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Original Research

Influences of dietary omega-3 fatty acids on haematologic and immunologic changes in rainbow trout milked regularly

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Abstract: In this study, it was examined the effects of dietary omega-3 fatty acids on the haematocrit (Ht), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), total protein (TP) and total immunoglobulin (TI) values, erythrocyte (RBC) and leucocyte (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), phagocytic index (PI) and nitroblue tetrazolium (NBT) activity in rainbow trout (*Oncorhynchus mykiss*) milked regularly. For this aim, one control and two experimental diets were prepared as isonitrogenous and isocaloric. Omega-3 fatty acid was not supplement to the control diet. However, the D1 and D2 diets included 1 and 3% omega-3 fatty acids, respectively. The Ht, Hb, RBC, WBC, MCV, MCH, MCHC, NBT, TP, TI and PI values of the rainbow trout fed the control diet were found significantly lower than these values in the fish fed the D2 and D1 diets at all sampling periods, too ($P < 0.01$). The highest levels for all these parameters were obtained from the fish fed the D2 diet at all sampling periods. These values in the rainbow trout fed the D2 diet were significantly higher than the values in the fish fed the D1 diet at all sampling periods ($P < 0.01$). However, the effect of sampling periods on these values in the fish fed the D1 and D2 diets was found insignificant ($P > 0.01$). In conclusion, the omega-3 fatty acid supplementation at 2% level to the diet can effectively support the haematological and immunological values in the rainbow trout under regular milking conditions, and ultimately protect the health.

Key words: Rainbow trout, fatty acids, haematologic values, immune response, regular milking.

Introduction

Poly unsaturated fatty acids (PUFAs) are very effective on the haematologic and immunologic values as well as its nutritional value (1-4). The taken together with diet in a sufffficient amount of omega-3 fatty acids is necessary for the alternate complement activity in fish such as Atlantic salmon *Salmo salar* (5) and rainbow trout *Oncorhynchus mykiss* (6, 7). The influence of dietary omega-3 PUFAs on the sperm quality and antioxidant defense in the rainbow trout brood under regular stripping conditions has been investigated by Köprücü et al. (8). Nevertheless, the effects of dietary omega-3 fatty acids on the haematologic and immunologic values in the rainbow trout milked regularly has been not examined. Thus, this study focused on the investigation of the effects of dietary omega-3 fatty acids on the haematocrit (Ht), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), total protein (TP) and total immunoglobulin (TI) values, erythrocyte (RBC) and leucocyte (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), phagocytic index (PI) and nitroblue tetrazolium (NBT) activity in the rainbow trout milked regularly.

Materials and Methods

Experimental Condition and Diets

This trial was carried at Fisheries Station of Aquaculture Faculty in Firat University, Elazig, Turkey. The rainbow trout brood in two-years-old were used in the study. The treatments were conducted with three replications. Each replicate had 12 fish (mean fish weight: 1 ± 0.1 kg). The experiments were performed in concrete tanks (3 x 2 x 1 m) subjected to the natural daily light regime. The tanks were continually supplied with 1.5 L min^{-1} of freshwater. The water temperature, pH and dissolved oxygen were measured daily during the trial. The mean temperature was 9 ± 2 °C; dissolved oxygen was 10.4 ± 0.2 mg L^{-1}; pH was 8 ± 0.3 (9).

The control, D1 and D2 diets used in this study were arranged as isocaloric (3,600 kcal kg^{-1} digestible energy) and isonitrogenous (45% crude protein). The omega-3 fatty acid was supplemented to the D1 and D2 diets at 1% and 2% ratios, respectively (Table 1). However, it was not supplemented to the control diet. The omega-3 fatty acids concentrated from the anchovy oil (Solgar OMEGA-3 700) was obtained from Solgar Corporation, Istanbul, Turkey. Other diet ingredients were bought from a local firm. Preparation of the experimental diets had previously been described by Köprücü et al. (8). The rainbow trout used in the experiment was fed three times in a day used to the free feeding technique for 60 days in the natural spawning season (10).

Sample collection and analysis

The sampling was permormed at start of the experiment and at the 30, 45 and 60th days post feeding. Blood sample (N = 12 for each sampling period) was provided from the caudal vein of the rainbow trout thereafter anesthetisation with benzocaine (25 mg L^{-1} water). Later, it was centrifuged at 1500 x *g* for 5 min at 4 °C. A divi-

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Table 1. Formulation of the experimental diets.

Diet ingredients (% as diet basis)	Control	D1	D2
Anchovy meal	35	35	35
Soybean meal	30	30	30
Corn gluten	5	5	5
Wheat starch	5	5	5
Soybean oil	15.2	14.2	13.2
Omega-3 fatty acid [1]	-	1	2
Vitamin mixture [2]	1	1	1
Mineral mixture [3]	1	1	1
Wheat bran	7.8	7.8	7.8

[1] Solgar OMEGA-3 700 (Solgar Corporation, Istanbul, Turkey): Omega-3 fatty acids concentrated from the anchovy oil containing 54.3% eicosapentaenoic acid, 37.1% docosahexaenoic acid and 8.6% other omega-3 fatty acids (Docosapentaenoic, linolenic, stearidonic acid).

[2] Vitamin mixture (IU or mg kg^{-1} diet): Vitamin A 2,500 IU, vitamin D_3 2,400 IU, vitamin E 100 mg, vitamin K 30 mg, vitamin B_1 1 mg, vitamin B_2 4 mg, niacin 10 mg, pantothenic acid 20 mg, vitamin B_6 3 mg, vitamin B_{12} 0.01 mg, folic acid 1 mg, d-biotin 0.2 mg, choline 1,000 mg, myoinositol 300, vitamin C 100 mg.

[3] Mineral mixture (mg kg^{-1} diet): Iron 60, zinc 30, manganese 13, copper 3, iodine 1.1, calcium 1, chlorine 0.9, potassium 0.7, phosphorus 0.6, sodium 0.6, selenium 0.3, cobalt 0.2, magnesium 0.05.

sion of the whole blood obtained from each fish individually was used for detecting the Ht, Hb, RBC, WBC, MCV, MCH, MCHC, NBTand PI levels. The remaining portion of the blood sample was used to the plasma separation. The TP and TI values were defined from the plasma samples.

The approximate contents of the diets were analysed according to AOAC (11) methods. The fatty acids in the diets were determined using the gas chromatographic methods noticed by Christie (12). The Ht, Hb, RBC, WBC, MCH, MCV and MCHC were analysed according to the haematological methods (4, 13). The immunologic (the WBC, NBT, TP, TI and PI) values were determined using the methods reported by Yonar (14).

Statistical analysis

The results provided from the trials were given as mean ± standard error. Two-way ANOVA was used for analysis of the Ht, Hb, RBC, WBC, MCV, MCH, MCHC, NBT, TP, TI and PI values using the dietary omega-3 fatty acid levels (0, 1 and 2%) and sampling periods (30, 45 and 60th day) as independent variables, followed by Duncan's new multiple range test for identified the significant ($P < 0.01$) differences between the means. The IBM SPSS 21.0 package program were used to the statistical analyses (SPSS Inc. Chicago, IL, USA).

Results

The approximate compositions and fatty acid contents of the experimental diets were showed in the Table 2 and Table 3, respectively.

In this study, the average amount of individual feed intake of the rainbow trout fed the control, D1 and D2 diets during 60 days was counted as 1200, 1225 and 1240 g, respectively. The difference between the feed intake amounts of the fish fed with the control and experimental diets was found insignificant ($P > 0.01$).

The Ht, Hb, RBC, MCV, MCH and MCHC values (Table 4) of the rainbow trout fed the omega-3 fatty acid insufficient control diet were significantly lower than these values in the fish fed the D2 and D1 diets at all sampling periods ($P < 0.01$). However, the dietary supplementation of the omega-3 fatty acids increased these

Table 2. Approximate composition and digestible energy value of the experimental diets.

Components (% in dry matter)	Control	D1	D2
Dry matter	95.95	95.96	95.98
Crude (digestible) protein	44.98 (35.7)	45.04 (35.7)	45.01 (35.7)
Crude fat	19.35	19.38	19.40
Ash	6.69	6.65	6.66
Fibre	2.58	2.56	2.57
Nitrogen-free extract	22.35	22.33	22.34
Digestible energy (kcal kg^{-1})[1]	3,600	3,600	3,600
Digestible protein/digestible energy (mg kg^{-1})	99.17	99.17	99.17

[1] The digestible protein and energy values were calculated from NRC (**14**).

Table 3. Fatty acid composition of the experimental diets.

Fatty acids (Percentage of total fatty acids)	Control	D1	D2
14:0	3.20	3.05	2.87
16:0	10.19	10.46	10.59
16:1 n-7	2.31	2.43	4.28
18:0	3.70	3.60	3.55
18:1 n-9	25.60	23.48	20.19
18:2 n-6	44.30	41.46	38.12
18:3 n-3	1.02	1.14	1.25
18:4 n-3	0.90	0.96	1.05
20:1 n-9	0.58	0.40	0.34
20:5 n-3	4.45	7.23	10.01
22:1 n-9	0.10	0.12	0.16
22:5 n-3	0.95	1.10	1.19
22:6 n-3	2.70	4.57	6.40
Total saturated fatty acids	17.09	17.11	17.01
Total monounsaturated fatty acids	28.59	26.43	24.97
Total omega-3 fatty acids	10.02	15.00	19.90
Total omega-6 fatty acids	44.30	41.46	38.12
Omega-3/Omega-6 ratio	0.23	0.36	0.52

Table 4. The Ht, RBC, Hb, MVC, MCH and MCHC values in blood of the rainbow trout fed the different levels of dietary omega-3 fatty acids under regular milking conditions.

Sampling period		Ht (%)	RBC (x10^6)	Hb (g dL^{-1})	MCV (μ^3)	MCH (µµg)	MCHC (%)
	Initial value	39.83 ± 1.58	1.54 ± 0.01	7.11 ± 0.22	258.64 ± 12.73	46.95 ± 3.78	18.44 ± 2.01
30th day	Control	35.09 ± 2.56^{a,x}	1.46 ± 0.05^{a,x}	6.27 ± 0.35^{a,x}	240.34 ± 16.25^{a,x}	42.54 ± 5.08^{a,x}	17.19 ± 2.56^{a,x}
	D1	41.51 ± 2.52^{b}	1.56 ± 0.05^{b}	7.55 ± 0.41^{b}	271.31 ± 18.09^{b}	49.36 ± 4.57^{b}	19.32 ± 2.65^{b}
	D2	46.30 ± 3.11^{c,x}	1.62 ± 0.07^{c,x}	8.32 ± 0.39^{c,x}	285.80 ± 14.52^{c,x}	53.67 ± 5.24^{c,x}	20.63 ± 2.92^{c,x}
45th day	Control	33.56 ± 3.69^{a,x}	1.45 ± 0.07^{a,x}	6.10 ± 0.23^{a,x}	231.45 ± 9.81^{a,x}	42.31 ± 4.08^{a,x}	17.06 ± 2.71^{a,x}
	D1	41.23 ± 2.56^{b}	1.53 ± 0.06^{b}	7.48 ± 0.33^{b}	269.48 ± 15.47^{b}	49.02 ± 4.94^{b}	19.03 ± 3.02^{b}
	D2	45.45 ± 3.06^{c,x}	1.61 ± 0.07^{c,x}	8.23 ± 0.45^{c,x}	282.30 ± 17.88^{c,x}	52.29 ± 5.32^{c,x}	20.15 ± 2.34^{c,x}
60th day	Control	33.17 ± 3.17^{a,x}	1.45 ± 0.06^{a,x}	6.01 ± 0.28^{a,x}	228.76 ± 14.28^{a,x}	40.03 ± 4.44^{a,x}	16.98 ± 2.60^{a,x}
	D1	40.58 ± 3.58^{b}	1.52 ± 0.06^{b}	7.39 ± 0.31^{b}	266.97 ± 14.09^{b}	48.76 ± 4.93^{b}	18.92 ± 2.69^{b}
	D2	45.04 ± 2.67^{c,x}	1.60 ± 0.07^{c,x}	8.00 ± 0.42^{c,x}	281.50 ± 13.72^{c,x}	51.75 ± 5.54^{c,x}	19.95 ± 3.52^{c,x}

Ht: Haematocrit, RBC: Erythrocyte counts, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration.

The control diet does not contain omega-3 fatty acid; the D1 and D2 diets are supplemented with the omega-3 series fatty acid at a 1% and 2% ratios, respectively.

^{a,b,c} The values (mean ± SE) in the same column with different letters are statistically significant ($P < 0.01$, N = 12).

^{x} Different from the initial value ($P < 0.01$).

values in the fish fed the D2 and D1 diets. The highest Ht, Hb, RBC, MCV, MCH and MCHC values were provided from the fish fed the D2 diet at all sampling periods, too. Furthermore, these values in the rainbow trout fed the D2 diet were significantly higher ($P < 0.01$) than the values in the fish fed the D1 diet at all sampling periods. However, the effect of sampling periods on these values of rainbow trout fed the D1 and D2 diets was insignificant ($P > 0.01$).

The WBC, NBT, PI, TP and TI values (Table 5) of the rainbow tout fed the control diet were found significantly lower than the values in the fish fed the D2 and D1 diets at all sampling periods ($P < 0.01$). On the contrary, the dietary supplementation of omega-3 fatty ac-

Table 5. The WBC, NBT, PI values in blood, the TP and TI levels in plasma of the rainbow trout fed the different levels of dietary omega-3 fatty acids under regular milking conditions.

Sampling period		WBC (x10^3)	NBT (mg ml^{-1})	TP (mg ml^{-1})	TI (mg ml^{-1})	PI (%)
	Initial value	39.25 ± 2.18	0.95 ±0.05	36.59 ± 2.76	15.33 ± 2.01	39.20 ± 1.75
30th day	Control	33.17 ± 2.10^{a,x}	0.86 ± 0.04^{a,x}	30.80 ± 2.60^{a,x}	12.30 ± 2.22^{a,x}	33.53 ± 2.65^{a,x}
	D1	40.29 ± 2.59^{b}	0.99 ± 0.06^{b}	37.11 ± 3.39^{b}	16.57 ± 1.80^{b}	39.91 ± 2.10^{b}
	D2	44.52 ± 3.21^{c,x}	1.18 ± 0.07^{c,x}	44.22 ± 4.13^{c,x}	20.15 ± 2.55^{c,x}	44.35 ± 2.64^{c,x}
45th day	Control	32.81 ± 3.05^{a,x}	0.84 ± 0.01^{a,x}	28.39 ± 2.82^{a,x}	12.24 ± 1.48^{a,x}	32.58 ± 2.23^{a,x}
	D1	41.10 ± 2.99^{b}	0.98 ± 0.05^{b}	36.63 ± 2.95^{b}	15.96 ± 2.14^{b}	40.10 ± 1.85^{b}
	D2	46.04 ± 4.51^{c,x}	1.14 ± 0.07^{c,x}	45.03 ± 3.85^{c,x}	21.70 ± 1.93^{c,x}	45.52 ± 2.55^{c,x}
60th day	Control	32.61 ± 3.07^{a,x}	0.83 ± 0.05^{a,x}	27.65 ± 2.67^{a,x}	11.87 ± 1.22^{a,x}	31.25 ± 1.65^{a,x}
	D1	40.53 ± 3.09^{b}	0.98 ± 0.06^{b}	36.24 ± 3.29^{b}	16.08 ± 1.84^{b}	38.40 ± 2.18^{b}
	D2	45.78 ± 4.16^{c,x}	1.15 ± 0.07^{c,x}	44.61 ± 4.56^{c,x}	20.93 ± 2.61^{c,x}	45.29 ± 2.29^{c,x}

WBC: Leucocyte counts, NBT: Nitroblue tetrazolium activity, PI: Phagocytic index, TP: Total protein, TI: Total immunoglobulin.
The control diet does not contain omega-3 fatty acid; the D1 and D2 diets are supplemented with the omega-3 fatty acid at a 1% and 2% ratios, respectively.
^{a,b,c} The values (mean ± SE) in the same column with different letters are statistically significant ($P < 0.01$, N = 12).
^{x} Different from the initial value ($P < 0.01$).

ids provided an increase in these values in the rainbow trout fed the D2 and D1 diets. The highest WBC, NBT, TP, TI and PI values were provided from the fish fed the D2 diet at all sampling times. Moreover, these values in the rainbow trout fed the D2 diet were significantly higher ($P < 0.01$) than the values in the fish fed the D1 diet at all sampling times, too. However, the effect of sampling periods on these values in the rainbow trout fed the D1 and D2 diets was insignificant ($P > 0.01$).

Discussion

Poly unsaturated fatty acids has been declared to be a potent immunostimulant in several fish species (3, 4). In the present study, the significant differences in the Ht, Hb, RBC, WBC, MCV, MCH, MCHC, NBT, TP, TI and PI values of the rainbow trout milked regularly were determined among the groups after feeding the control diet and omega-3 fatty acids supplemented experimental diets. The WBC level was higher in the D2 diet group followed by the D1 diet group, which were significantly higher ($P < 0.01$) than the WBC level of the rainbow trout in the control group at all sampling periods, too. The increase in the WBC level of the rainbow trout fed the D2 diet supplemented at 2% level of omega-3 fatty acids shows immunostimulant effect of their higher doses, which may be connected with the high NBT activity in the high omega-3 fatty acid fed groups. Our results are in conformity with the results of studies conducted by Mishra et al. (3), Kumar et al. (4) and Choudhury et al. (16).

The present study showed an important ($P < 0.01$) increase in the TP, TI and PI levels in the rainbow trout fed the diets supplemented omega-3 fatty acids. The highest TP, TI and PI values were provided from the rainbow trout fed the D2 diet followed by the D1 diet group, which were significantly higher than those of the fish fed the control diet at all sampling periods ($P < 0.01$). These results are supported by the increase in the PI and WBC levels. Puangkaew et al. (7) also reported that the higher WBC level is in correlation with the other immunological parameters. Wu et al. (2) showed that the dietary docosahexaenoic acid enhanced the phagocytic functions of leucocytes in the juvenile grouper (*Epinephelus malabaricus*). Our results also demonstrate that the dietary inclusion of omega-3 fatty acids could enhance the immune responses of the rainbow trout milked regularly. Sheldon and Blazer (17) determined that the increse of immune responses of the channel catfish (*Ictalurus puctatus*) when their diet contained the increased levels of dietary n-3 PUFAs. Mishra et al. (3) showed that up to 2% ratio of the dietary omega-3 PUFAs enhanced the immune responses of the rohu (*Labeo rohita*) juveniles.

However, the intake at higher rates than 2% of dietary omega- PUFAs has been well known to the reduce immune reactions in the *O. mykiss* (6), *S. salar* (5, 18) and Indian major carp (*Catla catla*) juveniles (4).

Blazer (1) reported that the increase of the respiratory burst activity can be related with the increase of the phagocytic activity. In our study, it was measured by the increase of the NBT activity. The highest NBT activity level was obtained from the rainbow trout fed the D2 diet supplemented omega-3 fatty acids at 2% ratio. This data compatible to the results of the studies conducted by Sheldon and Blazer (17) in the *I. puctatus* and Choudhury et al. (16) in the *L. rohita*.

In conclusion, the dietary supplementation of omega-3 fatty acids at 2% level shows better the haematological values and immunological responses in the rainbow trout milked regularly. The role of the omega-3 fatty acids as an potent immunostimulant clearly connected with the increase of the haematologic and immunologic values of the rainbow trout milked regularly. These results showed that the supplementation and balance of omega-3 fatty acids in the diets of the rainbow trout under regular milking conditions could improve the haematological and immunological values, and ultimately protect the health.

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The fish in this experiment were acted in agreement with the experimental protocol has been approved by

the Firat University Animal Experimentation Ethics Committee (FUAEEC) operating under the 2006 *Turkish code of practice for the care and use of animals for scientific purposes.*

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