Iranian Iranian Journal of Fisheries Sciences 21(1) 288-300 2022
DOI: 10.22092/ijfs.2022.125947

Research Article

Effects of turmeric (*Curcuma longa)* on growth parameters and expression of growth–related genes (GH and IGF) in juvenile sevruga (*Acipenser stellatus*)

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Received: December 2020 Accepted: December 2021

Abstract

Considering development of dense aquaculture systems and necessity of using herbs as growth stimulants, the present investigation aimed to explore the effect of different levels of turmeric feed supplement on growth indicators and expression of some growth genes in juvenile sevruga (*Acipenser stellatus*). Duration of the experiment was 60 days. For this purpose, 120 fish were distributed in to 12 fiberglass tanks (2000L) with average weight of 45±0.5 g and length of 26±0.5 cm. The fish were fed in 4 groups with 4 experimental diets containing different levels of turmeric with 0, 0.5, 1 and 2% formulations. At the end of the trial (60 day), growth indicators and expression of growth genes were evaluated. The results revealed that with increasing dosage of turmeric at concentration of 2%, dietary growth index increased significantly (p<0.05). Also, dose elevation of 2% turmeric in the diet increased expression of growth genes (GH, IGF), though no significant difference was observed among treatments (p>0.05). According to the results of this study, use of 2% turmeric powder in the diet is suggested to improve growth indicators and expression of growth genes in juvenile sevruga (*Acipenser stellatus*).

Keywords: Juvenile sevruga (*Acipenser stellatus*), Turmeric, Growth performance, Growth genes

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Introduction

Expansion of aquaculture has made it an important source of protein worldwide (Sánchez-Martínez *et al.*, 2015). Aquaculture industry is grown dramatically due to declining catches in the oceans, increased market demand, increased human population, and need for healthy human protein (FAO, 2018). Sevruga (*Acipenser stellatus* Pallas, 1771) is an important species of sturgeon that has a significant population in Caspian Sea. Advantages of this species over most sturgeon species, include high quality of meat, excellent marketability, shorter time to reach maturity and produce caviar, as well as higher rate of extracted caviar in relationship with body weight. Due to its early maturity, it is capable of producing caviar at an earlier age than species such as Russian and Persian sturgeons, so it is suitable for caviar production in breeding systems (Norouzi *et al.*, 2008). Use of some dietary supplements can improve the growth performance (Javed *et al.*, 2009). Medicinal herbs with active ingredients can be added to fish feed as growth stimulants; with aromatic substances or as digestive stimulants, they can be absorbed causing change in the nutritional pattern, as well as secretion of gastrointestinal fluids, bile, mucus and eventually more food consumption (Ganguly *et al.*, 2010).

Turmeric (*Curcuma longa)* belongs to Zingiberaceae family, and is a herbaceous and perennial plant used in food and pharmaceutical industries. It is native to warm Asian regions (Keys, 1976). Turmeric yellowness is due to presence of compounds, such as curcumin, methoxy base curcumin and methoxy curcumin (Boon and Wong, 2004). Numerous studies reported different effects of this plant on humans and other organisms, including antioxidant, antibacterial (Negi *et al.*, 1999; Masuda *et al.*, 2002), anti-inflammatory and anti-cancer (Duvoix *et al.*, 2005), antimicrobial, and anti-diabetic activity (Saravanan and Pari, 2005). In aquatic life, there have also been various studies on effects of edible turmeric supplementation on fish diets, which in many cases indicate increased growth rate (Saho *et al.*, 2008; Chen *et al.*, 2016; Midhun *et al.*, 2016; Sodamola *et al.*, 2016; Abdel-tawwab and Abbass, 2017; Mahmoud *et al.*, 2017; Nascimento *et al.*, 2019). There are several studies in World Gene Bank on sequencing or gene expression of GH and IGF of different species, including *Oncorhynchus mykiss* (Shamblott and Chen, 1992) and *Cyprinus carpio* (Liang *et al.*, 1996). However, very limited information is published on sturgeons, with the exception of *Acipenser ruthenus* (Wuertz *et al.*, 2007). Thus, given the importance of GH and IGF in various physiological processes, such as sexual maturation and growth, caviar production, and regulation of osmotic pressure, access to such information can provide better understanding of physiological state of growth and reproduction in *A. stellatus.*

Materials and methods

Fish husbandry, diet preparation and feeding experiment

This study was carried out in fall and winter of 2019 on a private fish farm of Mr. Eslami in Hossein Abad, Sari, Iran, for 60 days. Pure turmeric rhizomes were purchased from local market in Noor, Iran. The rhizomes were oven dried at 70°C for 24 hours and then powdered using a mill. The powder was sifted using a 30-mesh sieve to obtain a uniform particle powder. In order to prepare the ration, the required components of the ration were prepared (Table 1).

Table 1: Components of rations used in experimental diets.

Ingredients (g)	Groups			
	Control	T_1	T_2	T_3
Fish meal	32	32	32	32
Soybean meal	21	21	21	21
Meat meal	16	16	16	16
Wheat flour	24.8	24.8	24.8	24.8
Fish oil	4	4	4	4
Vitamin premix	0.045	0.045	0.045	0.045
Cellulose	0	1	1.5	2
Turmeric	0.5	1	2	0
Choline	0.155	0.155	0.155	0.155

Diets were supplemented with 0.5 (T1), 1 (T2) and 2% (T3) turmeric powder.

After sifting the required amount for each food item, they were weighed by a digital balance with accuracy of 0.01 g and added to a plastic pan. After mixing the materials inside pelletizing machine the made pellets were kept for 24 hours indoor for drying. Once dried, the pellets were packed in suitable plastic coatings and stored in freezer until use at -18°C. The required amount of food was withdrawn daily from the freezer and consumed. The diet was manually prepared with a pellet diameter of 2 mm. Chemical analysis and determination of percentages of these feed components were performed in laboratory of Caspian Ecology Research Institute in Sari, Iran.

Growth performance

Feeding was done in three daily meal times of morning, noon, and evening. Average weight of juveniles in each pond was measured once every 2 weeks to determine live mass of the ponds and to calculate the amount of feed. Amount of consumed feed was determined from live mass of each pond according to fish feeding tables (Chebanov and Galich, 2011). Growth parameters were measured according to the following equations (Tacon *et al.*, 2002):

Weight gain (g) = final body weight (g) –initial body weight (g);
Length gain (cm) = final body length (cm) – initial body length (cm);
FCR (feed conversion ratio) = feed intake (g) / weight gain (g);
SGR (specific growth rate) = (Ln final mean body weight – Ln initial mean body weight) / time periods (days) × 100;

K (condition factor) = (fish mass / fish total length^3) × 100;
BWI (body weight index) = mean final body weight (g) – mean initial body weight (g);
PBWI (percent body weight index) = mean final body weight (g) – mean initial body weight (g) × 100.

Gene expression

Five fish specimens were randomly captured from each tank and transferred to anesthetizing solution containing 150 mg/L clove powder. The fish were dissected on ice to collect their brain, kidney, and intestine tissues. The samples were immedietly transferred into liquid nitrogen and stored at -75°C until RNA extraction.

RNA extraction and cDNA synthesis

Total RNA was extracted from the brain, kidney, and intestine tissues of each sample using Gene Roll's RiboExTM Total RNA isolation solution kit with catalog number 001-301 made in South Korea. RNA extraction was performed according to manufacturer's instructions (Wang *et al.*, 2009). The isolated RNA was quantified by a spectrophotometer (NanoDrop device (USA, Thermo scientific, ND-1000) at 260/280 nm. Quality of the RNA was determined through electrophoresis on 1 % agarose gel stained with ethidium bromide. cDNA was synthesized using Bioneer AccuPower® CycleScript RT PreMix (dN6) reverse transcription kit with catalog number 2046-k made in USA. Real-time PCR reactions were run in triplicate using standard protocol (initial denaturation at 95°C for 10 min, 40 cycles of denaturation, annealing and extension at 95°C for 15 s) (Kolangi Miandare *et al.*, 2013).

Fish carcass analysis

At the end of the two-month feeding period, protein, fat, carbohydrate, and ash levels were measured to evaluate changes in tissue composition in different treatments. Three fish specimens were randomly selected from each replication, with carcass analysis performed according to AOAC (1995) method. Protein levels were measured by Kjeldahl method and fat by Soxhlet extraction and solvent ether. Humidity was determined by placing the samples in an oven at 105°C and weighing them after drying in a desiccator. The ashes of the samples were measured by burning the samples in a furnace at 550°C for 5 hours (AOAC, 1995).

Statistical Analysis

Data analysis in this study was performed using SPSS software version 23. One-way ANOVA and comparison of mean treatments by Duncan test were used to analyze the data. All statistical analyzes were performed at significant level of $p<0.5$ and mean data were presented with standard deviation (SD).

Results

Results of the data related to growth indicators are reported in Table 2. The results showed significant difference in mean values of weight and length among different experimental treatments ($p<0.05$). Comparison of mean weight in

different treatments of the experiment showed that with increasing dose, weight increased significantly (p<0.05), such that the highest weight was related to treatment 3. Also, the results of comparing mean length in different treatments indicated that with elevating dose, length increased significantly (p<0.05) with the highest value observed in treatment 3. Comparison of mean growth factors indicated that with increasing turmeric dose, FCR diminished, while PBWI, BWI, and SGR increased but the difference among treatments was not significant (p>0.05). Finally, the results related to K indicated that the amount of the intended factor increased significantly with elevating dose (p<0.05).

Table 2: Mean growth performance values of juvenile sevruga fed different doses of turmeric for 60 days (± standard deviation, sample size of each treatment was 3 replicates×12=36).

Parameters	Control	T_1	T_2	T_3
Weight (g)	54.00 ± 3.46 ^{a}	61.00 ± 3.60^{b}	65.33 ± 2.51^{ab}	69.66 ± 1.52 ^{c}
Length (cm)	31.63 ± 0.90 ^{a}	31.83 ± 0.20 ^{ab}	33.03 ± 0.20^{b}	33.30 ± 0.100 ^{bc}
FCR (%)	2.23 ± 0.78 ^{a}	2.10 ± 0.51^{b}	1.99 ± 0.30^{c}	1.68 ± 0.47^{d}
PBWI (%)	144.1 ± 5.85^{b}	144.01 ± 5.85^{b}	147.79 ±8.15^{c}	160.71 ± 1.07^{b}
BWI (g)	67.66 ± 4.58^{c}	67.66 ± 4.58^{c}	69.00 ± 4.58^{c}	75.00 ± 1.100 ^{bc}
SGR (%)	1.48 ± 0.40^{b}	1.48 ± 0.40^{b}	1.51 ± 0.05^{c}	1.59 ± 0.55 ^{bc}
CF (%)	0.27± 0.100^{ab}	0.27 ± 0.100^{ab}	0.29 ± 0.15^{ab}	0.30 ± 0.15^{b}

Diets were supplemented with 0.5 (T1), 1 (T2) and 2% (T3) turmeric powder. Different letters in each row indicate significant difference among treatments (p<0.05).

According to the results of this study, mean values (±SD) of carcass analysis parameters in juvenile sevruga (*Acipenser stellatus*) are summarized in Table 3. Values of carcass moisture decreased significantly with increasing dose (p<0.05), the highest amount of moisture was observed in control group and the lowest was in treatment 3. Also, the results of fat evaluation indicated that with raising dose, the amount of fat increased significantly (p<0.05) with the highest level observed in treatment 3. The amount of carcass protein rose significantly with elevating dose (p<0.05), with the highest amount observed in treatment 3 and the lowest in the control group. Mean comparisons among breeding treatments indicated that with incrementing dose, the amount of ash increased where the highest amount was observed in treatment 3 and the lowest in the control group; but there was no significant difference among experimental treatments (p>0.05).

Statistical results of evaluation of gene expression (IGF and GH) in this study indicated that feeding juvenile sevruga (*A. stellatus*) with turmeric powder enhanced expression of both growth-related genes compared to the control group (Fig. 1). Examination of expression pattern of these genes showed dose-dependent ascending pattern with elevation of dose. The amount of these genes increased and maximum was observed in treatment 3 with a dose of 2% turmeric but no significant difference was observed among treatments (p>0.05).

Table 3: Mean carcass analysis values of juvenile sevruga fed different doses of turmeric for 60 days (± standard deviation, sample size of each treatment was 3 replicates×12=36).

Parameters	Control	T_1	T_2	T_3
Moisture (%)	65.72 ± 0.76 [a]	63.89 ± 0.15 [b]	62.25 ± 0.47[c]	62.11 ± 1.74 [c]
Lipid (%)	11.32 ± 0.90 [c]	11.50 ± 0.15 [ab]	11.58 ± 0.03[b]	12.04 ± 0.06 [a]
Protein (%)	20.93 ± 0.33 [a]	22.79 ± 0.16 [b]	23.34± 0.17[b]	23.84 ±1.07[b]
Ash (%)	1.75 ± 0.13[a]	1.95 ± 0.76 [a]	2.11 ±0.06[a]	2.34 ± 0.29[a]

Diets were supplemented with 0.5 (T1), 1 (T2) and 2% (T3) turmeric powder. Different letters in each row indicate significant difference among treatments (p<0.05).

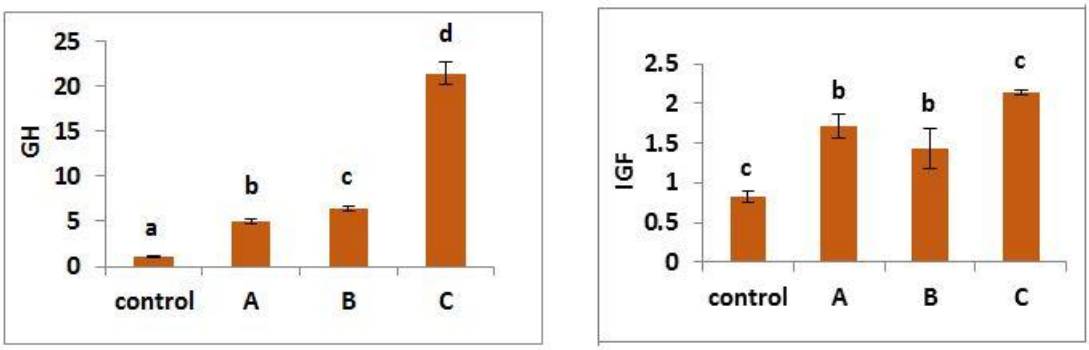

Figure 1: Effects of different levels of turmeric powder in diet of juvenile sevruga (*Acipenser stellatus*) on GH and IGF gene expression. Diets were supplemented with 0.5 (A), 1 (B) and 2% (C) turmeric powder. Error bars demonstrate standard deviation.

Discussion

Aquaculture is grown rapidly in the food production sector and is recognized as an important component in food security sector (Abdel-tawwab and Abbass, 2017). Turmeric is traditionally used as a medicinal plant for centuries due to its antioxidant and anti-inflammatory properties for treatment of different diseases (Nishikawa *et al.*, 2013). Adding turmeric to fish diets may have a positive effect on nutrient digestibility as well as improving nutrient utilization, which in turn can help boost fish farming and feed intake (Jiang *et al.*, 2016). The present study results indicated that addition of turmeric to diet of juvenile sevruga (*Acipenser stellatus)* increased growth performance at concentrations above 2% of diet. Abdel-tawwab and Abbass (2017) showed that use of 2 g/kg turmeric offered most promising results in growth indices in common carp (*Cyprinus carpio*). Sahu *et al.* (2008) reported that use of turmeric at 1 g/kg resulted in the best growth performance in rohu *(Labeo rohita*). Mahmoud *et al.* (2014) showed that use of 0.5% turmeric in diet improved growth indices in Nile tilapia (*Oreochromis niloticus*). Jiang *et al.* (2016) found that the best growth performance was obtained from the activity of intestinal digestive enzymes using 5 g/kg turmeric in Crucian carp (*Carassius auratus*). The results of the present study are consistent with these mentioned findings.

Mahmoud *et al.* (2017) examined daily effect of curcumin on growth of Nile tilapia (*Oreochromis niloticus*) at doses of 50, 150, and 200 mg curcumin per kg in diet. They found that 50 mg/kg curcumin added to diet increased daily growth rate, SGR, and survival rate while

reducing FCR and mortality. Also, results of the study of Jiang *et al.* (2016) who explored the effect of curcumin on growth performance in Crucian carp (*Carassius auratus*) at 3 doses of 0, 1 and 5 g/kg, revealed that with increasing dose, SGR increased causing reduced FE.

Enhanced growth and feed utilization following CUR dietary supplementation could be linked to three main issues. Firstly, CUR is reported to possess properties as digestive enhancer via improving trypsin and lipase activities in hepatopancreas and intestine, as well as amylase activity in hepatopancreas and thus improve growth performance (Liang *et al.*, 2008; Jiang *et al.*, 2016). Secondly, CUR is found to have the ability to boost activity of Na+/K+ -ATPase (NKA), intestinal alkaline phosphatase (AKP), gamma-glutamyl transpeptidase (γ-GT), and creatine kinase (CK). The former enzymes are located in intestinal brush border section and are responsible for final stages of nutrients degradation and assimilation. Consequently, CUR could enhance nutrient utilization to a great extent (Jiang *et al.*, 2014, 2016).

Chemical composition of the body is always affected by diet and even percentage and amount of daily nutrition. Different food compounds have different effects on carcass composition of fish (Hung *et al.*, 1987; Gawlicka *et al.*, 2002). In the present study, chemical composition of carcass analysis of juvenile sevruga (*A. stellatus*) under the influence of different doses of turmeric revealed that with increasing dose of turmeric, amount of protein, fat, and ash increased while the amount of moisture decreased. In case of increased protein, growth-promoting plants can elevate the amount of amino acids by stimulating RNA transcription, which in turn leads to higher protein production (Citarasu, 2010). Regarding the amount of moisture and fat, the results showed that there was a relationship between moisture and fat in the body so that with increasing fat, moisture decreased, which concurs with Wang *et al.* (2006) research. This is because catalyzed fats are replaced by an equal volume of water (Halver and Hardy, 2002). Multiple factors affect the amount of tissue moisture of a species, which depends on species, age, habitat, environmental and seasonal conditions, and even amount of food received as well as percentage of daily feeding (Shalaby *et al.*, 2006; Citarasu, 2010). Regarding the amount of ash, it seems that the minerals in turmeric play an important role in ash content in carcasses of juvenile sevruga (*A. stellatus)*; permanent access to food and absorption of minerals and elements in food affect the amount of carcass ash (Tacon *et al.*, 2002).

Growth hormone, or GH, is a single polypeptide protein hormone with a molecular weight of 21 to 23 kDa which is made by somatotropic cells in anterior pituitary gland (Canosa *et al.*, 2007). This hormone is involved in many physiological processes in living organisms, including body growth, metabolism of carbohydrates, fats, proteins, and energy balance (Parhar *et al.*, 2003). It is controlled by many factors, especially two neuropeptides

secreted by hypothalamus (GHRH) and somatostatin, which inhibit release of growth hormone. Growth stimulation during GH deficiency in humans and animals is well established (Schmid *et al.*, 2003). Study and identification of genes involved in aquatic growth play an important role in aquaculture and development of genetic science, especially during larval development of fish. Indeed, one of the most important indicators for balanced evolution and growth in organisms is growth hormone, which plays an effective role in the growth of body cells, basal metabolism, and osmotic regulation (Riley *et al.*, 2002). Growth hormone gene is used as a natural marker for evolutionary genetic studies of different species of fish due to its conserved sequences, sufficient length and minimal structural similarity (Pinheiro *et al.*, 2008). Accordingly, research on the sequence and structure of growth hormone, in addition to its fundamental and functional importance, useful information can be obtained about evolutionary process of vertebrates (Kocour and Kohlmann, 2011).

Results of the present study showed that addition of turmeric powder to diet of juvenile sevruga (*A. stellatus)* enhanced expression of GH and IGF genes significantly compared to the control group ($p<0.05$). Results of this study concur with results of the following studies. Midhun *et al.* (2016) explored the effect of curcumin on expression of GH, IGF-1, and IGF-2 genes plus digestive enzymes in Mozambique tilapia (*Oreochromis mossambicus*); Sruthi *et al.* (2018) examined the effect of curcumin on expression of GH, IGF-1, IGF-2, and leptin genes along with liver factors. However, results of Chen *et al.* (2016) on *Oreochromis niloticus* are in conflict with findings of the present study. In this study, no significant change was observed in expression of GH and IGF-I genes at 300 mg/g genistein, while among the fish fed with 3000 mg/g genistein expression of GH and IGF-I genes diminished.

Acknowledgments

The authors would like to express their sincere gratitude to the chairman of Eslami Sturgeon Stocks Reproduction Center as well as others who contributed to conduction of this research project.

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