Repeated intraperitoneal injection of ovine growth hormone accelerates growth in sub-yearling Siberian sturgeon *Acipenser baerii*

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**A B S T R A C T**

The role of growth hormone (GH) in chondrosteans is poorly understood, particularly with regard to its effects on growth. In this study, we examined the influence of exogenous GH on growth performance and body composition in juvenile Siberian sturgeon (*Acipenser baerii*). Fish with initial weight of 80.2 ± 0.1 g (mean ± S.E) were injected once every 10 days with either purified ovine GH (oGH) at 1, 2, 4, and 8 μg oGH/g body weight (BW) or with saline over a 50-day period. Treatment with the highest dose of oGH significantly enhanced growth performance (final body weight and length, body weight increase and specific growth rate, SGR). Notably, 8 μg oGH/g BW increased body weight by 33% and SGR by 141% compared to control fish. GH-stimulated (8 μg oGH/g BW) growth was accompanied by increased crude protein content; however, oGH treatment did not affect levels of total protein, total lipid, cholesterol, triglyceride, or glucose in plasma. oGH decreased plasma levels of thyroxine (at 4 μg oGH/g BW), but had no significant effect on plasma levels of triiodothyronine or cortisol compared to controls. These findings indicate that 8 μg oGH/g BW enhances somatic growth and synthesis of body protein in juvenile Siberian sturgeon and demonstrate the feasibility of exogenous oGH treatment in conservation and aquaculture programs for this ancient species.

1. Introduction

One of the key players regulating growth in all vertebrates is growth hormone (GH). Growth hormone was first isolated from the pituitary of mammals as a single chain peptide with 191 amino acids and found to influence growth via insulin-like growth factor-1 (IGF-1) (Wood et al., 2005) secreted from the liver and other tissues. GH is produced primarily in the pituitary under control of hypothalamic factors as well as peripheral factors (Fink, 2012). The production and release of GH from the pituitary of fish, as in mammals, is regulated by stimulatory (e.g., ghrelin, neuropeptide Y, cholecystokinin) and inhibitory (e.g., somatostatin, 5-hydroxytryptamine) hypothalamic factors (Chang and Wong, 2009; Sheridan, 2011, 2021; Reindl and Sheridan, 2012). The secretion of GH also is influenced by several peripheral factors, including leptin, thyroid hormones, corticosteroids and sex steroids, as well as by external environmental factors, including food quantity and quality, temperature, and photoperiod (Mommsen, 2001; Chang and Wong, 2009; Sheridan, 2011, 2021; Reindl and Sheridan, 2012). GH can act as a growth stimulator both directly through its own receptors in some tissues such as kidney, muscle, and intestine (e.g., stimulate amino acid uptake, protein synthesis) and indirectly via inducing the synthesis and secretion of IGFs (Wood et al., 2005; Reindl and Sheridan, 2012; Sheridan, 2021).

Growth hormone has been administrated to various species of fish to enhance growth rate and to optimize production efficiency in husbandry programs. Notably, heterologous GH, either bovine or ovine, has similar efficacy to the homologous protein in many species of fish (McLean and Donaldson, 1993; Silverstein et al., 1999, 2000; Linán-Cabello et al., 2013; Fenn and Small, 2015; Vélez et al., 2019). It is well documented that exogenous GH can improve growth of fish by stimulating appetite, food intake, and food conversion (Silverstein et al., 1999; Lorente et al., 2004; Rasholdt and McLean, 2004; Shepherd et al., 2007). In addition, the effect of GH on food intake can be mediated indirectly by other metabolic changes (e.g., an increase in nutrient utilization affecting hypothalamic regulation of energy balance) (Silverstein et al., 1999). Earlier studies in channel catfish (*Ictalurus punctatus*) indicated that recombinant bovine GH (rbGH) can increase both weight gain and fat...
deposition (Wilson et al., 1988; Silverstein et al., 2000). In another study, the growth rate of catfish was stimulated by GH injection, but no significant effect was observed on proximate composition or food conversion ratio (Peterson et al., 2004). The correlation between GH and somatic growth also has been reported in rainbow trout Oncorhynchus mykiss (Rensholtz and McLean, 2004; Gahr et al., 2008), coho salmon O. kisutch (Raven et al., 2012), and gilthead sea bream Sparus aurata (Velez et al., 2018, 2019). The improvement of production efficiency by GH demonstrates the feasibility of its use in the aquaculture of a wide array of teleosts. However, little is known about the specific effects of GH on growth performance or other physiological responses in sturgeon, which are considered prime candidates for aquaculture because of the high monetary value of flesh and caviar (Falahatkar et al., 2011; Bronzi et al., 2019).

Sturgeon GH was isolated and characterized from the pituitary and displayed structural similarity to GHs of teleosts and tetrapods (Farmer et al., 1981). Russian sturgeon Acipenser gueldenstaedtii possess two distinct GHs (I and II), each consisting of 190 amino acid residues, and both having greater amino acid identity to tetrapods (63–76%) than to teleosts (42–63%) (Yasuda et al., 1992). GH was detected early during embryogenesis in larvae of Chinese sturgeon, A. chinensis (Cao et al., 2011), and appears to be expressed equally in female and male Russian sturgeon ranging 1–5 years old (Yom Din et al., 2008). Expression of the GH gene also was detected in early embryogenesis and in the early life stage of Siberian sturgeon Acipenser baerii (Abdolahnejad et al., 2015). Previous studies on teleosts showed positive effects of exogenous GH treatment on growth performance (Silverstein et al., 2000; Peterson et al., 2004; Fenn and Small, 2015). To date however, there has been only one study in a chondrostean, the strictly freshwater shovelnose sturgeon Scaphirhinchus platorynchus that showed exogenous rbGH increased body weight and body length (Fenn and Small, 2015).

Sturgeon have been shown to be particularly amenable to aquaculture, displaying rapid growth and resistance to disease and disturbance conditions in a culture environment (Poursaied et al., 2012; Falahatkar and Poursaied, 2013). In recent years, Siberian sturgeon have attracted considerable attention due to its rapid growth, tolerance to aquaculture practices, and early maturity that allows farmers to obtain earlier, considerable attention due to its rapid growth and resistance to disease and disturbance conditions in a culture environment (Poursaied et al., 2012; Falahatkar and Poursaied, 2013). In recent years, Siberian sturgeon have attracted considerable attention due to its rapid growth, tolerance to aquaculture practices, and early maturity that allows farmers to obtain earlier, significant improvements in growth performance, body composition, and biochemical parameters of sub-yearling Siberian sturgeon.

2. Materials and methods

2.1. Fish and rearing system

The sub-yearling (F2) Siberian sturgeon used in this study were artificially produced in the Shahid Dr. Beheshti Sturgeon Fishes Restoration and Genetic Conservation Center (Rashid, Guilan, Iran) from cultured broodstock. The fish were acclimated to an artificial diet and then transferred to the aquaculture research laboratory at the Faculty of Natural Resources, University of Guilan (Sowmeh Sara, Guilan). The care and use of animals followed and approved the basic experimental principles by the University of Guilan animal ethics committee with confirmation that the study complies with all regulations for the present experiment. The fish were maintained in 2000-L fiberglass tanks and fed using commercial formulated diet (Faradaneh, Shahkord, Iran; 42% crude protein, 15% crude fat, 2% crude fiber, 9% ash, 10% moisture and 1% phosphorus). Following a 20-day acclimatization period, one hundred and fifty Siberian sturgeon with an average weight and total length of 80.2 ± 0.1 g (mean ± S.E) and 31.7 ± 0.1 cm, respectively, were randomly distributed into fifteen 500-L circular tanks (10 fish per tank; 30 fish per treatment). There were no significant differences in initial wet weight or initial total length among fish in the experimental tanks. The tanks were supplied with well water in a flow-through system at the rate of 7.5 ± 0.3 L/min. Continuous aeration was applied for each tank to maintain dissolved oxygen at 6.5–7.2 mg/L. Photoperiod was 12L:12D and the average water temperature, ammonia, and nitrite levels were 17.6 ± 0.5 °C, 0.01 mg/L and 0.01 mg/L, respectively. Tanks were cleaned when the fish were removed for each 10-day biometric and injection procedure; 20% of the tank volume was removed daily by siphoning each morning before the first feeding.

2.2. Experimental design

In this study, five treatments with three replicates for each treatment were used to assess the effects of oGH on growth of Siberian sturgeon over a 50-day period. At the onset of the study (day 0), fish were anaesthetized with 300 mg/L clove powder extract (Eslamloo et al., 2012), measured, and then injected intraperitoneally (IP) with 0.9% (w/v) saline (sham injected control) or with immuno-affinity purified ovine growth hormone (oGH; NIH National Hormone and Peptide Program; dissolved in 0.9% saline; Harbor-UCLA Research and Education Institute, Dr. Parlow, Torrance, CA, USA) at 1, 2, 4 or 8 μg/g body weight every 10 days for a 50-day period (at days 0, 10, 20, 30, and 40) at a volume of 1 mL/100 g fish. The range of doses used was based on studies in other species (Silverstein et al., 2000; Peterson et al., 2004). Fish were injected IP on the ventral midline after disinfection with alcohol. The injection procedure for each fish lasted less than 10 s. After injection, fish were replaced into their original tank and monitored for recovery. Fish were fed by hand to apparent satiation three times daily at 8:30, 12:00 and 15:30 with the same formulated diet to which they had been acclimatized. Uneaten pellets were removed every morning before the first feeding of fish and dried for calculation of the feed intake in each tank.

2.3. Sampling and analytical procedures

Feeding was suspended 24 h prior to each sampling. On days 10, 20, 30, and 40, the fish were anaesthetized, measured (body weight, total length) individually, and injected with saline or oGH as described above. At the end of the experimental period on day 50, two fish from each tank (6 fish per treatment) were randomly captured and sacrificed by an overdose of clove powder extract (1000 mg/L). Blood samples were taken from the caudal vein before fish was euthanized and then livers were dissected from each fish for calculation of hepatosomatic index (HSI). Sampling time was less than 1 min for each fish.

Growth parameters were calculated according to the following formulas (Falahatkar et al., 2013):

- **Body weight increase (BWI; %)** = \( \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100 \)
- **Specific growth rate for weight (SGRw; %/day)** = \( \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{days}} \)
- **Specific growth rate for length (SGRL; %/day)** = \( \frac{\text{Ln final length} - \text{Ln initial length}}{\text{days}} \)
- **Feed intake (FI; g/fish)** = feed consumed/number of fish
- **Feed conversion ratio (FCR)** = feed intake/weight gain
- **Hepatosomatic index (HSI; %)** = \( \frac{\text{liver weight/body weight}}{100} \)
- **Survival rate (%)** = \( \frac{\text{final fish number}/\text{initial fish number}}{100} \)

At the termination of the experiment, the whole body of each fish (6 fish per treatment) was used for proximate analysis. Briefly, after...
homogenizing the whole body, samples were weighed and dried (105 °C) to determine dry weight. Crude protein was determined from total nitrogen (N × 6.25) following Kjeldhal digestion, crude fat was determined gravimetrically using the Soxhlet method following the extraction of lipids, and ash content was obtained by furnace dry-ashing (AOAC, 1995).

Each blood sample was divided into two aliquots: the first (0.5 mL) was used for hemoglobin determination, and the second (1.5 mL) was centrifuged at 1500 g for 10 min and the plasma was separated and stored at -20 °C for later analyses.

Hemoglobin (Hb) concentration was measured by the cyanmethemoglobin method at a wavelength of 450 nm (Drabkin, 1945). Total protein and total lipid were assayed by the Biuret (Sandnes et al., 1988) and Folch (Parrish, 1999) methods, respectively. Cholesterol (product number: 1500010; detection range 5–500 mg/dL), triglyceride (product number: 1500012; detection range 5–700 mg/dL), and glucose (product number: 1500017; detection range 5–400 mg/dL) levels were determined using commercially available kits (Pars Azmun, Karaj, Iran) (using CHOL oxidase, GPO-PAP, and glucose-oxidase-peroxidase, respectively) following the manufacturer’s instructions. For validation of biochemical and hormone assays, serial dilutions of sturgeon plasma were examined for parallelism with standard curves for each parameter.

Cortisol was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Pomezia, Rome, Italy) according to Poursaeid et al. (2015) and validated for Siberian sturgeon. Briefly, 10 μL of plasma was added to each well and then 200 μL of conjugated enzyme was added and the mixture was shaken well for 15 s and then incubated for 1 h at 37 °C. The wells were washed four times with 300 μL of washing buffer. Two hundred microliters of chromogen solution were added to each well and then incubated in darkness at 37 °C for 15 min. Finally, 100 μL of stop solution was added to each well and the absorbance was read using an ELISA reader (Sirio, Radim, Italy) set to 450 nm wavelengths. Triiodothyronine (T3) and thyroxine (T4) concentrations were measured using available commercial kits (Immuno-tech, Marseille, France) based on radioimmunoassay (Poursaeid et al., 2015) and validated for Siberian sturgeon. In brief, to analyze T3 levels, 25 μL of plasma was added to each well and mixed with 200 μL of 125I-labeled T3. After 15 s of whirling, the mixture was shaken at room temperature for 1 h with a shaker at 400 g and the content was removed by aspiration. Bound radioactivity was determined with a gamma counter (LKB, Finland). The procedure for analyzing T4 was also performed to evaluate the relationship between oGH levels and Aspencser baerii injected every 10 days with different doses of ovine growth hormone (oGH) during 50 days of rearing. Different letters indicate significant differences in an experimental group over time calculated by two-way ANOVA; an asterisk (*) indicates significant differences among treatments for a given date (P < 0.05).

2.4. Statistical analysis

Assumptions for homogeneity of variances and normality of data were first examined by Levene’s test and the Shapiro-Wilk test, respectively. Nested-analysis of variance (ANOVA) was used to determine tank effects among replicates. When no tank effects were found, body characteristics over the course of the 50-day experiment were evaluated by Two-Way ANOVA (with oGH dose and time as main effects). One-Way ANOVA followed by Tukey’s test as post-hoc at the level of P < 0.05 was used to evaluate differences among treatments in measured parameters at the end of experiment. Linear and quadratic regression analyses also were performed to evaluate the relationship between oGH levels and

### Table 1. Growth performance (mean ± SE) of sub-yearling Siberian sturgeon Acipenser baerii injected every 10 days with different doses of ovine growth hormone (oGH) after 50 days of rearing (n = 30 fish for each treatment). Means within treatments not sharing similar superscripts show significant difference at P < 0.05.

| Treatments (μg oGH/g BW) | 0      | 1      | 2      | 4      | 8      |
|-------------------------|--------|--------|--------|--------|--------|
| Initial weight (g)      | 80.5 ± 0.2 | 80.2 ± 0.1 | 80.4 ± 0.1 | 79.9 ± 0.4 | 80.2 ± 0.3 |
| Final weight (g)        | 98.9 ± 3.2<sup>b</sup> | 98.1 ± 3.6<sup>b</sup> | 111.9 ± 4.5<sup>b</sup> | 106.8 ± 7.7<sup>b</sup> | 131.8 ± 7.2<sup>b</sup> |
| Initial length (cm)     | 31.5 ± 0.1  | 31.8 ± 0.1  | 31.6 ± 0.1  | 31.7 ± 0.2  | 31.9 ± 0.1  |
| Final length (cm)       | 34.7 ± 0.4<sup>b</sup> | 34.5 ± 0.6<sup>b</sup> | 35.7 ± 0.5<sup>b</sup> | 35.4 ± 0.4<sup>b</sup> | 37.2 ± 0.4<sup>b</sup> |
| BWI (%)                 | 22.8 ± 3.9<sup>b</sup> | 22.4 ± 4.6<sup>b</sup> | 39.2 ± 5.7<sup>b</sup> | 33.7 ± 8.9<sup>b</sup> | 64.5 ± 8.8<sup>b</sup> |
| SGR<sub>F</sub> (%/day) | 0.4 ± 0.06<sup>b</sup> | 0.4 ± 0.07<sup>b</sup> | 0.66 ± 0.08<sup>b</sup> | 0.57 ± 0.1<sup>b</sup> | 0.99 ± 0.1<sup>b</sup> |
| SGR<sub>L</sub> (%/day) | 0.19 ± 0.02<sup>b</sup> | 0.14 ± 0.04<sup>b</sup> | 0.25 ± 0.03<sup>b</sup> | 0.22 ± 0.02<sup>b</sup> | 0.31 ± 0.02<sup>b</sup> |
| FI (g/fish)             | 15.6 ± 0.8<sup>b</sup> | 16.1 ± 0.7<sup>b</sup> | 25.5 ± 0.2<sup>b</sup> | 25.5 ± 0.2<sup>b</sup> | 41.6 ± 0.2<sup>b</sup> |
| FCR                    | 0.85 ± 0.06 | 0.89 ± 0.06 | 0.81 ± 0.07 | 0.90 ± 0.01 | 0.74 ± 0.07 |
| HSI (%)                | 2.05 ± 0.26 | 2.07 ± 0.41 | 2.19 ± 0.44 | 2.48 ± 0.45 | 2.23 ± 0.26 |
| Survival (%)            | 100       | 100      | 100      | 100      | 100      |

BWI: body weight increase; SGRw: specific growth rate for weight; SGR<sub>L</sub>: specific growth rate for length; FI: feed intake; FCR: food conversion ratio; HSI: hepatosomatic index.
measured parameters. Data in figures and tables are shown as mean ± standard error (SE).

3. Results

3.1. Growth performance

Repeated injection of oGH significantly enhanced growth (Table 1). By the end of the 50-day trial, body length increased by 7.2% (P < 0.05) and body weight increased by 33% (P < 0.05) in fish treated with the highest dose of oGH (8 μg oGH/g BW). An interaction effect of oGH on fish weight and total length was found. Significant differences in weight and length between fish treated with 8 μg oGH/g BW and control fish were found after the third injection (day 30) onwards (Figure 1A and B). BWI, SGRw and SGRr were significantly increased in fish treated with the highest dose of oGH compared to control fish, but no significant differences were found in these parameters between fish injected with 1, 2 or 4 μg oGH/g BW and the control group. Fish treated with 8 μg oGH/g BW showed the highest feed intake. FCR and HSI were not affected by the oGH injections (Table 1). Increases in body weight, body length, BWI, SGRw, and feed intake were linearly and positively correlated with dose of oGH (P < 0.05; Table 2). Survival rate was 100% in all treatments.

3.2. Body composition

Differences in body composition of Siberian sturgeon treated with oGH also were evident. Notably, crude protein content increased nearly 14% (P < 0.05) in fish treated with 8 μg oGH/g BW compared to the control group (P < 0.05). No significant changes in crude fat and dry weight contents were observed (Table 3); however, the lowest and highest levels of ash content was observed in 4 and 8 μg/g oGH, respectively (P < 0.05).

3.3. Biochemical and hormone parameters

Concentrations of Hb were higher than 6 g/dL in all treatments and no significant variations were observed among treatment groups (Figure 2). oGH treatment did not affect levels of total protein, total lipid, cholesterol, triglycerides, or glucose in plasma (Table 4). However, a positive linear relationship between cholesterol levels and the dose of oGH was observed (P < 0.05); however, no significant differences were found between control and other oGH treatments.

4. Discussion

Growth hormone is well known to increase growth rate of teleost fish by increasing feed consumption and improving feed efficiency (McLean and Donaldson, 1993; Silverstein et al., 1999, 2000; Lorente et al., 2004; Peterson et al., 2004; Rasholdt and McLean, 2004; Shepherd et al., 2007; Sheridan, 2011; Fenn and Small, 2015; Velez et al., 2018, 2019). The current study extends our understanding of the effects of GH on growth performance in Chondrosteans and revealed that exogenous oGH improves growth performance of sub-yearling Siberian sturgeon. The effectiveness of the oGH treatment regime was evident despite, due to technical limitations, our inability to measure oGH.

Administration of the highest dose (8 μg oGH/g BW) of oGH resulted in a 33% increase in weight by the end of 50-day experiment. The improved growth performance also was reflected by GH-stimulated increases in both SGR and BWI. These findings are consistent with previous studies in which improvement in growth performance was observed after applying different types of recombinant GH. For example, a single injection of rbGH (2.23 mg/g BW) was effective at stimulating growth in coho salmon (Raven et al., 2012). Similarly, a single injection of 6 mg rbGH/g enhanced growth in gilthead sea bream juveniles (Velez et al., 2019). Biweekly intraperitoneal injection of shoelace sturgeon (Scaphirhynchus platorynchus) with 240 μg rbGH/g BW for 6 weeks induced a significant increase in weight gain, total length and increase in body protein content (Fenn and Small, 2015). A single injection of 6 mg rbGH/g enhanced growth in gilthead sea bream juveniles (Velez et al., 2019). Also, small doses of rbGH (0.1–2.5 μg/g BW) promoted growth of catfish (Wilson et al., 1988; Silverstein et al., 2000). A single injection of Posilac® (10–30 μg/g BW) increased growth performance of rainbow trout (Garber et al., 1995). Posilac® at the dose of 420–4200 μg/g BW also stimulated growth in coho salmon (McLean et al., 1997). With rbGH injection of 100 or 1000 μg/g BW or 1000 μg/g BW Posilac® in tilapia (Oreochromis niloticus), growth was promoted after 4 weekly injections (Leedom et al., 2002), but no growth enhancement was observed in this species when rhGH was administered at 0.1, 1 and 10 μg/g BW weekly injection after 8 weeks. In the above-mentioned studies, rbGH was used rather than oGH as it was used in the present study. Regardless, heterogeneous GH stimulated growth in all these cases, but with varying efficacies, which can be explained not only by the form of GH used, but also by the means of administration, dose, species of study and life history stage, physiologic state, and/or other environmental factors.

Although the highest dose of oGH used in this study (8 μg oGH/g BW) enhanced growth performance of Siberian sturgeon, lower doses failed to have such effect and we do not know the efficacy of higher doses. In previous studies, dose-related effects of exogenous GH treatment on growth have been observed in teleosts. For instance, Leedom et al. (2002) observed growth promotion after injection of 100 or 1000 μg rhGH/g BW in tilapia, whereas lower doses (0.1, 1 and 10 μg rhGH/g BW) had no effect on growth performance. Although circulating levels of GH were not measured in the current study, the lack of significant effects in fish treated with lower doses may be due to the dosage and metabolic clearance of administered GH, as reported previously for teleostean fishes (Garber et al., 1995; McLean et al., 1997; Leedom et al., 2002), as well as to the use of heterologous hormone, which may interact different-ly with the GH receptor than endogenous hormone. In addition, Peterson et al. (2004) injected 0, 30, 60 and 120 μg rbGH/g BW into channel catfish for each 3 weeks in a period of 9-week and found that

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Table 2. Body composition in wet weight (mean ± SE) of sub-yearling Siberian sturgeon Acipenser baerii injected every 10 days with different doses of ovine growth hormone (oGH) after 50 days of rearing (n = 6 fish for each treatment). Data are presented as % of wet-weight basis. Means within treatments not sharing similar superscripts show significant difference at P < 0.05.

| Treatments (μg oGH/g BW) | 0  | 1  | 2  | 4  | 8  |
|-------------------------|----|----|----|----|----|
| Dry weight (%)          | 19.6 ± 0.6 | 21.5 ± 0.3 | 19.9 ± 0.7 | 21.4 ± 0.7 | 19.5 ± 0.4 |
| Crude protein (%)       | 13.6 ± 0.4<sup>b</sup> | 14.3 ± 0.3<sup>ab</sup> | 15.2 ± 0.4<sup>ab</sup> | 13.9 ± 0.4<sup>b</sup> | 15.5 ± 0.1<sup>a</sup> |
| Crude fat (%)           | 5.5 ± 0.4 | 5.8 ± 0.2 | 5.6 ± 0.1 | 5.6 ± 0.1 | 5.3 ± 0.4 |
| Ash (%)                 | 4.7 ± 0.2<sup>ab</sup> | 4.5 ± 0.1<sup>ab</sup> | 4.4 ± 0.1<sup>ab</sup> | 4.2 ± 0.1<sup>b</sup> | 4.9 ± 0.1<sup>a</sup> |
both the frequency and the length of treatment were more important than the concentration of hormone for inducing growth.

The significant increase in feed intake by fish treated with 8 μg oGH/g BW suggests that GH-stimulated growth performance of Siberian sturgeon may, in part, be due to stimulation of appetite. Although the appetite-stimulating effects of GH have been reported in different species of teleosts (Silverstein et al., 2000; Leedom et al., 2002), the mechanism by which this occurs remains unclear. However, increased feed intake in the present study might be explained by metabolic changes such as increased nutrient utilization that feedback on appetite-regulating hypothalamic centers, as suggested previously by Silverstein et al. (2000) for catfish and Shepherd et al. (2007) for rainbow trout, as well as other humoral factors (Sheridan, 2021). GH also has been shown to improve nutrient absorption across intestinal epithelia (Petro-Sakuma et al., 2020). Such an action also may occur in Siberian sturgeon as evidenced by improved FCR in fish treated with the highest dose of GH, similar to what has been reported for some species of teleosts. For example, catfish injected with rbGH showed improved feed efficiency (Wilson et al., 1988; Silverstein et al., 2000); however, no significant changes in FCR or feed intake were reported in the same species by Peterson et al. (2004). The increase in food intake in GH-treated fish has been observed previously and it results from stimulation of the orexigenic center (Shepherd et al., 2007; Sheridan, 2021).

Ovine GH did not significantly affect HSI of Siberian sturgeon. A similar result was reported in catfish (Peterson et al., 2004) and gilthead sea bream treated with rbGH (Vélez et al., 2019). The same result also was noted in different strains of catfish that received rbGH after 9 weeks of rearing (Peterson et al., 2004). It is well known that GH enhances fat utilization for energy, resulting in decreased body fat content (Mommersen, 1998). Moreover, GH-induced lipolysis has been reported for some teleostean fish such as rainbow trout (Garber et al., 1995), catfish (Silverstein et al., 2000), and gilthead sea bream (Vélez et al., 2018, 2019). However, no significant difference was observed in body fat content between control and oGH-treated fish in the current experiment. It seems that oGH, at least in the doses used, has no lipolytic effects on Siberian sturgeon. This conclusion is supported by the lack of significant differences in HSI among experimental groups. Similarly, in shovelnose sturgeon, exogenous GH at a dose of 240 μg/g BW exerted no lipolytic effects (Fenn and Small, 2015). The lack of GH-induced lipolysis was also reported for rbGH-treated catfish (Peterson et al., 2004). In contrast, some studies have shown that GH injection caused accumulation of body fat (Adelman, 1977; Wilson et al., 1988; Wille et al., 2002). These conflicting results suggest that there are species-specific or other differences in the mechanisms regulating energy allocation (Bergan-Roller and Sheridan, 2018).

GH has been shown to have an insulin-like anabolic action and increases the synthesis of protein (Mommensen, 1998; Bergan-Roller and Sheridan, 2018). GH enhances the accumulation of protein in tissues and alters the whole-body composition of various fish (Farmanfarmaian and Sun, 1999; McLean and Devlin, 2000; Moody et al., 2000). Proximate analysis of the whole body of Siberian sturgeon in the present study indicated that oGH-treated fish had higher body protein content than that in the control group. Similarly, higher protein content was observed in turbot (Scophthalmus maximus) fed GH-transgenic algae (Liu et al., 2007) and in rbGH-treated shovelnose sturgeon (Fenn and Small, 2015). The exact mechanism that enhances protein synthesis is not known. However, possible mechanisms include GH-stimulated amino acid transport into cells, decreased catabolism of protein, and increased RNA translation (McLean et al., 1992; Breier, 1999; Farmanfarmaian and Sun, 1999; Walker et al., 2004; Guyton and Hall, 2006). The anabolic effects of GH also may result from increased binding to its cognate receptors and its interaction with other anabolic hormones (Proctor et al., 1998). The greatest ash content was observed in sturgeon injected with the highest oGH. The mechanism of action on ash content remains to be elucidated, but it may result from the effects of GH on cartilage and bone growth (Guyton and Hall, 2006) - an explanation which is consistent with the increased axial growth observed in the higher oGH-treated sturgeon.

Hematological and plasma biochemical indices have a close relationship with environmental and nutritional condition (Ballarin et al., 2004). Hb concentrations were not affected by GH treatment in Siberian sturgeon, suggesting that GH did not influence the hematopoietic tissue to change carrying capacity of oxygen in the blood. Similar results on hematological parameters were observed by Liu et al. (2007) in turbot. There were appreciable but statistically nonsignificant increases in plasma levels of glucose, total protein, total lipid, cholesterol, and triacylglycerides in Siberian sturgeon treated with oGH. Lorente et al. (2004) reported an increase in glucose and lactate levels with a slight variation in free fatty acids in rainbow trout injected by human recombinant GH. Such increases in glucose levels may arise from either decreased glucose uptake by adipose cells and skeletal muscles (Cameron et al., 1987; Sun, 1999; McLean and Devlin, 2000; Moody et al., 2000).

**Table 3.** Biochemical parameters (mean ± SE) in the plasma of sub-yearling Siberian sturgeon *Acipenser baerii* injected every 10 days with different doses of ovine growth hormone (oGH) after 50 days of rearing (n = 6 fish for each treatment). Means within treatments not sharing similar superscripts show significant difference at P < 0.05.

| Treatments (μg oGH/g BW) | 0     | 1     | 2     | 4     | 8     |
|-------------------------|-------|-------|-------|-------|-------|
| Total protein (g/dL)    | 2.8 ± 0.4 | 2.9 ± 0.4 | 3.0 ± 0.5 | 3.0 ± 0.3 | 3.1 ± 0.5 |
| Total lipids (mg/dL)    | 272.3 ± 49.2 | 308.2 ± 59.3 | 269.9 ± 49.3 | 416.1 ± 74.1 | 365.8 ± 76.6 |
| Cholesterol (mg/dL)     | 26.3 ± 4.2 | 30.9 ± 5.2 | 31.1 ± 6.2 | 50.8 ± 10.5 | 42.6 ± 11.4 |
| Triglyceride (mg/dL)    | 195.7 ± 39.7 | 206.3 ± 41.2 | 163.7 ± 52.4 | 269.2 ± 51.7 | 279.8 ± 64.4 |
| Cortisol (ng/mL)        | 5.2 ± 1.0ab | 4.8 ± 0.9ab | 3.2 ± 0.4ab | 6.0 ± 1.2ab | 8.4 ± 1.0ab |
| Glucose (mg/dL)         | 54.2 ± 3.6 | 62.0 ± 6.0 | 65.0 ± 8.9 | 68.9 ± 7.9 | 62.8 ± 7.3 |
| TG (g/g/L)              | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.15 ± 0.03 | 0.15 ± 0.04 | 0.11 ± 0.03 |
| T3 (μg/mL)              | 0.52 ± 0.11ab | 0.67 ± 0.14ab | 0.35 ± 0.12ab | 0.14 ± 0.06ab | 0.26 ± 0.05ab |
| T3/T4                   | 0.29 ± 0.16ab | 0.23 ± 0.08ab | 1.17 ± 0.55ab | 2.31 ± 0.79ab | 0.68 ± 0.3ab |

![Figure 2. Hemoglobin concentration of sub-yearling Siberian sturgeon *Acipenser baerii* injected every 10 days with different doses of ovine growth hormone (oGH) after 50 days of rearing. No significant difference was found in this parameter (P < 0.05).](image-url)
Table 4. Linear and quadratic regression of all measured parameters with $R^2$ and $P$-values.

| Parameter          | Regression | Equation                                           | $R^2$ | $P$-value |
|--------------------|------------|----------------------------------------------------|-------|-----------|
| Final weight       | Linear     | $y = 4.62x + 96.62$                               | 0.869 | 0.021     |
|                    | Quadratic  | $y = 0.402x^2 + 1.30x + 99.73$                    | 0.903 | 0.097     |
| Final length       | Linear     | $y = 0.37x + 34.40$                               | 0.845 | 0.027     |
|                    | Quadratic  | $y = 0.019x^2 + 0.21x + 34.55$                    | 0.856 | 0.144     |
| BWI                | Linear     | $y = 5.76x + 20.34$                               | 0.881 | 0.018     |
|                    | Quadratic  | $y = 0.45x^2 + 2.007x + 23.87$                    | 0.909 | 0.091     |
| SGRig             | Linear     | $y = 0.079x - 0.38$                               | 0.875 | 0.118     |
|                    | Quadratic  | $y = 0.005x^2 - 0.04x + 0.42$                     | 0.892 | 0.108     |
| SGRg              | Linear     | $y = 0.02x + 0.17$                                | 0.729 | 0.066     |
|                    | Quadratic  | $y = 0.001x^2 + 0.01x + 0.18$                     | 0.739 | 0.261     |
| FCR                | Linear     | $y = -0.14x + 0.88$                               | 0.427 | 0.232     |
|                    | Quadratic  | $y = -0.004x^2 + 0.02x + 0.84$                    | 0.654 | 0.346     |
| HSI                | Linear     | $y = 0.008x + 2.15$                               | 0.018 | 0.831     |
|                    | Quadratic  | $y = -0.023x^2 + 0.195x + 1.97$                   | 0.793 | 0.207     |
| Feed intake        | Linear     | $y = -6.3x + 3.94$                                | 0.944 | 0.010     |
|                    | Quadratic  | $y = 0.50x^2 + 0.48x + 0.04$                      | 1.000 | 0.000     |
| Dry weight         | Linear     | $y = 0.052x + 79.85$                              | 0.133 | 0.546     |
|                    | Quadratic  | $y = 0.042x^2 + 0.29x + 80.17$                    | 0.557 | 0.443     |
| Crude protein      | Linear     | $y = 0.18x + 13.97$                               | 0.452 | 0.214     |
|                    | Quadratic  | $y = 0.096x^2 + 0.12x + 14.02$                    | 0.455 | 0.545     |
| Crude fat          | Linear     | $y = -0.04x + 5.67$                               | 0.483 | 0.193     |
|                    | Quadratic  | $y = -0.01x^2 + 0.04x + 5.59$                     | 0.635 | 0.365     |
| Ash                | Linear     | $y = 0.03x^2 + 4.49$                              | 0.107 | 0.592     |
|                    | Quadratic  | $y = 0.03x^2 + 0.25x + 4.74$                      | 0.966 | 0.034     |
| Total protein      | Linear     | $y = 0.042x + 3.11$                               | 0.086 | 0.632     |
|                    | Quadratic  | $y = 0.057x^2 + 0.52x + 2.665$                    | 0.903 | 0.097     |
| Total lipids       | Linear     | $y = 22.9x + 240.99$                              | 0.552 | 0.150     |
|                    | Quadratic  | $y = -0.91x^2 + 30.56x + 233.70$                  | 0.556 | 0.444     |
| Cholesterol        | Linear     | $y = 1.81x + 26.98$                               | 0.89  | 0.016     |
|                    | Quadratic  | $y = 0.014x^2 + 0.62x + 28.11$                    | 0.919 | 0.081     |
| Triglyceride       | Linear     | $y = 12.62x + 185.08$                             | 0.643 | 0.103     |
|                    | Quadratic  | $y = -0.2x^2 + 14.24x + 183.56$                   | 0.644 | 0.356     |
| Cortisol           | Linear     | $y = 0.50x + 3.94$                                | 0.613 | 0.117     |
|                    | Quadratic  | $y = 0.11x^2 + 0.42x + 4.81$                      | 0.763 | 0.232     |
| Glucose            | Linear     | $y = 0.62x + 0.77$                                | 0.116 | 0.618     |
|                    | Quadratic  | $y = -0.58x^2 + 5.35x + 56.35$                    | 0.697 | 0.497     |
| T3                 | Linear     | $y = 0.006x + 0.089$                              | 0.219 | 0.427     |
|                    | Quadratic  | $y = -0.005x^2 + 0.04x + 0.05$                    | 0.801 | 0.199     |
| T4                 | Linear     | $y = -0.034x - 0.490$                             | 0.263 | 0.374     |
|                    | Quadratic  | $y = -0.016x^2 + 0.11x + 0.57$                    | 0.371 | 0.629     |
| T3-T4              | Linear     | $y = 0.02x - 0.87$                                | 0.007 | 0.892     |
|                    | Quadratic  | $y = -0.077x^2 + 0.66x + 0.27$                    | 0.416 | 0.584     |
| Hemoglobin         | Linear     | $y = 0.08x - 7.09$                                | 0.391 | 0.26      |
|                    | Quadratic  | $y = -0.03x^2 + 0.14x + 6.88$                     | 0.602 | 0.398     |

Goodman, 1993) or from increased glucose production by liver. Our result indicated that based on protein synthesizing effects of oGH, total protein concentration did not significantly increase in different doses of oGH treatments. Plasma proteins concentration could also be altered as a result of nutritional status, stress activity, immune status, hemococoncentration, hemodilution, and protein synthesis modifications in the liver (Hoseini and Tarkhani, 2013). However, the enhancement of crude protein in muscle of fish that received higher level of oGH was not correlated with plasma total protein, but high levels of proteins in the body and plasma might be the result of the influence of oGH on fish physiology and growth status. Elevation in plasma total lipid, cholesterol and triglycerides in oGH-treated Siberian sturgeon suggests that exogenous GH influenced lipid mobilization from storage depots (i.e., adipose tissue, liver) as reported previously for rainbow trout (Bergan-Roller and Sheridan, 2018).

Plasma levels of cortisol in all treatment groups of Siberian sturgeon in the present study were in the range of resting values demonstrated previously for this species (Eslamloo and Falahatkar, 2014). Therefore, the treatment regime did not appear to stress fish. Information on the potential effects of exogenous GH on the hypothalamus-pituitary-interrenal (HPI) axis in fish is limited. Studies in mammals indicated that GH alters the activity of certain enzymes involved in cortisol synthesis (Stewart et al., 2001). Further study is required to elucidate interaction(s) between the growth axis and HPI axis in fish.

Few studies have investigated the direct effects of exogenous GH on thyroid function in fish (Holloway et al., 1994). However, the effect of GH on peripheral metabolism of thyroid hormones has been well documented in mammals (Agha et al., 2007; Losa et al., 2008; Smyczynska et al., 2010). Reduced levels of plasma T4 combined with an increased T3:T4 ratio in fish treated with oGH suggest an interaction between GH and thyroid function in sturgeon. For example, whether or not the GH-mediated decrease in T4 levels results from an effect of GH on thyroid stimulating hormone production/secretion and/or altering activity of deiodinase enzymes in peripheral tissues will be evaluated for future studies.

Despite technical limitations that prevented the measurement plasma GH concentrations, the changes induced by oGH administration in growth and some metabolites (e.g., cortisol and T4) as well as numerical enhancement of other parameters (e.g., total protein, total lipid,
cholersterol, triglyceride, and glucose) indicate that exogenous oGH treatment was effective over the course of the experiment. Although our data indicate that high dose of oGH may be required for optimal growth performance, we recognize that conditions such as duration of the experiment and the interval of injections also may be important to growth acceleration. In the future, we hope to obtain purified oGH and its specific antisera so as to develop an immunoassay that enables us to determine the circulating levels and clearance rate of oGH in Siberian sturgeon.

5. Conclusion

The present study showed for the first time that oGH improved somatic growth performance and increased body protein synthesis in juvenile Siberian sturgeon. The relatively low minimum effective dose used to achieve enhanced growth (8 μg oGH/g BW) makes exogenous GH treatment feasible for accelerating production of Siberian sturgeon in an aquaculture setting. Although hormonal treatment may not be suitable for caviar production due to possible negative effects on humans, the GH-induced acceleration of growth and maturation may be highly valuable for sturgeon selection programs and development of new commercial breeds with highly desirable traits. Future research should examine the effects of GH on gonad development, fertility of fish, progeny viability, all of which will be important for breeding and conservation efforts of this species.

Declarations

Author contribution statement

Bahram Falahatkar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Samaneh Poursaeid: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mark A. Sheridan: Conceived and designed the experiments; Contributed reagents, materials; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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