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EVALUATION THE MEAT COMPOSITION AND IMMUNITY PARAMETERS OF RAINBOW TROUT (*Oncorhynchus mykiss*) FED BY DIETARY DIFFERENT OIL SOURCES, L-CARNITINE AND RACTOPAMINE SUPPLEMENT

Mojtaba Nazari Farsani[1], Sayed Mohammad Ali Jalali[1] and Mohesn Jafarian Dehkordi[2]
[1]Department of Animal Sciences, Faculty of Agriculture and Natural Resources, Islamic Azad University, Shahrekord Branch, Iran
[2]Department of Veterinary, Faculty of Veterinary, Islamic Azad University, Shahrekord Branch, Iran
Email: Fish.nutritionist@gmail.com

ABSTRACT

To study the effects of dietary oil sources, L-carnitine and Ractopamine supplement on the meat filet composition, hematological and immunological parameter of rainbow trout, 288 fish (initial body weight 90 ± 5 g) were fed by 8 dietary treatments as a 2×2×2 factorial experimental design. Dietary treatments contained fish oil or soybean oil and two levels of L-Carnitine (0 or 1g.kg^{-1}) and ractopamine (0 or 10 mg.kg^{-1}) supplement and fish fed for 8 week feeding trials. At the end of experiment, fish filet composition (protein, fat and ash), liver fat and hematological parameter (hematocrit, red blood cell, white blood cell, hemoglobin, phagocytosis and etc.) and also concentration of immunoglobulin M (Ig M) in blood of fish were measured. Results showed that replacement of fish oil by soybean oil significantly increased crud protein percentage of fish filet, lipid content of liver and hematocrit, red blood cell count, hemoglobin, phagocytosis activity and phagocytosed particles in blood fish as comparison of fish oil dietary treatment ($p<0.05$). Dietary L-carnitine supplementation reduced heterophils and phagocytosed particles but no change other hematological parameter and filet fish composition ($p<0.05$). Ractopamine supplement significantly reduced hematocrit, monocyte and Ig M but increased phagocytosed particles ($p<0.05$) in blood fish. Addition of L-carnitine plus ractopamine to soybean oil diet reduced fat content of filet and increased it in fish liver. Also addition of L-carnitine or ractopamine to fish oil diet increased lymphocyte and reduced Ig M in fish blood. Data of the present experiment showed that immunological and hematological response of rainbow trout to L-carnitine and ractopamine supplement were affected by dietary oil sources.

Keywords: fish oil, soybean oil, L-carnitine, ractopamine, rainbow trout.

INTRODUCTION

Advantages of aquatic protein in human nutrition than beef and sheep protein led to aquaculture industry have become as important and noteworthy as fishing in different areas of the world (FAO, 2012). Rainbow trout with scientific name of *Oncorhynchus mykiss*, is adapted to freshwater, also is one of the most important farmed fish and in 2010, about 0.7 million tons of this species were produced around the world (FAO, 2012). Benefits of cold water culture such as simplified artificial reproduction, lager larva than other freshwater fish, food reception, acceptable gain rate and good marketability has led to development of rainbow industry (Webster and Lim, 2002). Fish, like other animals, required energy and essential amino acids for growth and this could be met from feed sources. Typically, dietary protein is the most expensive dietary composition and it could be provided essential and nonessential amino acids for maximum growth (Lovell, 1998,). It is inevitable that some amino acids used for energy production through the direct oxidation of them in the Krebs cycle or after the conversion of them into glucose by gluconeogenesis process (Halver and Hardy, 2002). It seems it will be improved protein efficiency if a greater percentage of dietary protein allocated to growth and tissues recovery and energy supply from lipids (protein sparing action) (Halver and Hardy, 2002). Lipids are the major energy contribution in fish nutrition, and act as vectors for the absorption of lipid-soluble nutrients, including vitamins and carotenoid pigments (Turchini *et al.* 2010). Dietary lipids are an important source of essential fatty acids for regular growth, health, reproduction and bodily functions of fish (Turchini *et al.* 2009). Fish oils have traditionally been used as the sole dietary lipid source in commercial fish feeds and it is the high level of n-3 highly unsaturated fatty acids, which are known to be essential for the optimal growth and health of farmed 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 affected both innate and adaptive immunity and fish metabolism (Turchini *et al*. 2010). L-carnitine acts as a transmitter for long chain fatty acids translocation therefore is vital for fatty acids β-oxidation (Ozorio, 2001 and Foster, 2004) and it is a non-essential nutrient (Harpaz, 2005) and is synthesized from methionine and lysine (Arslan, 2006). Ractopamine, is a synthetic beta-adrenergic agonists which is characterized by a repartitioning agent of nutrients and increase growth efficiency of Japanese quail (Mirhendi *et al*. 2014) and rainbow trout (Jalali Haji Abadi, 2010.). These components are capable to repartitioning of energy between muscle and fat tissue, hence causing improvement of muscle to fat ratio (Watkins, 1990 and Ricke *et al*, 1999). L-carnitine and ractopamine are including supplements which may be affected on fat and protein metabolism of rainbow trout

(Jalali Haji-Abadi *et al*. 2010). This study occurred to evaluate the effects of alternative oil source and also dietary ractopamine and L-carnitine supplementation on meat quality and immune system of rainbow trout.

MATERIAL AND METHODS

Fish

In this experiment a total of 288 rainbow trout with average initial body weight of 90±5 g were randomly allotted in 8 treatments according a 2×2×2 factorial experimental design. Before starting the experiment, the pieces of fish randomly allocated to each of 32 experimental rearing tanks. In order to adaptation, fish were fed by basal diet up two weeks and after this period experimental diets were fed daily in the morning and evening (9:00 am and 17:00 pm) manually according to apparent saturation for 8 weeks.

Diet preparation and treatments

Basal diet formulated based on free oil feed GFT1 provided in Faradaneh Co. (Iran). Each of experimental diets composition analysis is presented in table 1. In this experiment according to supplementation, basal diet containing dietary soybean or fish oil up to 9.26%, also oil supplemented diets prepared again with addition two levels of L-Carnitine (0 and 1 gr.kg^{-1}) or two levels of ractopamine (0 and 10 mg. kg^{-1}) or both of L-carnitine and ractopamine (each one of oil sources plus both of L-carnitine and ractopamine).

Accordingly eight experimental diets were formulated. In terms of experimental diets, grounded L-carnitine tartrate, ractopamine hydrochloride, chalk and free L-carnitine supplement added to basal diet. As mentioned, different oil sources added into basal diets and were mixture for 30 minutes manually (Table-1).

Table-1. Ingredients and chemical composition of experimental diets.

Experimental diets								
L-Carnitine (g/ kg)	0	0	1	1	0	0	1	1
Ractopamine (mg/kg)	0	0	0	0	10	10	10	10
Oil source	soybean	fish	soybean	fish	soybean	fish	soybean	fish
Ingredients (g/kg)								
Basal diet[1]	886.5	886.5	886.5	886.5	886.5	886.5	886.5	886.5
Soybean oil	110	0	110	0	110	0	110	0
Fish oil	0	110	0	110	0	110	0	110
L-Carnitine[2]	0	0	2.5	2.5	0	0	2.5	2.5
Free L-Carnitin[3] supplement	1	1	0	0	1	1	0	0
Ractopamine(mg/kg)	0	0	0	0	10	10	10	10
chalk	2.5	2.5	1	1	2.5	2.5	1	1
Chemical composition (%)								
Dry mater	92.1	92.1	92.0	92.2	92.2	92.1	92.2	92.1
Crude protein[4]	36.95	36.95	36.95	36.9	36.9	36.95	36.9	36.95
Crude fiber[5]	18.52	18.52	18.52	18.52	18.52	18.52	18.52	18.52
Ash[6]	7.65	7.62	7.62	7.64	7.64	7.65	7.6	7.63

[1]. Contain: fish meal, soybean meal, wheat flour, choline chloride, D,L methionin, lysine hydrochloride, Vit. C, B complex supplement, Vit E, salt, mineral premix, vit premix, Di calcium phosphate, binder. [2]. L-carnitine complex containing 60% of L-carnitine tartrate (40 % pure L-carnitine).[3]. Lactose; starch, micro crystal cellulose, Mg stearate.

After the experimental period, all fish were anaesthetized by solution of clove powder in water (40 mg.lit^{-1}) and 3 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 °C until testing fat percentage. The fish fillet meat without skin and bone were isolated for measuring carcass parameters and stored at -80 °C until testing.

Immune parameters assay

At the end of trial, blood was connected from 3 fish of each tank and added to heparin and non-heparin tube. For Ig M measurement, (non-heparin) clouting blood centrifuged 2000 rpm for 25 min. and blood serum sample were taken and 3 serum of each tank were mixed to getter and stored at -70°C prior to analysis. Other part of blood (heparin) was used for immune parameter detection. Fish hematocrit (Hct) was detected base on Ruane *et al* (2001) description. Each of samples divided in to three heparinized capillary tube and centrifuged at 10000 rpm for 5 min by micro centrifuge adjusted for hematocrit. Hematocrit calculated based on the WBC were determined by using white melanjor pipette, neubauer lcm and Turke· s solution with 20:1 ratio. Hemoglobin was detected base on cyano-methemoglobin method and following equation:

Hb (mg.dl^{-1}) = sample absorbance × standard concentration / standard absorbance

The differential leukocyte count was done based on Rey V´azquez and Guerrero (2006) by an optical

microscope with ×40 and ×100 magnification. White and red blood cells (WBC and RBC) counts were achieved by Rawling, (2009) method.

Ig M measurement was performed using commercial kit (Minineph Ig M KIT, The Binding Site Group Ltd, United Kingdom) based on the reaction between Ig M (antibody) and antigen resulting creation of insoluble compound. Light scattering by insoluble compound is proportional to Ig M concentration. Phagocytosis was determined combining about 1cc staph emulsion to 1cc blood sample and incubated for 30 min at 37°C before being fixed and prepared smear stained by Giemsa solution. The phagocyte activity was expressed based on 100 phagocytizing neutrophil observed under microscope. Average number of particle beads ingested by phagocytosis process was determined based on average of 10 phagocyte neutrophil.

Meat parameters assay

Meat filleted of fish isolated and separated from the bones and skin, then converted into a homogeneous mixture. Parameters of dry matter, crude protein, ash and fat of meat were measured. Dry matter, ash, crud protein (Kjeldahl method) and crud fat (Ether extract method) were determined based on approximate analysis as describe in AOAC. The liver of fish in each replicate were also mixed and homogenized and fat percent of liver measured according ether extract method.

Statistical analysis

Experiment was done according to factorial 2×2×2 by a completely randomized design. Results were analyzed by GLM program in statistical software of SAS (Statistical Analysis System Institute, Cary, Nc, USA). Significance level was based on $p<0.05$). Differences between means were made with Duncan's multiple range tests at 0.05 level. Prior to analyze statistically, percentage data converted to Arc sin.

RESULTS AND DISCUSSIONS

The results of hematological parameters of rainbow trout are shown in Table-2 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 ($p<0.05$). Replacement of fish oil with 9.26% of soybean oil significantly increased hematocrit, RBC, hemoglobin, phagocytosis activity and phagocytized particle, but percentage of eosinophil was lower than fish oil treatment ($p<0.05$). Other parameters such as heterophil, lymphocyte, monocyte and also Ig M were no effected by dietary oil source ($p>0.05$). As shown in Table-2. L-carnitine supplementation significantly ($p<0.05$) reduced heterophils and phagocytized particles but other hematological factors of fish were not affected by it. Ractopamine supplement significantly reduced hematocrit, monocyte and Ig M but increased phagocytosed particles ($p<0.05$) in blood fish.

Soybean oil supplemented with both ractopamine and L-carnitine causes significantly decreased hematocrit, RBC and hemoglobin ($p<0.05$) of blood fish (Table-2). Fish oil with and without L-carnitine and ractopamine had no effect on hematocrit ($p>0.05$) but fish oil without supplementation highly significant reduced RBC level comparison to fish fed by fish oil containing L-carnitine or ractopamine. Hemoglobin level was significantly greater in fish blood which is fed by fish oil plus ractopamine comparison to other fish oil dietary treatment ($p<0.05$). There were no interaction effect between soybean oil and L-carnitine or ractopamine 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 treatment containing fish oil plus both supplements ($p<0.05$). Differential count of WBC indicated that soybean oil and supplements had no effect on heterophil and eosinophil count but soybean oil plus both supplements increased blood lymphocytes significantly in comparison to soybean oil dietary treatment without supplementation ($p<0.05$). Also, interaction between fish oil and two supplement had no effect on monocyte and neutrophil, but fish oil alone (without supplementation) significantly increased heterophil and decreased lymphocyte of fish blood compared to all other diet containing fish oil ($p<0.05$) (Table-2).

Phagocytosis and phagocytized particle were significantly higher in rainbow trout fed dietary containing soybean oil plus both supplements compared to fish fed by soybean oil plus L-carnitine ($p<0.05$) but not in other treatment. It seems addition f L-carnitine and ractopamine to soybean oil dietary treatment (but not in fish oil) reduce Ig M level ($p<0.05$). Ig M level of blood fish was positively affected by addition of L-carnitine plus ractopamine (Table-2). Also, addition of L-carnitine or ractopamine to fish oil dietary treatment reduced Ig M level in fish blood. The use of soybean oil to substitution fish oil in rainbow trout diet did not change in immune parameters such as heterophil, lymphocyte, monocyte or Ig M; although these parameters were slightly lower in fish oil treatments than soybean.

Table-2. Immunological and hematological parameters of rainbow trout fed different oil source supplemented by L-carnitine and Ractopamine.

Experimental diet/ parameters	HCT%	RBC (10^6/µl)	Hb (g/dl)	WBC (/µl)	Hetero%	Lym%	Mon%	Eos%	Phagocytosis%	phagocytized particle	IgM (g/L)
Main effects											
Oil source											
soybean	37.29^{a*}	3.21^{a}	9.45^{a}	25465	31.00	61.52	4.85	2.47^{b}	29.22^{a}	24.58^{a}	0.128
fish	31.93^{b}	2.75^{b}	8.87^{b}	25238	29.60	62.43	5.08	3.12^{a}	24.92^{b}	22.25^{b}	0.122
L-carnitine											
0(g/kg)	35.57	3.04	9.38	24860	32.21^{a}	60.29	4.77	2.81	27.00	25.17^{a}	0.129
1(g/kg)	33.53	2.91	8.94	25925	28.29^{b}	63.53	5.14	2.80	27.25	21.67^{b}	0.121
Ractopamine											
0(mg/kg)	35.93^{a}	3.06	9.22	26529	31.00	59.67	5.77^{a}	2.80	26.42	22.08^{b}	0.132^{a}
10(mg/kg)	33.20^{b}	2.89	9.08	24343	29.38	64.43	4.21^{b}	2.81	27.82	24.75^{a}	0.119^{b}
PSEM	1.697	0.128	0.278	1190.3	1.501	2.167	0.529	0.407	1.323	1.271	0.0067
P value											
Oil	0.0004	0.0001	0.0110	0.810	0.233	0.584	0.596	0.034	0.0009	0.038	0.194
L-carnitine	0.155	0.204	0.0554	0.270	0.003	0.054	0.382	0.907	0.819	0.003	0.112
Racopamin	0.0398	0.0806	0.4087	0.036	0.254	0.012	0.0009	0.904	0.208	0.021	0.011
oil× L-carnirtine	0.3001	0.109	0.326	0.247	0.545	0.992	0.047	0.323	0.819	0.038	0.035
oil× Ractopamine	0.0007	0.0056	0.0001	0.068	0.319	0.504	0.520	0.122	0.008	0.277	0.295
L-carnirtine× Ractopamine	0.0100	0.0001	0.0187	0.021	0.011	0.033	0.715	0.029	0.125	0.021	0.009
Soybean oil	41.00^{a}	3.27^{abc}	10.03^{a}	2257^{cb}	32.25^{ab}	58.50^{c}	5.00^{abcd}	3.25^{ab}	27.67^{cab}	24.67^{abc}	0.148^{a}
SO + L-carnitine	41.00^{a}	3.55^{a}	10.05^{a}	2785^{ab}	29.67^{bc}	59.25^{cb}	6.67^{a}	2.00^{b}	26.33^{cb}	20.67^{cd}	0.129^{ab}
SO + Ractopamine	37.25^{ab}	3.40^{ab}	9.55^{ab}	2512^{abc}	32.67^{ab}	61.67^{abc}	3.33^{d}	2.00^{b}	31.00^{ab}	25.67^{ab}	0.132^{ab}
SO + L-carnitine + Ractopamin	28.67^{c}	2.40^{c}	7.93^{d}	2542^{abc}	29.00^{bc}	66.75^{ab}	4.50^{bcd}	2.50^{ab}	32.33^{a}	37.33^{a}	0.108^{ab}
Fish oil	29.33^{c}	2.47^{c}	8.52^{cd}	2602^{cab}	36.00^{a}	55.00^{c}	6.33^{ab}	3.00^{ab}	26.67^{cb}	25.67^{ab}	0.143^{a}
FO + L-carnitine	32.00^{bc}	2.92^{cd}	8.47^{cd}	2867^{a}	25.70^{c}	68.00^{a}	5.33^{abc}	2.75^{ab}	25.00^{c}	17.33^{d}	0.113^{bc}
FO + Ractopamine	34.50^{bc}	3.00^{bcd}	9.63^{ab}	2469^{abc}	26.67^{c}	68.33^{a}	4.33^{bc}	3.00^{ab}	22.67^{c}	24.67^{abc}	0.102^{c}
FO +L-carnitine+ Ractopamin	31.25^{c}	2.57^{dc}	9.05^{bc}	2175^{c}	29.25^{bc}	61.25^{abc}	4.50^{bcd}	3.75^{a}	25.33^{c}	21.33^{bcd}	0.138^{a}

*Columns values with same superscript or not superscript are not significantly different (P<0.05). PSEM: Pooled Standard error of mean. FO: Fish Oil; SO: Soybean Oil

Table-3. The effects of oil source supplemented by L-carnitine and Ractopamine on rainbow trout carcass quality.

Experimental diets	Meat parameters (%)				Liver fat (%)
	Protein	Ash	Fat	DM	
Main effect					
Oil source					
Soybean	77.334[a]	6.653	15.112	14.769	3.261[a]
Fish	75.474 [b]	6.579	15.705	14.842	2.775[b]
L-carnitine					
0(g/kg)	75.679	6.348	15.828	14.761	2.985
1(g/kg)	77.257	6.888	15.049	14.856	3.0187
Ractopamine					
0(mg/kg)	76.301	6.222[b]	15.211	14.909[a]	2.865
10(mg/kg)	76.587	6.964[a]	15.691	14.724[b]	3.139
PSEM	0.992	0.468	1.078	0.111	0.259
P value					
Oil	0.0250	0.8312	0.4909	0.384	0.0180
L-carnitine	0.0632	0.1286	0.3738	0.2506	0.8622
Ractopamine	0.4837	0.0397	0.5771	0.0361	0.1578
Oil × L-carnitine	0.5721	0.2446	0.1361	0.0725	0.8698
Oil × Ractopamine	0.1204	0.9044	0.0533	o.7392	0.0103
L-carnitine× Ractopamine	0.8689	0.3435	0.6252	0.7308	0.4700
oil× L-carnitine× Ractopamine	0.0755	0.9623	0.9010	0.8763	0.2105
Interaction effect					
Soybean oil	75.86[ab]	6.34	17.10 [a]	14.75[ab]	2.98[b]
SO + L-carnitine	76.02[ab]	6.18	14.60[ab]	15.03[a]	2.74[b]
SO + Ractopamine	73.61[b]	6.81	15.42[ab]	14.55[b]	3.43 [ab]
SO + L-carnitine+ Ractopamine	77.03[ab]	7.28	13.53[b]	14.81[ab]	4.01[a]
Fish oil	75.47[ab]	5.89	14.78[ab]	4.93[ab]	2.83[b]
FO + L-carnitine	77.67[a]	6.46	14.83[ab]	14.92[ab]	2.94[b]
FO+ Ractopamine	78.52[a]	6.24	16.36[ab]	14.81[ab]	2.40[b]
FO + L-carnitine+ Ractopamine	78.18[a]	7.52	17.45[a]	14.73[ab]	2.64 [b]

***** Columns values with same superscript or not superscript are not significantly different (P<0.05). PSEM: Pooled Standard error of mean. FO: Fish oil; SO: Soybean oil

As mentioned in studies, many parameters are related to cell immunity and improving of disease resistance (Pablo *et al*, 2002). It seems that complete fish oil replacement with soybean oil can enhance cell immunity in rainbow trout. 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 ω-3 fatty acids on immune modulators both immune repressive and stimulator factors documented (Lall, 2000). 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 plus ractopamine to soybean oil diet reduced fat content of filet and increased it in fish liver (Table-3). Supplementation of ractopamine reduced meat DM, but significantly increased ash content of fish filet ($p<0.05$). 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.

In consistent with others, the percentage of ash, carcass fat and carcass DM had not affected by oil source (Greene and Selivonchick, 1990; Nanton *et al*. 2003). Liu *et al* (2004) concluded that under fish oil substitution with some vegetable oil (soybean and corn lecithin) condition, fat, protein, ash, phosphorous and moisture of tissues remained without any change. Given a change in the visceral composition by vegetable oil source may confirmed the effect of vegetable fatty acid profile on reduction of visceral fat and increasing moisture content in the rainbow trout. No significant change in mentioned parameters express the fact that some farmed species such as rainbow trout may be capable to exploit other fat source such as soybean oil beneficially, hence it is possible substitute them to providing commercial fish diet without any disturbance effect. In the past two decades, decreasing availability of protein sources coupled with the high price of this resource had forced the aquaculture industry to investigate the possibilities of reduction in fish meal, diet protein source any way (reference). In many carnivorous fish, implementation of high lipid, dense energy diet up to 35% fat or even higher is effort to reduce fish meal in aquaculture and improvement of protein efficiency in farmed carnivorous fish. In this way, L-Carnitine acting as a carrier in fatty acid β-oxidation was considered because increasing protein efficiency (Froyland *et al*, 1998). Rodehutscord (1995) in accordance with other studies even by different fish species reported that growth parameters of rainbow trout could not be changed by short time L-carnitine feeding (Dias *et al*, 2001., Yang *et al*, 2009) although fish fed L-carnitine at the 300 $mg.kg^{-1}$ during 48 days showed the better growth than control group. In contrast to these results, 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. Ractopamine, a β- adrenergic compound be used in muscling in poultry, animal and fish studies (Moloney *et al*, 1991. Vandenberg and Moccia, 1998). Thereby ractopamine facilitates protein accumulation (Mersmann, 1998). It is known that Ractopamine increasing lipolysis by lipase sensitive hormone (Mersmann, 2002). Hence ractopamine could be used to body fat reduction in farmed animal carcasses (Carr *et al*, 2008). It is noteworthy that the ractomaine have greater suppressive effect on lipogenesis than lipolysis (Mills *et al*, 2003). Simultaneously lipogenesis blocking by diet ractopamine supplementation have been confirmed in swine because of reduction in insulin sensitivity of fat cells (Liu and Mills, 1990). Nevertheless Vandenberg and Moccia, (1998) reported increasing in plasma free fatty acid in fish, because the neutralization of β-adrenergic receptors in adult fish but not in juvenile is too slowly. Besides well known β-adrenergic effect of ractopamine on growth and carcass composition, there is evidence that ractopamine also have an unknown effect on immune system. In agreement to other reports, the results of this experiment showed, administration of Ractopamine up to 10 $mg.kg^{-1}$ cause impressive decrease in hematocrit, monocyte and Ig M ($p<0.05$), but this observation only taken place in soybean oil plus both supplements (L-carnitine plus ractopamine). The main effects of oil source, L-carnitine and Ractopamine supplementation on meat filet quality parameters of fish are shown in Table-3.

CONCLUSION

Data of the present experiment showed that immunological and hematological response of rainbow trout to L-carnitine and ractopamine 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 in liver and muscle of rainbow trout.

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