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Full Length Research Paper

Effect of dietary citric acid supplementation and partial replacement of dietary fish meal with soybean meal on calcium and phosphorus of muscle, scute and serum of Beluga, *Huso huso*

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Beluga is one of the most important fishes in Caspian Sea. The purpose of this experiment were to evaluate the effect of soybean meal (SBM) as a fishmeal (FM) partial replacement and citric acid (CA) supplement on the calcium (Ca) and phosphorus (P) of muscle, scute and serum of Beluga diets. Three isonitrogenous and isoenergetic experimental diets were formulated, they are SBM_1 (soybean protein (SBP): fish protein (FP) = 1:3), SBM_2 (SBP : FP = 2:3) and SBM_3 (SBP : FP = 1:1). Diets were supplemented with CA at 0 and 3%. The fishes (average weigh 25.1±1.9 g) were fed at 3 to 4% of body weight for four times a day over 8 weeks. At the end of the feeding trial, Ca and P of muscle, scute and serum were determined. Ca and P of muscle and serum were significantly higher in 3% CA. P of scute was also significantly higher in 3% CA; however, dietary CA had no effect on Ca of scute. Although the results of this study showed that partial replacement of fish meal with soybean had no effects on the calcium and phosphorus of muscle, scute and serum in beluga.

Key word: Citric acid, soybean meal, calcium, phosphorus, *Huso huso*

INTRODUCTION

Beluga is one of the most important fishes in Caspian Sea and especially in coastal part of Iran and good prime candidate for aquaculture because of its high market price, fast growth, and reproduction in captivity and because of the accelerating decline of natural population as a result of over fishing (Mohseni et al., 2006). Beluga is generally regarded as a carnivorous species. Fishmeal (FM) has been a pro-minent protein source used in most feed formulations for this species. Due to the limited and unpredictable supply of FM, attention has been devoted to the possibility of increasing the inclusion of vegetable protein sources in diet for carnivorous fishes. Soybean meal is considered as a promising alternative protein source be-cause of its availability, high protein content and low P content rela-tive to FM. However, the content of anti-nutritional factors such as phytic acid, are major factor to restrict the use of soybean meal in diets of fish (Storebakken et al., 2000). Major vegetable portion of P (60 to 70%) is bounded to phytic acid. This reduces the availability of P in the fish (Storebakken et al., 1998), as well as the availability of other minerals such as zinc (Zn), magnesium (Mg) and calcium (Ca) (Denstadi et al., 2006; Fredlund et al., 2006).

A supplemental organic acid reduces intestinal pH and can also bind various cations along the intestine and may act as a chelating agent (Ravindran and Kornegay, 1993). Some authors have been studied the effects of diet acidi-fication on growth and mineral utilization in terrestrial animals (Han et al., 1998; Brenes et al., 2003). Previous research demonstrates that the inclusion of organic acid in diets may improve the level of mineral in beluga (Khajepour and Hosseini, 2011), also, supplementation of CA increased growth of beluga (Khajepour et al., unpublished). Like in other animals, P is an essential nutrient for fish, being a major constituent

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Abbreviations: SBM, Soyabean meal; **FM,** fish meal; **CA,** citric acid; **SBP,** soyabean protein; **FP,** fish protein.

Table 1. Formulaton of experimental diets (g Kg^{-1} dry matter basis).

Ingredient	SBM_1	SBM_2	SBM_3
Kilka fish meal	450	370	300
Soybean meal	200	310	415
Barley meal	76	42	23
Wheat meal	80	80	60
Cottonseed meal	50	50	50
Yeast	30	30	30
Lecitine	30	30	30
Vitamin permix[a]	10	10	10
Mineral permix[a]	10	10	10
Soybean oil	32	34	36
Kilka fish oil	32	34	36
Proximate composition(g kg^{-1})			
Dry matter (fresh matter basis)	915	907	909
Crude protein	439	437	441
Crude lipid	145	157	155
Ash	7.95	7.7	7.4
Phosphorus (g/kg diet)	14.3	12.3	11.2
Gross energy (kJ g^{-1})	19.44	18.94	19.40
SBP : FP[b]	1:3	2:3	1:1

[a]Premix detail in Khajepour and Hosseini (2011); [b]SBP (soybean protein) and FP (fish protein).

of skeletal tissues, nucleic acids DNA and RNA, energy transport compounds like ATP and phospholipids in cell membranes (Lall, 1991). Calcium, apart from its structural function also ensures: the coagulation of blood, muscular contraction, nerve transmissions and osmoregulation.

Phosphorus is necessary for a great number of the essential metabolic functions. It consists of adenosine triphosphate (ATP), phospholipids, DNA and RNA. P plays an important role in the energetic transformations, and in the control of permeability of the membranes.

Therefore, the purpose of this study was to evaluate the effect of soybean meal (SBM) as a FM partial replacement and citric acid supplement on the calcium (Ca) and phosphorus (P) of muscle, scute and serum in beluga diets.

MATERIALS AND METHODS

Fish, experimental condition and feeding

Beluga fingerlings were obtained from Sturgeon Propagation and Rearing Complex of Shahid Marjani (Gorgan, Iran). Four hundred fifty fish (average weigh 25.1±1.9 g) was randomly distributed in each rectangular tank (twenty five fish for each tank and 18 tanks filled with 1000 L water). Three isonitrogenous and isoenergetic experimental diets were formulated, as SBM_1 (SBP/FP = 1:3), SBM_2 (SBP/FP = 2:3) and SBM_3 (SBP/FP = 1:1) (Table 1). Diets were supplemented with citric acid at 0 and 3% (Monohydrous CA ($C_6H_8O_7(H_2O)$), analytical crystal, Jining Andy Trading Co., Ltd. China). Required amount of water was added to mixed diet ingredients, to form dough and pellets (2 mm) prepation. All tanks received continuous water flow (10 $Lmin^{-1}$) 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℃. The fish were fed at 3 to 4% of body weight, four times a day for 8 weeks (at hours 06:00, 12:00, 18:00 and 24:00).

Sample collection and chemical analysis

At the end of the feeding trial, blood samples from three fish per tank were taken for study of Ca and P, by caudal severance. Blood samples were aliquoted into non-heparinized tubes and left to clot for 12 h (at 4℃), prior to centrifugation at 3,000 g for 5 min in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany). Isolated serum was stored at -20℃ 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 was 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 scutes of fish were boiled for 20 min, the excess flesh was stripped off from them and the adhering flesh was removed by light brushing and rinsing in distilled water. The scutes were then dried for 2 h at 110℃ and extracted with anhydrous ethyl ether for 7 h, pulverized, dried again and weighed. The dried samples (muscle and scute) were ashed at 550℃ for 6 h. For Ca and P estimation, the ash of scute and muscle 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 expectrophotometrically using molybdovanadate method (Biochrom, Libra, UK) at 400 nm (AOAC, 1995).

Statistical analysis

Results were expressed as mean ± SD. All data were subjected to Two-way ANOVA. When significant differences ($P<0.05$) occurred, mean values of the groups were further compared with Duncan tests. All statistical analyses were performed using SPSS V.16 (SPSS, IL, USA).

RESULTS

The proximate compositions of the experimental diets are presented in Table 1. The effects of dietary CA on the Ca and P content in scute, muscle and plasma are summarized in Table 2. Ca and P content of serum were significantly increased in CA supplemented diets. No variation was observed for Ca content of scute among treatments. However, P content of scute significantly increased in the 3% CA fed fish as compared to other treatments. Also, Ca and P muscle significantly increased by additional 3% CA (Table 2).

DISCUSSION

This study showed that CA might have increased Ca and P content of muscle, scutes and serum in beluga, which

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

Item		SBM_1		SBM_2		SBM_3		P_{value}		
		0% CA	3% CA	0% CA	3% CA	0% CA	3% CA	CA	SBM	CA × SBM
Muscle	Ca (g 100 g^{-1})	2.76^{c}±0.11	3.66^{b}±0.08	2.73^{c}±0.11	3.60^{b}±0.10	2.6^{c}±010	3.96^{a}±0.11	0.00	0.39	0.41
	P (g 100 g^{-1})	6.03^{b}±0.11	7.33^{a}±0.02	5.93^{b}±0.23	7.28^{a}±0.12	6.12^{b}±0.28	7.38^{a}±0.12	0.00	0.37	0.90
Scute	Ca (g 100 g^{-1})	35.2^{a}±2.3	33.53^{a}±3.2	30.80^{a}±1.93	35.37^{a}±2.02	36^{a}±201	37.5^{a}±2.15	0.57	0.90	0.22
	P (g 100 g^{-1})	24.45^{b}±0.93	28.16^{a}±0.16	24.33^{b}±1.27	26.27^{a}±1.39	24.28^{b}±0.40	26.28^{a}±0.7	0.00	0.39	0.27
Serum	Ca (mg dl^{-1})	9.6^{b}±1.08	10.18^{a}±0.35	9.5^{b}±0.30	9.76^{a}±0.35	9.33^{b}±0.04	10.23^{a}±0.73	0.03	0.87	0.89
	P (mg dl^{-1})	8.26^{b}±0.70	8.73^{a}±0.40	7.69^{b}±0.68	10.53^{a}±2.21	7.93^{b}±0.56	8.63^{a}±0.55	0.02	0.39	0.14

Values are mean ± SD; Values in the same row with different superscripts are significantly different ($p<0.05$).
The interaction term was not significant.

is in agreement with other findings observed in red sea bream (Sarker et al., 2005) and rainbow trout (Vielma et al., 1999; Pandey and Satoh, 2008). These results 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 (Wolffram et al., 1990, 1992). Pileggi et al. (1956) further suggested that the antirachitogenic effects of CA in rats could be attributed to a reduction of the inhibitory effect of Ca on phytic acid hydrolysis. Erdman (1979) reviewed literature and suggested that the phytate molecule binds minerals such as Ca. Perhaps CA, a strong chelator of Ca, removes Ca from or decreases Ca binding to the phytate molecule, thus, making it less stable and more susceptible to endogenous phytase. 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. Higher muscle and scute ash content was determined in fish fed diets supplemented with CA in this study which suggests that CA enhanced the mineral utilization of fish meal and plant ingredient might have protected the inhibitory action of dietary components. Similar results were observed by other investigators (Sugiura et al., 1998). Practical diets for fish normally contain plant and animal protein sources; however, these contain mineral inhibitors, particularly phytate and tricalcium phosphate, respectively. Studies also revealed that the growth of rainbow trout and red sea bream might be affected by chemical form of trace minerals (Parveen, 1999). Pandey et al. (2008) showed that CA might have liberated adequate inorganic P from tricalcium phosphate to allow fish to grow. The exact mechanism by which citric acid acts in this study is unknown.

In conclusion, this study indicates that Ca and P content of muscle, scute and serum could be affected by CA diet, which encourages further investigations into the effects of organic acid on other minerals in the muscle, scute and serum of sturgeons.

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REFERENCES

AOAC (1995). Official Methods of Analysis of the Association Official Analytical Chemists, Vol. 1, 16th edn (ed. by Cunni PA). AOAC International. Arlington. USA.
Baginski ES, Marie SS, Clark WL, Zak B (1973). Direct micro-

determination of serum calcium. Clin. Chem. Acta, 46: p. 49.
Brenes A, Viveros I, Arija C, Centeno M, Pizarro M, Bravo C (2003). The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110: 201-219.
Denstadi V, Skrede A, Krogdahl A, Sahlstrom S, Storebakken T (2006). Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*salmo salar* L.) fed graded levels of phytic acid. J. Aquacult. 256: 365-376.
Erdman Jr. JW (1979). Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 56:736-741.
Fredlund K, Isaksson M, Rossander-Hulthen L, Almgren A, Sandberg AS (2006). Absorption of zinc and retention of calcium: dose-dependent inhibition by phytate. J. Trace Elem. Med. Biol. 20: 49-57.
Han YH, Roneker KR, Pond WG, Lei XG (1998). Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. J. Anim. Sci. 76: 2649-2656.
Jongbloed AW (1987). Phosphorus in the feeding of pigs. PhDThesis. Agricultural University of Wageningen, Wageningen.
Khajepour F, Hosseini SA (2011). Calcium and phosphorus status in juvenile Beluga (Huso huso) fed citric acid-supplemented diets. J. Aquacult. Res. doi:10.1111/j.1365-2109.2011.02843.x
Lall SP (1991). Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey CB, Cho CY (Eds.). Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Guelph, Ontario. Canada. pp. 21-35.
Pandey A, Satoh S (2008). Effects of organic acids on growth and phosphorus utilization in rainbow trout *Oncorhynchus mykiss*. J. Fish. Sci. 74: 867-874
Parveen J (1999). Reduction of environmental phosphorus loading from carp culture. Master's Thesis. Tokyo University of Fisheries. Tokyo.
Pileggi VJ, De Luca HF, Cramer JW, Steenbock H (1956). Citrate in the prevention of rickets in rats. Arch. Biochem. Biophys. 60: 52-57.
Sarker MSA, Satoh S, Kiron V (2005). Supplementation of citric acid and amino acid-chelated trace element to develop environment-friendly feed for red sea bream, *Pagrus major*. J. Aquacult. 248: 3-11.
Storebakken T, Refstie S, Ruyter B (2000). Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Drackley JK (Ed.), Soy in Animal Nutrition. Fed. Anim. Sci. Soc. Savoy. IL., USA. pp. 127-170.
Storebakken T, Shearer KD, Roem AJ (1998). Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. J. Aquact. 161: 365-379.
Ravindran V, Kornegay ET (1993). Acidification of weaner pig diets: a review. J. Sci. Food Agric. 62: 313-322.
Sugiura SH, Dong FM, Hardy RW (1998). Effects of dietary supplements on the availability of minerals in fish meal; preliminary observations. J. Aquact. 160: 283-303.
Thomasl L (1998). Clinical Laboratory Diagnosties. 1st ed. Frankfurt: TH-Books Verlagsgesellschsft. pp. 241-7.
Vielma J, Ruohonen K, Lall SP (1999). Supplemental citric acid and particle size of fish bone-meal influence the availability of minerals in rainbow trout *Oncorhynchus mykiss* (Walbaum). J. Aquacult. Nutr. 5: 65-71.
Wolffram S, Hagemann C, Grenacher B, Scharrer E (1992). Characterization of the transport of tri- and dicarboxylates by pig intestinal brush-border membrane vesicles. Comp. Biochem. Physiol. 101A: 759-767.
Wolffram S, B Bisang, B Grenacher, E Scharrer (1990). Transport of tri- and dicarboxylic acids across the intestinal brush border membrane of calves. J. Nutr. 120: 767-774.