

### The use of citric acid as attractant in diet of grand sturgeon *Huso huso* fry and its effects on growing factors and survival rate

<sup>1</sup>Mohammad Sudagar, <sup>1</sup>Hosseinpoor Zelti, and <sup>1</sup>Abbas Hosseini

<sup>1</sup>Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Corresponding author: M. Sudagar, sudagar\_m@yahoo.com

**Abstract.** In an 8-weeks feeding trial, attractant (citric acid) was added to juvenile beluga (*Huso huso*) diets at different levels in order to increase growth and survival. In this trial that was carried out in Shahid Marjani complex of sturgeon propagation and cultivation, three different dietary of attractants (0.5%, 1% and 1.5%) were taken into account. The trial was carried out in 500 liter PVC tanks which were filled with about 350 liter of water. 50 juvenile beluga (mean initial weight  $26.04 \pm 0.43$ g) were stocked in tanks and were fed up four meals a day. Growth and survival factors were analyzed at the end of trial period. After 54 days, weight gain, feed intake, feed conversion ratio (FCR), Daily Growth Index (DGI), Daily Growth Rate (DGR), Specific Growth Rate (SGR), Condition Factor (CF), were higher in beluga fed the three citric acid-added diets compared with control feed. Among the citric acid-added diets, juvenile beluga fed citric acid of 5, 10, 15 g Kg<sup>-1</sup> level showed highest weight gain (134 g) by registering 136.6 % increase in growth over control and higher feed intake (7.34) and good FCR (2.20). There was highly significant differences ( $P < 0.05$ ) in weight gain, feed intake, per day growth and FCR among treatment. There was no significant difference ( $P > 0.05$ ) in survival among treatment.

**Key Words:** Beluga, feeding, attractant, citric acid, growth.

در یک آزمایش 8 هفته ای به منظور افزایش رشد و بقا، فیل ماهیان جوان بوسیله جیره های با سطوح مختلف ماده جاذب غذایی (اسید سیتریک) تغذیه شدند. در این آزمایش که در مجموعه تکثیر و پرورش ماهیان خاویاری شهید مرجانی انجام شده بود، سه جیره غذایی مختلف از ماده جانب (0.5%، 1% و 1.5%) در نظر گرفته شده بود. آزمایش در تانک های 500 لیتری پی وی سی که با حدود 350 لیتر آب پر شده بودند انجام شد. 50 فیل ماهی جوان (متوسط وزن اولیه  $26.04 \pm 0.43$  گرم) در مخازن ذخیره شدند و 4 وعده در روز غذاهای شدند. فاکتورهای رشد و بقا در پایان دوره آزمایشی مورد تجزیه و تحلیل قرار گرفت. پس از 54 روز، وزن اضافه شده، غذای مصرف شده، ضریب تبدیل غذایی، شاخص رشد روزانه، نرخ رشد روزانه، نرخ رشد ویژه و فاکتور وضعیت در فیل ماهیانی که با سه جیره غذایی افزوده شده با اسید سیتریک تغذیه شده بودند نسبت به گروه شاهد بالاتر بود. فیل ماهیان جوان تغذیه شده با جیره های افزوده شده با اسید سیتریک در سطوح 5، 10 و 15 گرم در کیلوگرم بالاترین وزن بدست آمده (134 گرم) با ثبت 136.6 درصد افزایش در رشد، مصرف خوراک بالاتر (7.34) و ضریب تبدیل خوب (2.20) را نسبت به گروه شاهد نشان دادند. میان تیمارها اختلاف معنادار قابل توجهی ( $p < 0.05$ ) در وزن بدست آمده، میزان غذای مصرف شده، رشد روزانه ضریب تبدیل غذایی وجود داشت. اختلاف معناداری میان تیمارها در میزان بقا وجود نداشت ( $p > 0.05$ ).

**کلمات کلیدی:** فیل ماهی، تغذیه، ماده جاذب، اسید سیتریک، رشد

**Introduction.** Sturgeon (Acipenseridae) are prehistoric fish that have evolved over 80 million years ago. Beluga *Huso huso* (Linnaeus, 1758) has a wide distribution. It occurs in the Caspian Sea, Black Sea, and the Sea of Azov and many of the tributaries of these seas. About 90 % of world sturgeon population is in the Caspian Sea. Beluga is the largest species of Acipenseriformes reaching a length of size six meters and a weight of more than one tone (Berg 1948).

In recent years over exploitation, environmental pollutions and poaching have caused beluga to be listed as a threatened species. Approximately 90 % of spawning grounds in the Caspian Sea have been lost and 91 % of each generation now originated from hatcheries (Barannikova et al 1995). Millions of beluga *Huso huso* have been artificially reared in Iranian hatcheries and then fingerlings weight 3-5 g released into the Caspian Sea. It has never been farmed as marketable size or producing golden eggs. But at the moment the new proposal is suggested to raise beluga in the farms, outdoor tanks and terrestrial pond or raceways and pens, also considering in aquaculture costs associated with feeding generally constitute 50 % of total production cost, it is necessary to have a serious attention and use artificial feed in sturgeons farms.

Sturgeons have a weak vision but their olfactory and gustatory senses are well developed because of possessing chemo-receptors. Indeed, these are principal senses of

sturgeons for feeding, spawning, migration and orientation. These fish have many taste buds inside and outside of the mouth, along with barbells and on the belly that possess chemo-receptors. Extra-oral and intra-oral chemo-receptors are different in threshold concentration of attractants so that sensitivity of extra-oral is 10 times more than intra-orals (Kasumyan 1999). Then, gustatory reception has a controller role of final phase of feeding behavior in this species. Indeed, beluga uses its extra-oral gustatory reception for hunting and snacking the prey (Atemia 1988). When food is in the mouth cavity, intra-oral gustatory receptors determine its quality and make final decision for swallowing or rejecting (Kasumyan & Morsy 1997). The addition of attractants can stimulate receptors and be effective on food swallowing (Kasumyan 1999).

The use of dietary feeding attractants within compound aqua-feeds has received considerable attention in recent years. The rationale behind their use has been to improve dietary food intake, and at the same time by promoting quicker food intake, minimizing the time the feed remains in water and thereby the leaching of water soluble nutrients, and at the same time providing additional nutrients for protein and energy metabolism. It follows therefore that if aqua-feed are ingested with minimum wastage and feed efficiency therefore maximized, that feed wastage and water pollution will be minimized (Polat & Bekievik 1999). Free amino acids are among the adequate highly effective gustatory stimuli.

**Material and Method.** This trial was carried out in Shahid Marjani complex of sturgeon propagation and cultivation (Golestan Province) for a period of 54 days during summer (5 July to 27 August 2006). It was carried out in outdoor circular fiber glass-reinforced plastic (FRP) tanks of 2 m<sup>2</sup> area each. Water level was maintained at 50±2 cm level throughout the experimental period. Each tank was provided with one PVC pipes of 3 cm diameter, 80 cm length. Water was exchanged at a rate of 4 per day. The juvenile of beluga were procured from Shahid Marjani complex of sturgeon propagation and cultivation located at Golestan Province, Gorgan, Iran, and they were reared for about 2 months in 2000 L capacity FRP tanks to attain juveniles of above 26 g size. The nursery-reared juvenile beluga (*H. huso*) with a size range of 25.72±0.6–26.75±1.5 g were stocked in the FRP tanks at a rate of 50 juvenile beluga per tank. The juvenile of beluga were weighed initially and thereafter fortnightly until the end of the experiment digital scale. The samples for water quality parameters were taken at daily intervals between 8.30 and 9.00 hours. The temperature, pH, dissolved oxygen, salinity of the water ranged 24–28°C, 7.5–7.9, 5.5–7.1 (mg L<sup>-1</sup>), and 0.9–1.1 (ppt), respectively.

**Diet preparation.** Diets were prepared from local raw materials and formulated by Lindo software (copyright 1995). The raw materials included fish meal, soybean meal, fish oil, lecithin, vitamin supplements, wheat meal, mineral supplement, and other additives. For diet preparation, dry raw materials and attractant (citric acid) at three different dietary levels (0.5 %, 1 %, 1.5 %) were balanced and mixed. Then, liquid raw materials like molasses, fish oil and lecithin were added to dry materials. Outcome paste was passed through a mince 2.5 mm screen and transformed to spaghetti. This spaghetti-form paste was chopped a little by hand and then dried. Finally, dried strings of the paste were grinded to a proper size and packed and maintained in a refrigerator. The percentage composition of ingredients and the proximate composition of the diets are presented in Tables 1, 2 and 3 respectively.

**Feeding.** Beluga juveniles were maintained in tanks for a week and fed diets containing no citric acid for adaptation. They were then weighed and randomly stocked in the tanks at a density of 50 juveniles (with average of 26.04±0.43 g). The trial was accomplished in a salon and under random blocks. Three repeats were taken into account for each treatment. Beluga juveniles were fed up 4 meals throughout the trial. Feces and other wastes were discharged every morning and 50 % of water was exchanged before feeding. Biometry was done every two weeks. For this purpose, juveniles were removed from the tanks and weighed. Also, tanks and air stone were fully washed and cleaned. Quality parameters of water like temperature, dissolved oxygen, PH, were 19.5–27°C, 4.5–7 ppm and 8.1–8.3, respectively. Beluga juveniles were maintained in tanks for 54 days and fed diets containing different levels of citric acid. At the end of trial period, body

weight increase (gr), growth coefficient (GF), specific growth rate (SGR), food conversion ratio (FCR) and condition factor(CF), daily growth index, daily growth rate, were calculated through following equations (Tacon 1990).

Table 1

Proximate composition of the ingredient (g kg<sup>-1</sup> dry weight) used in the experimental diets

<i>Ingredients</i>	<i>Wheat meal</i>	<i>Rice bran</i>	<i>Soybean meal</i>	<i>Corn meal</i>	<i>Fish meal</i>
Moisture (%)	12.435	8.516	13.043	11.916	10.702
Crude protein (%)	11.002	9.265	44.403	7.425	66.017
Ether extract (%)	1.602	9.495	1.598	4.028	8.339
Total ash (%)	0.445	13.662	5.495	1.228	14.677
Fiber (%)	1.339	21.530	6.147	2.302	1.447
NFE (%)	73.15	37.53	29.32	73.10	1.18
Gross energy (kcal/kg)	3173.03	2701.78	2947.21	3340.30	3041.71

Table 2

Percentage composition of ingredients

<i>Ingredients</i>	<i>%</i>
Fish meal	45
Wheat meal	10.62
Rice bran	1.63
Soybean meal	15
Fish oil	5
Soybean oil	5
Corn meal	9.88
Binder	2
Vitamin C	0.1
Vitamin supplement	2
Mineral supplement	2
Antioxidant	0.02
Anti-fungi	0.25

Table 3

Proximate composition of the experimental diets

<i>Analyzed</i>	<i>%</i>
Moisture	10
Protein	45
Fat	15
Carbohydrate	20
Ash	7.3
Fiber	2.5
Energy(Kcal kg <sup>-1</sup> )	3470

**Growth analysis.** The growth parameters were calculated by using the following formulae:

- Mean weight gain (g) =  $W_f (g) - W_i (g)$  (Tacon 1990);
- Food Conversion Ratio (FCR) = total feed consumed (g)/(initial number of fishes/final number of fishes) (De Silva & Anderson 1995);
- Per day growth (g) = mean weight gain (g)/number of days (Tacon 1990);
- Survival (%) = number of fishes survival at the end of the experiment/number of fishes stocked at the start of the experiment (Ai et al 2006);

- Percentage of mean weight gain (PMWG) =  $((W_f (g)-W_i (g))/W_i (g))\times 100$  (Bekcan et al 2006);
- Specific growth rate (SGR) =  $\{(\ln W_f (g)-\ln W_i (g))/t\}$  (Hevroy et al 2005);
- Condition factor (CF) =  $W\times 100/L^3$  (Ai et al 2006);
- Daily growth rate (DGR) =  $\{100\times(\text{final weight (g)}-\text{initial weight (g)})/(\text{days}\times\text{initial weight (g)})\}$ ; (De Silva & Anderson 1995);
- Daily growth index (DGI) =  $\{100\times(W_f^{1/3}-W_i^{1/3})/\text{days}\}$  (De Silva & Anderson 1995);
- Growth coefficient (GC) =  $\{100\times(W_f^{1/3}-W_i^{1/3})/\Sigma\theta\}$  (De Silva & Anderson 1995), where  $W_f$  = Final weight,  $W_i$  = Initial weight,  $\Sigma\theta$  = sum of average daily temperature in °C.

**Proximate analysis.** The moisture, crude protein, lipid, ash and crude fiber contents in the feed ingredients and test diets were analyzed according to the standard procedures of Association of Official Analytical Chemist (1980). Moisture was determined by oven drying at 105°C for 24 h and protein by Kjeldahl method (Mehrad & Sudagar 2010) after acid digestion. Lipid was determined in Soxhlet apparatus by extracting the residue with 40–60°C petroleum ether for 8 h. Crude fiber was determined as loss on ignition of dried lipid-free residues after digestion with 1.25 % H<sub>2</sub>SO<sub>4</sub> and 1.25 % NaOH, and ash was determined by ignition at 550°C in a muffle furnace to constant weight. The nitrogen free extract (NFE) was calculated by using the following formula:

$$\text{NFE [1000 (crude protein+crude lipid+ash+crude fiber+moisture)] gkg}^{-1}$$

**Statistical analysis.** Data were analysed using One-way analysis of variance and significance of difference between treatments ( $P<0.05$ ) were assessed by Duncan multiple range test (Hoshmand 1994).

**Results.** The growth parameters recorded in this experiment are presented in table 4. Among the three different levels used citric acid incorporated diet at 10 gkg<sup>-1</sup> level performed well in all the growth-related parameters.

The beluga juvenile fed diet with citric acid 10 g kg<sup>-1</sup> showed a higher mean weight gain (135.80 g) increase in growth over control. Daily growth index, percentage of growth coefficient, condition factor and percentage of specific growth rate had significantly difference ( $p<0.05$ ) with other groups. The best feed conversion ratio was found in group was fed supplemented diet with citric acid 15 g kg<sup>-1</sup> ( $p<0.05$ ) however, there was no significant difference between this group and group was fed supplemented diet with citric acid 10 g kg<sup>-1</sup> ( $p>0.05$ ).

Table 4

Growth parameters in beluga juvenile fed supplemented diets with different levels of citric acid

Growth Indexes	Control	Citric acid 0.5 %	Citric acid 1%	Citric acid 1.5 %
Initial Weight (g)	26.75±1.5 <sup>a</sup>	25.22±1.5 <sup>a</sup>	26.08±1.1 <sup>a</sup>	26.17±0.6 <sup>a</sup>
Final Weigh (g)	126.46±8.7 <sup>a</sup>	143.75±5.2 <sup>ab</sup>	159.70±7.6 <sup>b</sup>	143.75±5.2 <sup>ab</sup>
Mean Weight Gain (g)	99.73±7.2 <sup>a</sup>	115.33±7.1 <sup>ab</sup>	135.80±9.8 <sup>b</sup>	117.72±6.9 <sup>ab</sup>
Daily Growth Index	4.04±0.18 <sup>a</sup>	4.56±0.19 <sup>ab</sup>	5.17±0.53 <sup>b</sup>	4.79±0.22 <sup>b</sup>
Growth Coefficient (%)	7.09±0.5 <sup>a</sup>	8.93±0.67 <sup>ab</sup>	11.68±0.91 <sup>b</sup>	9.76±0.38 <sup>ab</sup>
Condition Factor	373.2±21 <sup>a</sup>	421.4±18 <sup>ab</sup>	477.1±28 <sup>b</sup>	442.0±20 <sup>b</sup>
Specific Growth Rate (%)	3.05±0.21 <sup>a</sup>	3.43±0.12 <sup>ab</sup>	3.86±0.19 <sup>b</sup>	3.58±0.11 <sup>b</sup>
Feed Conversion Ratio	2.86±0.01 <sup>a</sup>	2.86±0.01 <sup>a</sup>	2.47±0.01 <sup>ab</sup>	2.20±0.01 <sup>b</sup>

**Discussions and conclusions.** The food of fish consists of a variety of prey organisms and aquatic plants. All these feeds contain a lot of free amino acids (Dabrowski & Rusiecki 1983, De la Noue & Choubert 1985). Fish taste receptors are highly sensitive to free amino acids and induce strong electrophysiological responses in recordings of the nervous activity from gustatory fibers (Marui & Caprio 1992).

The attractant ability of citric acid was evident from the better growth and higher feed intake recorded by beluga juvenile fed with citric acid-added diets over control feed.

The results of the present study also suggest that the efficiency of attractant, citric acid, may vary with the level of inclusion. The attractant ability of citric acid in fishes has

been reported by several authors. The attractant was shown to act as a dietary feeding attractant in rainbow trout (Sudagar et al 2004). Based on comparison of taste performance in herbivorous and carnivorous fish, it was suggested that these two groups of fish have specific amino acids that stimulate feeding (Johnsen & Adams 1986).

In a similar study on *Tilapia sp.* and grass carp L-cysteine and glycine, the indifferent taste substances for tilapia, were stimulants for grass carp (Kasumyan & Morsy 1997, Kasumyan & Morsy 1998). Sturgeon juvenile are not only slow eaters but also nibble the feed for a longer time. The pace at which animal reach and consume their feed is important when artificial pellet are fed, because they rapidly disintegrate in the water. Addition of an attractant in the water stimulates the beluga juvenile to search for feed (Kasumyan & Doving 2003). The growth results in juvenile sturgeon presented here clearly show that the attractability enhanced higher food conversion, feed intake and growth.

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Authors:

Mohammad Sudagar, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: sudagar\_m@yahoo.com

Hosseinpoor Zelti, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: hosseinpoorzelti@yahoo.com

Abbas Hosseini, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: hoseini-abbas@yahoo.com

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