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Effects of rosemary essential oil on growth performance and hematological parameters of young great sturgeon (*Huso huso*)

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ABSTRACT

This study was conducted to investigate the immunological effects of rosemary (*Rosmarinus officinalis*) essential oil (RO) in young Beluga (*Huso huso*). Six groups (three replicates for each group) of 180 fish with initial weight of 130.94 ± 5.28 g were feed with diets containing 0 (control), 0.01, 0.1, 1 and 2% of RO and a group of oxytetracycline (OT) (0.003%). Then they were randomly distributed to 18 tanks (ten fish per tank). Fish were acclimatized for two weeks and then fed with prepared diets based on 2% of their body weights per day for 8 weeks. At the end of the experiment, some immuno-hematological parameters, and serum metabolic products including cholesterol, glucose, total protein, albumin, globulin, triglyceride as well as some liver enzymes such as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were examined. Survival was not significantly different among all the treatments. Growth parameters remained unaffected at the end of the trial. However, the lowest BWI, SGR, and CF were observed in fish fed on 2% RO ($P < .05$). Significant improvements of the immune system and physiological condition were observed in young beluga fed on some levels of RO. Total protein and albumin were elevated significantly in OT group as well as all the groups fed with RO except for the one treated with 2% RO whereas glucose, liver enzymes and hemoglobin were decreased by using 0.01, 0.1, and 1% RO ($P < .05$). In conclusion, it can be suggested that rosemary essential oil can be used in the diet to improve hematological parameters and enhance the immunity of young beluga (*Huso huso*). Moreover it can be a suitable replacement for oxytetracycline in the diet of young beluga.

1. Introduction

When living in unfavorable environments, fish are exposed to various pathogenic microorganisms against which their defense system plays a major role (Mohebbi et al., 2011; Dorucu et al., 2009). Although bacterial diseases in aquaculture are mainly controlled by antibiotics, continuous intensive use of antibiotics is undesirable as it causes drug resistance and reduces the efficacy of the drugs (Vijayan et al., 2017). In the public health context, antibiotic resistance can be transferred to the environment and human pathogenic bacteria. In addition, antibiotics accumulated in the fish will pose a potential risk to both the consumers and the environment (Mohan et al., 2019; Abutbul et al., 2004). Increased public awareness about the adverse effects of overexposure to synthetic chemicals has promoted the interest in green solutions such as organic and chemical-free food products (Agoba et al., 2017).

Organic fish production entails the development of natural antibacterial treatments (Vallejos-Vidal et al., 2016; Abutbul et al., 2004). Plant secondary metabolites, such as essential oils and flavonoids have been widely studied for their antimicrobial, antifungal, and antibacterial activities (Hassan et al., 2018; Zaouali et al., 2010). The use of immunostimulants can enhance activities in the nonspecific defense mechanism, increase resistance to infectious diseases by enhancing innate humoral and cellular defense mechanisms, and indirectly improve growth in fish (Mohan et al., 2019; Yousefi et al., 2019; Safarpour et al., 2011).

Rosemary (*Rosmarinus officinalis* L.) is globally known as a natural preservative due to its high antioxidant, antifungal, and antimicrobial activities (Sarabi et al., 2017; Oliveira et al., 2016; Zaouali et al., 2010) which can be justified by the existence of phenolic compounds among which phenolic diterpenes, carnosol, carnosic acid, and rosmarinic acid are known as the main compounds responsible for the antioxidant activity of rosemary (Shi et al., 2019). Furthermore the antimicrobial activity primarily causes by alpha-Pinene, bornyl acetate, camphor, and 1,8-cineole (Genena et al., 2008).

All species of sturgeon were included in the Convention on International Trade in Endangered Species (CITES) of wild fauna and

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Table 1
Main compounds of rosemary essential oil (gas chromatography-mass analysis report).

Compounds	% (by weight)
Alpha-Pinene	21.523
Camphene	6.276
Verbenene	0.395
Beta-Pinene	3.499
Beta-myrcene	1.638
P-Cymene	2.980
1,8-Cineole	15.171
Camphor	6.804
Borneol	8.619
Verbenone	8.563
Borneol acetate	6.077

flora appendices in 1997 (Jalali et al., 2009). Farming beluga up to a marketable size can contribute to fulfilling global needs for its meat and caviar and thus reduce pressure on the natural populations of the species in the Caspian Sea (Hoseinifar et al., 2011). Although several studies have evaluated the use of medicinal plants as growth promoters and immunostimulants in fish (Öz, 2018; Etyemez et al., 2018; Hoseini et al., 2018; Akrami et al., 2015; Nya and Austin, 2011; Dorucu et al., 2009; Diab et al., 2008), no studies were found on investigating the effects of rosemary essential oil on beluga. Therefore, the present study was conducted to determine the effects of dietary rosemary essential oils on the growth performance, immune parameters and hematological indices of great sturgeon (*Huso huso)*.

2. Materials and methods

2.1. Rosemary essential oil

Rosemary essential oil (RO) was purchased from Barij Essence Pharmaceutical Company (Kashan, Iran). The gas chromatography-mass analysis of the RO is done and prepared by Barij Essence Laboratory (Table 1).

2.2. Diet preparation

From the six designed diets, four were supplemented with 0.01, 0.1, 1, and 2% rosemary essential oil (RO) and one with 0.003% oxytetracycline (OT) respectively. One group was not supplemented and served as the control group. Protein, lipid, and carbohydrates were provided by fish meal and soybean meal, fish oil, and wheat grain, corn flour, and corn gluten meal, respectively. All ingredients were milled into fine powder and blended with 100ml of water per 1 kg of diet. Molasses was also added to obtain a homogenous mixture (Table 2).

The dough was then passed through a meat grinder equipped with a 2 mm screen to obtain uniform pellets. The pelleted diets were air-dried in 30 °C for 48 h (moisture content of about 7%) and then packed in plastic bags at −2 °C until use. The proximate composition of the treatments is presented in Table 2.

2.3. Culture system and feeding regime

Young Belugas were obtained from Shahid Marjani Center (Golestan Province, Iran) where they were originally hatched and reared. The experimental fish had no clinical signs of any diseases before the stocking. During a two-week acclimatization period, the fish were kept in two fiberglass tanks (1000l) and fed with the control diet. Tanks' water was adjusted in a semi-recirculating system with a flow rate of one liter per minute and an average temperature of 20.0 ± 1.8 °C (mean ± standard error). Each tank had a separate aeration system. In addition, the remaining excreta in the tanks were flushed twice daily in order to maintain a better water quality. To begin the trial 180 fish with the initial mean body weight of 130.94 ± 5.28 g were randomly distributed among eighteen 100l circular fiberglass tanks (six groups of three replicates, 10 fish /tank) with a flow rate of about 0.70 ± 0.08 l/min. Fish were fed with the experimental diets (2% of the body weight per day) for eight weeks. They were fed four times daily at 08:00 am, 12:00 pm, 04:00 pm, and 08:00 pm. The natural light/dark cycle (14 h/10 h) was maintained during the experiment. Water aeration was also provided to prevent the accumulation of ammonia and other toxic metabolites.

Table 2
Formula and proximate composition of the experimental diets (% of dry matter)[a].

Ingredients	Control	0.01(RO)	0.1(RO)	1(RO)	2(RO)	0.003(OT)
Fish meal	20	20	20	20	20	20
Canola	20	20	20	20	20	20
Soybean flour	20	20	20	20	20	20
Wheat flour	8.7	8.69	8.6	7.7	7.7	8.69
Corn flour	8.7	8.7	8.7	8.7	7.7	8.7
Cotton seed	8.7	8.7	8.7	8.7	8.7	8.7
Fish oil	6	6	6	6	6	6
Mineral and vitamin Premix[b]	1	1	1	1	1	1
Beet molasses	2.5	2.5	2.5	2.5	2.5	2.5
Corn gluten	4.4	4.4	4.4	4.4	4.4	4.4
Rosemary oil	0	0.01	0.1	1	2	0
Oxytetracyclin	0	0	0	0	0	0.003
Protein	31.87	31.89	31.84	31.92	31.87	31.95
Lipid	12.05	12.14	12.21	12.3	12.41	12.08
Moisture	6.73	6.82	6.64	6.95	6.67	6.66
Ash	15.86	15.63	15.92	15.61	15.57	15.71

[a] Diet abbreviations are as follows: 0.01(RO) = 0.01% rosemary oil; 0.1(RO) = 0.1% rosemary oil; 1(RO) = 1% rosemary oil; 2(RO) = 2% rosemary oil; 0.003(OT) = 0.003% oxytetracycline.

[b] Each 1000 g of the mineral and vitamin premix contained vitamin A (4.8 IU), vitamin E (0.8 IU), vitamin K (4 g), vitamin B_1 (0.8 g), vitamin B_2 (0.4 g), vitamin B_6 (16 g), vitamin B_{12} (0.6 g), pantothenic acid (4 g), nicotinic acid (4 g), folic acid (0.8 g), biotin (20 g), choline chloride (0.2 g), copper (4 g), iodine (0.8 g), ferrous (12 g), manganese (22 g), and selenium (0.04 g).

2.4. Water quality analysis

Throughout the experiment, water quality parameters were measured weekly from the outflow of each experimental tank. Hardness and pH were measured by a pH-meter. Dissolved oxygen and temperature were assessed daily. The mean dissolved oxygen, hardness, pH, and ammonium (NH_4) concentration in all treatments were 8.0 ± 0.7 mg/l, 430 ± 30 μz, 7.00 ± 0.08, and 0.44 ± 0.15 mg/l, respectively. The tanks had no significant differences in ammonia (NH_3) concentration.

2.5. Growth, hematological, and biochemical analyses

In order to determine growth indices, total fish length and weight were measured after 24 h of starvation. The growth performance of juveniles such as body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), hepatosomatic index (HSI), feed efficiency (FE) and survival rate were calculated based on standard formulae (Ta'ati et al., 2012; Lee et al., 2012).

$$\text{BWI} = (\text{final body weight} - \text{initial body weight}) \times 100/\text{initial body weight}.$$

$$\text{SGR} = (\ln\ \text{final weight} - \ln\ \text{initial weight}) \times 100/\text{days}.$$

$$\text{FCR} = \text{feed consumption}/\text{body weight gain}.$$

Table 3
Growth parameters of young beluga with diets containing different levels of rosemary oil and oxytetracycline for eight weeks[a].

	Control	0.01(RO)	0.1(RO)	1(RO)	2(RO)	0.003(OT)
Initial weight(g)	129.37 ± 6.86	130.33 ± 9.54	130.43 ± 4.98	132.86 ± 2.98	133.00 ± 0.94	129.68 ± 6.42
Final weight(g)	224.08 ± 24.35	238.13 ± 14.21	235.01 ± 11.35	234.90 ± 21.94	216.46 ± 5.24	246.97 ± 13.27
CF	0.38 ± 0.01	0.43 ± 0.02	0.40 ± 0.00	0.40 ± 0.00	0.38 ± 0.01	0.42 ± 0.01
BWI (%)	72.06 ± 12.17^{ab}	83.11 ± 4.37^{ab}	80.05 ± 2.50^{ab}	74.68 ± 13.9^{ab}	62.78 ± 4.47^{a}	90.95 ± 4.20^{b}
SGR (%/day)	1.81 ± 0.36	2.06 ± 0.11	2.00 ± 0.12	1.92 ± 0.39	1.60 ± 0.11	2.26 ± 0.14
FCR	1.39 ± 0.26	1.30 ± 0.05	1.27 ± 0.04	1.35 ± 0.24	1.51 ± 0.08	1.20 ± 0.06
FE (%)	12.17 ± 0.43	12.38 ± 0.97	11.10 ± 0.33	10.35 ± 2.66	9.46 ± 0.59	13.37 ± 0.86
HSI (%)	2.17 ± 0.07^{a}	2.79 ± 0.13^{b}	2.83 ± 0.05^{b}	2.93 ± 0.24^{b}	2.06 ± 0.06^{a}	2.91 ± 0.17^{b}
PER	2.45 ± 0.38	2.47 ± 0.10	2.52 ± 0.08	2.71 ± 0.58	2.13 ± 0.13	2.69 ± 0.12

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < .05$).
CF, condition factor, BWI, body weight increase, SGR, specific growth rate, FCR, feed conversion ratio, FE, feed efficiency, HIS, hepatosomatic index, PER, protein efficiency ratio.

[a] Diet abbreviations are as follows: 0.01(RO) = 0.01% rosemary oil; 0.1(RO) = 0.1% rosemary oil; 1(RO) = 1% rosemary oil; 2(RO) = 2% rosemary oil; 0.003(OT) = 0.003% oxytetracycline.

$$\text{PER} = \text{weight gain/protein intake},$$

$$\text{CF} = (\text{body weight/body length}^3) \times 100.$$

$$\text{HSI} = (\text{liver weight/body weight}) \times 100.$$

$$\text{FE} = \text{wet weight gain (g)} \times 100/\text{feed intake (g, DM)}.$$

$$\text{Survival rate} = (\text{final number of fish/initial number of fish}) \times 100.$$

In order to evaluate the humoral system factors, two fish were sampled from each tank at the end of the trial after they had been starved for 24 h and anesthetized with 100 mg/l clove oil (Heidarieh et al., 2011). Approximately 3 ml of blood were taken from the caudal vein of each fish using a 5 ml heparinized syringe (Falahatkar et al., 2009). About 2 ml of blood were transferred into a plastic tube and left to clot for 12 h at 4 °C. The samples were then centrifuged at 5000 rounds per minute (rpm) for 5 min and their serum was removed with a disposable pipette (Akrami et al., 2009). The remaining blood was maintained in heparinized tube and used in the assessment of hematocrit, hemoglobin, and differential leukocyte counts. The plasma was stored at −80 °C for cholesterol, glucose, total protein, albumin, globulin, triglyceride, and some liver enzymes such as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) measurements. Total protein (TP) was measured using the biuret test at a wavelength of 520–560 nm using a commercial kit (ZiestChemie Diagnostics, Tehran, Iran). A bromocresol green albumin assay kit (ZiestChemie Diagnostics, Tehran, Iran) was used to determine albumin levels at a wavelength of 630 nm. A calorimeter (Pars Azmoon, Tehran, Iran) was employed to calculate cholesterol and triglyceride concentrations at a wavelength of 546 nm. All biochemical blood serum parameters were analyzed with an auto-analyzer (Selectra-xl, Netherlands). The hematocrit test involved whole blood centrifugation (Hettich-EBA20, Germany) in heparinized microhematocrit capillary tubes at 13,000*g* for 3 min and then measuring the packed cell volume. The cyanmethemoglobin method was applied in determination of hemoglobin (Hb).

Differential leukocyte counts (as % white blood cells) were computed using blood smears under a light microscope. Leukocytes in blood smears were categorized into lymphocytes, neutrophils, eosinophils, and monocytes (Ta'ati et al., 2011). A minimum of 100 cells per slide were counted in each smear.

Chemical analyses of the feeds and the fish were performed according to approved Association of Official Analytical Chemists (AOAC International, 1997) protocols. At the beginning of the trial, three fish were kept at −20 °C. At the end of the trial, these fish were compared with the control group in terms of muscle composition and liver lipids. In addition, at the end of the experimental period, two fish from each tank were starved for 24 h and killed by physical destruction of the brain. Muscles around the spine were then removed to assess muscle composition. Moreover, the liver was excised and weighed to determine liver lipid concentrations and calculate the hepatosomatic index (HSI). Crude protein, crude lipid, moisture, and ash contents were respectively calculated by using Kjeldahl method (Auto Kjeldahl System, DISTYL EM S, India), Soxhelt apparatus (extraction with petroleum ether), a dry oven (105 °C for 24 h), and a muffle furnace (550 °C for 4 h) (AOAC International, 1997).

3. Results

3.1. Growth performance and survival rate

At the end of the experiment, there were no significant differences in the final weights and fish mortality among the treatments and no malformation or illness was observed during the experimental period. The lowest BWI, SGR, and CF were observed in fish fed on 2% RO ($P < .05$). The amounts of FCR, PER, and FE showed no significant differences among the treatments comparing to the control. According to our results, PER and FE remained unaffected at the end of the experimental period ($P < .05$). The result of growth performances of the young beluga fed on the experimental diets is presented in Table 3.

The HSI was significantly different among the treatments. In fact, while the fish fed on 0.01, 0.1, and 1% RO as well as the OT group showed the highest HSI, the control group and those supplemented with 2% RO had the lowest HSI.

3.2. Hematological parameters

As seen in Table 4, at the end of the experiment glucose, GOT, and GPT were significantly lower in all treatments comparing to the control group except for 2% RO treatment which was just significantly lower in the glucose level than the control. Albumin and TP levels showed a significant increase in groups 0.01, 0.1 and 1% RO, and 0.003% OT comparing to the control. The amount of globulin was just higher in the OT group than the control ($P < .05$). No significant triglyceride, cholesterol, and hematocrit differences were observed.

3.3. Differential count

Differential leukocyte counts of the fish are presented in Table 5. At the end of the trial, the experimental groups had significant differences in neutrophil, eosinophil, and lymphocyte counts. However, monocyte counts were not affected by the treatments. The control group and the fish receiving 2% RO revealed significantly lower lymphocyte counts comparing to other groups. Moreover, lymphocyte count was significantly lower in the control group than in the fish supplemented with 2% RO.

Table 4
Hematological and biochemical parameters of young beluga fed with diets containing different levels of rosemary oil and oxytetracycline for eight weeks[a].

	Control	0.01(RO)	0.1(RO)	1(RO)	2(RO)	0.003(OT)
Glucose (mg/dl)	57.33 ± 1.92[b]	42.16 ± 3.57[a]	41.66 ± 2.85[a]	40.16 ± 3.34[a]	46.00 ± 4.27[a]	45.00 ± 3.15[a]
GOT (IU/l)	327.66 ± 17.09[b]	269.33 ± 14.75[a]	267.66 ± 25.07[a]	269.16 ± 15.16[a]	286.66 ± 15.50[ab]	252.33 ± 8.92[a]
GPT (IU/l)	44.50 ± 1.58[b]	34.33 ± 3.81[a]	34.66 ± 1.74[a]	33.16 ± 2.77[a]	38.83 ± 1.68[ab]	33.33 ± 3.23[a]
Albumin (g/dl)	0.38 ± 0.02[a]	0.56 ± 0.02[b]	0.53 ± 0.02[b]	0.56 ± 0.01[b]	0.38 ± 0.02[a]	0.51 ± 0.06[b]
Total protein (g/dl)	1.19 ± 0.02[a]	1.49 ± 0.02[b]	1.50 ± 0.01[b]	1.45 ± 0.01[b]	1.23 ± 0.02[a]	1.49 ± 0.01[b]
Globulin (g/dl)	0.81 ± 0.02[a]	0.93 ± 0.02[ab]	0.93 ± 0.01[ab]	0.83 ± 0.07[a]	0.84 ± 0.03[ab]	0.97 ± 0.05[b]
Cholesterol (mg/dl)	70.83 ± 8.47	61.00 ± 4.45	58.66 ± 6.30	59.66 ± 7.94	70.66 ± 7.27	71.66 ± 13.77
Triglyceride (mg/dl)	464.66 ± 37.03	375.33 ± 32.52	366.00 ± 31.66	410.00 ± 56.09	412.83 ± 42.70	334.66 ± 64.54
Hematocrit (%)	31.33 ± 2.33	18.66 ± 3.28	18.66 ± 3.75	26.00 ± 8.50	27.00 ± 4.16	22.00 ± 5.50
Hemoglobin (g/dl)	11.86 ± 1.50[ab]	5.76 ± 1.16[ab]	4.36 ± 0.84[b]	8.46 ± 1.22[ab]	8.73 ± 3.26[a]	8.33 ± 3.33[ab]

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < .05$).
GOT, glutamic oxaloacetic, GPT, glutamic pyruvic transaminase.
[a] Diet abbreviations are as follows: 0.01(RO) = 0.01% rosemary oil; 0.1(RO) = 0.1% rosemary oil; 1(RO) = 1% rosemary oil; 2(RO) = 2% rosemary oil; 0.003(OT) = 0.003% oxytetracycline.

According to evaluations of differential leukocyte counts in the current research, the control group and the fish supplemented with 2% RO showed significantly higher neutrophil and eosinophil counts compared to the other groups. Receiving 0.01and 0.1% RO led to the lowest eosinophil and neutrophil counts, respectively. On the other hand, the highest eosinophil count was observed in the control group.

Finally, as Table 5 shows, there were no significant differences in monocyte content among the six groups.

3.4. Muscle composition

Muscle proximate composition of the studied groups is presented in Table 6. A significant difference was observed in muscle protein content of the young beluga fish at the end of the eight-week feeding trial. More precisely, while the highest protein levels were observed in the fish fed with 0.01% RO, the lowest levels were detected in the control group ($P < .05$).

Furthermore, in comparison with the control group, moisture and ash contents did not differ significantly following the consumption of rosemary essential oil ($P < .05$). Also the supplemented diets could not generally affect muscle lipid content in beluga fish.

4. Discussion

Various dietary herbal extracts and spices have been reported to improve animal performance by stimulating intestinal secretions or by exerting direct bactericidal effects on intestinal microflora. They have also been found to enhance digestive enzyme secretion and promote growth through increasing protein synthesis (Adeshina et al., 2018; Akrami et al., 2015; Lee et al., 2012). The present study was conducted to investigate the effects of rosemary essential oil on growth performance and immune system function of young beluga.

No significant growth was observed at the end of this study. Likewise, no significant effects were recorded in gilthead seabream growth and feed intake fed on rosemary (Hernández et al., 2015). But Hasan et al. (2018) reported significant changes in the WG, SGR, and PER in Nile tilapia by using 1% rosemary supplementation. As well using 3% rosemary leaf powder led to a significant increase in final weight, WG and SGR in common carp (Yousefi et al., 2019). Some other previous studies have also indicated significant effects of immunostimulants on fish growth and immune system improvement (Ta'ati et al., 2012; Hoseinifar et al., 2011; Jalali et al., 2009). These differences in the growth performance may relate to the fish species used for the trials and the part of the plants used in the experiments. To cite an instant, an improvement in WG and SGR of gilthead sea bream reported after administration of a diet containing 2% dried basil seeds and 2% soaked and dried basil seeds while there was no response shown in the group fed with 2% of basil leaf after 84 days (Awad and Awaad, 2017). The lowest BWI, SGR, and CF in fish fed on 2% RO can be justified by the fact that large amount of rosemary essential oil and the consequent pungent smell of the food might have prevented the fish from consuming adequate food.

The fish living in poor environments usually have smaller livers with lower amount of reserved energy. In line with our results, Safarpour et al. (2011) reported significant increase in the HSI of young beluga fish supplemented with vitamin E. Therefore, the significant increase of HSI in the fish receiving 1% RO compared to the control group might suggest enhanced food digestion and energy uptake in the liver and thus insignificant greater growth in this group.

Zoral et al. (2018) found that feeding common carp with rosemary aqueous extract for 20 days caused some dose-dependent histopathological alterations in liver and kidney. They concluded that using more than 20 ml/100 g rosemary extract leads to hepatotoxicity and nephrotoxicity against fish. Similarly, the lowest HSI observed in the highest level of the used RO. This may be due to toxic compounds in the rosemary extract. So, the exact determination of a safe dosage for each species before administration is of a great important.

In our study, the highest serum GOT and GPT levels were observed in the control group and the group supplemented with 2% RO compared to the other groups. While GOT and GPT activities mainly belong to the cytoplasmic compartment, increments in these activities indicate lysis of the hepatic origin (Lemairea et al., 1991). In other words, the significant reductions in GOT and GPT values in fish fed with rosemary essential oil highlighted the positive impact of rosemary essential oil on the health status of liver cells in young beluga fish.

Hematological indices reflect the type, quality, and amounts of

Table 5
Differential leukocyte count of young beluga fed with diets containing various levels of RO and OT for eight weeks.

	Control	0.01(RO)	0.1(RO)	1(RO)	2(RO)	0.003(OT)
Lymphocyte	68.41 ± 0.26[a]	75.00 ± 0.31[c]	75.55 ± 0.32[c]	74.61 ± 0.52[c]	69.57 ± 0.32[b]	75.18 ± 0.28[c]
Monocyte	2.23 ± 0.04	1.72 ± 0.04	1.91 ± 0.09	1.92 ± 0.35	2.22 ± 0.18	1.87 ± 0.21
Eosinophil	7.23 ± 0.02[c]	4.31 ± 0.24[a]	4.34 ± 0.28[a]	4.87 ± 0.01[a]	6.46 ± 0.16[b]	4.51 ± 0.32[a]
Neutrophil	22.12 ± 0.20[b]	18.95 ± 0.31[a]	18.18 ± 0.04[a]	18.58 ± 0.50[a]	21.73 ± 0.10[b]	18.43 ± 0.27[a]

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < .05$).

Table 6
Muscle proximate composition of young beluga (mean ± SE).

Parameters	Baseline	Control	0.01(RO)	0.1(RO)	1(RO)	2(RO)	0.003(OT)
Moisture (%)	82.16 ± 0.59	77.70 ± 0.07	76.53 ± 0.29	76.66 ± 0.40	76.46 ± 0.27	77.58 ± 0.02	76.68 ± 0.68
Protein (%)	12.39 ± 0.26[c]	14.13 ± 0.04[a]	14.69 ± 0.24[b]	14.63 ± 0.19[ab]	14.65 ± 0.17[ab]	14.12 ± 0.03[a]	14.58 ± 0.16[ab]
Lipid (%)	3.06 ± 0.16	4.15 ± 0.06	4.73 ± 0.06	4.76 ± 0.21	5.00 ± 0.07	4.24 ± 0.04	4.77 ± 0.56
Ash (%)	2.35 ± 0.14	4.00 ± 0.07	4.01 ± 0.02	3.91 ± 0.11	3.83 ± 0.03	4.03 ± 0.05	3.92 ± 0.03

Values in the same row with different superscripts are significantly different ($P < .05$).

dietary intakes available to meet the physiological, biochemical, and metabolic needs of an animal (Mostafa et al., 2009). In this study serum glucose levels were significantly decreased by using rosemary essential oil and oxytetracycline compared to the control group among which the 1% RO treatment showed the lowest level. Metwally (2009) related the reduction in serum glucose concentration of fish supplemented with medicinal herbs to improved antioxidant defense system and thus insulin production in the pancreas. Blood serum protein is a responsive biochemical system whose alteration is a reflection of an organism's response to assorted internal and external circumstances. The increase in the levels of serum protein, albumin and globulin in fish is thought to be associated with a stronger innate immunity response suggesting improved function of organs involved in protein production including the liver (Akrami et al., 2015; Lee et al., 2012). As stated in the results, total serum protein of the young beluga fish supplemented with 0.01, 0.1, and 1% RO and 0.003% OT was significantly higher comparing to that of the control group and the fish receiving 2% RO. In addition, the highest serum total protein level was observed following the use of 0.1% RO. In a study on Nile tilapia, the aflatoxin B1 contaminated diet supplemented with 0.5% rosemary exhibited more effective increases in serum total protein and globulin levels than the other treatments, leading to activated immunity and enhanced fish health. In the current research, the highest albumin and globulin levels were observed in the fish supplemented with 0.01% RO and oxytetracycline. Several studies demonstrated that different herbal materials simultaneously increased plasma total protein, albumin and globulin levels in different fish species (Yousefi et al., 2019; Hoseini et al., 2018; Akrami et al., 2015). Albumin and globulin concentrations are commonly used for evaluating the effect of nutrients on fish immunity. Elevated serum protein and globulin levels are believed to be associated with a stronger innate immune response in fish (Metwally, 2009). Increased levels of serum albumin and globulin levels in the rosemary essential oil-supplemented groups compared to the control group may imply enhanced immune system function of young beluga after receiving the two supplements.

According to Awad and Awaad (2017) 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 considered as back-bone of all immune responses comprise of lymphocytes and phagocytes (monocytes and neutrophils). Lymphocytes not only produce antibodies through specific defense mechanisms, but also manifest macrophage activity. In other words, increased number of these cells will promote the defense system of fish. Based on our results, lymphocyte was significantly higher in all treatments comparing to the control.

In the present study, the fish supplemented with 0.1% RO showed the highest increase in lymphocyte count, i.e. this dose of rosemary essential oil seems appropriate for boosting the immune system function of beluga fish. Peripheral blood neutrophils are highly motile cells which rapidly gather at sites of infection and play a major role in nonspecific defense mechanisms by producing adhesive proteins and adhering to tissue surfaces. They also contribute to eliminating pathogenic microorganisms through involvement in O_2^- and OH^- production during respiratory burst (Dorucu et al., 2009). Like neutrophils, eosinophils are responsible for combatting parasitic infections. Jalali et al. (2009) reported significant increments in neutrophil and eosinophil counts in young beluga fish fed with AQUAVAC® Ergosan™. Similar results were also obtained in young beluga fed on diets containing various levels of fiber and alginic acid (Heidarieh et al., 2011). In juvenile great sturgeon (*Huso huso*) fed on a microalga (*Spirulina platensis*) no significant differences were observed in the number of blood monocytes and eosinophils, but the number of neutrophils and lymphocytes was increased and decreased, respectively, compared to control fish (Adel et al., 2016). However, our findings suggested eosinophil counts to have a decreasing pattern similar to that of neutrophils. The control group had a significantly higher eosinophil and neutrophils count compared to the other groups.

In the present study body protein level is significantly higher in all treatments comparing to the control. Likewise, Nile tilapia fish fed diet contaminated with aflatoxin and supplemented with 0.5% rosemary showed significantly higher crude protein and fat content than the group contaminated with aflatoxin but fed on a diet without rosemary supplement (Naiel et al., 2019). On the other side, Yousefi et al. (2019) found no significant differences in the body composition among common carp fingerlings fed on rosemary leaf powder. In line with our findings, some previous studies on other immunostimulants, e.g. brewer's yeast and vitamin E, in young beluga did not notice any significant differences in body ash content (Hoseinifar et al., 2011; Safarpour et al., 2011).

5. Conclusion

In conclusion, rosemary essential oil had positive effects on some biochemical and hematological parameters in the young beluga fish. Since the most growth and hematological indices of fish fed rosemary essential oil are practically the same as Oxytetracycline fed groups, it is suggested to use 0.01% rosemary essential oil to improve growth parameters and enhance immune system function in beluga. However, further studies are needed on the effective compounds of rosemary essential oil on some factors such as triglyceride and cholesterol.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

A.O.A.C, 1997. Association of official analytical. In: Cunniff, P.A. (Ed.), Official Methods of Analysis of AOAC International, 16th edn. AOAC International, Arlington.

Abutbul, S., Golan-Goldhirsh, A., Barazani, O., Zilberg, D., 2004. Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture. 238, 97–105.

Adel, M., Yeganeh, S., Dadar, M., Sakai, M., Dawood, M.A.O., 2016. Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso* Linnaeus, 1754). Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2016.08.003.

Adeshina, I., Jenyo-Oni, A., Emikpe, B.O., Ajani, E.K., Abdel-Tawwab, M., 2018. Stimulatory effect of dietary clove, Eugenia caryophyllata, bud extract on growth performance, nutrient utilization, antioxidant capacity, and tolerance of African catfish, *Clarias gariepinus* (B.), to Aeromonas hydrophila infection. J. World Aquacult. Soc. https://doi.org/10.1111/jwas.12565.

Agoba, E.E., Adu, F., Agyare, C., Boama, V.E., 2017. Antibiotic use and practices in selected fish farms in the Ashanti region of Ghana. J. Infec. Dis. Treat. 3 (2), 9.

Akrami, R., Hajimoradloo, A., Matinfar, A., Abedian Kinari, A., 2009. Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and Hematological parameters of young Beluga, *Huso huso* (Linnaeus, 1758). J. World Aquacult. Soc. 40, 771–779.

Akrami, R., Gharaei, A., Razeghi Mansour, M., Galeshi, A., 2015. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hematobiochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish Shellfish Immunol. 45, 828–834.

Awad, E., Awaad, A., 2017. Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol. 67, 40–54.

Diab, A.S., Aly, S.M., John, G., Abde-Hadi, Y., Mohammed, M.F., 2008. Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, *Oreochromis niloticus* (Teleosti: Cichlidae), and their response to artificial infection with *Pseudomonas fluorescens*. Afr. J. Aquat. Sci. 33, 63–68.

Dorucu, M., Colak, S.O., Ispir, U., Altinterim, B., Celayir, Y., 2009. The effect of black cumin seeds, Nigella sativa, on the immune response of rainbow trout, *Oncorhynchus mykiss*. Medit. Aquacult. J. 2, 27–33.

Etyemez, B.M., Balcázar, J.L., Demirkale, İ., Dikel, S., 2018. Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 486, 170–174.

Falahatkar, B., Poursaeid, S., Shakoorian, M., Barton, B., 2009. Responses to handling and confinement stressors in young great sturgeon (*Huso huso*). J. Fish Biol. 75, 784–796.

Genena, A.K., Hense, Haiko, Smania Junior, A., Souza, S.M., 2008. Rosemary (*Rosmarinus officinalis*) a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Cienc. Tecnol. Aliment. 28, 463–469.

Hassan, A.A.M., Yacout, M.H., Khalel, M.S., Hafsa, S.H.A., Ibrahim, M.A.R., Mocuta, D.N., Turek Rahoveanu, A., Dediu, L., 2018. Effects of some herbal plant supplements on growth performance and the immune response in Nile Tilapia (*Oreochromis Niloticus*). Agricul. Life Life Agric. Conf. Proc. 1, 134–141. https://doi.org/10.2478/alife-2018-0020.

Heidarieh, M., Soltani, M., Tamimi, A.H., Toluei, M.H., 2011. Comparative effect of raw fiber (Vitacel) and alginic acid (Ergosan) on growth performance, Immunocompetent cell population and plasma lysozyme content of giant sturgeon (*Huso huso*). Turk. J. Fish. Aquat. Sci. 11, 445–450.

Hernández, A., García García, B., Caballero, M.J., Hernández, M.D., 2015. Preliminary insights into the incorporation of rosemary extract (Rosmarinus officinalis L.) in fish feed: nfluence on performance and physiology of gilthead seabream (*Sparus aurata*). Fish Physiol. Biochem. 41, 1065–1074. https://doi.org/10.1007/s10695-015-0069-2.

Hoseini, S.M., Taheri Mirghaed, A., Iri, Y., Ghelichpour, M., 2018. Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 495, 766–772.

Hoseinifar, S.H., Mirvaghefi, A., Merrifield, L.D., 2011. The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. ellipsoideus on the growth, physiological responses and gut microbiota of young beluga (*Huso huso*). Aquaculture 318, 90–94.

Jalali, M.A., Ahmadifar, E., Sudagar, M., Azari, T.G., 2009. Growth efficiency, body composition, survival and haematological changes in great sturgeon (*Huso huso* Linnaeus, 1758) youngs fed diets supplemented with different levels of Ergosan. Aquac. Res. 40, 804–809.

Lee, D.H., Ra, C.S., Song, Y.H., Sung, K.I., Kim, J.D., 2012. Effects of dietary garlic extract on growth, feed utilization and whole body composition of young Sterlet sturgeon (*Acipenser ruthenus*). Asian-Aust. J. Anim. Sci. 25, 577–583.

Lemairea, P., Drai, P., Mathieu, A., Lemaireb, S., Carrikre, S., Giudicelli, J., Lafaurie, M., 1991. Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (cholesterol, triglycerides) of sea-bass (*Dicentrarchus labrax*). Aquaculture. 93, 63–75.

Metwally, M.A.A., 2009. Effects of garlic (*Allium sativum*) on some antioxidant activities in Tilapia Nilotica (*Oreochromis niloticus*). World J. Fish Mar. Sci. 1, 56–64.

Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Seedevi, P., Karthick Rajan, D., 2019. Potential uses of fungal polysaccharides as immunostimulants in fish and shrimp aquaculture: a review. Aquaculture. 500, 250–263.

Mohebbi, A., Nematollahi, A., Ebrahimi Dorcheh, E., Goodarzian Asad, F., 2011. Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquac. Res. 1–10. https://doi.org/10.1111/j.1365-2109.2011.02922.x.

Mostafa, A.A.Z., Ahmad, M.H., Mousallamy, A., Samir, A., 2009. Effect of using dried fenugreek seeds as natural feed additives on growth performance, feed utilization, whole-body Composition and Entropathogenic *Aeromonas hydrophila*-challinge of Monsex Nile Tilapia *O. niloticus* (L) Fingerlings. Aust. J. Basic Appl. Sci. 3, 1234–1245.

Nya, E.J., Austin, B., 2011. Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish Shellfish Immunol. 30 (3), 845–850.

Oliveira, G., de Oliveira, A.E., da Conceição, E.C., Leles, M.I., 2016. Multiresponse optimization of an extraction procedure of carnosol and rosmarinic and carnosic acids from rosemary. Food Chem. 211, 465–473.

Öz, M., 2018. Effects of garlic (*Allium sativum*) supplemented fish diet on sensory, chemical and microbiological properties of rainbow trout during storage at −18°CL,W T. Food Sci. Technol. 92, 155–160.

Safarpour Amlashi, A., Falahatkar, B., Sattari, M., Tolouei Gilani, M.H., 2011. Effect of dietary vitamin E on growth, muscle composition, hematological and immunological parameters of sub-yearling beluga *Huso huso* L. Fish Shellfish Immunol. 30, 807–814.

Sarabi, M., Keramat, J., Kadivar, M., 2017. Antioxidant effect of rosemary extract and BHT on the quality of coated fried Escolar (*Lipidocybium flavobrunium*) fish fillets during frozen storage. Int. Food Res. J. 24 (2), 525–533.

Shi, J., Lei, Y., Shen, H., Hong, H., Yu, X., Zhu, B., Luo, Y., 2019. Effect of glazing and rosemary (*Rosmarinus officinalis*) extract on preservation of mud shrimp (*Solenocera melantho*) during frozen storage. Food Chem. 272, 604–612.

Ta'ati, R., Soltani, M., Bahmani, M., Zamini, A.A., 2011. Growth performance, carcass composition, and immunophysiological indices in young great sturgeon (*Huso huso*) fed on commercial prebiotic, immunoster. Iran. J. Fish. Sci. 10, 324–335.

Ta'ati, R., Abolghasemi, S.J., Tatina, M., Tajan, M.N., 2012. Influence of prebiotic Immunowall on growth performance, body composition and immunophysiological variables in young great sturgeon, *Huso huso*. Ann. Biol. Res. 3, 4435–4441.

Vallejos-Vidal, E., Reyes-López, F., Teles, M., MacKenzie, S., 2016. The response of fish to immunostimulant diets. Fish Shellfish Immunol. 56, 34–69.

Vijayan, K.K., Makesh, M., Otta, S.K., Patil, P.K., Poornima, M., Alavandi, S.V. (Eds.), 2017. Compendium on Prophylaxis in Aquaculture. Central Institute of Brackish water Aquaculture Indian Council of Agricultural Research.

Yousefi, M., Hoseini, M., Vatnikov, Y.A., Kulikov, E.V., Drukovsky, S.G., 2019. Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. Aquaculture. 505, 473–480.

Zaouali, Y., Bouzaine, T., Boussaid, M., 2010. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem. Toxicol. 48, 3144–3152.

Zoral, M.A., Ishikawa, Y., Ohshima, T., Futami, K., Endo, M., Maita, M., Katagiri, T., 2018. Toxicological effects and pharmacokinetics of rosemary (*Rosmarinus officinalis*) extract in common carp (*Cyprinus carpio*). Aquaculture. 495, 955–996.