from the control group at 1.0 and 2.5 g/kg dose groups, and mathematical differences were observed in other parameters. And blood parameters determined in 60-day feeding experiment are shown in Table 9. At the doses of 1.0 and 2.5 g/kg, many parameters were differed from the control group and other groups.

Challenge

The differences in blood parameters determined by dose groups were statistically determined and summarized in Table 10. When we look at the blood parameters determined in fish feeding with black cumin seed powder after *Yersinia ruckeri* infection, at doses of 0.5 and 1 g/kg, many parameters were differ from the control group.

Discussion

The application of medicinal plants and their derivates as immunostimulants in fish culture is an effective and safe method to enhance the immune responses against pathogens during periods of stress, such as intensive farming culture, grading, vaccination and reproduction. Immunostimulants and adjuvants used in fish vaccines are of interest, as they offer an alternative to the drugs, chemicals and antibiotics currently used in fish culture to control disease. The positive effects of immunostimulants on the immune system of fish are obvious. The major components of the innate immune system (nonspecific) are macrophages, monocytes, granulocytes and humoral elements, like lysozyme or complement system (Secombes &Fletcher, 1992; Magnado´ ttir, 2006). Hematological parameters provide an index of the physiological status of fish. (Fadeifared *et al.*,2018).

It is very important to determine the ideal dose to strengthen the immune system of fish and stand aside the risk of immunosuppression (Awad, & Awaad, 2017). There are many studies on the effects of medicinal plant on the growth performance and immun system of fish (Awad *et al.*, 2013; Bilen, Bulut, & Bilen, 2011; Adel, Safari, Pourgholam, Zorriehzehra, Angeles Esteban, 2015; Talpur, 2013) and some studies on black cumin seed. Çelik Altunoğlu *et al.* (2017) have studied immune responses to methanolic extract of black cumin (*Nigella sativa*) in rainbow trout (*Oncorhynchus mykiss*). Öz, Dikel and Durmuş (2018) have studied Effect of black cumin oil (Nigella sativa) on the growth performance, body composition and fatty acid profile of rainbow trout (*Oncorhynchus mykiss*), Khandoker *et al.* (2016) have investigated the effect of *N. sativa* to Enhance the Immunity of Common Carp (*Cyprinus carpio*) Against *Pseudomonas fluorescens*. Besides, *Khatun et al.* (2015) and Elkamel and Mosaad (2012) have studied on *N. sativa* as immunstimulator in fish.

In this study, we intended to test powder of *N. sativa* as a feed additive for growth promotion and immune stimulation in rainbow trout. We think present study is different from another study in accordance feed additive ratio, number of samples worked, use of the plant itself, optimal dose, specific and non specific immunological parameters. Furthermore, we analyzed non specific immunological parameters periodically (such as 7, 15, 25, 40 and 60 day periods) so it is more broad and detailed study and it is difficult evaluate and compare all parameters with another studies.

In this study, when we look at the weight measurements after feeding with *N. sativa* powder supplementation for 7, 15, 25, 40 and 60 days, there were difference in the amount of 2.5 g/kg but not statistically significant, this became statistically significant after 60 days of feeding and the highest growth was in this group (Table 1). There was no statistical difference among the other groups. Also statistical difference was not obsorved in the total length (Table 2). These results are different from Çelik Altunoglu, Bilen, Ulu, and Biswas (2017) who added methanolic extract of *N. sativa* to feed at a rate of 0,1 and 0,5g/kg. Moreover, the use of black cumin seeds showed an enhancement in growth performance and immunity of fish and decrease in FCR. (Abd Elmonem *et al.*, 2002; Atwa, 1997; John *et al.*, 2007; Abdelwahab abd El-Bahr, 2012; Diab, Aly, John, Abdel-Hadi, &

Table 5. Blood parameters determined in 7-day feeding experiment

Parameters	Dose groups (g/kg feed black cumin seed powder additive)						
	0	0,5	1,0	2,5	5,0	10,0	20,0
Lizozim	10,1 ± 0,9^{ab}	9,5 ± 1,1^{a}	9,8 ± 0,9^{ab}	9,6 ± 0,9^{a}	10,1 ± 0,9^{ab}	10,4 ± 1,0^{b}	9,6 ± 0,7^{a}
Protein	53,9 ± 6,9^{ab}	58,2 ± 11,6^{bc}	56,9 ± 9,9^{bc}	60,5 ± 9,5^{c}	54,0 ± 7,4^{ab}	57,2 ± 8,5^{bc}	51,8 ± 10,6^{a}
WBC	124,4 ± 11,9^{b}	116,4 ± 6,5^{a}	121,1 ± 9,1^{ab}	119,4 ± 9,1^{ab}	123,2 ± 7,2^{ab}	117,1 ± 9,8^{a}	121,4 ± 7,5^{ab}
LYM	100,4 ± 6^{b}	95,2 ± 4,3^{a}	97,5 ± 5,5^{ab}	97,2 ± 4,1^{ab}	99,2 ± 4,0^{b}	95,2 ± 7,1^{a}	98,1 ± 2,9^{ab}
MID	12,6 ± 2,9	11,3 ± 2,0	12,3 ± 1,9	12,1 ± 2,4	12,4 ± 2,0	11,5 ± 2,3	12,4 ± 2,1
GRAN	11,4 ± 3,8	10,1 ± 3,8	11,1 ± 2,9	10,9 ± 3,2	11,6 ± 3,4	10,8 ± 3,8	10,9 ± 2,9
RBC	1,2 ± 0,2	1,2 ± 0,2	1,1 ± 0,2	1,1 ± 0,1	1,3 ± 0,2	1,2 ± 0,2	1,2 ± 0,2
HGB	10,1 ± 2,1^{ab}	9,4 ± 1,5^{a}	10,4 ± 1,5^{bc}	10,0 ± 1,3^{ab}	10,2 ± 1,8^{abc}	10,1 ± 1,6^{ab}	11,0 ± 1,4^{c}
HCT	17,1 ± 3,5^{d}	14,3 ± 1,7^{a}	15,7 ± 2,1^{bcd}	15,4 ± 2,3^{abc}	17,0 ± 2,7^{d}	14,5 ± 2,3^{ab}	16,7 ± 2,5^{cd}
MCV	142,4 ± 6,6^{b}	136,3 ± 2,9^{a}	136,4 ± 3,3^{a}	136,6 ± 3,2^{a}	143,1 ± 5,6^{b}	136,7 ± 5,1^{a}	141,6 ± 2,5^{b}
MCH	83,1 ± 5,1^{a}	89,6 ± 8,6^{bcd}	90,0 ± 4,6^{bcd}	88,8 ± 4,9^{abc}	85,2 ± 7,6^{ab}	95,8 ± 14,7^{d}	93,6 ± 5,8^{cd}
MCHC	58,7 ± 4,9^{a}	65,8 ± 6,3^{bc}	66,3 ± 4,6^{bc}	65,3 ± 4,9^{b}	59,8 ± 5,8^{a}	70,5 ± 11,4^{c}	66,3 ± 4,2^{bc}
RDW_SD	92,5 ± 4,5^{c}	82,1 ± 8,0^{b}	80,8 ± 6,6^{ab}	80,3 ± 7,8^{ab}	83,1 ± 12,2^{b}	78,1 ± 6,5^{ab}	73,8 ± 7,7^{a}
RDW_CV	20,6 ± 2,8^{d}	16,9 ± 1,8^{b}	16,5 ± 1,5^{b}	16,4 ± 1,8^{b}	18,3 ± 3,0^{c}	16,4 ± 2,3^{b}	14,5 ± 1,4^{a}
PLT	10,1 ± 3,9	10,8 ± 4,2	10,0 ± 4,4	11,8 ± 5,3	10,8 ± 3,8	11,8 ± 10,4	8,4 ± 2,4
MPV	9,7 ± 0,4^{a}	10,5 ± 0,5^{bc}	10,4 ± 0,5^{bc}	10,5 ± 0,5^{bc}	9,9 ± 0,8^{ab}	10,4 ± 1,0^{bc}	11,0 ± 0,7^{c}
PDW	10,8 ± 1,5^{a}	12,1 ± 2,0^{bc}	11,8 ± 1,4^{abc}	12,4 ± 1,9^{c}	10,9 ± 2,4^{ab}	12,1 ± 2,7^{bc}	13,0 ± 1,5^{c}

Plasma protein, MCH, MCHC, RDW_SD and RDW_CV, MPV values at 1.0 and 2.5 g/kg dose groups differed favorably (significant difference) from the control group.

Table 6. Blood parameters determined in 15-day feeding experiment

Parameters	Dose Groups (g/kg feed black cumin seed powder additive)						
	0	**0,5**	**1,0**	**2,5**	**5,0**	**10,0**	**20,0**
Lizozim	10,2 ± 1,1	10,0 ± 0,7	10,3 ± 1,0	10,4 ± 1,1	9,9 ± 0,7	9,8 ± 0,8	10,0 ± 0,7
Protein	53,8 ± 5,0^{ab}	55,6 ± 7,6^{b}	58,0 ± 9,3^{bc}	55,4 ± 7,0^{b}	60,5 ± 11^{c}	53,9 ± 7,7^{ab}	50,5 ± 9,5^{a}
WBC	123,2 ± 7,2^{bc}	126,5 ± 8,0^{c}	123,7 ± 7,9^{bc}	125,4 ± 7,1^{bc}	121,6 ± 9,0^{ab}	125,4 ± 7,4^{bc}	117,6 ± 8,2^{a}
LYM	99,5 ± 3,4^{a}	101,4 ± 4,9^{a}	100,0 ± 4,2^{a}	100,9 ± 3,3^{a}	98,5 ± 3,9^{a}	100,9 ± 2,6^{a}	106,2 ± 6,9^{b}
MID	12,5 ± 1,9^{b}	13,2 ± 2,4^{b}	12,6 ± 2,0^{b}	12,9 ± 1,9^{b}	12,1 ± 2,5^{b}	12,9 ± 2,2^{b}	7,0 ± 3,5^{a}
GRAN	11,1 ± 2,7^{b}	11,9 ± 3,4^{b}	11,1 ± 2,6^{b}	11,5 ± 2,5^{b}	11,0 ± 3,4^{b}	11,6 ± 3,2^{b}	4,4 ± 4,1^{a}
RBC	1,2 ± 0,2^{ab}	1,2 ± 0,2^{ab}	1,1 ± 0,2^{a}	1,2 ± 0,2^{ab}	1,1 ± 0,2^{a}	1,2 ± 0,2^{ab}	1,3 ± 0,1^{b}
HGB	9,7 ± 1,4	10,1 ± 1,5	9,6 ± 1,2	9,7 ± 1,2	9,4 ± 1,4	9,9 ± 1,3	10,2 ± 0,9
HCT	18,1 ± 2,6	18,5 ± 2,7	17,4 ± 2,7	17,9 ± 2,4	17,3 ± 3,5	18,7 ± 2,6	18,1 ± 1,6
MCV	152,7 ± 3,8^{b}	152,6 ± 3,7^{b}	153,7 ± 4,8^{b}	153,8 ± 3,3^{b}	153,4 ± 5,9^{b}	153,7 ± 2,9^{b}	142,5 ± 5,3^{a}
MCH	81,7 ± 6,0	83,1 ± 4,8	85,2 ± 7,7	82,9 ± 4,7	85,7 ± 21,1	81,2 ± 4,9	80,0 ± 4,7
MCHC	53,6 ± 3,7	54,6 ± 3,4	55,6 ± 5,3	54,0 ± 3,4	55,7 ± 11,5	52,7 ± 3,8	56,4 ± 4,6
RDW_SD	88,4 ± 5,2	85,9 ± 6,0	86,1 ± 7,1	83,5 ± 7,1	83,3 ± 7,1	85,6 ± 7,9	87,9 ± 6,8
RDW_CV	16,4 ± 1,2	15,8 ± 1,4	15,9 ± 1,7	15,5 ± 1,8	16,3 ± 2,1	16,0 ± 2,0	16,6 ± 1,5
PLT	12,2 ± 6,8	12,5 ± 8,4	10,9 ± 6,7	10,4 ± 7,8	14,4 ± 16,5	10,2 ± 3,8	16,3 ± 6,9
MPV	9,9 ± 0,9	10,1 ± 0,6	10,1 ± 0,6	10,3 ± 0,6	9,8 ± 0,7	10,0 ± 0,4	10,0 ± 0,6
PDW	10,2 ± 3,5^{ab}	11,4 ± 1,5^{ab}	11,8 ± 2,2^{b}	11,4 ± 1,8^{ab}	9,4 ± 1,8^{a}	10,7 ± 1,8^{ab}	9,7 ± 1,7^{ab}

Although the statistical difference is not in any group, there is a positive mathematical difference in almost all parameters in the 2.5 g/kg dose group according to the control group.

Mohammed 2008). On the contrary Bilen *et al.* (2011) who studied the immunostimulant effects of *Cotinus coggyria* on rainbow trout (*Oncorhynchus mykiss*) and they didn't acquired any significant increase in growth rate and average weight like similar us. In another study, John, Mesalhy, Rezk, El-Naggar and Fathi (2007) used 3% N. sativa on the growth of *Oreochromis niloticus*. By end of second phase (winter) phase, the observed mean final weights in all treatments were higher than the control. According to our result, it is necessary to increase the amount of feed proportional to body weight of fish feeding with lower dose of *N. sativa*. It is therefore necessary to increase the amount of feed given to fish in proportion to their body weight at lower doses with better growth, and to reduce the amount of feed given to fish in proportion to their body weight at higher doses that appear lower growth. Here the important point is feed cost due to the rate of feed evaluation. Feed conservation rate (FCR) was determined after 60 days feeding. The FCR results are shown in Figure 1. The difference between the groups insignificant when the importance level is taken as 0.05. However, it is important that, the emerges mathematical difference confirms the growth values and FCR has not increased, in order to recommend the *N. sativa* to the producers. The low FCR in low-dose groups fed with *N. sativa* was statistically insignificant in terms of analysis but in the case of lower error level studies, it is important that the likelihood of difference being significant is high. So more detailed work can be done in this regard. Our result is different from Talpur (2013) who gained significant enhancement in FCR in Asian seabass, Lates calcarifer (Bloch) fed with *Mentha piperita* (Peppermint) but similar with Khatun *et al.* (2015) who studied Effect of Black Cumin Seed Oil (*Nigella sativa*) on Enhancement of Immunity in the Climbing Perch, *Anabas testudineus*. Adel, Safari, Pourgholam, Zorriehzahra, & Esteban, (2015) also have gained increase in growth rate but decrease in FCR like our results.

According to our knowledge, there is little information in the literature about the use of *N. sativa* seed powder in rainbow trout in the meaning of both growth and broad immun parameters. This study is unique in this respect and it provides the detailed valuable information on the subject in the literature. The innate immune system in fish is an important defense mechanism of the body against pathogens. The immune system consists of macrophages, monocytes, granulocytes, and humoral elements, such as lysozymes, immunoglobulins and the complement system. Lysozyme level or activity is an important index of innate immunity of fish. Lysozym plays an important role in non-specific immune system and perform defence against pathogen bacteria (Saurabh, & Sahoo, 2008). In terms of the lysozyme activity and plasma protein, in 7-day feeding experiment, the control group showed similarities in all the groups in accordance lysozyme activity, but differences were observed between the experimental groups. The best plasma protein values were found at 0.5 and 2.5 g/kg dose groups and the difference was significant (Table 5). In 15-day feeding experiment, the highest amount of plasma protein was detected in the dose group of 0.1 and 5.0 g/kg. Lysozyme activity was mathematically was high at the doses of 1.0 and 2.5 g/kg (Table 6). In 25-day feeding experiment the best lysozyme result obtained from 0.5g/kg, Çelik Altınoğlu *et al.* (2017) determined significant increase in lysozyme in 0.1 and 0.5 g/kg dose group and this result is different from us (Table 7). In 40-day feeding experiment, lysozyme activity were homogeneous

Table 7. Blood parameters determined in 25-day feeding experiment

Parameters	Dose Groups (g/kg feed black cumin seed powder additive)						
	0	0,5	1,0	2,5	5,0	10,0	20,0
Lizozim	10,3 ± 0,9^{ab}	10,1 ± 1,0^{ab}	10,2 ± 1,1^{ab}	10,6 ± 1,0^{b}	10,3 ± 1,0^{ab}	10,3 ± 0,7^{ab}	9,8 ± 0,7^{a}
Protein	53,9 ± 9,2^{bc}	54,7 ± 5,8^{bc}	55,9 ± 9,4^{cd}	58,2 ± 7,2^{cd}	59,2 ± 9,3^{d}	51,0 ± 4,1^{b}	47,0 ± 7,5^{a}
WBC	126,5 ± 9,8	128,0 ± 4,6	127,0 ± 6,7	127,8 ± 6,7	126,1 ± 5,2	123,8 ± 7,5	128,6 ± 5,1
LYM	100,6 ± 5,2^{ab}	101,9 ± 2,0^{ab}	101,1 ± 2,8^{ab}	101,1 ± 3,5^{ab}	101,9 ± 2,1^{ab}	99,8 ± 3,7^{a}	102,5 ± 1,9^{b}
MID	13,4 ± 2,4	13,6 ± 1,3	13,4 ± 1,9	13,8 ± 1,8	12,9 ± 1,5	12,7 ± 2,1	13,7 ± 1,6
GRAN	12,5 ± 3,5	12,4 ± 2,1	12,5 ± 3,2	12,9 ± 3,1	11,3 ± 2,0	11,3 ± 3,7	12,4 ± 2,3
RBC	1,2 ± 0,2	1,2 ± 0,1	1,2 ± 0,2	1,2 ± 0,2	1,2 ± 0,1	1,1 ± 0,1	1,2 ± 0,1
HGB	9,7 ± 1,7	9,8 ± 0,9	10,2 ± 1,6	10,2 ± 1,3	9,8 ± 1,1	9,6 ± 1,0	10,4 ± 1,4
HCT	17,8 ± 3,9	18,3 ± 1,9	18,4 ± 3,0	17,9 ± 2,8	19,2 ± 2,3	17,7 ± 2,0	18,8 ± 2,3
MCV	151,5 ± 8,1^{a}	155,3 ± 3,6^{b}	153,0 ± 2,7^{ab}	151,8 ± 4,3^{ab}	155,5 ± 3,2^{b}	152,8 ± 9,0^{ab}	152,7 ± 4,2^{ab}
MCH	82,9 ± 6,0^{ab}	83,0 ± 3,6^{ab}	84,6 ± 5,1^{bc}	87,4 ± 14,1^{c}	79,5 ± 3,6^{a}	85,0 ± 10,2^{bc}	84,4 ± 4,7^{bc}
MCHC	55,3 ± 8,5^{bc}	53,6 ± 3,0^{ab}	55,5 ± 3,8^{bc}	57,7 ± 8,1^{c}	51,3 ± 3,1^{a}	56,1 ± 10,9^{bc}	55,5 ± 3,5^{bc}
RDW_SD	87,8 ± 6,2	87,0 ± 5,9	85,9 ± 6,9	87,5 ± 6,7	86,9 ± 6,0	87,8 ± 6,8	88,2 ± 6,9
RDW_CV	17,2 ± 2,6	16,0 ± 1,8	16,5 ± 1,8	16,3 ± 1,6	16,7 ± 2,2	16,0 ± 1,5	17,4 ± 2,7
PLT	9,7 ± 2,5^{a}	10,1 ± 3,1^{ab}	11,0 ± 5,9^{abc}	11,9 ± 5,1^{abc}	13,7 ± 4,6^{bc}	8,3 ± 2,9^{a}	14,2 ± 6,4^{c}
MPV	9,9 ± 0,4^{a}	10,0 ± 0,4^{ab}	9,9 ± 0,4^{ab}	10,5 ± 0,7^{d}	9,8 ± 0,3^{a}	10,2 ± 0,6^{bc}	10,3 ± 0,5^{cd}
PDW	11,2 ± 1,8	11,1 ± 1,8	10,6 ± 1,9	10,8 ± 2,2	11,1 ± 1,5	10,9 ± 2,2	11,6 ± 1,7

In the 2.5 g/kg dose group, there is a positive mathematical difference according to control group in almost all parameters and statistically significant difference between MCH and MPV values.

Table 8. Blood parameters determined in 40-day feeding experiment

Parameters	Dose Groups (g/kg feed black cumin seed powder additive)						
	0	0,5	1,0	2,5	5,0	10,0	20,0
Lizozim	9,8 ± 1,2^{ab}	10,5 ± 1,2^{c}	10,2 ± 1,0^{bc}	10,2 ± 0,9^{bc}	9,2 ± 1,0^{a}	10,1 ± 0,8^{bc}	9,8 ± 0,9^{ab}
Protein	54,4 ± 8,7^{ab}	56 ± 11,7^{abc}	58,1 ± 9,8^{bc}	53,2 ± 9,2^{ab}	60,5 ± 6,8^{c}	51,3 ± 8,2^{a}	53,8 ± 9,0^{ab}
WBC	132,5 ± 5,0^{b}	131,9 ± 6,9^{b}	134,0 ± 7,1^{b}	132,8 ± 5,3^{b}	130,3 ± 7,6^{ab}	134,1 ± 4,1^{b}	125,8 ± 9,7^{a}
LYM	103,1 ± 1,7^{a}	102,4 ± 2,7^{a}	101,3 ± 5,7^{a}	103,0 ± 1,4^{a}	112,6 ± 9,4^{b}	103,9 ± 1,0^{a}	110,2 ± 12,3^{b}
MID	15,1 ± 1,7^{b}	14,9 ± 2,0^{b}	15,9 ± 1,5^{b}	15,1 ± 1,7^{b}	9,7 ± 5,1^{a}	15,4 ± 1,3^{b}	8,9 ± 3,9^{a}
GRAN	14,5 ± 2,7^{b}	14,5 ± 2,8^{b}	16,8 ± 2,8^{b}	14,7 ± 2,9^{b}	8,0 ± 6,7^{a}	14,9 ± 2,3^{b}	6,8 ± 5,2^{a}
RBC	1,4 ± 0,2^{c}	1,2 ± 0,1^{a}	1,2 ± 0,1^{ab}	1,2 ± 0,1^{a}	1,3 ± 0,2^{bc}	1,2 ± 0,1^{ab}	1,3 ± 0,3^{ab}
HGB	11,3 ± 2,2	10,6 ± 0,8	10,8 ± 0,8	10,9 ± 0,9	11,1 ± 1,2	10,9 ± 0,9	10,7 ± 1,1
HCT	18,3 ± 3,2	19,1 ± 1,7	19,3 ± 1,6	18,7 ± 2,3	19,4 ± 2,4	19,4 ± 1,8	17,8 ± 3,4
MCV	142,6 ± 17,9^{a}	157,3 ± 3,8^{c}	155,2 ± 4,1^{bc}	155,1 ± 4,6^{bc}	147,8 ± 9,7^{ab}	156,4 ± 3,6^{c}	142,7 ± 13,8^{a}
MCH	82,5 ± 15,0	78,4 ± 9,0	82,7 ± 4,1	85,0 ± 5,9	77,8 ± 3,7	77,9 ± 4,0	77,5 ± 3,9
MCHC	54,6 ± 7,4	50,5 ± 6,6	52,9 ± 2,7	54,7 ± 4,6	54,4 ± 3,1	51,7 ± 4,0	50,6 ± 3,6
RDW_SD	81,3 ± 20,5	90,9 ± 6,6	89,9 ± 4,8	92,6 ± 5,0	91,0 ± 4,1	91,4 ± 5,6	87 ± 10,6
RDW_CV	22,1 ± 3,8^{c}	17,2 ± 2,1^{a}	18,2 ± 2,4^{ab}	17,3 ± 1,5^{a}	18,7 ± 2,4^{ab}	17,6 ± 1,9^{a}	20,0 ± 3,3^{bc}
PLT	15,1 ± 7,5^{b}	8,9 ± 5,9^{ab}	6,7 ± 1,4^{a}	7,2 ± 2,4^{a}	11,0 ± 4,5^{ab}	10,5 ± 10,5^{ab}	11,6 ± 4,1^{ab}
MPV	10,0 ± 0,9^{abc}	10,1 ± 0,5^{bc}	10,5 ± 0,6^{c}	10,1 ± 0,7^{bc}	9,4 ± 0,6^{a}	9,5 ± 0,8^{ab}	9,6 ± 0,4^{ab}
PDW	11,9 ± 3,6	11,2 ± 1,3	11,2 ± 2,3	11,7 ± 0,9	9,7 ± 1,9	10,0 ± 1,3	10,0 ± 1,7

Plasma protein, MCV, RDW_CV, PLT values differed positively from the control group at 1.0 and 2.5 g/kg dose groups, and mathematical differences were observed in other parameters.

Table 9. Blood parameters determined in 60-day feeding experiment

Parameters	Dose Groups (g/kg feed Black cumin seed powder additive)						
	0	0,5	1,0	2,5	5,0	10,0	20,0
Lysozyme	9,6 ± 0,9	9,0 ± 0,8	9,3 ± 0,9	9,5 ± 1,2	9,3 ± 1,0	9,6 ± 0,8	9,4 ± 0,7
Protein	53,8 ± 5,5^{a}	59,5 ± 9,4^{b}	59,1 ± 8,0^{b}	62,0 ± 7,4^{b}	60,4 ± 6,9^{b}	55,2 ± 7,6^{a}	51,9 ± 5,8^{a}
WBC	95,3 ± 7,6^{ab}	97,5 ± 5,6^{bc}	102,4 ± 4,6^{de}	104,1 ± 5,1^{e}	98,1 ± 6,6^{bc}	100,5 ± 5,9^{cd}	93,6 ± 5,0^{a}
LYM	87,2 ± 5,5^{ab}	89,0 ± 4,0^{bc}	91,6 ± 3,0^{cd}	92,8 ± 3,1^{d}	89,4 ± 5,1^{bc}	90,6 ± 4,3^{bcd}	85,2 ± 5,1^{a}
MID	5,3 ± 1,4^{ab}	5,6 ± 1,0^{bc}	6,8 ± 1,1^{d}	7,1 ± 1,2^{d}	5,7 ± 1,1^{bc}	6,4 ± 1,0^{cd}	4,7 ± 0,8^{a}
GRAN	2,8 ± 1,0^{a}	2,8 ± 0,7^{a}	4,0 ± 1,0^{c}	4,2 ± 1,0^{c}	3,1 ± 0,7^{ab}	3,5 ± 0,8^{bc}	3,0 ± 0,9^{ab}
RBC	1,8 ± 0,3^{b}	1,6 ± 0,2^{a}	1,7 ± 0,2^{ab}	1,7 ± 0,2^{a}	1,8 ± 0,2^{ab}	1,6 ± 0,2^{a}	1,7 ± 0,2^{ab}
HGB	10,8 ± 1,8^{abc}	10,2 ± 1,0^{ab}	11,0 ± 1,1^{bc}	11,2 ± 1,1^{c}	10,0 ± 1,1^{a}	10,5 ± 0,8^{abc}	10,2 ± 1,1^{ab}
HCT	27,0 ± 3,9^{bc}	23,6 ± 2,8^{a}	25,3 ± 2,8^{ab}	25,4 ± 3,5^{ab}	27,9 ± 2,9^{ab}	24,2 ± 2,5^{c}	25,8 ± 2,7^{abc}
MCV	146,9 ± 4,7^{ab}	145,6 ± 4,5^{a}	147,7 ± 3,5^{ab}	151,3 ± 2,7^{c}	158,0 ± 5,3^{d}	148,5 ± 5,6^{b}	153,6 ± 7,3^{c}
MCH	58,5 ± 2,2^{ab}	63,1 ± 5,8^{bcd}	64,0 ± 5,0^{cd}	67,6 ± 12,1^{d}	56,3 ± 3,7^{a}	64,8 ± 5,9^{cd}	60,4 ± 2,9^{abc}
MCHC	39,9 ± 1,3^{b}	43,5 ± 3,8^{c}	43,4 ± 3,0^{c}	44,7 ± 7,8^{c}	35,7 ± 2,4^{a}	43,7 ± 3,2^{c}	39,5 ± 2,2^{b}
RDW_SD	77,2 ± 5,2^{bc}	68,7 ± 6,8^{a}	72,4 ± 5,7^{ab}	76,1 ± 7,3^{b}	92,0 ± 4,7^{d}	74,6 ± 8,0^{b}	82,9 ± 8,0^{c}
RDW_CV	14,8 ± 1,5^{c}	13,1 ± 1,0^{a}	13,7 ± 0,9^{ab}	14,0 ± 1,4^{b}	17,7 ± 1,3^{e}	14,0 ± 1,1^{ab}	16,4 ± 2,9^{d}
PLT	11,6 ± 5,8^{ab}	7,4 ± 2,1^{a}	9,5 ± 3,9^{ab}	10,1 ± 3,6^{ab}	19,4 ± 10,6^{c}	8,4 ± 3,2^{ab}	13,4 ± 4,7^{b}
MPV	10,6 ± 0,6^{b}	10,4 ± 0,5^{b}	11,5 ± 0,7^{c}	11,6 ± 0,7^{c}	9,7 ± 0,4^{a}	11,6 ± 0,4^{c}	10,6 ± 0,6^{b}
PDW	12,0 ± 3,1^{ab}	12,3 ± 2,0^{abc}	14,0 ± 3,7^{bcd}	16,0 ± 3,5^{d}	11,1 ± 1,4^{a}	14,7 ± 3,6^{cd}	11,8 ± 2,3^{ab}

At the doses of 1.0 and 2.5 g/kg, many parameters were differed from the control group and other groups.

among all experimental groups. 0.5, 1.0 and 2.5 g/kg dose groups were positive for the other groups; The differentiation in the 5.0, 10.0 and 20.0 g/kg dose groups was clarified in a negative sense. In this context Christybapita, Divyagnaneswari, & Michael, (2007) have studied an aqueous extract of false daisy, Eclipta alba leaf incorporated into the diet and fed for 2 and 3 weeks, they obtained significant increase in the lysozyme activity of common tilapia, *Oreochromis mossambicus*. Lysozyme was found to be significantly higher in the 0.5 g/kg dose group than in the control group, but similar both in the 1.0, 2.5 and 10.0 g/kg dose groups and the 0.5 g/kg dose group according to control group. The plasma protein level was found to be significantly higher in the 5.0 g/kg dose group than in the control group, while the 0.5 and 1.0 g/kg dose groups were found to be similar both in the 5.0 g/kg dose group and the control group (Table 8). In 60-day feeding experiment, plasma protein levels were significantly higher in the 0.5, 1.0, 2.5 and 5.0 g/kg dose groups compared to the other groups. Mathematically, the highest value was recorded in the 2.5 g/kg dose group (Table 9). Awad *et al.* (2013),

recorded an enhancement in total protein and lysozyme activity in groups fed with *N. sativa* oil and Quercetin, especially with higher doses that recorded the highest significant value compared to control. Also, Dügenci, Arda and Candan (2003) who studied with ginger (0,1% and 1%) and Bilen *et al.* (2011) who studied with *Cotinus coggyria* (at a rate of 0.5% and 1%) have been obtained similar results. Dorucu *et al.* (2009) used 1%, 2.5% and 5% of *N. sativa* on the immun response of *O. mykiss* and they reported serum protein levels significantly (P <0.05) higher than those of the control groups. According to the these results we can say that these results are copmatible with our studies in general mean especially in 2.5g/kg dose group.

The most important way to prevent fish diseases is strengthening the immune system. In this study the major immun parameters of fish studied detailed for giving us an opinon health status of *O. mykiss*. we studied non specific parameters such as WBC (leucocyte), LYM (lymphocyte), MID (monocyte), GRAN(granulocytes), RBC (erythrocyte), HGB(hemoglobin), HCT (hematocrit), MCV (mean cell vloume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), RDW-SD (red cell distribution width), RDW-CV, PLT (platelet), MPV (mean platelet volume), PDW (platelet distribution width). From the immun parameters White blood cells (WBCs) of fish have a critical importance in the cellular immunity and resistance to infectious diseases (Whyte, 2007). Many studies recorded that the medical plants could act as immunostimulants and increase the WBC count (Kumar *et al.*, 2013; Baba, Acar, Öntaş, Kesbiç, &Yılmaz, 2012).

When we look at the hematological parameters, in the 7-day feeding experiment, MID, GRAN, RBC and PLT parameters were statistically homogeneous across all groups. This result is compatible in accordance RBC but incompatible HCT with Altınterim and Dörücü (2009). HGB value was found to be significantly higher in the 20.0 g/kg dose group than the control group. Higher HCT levels were found at doses of 0.5, 2.5 and 10.0 g/kg. Like our result, John *et al.* (2007) recorded significant hematocrit values changes in *O. niloticus* fed with 3*% N. sativa*. The high level of MCV was statistically significant in comparison with the groups other than the dose groups of 5.0 and 20.0 g/kg. On the other hand, the MCH, MCHC, RDW-SD, RDW-CV and MPV values differed significantly from the control group in all experimental groups. For this reason, it will be useful for producers to use 1.0 and 2.5g/kg for 7 days use (Table 5). In the 15-day feeding experiment, HGB, HCT, MCH, MCHC, RDW-SD, RDW-CV, PLT, MPV values were homogeneous in control and experimental groups. Among these homogeneous parameters, the best mathematically acceptable values were found at 0.5 and 10.0 g/kg dose groups for HCT. Especially; WBC, LYM, MID, GRAN and MCV parameters were statistically different from the other groups and significant decreases occurred in 20.0 g/kg dose group (Table 6). Similar results recorded by Altınterim and Dörücü (2009) and Dorucu *et al.* (2009) for WBC. The highest mathematical values were found in the 1.0 and 2.5 g/kg dose groups for MCV and in the 0.5 and 2.5 g/kg dose groups for WBC, LYM, MID and GRAN. The increase in WBC was recorded by Elkamel and Mosaad (2012). As a result of the 15-day feeding trial, generally positive results were obtained in the 2.5 g/kg dose group. Govind, Madhuri, and Mandloi, (2012) have studied the immunostimulant effect of Medicinal plants on fish. They recorded increase in WBC, MCV, MCH and MCHC;

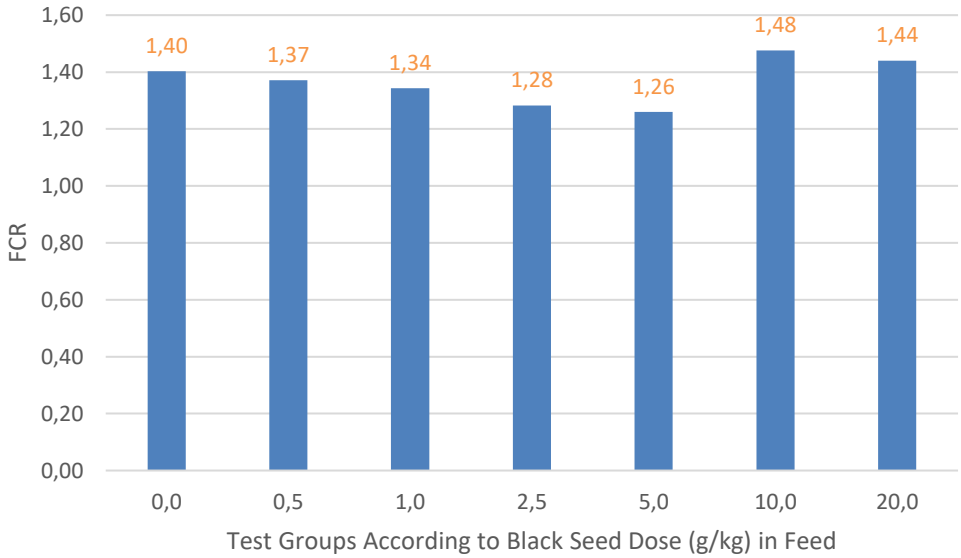

Figure 1. FCR results according to the black seed dose (g/kg) in feed. Although there is no difference in the FCR values according to the significance level p = 0.05, the mathematical difference gives hope for decreasing the feed costs in aquaculture.

Table 10. Blood parameters determined in fish feeding with black cumin seed powder after *Yersinia ruckeri* infection

Parameters	Dose Groups (g/kg feed Black cumin seed powder)						
	0	0,5	1,0	2,5	5,0	10,0	20,0
Lysozyme	10,6 ± 1,2^{c}	9,4 ± 0,8^{ab}	9,1 ± 0,7^{a}	9,4 ± 1,1^{ab}	9,9 ± 0,8^{bc}	9,9 ± 1,0^{b}	9,0 ± 0,7^{a}
Protein	44,7 ± 7,9^{a}	48,1 ± 9,3^{ab}	48,5 ± 7,9^{abc}	54,3 ± 8,5^{c}	53,4 ± 11,9^{bc}	50,9 ± 7,9^{bc}	49,5 ± 9,8^{abc}
WBC	92,4 ± 17,1^{a}	124,5 ± 5,8^{b}	121,3 ± 9,5^{b}	87,8 ± 17,4^{a}	91,8 ± 9,7^{a}	91,7 ± 5,8^{a}	90,4 ± 6,3^{a}
LYM	84,3 ± 15,6^{a}	100,6 ± 2,1^{b}	97,9 ± 5,2^{b}	79,7 ± 13,3^{a}	82,6 ± 8,4^{a}	83,7 ± 4,6^{a}	83,2 ± 4,9^{a}
MID	5,0 ± 1,3^{a}	12,6 ± 1,8^{b}	12,1 ± 2,2^{b}	5,0 ± 2,5^{a}	5,5 ± 1,1^{a}	5,0 ± 1,1^{a}	4,7 ± 0,9^{a}
GRAN	3,1 ± 1,1^{a}	11,2 ± 2,6^{b}	11,3 ± 3,0^{b}	3,1 ± 2,3^{a}	3,7 ± 1,4^{a}	3,0 ± 1,0^{a}	2,5 ± 0,7^{a}
RBC	1,4 ± 0,3^{bc}	1,2 ± 0,1^{a}	1,1 ± 0,2^{a}	1,4 ± 0,2^{b}	1,4 ± 0,2^{bc}	1,5 ± 0,2^{c}	1,4 ± 0,2^{bc}
HGB	8,9 ± 1,6	9,4 ± 0,9	9,1 ± 1,3	9,1 ± 1,3	8,6 ± 1,5	9,1 ± 1,1	8,9 ± 1,0
HCT	20,5 ± 3,9^{bc}	18,6 ± 1,9^{ab}	17,7 ± 2,9^{a}	19,8 ± 2,8^{abc}	22,3 ± 3,3^{c}	21,6 ± 2,7^{c}	21,4 ± 2,5^{c}
MCV	149,2 ± 6,1^{a}	156,2 ± 2,3^{b}	157,8 ± 5,0^{bc}	147,3 ± 7,9^{a}	161,1 ± 3,1^{c}	145,8 ± 6,8^{a}	148,3 ± 5,2^{a}
MCH	65,2 ± 9,3^{a}	78,2 ± 3,4^{ba}	81,9 ± 11,7^{b}	67,3 ± 6,8^{a}	61,7 ± 3,3^{a}	61,4 ± 3,1^{a}	61,4 ± 4,0^{a}
MCHC	44,0 ± 7,5^{bc}	50,2 ± 2,6^{d}	51,9 ± 6,1^{d}	45,8 ± 3,6^{c}	38,4 ± 2,2^{a}	42,3 ± 2,5^{abc}	41,5 ± 2,3^{ab}
RDW_SD	76,9 ± 8,4^{bc}	83,4 ± 6,7^{cd}	86,4 ± 6,3^{d}	72,4 ± 13,1^{ab}	85,0 ± 7,2^{d}	68,8 ± 6,1^{a}	68,2 ± 5,0^{a}
RDW_CV	14,5 ± 2,0^{bc}	14,9 ± 1,2^{bc}	15,5 ± 1,6^{c}	13,6 ± 1,8^{ab}	14,8 ± 1,3^{bc}	13,1 ± 0,9^{a}	12,8 ± 0,7^{a}
PLT	13,2 ± 6,5	8,3 ± 2,8	7,8 ± 2,5	8,3 ± 2,5	9,9 ± 3,3	6,9 ± 1,2	7,2 ± 1,6
MPV	9,5 ± 0,5	10,1 ± 0,6	10,3 ± 1,0	9,9 ± 0,5	9,1 ± 0,4	9,3 ± 0,3	8,9 ± 0,2
PDW	9,6 ± 1,3	10,8 ± 1,4	11,6 ± 2,1	11,2 ± 2,3	8,8 ± 1,6	9,2 ± 1,3	8,7 ± 0,6

At doses of 0.5 and 1 g/kg, many parameters differ from the control group. Positive statistically significant differences are observed in blood parameters.

decrease in RBC, Hb, haematocrit. These results are compatiple with our results in general means. In the 25-day feeding experiment, MID, GRAN, RBC, HGB, HCT, RDW-SD, RDW-CV and PDW values were found homogeneous in control group and all experimental groups. This results are compatible in accordance WBC and MCV with our results. The highest value were observed in 2,5 g/kg dose group for MID and GRAN, in 1,0 ve 2,5 g/kg for HGB; in 5 g/kg for HCT; the lowest value in 1.0g/kg for RDW-SD; in 0.5g/kg for RDW-CV, in 1,0 g/kg for PDW. The best result for WBC was taken from groups using 0.5g/kg dose (Table 7). In the 40-day feeding experiment, HGB, HCT, MCH, MCHC, RDW-SD and PDW values were found homogeneous in the control group and all experimental groups. The highest values for these parameters were found at 1.0, 5.0 and 10.0 g/kg dose groups for HCT; in a dose group of 2.5 g/kg for MCH and MCHC. RDW-CV was found to be positively and significantly lower in the 20.0 g/kg dose group compared to the control group. The lowest value in the RDW-SD was obtained from control group and in the PDW at the 5.0 g/kg dose group. WBC, LYM, MID, and GRAN values were homogeneous in all groups except for the 5.0 and 20.0 g/kg dose groups and differed in these groups. MCV values were significantly higher in the 0.5, 1.0, 2.5 and 10.0 g/kg dose groups than in the control group, and PLT values were significantly lower in the 1.0 and 2.5 g/kg dose groups. The difference between 0.5, 1.0 and 2.5 g/kg dose groups and 5.0, 10.0 and 20.0 g/kg dose groups was more evident after 40 days of feding (Table 8). In the 60-day feeding experiment, 1.0 and 2.5g/kg dose groups gave the most different results in terms of HGB among the experimental groups. It was observed that at 1.0 and 2.5 g/kg dose groups gave more positive results than the others. WBC, LYM, MID, GRAN, MCH, MPW values were higher than the other groups and the difference statistically significant (Table 9). This result is insimilar in WBC in study conducted by Fadeifard *et al.* (2018) who studied Effects of black seed (*Nigella sativa*), ginger (*Zingiber officinale*) and cone flower (*Echinacea angustifolia*) on the immune system of rainbow trout, *Oncorhynchus mykiss* but similar in haematological parameters including RBC, Hb, PCV, MCV, MCH and MCHC of fish fed the diets containing essential oils and the control group (P>0.05). Furthermore, Talpur (2013) have used Mentha piperita as feed additive in Asian seabass, *Lates calcarifer* (Bloch) for enhance growth performance, survival, immune response and disease resistance. They reported significantly increase in erythrocytes, leucocytes, haematocrit, haemoglobin, phagocytic activity, respiratoryburst, lysozyme, anti-protease and bactericidal activities in treated fish. Significantly higher ($p < 0.05$) serum protein and globulin levels in treated fish groups over the control. MCV levels were observed statistically significant in the 2.5, 5.0 and 20.0 g/kg dose groups; MCHC values were high and significant at doses of 0.5, 1.0, 2.5 and 10.0 g/kg. RDW-CV in all groups except 10.0 g/kg; RDW-SD was significantly and positively low in the 0.5 g/kg dose group compared to the control group. The PLT value was positively low in the 0.5 g/kg dose group, and was significantly higher in the dose group of 20.0 g/kg than in the dose group of 5.0 g/kg. Altınterim and Dörücü (2013) have investigated the effect of *N. sativa* oil on non-spesific immun defense mechanism such as hematocrit, leucocrit, erythrocyte, leucocyte, nitroblue tetrazolium activity, protein level and total

immunoglobulin levels of *O. mykiss* for 21 days. They determined significance in leucocyte, erythrocyte and insignificance in hematocrit and leucocrit. Our results are similar in accordance to leucocyte but difference with another parameter.

As a result of the blood parameters in the post-infection recovery period, the favorable outcomes were concentrated in the 0.5 and 1.0 g/kg dose groups, and the 1.0 g/kg dose group appeared in the forefront of these two groups. The highest lysozyme activity was recorded in control group, while the 5.0 g/kg dose group was found to be similar to the control group and 0.5 and 10.0 g/kg dose groups. Plasma protein levels were significantly higher in the 2.5, 5.0 and 10.0 g/kg dose groups. WBC, LYM, MID, GRAN, MCV and MCHC values were highest in the 0.5 ve 1.0 g/kg dose groups and MCHC in the 1,0 g/kg dose group. And also the best result obtained from 1.0 g/kg dose group. Continuation of *N. sativa* feed supplementation with 1.0 g/kg dose during a possible infection showed that non-specific immun system parameters and blood chart improved faster recovery (Table 10). Chang *et al.* (2012) examined the effects of zingeron, one of the ginger active ingredients, on growth, immune and disease in Pacific white shrimp (*Litopenaeus vannamei*). Experimental results reported an increase in excess weight and benefit from diet in shrimp fed on 2.5 and 5.0 mg/kg diet. Experimental infection of *V. alginolyticus* was established after 56 days feeding and it was reported that after 24-72 hours, survival rates were higher than control group. In general, these results are compatible with our study.

In our study, the doses recommended in the study are safe to use, and even positivie results in some blood parameters which are indicators of immunosystem support both studies.

Suggestions for manufacturers

According to the findings obtained within the scope of this study, it has been determined that *N. sativa* can be given to rainbow trout (*O. mykiss*) weight of 100-250 g individuals. From the results it has been understood that the feed additives used in the recommended doses will have a positive effect on feed evaluation and growth as well as immunostimulant effects. Also, after experimental infection for 20 days the results showed that the positive blood results obtained from fish groups fed with *N. sativa* according to control group. It has been determined that the use of *N. sativa* supplement at higher ratio than recommended doses results in a decrease in growth and feed conversion rate and a deterioration of the blood results. The recommended doses for *N. sativa* are given in Table 11.

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Table 11. Recommended dosed to producers for *N. sativa*

Supplement	Feeding period (day)	Dose			
		(g/kg) feed		(mg/kg) alive weight	
	7	1,0	2,5	20	50
	15		2,5		50
N.sativa seed powder	25		2,5		50
	40	1,0	2,5	20	50
	60	1,0	2,5	20	50

It can be recommended to use 2.5 g / kg or 50mg / kg body weight in feed. It is considered that the proposed dose is suitable for growth parameters, feed evaluation and immune parameters.

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