Effects of three beta adrenergic receptor agonists on growth performance, blood biochemical parameters, fatty acids composition and carnitine palmitoyltransferase I gene expression of rainbow trout *Oncorhynchus mykiss*

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Abstract

Different beta 1 and 2 adrenergic receptors agonists might have various biological and physiological effects on fish species. An experiment was designed to study the effects of feeding ractopamine, terbutaline and metaproterenol; as beta1, beta2 and less selective beta2 adrenergic receptor agonists, respectively; on body weight gain, feed conversion rate, concentration of biochemical parameters in the serum, gene expression of carnitine palmitoyltransferase I in liver and meat and also, fatty acids profile of filet of the rainbow trout. One hundred ninety two juvenile rainbow trout were randomly assigned into 16 fiber glass tanks and fed one of four dietary treatments containing control (0), ractopamine, terbutaline and metaproterenol at level of 10 ppm in diet for 8-week feeding trials. The results showed metaproterenol and ractopamine improved final body weight, body weight gain and feed conversion rate of fish. The serum concentrations of phosphorus and albumin were also significantly increased by all beta adrenergic agonists and ractopamine reduced triglyceride level (p<0.05). The fatty acids level of fish filet was significantly increased by the dietary supplement of various beta adrenergic agonists (p<0.05) but ractopamine had a greater effect. The gene expression of carnitine palmitoyltransferase I in liver was significantly (p<0.05) increased by all beta adrenergic agonists. The present study showed that various beta1 and beta2 adrenergic receptor agonists had same physiological effects on rainbow trout but it seems ractopamine, as a beta1 adrenergic receptor, had more potential on fatty acid metabolism and growth response of rainbow trout.

Keywords: Ractopamine, Terbutaline, Metaproterenol, Fish

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Introduction

Oral administration of beta adrenergic agonists has been shown to increase muscle protein, decrease muscle fat, and improve the growth performance of poultry (Kheiri et al., 2011; Zareal., 2012; Mirhendi-Shahneh etEsfahani et al., 2014;), pig, cattle (Etherton, 2009) and fish (Salem et al., 2006; Jalali Haji-Abadi et al., 2010). The physiological action of beta agonists is similar to adrenergic phenethanolamines where physiological responses occur upon ligand-receptor binding (Mersmann, 1998). It is clear that beta adrenergic agonists do play a critical role in lipid metabolism and mobilization of lipid stores from liver and adipose tissues of fish by activating hormone-sensitive triacylglycerol lipase (Tocher, 2003) through a cAMPdependent protein kinase (PKA), resulting in free fatty acids release (Mersmann, 1998). For beta oxidation of free fatty acids, they must be transferred in to mitochondria and this process can be done by carnitine palmitoyltransferase I (CPT I), which is regulatory the main enzyme mitochondrial fatty acid oxidation (Kerner and Hoppel, 2000; Ramsay et al., 2001).

The beta adrenergic agonists are classified as the base on the binding type of beta adrenergic receptor and according to this ability, ractopamine is a selective beta 1 adrenergic receptor agonist (Moody *et al.*, 2000); on the other hand, terbutaline and metaproterenol are selective beta 2 adrenergic receptor agonists. However, metaproterenol appears less selective

compared to terbutaline (Westfall and 2011). Westfall. The most adrenergic receptor agonists studies on growth performance of fish species; except two reports (Lortie et al., 2004; Salem et al., 2006); are related to one specific beta adrenergic agonists. For example, salbutamol (a beta adrenergic agonist) was used for rohu fish, Labeo rohita (Satpathy et al., 2001), L644,969 (a beta 2 adrenergic agonist) was employed for blue catfish, Ictalurus furcatus (Webster et al., 1995) and ractopamine were used for channel catfish, I. punctatus (Mustin and Lovell, 1993), pacu, Piaractus mesopotamicus (Bicudo, 2012) and rainbow trout, Oncorhynchus mykiss (Jalali Haji-Abadi et al., 2010; Nazari-Farsani et al., 2015). On the other hand, the effects of beta adrenergic agonists on protein metabolism were more interested for researchers than fatty acid metabolism in fish. According to this, Lortie et al. (2004) and Salem et al. (2006) reported that muscle protein metabolism of rainbow trout, O. mykiss, change by using two beta adrenergic receptor agonists (clenbuterol and ractopamine). Different beta 1 and 2 adrenergic agonists are not equally potent and also, the expression of beta adrenergic receptor types varies in different cells and tissues. For the same reason, the metabolic effects of them, fat metabolism, protein such as synthesis and growth promotor, may be different (Strydom et al., 2009). The current trial was designed to compare the effects of ractopamine, terbutaline and metaproterenol (as beta 1, 2 and 2 with less selective receptor adrenergic agonists, respectively) on growth performance, blood biochemical parameters, fatty acids composition of meat, and gene expression of carnitine palmitoyltransferase I (CPT I) in rainbow trout.

Materials and methods

Experimental fish and diets

One hundred ninety two juvenile rainbow trout with 121±3 g. initial body weight were randomly assigned to 16 fiber glass tanks (500 L) allotted to four dietary treatment groups. Each dietary treatment was replicated in four tanks with 12 fish on each. Fish were adapted to the experimental conditions for 2 weeks before start of the experiment, and during the adaptation period fed by control diet without beta adrenergic agonists. Fish were fed twice daily (9:00 am and 5:00 pm) by hand (Nazari-Farsani et al., 2015) at the rate of 1.5% of body weight per day (NRC, 2011) for the adaptation period and 8 weeks feeding trial. Every two weeks, feeding was stopped for 24 h before mass weighing all fish in each tank, to adjust the daily ration for the next period according to Jalali Haji-Abadi et al. (2010). The four dietary treatments contained control (0), ractopamine, terbutaline and metaproterenol at level of 10 ppm in diet. Basal commercial pellet diet was formulated based on free oil feed (GFT1, Grower Feed Trout at 5mm diameter, Faradaneh Co., Iran) and beta adrenergic agonists were added to the experimental diet by mixing them with oil (1:1 ratio of fish and soybean oil added at 5.5% in basal diet). The crude protein and the crude fat of experimental diets were 44.3 and 15.3% as dry matter, respectively. The water temperature ranged from 12.5 to 13.8°C during the 10-week study period. Water quality parameters were monitored every 2 weeks (pH=7.8± 0.2; dissolved O2 =9.6±0.5 mg L⁻¹; total ammonia nitrogen= 0.61±0.06 mg L⁻¹).

Data collection

Performance and blood parameters

For the growth performance of fish, the following parameters were calculated at the end of the experiment (Jalali Haji-Abadi *et al.*, 2010):

weight gain, specific growth rate [SGR=(In final weight-In initial weight) $\times 100/\text{days}$], feed conversion [FCR= feed intake (g)/ weight gain (g)], protein efficiency rate [PER= weight gain (g)/protein intake (g)], condition $[K=BW\times100/TL^3]$ factor where BW=body weight (g) and TL=total length (cm)], hepatosomatic index [HSI=LW \times 100/BW, where LW=liver weight (g)], and carcass Index =CW (g) $\times 100/$ BW (g), where CW= the weight of carcass without internal organ.

At the end of the experiment, all fish were anaesthetized by MS222 (100 ppm) and individually weighed (0.1g); also, the length was measured (0.1cm). Four anaesthetize fish were randomly selected from each tank and blood samples were collected from their caudal vein. Then fish killed by a blow to the head and after the removing internal organ (liver, intestine, heart, pyloric caeca, kidney and etc.), slices of liver and meat filet were removed and immediately transferred and stored in liquid nitrogen for the analysis of CPT I

gene expression. The blood allowed to coagulate for 20 minutes and the serum was collected by 10 minutes centrifugation at 2000 g. Serum was separated and stored at -20 °C before being analyzed. Concentrations of albumin, glucose, total protein, triglyceride, cholesterol, calcium and phosphorus (Manera and Britti, 2006) in the serum were measured based on ZiestChem Diagnostics Kits (Technicon RA1000, Tehran, Iran). Sodium and potassium in the sera of fish were directly assessed by using Sherwood Model 410 Classic Flame Photometer Analyzer (Hald, 1946).

Analysis of CPT I gene expression RNA isolation

Total RNA was isolated from tissue samples using RNA Isolation Kit (Qiagen, Limburg, Netherlands), according the manufacturer's instructions, a DNase treatment was performed. The RNA concentrations and purities were determined using Picodrop P200 system (Alpha Biotech Ltd., Glasgow, UK). First-strand cDNA was synthesized from 1 µg of total RNA preparations (Jalali et al., 2011). transcription The reverse was performed according the manufacturer's instructions (Thermo Scientific, Bremen, Germany). Finally, the reverse transcription products were stored at -20°C for PCR.

Semi quantitative reverse-transcription PCR

The primer sequences for the PCR reactions are listed in Table 1. Primers for CPT1 and β-actin were designed from by Primer3web software (Ver. 4.0). PCR reaction conditions were optimized for each primer pairs to obtain a linear relationship between the input RNA and the final PCR product. The PCR was performed with a total volume of 25 µL containing 3 mM MgCl₂, 0.1 mM of each dNTP, 0.1 mM of each primer, 5 µL cDNA, and 25 U Taq polymerase. The PCR program for CPT1 and β-actin consisted of 3 min at 94°C, with 30 cycles of amplification (45 s at 94°C, 60 s at 56°C and 60 s at 72°C), and continued with 5 minutes at 72°C for one cycle. PCR products were loaded on 1 % agarose gels in a TAE buffer (1X) and stained with 0.5 µg mL ethidium bromide. Pictures were captured by Sony XC-75 CE camera (Vilber Lourant Inc. Cedex, France) the density of bands determined by Photo-Capt v.99 Image software (Vilber Lourant Inc. Cedex, France); also, relative densities were expressed as CPT1/β-actin density (Jalali et al., 2011). The stability of βactin was analyzed by comparing βactin densities between the experimental and control groups. Normalization of the samples was accomplished using RT-PCR for the housekeeping gene, β-actin, to control the efficacy of the RNA extraction, integrity and the amount of CPT1 mRNA present in the samples.

Table 1: Forward (F) and reverse	(P) companees of primare us	ead in comi-quantitative RT-PCR
Table 1: Forward (F) and reverse	(R) sequences of brimers us	ea in seim-auanutauve K i -PCK.

Gene	Primer name	Sequence (5'-3')	Product length (bp)
CPT I	CPT I -F	5- TGAAGATGCTCTCTGGGCGC -3	336
Gene Bank: NM_001124735.1	CPT I -R	5- GTGTGGGAGTCACGTACAGC -3	
β-actin	β-actin-F	5- ATGGGCCAGAAAGACAGCTA -3	150
Gene Bank:NM_001124235.1	β-actin-R	5-CACTCGCAGCTCGTTGTAGA -3	

Fatty acids composition of meat

Fillets of four fish from each tank were minced and homogenized for fatty acids analyses. The homogenized fish fillets were freeze dried (Heto Power Dry DW8, freeze dryer, Allerod, Denmark) and fish fillet lipids were extracted according to the procedure developed al. Folch (1957)by chloroform/methanol (2:1 v v⁻¹). The organic solvent was evaporated under a stream of nitrogen and the lipid was dissolved in n-hexane and then stored at -20 °C.

For the analysis of fatty acids composition of the filet meat, fatty acids methyl esters of extracted lipids were prepared according to the method of Christie (1990). Fatty acid methyl esters were separated and quantified by gas chromatography (Agilent model 6890, USA) equipped with a Flame ionization detector (FID) and a SGE BPX70 capillary column system (BPX-70: 120 m \times 0.25 mm, 250 um I.D., 0.2 film thickness, Agilent um Technologies). Nitrogen was used as a carrier gas at 0.9 ml min⁻¹ flow rate. The injector and detector temperatures were 260 and 300°C, respectively. Individual methyl esters were identified in comparison with known mix fatty acids methyl standards and quantified by comparing their peak area with the external standard (Methyl Erucate).

Atherogenicity index (AI) and thrombogenicity index (TI) were calculated by fatty acids concentration and the following equations (Tonial *et al.*, 2014):

AI=[(C12:0)+(4×C14:0)+ (C16:0)]/[PUFA+MUFA]; TI=[(C14:0)+(C16:0)+(C18:0)]/[(0.5× MUFA)+(0.5×n-6)+(3×n-3)+(n-3/n-6)] PUFA: poly unsaturated fatty acids; MUFA: mono unsaturated fatty acids

Statistical analysis

The experiment was conducted using a completely randomized design with four treatments and data were analyzed using the GLM procedure of the SAS software for the analysis of variance (SAS, 2002). Significant differences among treatment means were determined by using Duncan's multiple range tests at 0.05 levels and all data presented are average ± standard error of mean (SEM).

Results

The results showed that the metaproterenol and ractopamine supplements improved body weight gain (10.8%), feed conversion rate (9%) and final body weight of fish (Table 2).

Table 2: Effects of dietary supplemental of three types of beta adrenergic agonist on growth performance (SEM*) of rainbow trout.

•	IBW ¹	EBW ²	BWG ³	FI ⁴	FCR	PER ⁶	SGR ⁷	K ⁸	HSI ⁹	Carcass Index
Beta agonist	g	g	g .(fish.day) ⁻¹	g.(fish.day) ⁻¹					%	%
Control	131.4 (0.41)	229.9 ^b (1.7)	1.75 ^b (0.03)	2.53 (0.026)	1.44 ^a (0.023)	1.542 ^b (0.024)	0.998 (0.016)	1.31 (0.007)	0.98 (0.039)	84.26 (1.47)
Metaproterenol	131.2 (1.9)	240.1 ^a (4.2)	1.94 ^a (0.09)	2.53 (0.033)	1.31 b (0.061)	1.736 a (0.081)	1.078 (0.047)	1.35 (0.009)	1.09 (0.163)	82.61 (2.52)
Ractopamine	132.29 (0.55)	241.2 a (1.9)	1.94 ^a (0.02)	2.56 (0.017)	1.32 b (0.007)	1.725 ab (0.009)	1.072 (0.007)	1.33 (0.015)	1.08 (0.041)	82.74 (0.92)
Terbutaline	131.87 (0.62)	234.5 ab (1.56)	1.83 ^{ab} (0.03)	2.54 (0.021)	1.39 ^{ab} (0.024)	1.626 ab (0.028)	1.028 (0.015)	1.34 (0.007)	0.99 (0.022)	83.43 (0.924)

Columns values with the same superscript or not superscript are not significantly different (p>0.05). 1. IBW: Initial body weight; 2.EBW: End body weight; 3. BWG: Body weight gain; 4. FI: Feed intake; 5.FCR: Feed conversion rate; 6. PER: Protein efficiency ratio; 7. SGR: Specific growth rate; 8. K: condition factor; 9: HSI: hepatosomatic index.*SEM: Standard error of mean.

Feed intake, specific growth rate, condition factor, hepatosomatic and carcass index were not significantly affected by and beta 1 and 2 adrenergic agonist (Table 2).

The metaproterenol and terbutaline supplements significantly (p<0.05) increased the albumin level of fish

serum and ractopamine supplement reduced the triglyceride blood level. All beta adrenergic agonist supplements increased phosphorous level in the blood serum of fish. The concentrations of glucose, cholesterol, total protein, Na and K in fish serum were not affected by dietary treatments (Table 3).

Table 3: Effects of dietary supplemental of three types of beta adrenergic agonist on blood biochemical parameter (SEM*) of rainbow trout.

	GLU^1	CHO ²	TG ³	ALB ⁴	TP⁵	Na ⁶	K ⁷	Ca ⁸	P ⁹
Beta agonist	mg dL ⁻¹	mg dL ⁻¹	mg dL ⁻¹	$g~dL^{\text{-}1}$	$g\ dL^{\text{-}1}$	mmol L ⁻¹	mmol L ⁻¹	mg dL ⁻¹	mg dL ⁻¹
Control	101.0 (20.5)	340.6 (18.0)	353 a (33.8)	2.12 b (0.16)	4.06 (0.56)	162.3 (1.20)	1.60 (0.17)	11.9 ab (0.13)	17.0 b (0.54)
Metaproterenol	86.6 (3.75)	334.3 (28.41)	331 ^a (26.1)	2.96 a (0.12)	3.20 (0.11)	162.3 (0.66)	1.30 (0.05)	12.9 ^a (0.45)	20.6 a (0.63)
Ractopamine	93.3 (0.88)	304.6 (9.83)	204 ^b (7.5)	2.52 ab (0.35)	3.73 (0.54)	160.0 (1.73)	1.50 (0.05)	11.1 b (0.40)	19.9 a (0.53)
Terbutaline	75.0 (10.2)	296.6 (1.76)	300°a (26.2)	3.06 a (0.08)	3.73 (0.53)	161.6 (1.6)	1.16 (0.21)	11.7 ab (0.06)	19.2 a (0.62)

Columns values with the same superscript or not superscript are not significantly different (p>0.05)

The CPT I gene expression of liver was significantly (p<0.05) increased by all beta adrenergic agonists, and the different types of beta adrenergic agonists had the same effect on CPT I

gene expression. Expression of CPT I in fish filet was not significantly affected by any of the tested beta adrenergic agonists (Table 4).

^{1.} GLU: Glucose; 2. CHO: Cholesterol; 3. TG; Triglycerides; 4. ALB: Albumin; 5: TP: Total protein; 6. Na: Sodium; 7. K: Potassium; 8. Ca: Calcium; 9. P: Phosphorous.

^{*}SEM: Standard error of mean.

Table 4: Effects of dietary	supplemental of three	types of beta	adrenergic	agonist on	CPTI gene
expression (SEM*)	of rainbow trout.				

Beta agonist	Liver	Filet meat
Control	0.561 ^b	0.620
Control	(0.003)	(0.02)
Matamatananal	1.014 ^a	0.589
Metaproterenol	(0.045)	(0.037)
D	0.939 a	0.569
Ractopamine	(0.064)	(0.04)
T. 1. (.1	0.946 ^a	0.491
Terbutaline	(0.010)	(0.02)

Columns values with the same superscript or not superscript are not significantly different (p> 0.05). *SEM: Standard error of mean.

All three types of beta adrenergic agonists increased fatty acids levels in trout muscle. However, ractopamine was the most effective supplement. Ractopamine also significantly (p<0.05)

increased eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids. The highest thrombogenicity index of fish filet (muscle) was found with terbutaline treatment (Table 5).

Table 5: Effects of dietary supplemental of three types of beta adrenergic agonist on fatty acid composition (SEM*) of rainbow trout fillet.

Fatty acids (mg g ¹)																
Beta agonist	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	C20:5	C22:6	Sat.	MUFA	n-6	n-3	AI	TI
Control	0.125 d (0.011)	3.88° (0.11)	1.12 ^d (0.05)	0.71 ^d (0.08)	8.64 ^d (1.0)	5.80° (0.58)	1.45 ^d (0.14)	0.26° (0.03)	0.33 b (0.018)	0.28 ^b (0.05)	4.72° (0.17)	9.77 ^d (1.05)	6.06° (0.607	2.11° (0.105)	0.254 (0.022)	0.329ab (0.021)
Metaproterenol	0.286 ^b (0.008)	6.60 ^b (0.39)	2.18 ^b (0.03)	1.67 ^b (0.07)	17.93 ^b (0.80)	9.72 ^b (1.14)	2.84 ^b (0.18)	0.57 ab (0.028)	0.32 ^b (0.015)	0.39 ab (0.07)	8.56 ^b (0.41)	20.12 ^b (0.79)	10.29 b (1.17)	3.56 ^b (0.12)	0.239 (0.006)	0.339ab (0.004)
Ractopamine	0.360 a (0.004)	9.10 ^a (0.16)	2.75a (0.13)	2.14 ^a (0.09)	22.87 ^a (0.62)	14.40 ^a (0.49)	4.82 ^a (0.16)	0.65ª (0.051)	0.45ª (0.065)	0.53* (0.05)	11.61 ^a (0.24)	25.62a (0.71)	15.05ª (0.53)	5.81ª (0.23)	0.230 (0.003)	0.304b (0.003)
Terbutaline	0.245¢ (0.01)	6.30 b (0.13)	1.65¢ (0.07)	1.45° (0.05)	14.19° (1.02)	9.20 ^b (0.52)	2.46° (0.06)	0.48 ^b (0.029)	0.34 ^b (0.006)	0.33 ^b (0.07)	8.00 ^b (0.16)	15.85° (1.01)	9.69 ^b (0.52)	3.09b (0.13)	0.257 (0.015)	0.36 ^a (0.017)

Columns values with the same superscript or not superscript are not significantly different (p>0.05). Sat.: Saturated fatty acids, MUFA: Mono unsaturated fatty acids, AI: Atherogenicity index, TI; Thrombogenicity index.

Discussion

The increased growth (10.8%) or final body weight (4.4-5%) of fish and improved feed conversion rate (9%) of fish (Table 2) as a result of ractopamine and metaproterenol supplements might be due to the repartitioning effects of them, leading to redirecting the nutrient away from adipose to muscle tissue (Sillence, 2003). Our results were in close to the results of Mustin and Lovell (1993) on channel catfish, *I. punctatus* (20 ppm ractopamine, 17% rise in gain), Satpathy *et al.* (2001) on

rohu, *L. rohita* (6 ppm salbutamol; a beta 2 adrenergic agonist; 12% rise in growth), Van den Berg and Moccia (1998) and Jalali Haji-Abadi *et al.* (2010) on rainbow trout, *O. mykiss*, (10 ppm ractopamine, 9.3% and 6.6% rise feed efficiency and gain, respectively). In contrast, other studies such as Webster *et al.* (1995) on blue catfish, *I. furcatus*, and Bicudo *et al.* (2012) on pacu, *P. mesopotamicus*, showed no changes in growth performance by feeding 3 ppm L644,969 and 40 ppm ractopamine, respectively. The reason

^{*}SEM: Standard error of mean.

for the existing discrepancy between these results could be related to the differences in the fish species, initial body weight, type of beta adrenergic agonists, dietary level of them and the nutrient composition of basal diets. According to our findings, it appeared that terbutaline (a beta 2 adrenergic receptor agonist) supplement had a lower effect on the growth performance of fish, as compared with ractopamine and metaproterenol (a beta 1 and nonselective beta 2 adrenergic receptor agonist) supplement (Table 2). This may be due to the different efficiency and affinity of them to the beta adrenergic receptors (Moody et al., 2000) of fish. According to Törneke et al. (1998), three beta 2 adrenergic agonists receptor (clenbuterol. salbutamol and terbutaline) had different affinity on beta adrenergic receptor of equine tracheal muscle. The other reason may be related to dietary concentration and intake of terbutaline which may result in internalization and down-regulation of beta adrenergic receptors (Moody et al., 2000).

The supplementation of beta adrenergic agonists, especially metaproterenol. terbutaline and increased albumin in the blood serum of fish; and the lowest concentration was observed in fish fed control diet (Table 3). Albumin in fish blood is bound to free fatty acids, transporting them (Rodnick and Williams, 1999). The observed higher albumin levels of serum terbutaline blood in metaproterenol supplemented diet could be attributed to the improved capacity for fatty acids transport and the response the enhanced protein to (Jalali Haji-Abadi synthesis 2010). The supplementation of ractopamine decreased the level of triglycerides in blood serum. This finding was consistent with those of Bicudo et al. (2012) in juvenile pacu (P. mesopotamicus), who reported reduced levels of triglycerides in blood when ractopamine was added to the fish diet. However, previous work by the authors has shown that blood triglycerides were not affected ractopamine (Jalali Haji-Abadi et al., 2010). The terbutaline and metaproterenol supplement also reduced triglycerides blood levels, but it was not significantly different from control group. The electrolytes (Na and K) in blood serum were not affected and the phosphorus was increased by adrenergic different beta receptor agonists supplement (Table 3). These results differed with those of other studies on mammalian animal species and human. Epinephrine, a natural beta agonist, has been characterized as a hypophosphatemic hormone in human (Liamis et al., 2010) and according to Mostafa et al. (2006), beta adrenergic agonists shift phosphorous extracellular to intracellular parts; also, in some reports, ractopamine reduced phosphorus excretion by urine in cow and pig has been observed. The present data did not support this hypothesis in fish and the reason is not known. Overall, the related to the blood biochemical parameters showed that various beta adrenergic agonists had the effect on some of blood parameters, such as phosphorus, and different effects on others like calcium, albumin and triglycerides.

According to the data, CPTI gene expression of liver was increased by supplementing various beta adrenergic agonists and filet meat was unaffected (Table 4). by them Carnitine palmitoyltransferase I (CPT I) is the main enzyme in mitochondrial fatty acid oxidation (Kerner and Hoppel, 2000; Ramsay et al., 2001). Although some tissues such as liver, red and white muscles are the most important organs of fish for fatty acids oxidation (Tocher, 2003), it seems. adrenergic agonists affect the gene expression of lipid metabolism (such as CPT I) in the liver more than muscles. Furthermore, the studies have shown the gene expression of CPT in the liver of rat is increased by catecholamines (Barke et al., 1993) and the incubation of rainbow trout muscle cells with catecholamines does not affect oleic acid metabolism (Sánchez-Gurmaches et al., 2010). The other reason for increasing the gene expression of CPT I in liver may be related to lipolysis action of beta adrenergic agonists and the improved availability of fatty acids by them. According to Morash and McClelland (2011) the fatty acid oxidation in the isolated mitochondria of trout liver was increased during fasting and the sensitivity of CPT I in liver was higher than in red muscle. On the other hand, researchers have shown that dietary polyunsaturated fatty acids affected carnitine palmitoyltransferase I and increase CPT-I mRNA expression in liver tissue (Morash et al., 2009).

All long chain fatty acids in fish fillet were found to be increased by three types of beta adrenergic agonists and ractopamine supplement had higher effects on them (Table 4). This could be related to lipolysis action of beta adrenergic agonists on the adipose tissue (Tocher, 2003; Dugan et al., 2003) and liver (Van Heeswijk, 2006) and released fatty acids from them. On the other hand, beta adrenergic agonists could act as repartition agents by which nutrients were distributed to muscle tissues (Moody et al., 2000). Previous studies showed that the long chain fatty acids in trout filet were increased by feeding ractopamine treatment (Jalali Haji-Abadi et al., 2010); also, Van Heeswijk et al., 2006 showed that the stimulation of adrenoceptor in trout liver increased the fatty acids release. Apple et al. (2008) also showed that fatty acids composition of pork back-fat were affected by ractopamine supplement.

The fillet muscle is an important section of fish which is used for human feeding and reduction in the content of EPA and DHA (C20:5 and C22:6, respectively) can reduce its benefit for human health (Reddy, 2001). Results showed that terbutaline metaproterenol had no effect on these fatty acids. However, more importantly, significantly ractopamine increased C20:5 and C22:6 in trout fillet. The nutritional quality of the fatty acids in fish filet was assessed by the atherogenicity (AI) and thrombogenicity indexes (TI). These indexes indicated that the amount of fatty acids might have affected human

health and low values of them could reduce the potential risk of coronary heart disease (Menezes et al., 2009) by reducing atheroma and thrombus formation (Garaffo et al., 2011). The present data do not show any effect of adrenergic agonists on atherogenicity index of the fish fillet. However, the thrombogenicity index of filet meat was reduced by ractopamine supplement (Table 5), which improved the nutritional quality of fish meat.

To conclude, this study showed that ractopamine; as a beta 1 adrenergic receptor agonists; and terbutaline and metaproterenol; as beta 2 adrenergic receptor agonists; had same physiological effects such as gene of **CPTI** expression liver. concentration of phosphorus in blood and level of fatty acids in filet muscle of rainbow trout. The present study also showed that ractopamine had more potential on fatty acid metabolism and growth response of rainbow trout.

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