Iranian Journal of Fisheries Sciences 18(4) 605-618 2019
DOI: 10.22092/ijfs.2018.116981

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*

Jalali S.M.A.[1]*; Jalali S.A.H.[2,3]; Yadollahi F.[4]

Received: October 2016 Accepted: June 2018

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

1-Department of Animal Sciences, Faculty of Agriculture and veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

2-Department of Natural Resources, Isfahan University of Technology, Isfahan 84156–83111, Iran.

3-Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156–83111, Iran.

4-Department of Food Sciences, Faculty of Agriculture and veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

*Corresponding author's Email: Fish.nutritionist@gmail.com

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; Zare-Shahneh *et al.*, 2012; Mirhendi-Esfahani *et al.*, 2014;), pig, cattle (Etherton, 2009) and fish (Salem *et al.*, 2006; Jalali Haji-Abadi *et al.*, 2010). The physiological action of beta adrenergic agonists is similar to 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 a hormone-sensitive triacylglycerol lipase (Tocher, 2003) through a cAMP-dependent 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 the main regulatory enzyme in 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 Westfall, 2011). The most beta 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 2 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, such as fat metabolism, protein 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=(ln final weight-ln initial weight) ×100/days], feed conversion rate [FCR= feed intake (g)/ weight gain (g)], protein efficiency rate [PER= weight gain (g)/protein intake (g)], condition factor [$K=BW\times100/TL^3$, where BW=body weight (g) and TL=total length (cm)], hepatosomatic index [HSI=LW × 100/BW, where LW=liver weight (g)], and carcass Index =CW (g) ×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 was 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 glucose, albumin, 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 to 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). The reverse transcription was performed according to 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 $MgCl_2$, 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^{-1} ethidium bromide. Pictures were captured by Sony XC-75 CE camera (Vilber Lourant Inc. Cedex, France) and the density of bands were 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 β-actin was analyzed by comparing β-actin 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 (R) sequences of primers used in semi-quantitative RT-PCR.

Gene	Primer name	Sequence (5'-3')	Product length (bp)
CPT I Gene Bank: NM_001124735.1	CPT I -F	5- TGAAGATGCTCTCTGGGCGC -3	336
	CPT I -R	5- GTGTGGGAGTCACGTACAGC -3	
β-actin Gene Bank:NM_001124235.1	β-actin-F	5- ATGGGCCAGAAAGACAGCTA -3	150
	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 by Folch *et al.* (1957) with 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 × 0.25 mm, 250 um I.D., 0.2 um film thickness, Agilent 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.

Beta agonist	IBW[1] g	EBW[2] g	BWG[3] g .(fish.day)$^{-1}$	FI[4] g.(fish.day)$^{-1}$	FCR	PER[6]	SGR[7]	K[8]	HSI[9] %	Carcass Index %
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.

Beta agonist	GLU[1] mg dL^{-1}	CHO[2] mg dL^{-1}	TG[3] mg dL^{-1}	ALB[4] g dL^{-1}	TP[5] g dL^{-1}	Na[6] mmol L^{-1}	K[7] mmol L^{-1}	Ca[8] mg dL^{-1}	P[9] 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)
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.

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).

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] (0.003)	0.620 (0.02)
Metaproterenol	1.014 [a] (0.045)	0.589 (0.037)
Ractopamine	0.939 [a] (0.064)	0.569 (0.04)
Terbutaline	0.946 [a] (0.010)	0.491 (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 [c] (0.11)	1.12 [d] (0.05)	0.71 [d] (0.08)	8.64 [d] (1.0)	5.80 [c] (0.58)	1.45 [d] (0.14)	0.26 [c] (0.03)	0.33 [b] (0.018)	0.28 [b] (0.05)	4.72 [c] (0.17)	9.77 [d] (1.05)	6.06 [c] (0.607)	2.11 [c] (0.105)	0.254 (0.022)	0.329 [ab] (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)	0.339 [ab] (0.004)
Ractopamine	0.360 [a] (0.004)	9.10 [a] (0.16)	2.75 [a] (0.13)	2.14 [a] (0.09)	22.87 [a] (0.62)	14.40 [a] (0.49)	4.82 [a] (0.16)	0.65 [a] (0.051)	0.45 [a] (0.065)	0.53 [a] (0.05)	11.61 [a] (0.24)	25.62 [a] (0.71)	15.05 [a] (0.53)	5.81 [a] (0.23)	0.230 (0.003)	0.304 [b] (0.003)
Terbutaline	0.245 [c] (0.01)	6.30 [b] (0.13)	1.65 [c] (0.07)	1.45 [c] (0.05)	14.19 [c] (1.02)	9.20 [b] (0.52)	2.46 [c] (0.06)	0.48 [b] (0.029)	0.34 [b] (0.006)	0.33 [b] (0.07)	8.00 [b] (0.16)	15.85 [c] (1.01)	9.69 [b] (0.52)	3.09 [b] (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.
*SEM: Standard error of mean.

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

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 non-selective 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 receptor agonists (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 terbutaline and metaproterenol, 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 blood serum in terbutaline and metaproterenol supplemented diet could be attributed to the improved capacity for fatty acids transport and the response to the enhanced protein synthesis (Jalali Haji-Abadi *et al.*, 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 by 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 different beta adrenergic 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 from 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 same 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 by them (Table 4). 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, beta 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 and metaproterenol had no effect on these fatty acids. However, more importantly, ractopamine significantly increased C20:5 and C22:6 in trout fillet. The nutritional quality of the fatty acids in the fish filet was assessed by 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 beta adrenergic agonists on the 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 expression of CPTI in 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.

Acknowledgements

This experiment was carried out at the Aquaculture Center of Islamic Azad University, Sharekord branch, Iran. This research project was also funded and supported by Islamic Azad University, Shahrekord branch. The authors are indebted to Faradaneh Company, an Iranian Aquatic Animals Feed Producer, for providing fish and feed.

References

Apple, J.K., Maxwell, C.V., Kutz, B. R., Rakes, L.K., Sawyer, J. T., Johnson, Z.B., Armstrong, T.A., Carr, S.N. and Matzat, P.D. 2008. Interactive effect of ractopamine and dietary fat source on pork quality characteristics of fresh pork chops during simulated retail display. *Journal of Animal Science,* 86, 2711–2722.

Barke, R.A., Brady, P.S. and Brady, L.J., 1993. The regulation of mitochondrial fatty acid oxidation and hepatic gene expression by catecholamine. *Journal of Surgical Research,* 54, 95-101.

Bicudo, A.J.A., Sado, R.Y. and Cyrino, J.E.P., 2012. Growth, body composition and hematology of juvenile pacu (*Piaractus mesopotamicus*) fed increasing levels of ractopamine. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* 64, 1335-1342.

Christie, W.W. 1990. The analysis of fatty acids, preparation of methyl ester and other derivatives. In: Gas chromatography and lipids. A practical guide, (Christie, W.W. ed.), pp. 36-47. Oily Press, Bridgwater.

Dugan, S.G., Lortie, M.B., Nickerson, J.G. and Moon, T.W., 2003. Regulation of the rainbow trout (*Oncorhynchus mykiss*) hepatic b_2-adrenoceptor by adrenergic agonists. *Comparative Biochemistry and Physiology Part B,* 136, 331–342.

Etherton, T.D., 2009. Animal growth and development research: Historical perspectives. *Journal of Animal Science*, 87, 3060–3064.

Folch, J., Lees, M. and Stanley, S.G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal*

of Biological Chemistry, 226, 497-509.

Garaffo, M., Agius, R., Nengas, Y., Lembo, E., Rando, R., Maisano, R., Dugo, G. and Giuffrida, D., 2011. Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid índices, of raw roe of Blue Fin Tuna (*Thunnus thynnus* L.) and their salted product "Bottarga". *Food and Nutrition Sciences*, 2, 736-743.

Hald, P.M., 1946. The flame photometer for the measurement of sodium and potassium in biological materials. *Journal of Biological Chemistry*, 167, 499–510.

Jalali Haji-Abadi, S.M.A., Mahboobi soofiani, N., Sadeghi, A.A., Chamani, M. and Riazi, G.H., 2010. Effects of supplemental dietary L-carnitine and ractopamine on the performance of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research*, 41, 1582-1591.

Jalali, S.A.H., Nikbakht, G., Mahboobi Soofiani, N. and Jalali, S.M.A., 2011. Optimization of semi-quantitative RT PCR analysis for CPT I gene expression in rainbow trout (*Oncorhynchus mykiss*). *Iranian Journal of Fisheries Sciences*, 10, 749-752.

Kerner, J. and Hoppel, C., 2000. Fatty acid import into mitochondria. *Biochimica et Biophysica Acta*, 1486, 1–17.

Kheiri, F., Pourreza, J., Ebrahimnezhad, Y. and Jalali Haji-Abadi, S.M.A., 2011. Effect of supplemental ractopamine and L-carnitine on growth performance, blood biochemical parameters and carcass traits of male broiler chicks. *African Journal of Biotechnology*, 68, 15450-15455.

Liamis, G., Milionis, H.J. and Elisaf, M., 2010. Medication-induced hypophosphatemia: A review. *Quarterly Journal of Medicine*, 103,449–459.

Lortie, M.B., Arnason, T, Dugan, S.G., Nickerson, J.G. and Moon, T.W., 2004. The impact of feeding β2-adrenergic agonists on rainbow trout muscle β2-adrenoceptors and protein synthesis. *Journal of Fish Biology*, 65, 769–787

Manera, M. and Britti, D., 2006. Assessment of blood chemistry normal ranges in rainbow trout. *Journal of Fish Biology*, 69, 1427-1434.

Menezes, M.E.S., Lira, G.M., Omena, C.M.B., Freitas, J.D. and Sant'ana, A.E.G., 2009. Nutritive values of fishes from maritime coast of Alagoas. *Brazilian Revista do Instituto Adolfo Lutz*, 68, 21-28.

Mersmann, H.J., 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. *Journal of Animal Science*, 76, 160-172.

Mirhendi-Esfahani, S.R., Jalali, S.M.A. and Zare-Shahneh A., 2014. Effects of dietary ractopamine on growth performance and blood biochemical parameters in male Japanese quail (*Coturnix japonica*). *Research Opinions in Animal and Veterinary Sciences*, 4, 442-445.

Moody, D.E., Hancock, D.L. and Anderson, D.B., 2000. Phenethanolamine repartitioning agents. In *Farm Animal Metabolism and Nutrition*. (D'Mello, J.P.F. ed.), New York, CAB International. pp. 65-96.

Morash, A.J., Bureau, D.P. and McClelland, G.B., 2009. Effects of dietary fatty acid composition on the regulation of carnitine palmitoyltransferase (CPT) I in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part B*, 152, 85–93.

Morash, A.J. and McClelland, G.B., 2011. Regulation of carnitine palmitoyltransferase (CPT) I during fasting in rainbow trout (*Oncorhynchus mykiss*) promotes increased mitochondrial fatty acid oxidation. *Physiological and Biochemical Zoology*, 84, 625-633.

Mostafa, G., Lamont, C. and Greene F.L., 2006. Review of surgery: basic science and clinical topics for ABSITE. NY, USA. Springer Publishing, 456.

Mustin, W.T. and Lovell, R.T., 1993. Feeding the repartitioning agent ractopamine to channel catfish (*Ictalurus punctatus*) increases weight gain and reduces fat deposition. *Aquaculture*, 109, 145-152.

Nazari-Farsani, M., Jalali, S.M.A. and Jafarian-Dehkordi, M., 2015. Evaluation the meat composition and immunity parameters of rainbow trout (*Oncorhynchus mykiss*) fed by dietary different oil sources, L-carnitine and ractopamine supplement. *ARPN Journal of Agricultural and Biological Science*, 10, 108-115.

NRC (National Research Council)., 2011. Nutrient requirements of fish. National Academy Press, Washington, DC.

Ramsay, R.R., Gandour, R.D. and Van der Leij, F.R., 2001. Molecular enzymology of carnitine transfer and transport. *Biochimica et Biophysica Acta*, 1546, 21-43.

Reddy, D.V., 2001. Principle of animal nutrition and feed technology. New Delhi, India. Oxford and IBH Publishing Company. 376.

Rodnick, K.J. and Williams, S.R., 1999. Effects of body size on biochemical characteristics of trabecular cardiac muscle and plasma of rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A*, 122, 407–413.

Salem, M., Levesque, H., Moon, T.W., Rexroad, C.E. and Yao, J., 2006. Anabolic effects of feeding B2-adrenergic agonists on rainbow trout muscle proteases and proteins. *Comparative Biochemistry and Physiology Part A*, 144, 145-154.

Sánchez-Gurmaches, J., Cruz-Garcia, L., Gutiérrez, J. and Navarro, I., 2010. Endocrine control of oleic acid and glucose metabolism in rainbow trout (*Oncorhynchus mykiss*) muscle cells in culture. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299, 562-572.

SAS, 2002. Statistical analysis system, Institute Inc. SAS/STAT User's Guide. Version 9.1th edn. SAS Institute Inc, Cary, NC, USA.

Satpathy, B.B., Mukherjee, D. and Ray, A.K., 2001. Effects of dietary inclusion of the beta-adrenergic agonist, salbutamol, on growth performance and whole body composition of rohu, *Labeo rohita* (Hamilton) fingerlings fed diets containing two protein levels. *Aquaculture Research*, 32, 739-747.

Sillence, M.N., 2003. Technologies for the control of fat and lean deposition in livestock. *The Veterinary Journal*, 167, 242-257.

Strydom, P.E., Frylinck, L., Montgomery, J.L. and Smith, M.F., 2009. The comparison of three b-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. *Meat Science*, 81, 557–564.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, 11, 107-184.

Tonial, I.B., Oliveira, D.F., Coelho, A.R., Matsushita, M., Coró, F.A.G., De Souza, N.E. and Visentainer, J.V., 2014. Quantification of essential fatty acids and assessment of the nutritional quality indexes of lipids in tilapia alevins and juvenile tilapia fish (*Oreochromis niloticus*). *Journal of Food Research*, 3, 105-113.

Törneke, K., Ingvast Larsson, C. and Appelgren, L.E., 1998. A comparison between clenbuterol, salbutamol and terbutaline in relation to receptor binding and in vitro relaxation of equine tracheal muscle. *Journal of Veterinary Pharmacology and Therapeutics*, 21, 388-392.

Van den Berg, G.W. and Moccia, R.D., 1998. Growth performance and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed the b-agonist ractopamine. *Aquaculture Research*, 29, 469-479.

Van Heeswijk, J.C.F., Vianen, G.J. and Van den Thillart, G.E.E.J.M., 2006. The adrenergic control of hepatic glucose and FFA metabolism in rainbow trout (*Oncorhynchus mykiss*): Increased sensitivity to adrenergic stimulation with fasting. *General and Comparative Endocrinology*, 145, 51–61.

Webster, C.D., Tui, L.G., Tidwell, J.H. and Reed, E.B., 1995. Effects of feeding the repartitioning agent L644, 969 on growth and body composition of blue catfish, *Ictalurus furcatus*, fed diets containing two protein levels reared in cages. *Aquaculture*, 134, 247-256.

Westfall, T.C. and Westfall, D.P. 2011. Adrenergic agonists and antagonists. In Goodman and Gilman's the pharmacological basis of therapeutics. (Brunton, L.L., Chabner, B.A. and Knollmann, B.C. eds.), 12th ed., New York, NY, McGraw Hill Medical Companies. pp. 277-333.

Zare-Shahneh, A., Beigi-Nejhad, H.A., Rowghani, E., Eilami, B. and Rowghani, S., 2012. Effects of

salbutamol on growth performance and carcass characteristics of Japanese quail (*Coturnix japonica*). *Iranian Journal of Veterinary Research*, 13, 112-119.