Effect of partial replacement of dietary fish meal with soybean meal on some hematological and serum biochemical parameters of juvenile beluga, *Huso huso*

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Abstract

This study was conducted to investigate the effects of partial replacement of dietary fish meal with soybean meal (SBM) on some blood and serum parameters of Beluga (*Huso huso*) juveniles. Three isonitrogenic and isoenergetic diets, as SBM_1 (Soybean meal protein (SBP): Fishmeal Protein (FP) =1:3), SBM_2 (SBP: FP = 2: 3) and SBM_3 (SBP: FP =1: 1) were fed to triplicate groups of fish. After 8 weeks feeding on the experimental diets, blood parameters were measured. The results revealed that of partial replacement of dietary fish meal with soybean meal had no effect on, leukocyte (WBC) levels, red blood cell counts (RBC), mean corpuscular volume (MCV), mean cellular hemoglobin (MCH) or mean cell hemoglobin concentration (MCHC) ($P>0.05$). However, hemoglobin (Hb) concentration and hematocrit (Hct) values were significantly decreased by increasing dietary soybean meal ($P<0.05$). Serum glucose had significantly affected by increasing soybean meal. While total protein, phosphorus or calcium remained unaffected between groups. These results indicated that partial replacement of dietary fish meal with soybean meal could affect on some haematological and biochemical parameters in beluga which should be studied in future.

Keywords: Beluga, Blood parameters, Soybean meal, Fish meal

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Introduction

Culture of critically endangered sturgeon species such as beluga to marketable size reduces pressure on wild populations. Fish meal has been a prominent protein source used in most feed formulations for this species. Due to the limited and unpredictable supply of fish meal, attention has been devoted to the possibility of increasing the inclusion of vegetable protein sources in diet. Soybean meal is considered a promising alternative protein source because of its availability, high protein content and low phosphorus (P) content relative to fish meal (Storebakken et al., 2000). However, the content of anti-nutritional factors such as phytic acid, are major factor to use soybean meal in diets for fish (Storebakken et al., 2000). Major vegetable portion P (60-70%) is bounded to phytic acid. Like other mono-gastric organisms, carnivorous fish lack the enzymes capable of releasing P from phytic acid by hydrolysis. This reduces the availability of P in the fish (Storebakken et al., 1998), as well as the availability of other mineral such as zinc (Zn), magnesium (Mg) and calcium (Ca) (Denstadli et al., 2006; Fredlund et al., 2006). Haematological parameters (such as erythrocyte numbers and hemoglobin) are regarded as valuable tools for assessing fish health and are reported to be affected by other dietary (Ferguson et al., 2010). However, in applied physiology, nutrition and growth are two closely related and complementary subjects which are considered with each other and applying different strategies in this relation have considerably improved animal production (Rahmani and Speer, 2005). There is any information on the effect of soybean on the haematological indices in beluga. In view of the increasing use of soybean as a major source of protein in several communities, there is a need to revisit its effect on some haematological and biochemical parameters with a view to ascertain the quantity of soybean in the diet that can have deleterious effect on the body systems. The present study was therefore designed to investigate the effects of different increasing soybean diet on some haematological and biochemical indices in the beluga.

Materials and methods

Diets and experimental design

Three isonitrogenous (450 g CP kg^{-1} dry matter) and isoenergetic (18.9 MJ gross energy kg^{-1} dry matter) experimental diets were prepared according to the basal diet, as SBM_1 (Soybean Meal Protein (SBP): Fishmeal Protein (FP) =1:3), SBM_2 (SBP: FP = 2: 3) and SBM_3 (SBP: FP =1: 1) (Table 1).

Table 1: Formulation of experimental diets (g kg^{-1} diets)

Ingredients	SBM_1	SBM_2	SBM_3
Kilka fish meal (645)[X]	450	370	300
Soybean meal (485)[X]	200	310	415
Barley meal	76	42	23
Wheat meal	80	80	60
Cottonseed meal	50	50	50
Yeast	30	30	30
Lecithin	30	30	30
Vitamin Permix[Y]	10	10	10
Mineral Permix[Z]	10	10	10
Soybean oil	32	34	36
Kilka fish oil	32	34	36
Chromic oxide	10	10	10
Proximate composition of diets (g Kg^{-1})			
Dry matter	915	907	909
Crude protein	439	437	441
Crude lipid	145	157	155
Ash	8.0	7.7	7.4
Phosphorus	14.3	12.3	11.2
Calcium	19.20	13.79	12.01
Lysine	20.00	19.79	19.67
Methionine	7.10	6.94	6.83
Gross energy (KJ g^{-1}DM)	19.4	18.9	19.4

[X] Values in parentheses are the crude protein content (g kg^{-1}).

[Y] Vitamin mixture (mg or IU if mentioned/kg diet): 5000 IU retinylacetate,; 2000 IU cholecalciferol; 80 IU all-rac-a-tocopheryl acetate; 10 menadione sodium bisulfite; 10 thiamin; 5 riboflavin; 10 pyridoxine; 50 d-calcium pantothenate; 120 niacin; 500 choline; 1 biotin; 5 folic acid; 400 myoinositol; 50 vitamin C,; 0.05 vitamin B12. [Z] Mineral mixture (mg/kg diet): 40 Fe; 150 Zn; 25 Mn; 3 Cu; 5 K; 0.09 Na.

The protein content of the diets was balanced by adjusting barley and wheat meal levels. Fish and soybean oil (V/V = 1:1) were added to keep lipid and energy constant in all treatments. Oil was added to the dry ingredients and dough was prepared with the required amount of water. Pellets (2mm) were prepared using a hand palletizer, air-dried to about 10% moisture and sealed in vacuum-packed bags and frozen (-18 ^{o}C). Beluga fingerlings were obtained from Sturgeon Propagation and Rearing Complex of Shahid Marjani (Gorgan, Iran). Twenty five fish (average weigh 25.15±1.9 g and total length 18.2 ± 3.2 cm) was randomly distributed in each of the 9 rectangular tanks (filled by 1000 L water). The tanks were divided into three groups (each consisted of three tanks). 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.

Blood Samples

Differential leucocyte counts (Neutrophil, eosinophils, lymphocytes and monocytes) were conducted on May-Grunwald-Giemsastained blood smears. For serum isolation, blood samples were into non-heparinized tubes and left to clot for 12 h (at 4ºC), prior to centrifugation at 3,000g for 5 min in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany). Isolated serum was stored at -20ºC until further analysis. Glucose levels were determined by the glucose oxidase method (Asadi et al., 2009). Serum protein was estimated by the Biuret and bromocresol green (BCG) dye binding method (Reinhold, 1953) using a kit (Pars Azmoon, Tehran, Iran). Serum P levels were analyzed according to method described by Thomasl (1998) using inorganic phosphorus kit (Pars Azmon, Tehran, Iran) and serum levels of Ca determined according to the methods described by Baginski (1973).

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.

Results

The effect of partial replacement of dietary fish meal with soybean meal on beluga blood profiles is displayed in Table 2. Erythrocyte levels (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not significantly ($P>0.05$) affected by increasing soybean meal. However, haematocrit (Hct) and total blood hemoglobin content (Hb) were significantly higher in the SBM_1 fed fish compared to SBM_2 and SBM_3 groups ($P<0.05$). Leucocytes (WBC) levels were not significantly influenced by replacement. Additionally, leucocytes types were no affected by replacement. Serum glucose significantly differs between treatments. Fish fed SBM_3 displayed significantly higher ($P<0.05$) Serum glucose levels compared to other groups, but no significant differences serum of total protein levels or phosphorus or calcium were observed ($P>0.05$).

Table 2: Effect of partial replacement of dietary fish meal with soybean meal on some haematological and serum biochemical parameters of juvenile Beluga

Items	SBM_1[1]	SBM_2[2]	SBM_3[3]
Erythrocyte count ($\times 10^6$ μ^{-1})	$0.99^a \pm 0.21$	$0.95^a \pm 0.17$	$0.79^a \pm 0.28$
Hemoglobin (g dl^{-1})	$8.70^a \pm 0.66$	$7.70^{ab} \pm 0.10$	$7.17^b \pm 0.77$
Hematocrit (%)	$26.00^a \pm 2.60$	$24.16^{ab} \pm 1.6$	$22.10^b \pm 1.29$
MCH[4] (pg)	$90.20^a \pm 18.49$	$82.96^a \pm 17.44$	$90.10^a \pm 2.70$
MCHC[5] (g dl^{-1})	$34.13^a \pm 1.17$	$31.93^a \pm 1.67$	$31.43^a \pm 1.58$
MCV[6] (fl)	$265.00^a \pm 58.00$	$259.73^a \pm 51.32$	$291.76^a \pm 73.80$
Leucocyte count ($\times 10^3$ μ^{-1})	$24.00^a \pm 1.99$	$24.50^a \pm 3.57$	$23.10^a \pm 2.16$
Lymphocyte (%)	$71.32^a \pm 4.93$	$68.33^a \pm 4.93$	$69.66^a \pm 3.78$
Neutrophil (%)	$19.33^a \pm 3.05$	$20.00^a \pm 1.00$	$21.33^a \pm 3.78$
Monocyte (%)	$3.33^a \pm 1.52$	$3.66^a \pm 2.51$	$1.60^a \pm 0.52$
Eosinophil (%)	$5.33^a \pm 1.52$	$7.66^a \pm 2.08$	$7.00^a \pm 1.00$
Glucose(mg dl^{-1})	$34.00^b \pm 4.29$	$40.00^{ab} \pm 6.24$	$47.33^a \pm 7.5$
Total protein (mg dl^{-1})	$2.62^a \pm 0.66$	$2.3^a \pm 0.16$	$2.47^a \pm 0.36$
Calcium(mgdl^{-1})	$8.26^a \pm 0.70$	$7.70^a \pm 0.70$	$8.26^a \pm 0.85$
Phosphorus (mg dl^{-1})	$9.60^a \pm 1.08$	$9.50^a \pm 0.60$	$9.33^a \pm 0.94$

[a, b, c] Values in the same row different superscripts are significantly different ($p < 0.05$)

[1] Soybean meal protein: Fishmeal Protein =1:3

[2] Soybean meal protein: Fishmeal Protein = 2: 3

[3] Soybean meal protein: Fishmeal Protein =1: 1

[4] MCH = Hb/RBC

[5] MCHC = (Hb × 10)/ Hct

[6] MCV = (PCV × 1,000)/ RBC

Discussion

The haematological parameters of fish are reported to be affected by a range of factors, which include species, size, age, physiological status, environmental conditions and dietary regime (e.g. quality and quantity of food, dietary ingredients, protein sources, vitamins, probiotics) (Barnhart, 1969; Osuigwe et al., 2005). However, to our knowledge, few studies have extensively assessed the haematological factors of fish, particularly sturgeon, in response to partial replacement of dietary fish meal with soybean meal. The results of the present study showed that dietary soybean meal had no effects on WBC, RBC count and serum of total protein levels. Similar results have not been reported. On the contrary, Alada et al. (2004) showed the significant increase in total plasma protein observed in rats fed soybean diet preparation as compared with those fed normal rat diet, similar to Bolarinwa et al. (1991). Yue and Zhou (2008) showed replacing soybean meal with cottonseed meal decreases WBC and RBC at juvenile hybrid tilapia. In the present study, Hct and Hb were significantly lower by increasing soybean meal of diets.

Similarly, Yue and Zhou (2008) showed replacing soybean meal with cotton seed meal decreases Hct and Hb at juvenile hybrid tilapia. Olaleye et al. (1999) showed either a decrease or no effect on the haematocrit and hemoglobin concentrations of rats fed soybean diets. Indeed, animals fed protein calorie malnourish diets have been reported to have significant reduction in haematocrit and hemoglobin concentration (Bolarinwa et al., 1991). Olaleye et al. (1999) showed that raw soy bean reduced red cell osmotic fragility and could also reduce the haematocrit in the rat depending on the processing methods applied. The presence of trypsin inhibitor in soy protein was ascribed to be responsible for these effects.

The present study revealed that partial replacement of dietary fish meal with soybean meal had adverse effects on some haematological parameters of beluga. These results may be attributed to the presence of antinutrional factors in SBM and an adverse effect of phytate on bioavailability of various dietary components and nutrient digestibility, which is in agreement with other findings observed in beluga (Khajepour and Hosseini, 2011a, 2011b, 2011c; Khajepour et al., 2011), rainbow trout (Sugiura et al., 2001), red sea bream (Biswas et al., 2007) and Korean rock fish (Lim et al., 2003). However, the few data that have been published on the effect of the dietary soybean on haematocrit and hemoglobin values are not always consistent. Serum glucose levels significantly were decreased by dietary soybean. Serum total protein, phosphorus and calcium were no affected significantly by increasing dietary soybean. The exact mechanism by which dietary soybean on the serum biochemical parameters affect in the present study is unknown.

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