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Study on Some Hematological and Biochemical Parameters of Juvenile Beluga (*Huso huso*) Fed Citric Acid Supplemented Diet

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Abstract: A study was conducted to investigate the effects of dietary citric acid (CA) (0 and 3%) on some blood parameters of Beluga (*Huso huso*) juveniles (25.1±1.9 g). After 8 weeks feeding on the experimental diets, blood haematological parameters, serum levels of glucose, total protein, calcium (Ca) and phosphorus (P) were measured. The results revealed that dietary CA had no effect on leukocytes (WBCs) count red blood cell counts (RBCs), mean corpuscular volume (MCV), mean cellular haemoglobin (MCH) or mean cell haemoglobin concentration (MCHC) (P>0.05). However, haemoglobin (Hb) concentration and haematocrit (Hct) values were significantly higher (P<0.05) in the 3% CA group, compared to control. While serum glucose and total protein remained unaffected, levels of Ca and P were significantly higher in the CA groups compared to control (P<0.05) but these parameters were not significantly between 3% CA group and control group. These results indicate that CA could improve some haematolgical and biochemical parameters in Beluga which should be taken into account in future studies.

Key words: Beluga · Citric Acid · Haematology · Biochemistry

INTRODUCTION

Great sturgeon or Beluga (*Huso huso*) is one of the sturgeon species with high growth rates and appears to be very suitable for aquaculture. The beneficial effects of acids preserved products caught the attention of the scientific community to investigate the effects of short-chain acids onto the fish feed directly [1]. Some authors worked on the effects of diet acidification on growth and mineral utilization in terrestrial animals [2]. A study by Khajepour & Hoseini [3] on Beluga *H. huso* has shown that citric acids have positive influences on mineral status at serum, muscle and Scute. Previous research demonstrate that organic acid may improve the production of rainbow trout, *Onchorhynchus mykiss* [4] and red sea bream, *Pagrus major* [5]. Haematological parameters (such as erythrocyte numbers and haemoglobin) are regarded as valuable indices for assessing fish health and are reported to be affected by other dietary additives. The effect of dietary organic acid and particularly CA on haematological parameters of juvenile Beluga, *Huso huso* has not been studied. Thus, the aim of this study was to investigate the effects of CA supplementation on the some hematological and biochemical parameters of juvenile Beluga.

MATERIALS AND METHODS

The control diet (Table 1) was formulated using fish meal and plant meal as protein sources. The experimental diet was prepared by adding 3% CA to the control diet which lacks of any CA (Monohydrous citric acid ($C_6H_8O_7$ (H_2O)), analytical crystal, Jining Andy Trading Co. Ltd. China).

Beluga fingerlings were obtained from Sturgeon Propagation and Rearing Complex of Shahid Marjani þ (Gorgan, Iran). Twenty five fish (average weigh 25.15±1.9 g) was randomly distributed in each of the 6 rectangular tanks (filled by 1000 L water). The tanks were divided to two 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

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Table 1: Formulation of experimental diet (g kg dry matter basis^{-1})

Ingredients	Diet
Kilka fish meal	30
Soybean meal	41.5
Barley meal	2.3
Wheat meal	6
Cottonseed meal	5
Yeast	3
Lecithin	3
Vitamin permix[a]	1
Mineral permix[a]	1
Soybean oil	3.6
Kilka fish oil	3.6
Proximate composition(g Kg^{-1})	
Dry matter (fresh matter basis)	909
Crude protein	441
Crude lipid	155
Ash	7.4
Gross energy (KJ g^{-1})	19.40

a Premix detailed by Jalali *et al.* 2009.

Table 2: The effects of dietary citric acid on growth performance, haematological and serum biochemical parameters of beluga juveniles, *Huso huso*. (n=9)

Items	Control	3% CA	P_{value}
Erythrocyte count ($\times 10^6$ μ^{-1})	$0.95^a \pm 0.23$	$0.97^a \pm 0.17$	0.83
Haemoglobin (g dl^{-1})	$7.44^b \pm 0.59$	$8.31^a \pm 0.7$	0.049
Haematocrit (%)	$22.33^b \pm 1.63$	$25.08^a \pm 2.20$	0.034
MCH[1] (pg)	$82.06^a \pm 18.89$	$86.58^a \pm 16.55$	0.66
MCHC[2] (g dl^{-1})	$33.35^a \pm 1.97$	$33.03^a \pm 1.76$	0.77
MCV[3] (fl)	$246.71^a \pm 61.31$	$262.36^a \pm 49.17$	0.63
Leucocyte count ($\times 10^3$ μ^{-1})	$23.800^a \pm 2.75$	$24.866^a \pm 2.17$	0.47
Lymphocyte (%)	$69.00^a \pm 4$	$70.5^a \pm 3.98$	0.53
Neutrophil (%)	$20.66^a \pm 2.58$	$20.66^a \pm 3.61$	1.00
Monocyte (%)	$2.63^a \pm 1.98$	$4.33^a \pm 1.75$	0.14
Eosinophil (%)	$7.33^a \pm 1.5$	$6.16^a \pm 2.85$	0.39
Glucose (mg dl^{-1})	$37.00^a \pm 6.13$	$44.5^a \pm 9.39$	0.13
Total protein (mg dl^{-1})	$2.38^a \pm 0.26$	$2.28^a \pm 0.27$	0.53
Calcium (mg dl^{-1})	$7.98^b \pm 0.76$	$9.73^a \pm 1.71$	0.046
Phosphorus (mg dl^{-1})	$9.20^b \pm 0.53$	$10.11^a \pm 0.62$	0.021

[1] MCH = Hb/RBC [2] MCHC = (Hb × 10)/ Hct [3] MCV = (PCV × 1000)/ RBC

Values are represented as mean ± SD. Values in the same row with

oxygen was measured fortnightly. After 8 week, blood samples from three fish per tank were taken for haematological analysis, by caudal severance. Whole blood was suspended in diluents [6] for erythrocytes (RBCs) and total leukocytes Count (WBCs) using a haemocytometer. Haematocrit (Hct) was determined by the microhaematocrit method as described by Brown [7] and reported as percentage packed cell volume (% PCV). Haemoglobin (Hb) levels were estimated using Sahli's method [8]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from RBC, Hct and Hb according to the following formulae [9] (Table 2).

Differential leucocyte counts (Neutrophil, eosinophils, lymphocytes and monocytes) were conducted on May-Grunwald-Giemsastained blood smears. For serum isolation, blood samples were aliquoted into non-heparinized tubes and left to clot for 12 h (at 4° C), prior to centrifugation at 3000 g for 5 min in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany). Isolated serum was stored at -20°C until further analysis. Glucose levels were

determined by the glucose oxidase method [10]. Serum protein was estimated by the Biuret and bromocresol green (BCG) dye binding method [11] using a kit (Pars Azmoon, Tehran, Iran). Serum P levels were analyzed according to method described by Thomasl [12] using inorganic phosphorus kit (Pars azmon, Tehran, Iran) by Spectophotomerer (Libra, S12, Biochrom, Cambridge, UK) and serum levels of Ca determined according to the methods described by Baginski [13].

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).

RESULTS AND DISCUSION

The effects of CA, on the hematological and biochemical parameters of juvenile Beluga (*H. huso*) are summarized in Table 2. The results of the present study showed that dietary CA had no effects on RBCs and WBCs count (P>0.05). Similar results have been reported in Rohu, *L. Rohita* [14]. WBCs play an important role in nonspecific or innate immunity and their count can be considered as an indicator of the health status of fish. In stress conditions (due to infection, dietary imbalance, etc.), the WBCs count will increase [15]. Lowering the intestinal pH through dietary CA might be stressful to the fish. Data showed that dietary addition of CA (3%) significantly increased (P<0.05) the blood Hb and Hct compare to control diets, in agreement with the results of Baruah *et al.* [14] in Rohu; *L. Rohita.* Acidifying the diet by adding 3% CA reduced the pH of intestinal digesta. So the increase in Hb and Hct levels in the group fed diet containing CA might be the effect of maximum liberation of Ca, P, Fe and Cu from the phytic acid complex by the action of CA, in agreement with the results of Khajepour & Hoseini [3-16] in Beluga *H. huso* and Baruah *et al.* [17] in Rohu, *L. Rohita.* Moreover, blood glucose level also did not vary significantly among the treatments. Similar results were also obtained by Baruah *et al.* [14] in Rohu, *L. Rohita*. These mean that there is no metabolic stress mediated by the experimental diets. The concentration of total serum protein is used as a basic index for health status of fish [18], which plays a significant role in the immune response. In this study, fish fed dietary CA showed no significant differences of total serum protein levels compared with those of control diet (P>0.05). However, in the study carried out by Baruah *et al.* [14] on Rohu, *L. Rohita,* significant increase in total serum protein was found when fed diet supplemented with CA, which is in contrast to our results. In the present study, serum P levels and serum Ca were significantly higher in 3% CA. There is no clear evidence in the literature about the influence of CA on the serum P and Ca of Beluga, *H. huso* or other fish species. Erdman & Jr [19] 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. In conclusion, the present study indicates that fish blood parameters could be affected by CA, which encourages further investigations into the effects of organic acid on physiological and immunological parameters of sturgeons.

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