Research Article

Effect of time-dependent protein restriction on growth performance, immunity response, and body composition in the stellate sturgeon (*Acipenser stellatus*)

S. A. A. Bagheri Khamkhane[1*], A. Ehsanfar[2], S. S. Mirkhataminasab Langerodi[3], A. Keramat[4], H. Oraji[5], A. Abedian kenari[6]

[1, 2]Shahid Rajaee Sturgeon Hatchery Center, Sari, Mazandaran, Iran
[3]Islamic Azad University of Lahijan, Lahijan, Guilan, Iran
[4, 5]Fisheries Department, Faculty of Animal Sciences and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
[6]Department of Aquaculture, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Mazandaran, Iran

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Abstract

One hundred and fifty stellate sturgeon (*Acipenser stellatus*, 250 ± 3.24 g) were assembled into 15 round concrete tanks. The tanks were allocated to five treatments with three replications: fed with a diet containing 30% protein for eight weeks (T1); fed with a diet containing 30% protein level in weeks 1, 3, 5, and 7 and fed with a diet containing 35% protein level in weeks 2, 4, 6, and 8 (T2); fed with a diet containing 30% protein in weeks 1, 2, 5, and 6, and fed with a diet containing 35% protein in weeks 3, 4, 7, and 8 (T3); fed with a diet containing 30% in the diet in weeks 1, 2, 3, and 4, and fed with a diet containing of 35% protein in weeks 5, 6, 7, and 8 (T4); and fed with a diet containing 35% for eight weeks (T5). Remarkable effects were recorded in growth efficiency, and T2 and T5 had higher growth than other treatments. No marked difference was seen in the whole body composition. Different feeding strategies affected RBC, WBC, and MCV, and RBC and WBC of fish in the T4 were notably upper than in the other groups. Different feeding strategies had marked differences in lysozyme and ACH50 activity. These results demonstrated that T2 could use as a feeding strategy for stellate sturgeon.

[*]**Corresponding author's email:** aa_bagheri61@yahoo.com

Keywords: Stellate sturgeon, Feeding strategy, Protein restriction time

Introduction

One of the main sources of food supply for humanity is the aquaculture industry, which provides high-quality protein (Dawood, 2021; Yu *et al.,* 2021). The success of the aquaculture industry depends on minimizing production costs. One of the most effective factors in the total breeding cost is the feed cost (Zhao *et al.,* 2021). The most expensive component in the formulated diet is protein, and it is a major item affecting fish growth, feed intake, and feed cost. Many studies have reported better growth efficiency resulting from optimal dietary protein levels in different species (Jin *et al.,* 2013; xu *et al.,* 2016; Zhao *et al.,* 2021). Although, if the diet contains excess protein, it will be metabolized as a source energy supply instead of used for growth and may enhance nitrogen excretion, thereby declining water quality and growth efficiency. Although, if the diet contains excess protein, it is metabolized as an energy source rather than used for growth, and it may increase nitrogen excretion in the aquatic environment, thereby reducing water quality and growth performance (Dong *et al.,* 2013). Therefore, a proper protein management strategy is of exclusive importance for the supportable increase of the aquaculture industry (Zhao *et al.,* 2021). Developing strategies to decrease the protein content of fish diets is essential for more effective aquaculture activities. Protein-restricted diets are now widely used to enhance protein intake and have attracted the attention of aquatic nutritionists (Zhao *et al.,* 2021). In aquaculture, diets with protein restriction for short times are presented as a nutritional plan to overcome different problems such as poor water quality and ultimately reduce feed costs (Hosseinpour Aghaei *et al.,* 2018). Therefore, this strategy may create a more effective and supportable way to develop aquaculture.

Many researches have been performed to demonstrate the role of protein restriction as a main achievement in nutrition strategy (Wu and Dong, 2002; Wu *et al.,* 2006; Hosseinpour Aghaei *et al.,* 2018; Zhao *et al.,* 2021). Sturgeon is one of the oldest vertebrates on earth and one of the earliest ancestors of teleost. Because sturgeons live longer than other fish, they experience regular periods of low food availability, which make them suitable species for studies on feed deprivation or restriction (Falahatkar, 2012). Survival rates during periods of feed restriction vary depending on the fish's ability to adapt the metabolic activity to reallocate reserves to meet different tissue needs. The nature of this metabolic organization in sturgeon is not well known. Therefore, studying various nutritional strategies, including food restriction, helps to make sturgeon farming economical on a large scale. The role of protein restriction has been investigated in common carp (*Cyprinus carpio*), Chinese shrimp (*Fenneropenaeus chinensis*), Japanese flounder (*Paralichthys olivaceus*), yellow catfish (*Pelteobagrus fulvidraco*), beluga (*Huso huso*) and siberian sturgeon (*Acipenser baerii*) (Wu & Dong, 2002; Wu *et al.,* 2006; Dong *et al.,* 2013; Mohseni *et al.,* 2014; Hosseinpour Aghaei *et al.,* 2018). However, to date, information the role of protein restriction on growth performance and immunity factors in stellate sturgeon (*Acipenser stellatus*) is limited. So, this study was performed in order to investigate the efficacy of the frequency of protein restriction on the growth efficiency, body composition, and biochemical parameters of the stellate sturgeon.

Material and methods

Preparation of experimental diets

Two experimental diets were prepared to contain 30% and 35% protein (Table 1). The dry ingredients (Mazandaran Animal and Aquatic Feed, Sari, Mazandaran, Iran) were softly blended together and then, water (30-40%) was added to the mixture. The wet powder mixture was pelleted in an electric meat grinder and the feed was broken down according to the size of the fish's mouth. The pellets were spread out in the room to dry at room temperature, and subsequently, stored at –20°C until using and daily food was kept at 4 °C.

Table 1. Ingredients and proximate composition of excremental diets

Compounds	30 % protein	35 % protein
Kilka fish meal	29.4	33
wheat flour	32.7	28
Soybean meal	20.4	20.49
Fish oil	6.54	5.87
Soybean oil	0.654	5.87
Antioxidants	0.2	0.2
Antifungal	0.25	0.25
Mono calcium phosphate	1	1
Binder (Artfire)	1.5	1.5
Vitamin and mineral premix[a]	2	2

[a] Vitamin premix (Science Laboratories Company, Qazvin, Iran). Mineral premix (Science Laboratories Company, Qazvin, Iran).

Fish rearing condition

The trial was done on Shahid Rajaee sturgeon farm (Sari, Iran). One hundred and fifty stellate sturgeon (250 ± 3.24 g) assembled into 15 tanks (10 fish per tank) and adapted to rearing condition for 14 days. During this time, these fish were fed twice a day with a diet (35 % protein). After two weeks, the tanks were allocated to five treatments with three replications: fed with a diet containing 30% protein for eight weeks (T1); fed with a diet containing 30% protein level in weeks 1, 3, 5, and 7 and fed with a diet containing 35% protein level in weeks 2, 4, 6, and 8 (T2); fed with a diet containing 30% protein in weeks 1, 2, 5, and 6, and fed with a diet containing 35% protein in weeks 3, 4, 7, and 8 (T3); fed with a diet containing 30% in the diet in weeks 1, 2, 3, and 4, and fed with a diet containing of 35% protein in weeks 5, 6, 7, and 8 (T4); and fed with a diet containing 35% for eight weeks (T5). (Table 2). Fish were hand-fed diets three times a day at the rate of 3% body weight for 8 weeks. Water quality factors were regularly measured (temperature 19.25 ± 0.6 °C; dissolved oxygen 11.5 ± 0.8 mg/L; photoperiod was maintained at 12D:12L).

Table 2. Experimental treatments

Treatments	Feed Protein levels	
	30%	**35%**
T1	8 weeks	-
T2	Weeks 1, 3, 5, 7	Weeks 2, 4, 6, 8
T3	Weeks 1, 2, 5, 6	Weeks 3, 4, 7, 8
T4	Weeks 1, 2, 3, 4	Weeks 5, 6, 7, 8
T5	-	8 weeks

Growth efficiency

After 8 weeks, feeding was stopped for 24 hours and all fish were anesthetized using clove powder, and weighted. The growth indexes weight gain, Survival rate, specific growth rate (SGR), Feed Conversion Ratio (FCR), protein efficiency (PER) were calculated by standard formulas.

Sampling

After 8 weeks, nine fish (per treatment) were sampled and were anesthetized with clove powder (100 mg/L). Blood samples were collected and presented to both heparinized and nonheparinized tubes. Serum was collected by centrifuging blood samples at 1,600 g for 10 min and it was held at -70°C for future analysis.

Proximate chemical analyses

Nine fish were randomly selected from each treatment to perform a chemical analysis of body composition, such as protein, fat, moisture, and ash, and were anesthetized with clove powder; then, samples were homogenized with a meat grinder. The AOAC standard method (1990) was used to analysis of fish samples. Briefly, Moisture was obtained by drying the samples at 105 ° C for 24 hours. Crude protein (total nitrogen × 6.25) was determined by the Kjeldahl method. Fat was extracted by using a Soxtec extraction. Ash content was obtained by a furnace at 550°C for 6 hr.

Hematological parameter

The whole blood was suspended in the diluent (Natt and Herrick, 1952) for determining red and white blood cell levels using a hematocytometer. A commercial kit (Ziest Chem) was prepared to detect haemoglobin concentration (Hb). Then whole blood was centrifuged in heparinized capillary tubes at 3500×g for 10 min to evaluate Hematocrit (Hct %). Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular haemoglobin (MCH) were measured according to the following formulas:

$$\text{MCV (fl)} = (\text{Hct} / \text{RBC}) \times 10$$

$$\text{MCHC (\%)} = (\text{Hb} / \text{Hct}) \times 100$$

$$\text{MCH (fl)} = (\text{Hb} / \text{RBC}) \times 10$$

Immunological assays

The modified turbidimetric method was applied to detect lysozyme activity based on a pervious method (Ellis, 1990). We used the method of Yano et al. (1988) to measure serum alternative complement (ACH50) activity.

Statistical analysis

All data were analyzed using SPSS version 22 software at a 95% confidence level. First, the normality and homogeneity of the variance were checked using Kolmogorov-Smirnov test and Levene's test respectively. After both conditions were satisfied, the one-way analysis of variance (ANOVA) was carried out, Tukey's test was used to specify the differences between treatments. All data were reported as mean ± SD.

Results

Growth efficiency

The growth efficiency of stellate sturgeon subjected to different protein restriction strategy was showed in Table 3. A notable effect was obtained in growth indexes like final weight, WG, FCR, SGR, and PER of stellate sturgeon fed with different feeding strategies ($p < 0.05$). The highest WG and the best PER were observed in T5 without difference between T2, T4, and T5 treatments ($p > 0.05$). The highest and lowest SGR were obtained in T5 and T1, respectively. A significant decline was seen in FCR in T5 compared to T1 ($p < 0.05$). No mortality was seen during 8 weeks.

Table 3. Growth indexes of stellate sturgeon after 8 weeks

Treatment/factor	WG (g)	SGR	PER (%)	FCR	Survival (%)
T1	59.30 ± 9.40 ^{b}	0.33 ± 0.04 ^{b}	0.44 ± 0.07 ^{b}	5.08 ± 0.84 ^{a}	100
T2	76.26 ± 2.67 ^{ab}	0.41 ± 0.03 ^{ab}	0.57 ± 0.05 ^{ab}	3.90 ± 0.33 ^{ab}	100
T3	64.73 ± 6.01 ^{b}	0.35 ± 0.03 ^{ab}	0.48 ± 0.04 ^{b}	4.60 ± 0.41 ^{ab}	100
T4	70.16 ± 13.75 ^{ab}	0.37 ± 0.07 ^{ab}	0.53 ± 0.10 ^{ab}	4.33 ± 0.84 ^{ab}	100
T5	86.13 ± 12.33 ^{a}	0.45 ± 0.06 ^{a}	0.64 ± 0.0 ^{a}	3.49 ± 0.53 ^{b}	100

Different letters designate significant differences.

Proximate chemical analyses

The whole body composition of stellate sturgeon at the end of feeding trial were showed in 4. No marked effect in protein and fat were obtained between treatments (Table 4, $p > 0.05$). Protein restriction had no meaningful effects on ash and moisture ($p > 0.05$).

Table 4. Whole body composition in stellate sturgeon after 8 weeks

Treatment/factor	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
T1	50.6 ± 3.5	31.4 ± 2.0	77.8 ± 0.4	4.9 ± 0.7
T2	55.6 ± 7.1	28.7 ± 3.0	76.3 ± 1.3	7.0 ± 1.3
T3	48.2 ± 11.3	30.4 ± 1.8	77.5 ± 0.9	6.4 ± 1.6
T4	58.9 ± 6.0	30.8 ± 5.0	78.5 ± 0.3	6.3 ± 1.8
T5	60.3 ± 2.7	28.0 ± 2.5	75.4 ± 0.6	4.9 ± 0.6

Different letters designate significant differences as determined by Tukey's post-hoc tests.

Hematological parameter

RBC, WBC, and MCV were affected by different feeding strategies ($p < 0.05$, Table 5). The RBC and WBC of fish in the T4 were notably higher than in the other treatments. The lowest amount of MCV was seen in T4 and it had a significant difference from other treatments except T5 $p < 0.05$, Table 5). There was no marked effect in HB, Ht, MCH, and MCHC between treatments ($p > 0.05$).

Table 5. The hematological parameter in stellate sturgeon after 8 weeks

Treatment / factor	**RBC (×10^6/mm$^{3)}$**	**WBC (10^3/mm^3)**	**Hb (g/dI)**	**Ht (%)**	**MCV (fI)**	**MCH (pg)**	**MCHC (%)**
T1	0.9 ± 0.03^{b}	22.7 ± 1.4^{d}	6.9 ± 0.2	27.0 ± 2.3	293.6 ± 36.6^{a}	75.2 ± 5.1	25.7 ± 1.4
T2	0.9 ± 0.07^{b}	25.7 ± 0.9^{cd}	7.6 ± 0.1	29.0 ± 1.5	303.0 ± 40.6^{a}	26.2 ± 1.9	25.7 ± 0.9
T3	0.9 ± 0.02^{b}	28.7 ± 2.2^{bc}	7.3 ± 0.04	27.1 ± 1.4	292.0 ± 24.7^{a}	27.0 ± 1.6	28.7 ± 2.3
T4	1.0 ± 0.06^{a}	36.6 ± 1.8^{a}	7.86 ± 0.4	24.9 ± 0.01	202.6 ± 10.5^{b}	31.4 ± 1.9	36.6 ± 1.8
T5	1.1 ± 0.1^{a}	32.3 ± 0.4^{b}	7.0 ± 0.7	28.6 ± 0.2	249.5 ± 38.8^{ab}	31.6 ± 1.9	32.3 ± 0.4

Different letters designate significant differences as determined by Tukey's post-hoc tests (means ± SD).

Immunological assays and protease activity

Different feeding strategies had marked differences in lysozyme (Figure 1A) and ACH50 activity (Figure 1B) ($p < 0.05$). The highest and lowest lysozyme activity was obtained in T5 and T2, and T1, respectively.

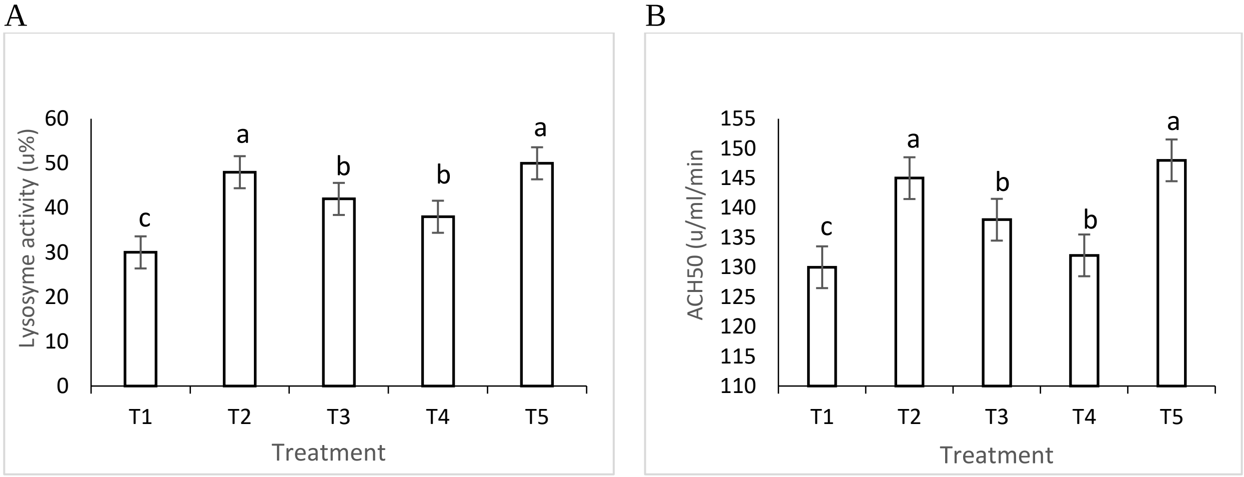

Figure 1. Lysozyme (Figure 1A) and ACH50 activity (Figure 1B) in stellate sturgeon **after 8 weeks**. Different letters designate significant differences as determined by Tukey's post-hoc tests (means ± SD).

Discussion

The results indicated that growth performance increased with an increased protein level in the diet (35% compared to 30%). The present study showed that a 35% protein diet was appropriate for optimum growth in stellate sturgeon. The highest WG was seen in fish fed at 35% protein level for eight weeks, and different studies indicated that 35-40% protein was suitable for the best growth performance for sturgeons (Mohseni *et al.,* 2007; Guo *et al.,* 2012; Hosseinpour Aghaei *et al.,* 2018). Moreover, increasing protein restriction for one other week (T2) had no adverse effect on growth performance. However, with increasing protein restriction time for two other weeks (T3) and four weeks (T4), the WG and SGR declined, and FCR increased. In consist with our results, growth reduced in Rainbow trout (*Oncorhynchus mykiss*) hybrid tilapia (*Oreochromis mossambicus*, *O. niloticus*) and Siberian sturgeon (*Acipenser baerii*) with increasing protein restriction times (Wang *et* *al.*, 2000; Sevegili *et al.*, 2012; Hosseinpour Aghaei *et al.*, 2018). The decrease in SGR, PER, and raised FCR with rising protein restriction time may be related to extreme protein shortage. The present result, protein deficiency might occur in T1 and T3. It has been reported in hybrid tilapia, Siberian sturgeon, and yellow catfish (Wang *et al.*, 2000; Tian and Qin, 2004; Hosseinpour Aghaei *et al.*, 2018). In groups with high protein restriction time, fish have to increase feeding to provide sufficient protein.

The present study showed significant difference in whole-body composition between treatments. In agreement with these results, Sevegili et al. (2012) and Hosseinpour Aghaie et al. (2018) reported that the whole body composition was not affected by moderate protein restriction time. In the present research, no significant difference was obtained in the protein level. However, the protein levels decreased by increasing protein restriction. Similar results have been observed by Zhao et al. (2021), who showed that 15% protein level restriction and further improvement declined the protein content of juvenile white shrimp (*Litopenaeus vannamei*) also Hosseinpour Aghaei et al. (2018) indicated that 30% protein level restriction decreased the protein content in Siberian sturgeon. No significant change in protein level can be related to supplying the minimum requirements of fish by different food treatments. In this study, a small increase was observed in fat content, in agreement with other research (Luo *et al.*, 2004; Zhao *et al.*, 2021). Restriction can reduce protein levels and increase body fat (Wu and Dong, 2002). In this study, an isoenergetic diet was applied. Therefore, this result indicated that dietary protein levels could directly affect the amount of protein and fat body. These results have been expressed in other research (Hafech *et al.*, 1999; Pérez-Sánchez, 2000).

A balanced diet is assessed by measuring blood parameters in fish, and nutritional deficiencies and insufficient protein may cause severe anemia due to decreased hematocrit and hemoglobin levels. In the current experiment, no meaningful effect were observed in hematocrit, hemoglobin, MCH, and MCHC between treatments. RBC, WBC, and MCV were affected by different feeding strategies. The level of WBC in the treatment fed with low protein level (T1) was the lowest and the treatment fed with protein restriction time for four weeks (T4) and 35% protein level (T5) was the highest level. These results indicated that total leukocyte values are so sensitive to feed restriction. WBCs are considered the immunocompetent cells that take part in immunological resistance. Therefore, an expansion of the duration of feed restriction may cause a significant increase in the number of white blood cells, affecting immune responses in fish. The number of RBCs can have a considerable effect on the energy level in an organism. Whenever, fish activity reduces due to several factors such as a decrease in water temperature and consequently a decline in metabolism, the number of RBC cells decreases, and the plasma concentration rise. The RBC decreased by increasing protein restriction time and the lowest value was seen in fish fed 30% protein for 8 weeks. This result may be related to food restriction, reduced nutrition, temperature and generally reduced body metabolism (Sattari *et al.*, 2002).

Fish immunity is affected by dietary protein, and protein deficiency may impair immune function, in which animals are more susceptible to infectious diseases (Li *et al.*, 2007). In addition, lysozyme activity decreases at low protein levels compared to higher levels (Khalil *et al.*, 2016). In the current study, lysozyme was lower T1, T3, and T4 than T2 and T5. Protein restriction displayed a decline in immune function (Venesky *et al.*, 2012). In this content, lysozyme activity decreased in starved blackspot sea bream, *Pagellus bogaraveo* (Caruso *et al.*, 2011). Hosseinpour aghaei et al. (2018) have demonstrated that lysozyme activity in fish fed low protein diet or restricted protein for a long time was not as robust as fish fed with high protein diets. These findings are consistent with our study. In contrast to our results, lysozyme activity increased in sea bass, *Dicentrarchus labrax*, after 31 days starvation (Caruso *et al.*, 2011). Difference results may be related to differences in age, size and sex of the organisms, and type of the examined tissue (Bodin, 2009). In the present study, ACH50 had a similar pattern and in fish restricted of protein for long time (T1, T3, and T4) decreased. In consistent with our study, Hosseinpour aghaei et al. (2018) reported a decline in ACH50 in fish fed low protein or restricted protein for a long time. There was no significant difference between fish with one other week restriction (T2) and T5. Regarding lysozyme and ACH50, it was demonstrated that one week was adequate time for protein restriction and the immune system was successfully adapted to this situation.

In conclusion, the present results demonstrated that increasing protein restriction times (T1 and T3 and T4 groups) reduced growth performance, negatively result the quality of the fish body composition, and had negative effect on fish health. T2 treatment can be applied in the culture of Persian sturgeon fish without any destructive results. To use these nutritional management strategies may improve economic productivity and efficiency in sturgeon aquaculture.

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Conflict of interest

The authors have no conflict of interest in this work.

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