Effects of supplemental dietary L-carnitine on growth and body composition of beluga (*Huso huso)* juveniles

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Abstract: The effect of feed supplemented with L-carnitine at four levels of 0, 300, 600 and 900mg kg^{-1} on growth performance and body composition of beluga, *H. huso*, juveniles was evaluated in two separate trials. In the first trial, 180 juveniles (525±9g) were fed with the test diet for a period of 71 days, and the trial extended to another 126 days in the second phase by randomly selecting 120 juveniles (870.7±32g) and rearing them with similar diets. All trials, in triplicates, were conducted in circular fiberglass tanks (200cm diameter, 40cm height). The fish were fed 4 times daily to apparent satiation.

In the first phase, L-carnitine did not significantly affect mean final weight, feed conversion ratio (FCR) or specific growth rate (SGR) of the fish, however, the fish receiving 900mg kg^{-1} L-carnitine showed better growth increment, SGR and feed efficiency (FE) than those in control group ($P \geq 0.05$); neither did it significantly affect protein, lipid, moisture or ash as well as hepatosomatic index (HSI) ($P \geq 0.05$). The protein efficiency ratio (PER) was significantly affected by L-carnitine supplemented diet, particularly at 900mg kg^{-1} ($P \leq 0.05$). In the second phase, fish fed L-carnitine-supplemented diets produced significantly higher W2, SGR and PER than the control group ($P \leq 0.05$), but HSI decreased significantly ($P \leq 0.05$). At the end of the trial, the whole body composition of fish did not differ significantly among experimental treatments, but slightly reduced lipid content were observed at 600 and 900mg kg^{-1} L-carnitine ($P \geq 0.05$). The results of this study indicated that supplementation of diets with 300-600mg carnitine kg^{-1} improved growth rate, feed utilization and stimulated protein-sparing effect in this species.

Keywords: L-carnitine, Growth rate, Body composition, Beluga, *Huso huso*

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Introduction

Beluga, *Huso huso,* is considered as a good candidate for meat production purposes among sturgeons of the southern Caspian Sea for having valuable characteristics such as fast growth, reproduction in captivity, toleration to unfavorable rearing conditions, such as fluctuations in water temperature and dissolved oxygen, and stocking density (Mohseni *et al.,* 2000). Several countries, such as USA, Russia, Italy, and France are currently involved in sturgeon rearing for caviar and meat production purposes (Rosenthal, 2000). Feed efficiency and feeding percentage are considered as the main economic factors determining potential of commercial sturgeon production (Gershanovich & Taufik, 1992). Production efficiency depends upon the type of diet and its preparation method, which in turn is controlled by factors including energy, feed composition, protein, fat, vitamins, minerals, digestibility of feed components and their continuous availability (Mohseni *et al.,* 2007). For the very same reason, application of new materials and methods leading to higher growth in sturgeon, particularly at early stages has become attractive in Iran (Abedian *et al.*, 2007). Therefore, the economic evaluation of feed and establishment of feed requirements are crucial to increase production results and benefits (Vasilieva, 2000).

L-carnitine is a vitamin like compound ($C_7H_{15}NO_3$), which is naturally synthesized in animals, mostly in the liver and kidneys (Harpaz, 2005). The history of L-carnitine in fish biology started with the studies of Bilinsky and Jonas (1970), which showed that L-carnitine had an intermediary function in transporting long chained fatty acids (LCFA) for oxidation in mitochondria of rainbow trout. Further studies showed that in the absence of L-carnitine the organism was unable to break down LCFA for energy production (Baumgartner & Blum, 1997). Dietary L-carnitine supplementation was found to improve growth performance in several fish species, such as the red seabream, *Pagrus major* (Chatzifotis *et al.*, 1995), common carp, *Cyprinus carpio* (Becker & Foken, 1995, Seyfabadi *et al.*, 2006), Indian white shrimp, *Penaeus indicus* (Jayaprakash & Sambhu, 1996), beluga, *H. huso* (Mohseni *et al.,* 2002) and Persian sturgeon *Acipenser persicus* (Seyfabadi *et al.*, 2005). By contrast, supplemented L-carnitine did not affect growth performance

in rainbow trout, *Oncorhynchus mykiss* (Rodehutscord, 1995; Chatzifotis *et al.*, 1997; Hosseini *et al.*, 2002), Atlantic salmon, *Salmo salar* (Ji *et al.*, 1996), and kutum, *Rutilus frisii kutum* (Seifabadi *et al.*, 2002). Due to this variability of responses to carnitine feeding in fish, it is still difficult to explain the underlying mechanisms by which supplemental dietary L-carnitine may exert such possible beneficial effects in terms of growth performance and lipid deposition.

Beluga farming is a priority to the Iranian fisheries. The duration in which beluga reaches marketable size (3-4kg) is approximately 17-20 months (Mohseni *et al.*, 2004). In this period 98% of food is composed of concentrated diet, which requires considerable expenditure. Therefore, the objective of the present study was to investigate the effect of L-carnitine on growth performance and body composition of the beluga when fed on a high-fat diet.

Materials and methods

The study was conducted in two phases from January 4th to March 16th 2003 and March 19th to July 21st 2004, at the International Sturgeon Research Institute in northern Iran. In the first phase, 180 beluga juveniles (525±9g), distributed in 12 fiberglass tanks (15 fish in each tank) reared for 71 days with four experimental diets in three replicates. In the second phase, 120 juveniles of beluga (870.7±32g), randomly selected from the first phase, were reared for 126 days under similar conditions as the first phase, except for the number of fish per tank that was 10 fish in the second phase.

The experiments were conducted under identical conditions, following completely randomized design. The tanks (200cm diameter, 40cm height and 1600 l volume), were supplied with the water from the Sefidroud River; water in the tank was well-aerated (7.8±0.5mg L^{-1} dissolved oxygen) and exchanged at a rate of 35% h^{-1}. Water temperature ranged from 9.8 to 10.5°C in January, 8.5 to 10°C in February, 11 to 13°C in March, 14-16°C in April, 17-20°C in May and 20-24°C in July, and the pH of 7.3±0.2. All tanks were maintained under natural photoperiod of 12 D:12 L.

Fish were initially adapted to experimental conditions for two weeks. Four isonitrogenous (40% crude protein), isolipidic (15% crude fat), moisture (9%), ash

(10%), and isocaloric (21.5 $MJ.kg^{-1}$) diets were formulated containing 0, 300, 600 and 900 mg kg^{-1} L-carnitine (Lohmann, GmbH Co. Germany). The desired amount of L-carnitine was weighed using a digital balance and dissolved in 50ml water and mixed homogeneously with 1kg of diet.

Dry ingredients were finely ground (<800µm) in a Damico mill (Damicar Co., Tehran, Iran), before being combined with the wet ingredients (maize and anchovy oils). Micronutrients (vitamins, minerals and L-carnitine) were pre-mixed with ground wheat as a base, using a twin-shell blender (Pooya Notash Machinery Co., Mashhad, Iran) prior to being added to the main ingredient mixture. After that, all ingredients were mixed for 10 minutes in a Pooya mixer and steam-pelleted (screen diameter: 6.0mm) using a CPM meat grinder (California Pellet Mill Co., Sanfrancisco, CA, USA). Pellets were then dried in an air-convection drier at 30°C (until the moisture content was reduced to less than 10%) to form sinking pellets and stored in air-tight containers at -18°C until use. Diets were screened to remove fine dust prior to feeding.

The daily ration was adjusted based on fish biomass determined after each bioassay. Water temperature and dissolved oxygen were measured daily. Fish were hand-fed four times daily (08:00, 13:00, 18:00 and 23:00) to apparent satiation to avoid any uneaten feed. Each day, before the first feeding, the tanks and fecal collection columns were thoroughly cleaned with a brush to remove any residual particulate matter. Fish were measured every two weeks using digital balance (0.1g precision) and based on that diets were adjusted for the subsequent two weeks. Fish were anesthetized in 200mg lit^{-1} of clovetree *(Syzyglum aromaticum)* before biometry. Following biometry, feeding was stopped for one day to decrease stress (Mohseni *et al.*, 2004). Using data on length and weight of fish in each tank, the following formulas were used to determine specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and daily feed consumption (percent of body weight) (DFC).

FCR ($g\ g^{-1}$) = total feed intake (g) / total weight gain (g)

SGR (% BW day^{-1}) = 100 x [(Ln final weight – Ln initial weigh)/day]

PER ($g\ g^{-1}$) = fish wet weight gain (g) / fish crude protein consumption (g)

DFC (% BW day $^{-1}$) = (Feed intake / (initial weight +final weight)/number of days × 100
Hepatosomatic index (HSI) = (liver weight/total fish body weight) ×100)]

At the end of the trial, two fish from each tank (24 in total) were randomly sampled after 24h food deprivation and killed with an overdose of clovetree; the liver removed and weighed to calculate HSI, the liver put back with the carcass, frozen on dry ice and stored at –20°C until further analyses. Chemical analysis of diets and fish whole-body was performed according to standard methods: dry matter (drying at 105°C for 6h to constant weight); ash (AOAC, 1995); crude fat (Soxhlet extraction method); protein (Kjeldahl using a selenium catalyst [N×6.25]) and energy (bomb calorimeter, calibrated with benzoic acid).

Data on final weight (W2), SGR, PER, DFC, survival rate and proximate analysis of the whole body of each treatment were tested using one-way ANOVA (Super-ANOVA, Abacus Concepts, Berkeley, USA). Significant differences among treatments were evaluated by Duncan's new multiple range test. Statistical significance of differences was determined by setting the error at 5% ($P \leq 0.05$) for each set of comparisons. All statistical tests were performed using SPSS (version 10.0) statistical package.

Results

Experiment 1:

Results of the 71-days feeding trial with L-carnitine are presented in Table 1. Fish fed on the diets containing 600 and 900mg kg^{-1} L-carnitine showed a higher specific growth rate. The fish receiving 900mg kg^{-1} L-carnitine exhibited superior growth throughout, showing 6.68% increase in the mean weight over the untreated fish ($P \geq 0.05$). L-carnitine seemed to increase the feed efficiency, since the fish fed on 600 and 900mg kg^{-1} L-carnitine showed superior feed efficiency ($P \geq 0.05$). Although daily feed consumption (DFC) did not differ much among groups, a slight decrease was observed in the fish that received 600 and 900mg kg^{-1} L-carnitine ($P \geq 0.05$). Increase in L-carnitine significantly influenced ($P \leq 0.05$) protein efficiency ratio (PER), which peaked in the fish receiving 900mg kg^{-1} L-carnitine ($P \leq 0.05$). No mortality was observed during the feeding trial in this phase.

Table 1: Effect of dietary L-carnitine on final weight, daily feed consumption, feed conversion ratio, specific growth rate, feed efficiency and protein efficiency ratio of beluga, *Huso huso*, grown over 71 days (mean ± SE, n=3).

Levels of L-carnitine ($mg.kg^{-1}$)	Initial weight (g)	Final weight (g)	DFC (% day^{-1})	FCR ($g\,g^{-1}$)	SGR (% day^{-1})	FE ($g\,g^{-1}$)	PER ($g\,g^{-1}$)
Control	524.4±2.9	837.6±14.5	13.9±0.13	2.8±0.1	0.68±0.03	48.19±1.84	0.52±0.01b
300	518.1±2.4	836.1±24.7	13.8±0.04	2.6±0.14	0.70±0.05a	49.25±3.44	0.58±0.01b
600	528.1±3.9	886.7±7.6	13.3± 0.02	2.7±0.16	0.79±0.02	55.34±1.63	0.78±0.01a
900	529.3±3.2	897.5±46.1	13.4±0.12	2.5±0.14	0.86±0.07	59.62±3.83	0.82±0.02a

Means with same letter not significantly different (Duncan's new multiple range test).

The L-carnitine supplementation did not significantly affect ($P \geq 0.05$) the body composition and the hepatosomatic index (HSI) (Table 2). The lowest mean HSI and lipid content were found in the fish group fed the diet containing 900 $mg.kg^{-1}$ L-carnitine, though not significantly different from the other groups ($P \geq 0.05$).

Table 2: Whole-body composition (%wet weight) of beluga, *Huso huso*, fed on the experimental diets for 71 days (mean ± SE, n= 3)

Levels of L-carnitine ($mg.kg^{-1}$)	Moisture	Crude protein	Crude lipid	Crude ash	HSI (%)
Control	75.1 ± 0.5	17.9 ± 0.4	5.9 ± 0.19	1.9 ± 0.06	2.5 ± 0.2
300	75. 3 ± 0.7	17.3 ± 0.6	5.6 ± 0.22	1.7 ± 0.07	2.4 ± 0.21
600	75.5 ± 0.5	17.6 ± 0.7	5.6 ± 0.11	1.8 ± 0.06	2.3 ± 0.22
900	75.7 ± 0.7	17.6 ± 0.5	5.1± 0.05	1.9 ± 0.08	2.2 ± 0.18

Means with same letter not significantly different (Duncan's new multiple range test).

Experiment 2:

Data on growth performance, feed and protein efficiency of beluga fed on different experimental diets are presented in Table 3. L-carnitine supplementation significantly enhanced SGR, DFC and PER ($P \leq 0.05$), but no significant differences among different levels of L-carnitine were evident ($P \geq 0.05$). Although FCR and FE were not significantly affected by the L-carnitine supplementation ($P \geq 0.05$), they improved in the fish fed on diets containing 300 and 900mg kg^{-1} L-carnitine. No mortality was observed during the feeding trial in this phase.

Table 3: Effect of dietary L-carnitine on final weight, daily feed consumption, feed conversion ratio, specific growth rate, feed efficiency and protein efficiency ratio of beluga, *Huso huso*, grown over 126 days (mean ± SE, n= 3).

Levels of L-carnitine ($mg.kg^{-1}$)	Initial weight (g)	Final weight (g)	DFC (% day^{-1})	FCR ($g\,g^{-1}$)	SGR (% day^{-1})	FE ($g\,g^{-1}$)	PER ($g\,g^{-1}$)
Control	869.6±31.5	1673.9±63.1^{b}	21.2±0.26^{b}	1.93±0.11	0.60±0.02^{b}	47.5±2.77	0.62±0.01^{b}
300	859.2±35.4	2018.5±56.1^{a}	18.7±0.33^{a}	1.56±0.04	0.81±0.00^{a}	54.7±2.22	0.85±0.00^{a}
600	859.7±28.2	1974.7±58.2^{a}	19.5±0.33^{a}	1.71±0.0	0.76±0.00^{a}	49.8±3.82	0.81±0.00^{a}
900	867.4±33.3	2016.6±65.1^{a}	18.4±0.09^{a}	1.62±0.01	0.76±0.00^{a}	52.5±3.32	0.86±0.00^{a}

Means with same letter not significantly different (Duncan's new multiple range test).

Fish fed the diet without L-carnitine supplementation did not show any external signs of deficiency, but had significantly heavier liver than fish fed diets with supplemental L-carnitine ($P \leq 0.05$). L-carnitine did not significantly affect crude protein, crude lipid, moisture and ash contents of the whole body among groups, but crude lipid content was slightly lower in fish fed 600 and 900mg kg^{-1} L-carnitine (Table 4).

Table 4: Whole-body composition (%wet weight) of beluga, *Huso huso*, fed experimental diets for 126 days ;(mean ± SE, n= 3)

Levels of L-carnitine ($mg\,kg^{-1}$)	Moisture	Crude protein	Crude lipid	Crude ash	HSI (%)
Control	75.10 ± 0.50	16.08 ± 0.6	5.22 ± 0.19	1.4 ± 0.05	2.65 ± 0.22^{b}
300	75. 3 ± 0.66	15.99 ± 0.5	4.98± 0.22	1.8 ± 0.04	2.04 ± 0.21^{a}
600	75.5 ± 0.5	15.88 ± 0.7	4.62± 0.11	1.6 ± 0.06	1.99 ± 0.19^{a}
900	75.7 ± 0.72	16.04 ± 0.6	4.55± 0.05	1.7 ± 0.07	1.89 ± 0.20^{a}

Means with same letter not significantly different (Duncan's new multiple range test).

Discussion

It is difficult to ascertain with any certainty the beneficial effects of dietary L-carnitine supplements on growth performance and lipid metabolism in fish, as data available in the literature show that apart from possible species differences, the developmental stage of fish, environmental conditions and diet composition (i.e. fat and lysine content or even L-carnitine addition level) may also condition the animals response (Dias *et al.*, 2001).

In the first phase of the study, the juvenile beluga (525±9g) fed with supplementary L-carnitine showed improved mean body weight (BW), feed efficiency (FE), specific growth rate (SGR), feed conversion ratio (FCR) and hepatosomatic index, though not significantly ($P \geq 0.05$), within 71 days feeding trial (Tables 1 & 2), so that the growth improved by 7.2% in treatment receiving 900mg kg^{-1} L-carnitine as compared to control group (Table1). The effect of L-carnitine treated feed on the growth parameters and HSI of the juvenile beluga (870.7±32g) reared for another 126 days in the second phase of the study was, however, more conspicuous ($P \leq 0.05$) than the control group (Table 3 & 4). The results in the first phase are comparable with those of other studies on various species, among which hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, (Becker *et al.*, 1999), beluga, *Huso huso* (Mohseni *et al.*, 2002), African catfish, *Clarias gariepinus* (Ozorio *et al.*, 2003,2005), and the Persian sturgeon, *Acipenser persicus* (Seyfabadi *et al.*, 2005) could be addressed. As for the results in the second phase, similarities could be traced with other studies on the African catfish (Torreele *et al.*, 1993), channel catfish, *Ictalurus punctatus* (Burtle & Liu, 1994), and common carp, *Cyprinus carpio* (Becker & Focken, 1995; Seyfabadi *et al.*, 2006), red sea bream, *Pagrus major* (Chatzifotis *et al.*, 1995), where L-carnitine has been shown to enhance growth significantly. In several other studies, never-theless, no traceable effects of supplementary L-carnitine have been observed, as in the Atlantic salmon, rainbow trout (Chatzifotis *et al.*, 1997; Rodehutscord, 1995; Ji *et al.*, 1996; Hosseini *et al.*, 2002) and kutum, *Rutilus frisii kutum* (Seifabadi *et al.*, 2002).

Protein efficiency ratio (PER) was significantly affected by supplementary L-carnitine in both phases of the experiment (Tables 1 & 3), reaching the highest value in fish fed high L-carnitine (900 mg kg^{-1}), although no significant difference above

600 mg kg^{-1} was evident. Several other studies have also shown that supplementary L-carnitine has significant influence on energy production (Harpaz, 2005) as well as significant increase in PER ($P \leq 0.05$). This is may be due to better protein sparing effect under the influence of L-carnitine. Providing suitable energy sources, protein is not utilized as energy source thus the dietary protein is completely used for growth resulting in higher growth, increased protein efficiency, lower FCR and, ultimately, reduced production costs.

Our results show that the dietary L-carnitine supplementation did not affect the whole-body crude protein and moisture contents in both phases (Tables 2 & 4), but some decrease ($P \geq 0.05$) in the crude fat in both phases and slight increase in the crude ash in the second phase could be traced. Whereas Rodehutscord (1995) reported that supplemental dietary L-carnitine was ineffective for reducing body fat in rainbow trout. Ji *et al.* (1996) found that the Atlantic salmon fed with carnitine exhibited a decrease in the lipid content in white muscle and viscera by as much as 73 and 43%, respectively. The absence of a lipotropic action of L-carnitine feeding had also been reported in red sea bream (Chatzifotis *et al.*, 1995), hybrid tilapia (Becker *et al.*, 1999), and hybrid striped bass, *Morone saxatilis* male × *M. chrysops* female (Twibell & Brown, 2000). Whether such variability in terms of the body lipid lowering effect of supplemental L-carnitine is associated with dietary lipid level (and indirectly to the energy density of the diet) remains unclear. A more detailed study of the fat composition, i.e. long-chain, medium-chain and short-chain fatty acids values would be advantageous.

Application of high doses of L-carnitine exerts extra production costs and therefore must be calculated properly. According to our calculations, despite the high price of L-carnitine, the farmer would still benefit from supplementing the diet with L-carnitine. The proper dosage of the L-carnitine depends on several integrated or separate factors. In addition to dietary factors (i.e., energy, lysine and L-carnitine dietary levels) and possible species differences, the effectiveness of dietary L-carnitine supplements is also affected by variations in biochemical, metabolic and physiological activities, as in the case of the distinct life stages of fish. Interestingly, the level of supplementary L-carnitine, which exhibited a

positive growth enhancing effect in some fish species, was found to be limited to a certain concentration, out of the range tested, while the other concentrations tested did not show such positive results (Torreele *et al.*, 1993; Chatzifotis *et al.*, 1995; Becker *et al.*, 1999).

In conclusion, the results of this study indicated that supplementation of diets with 300-600mg carnitine kg^{-1} improved growth rate, feed utilization and stimulated protein-sparing effect in juvenile beluga under intensive culture conditions, but very little is known about the long-run practical effects of feeding L-carnitine to beluga sturgeon, which should be pursued in the future research.

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