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Determination the impact of use of dietary vegetable oil source and different levels of L-carnitine supplementation on hematological and immunological parameters of rainbow trout (*Oncorhynchus mykiss*)

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Abstract: The aim of current study was to determination the impact of different dietary vegetable oil source and L-carnitine supplementation on hematological and immunological parameters of rainbow trout. The number of 288 rainbow trout fish were fed by 2×2 randomized experimental factorial designs were used with 4 replicate each. Dietary treatments contained fish oil or vegetable oil and two dietary levels of L-carnitine (0.6 or 1.2 $g.kg^{-1}$) supplement respectively. Experiment period of trials lasted for 9 weeks. At the end of experiment period, hematological and immunological parameters such as immunoglobulin M (IgM) concentration of fish blood were determined. Results of study showed that replacement of fish oil by vegetable oil significantly increased blood hematocrit, red blood cell count, hemoglobin in comparison with fish oil dietary treatment ($p \leq 0.05$). Dietary L-carnitine supplementation reduced heterophils ($p \leq 0.05$) and L-carnitine supplementation with fish oil diets increased lymphocyte and reduced IgM and IgG significantly instead ($p < 0.05$). In conclusion we may demonstrate that use of dietary vegetable oil sources with different levels of L-carnitine supplementation has beneficial acts on immunological response and hematological traits of rainbow trout Fish.

Key words: L-carnitine, Rainbow trout fish, Vegetable oil, Hematological parameters, Immunological parameters.

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Introduction

Rainbow trout fish required energy and essential amino acids for growth and this could be met from feed sources (RODEHUTSCORD, 1995). The dietary protein is the most expensive and it could be provided essential and non-essential amino acids for maximum growth in fish diet (LOVELL, 1998,) It will be improved protein efficiency if a greater percentage of dietary protein allocated to growth and tissues recovery and energy supply from lipids and protein sparing action (HALVER AND HARDY, 2002).

Lipids also are the major energy contribution in fish nutrition, and increase the absorption of lipid-soluble nutrients, affected both innate and adaptive immunity and fish metabolism (TURCHINI et al 2010).

Carnitine, derived from an amino acid, is the generic term for several compounds, including L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine. It is an essential cofactor that helps transport long-chain fatty acids into the mitochondria so that they can be oxidized to produce energy in the form of adenosine triphosphate and Carnitine also helps transport some toxic compounds out of the mitochondria. L-carnitine helps the body turn fat into energy. It is an important such as vitamins and carotenoid pigments (Turchini et al 2010). They are as an important source of essential fatty acids for regular growth, health, reproduction and important functions of fish body (TURCHINI et al 2009).

Fish oils have high level of n-3 highly unsaturated fatty acids and traditionally have been used as the important dietary lipid source in commercial fish feeds which are known to be essential dietary source for the optimal growth and health for fish. Soybean oil is a vegetable oil which is high level of n-6 poly unsaturated fatty acids and changes in dietary fatty acid compositions by replacing fish oil by vegetable oil may be material for heart and brain function, muscle movement, and many other body processes and its acts as a transmitter for long chain fatty acids translocation therefore is vital for fatty acids β-oxidation (MOHSENI AND OZORIO 2014).

It is a non-essential nutrient (Harpaz, 2005) and is synthesized from methionine and lysine (Arslan, 2006). It is also capable to repartitioning of energy between muscle (Foster, 2004) and fat tissue, hence causing improvement of muscle to fat ratio (Ricke et al, 1999). L-carnitine is including supplements which may be affected on fat and protein

metabolism of trouth (KERAMAT and ABOLFAZLI, 2017).

Many investigations showed that different results obtained from different levels of supplementation of L-carnitine in rainbow trout and typically linear dose or response relations have been observed in a few studies when growth parameters are taken into account (Haji Abadi et al., 2010 , Arslan , 2006) but so far, a relatively small amount of work has been done on the effects of L-carnitine on hematological and immunological parameters of rainbow trout fish, hence the aim of this study was to determine the impact of comparison between fish oil and vegetable oil with different levels of L-carnitine supplementation on some hematological and immunological parameters of rainbow trout.

Material and methods

A total of 288 rainbow trout with an average initial body weight of 85±10 g was randomly allotted in 4 treatments according to the 2×2 randomized experimental factorial design and the fish randomly allocated to each of 16 experimental rearing tanks before starting the experiment. In order to diet adaptation, experimental fish were fed by basal diet up two weeks and after this period experimental diets were fed daily in the morning and evening (8:00 am and 16:00 pm) manually according to apparent saturation for 9 weeks.

Diet preparation and treatments

Basal diet formulated based on free oil feed GFT_1 provided in Mahan Company Iran. According to supplementation, basal diet containing dietary soybean or fish oil and also oil supplemented diets prepared again with addition two levels of L-Carnitine (0.6 and 1.2 $gr.kg^{-1}$). Accordingly, 4 experimental diets were formulated. In terms of experimental diets, free L-carnitine supplement added to basal diet. The different oil sources added into basal diets and were mixture for 45 minutes manually. After the experimental period, all fish were anaesthetized by solution of clove powder in water (45 $mg.lit^{-1}$) and 4 fish randomly selected from each tank and blood sampling was taken by caudal vein and then killed by a blow to the head. The liver removed and stored at -20$^{\circ C}$ until testing fat percentage. The fish fillet meat without skin and bone were isolated for measuring carcass parameters and stored at -80 $^{\circ C}$ until testing.

Blood hematology parameters

At the end of study period the blood samples were taken from 4 fish of each tank and added to heparin and non-

heparin tubes. To measure the Ig M, none-heparin clouting blood samples were centrifuged at 2000 rpm for 25 min and stored at -80°C prior to analysis. Other part of blood heparin was used for immune parameter detection.

The fish hematocrit (Hct) was detected based on (Ruane et al, 2001) description. Each of samples divided in to four heparinized capillary tube and centrifuged at 10000 rpm for 5 min by micro centrifuge adjusted for hematocrit value. The hematocrit value was calculated based on the white blood cells were determined by using white melanjor pipette, neubauer 1 cm and Turke' s solution with 20:1 ratio. Hemoglobin value was determined based on cyanmethemoglobin method.

The differential leukocyte count was done based on (Rey Vazquez and Guerrero, 2006) by an optical microscope with 40× and 100× magnification. The white and red blood cells WBC and RBC counts were achieved by (Rawling, 2009) method.

Immunological parameters assay

To determine the IgG and IgM the commercial kit (Zist Chemistry Company) based on the reaction between IgM and IgG antibody and antigen resulting creation of insoluble compound were applicated. The light scattering by insoluble compound is proportional to IgM and IgG concentration.

Statistical analysis

Results were analyzed by general linear model program in statistical software of SAS (9.1) version at the significance level was based on ($p \leq 0.05$) and differences means between groups were compared by Duncan's multiple range tests at 0.05 levels.

Results

The results of hematological parameters of experimental rainbow trout are shown in Table 1 as main and interaction effect. Based on results replacement of fish oil by soybean oil (or generally oil source) had significant effect on hematocrit, RBC, hemoglobin, percentage of eosinophil, phagocytosis and average phagocytosis particle in rainbow trout ($p \leq 0.05$).

Replacement of fish oil of soybean oil significantly increased hematocrit, RBC, hemoglobin, phagocytosis activity and phagocytized particle, but percentage of eosinophil was lower than fish oil treatment ($p \leq 0.05$).

Other parameters such as heterophils, lymphocytes, monocytes and also IgM and IgG were no affected by dietary oil source. As shown in table 1 the

L-carnitine supplementation significantly reduced heterophils but other hematological factors of fish were not affected. The soybean oil supplemented with L-carnitine causes significantly decreased hematocrit, RBC and hemoglobin ($p \leq 0.05$) of blood fish.

The fish oil with and without L-carnitine had no effect on hematocrit ($p \geq 0.05$) but fish oil without supplementation highly significant reduced RBC level comparison to fish fed by fish oil containing L-carnitine.

The hemoglobin values were significantly greater in fish blood which is fed by fish oil and L-carnitine comparison to other treatment ($p<0.05$). There was no interaction effect between soybean oil and L-carnitine supplements on WBC level of blood fish, but fish oil treatments, the interaction was effective, so that fish oil treatment supplied L-carnitine was differed from other treatment ($p<0.05$).

Differential count of WBC indicated that soybean oil and supplements had no effect on heterophil and eosinophil count but soybean oil and L-carnitine supplements increased blood lymphocytes significantly in comparison to the others ($p<0.05$). The interaction between fish oil and L-carnitine supplement had no effect on monocyte and neutrophil, but fish oil alone without L-carnitine supplementation significantly increased heterophil and decreased lymphocyte as shown in table 1 ($p \leq 0.05$). It seems that the addition of L-carnitine to soybean oil dietary regimes lead to reduce IgM and IgG level ($p \leq 0.05$).

Table 1. Hematological parameters of rainbow trout fed by different vegetable oil source and L-carnitine different levels supplementation

Treatments		HCT %	RBC (10^6/µl)	Hb (g/dl)	WBC (10^6/µl)	Het %	Lym %	Mon %	Eos %
Vegetable oil		37.28^{a**}	3.21^{a}	9.45^{a}	25458	31.04	61.52	4.85	2.47^{b}
Fish oil		31.94^{b}	2.75^{b}	8.87^{b}	25220	29.62	62.43	5.08	3.12^{a}
P-Value		**	**	**	n.s	n.s	n.s	n.s	**
L-carnitine	0.6	35.59	3.05	9.38	24852	32.22^{a}	60.29	4.77	2.81
	1.2	33.52	2.94	8.94	25951	28.29^{b}	63.56	5.15	2.82
P-Value		n.s	n.s	n.s	n.s	**	n.s	n.s	n.s
Oil		0.155	0.205	0.0501	0.252	0.001	0.028	0.294	0.821
L-carnitine		0.168	0.217	0.0559	0.270	0.003	0.055	0.381	0.914
Oil × L-carnirtine		0.3006	0.112	0.326	0.252	0.546	0.995	0.047	0.331
Vegetable oil		41.04^{a}	3.29^{bc}	10.08^{a}	2262^{cb}	32.25^{ab}	58.51^{c}	5.01^{abcd}	3.29^{ab}
Vegetable oil × L-carnitine		41.09	3.58^{a}	10.07^{a}	2725^{ab}	29.68^{bc}	59.25^{cb}	6.68^{a}	2.02^{b}
Fish oil		29.32^{c}	2.49^{c}	8.55^{cd}	2622^{cab}	36.02^{a}	55.00^{c}	6.35^{ab}	3.01^{ab}
Fish oil × L-carnitine		32.04^{bc}	2.92^{cd}	8.48^{cd}	2878^{a}	25.71^{c}	68.00^{a}	5.34^{abc}	2.76^{ab}
P-Value		**	**	**	**	**	**	**	**

**a,b,c: The means with different letters super scripted in each letter are different ignificantly($p \leq 0.05$)

The IgM and IgG level was positively affected by addition of L-carnitine and also, the addition of L-carnitine to fish oil dietary treatment reduced IgM and IgG level in fish blood serum. Data showed that the use of soybean oil to substitution fish oil in rainbow trouth diet did not change in immune parameters such as heterophil, lymphocyte, monocyte or IgM and IgG, although the mentioned parameters were slightly lower in fish oil treatments than soybean oil (Table 2).

According to the current data it seems that complete fish oil replacement with soybean oil can enhance cell immunity in experimental trout compared to the control. Although various studies have reported non-stationary effect of dietary oil sources on immunity of fish (BLAZER et al, 1991). In agreement with results of this study, Montero et al (2008) reported that replacing dietary fish oil with various vegetable oil such as soybean and mustard oil; in a short period of feeding; had no significant effect on immune function. Nevertheless, the effect of lipid composition especially omega-3 fatty acids on immune modulators both immune repressive and stimulator factors documented (LALL, 2000).

Table 2. Immunological parameters of rainbow trouth fed by different vegetable oil source and L-carnitine different levels supplementation

Treatments		Ig G	IgM
Vegetable oil		0.138	0.132
Fish oil		0.132	0.125
P-Value		n.s	n.s
L-carnitine	0.128	0.131a	0.128^{a}
	0.126	0.128b	0.126^{b}
P-Value		**	**
Oil		0.116^{b}	0.111^{b}
L-carnitine		0.121^{a}	0.115a
Oil × L-carnirtine		0.039	0.035
Vegetable oil		**	**
Vegetable oil × L-carnitine		0.151^{a}	0.147^{a}
Fish oil		0.142^{ab}	0.131^{ab}
Fish oil × L-carnitine		0.148^{a}	0.142^{a}
P-Value		**	**

**a,b,c: The means with different letters super scripted in each letter are different significantly($p \leq 0.05$)

Generally, based on studies accepted that diets containing vegetable oil compare to fish oil diets, increase levels of mono unsaturated fatty acids and poly unsaturated fatty acids particularly n-6 series and decrease the level of totally n-3 poly unsaturated fatty acids (LALL, 2000).

Evidence suggests that diverse fatty acid profile related to vegetable or fish oil can influence on immune function by changing the physiological process and eicosanoid and prostaglandin synthesis or even physiology of the cell membrane (ASHTON et al, 1994).

Previously shown that immune parameters related to humeral and cell immunity such as phagocytosis highly altered by level and profile of fatty acids, mainly due to change in membrane fluidity, intracellular signaling pathways and trans membrane receptors involved in complement activity, MONTERO et al (2008).

Unlike the results of this study, Montero et al (2008) reported that replacement of fish oil with vegetable oil adversely reduced phagocytosis activity in sea bream. Also, partial replacement fish oil up to 60% by vegetable oils reduced RBC counts, Montero et al (2008) while our results showed that the higher hematological values such as hematocrit, RBC, hemoglobin, phagocytosis and average phagocytised particle resulted in feeding of vegetable oil diets. Nonetheless different experimental condition, fish species and amount of substituted vegetable oil are responsible for inconsistent results reported by different articles.

Otherwise in opposition with some researchers based on potentiality to replacing fish oil with a vegetable oil in the feeds of farmed fish without compromising growth, non-specific immune function and overall histological appearance. Other observation focused on differential effects of fish oil and vegetable oil sources on growth trend and carcass and fillet composition; hence this led to some researchers reject substitution of vegetable oil instead of fish oil as an economical solution to compensate lack of dietary fish oil in developing aquaculture (SARGENT et al, 2002).

Based on our results (table 3), liver fat and carcass protein affected by oil source, so that in the fish oil diet these were significantly low levels ($p<0.05$). Dietary L-carnitine supplementation was not affected meat filet composition but addition of L-carnitine to soybean oil diet reduced fat content of filet and increased it in fish liver (table 3). In fish fed diet

containing soybean oil plus L-carnitine and soybean oil plus both supplements, reduction in fat content appeared to be significant (table 3), but liver fat increased in treatment.

Akbari Azad et al (2010) reported L-carnitine in doze dependently increasing immune function particularly Hb in broilers fed L-carnitine supplement up to 375 $mg.kg^{-1}$. Also, L-carnitine caused the increasing in WBC and decreasing in RBC level in broiler chicks (Akbari Azad et al, 2010) whereas in this study there were no effects on WBC or RBC counts. There has been no clear effect of L-carnitine supplementation on immune parapets and a few reported results are related to fat metabolism parameters such as cholesterol, triglyceride and total body fat.

Conclusion

In conclusion we may demonstrate that the immunological and hematological response of rainbow trouth to L-carnitine and supplement were affected by dietary oil sources. It seems that replacement of dietary fish oil by soybean oil may be changed fatty acid and lipid metabolism and immunity response of experimental fish trouth. The future studies are needed for more explanation.

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