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Effects of dietary L-carnitine and chromium picolinate supplementations on performance and some serum parameters in rainbow trout (*Oncorhynchus mykiss*)

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Abstract An experiment was conducted to determine the effects of supplemental dietary L-carnitine, chromium picolinate (Cr-Pic) and their combination on growth performance and serum total protein, cholesterol, triglyceride and glucose of rainbow trout (*Oncorhynchus mykiss*). A total of 276 rainbow trout were randomly allocated to four groups. Fish (average initial body weight = 151 ± 1.69 g) were fed a basal diet without supplemental L-carnitine and chromium picolinate in the control group. The basal diet was supplemented with either 500 mg/kg L-carnitine, 1.6 mg/kg chromium picolinate or 500 mg/kg L-carnitine plus 1.6 mg/kg chromium picolinate for experimental groups 1 (C), 2 (Cr-Pic) and 3 (C + Cr-Pic), respectively. Fish were fed twice a day to apparent satiation for 58 days. Weight gain, growth rate, feed consumption and feed conversion ratio (feed/gain) were calculated for the whole period. At the end of the study, six fish were chosen randomly from each tank to represent the experiment and frozen at −20°C for subsequent dorsal muscle analysis. The results showed no significant differences in final body weight, weight gain or feed conversion ratio among groups. There were also no significant differences in serum total protein, cholesterol, triglyceride or glucose concentrations among groups. Significantly higher lipid concentration of dorsal muscle was observed in experimental groups 1 and 3. In conclusion, supplemental dietary L-carnitine, chromium picolinate and their combination have no beneficial effects on improving growth

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performance and feed conversion ratio in rainbow trout. However, dietary L-carnitine slightly increased lipid concentration in dorsal muscle of rainbow trout.

Keywords Chromium picolinate · L-Carnitine · Performance · Rainbow trout

Introduction

L-Carnitine (*β*-OH-*γ*-*N*-trimethylaminobutyric acid) has a low molecular weight and is a water-soluble quaternary amine naturally found in microorganisms, plants and animals (Bremer 1983). It is supplied by both dietary sources and biosynthesis, with lysine and methionine as precursors, in animals. L-Carnitine performs a key role in energy metabolism and promotes the mitochondrial *β*-oxidation of long-chain fatty acids by admitting their transfer across the interior mitochondrial membrane (Rebouche 1992). Dietary L-carnitine supplementation improved growth in some fish species such as European seabass (Santulli and d'Amelio 1986), African catfish (Torreele et al. 1993), common carp (Becker and Focken 1995; Focken et al. 1997), Indian carp (Keshavaneth and Renuka 1998) and hybrid tilapia (Becker et al. 1999). However, carnitine supplementation to diet did not affect growth performance in Atlantic salmon (Ji et al. 1996) and rainbow trout (Rodehutscord 1995; Chatzifotis et al. 1997). Rodehutscord (1995) reported that dietary carnitine supplementation did not decrease the lipid content of the whole body in rainbow trout.

Previous experiments have suggested that chromium is an essential element for optimum lipid, protein and carbohydrate metabolism in animals (Anderson 1987; McCarty 1991; Mertz 1993). Chromium supplementation to diets improved the growth rate and feed conversion ratio in rats, poultry and pigs (Anderson 1987; NRC 1997; Lien et al. 1999). Kornegay (1996) reported that dietary organic chromium supplementation was more effective than dietary inorganic chromium supplementation in swine and poultry. Supplemental dietary organic chromium as chromium picolinate (Cr-Pic) decreased mortality and altered glucose metabolism in poultry, pigs and fish (NRC 1997).

The aim of the present study was to determine the effects of dietary L-carnitine and chromium picolinate supplementations on growth performance and serum total protein, cholesterol, triglyceride and glucose concentrations of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Animals and experimental design

The trial was set up in a flowthrough water system in an indoor facility in the Sinop University Fisheries Faculty in Sinop (Turkey). Rainbow trout with an initial mean fish weight of 151 ± 1.69 g obtained from a commercial farm (Kuzey Su Urunleri, Inc.) in Bafra-Samsun were used in the study. Fish were allowed to acclimate to the experimental conditions for 2 weeks before starting the study. Fish were selected from a large population for uniform size, weighted individually and randomly stocked into centrally drained circular fibreglass experimental tanks (water depth 80 cm; volume 300 l). A total of 276 rainbow trout were randomly allocated to four groups. Each group contained three tanks (three replicates) and each tank included 23 fish. Water flow was 4 l/min and supplemental

aeration was provided by air-stone diffusers. Water quality parameters were monitored daily during the experimental period. Water temperature was 14.0 ± 1.1°C, pH 7.5 and dissolved oxygen 6.6 ± 0.3 mg/l. The feeding trial was conducted under natural photoperiod. At the end of the study, six fish were chosen randomly from each tank to represent the experiment and frozen at −20°C for subsequent dorsal muscle analysis. Experimental fish were weighted at the beginning and end of the study. Weight gain, growth rate, feed consumption and feed conversion ratio (feed/gain) were calculated for the whole period (58 days).

Experimental diets

Fish were fed with a basal diet without supplemental L-carnitine and chromium picolinate in the control group. The basal diet was supplemented with 500 mg/kg L-carnitine (Carniking; a commercial product used in animal feeds containing 50% L-carnitine), 1.6 mg/kg chromium picolinate (Chromax; Prince Agri Products, Inc.) and 500 mg/kg L-carnitine plus 1.6 mg/kg chromium picolinate for experimental groups 1 (C), 2 (Cr-Pic) and 3 (C + Cr-Pic), respectively. The basal diet was formulated according to the NRC guidelines (NRC 1993). Feed ingredients and chemical composition of the basal diet are shown in Table 1. Proximate compositions of the experimental diets are presented in Table 2. Fish were fed twice a day to apparent satiation for 58 days.

Sample collection and laboratory analysis

At the end of the study 21 fish were randomly chosen from each treatment and blood samples were collected by syringe from caudal vein and transferred immediately and gently into tubes to avoid hemolysis. The tubes containing blood samples were centrifuged at 3,000 rpm for 10 min. Serum concentrations of total protein, cholesterol, triglyceride and glucose were analysed using commercially available kits (Sigma–Aldrich Chemie GmbH, Eschenstraße 5, 82024 Taufkirchen, Germany) according to the autoanalyser manufacturer's instruction (Autolab, AMS Srl, Selective Access). Chemical compositions of the basal diet and dorsal muscle were analysed according to AOAC (1990). Crude

Table 1 Composition of the basal diet

Ingredients	g/kg
Peruvian fish meal	166
Anchovy fish meal	107
Full-fat soybean meal	59
Extracted soybean meal	194
Maize gluten	217
Wheat meal	72
Sunflower meal	40
Fish oil	30
Soybean oil	110
Vitamin premix[a]	2
Mineral premix[b]	1.5
Choline	1.5
Total	1,000

[a] Provided per kilogram of feed: vitamin A, 12,500 IU; vitamin D3, 2,500 IU; vitamin K3, 10 mg; vitamin B1, 10 mg; vitamin B2, 20 mg; vitamin B6, 15 mg; vitamin B12, 0.03 mg; vitamin C, 250 mg; niacin, 200 mg; biotin, 1 mg; folic acid, 10 mg; pantothenic acid, 60 mg

[b] Provided per kilogram of feed: calcium, 1,000 mg; ethoxyquin, 130 mg; magnesium, 600 mg; potassium, 450 mg; zinc, 90 mg; manganese, 12 mg; copper, 5 mg

Table 2 Proximate composition of the experimental diets

Proximate composition (%)	Control	C	Cr-Pic	C + Cr-Pic
Dry matter	93.6	94.0	94.0	94.7
Crude protein	45.1	44.3	45.6	45.2
Crude lipid	25.3	24.5	25.3	25.2
Crude ash	6.6	6.7	6.7	6.7
NFE + fiber[a]	23.0	24.4	22.4	22.9
Gross energy (kJ g^{-1})	24.7	24.5	24.7	24.7

[a] NFE + fiber: nitrogen-free extract (calculated by difference)

protein was analysed according to the Kjeldahl method (N × 6.25), crude lipid by petroleum ether extraction in a Soxhlet apparatus and ash by incineration at 550°C in a muffle furnace. The nitrogen-free extract (NFE) + fibre component was determined by the equation: NFE + fibre = 100 − (% protein + % lipid + % ash).

Statistical analysis

A one-way analysis of variance model was used to determine differences among groups. The significance of differences among means was compared by Duncan's multiple range test (Duncan 1955). All statistical analyses were performed using the SAS (2007) statistical package.

Results

Final body weight, weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and composition of dorsal muscle in rainbow trout fed the control and experimental diets are presented in Tables 3 and 4, respectively. Average final body weights and feed conversion ratios were 272.5, 282.0, 286.0 and 275.6 g and 1.0, 1.0, 1.0 and 1.0 in the control, C, Cr-Pic and C + Cr-Pic groups, respectively. There was no significant difference ($P > 0.05$) among groups in terms of growth performance and feed conversion ratio. There was no significant difference ($P > 0.05$) in the muscle compositions among groups for dry matter, crude protein and ash except for lipid ($P < 0.05$). Blood

Table 3 Growth performance and feed conversion ratio in fish fed the experimental diets

	Control	C	Cr-Pic	C + Cr-Pic
Initial body weight (g)	151.5 ± 1.69	150.7 ± 1.69	151.3 ± 1.69	150.7 ± 1.69
Final body weight (g)	272.5 ± 5.02	282.0 ± 4.83	286.0 ± 5.06	275.6 ± 5.06
Specific growth rate (%)[a]	1.0 ± 0.04	1.1 ± 0.03	1.1 ± 0.06	1.0 ± 0.01
Feed conversion ratio[b]	1.0 ± 0.03	1.0 ± 0.01	1.0 ± 0.03	1.0 ± 0.02
Protein efficiency ratio[c]	2.1 ± 0.06	2.2 ± 0.01	2.2 ± 0.07	2.2 ± 0.04

Values are means from triplicate groups of fish (mean ± SEM)

[a] SGR, specific growth rate (%) = 100 × [(In final body weight − In initial body weight)/58 days]

[b] FCR, feed conversion ratio = total diet fed (g)/total weight gain (g)

[c] PER, protein efficiency ratio = weight gain (g)/protein intake (g)

Table 4 Dorsal muscle composition of fish fed the experimental diets (%)

	Control	C	Cr-Pic	C + Cr-Pic	*P* value
Dry matter	25.7 ± 0.47	26.7 ± 0.19	26.3 ± 0.26	26.5 ± 0.10	NS
Crude protein	19.7 ± 0.26	19.7 ± 0.10	19.8 ± 0.09	19.5 ± 0.21	NS
Crude lipid	3.6 ± 0.09[a]	4.4 ± 0.26[b]	4.0 ± 0.16[ab]	4.3 ± 0.10[b]	*
Ash	1.4 ± 0.06	1.3 ± 0.03	1.4 ± 0.02	1.3 ± 0.04	NS

NS: $P > 0.05$ results are not significantly different

[a, b] Means of same letters in rows are insignificant

* $P < 0.05$ results are significant different

Table 5 Effects of supplemental L-carnitine and chromium picolinate on blood serum metabolites of rainbow trout

	Control	C	Cr-Pic	C + Cr-Pic
Total protein (g/dl)	3.2 ± 0.11	3.3 ± 0.11	3.3 ± 0.11	3.5 ± 0.11
Cholesterol (mg/dl)	228.9 ± 9.67	221.6 ± 9.67	225.5 ± 9.67	237.1 ± 9.67
Triglyceride (mg/dl)	211.7 ± 13.73	217.4 ± 13.73	246.2 ± 13.73	234.6 ± 13.73
Glucose (mg/dl)	28.6 ± 1.98	27.9 ± 1.98	26.4 ± 1.98	24.4 ± 1.98

Values are means from triplicate groups of fish (mean ± SEM)

serum total protein, cholesterol, triglyceride and glucose concentrations of rainbow trout are shown in Table 5. Dietary supplemental L-carnitine and chromium picolinate did not significantly affect ($P > 0.05$) blood serum total protein, cholesterol, triglyceride and glucose concentrations between the control, C, Cr-Pic and C + Cr-Pic groups.

Discussion

The effect of L-carnitine on fatty acids and energy metabolism has been studied intensively in animals and fish. Harpaz (2005) mentioned that the required quantities of carnitine in fish during their developmental stages may follow a similar pattern to those reported in other vertebrates. Lin and Odle (2003) reported that hepatic carnitine palmitoyltransferase activity was low at birth, increased by 100% during suckling and then reduced to adult levels after weaning in dogs. However, carnitine palmitoyltransferase activity in the muscle continued to increase with age, reaching adult levels after 9 weeks. Rincker et al. (2003) showed that the supplementation of 50–100 mg L-carnitine/kg diet had only minor effects on growth during phases 1 and 3 of their growth while it improved growth performance of weanling pigs during phase 2 of growth. Ji et al. (1996) reported that supplemental dietary carnitine did not affect growth performance in Atlantic salmon. Rodehutscord (1995) and Chatzifotis et al. (1997) showed that dietary carnitine supplementation did not change growth performance in rainbow trout. Gaylord and Gatlin (2000a) determined that supplementation level or type of carnitine did not appear to be beneficial to hybrid striped bass based on growth performance. Gaylord and Gatlin (2000b) observed that L-carnitine supplementation did not affect weight gain in hybrid striped bass. Li et al. (2007) showed that L-carnitine supplementation at 500 mg/kg in the diet did not influence feed consumption, weight gain and feed efficiency in channel catfish × blue

catfish hybrids. Jayaprakas et al. (1996) showed that the effects of four levels (300, 500, 700 and 900 mg/kg) of dietary L-carnitine supplementation on growth and reproductive performance of male Mossambique tilapia. The study was carried out with juvenile (2.2 g) fish for a period of 252 days on the experimental diet administered at a level of 5% of fish biomass. The results showed an effect on both growth and reproductive performance of the fish, this effect being correlated with the level of carnitine supplementation.

A promoting effect of additional dietary L-carnitine has also been reported in some marine and freshwater species (Santulli and d'Amelio 1986; Torreele et al. 1993; Chatzifotis et al. 1995; Becker et al. 1999). By contrast, the results of the present study showed that supplemental dietary L-carnitine has no beneficial effects on weight gain and feed conversion ratio in rainbow trout. It can be stated that the effect of L-carnitine may be different between fish species and even in different life stages of the same species. On the other hand, dietary factors, possible species differences and variations in biochemical, metabolic and physiological activities in the distinct life stages of fish may also affect the effectiveness of dietary L-carnitine supplementation. Harpaz (2005) mentioned that initial size of the examined fish was important in the outcome of the growth due to growth decrease when fish increase in size. This can explain the different results among studies. Although many studies (Torreele et al. 1993; Chatzifotis et al. 1995, 1996; Keshavaneth and Renuka 1998) carried out with young, fast-growing fish (from larval stages to 15 g) have demonstrated that dietary L-carnitine has beneficial effect on growth performance, in studies of fish with higher initial body weight (15–45 g) the improvement effects of L-carnitine feeding were less evident. Some studies (Santulli and d'Amelio 1986; Chatzifotis et al. 1996) confirmed this result but others did not (Rodehutscord 1995; Ji et al. 1996; Chatzifotis et al. 1997). The findings relating to growth performance and feed conversion ratio are consistent with those of Rodehutscord (1995), Ji et al. (1996), Chatzifotis et al. (1997) and Dias et al. (2001).

Chromium in fish nutrition is important due to its participation in carbohydrate, protein and fat metabolism (Anderson 1987). Ng and Wilson (1997) mentioned that dietary trivalent chromium supplementation for channel catfish did not improve growth performance, which is in agreement with the present study. It is also reported that dietary trivalent chromium supplementation did not improve growth performance and feed efficiency in rainbow trout (Tacon and Beveridge 1982). The results of the present study relating to growth performance were in agreement with the results of other studies with rainbow trout (Tacon and Beveridge 1982; Bureau et al. 1995) in which no significant effect of additional dietary chromium had been observed.

The results of the present study indicated that supplementation of dietary L-carnitine had no effect on total cholesterol concentration in serum, as confirmed by Dias et al. (2001). However, Santulli et al. (1988) observed that carnitine feeding reduced the level of total cholesterol in plasma. The supplementation of Cr-Pic increased serum total protein whereas it decreased cholesterol concentrations in chickens, rats and calves (Anderson 1987; Mertz 1993). Küçükbay et al. (2006) reported that serum glucose and cholesterol concentrations reduced with higher dietary chromium supplementation (0, 0.4, 0.8 or 1.6 mg/kg diet) in rainbow trout. Hertz et al. (1989) and Shiau and Liang (1995) reported that chromium salts improved glucose utilisation in common carp juveniles and tilapia, respectively, whereas Ng and Wilson (1997) mentioned the opposite response in channel catfish. However, there was no difference in the findings of the present study for serum total protein, cholesterol, triglyceride and glucose concentration among all groups. Experiments (Shiau and Chen 1993; Shiau and Lin 1993; Pan et al. 2002) in hybrid tilapia suggested that chromium nicotinic acid or Cr-Pic reduced glucose concentration. Pan et al. (2003) reported there was no significant effect of supplementary Cr-Pic at 2 mg/kg diet on

growth and carbohydrate utilisation in tilapia. Experimental designs and conditions of these studies, such as use of static water system, closed water recirculation rearing system or flowthrough system, may be responsible for these different results. In studies (Shiau and Chen 1993; Shiau and Lin 1993; Pan et al. 2002) conducted in a static water system or a closed water recirculation rearing system, some Cr recycling might occur (Ng and Wilson 1997), thus resulting in additional bioaccumulation of Cr by fish from water. The present study was carried out in a flowthrough system. However, in a flowthrough rearing system used by Tacon and Beveridge (1982), Bureau et al. (1995) and the present study, excreted Cr is quickly removed from the medium.

There are varying results on the effects of L-carnitine on muscle composition. Dietary L-carnitine supplementation did not affect muscle composition in rainbow trout (Rodehutscord 1995) or hybrid tilapia (Becker et al. 1999). It was reported that the relative quantities of triglycerides, free fatty acids and phospholipids in muscle and liver were not influenced by carnitine level or type in hybrid striped bass (Gaylord and Gatlin 2000a). It has been reported that there was a trend toward a reduction in fillet lipid level in channel catfish × blue catfish hybrids fed diet containing 500 mg/kg L-carnitine supplementation (Li et al. 2007). On the other hand, addition of dietary L-carnitine reduced lipid content of muscle in Atlantic salmon (Ji et al. 1996), channel catfish (Burtle and Liu 1994) and European seabass juveniles (Santulli and d'Amelio 1986). Dias et al. (2001) reported that dietary supplementation of L-carnitine did not affect whole-body composition of 250 g seabass. Similar findings have been reported in red seabream, rainbow trout and hybrid tilapia (Chatzifotis et al. 1995, 1996, 1997; Rodehutscord 1995; Becker et al. 1999). Rodehutscord (1995) mentioned that additional dietary L-carnitine was not effective for decreasing body fat in rainbow trout fed high-fat diets (26%). Lipid content of dorsal muscle in rainbow trout was not reduced by L-carnitine or Cr-Pic supplementation in the present study. Contrarily, carnitine and the combination of carnitine and chromium picolinate supplementations slightly increased lipid content and did not affect protein content in the dorsal muscle of rainbow trout. These results of the present study are consistent with Chatzifotis et al. (1995), who reported higher lipid content in muscle of fish fed on feed supplemented with L-carnitine compared with unsupplemented group.

In conclusion, the results of the present study showed that supplemental dietary L-carnitine, Cr-Pic and their combination have no beneficial effects on improving growth performance, feed conversion ratio, reducing in lipid deposition of dorsal muscle in practical feeding of rainbow trout. Further investigation may be undertaken to assess the possible beneficial effects of L-carnitine and Cr-Pic supplementation on the performance and lipid deposition in some fish species, the distinct life stages of the same species and different experimental designs and conditions of studies such as static water system, closed water recirculation rearing system or flowthrough system.

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