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RESEARCH ARTICLE

Dietary vitamin E requirements and growth performance of young-of-the-year beluga, *Huso huso* (L.) (Chondrostei: Acipenseridae)

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Abstract. An eight-week feeding trial was conducted using a flow-though system to determine the dietary requirements for vitamin E of young-of-the-year (YOY) beluga, *Huso huso* (L.), in relation to growth performance. Six diets, including the control diet (basal diet without vitamin E supplementation) and five diets supplemented with 25, 50, 100, 200, and 400 mg vitamin E (Dl-all-rac-α-tocopherol acetate) kg^{-1}, were fed to triplicate groups of fish (mean initial body weight 49.7 ± 0.1 g). At the end of the trial, growth factors such as final body weight, weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), daily growth rate (DGR), and survival rate were determined. The values of WG, SGR, and DGR in the fish fed diet not supplemented with vitamin E were significantly lower than those of the fish fed the five supplemented diets. The final body weight and PER in fish fed diet not supplemented with vitamin E were significantly lower than fish fed diets supplemented with 25, 50, 100, and 200 mg vitamin E kg $diet^{-1}$, but were similar in the control group and the group fed feed with 400 mg vitamin E kg $diet^{-1}$. Moreover, the FCR in the fish fed the diet that was not supplemented with vitamin E was significantly higher compared to other groups. Based on observations, no mortality or any signs of deficiency were observed among the fish fed the control and other diets. Based on broken-line analysis, the dietary vitamin E requirement is in the range of 26.6-29.6 mg kg^{-1} dry diet based on the maximum values of WG, SGR, and minimum FCR, and can be added to the diet for the improved growth of this species.

Keywords: sturgeon, growth, requirement, vitamin E

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Introduction

Vitamins are organic substances that are essential for growth and maintenance in animals, but are required in small amounts. Since fish cannot synthesize vitamins at all or can only synthesize them in insufficient quantities for normal development, growth and maintenance, they must be supplied by the diet (Falahatkar et al. 2011).

Unlike some vitamins, which consist of a single compound, vitamin E consists of eight different compounds, four tocopherols and four tocotrienols (designated as alpha, beta, gamma, and delta). Vitamins E (α-tocopherol) and C (ascorbic acid) are among the most important nutrients influencing the immune system, and the supply of them can reduce fish mortality and improve growth performance (Montero et al. 2001, Shiau and Hsu 2002). Vitamin E functions as a lipid-soluble antioxidant, protecting biological membranes, lipoproteins, and lipid stores against oxidation (Lee and Shiau 2004). The antioxidative functions of vitamin E include scavenging of free radicals to terminate lipid peroxidation, which can initiate damage to unstable intracellular components including membranes, nucleic acids and enzymes, thereby result in pathological conditions and indirectly result in reduced growth (Agradi et al. 1993, Paul et al. 2004). Lipid peroxidation in body tissues decreases when dietary vitamin E increases (Huang et al. 2004).

In comparison to the information available on the vitamin requirements of terrestrial animals, aquatic species still lacks depth. However, the dietary requirement of vitamin E has been demonstrated in a number of fish, such as 28 mg kg^{-1} for hybrid striped bass, *Morone chrysops* ♀ × *M. saxatilis* ♂ (Kocabas and Gatlin 1999), 30-50 mg kg^{-1} for channel catfish, *Ictalurus punctatus* (Raf.) (Murai and Andrews 1974, Wilson et al. 1984), 99 mg kg^{-1} for mrigal, *Cirrhinus mrigala* (Bloch) (Paul et al. 2004), 120 mg kg^{-1} for Atlantic salmon, *Salmo salar* L. (Hamre and Lie 1995), 131.9 mg kg^{-1} for rohu, *Labeo rohita* (Hamilton) (Sau et al. 2004), and 200-300 mg kg^{-1} for common carp, *Cyprinus carpio* L. (Watanabe et al. 1977).

Sturgeon species are valuable and many of then are currently highly endangered (Sturgeon Specialist Group 2012). Rearing these species has progressed considerably in recent years. One of the highest growth rates among the sturgeon is noted for beluga, *Huso huso* (L.), and it appears to be very suitable for aquaculture (Falahatkar et al. 2006). Despite the economic importance of sturgeon, there is little information about its nutrient requirements, but some studies on vitamin requirements in these species are available (Moreau et al. 1999a,1999b, Moreau and Dabrowski 2003, Falahatkar et al. 2006, Wen et al. 2008, Tatina et al. 2010).

Therefore, the objectives of the present study were to the determine dietary requirements for vitamin E and the effect of dietary vitamin E on the growth factors of beluga, which is a valuable species for the production of meat and caviar, and a good candidate species for sturgeon aquaculture.

Materials and methods

Experimental diet

The composition of the basal diet and proximate analysis are presented in Tables 1 and 2, respectively. Graded levels of Dl-all-rac-α-tocopherol acetate (Sigma Chemical Co., Steinheim, Germany) were added to the basal diet at the expense of small amounts of soybean oil to provide the desired levels of 0, 25, 50, 100, 200 and 400 mg kg^{-1} diet. Vitamin E supplementation plus soybean oil was equal to 0.1% of the diet and only soybean oil was added in the control diet in the amount of 0.1%. The basal diet without the addition of Dl-all-rac-α-tocopherol acetate is referred to as the control diet.

Dl-all-rac-α-tocopherol acetate was chosen as the source of vitamin E because of its stability and bioavailability to fish (NRC 1993). The vitamin premix did not contain vitamin E and was provided according to the nutritional requirements of the fish for this experiment (NRC 1993). The ingredients were ground into a fine powder through a 1 mm mesh. All of the ingredients were mixed thoroughly and then pelleted into 3-mm diameter strand with an experimental feed mill and dried in a warm air cabinet (40°C, 48 h). The diets were stored in a freezer at -17°C until feeding. At the time of feeding, the strands were broken into the appropriate size before they were fed to the fish. The analysis of dry matter (at 105°C for 24 h), crude protein (Kjeldahl apparatus, nitrogen × 6.25), crude fat (extraction with petroleum ether by Soxhlet apparatus), ash (incineration at 600°C for 6 h), and energy (bomb calorimeter) were performed to assess the proximate composition of the diets (AOAC 1995).

Fish and experimental conditions

Young-of-the-year (YOY) belugas were obtained from the Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Complex in Guilan, Iran, where they had been hatched and reared. Based on our observations (but not immunological analysis), the experimental fish had no clinical signs of disease before

Table 1
Main ingredients in the experimental diet

Ingredients	Diet (g kg^{-1} dry weight)
Fish meal	610.0
Meat powder	60.0
Wheat meal	100.0
Soybean cake	50.0
Fish oil	45.0
Soybean oil	45.0
Lecithin	30.0
Beet molasses	15.0
Vitamin mixture[1]	25.0
Mineral mixture[2]	15.0
Salt	4.0
Vitamin E and carrier[3]	1.0

[1]Vitamin mixture was manually provided according to feed requirements of the fish (NRC 1993) and ingredients were obtained from Science Laboratories (Qazvin, Iran); which each 1000 g vitamin mixture provides vitamin A, 1 600 000 I.U; vitamin D_3, 400 000 I.U; thiamin, 6 g; riboflavin, 8 g; niacin, 12 g; pantothenic acid, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; vitamin C, 60 g; vitamin K_3, 2 g; biotin, 240 mg and inositol, 20 g

[2]Aquatic mineral premix, for cold and warm water fish, is manufactured by Science Laboratories (Qazvin, Iran); which each 1000 g contains mineral trace elements including ferrous, 6 g; zinc, 10 g; selenium, 20 mg; cobalt, 100 mg; copper, 600 mg; magnesium, 5 g; iodine, 600 mg; choline chloride, 6 g

[3]Supplemented as Dl-all-rac-α-tocopherol acetate (Sigma Chemical Co. Steinheim, Germany), purity ≥ 96% and mixed with soybean oil to 0.1 percent of diet

Table 2
Proximate compositions in the experimental diet

Ingredients	Diet (% dry weight)
Crude protein	46.18
Crude lipid	19.64
Moisture	10.25
Ash	11.50
Crude energy (kcal kg^{-1})	3021

stocking. Prior to the beginning of the experiment, the fish were acclimated to experimental conditions for two weeks in six separate 785 l circular concrete tanks and fed the diet without vitamin E supplementation (basal diet) to acclimatize them to the experimental feed. At the beginning of the experiment, 360 fish with a mean weight of 49.7 ± 0.1 g (mean ± SE) were stocked randomly into eighteen 785 l circular concrete tanks (20 fish in each tank with a near uniform biomass of 994.0 ± 2.8 g $tank^{-1}$) with a flow rate of 13.1 ± 0.9 l min^{-1} for a period of eight weeks. The six dietary treatment groups of fish, each in three replicates (n = 3), were hand-fed four times daily (06:00, 12:00, 18:00, 24:00) to satiation.

During the experimental period, the water temperature, dissolved oxygen content, pH, and nitrite were 16.8 ± 0.5°C, 6.8 ± 0.1 mg l^{-1}, 8.1 ± 0.1 and 0.035 ± 0.002 mg l^{-1}, respectively. The tanks were located indoors, and the light cycle was 12 h/12 h light/dark. The water used, which originated from Sefidrood River, was supplied through a flow-through system and inlet water was filtered with a sand filter. All the fish from each tank were counted and weighed at two weeks intervals, at least 12 h after the last feeding. Mortality, general health, abnormal swimming behavior, and specific signs of vitamin-E-deficiency, if noted, were recorded.

Growth data analysis

Every two weeks, all of the fish from each tank were counted and weighed to monitor the growth rate. Growth parameters and feed utilization rates such as weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), daily growth rate (DGR), and survival rate were calculated according to Imsland et al. (2006) and Abdel-Hakim et al. (2009) as follows:

$$\text{WG (g)} = W_f - W_i$$

$$\text{FCR} = \text{DFI (g) WG (g)}^{-1}$$

$$\text{PER} = \text{WG (g) TFPI (g)}^{-1}$$

$$\text{SGR (\% day}^{-1}) = 100 \times (\ln W_f - \ln W_i)\, t^{-1}$$

$$\text{DGR (g day}^{-1}) = \text{WG (g)}\, t^{-1}$$

where W_f and W_i were the final and initial fish weights, respectively; DFI is dry feed intake; TFPI is total feed protein intake, and t is experiment duration in day.

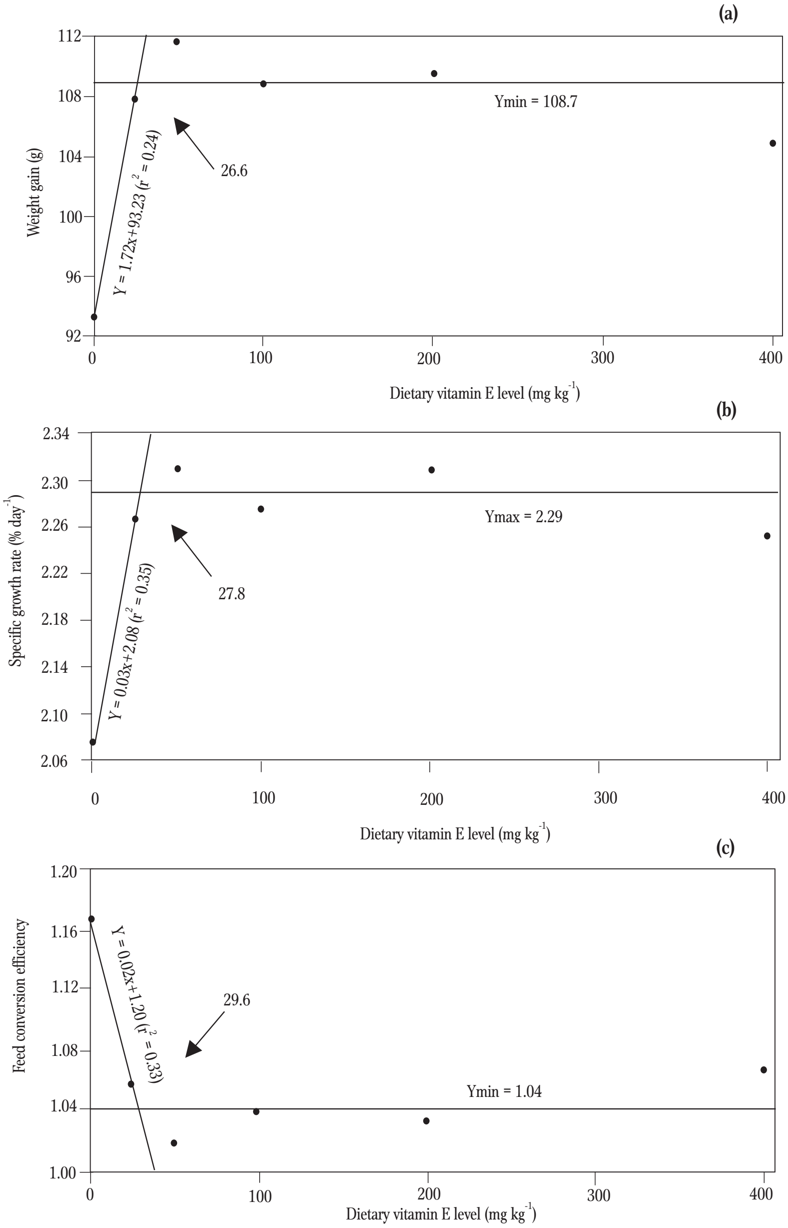

Figure 1. Broken-line analysis of weight gain (a), specific growth rate (b), and feed conversion ratio (c) on concentrations of dietary vitamin E indicates that the optimal vitamin E levels for maximal growth of YOY beluga, *H. huso*, are 26.6, 27.8 and 29.6 mg kg diet^{-1}, respectively. Each point represents the mean of three groups of fish with 20 fish per group (mean ± SE, n=3).

Statistical analysis and model evaluation

The Kolmogorov-Smirnov test was applied first to check the normality of the data. One-way analysis of variance (ANOVA), where appropriate, using SPSS 13.0 (Chicago, IL) was applied to study the effect of dietary levels of vitamin E on growth factors. All percentage data were transformed to arc-sin prior to statistical analysis and then ANOVA was applied. When significant differences were found, the means among treatments were compared using Tukey's test with a 95% level of significance. The broken-line regression method (Robbins et al. 2006) was used to determine the breakpoint that represents the dietary vitamin E requirement of YOY beluga based on maximum WG, SGR, and minimum FCR. All assays were performed in triplicate and the data are presented as mean ± SE for each dietary group.

Table 3
Growth factors of YOY beluga *H. huso* fed diets supplemented with different vitamin E levels for eight weeks

	Dietary treatments[1]					
Parameters	0	25	50	100	200	400
Initial body weight (g)	49.5 ± 0.4	49.5 ± 0.4	49.6 ± 0.3	49.7 ± 0.4	50 ± 0.4	49.9 ± 0.3
Final body weight (g)	142.4 ± 3.1[b]	156.9 ± 1.5[a]	160.9 ± 2.3[a]	158.1 ± 1.4[a]	159.1 ± 3.5[a]	154.4 ± 2.8[ab]
Wight gain WG (g)	92.9 ± 3.0[b]	107.4 ± 1.2[a]	111.3 ± 1.8[a]	108.4 ± 0.9[a]	109.1 ± 3.4[a]	104.5 ± 2.8[a]
Feed conversion ratio FCR	1.17 ± 0.03[a]	1.05 ± 0.00[b]	1.02± 0.01[b]	1.04 ± 0.01[b]	1.05 ± 0.02[b]	1.09 ± 0.02[b]
Protein efficiency ratio PER	2.06 ± 0.05[b]	2.29 ± 0.01[a]	2.36 ± 0.02[a]	2.32 ± 0.01[a]	2.30 ± 0.05[a]	2.22 ± 0.05[ab]
Specific growth rate SGR (% day^{-1})	2.07 ± 0.04[b]	2.26 ± 0.01[a]	2.31 ± 0.01[a]	2.27 ± 0.01[a]	2.27 ± 0.04[a]	2.21 ± 0.04[a]
Daily growth rate DGR (g day^{-1})	1.82 ± 0.06[b]	2.11 ± 0.02[a]	2.18 ± 0.04[a]	2.13 ± 0.02[a]	2.14 ± 0.07[a]	2.05 ± 0.05[a]

[1]Six dietary treatments were prepared with the addition of different amounts of vitamin E in the basal diet. Values are mean ± S.E. of triplicate groups of fish, with 60 fish per group. Values in the same row with different superscript letters are significantly different ($P < 0.05$)

Results

Based the observations, no mortality, deficiency, or illness attributable to the treatments were observed over the eight weeks of the experimental period. The growth factors of fish fed the experimental diets for eight weeks are presented in Table 3. The results show that the growth of beluga was significantly affected by dietary vitamin E ($P < 0.05$). Most of growth factors of YOY beluga including final body weight, WG, PER, SGR, and DGR in groups fed diets not supplemented vitamin E were significantly lower than those of the fish fed the other five diets. However, the final body weight and PER in the fish fed the control diet (not supplemented with vitamin E) was not significantly different than the fish fed 400 mg kg^{-1} vitamin E in diet. The highest final body weight, WG, PER, SGR, and DGR of the fish fed different levels of vitamin E were observed in those fed the diet supplemented with 50 mg kg^{-1} vitamin E . The FCR in fish fed the control diet was significantly different from the other treatments. The best and poorest FCR were observed in the diet supplemented with 50 mg kg^{-1} vitamin E, and in the fish fed the diet without vitamin E supplementation, respectively.

The dietary requirement based on WG, SGR, and FCR, analyzed with broken-line regression, indicated that the dietary vitamin E requirement is 26.6 ± 5.8, 27.8 ± 3.1 and 29.6 ± 3.6 mg kg^{-1}, respectively (Fig. 1). The results show that the requirement of dietary vitamin E for optimal growth in YOY beluga is in the range of 26.6-29.6 mg kg^{-1}.

Discussion

Different dietary levels of vitamin E had no impact on fish mortality, which was likely because of the high resistance of beluga and the likelihood of vitamin E content in ingredients, and a reduction in dietary levels of vitamin E alone cannot result in fish mortality in this species after a short period of eight weeks. This is confirmed by the results of Moreau and Dabrowski (2003) who observed that dietary vitamin E has no significant effect on the survival of white sturgeon, *Acipenser transmontanus* Richardson, with body weights of 3-5 g after eight weeks of an experiment. Both that and the present results indicate that sturgeon tolerate vitamin E deficiency in the short term without any mortality or signs of deficiency. Similar results have been reported for teleost fish. Montero et al. (2001) observed no effect in the mortality of sea bream, *Sparus aurata* L., juveniles (initial weight of 22 g) fed a diet not supplemented with vitamin E for 15 weeks when compared with fish fed 150 mg kg^{-1} of α-tocopherol acetate with the diet under stressed conditions. However, the survival rate was lower in sea bream fed with a diet supplemented with 0 mg vitamin E kg^{-1} compared to 1000 mg kg^{-1} after eight weeks of the experiment (Tocher et al. 2002).

The present study clearly demonstrates that dietary vitamin E is essential for normal growth in beluga. This vitamin could be involved indirectly in modulating growth by its effect on immunological factors. All Adriatic sturgeon, *Acipenser naccarii* Bonaparte, juveniles (initial weight 74.6 g) exhibited significant increases in weight and length when fed the diet supplemented with dietary vitamin E as compared to the fish fed the diet that was not supplemented (Agradi et al. 1993). Moreover, similar results have been reported for hybrid striped bass juveniles (Kocabas and Gatlin 1999), sea bream juveniles (Tocher et al. 2002), rohu fry (Sau et al. 2004), and mrigal fry (Paul et al. 2004). However, other studies have demonstrated that diets deficient in vitamin E have no significant impact on the growth of different species, such as sea bream juveniles (Montero et al. 2001), juveniles turbot, *Scophthalmus maximus* (L.), juveniles halibut, *Hippoglossus hippoglossus* (L.), (Tocher et al. 2002), three-month old white sturgeon (Moreau and Dabrowski 2003), and juveniles coho salmon, *Oncorhynchus kisutch* (Walbaum) (Huang et al. 2004). Differences in diet formulation, such as type and quantity of oil used, species and fish size, could have resulted in the discrepancies observed in this trial as compared to earlier studies. It has been shown that high levels of dietary lipids, especially those rich in polyunsaturated fatty acids, increase the susceptibility of diets to autoxidation and tissue lipid peroxidation, which have been shown to be responsible for detrimental changes in fatty acids composition of tissues (Lim et al. 2009). The tissue accumulation of oxidized breakdown products of lipids can have deleterious consequences for cell and organ functions, as well as lead to the depletion of tissue vitamin E concentrations (Tocher et al. 2002). A dietary lipid level in excess of 12% depressed growth in juvenile *O. aureus* × *O. niloticus* hybrids (Jauncey 2000). However, Hung et al. (1997) demonstrated that sub-yearlings white sturgeon can use salmon diets with high lipid contents (25.8–35.7%), and that they display fast growth and good feed utilization. In the present experiment, dietary lipid was 19.6% and this can affect growth performance and the dietary requirements of fish. Future studies are required to detect dietary vitamin E requirement at different levels of dietary lipids.

Based on broken-line analysis of the weight gain, feed conversion ratio, and specific growth rate data, it was concluded that the dietary requirement of YOY beluga is 26.6-29.6 mg vitamin E kg^{-1} diet. This value is in general agreement with vitamin E requirements for channel catfish (Wilson et al. 1984, Lovell et al. 1984), rainbow trout, *Oncorhynchus mykiss* (Walbaum), (Hung et al. 1980, Cowey et al. 1983), Korean rockfish, *Sebastes schlegeli* Hilgendorf, (Bai and Lee 1998), hybrid tilapia (Shiau and Shiau 2001), and Atlantic salmon (Hamre and Lie 1995) that were reported as 25-50, 25-50 45, 42-66, and 60 mg vitamin E kg^{-1} diet, respectively. However, higher requirements for this vitamin have been noted in mrigal (Paul et al. 2004), rohu (Sau et al. 2004), catla, *Catla catla* Hamilton (Sinha and Sinha 1994), and common carp (Watanabe et al. 1977) that were 99, 131.91, 150, and 200-300 mg kg^{-1} of diet, respectively.

Requirements of dietary vitamin E is sometimes related to the presence and amounts of other dietary nutrients; such as selenium (Poston et al. 1976), linoleic acid (Watanabe et al. 1977), vitamin C (Shiau and Hsu 2002), and specially dietary lipids (Lin and Shiau 2005). The dietary vitamin E requirement of blue tilapia, *Oreochromis aureus* (Steindachner), was estimated at 10 and 25 mg kg^{-1} Dl-α-tocopheryl acetate of diets with 3% and 6% lipid contents, respectively (Roem et al. 1990). Meanwhile, optimum dietary vitamin E requirements of juvenile grouper were 61-68 and 104-115 mg kg^{-1} of diet with 4 and 9% lipid contents, respectively (Lin and Shiau 2005). Similar results were noted in juvenile hybrid tilapia (Shiau and Shiau 2001), carp (Watanabe et al. 1981, Schwarz et al. 1988), and Atlantic salmon (Lall et al. 1988). Dietary polyunsaturated fatty acid content is one of the most important effective factors on dietary vitamin E requirements in fish, which means the elevation of unsaturated fatty acids can result in increased requirements for vitamin E as intracellular antioxidants to protect the cells (Halliwell and Chirico 1993, Lim and Webster 2006).

Conclusions

From the results of present study, it can be concluded that vitamin E has a direct impact on growth performance, and that supplementing beluga feed with vitamin E is necessary to prevent deteriorating growth. The dietary requirements of vitamin E in YOY beluga is in the range of 26.6-29.6 mg kg^{-1} under the present experimental conditions and with levels of 19.6% crude lipids in the diet. However, a number of factors, including dietary levels of unsaturated fatty acids, vitamin C, and selenium, as well as the age of fish, species-specific responses, and other conditions can influence the requirement for dietary vitamin E in this species. Further studies are needed to find the interaction between ingredients and rearing conditions.

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