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# Mineral Status of Juvenile Beluga (*Huso huso*) Fed Citric Acid Supplemented Diets

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**Abstract:** A study was conducted to investigate the effects of dietary citric acid (CA) (0, 1, 2 and 3%) on the Calcium(Ca) and Phosphorus(P) content of muscle, scute and serum of Beluga (Huso huso) juveniles (25.1±1.9 g). After 8 weeks feeding on the experimental diets, Calcium(Ca) and Phosphorus(P) content of muscle, scute and serum were measured. The results revealed that addition with 2% and 3% CA significantly increased the Ca and P content of muscle and serum. Dietary CA had no effect on Ca content of scute, but P content of scute was significantly higher compared to additional 1% CA and control. These results indicate that addition of CA to diet of Beluga increased the bioavailability of mineral, thereby increasing muscle and scute mineralzation.

**Key words:** Beluga · Citric acid · Calcium · Phosphorus · Scute · Muscle · Serum

## INTRODUCTION

Among sturgeon of the southern Caspian sea Beluga sturgeon (*Huso huso*) is a good prime candidate for aquaculture because of its high market price, fast growth, reproduction in captivity and because of the accelerating decline of natural population as a result of over fishing [1]. It is a well established fact in the field of aquaculture, that the use of antibiotic growth promoters as an in-feed additive for the diets of fish may promote growth and feed conversion as well as improve survival rates. However public concerns on the development of cross-resistances to humans, have led to a ban or decrease in the use of antibiotic growth promoters as an in-feed additive for the diets of fish worldwide. Consequently, researches have been focusing on other additives in order to maintain performance parameter and high survival rates in aquaculture. The beneficial effects of acid preserved products caught the attention of the scientific community to investigate the effects of short-chain acids onto the fish feed directly [2]. Some authors worked on the effects of diet acidification on growth and mineral utilization in terrestrial animals [3, 4]. A supplemental organic acid reduces intestinal pH and can also bind various cations along the intestine and may act as a chelating agent [5]. Previous research demonstrates that organic acid may improve the production of rainbow trout, Onchorhynchus mykiss [6, 7], red sea bream, *Pagrus major*, [8] and Rohu, *Labeo Rohita* [9]. However, the effect of dietary organic acid and particularly CA on juvenile Beluga, *Huso huso* has not been studied. Like in other animals, P is an essential nutrient for fish, being a major constituent of skeletal tissues, nucleic acids DNA and RNA, energy transport compounds like ATP and of phospholipids in cell membranes [10]. The mechanism of P absorption and transport in fish has not been well studied. Information concerning P metabolism of fish and crustacean is rather limited [11]. Fish meal is the source of most dietary P in fish diets, wherein it exists as hydroxyapatite and/or tricalcium phosphate (TCP). Due to its structural complexity, P and Ca from TCP have been reported to be less available to some fish species [12-14] as a result of which, large amounts of P are excreted in feces, leading to wastage and environmental pollution. Ca, apart from its structural function it also ensures: the coagulation of blood, muscular contraction, nerve transmissions and osmoregulation. Phosphor is necessary for a great number of the essential metabolic functions. It consists of adenosin triphosphate (A.T.P), phospholipids, DNA and RNA. P plays a part in the energetic transformations, the control of permeability of the membranes.

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Boiling *et al.,* (2000) observed that citric acid (2-6%) is very effective in improving P utilization in chickens fed on maize-soybean meal diets containing no supplemental P. however, studies on fish are very limited. Numerous factors have been shown to affect gastrointestinal absorption of mineral elements in fish [6]. Sugiura *et al.,* (1998) also observed an increase in the apparent availability of Ca, P, Mg, manganese (Mn) and iron (Fe) in rainbow trout fed fish meal based diets supplemented with citric acid. Therefore, the current study aimed to investigate the effect of citric acid supplementation on the utilization of phosphorous, calcium by Beluga, *Huso huso*.

## MATERIALS AND METHODS

**Diets and Experimental Design:** The diet (Table 1) was formulated using fish meal and plant meal as protein sources and was supplemented with CA (0, 1, 2 and 3%) (Monohydrous citric acid ($C_6H_8O_7(H_2O)$), analytical crystal, Jining Andy Trading Co., Ltd. China). Required amount of water was added to mixed diet ingredients, to from a dough and pellets (2mm) were prepared using a hand pelletizer. Thereafter, the pellets were dried (10% moisture) using air blower. Achived pellets were sealed in vacuum-packed bags and frozen (-18°C) until use.

**Fish, Experimental Condition and Feeding:** Beluga fingerlings were obtained from Sturgeon Propagation and Rearing Complex of Shahid Marjani þ (Gorgan, Iran). Twenty five fish (average weigh 25.1±1.9 g) was randomly distributed in 12 rectangular tanks (filled by 1000 L water). The tanks were divided to four groups (each consisted of three tanks) as control and CA group. All tanks were received continuous water flow (10 L/min) and aeration during the experimental period. Important water quality parameters such as temperature, PH and salinity were monitored daily and dissolved oxygen was measured fortnightly. Average daily water temperature was 28.9±1.0°C. The fish were fed at 4-5% of body weight, four times a day over 8 weeks.

**Sample Collection and Chemical Analyses:** At the end of the feeding trial, blood samples from three fish per tank were taken for study Ca and P, by caudal severance. blood samples were aliquoted into non-heparinized tubes and left to clot for 12 h (at 4°C), prior to centrifugation at 3,000g for 5 min in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany). Isolated serum was stored at -20°C until further analysis. Serum P levels were analyzed according to method described by Thomasl (1998) using inorganic phosphorus kit (Pars azmon, Iran) and serum levels of Ca determined according to the methods described by Baginski (1973). Dorsal muscle with scutes of beluga was removed from each group of fish and pooled for Ca and P analysis. The scute of fish were boiled for 20min, the excess flesh stripped off from them and the adhering flesh removed by light brushing and rinsing in distilled water. The scutes were then dried for 2 h at 110°C and extracted with anhydrous ethylether for 7 h, pulverized, dried again and weighed. The dried samples (muscle and scute) were ashed at 550°C for 6h. For Ca and P estimation, the ash of scute, muscle and faeces were digested in a boiling nitric acid and perchloric acid mixture (2:1) according to AOAC (1995). After appropriate dilution Ca content was estimated by atomic absorption spectrophotometer (Unicam, England, 919), while P was estimated spectrophotometrically using molybdovanadate method (Biochrom, Libra, S12) at 400 nm [18].

**Statistical Analysis:** Results are expressed as mean±SD. All data were subjected to One-way ANOVA. When significant differences ($P<0.05$) occurred, the group means were further compared with Duncan tests. All statistical analyses were performed using SPSS V.16 (SPSS, IL, USA).

Table 1: Formulation of experimental diet (g/kg dry matter basis)

| Ingredients | Diet |
|---|---|
| Kilka fish meal | 30.0 |
| Soybean meal | 41.5 |
| Barley meal | 2.3 |
| Wheat meal | 6.0 |
| Cottonseed meal | 5.0 |
| Yeast | 3.0 |
| Lecitine | 3.0 |
| Vitamin permix[a] | 1.0 |
| Mineral permix[a] | 1.0 |
| Soybean oil | 3.6 |
| Kilka fish oil | 3.6 |
| Proximate composition (g $Kg^{-1}$) | |
| Dry matter (fresh matter basis) | 909.0 |
| Crude protein (%) | 441.0 |
| Crude lipid (%) | 155.0 |
| Ash (%) | 7.4 |
| Phosohorus (%) | 11.2 |
| Gross energy (cal $g^{-1}$) | 4627.0 |

a Premix detailed by Jalali *et al.,* (2009)

Table 2: Ca and P of muscle, scute and serum of Beluga, *Huso huso* fed on experimental diets

| Parameters | $CA_0$ | $CA_1$ | $CA_2$ | $CA_3$ | P value |
|---|---|---|---|---|---|
| Muscle | | | | | |
| Ash (g 100g wet $wt^{-1}$) | 1.9±0.42$^{b}$ | 2.05±0.21$^{b}$ | 2.6±0.35$^{a}$ | 2.8±0.07$^{a}$ | 0.03 |
| Ca (g $100gr^{-1}$) | 2.78±0.07$^{b}$ | 2.71±0.1$^{b}$ | 3.6±0.1$^{a}$ | 3.76±0.3$^{a}$ | 0.00 |
| P (g $100gr^{-1}$) | 6.00±0.1$^{b}$ | 5.96±0.2$^{b}$ | 7.28±0.12$^{a}$ | 7.33±0.03$^{a}$ | 0.00 |
| Scute | | | | | |
| Ash (g 100g wet $wt^{-1}$) | 57.06±3.05$^{c}$ | 59.00±1.00$^{bc}$ | 61.73±0.37$^{ab}$ | 62.53±0.51$^{a}$ | 0.01 |
| Ca (g $100gr^{-1}$) | 78.20±2.31$^{a}$ | 79.80±1.93$^{a}$ | 82.36±2.02$^{a}$ | 82.53±3.02$^{a}$ | 0.12 |
| P (g $100gr^{-1}$) | 48.91±0.93$^{b}$ | 49.67±1.28$^{b}$ | 53.57±1.39$^{a}$ | 54.40±0.50$^{a}$ | 0.00 |
| Serum | | | | | |
| Ca (mg $dl^{-1}$) | 9.61±1.08 | 9.75±0.31 | 10.18±0.35 | 10.23±0.73 | 0.03 |
| P (mg $dl^{-1}$) | 7.69±0.68$^{b}$ | 8.26±0.70$^{ab}$ | 8.73±0.41$^{ab}$ | 11.53±2.21$^{a}$ | 0.04 |

Values are mean±SD. Values in the same row different superscripts are significantly different ($p< 0.05$)

## RESULT

The proximate composition of the experimental diets are presented in Table 1. All the water quality parameters were observed to be within the acceptable limit for culture. The effects of dietary citric acid on the Ash, Ca and P content of scute, muscle and plasma are summarized in Table 2.

Ca (P=0.03) and P (P=0.04) content of serum were significantly increased in CA supplemented diets (2 and 3% CA) (Table 2).

Ash (P=0.01)of scute and muscle (P=0.03) were significantly higher in the 2% and 3% CA fed fish compared to 1% CA and control fed fish. No variation was observed for Ca content of scute (P=0.12) among treatments. However P content scute (P=0.00) significantly increased in the 2% and 3% CA fed fish compared to other treatments. Also, Ca (P=0.00) and P (P=0.00) muscle significantly increased by additional 2% and 3% CA (Table 2).

## DISCUSSION

This study showed that in the CA group, 2 and 3% CA might have increased Ca and P content of muscle, scutes and serum in beluga. Similar results were observed by other investigators [19, 20, 21]. Vielma *et al.*, (1999) reported that dietary acidification by citric acid significantly increased whole body iron in fish. Citric acid supplementation to rainbow trout diet chelates Ca and P, which increases the solubility of Ca and P and improves mineral utilization [7]. This result could be attributed to two related factors; (1) effect of dietary acidification and solubilization and (2) effect of subsequent chelation of released cations. It has been shown that CA is absorbed across the intestinal brush border membrane via a $Na^{+}$ dependent transport mechanism that seems to be specific for tri- and dicarboxylic acids [22, 23]. Jongbloed (1987) reviewed that lowered intestinal pH increases the solubility of P and phytate and improves P absorption in the intestine. A supplemental organic acid reduces intestinal pH and can also bind various cations along the intestine and may act as a chelating agent. Erdman (1979) reviewed literature and suggested that the phytate molecule binds minerals such as Ca and P. Perhaps CA, a strong chelator of Ca and P, removes them from or decreases Ca and P binding to the phytate molecule, thus making it less stable and more susceptible to endogenous phytase. It shouldn´t be forgotten that 50% of the experimental dietary protein was supplied with plant protein sources that contain phytate, itself. The exact mechanism by which citrate acts in the present study is unknown. In conclusion, the present study indicates that Ca and p content of muscle, scute and serum could be affected by CA, which encourages further investigations into the effects of organic acid on other mineral at the muscle, scute and serum of sturgeons.

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