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# EFFECTS OF SOYBEAN MEAL BASED DIET ON GROWTH PERFORMANCE AND HEMOLYMPH BIOCHEMICAL PARAMETERS OF NARROW-CLAWED CRAYFISH (*Astacus leptodacylus* ESCHSCHOLTZ, 1823)

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**ABSTRACT**

Like other crustaceans, narrow-clawed crayfish (*Astacus leptodacylus* Eschscholtz, 1823) can change its diet to the available food during varied life cycles. Diet alteration can affect different biological indices of this species, therefore this study aims at studying changes in growth indices, hemolymph biochemical parameters and biochemical quality of its carcasses, which might occur during diet change of crayfish. The purpose of this experiment was to evaluate the effects of partial replacement of fish meal with soybean meal on growth performance, carcass quality and hemolymph biochemical parameters of narrow-clawed crayfish. 90 healthy adult narrow-clawed crayfish (W=35.50±4.05 g; TL=16.96±1.92 cm) were randomly distributed into 9 fiberglass tanks (200 L) and were fed for 45 days with three varied diets including: commercial shrimp diet, fishmeal-based diet (A) and soybean meal-based diet (B). The results show that changing the diet from animal protein to plant protein caused a significant decrease in the cholesterol and triglyceride levels in hemolymph, as well as carcass fat of the crayfish nourished with B diet when compared with the crayfish fed with A diet. No significant changes of hemolymph levels of glucose, AST and ALT were found between different treatments during this experimental period. In conclusion, it was found that though growth performance reduced, the increased rate of soybean meal in diet from 0.0% to 76% had no adverse effects on biochemical parameters.

## INTRODUCTION

Narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823) is the largest known invertebrate in freshwater ecosystems. It constitutes a significant biomass of benthic species (Holdich, 2002) and is an endemic crustacean in Iran inhabiting only the River Aras in West Azarbaijan, the Anzali Lagoon and also some rivers in Gilan province (Nekuie Fard et al., 2011). This species is also found in many Eastern European countries, Turkey and Australia (Sepici-Dinçel et al., 2013). Most species of narrow-clawed crayfish are nocturnal and find their fellows, food and prey via their chemical and mechanical sensors (Nystrom, 2002). Like other crustaceans, this species can change its diet alternatively at different times, which means that determining the status of this species in the food chain depends on the amount and type of available food. The natural food preference of crayfish depends on their age and stage of life cycles. Their larvae are carnivorous animals feeding primarily on zooplankton, especially small crustaceans, while the post-larvae and adults are omnivorous animals feeding on algae, aquatic plants, carcasses of died aquatic animals, aquatic invertebrate animals such as molluscs, aquatic insects, worms and smaller crustaceans (Nystrom, 2002; Figueiredo and Anderson, 2003; Johnston and Robson, 2009).

Studies show that the change of biological and non-biological factors may cause alterations in growth indices, hemolymph biochemical indices and biochemical analysis of their carcasses. The change of hemolymph biochemical indices of freshwater crayfish (*Parastacus defossus*) in varied seasons for both males and females (Buckup et al., 2008) confirms that biological conditions such as life cycle, sexual puberty and reproduction, molting and accessibility of food, and non-biological factors such as light period, temperature, pH and soluble oxygen in water can severely affect physiological and biological parameters of crustaceans (Rosa and Nunes, 2003a,b; Vinagre et al., 2007). However, the results of most studies show that feeding this species with plant protein sources, such as soybean, does not have an adverse effect on its growth indices (Thompson et al., 2005; McClain and Romaire, 2009). Among plant protein sources, soybean is regarded as a good candidate for substituting fish meal in aquaculture diet due to having favourable amino acid profile as well as abundance and appropriate price (Dersjan-Li, 2002; Watanabe, 2002). However, it is worth noting that, like any other plant protein source, soybean has many anti-nutritional compounds including trypsin inhibitors, lectins, oligosaccharides, soy antigens, phytoestrogens, phytic acid, antivitamins and saponins (Francis et al., 2001; Dersjant-Li, 2002) which can adversely affect the growth performance, food digestibility, physiological status and general health of aquatic species.

In many cases, however, using soya in diet has no adverse effect on the growth and health of aquaculture (Khan et al., 2003a,b) and is favourable to aquacultures and its usage has increased in some species (Tomás et al., 2005; Ogunkoya et al., 2006). Recently, practical efforts have been done at the optimal utilization of soybean meal in the feeding of aquatic species. For example, soybean meal has been used in diets for sea turbout, *Psetta maeotica*, (Yigit et al., 2010), white shrimp, *Litopenaeus schmitti*, (Alvarez et al., 2007), sharpsnout sea bream, *Diplodus puntazzo*, (Hernandez et al., 2007), Cobia, *Racycentron canadum*, (Hsu, 2005), Japanese flounder, *Paralichthys olivaceus,* (Masumoto et al., 2001), Pacific white shrimp, *Litopenaeus Vannamei*, (Davis and Arnold, 2000; Samocha et al., 2004; Suárez et al., 2009), European sea bass, *Dicentrarchus labrax*, (Tulli et al., 2000), gilthead sea bream, *Sparus aurata*, (Kissil et al., 2000), Dentex, *Dentex dentex* (Tomás et al., 2009), *Catla catla* (Priyadarshini et al., 2011) and red sea bream, *Pagrus major* (Takagi et al., 2001). Nevertheless, variation in diet formulation as well as individual features of the specific species are among the most important reasons for difference in the diet response of aquaculture to soybean in their diet. In other words, varied species sensitivity and their ability to use this protein source are so different (Refstie et al., 2000).

Since our knowledge on nutrition requirements of cultured species of freshwater narrow-clawed crayfish is so limited, most farmers produce diet for this species based on their diet in nature. Therefore, with regard to freshwater narrow-clawed crayfish compatibility in using animal and plant protein sources and considering difficulties in supplying raw food materials, farmers prefer to use low-cost sources for cultivating this species. Due to lack of knowledge on the effects of using plant protein sources on growth index, biochemical and physiological factors and carcass quality of narrow-clawed crayfish, conducting studies on this issue is a necessity. Therefore, studying changes in growth indices, hemolymph biochemical indices, as well as carcass quality of this species can not only be effective in monitoring and evaluating the health of this species, but also it is effective in economic justification of cultivating this species with low-cost diets. Therefore, this study aims at investigating growth indices, hemolymph biochemical indices and carcass quality of narrow-clawed crayfish (*A. leptodacylus* Eschscholtz, 1823) treated with different proportions of animal and plant protein in diet.

## MATERIALS AND METHODS

### *Animal*

Healthy adult crayfish (average weight and length 35.50±4.05 g; 16.96±1.92 cm, respectively) were purchased from Maku, West Azerbaijan Province, Aras Shrimp Company, Iran and were transferred to the aquaculture laboratory of the Natural Resource Faculty, Bahbahan University of Technology, Iran. The crayfish were randomly divided into nine groups (three treatment with triplicate) each consisting of 10 animals and were stocked in the 200 L fiberglass tanks with semi-closed water recirculating systems for at least two weeks to acclimate to laboratory conditions (17±2 °C; 7.4±0.2 pH; light regime of 8 hours ligh: 16 hours dark; 20% water exchange rate/day) prior to experiments. The floor of the tanks was covered with gravel, broken bricks and lengths of drainpipe to act as shelters, and limestone chippings in order to maintain calcium concentrations in water. During acclimation, crayfish were fed with the fresh fish meal based on the recommendations of Aras Shrimp Co experts.

### Diet preparation

The feed ingredients chosen for preparing the experimental diets were fresh fish meal (Kilka fish, Clupeonella cultriventris) and soybean meal as protein source, wheat flour, corn flour and rice flour as carbohydrate source and sunflower oil as lipid source, albumen as blender and zeolite as filler. These materials were purchased from the local market. Supplementary minerals and vitamins were obtained from the Razak Damloran Drug Co. Tehran, Iran.

The minced fresh fish meal and the dry pulverized feed ingredients such as soybean meal, wheat flour, corn flour and rice flour were accurately weighed and mixed in a blender. The mixture was moistened with water containing the required amount of gelatin and steam cooked for 30 minutes. After cooling, sunflower oil, vitamins and minerals were mixed uniformly in a kneader. The albumen was used as plate binder. The dough baked was passed through a meat grinder, producing extruded string shapes approximately 10 mm long which were dried in oven at 55 °C overnight. After drying, the pellets were broken into small crumbs and were packed in airtight containers and stored at -18 °C in a freezer until used. The prepared feeds were analyzed for proximate composition. Commercial formulated shrimp food used as formulated crayfish food in Iran was used as control (reference diet) with composition and proximate analysis reported in Table 1.

### Feeding trial

At the end of this adaptation period, twelve crayfish were randomly sampled from each treatment for biometric analyses before initial feeding trial. Following adaptations, all animals were fed experimental diets (commercial shrimp pellets as reference diet, A diet and B diet) at 5% of their wet body weight, twice a day for 45 days (Table 1). Mortalities were recorded daily. Uneaten feed and feces were removed by siphoning as required. Animals were fed at least 24 hours before sampling. At the end of the experimental period, 12 crayfish per treatment were randomly captured, then the total length and total weight were determined for each individual crayfish; hemolymph was collected from the pericardium using a 1 cc syringe. After bleeding, they were sacrificed and their exoskeleton was removed under sterile conditions and washed in normal saline solution. Then, crayfish carcasses (gutted body) were immediately pooled, minced, dried and ground to be analyzed for the final whole body composition after washing and weighting.

Table 1. Ingredients and proximate nutrient composition of experimental diets (values expressed as feed basis, g/100 g)

| Ingredient | Reference diet[a] | A diet | B diet |
|---|---|---|---|
| Fish meal | 60 | 51.5 | 18 |
| Soybean meal | 0 | 12 | 57 |
| Corn grain | 10 | 6.5 | 6 |
| Wheat flour | 12 | 17 | 5 |
| Rice bran | 0 | 6 | 6 |
| Safflower oil | 2 | 2 | 2 |
| Vitamin premix[b] | 1 | 1 | 1 |
| Mineral premix[c] | 1 | 1 | 1 |
| Egg | 0 | 1 | 1 |
| Spinach | 0 | 0 | 1 |
| Zeolite | 2 | 2 | 2 |
| DL-Methionine | 0.159 | 0 | 0 |
| L-Lysine | $5.7 \times 10^{-3}$ | 0 | 0 |
| Proximate composition | | | |
| Dry material (%) | 92.54 | 93.44 | 93.31 |
| Metabolic energy (Kcal/g) | 350.24 | 323.16 | 354.58 |
| Crud protein (%) | 40.22 | 40.74 | 40.26 |
| Ether extract (lipid) (%) | 10.49 | 8.04 | 13.3 |
| Ash (%) | 7.86 | 9.83 | 7.13 |
| Crude fiber (%) | 5.79 | 7.10 | 8.70 |
| Carbohydrate | 27.56 | 27.85 | 24.26 |

[a]Dietary reference componentes are written according to personal correspondence with experts from the Aras Shrimp Company, Iran.
[b]Mineral mix (g/kg diet): 0.5 KCl; 0.5 $MgSO_4.7H2O$; 0.09 $ZnSO_4.7H_2O$; 0.00234 $MnCl_2.4H_2O$; 0.005 $CuSO_4.5H2O$; 0.005 KI; 0.00025 $CoCl_2.2H_2O$; 2.37 $Na_2HPO_4$.
[c]Vitamin mix (unit in mg/kg except where given): 5000 IU retinol; 4000 IU cholecalciferol; 100 mg $\alpha$-tocopherol acetate; 5 mg menadione; 60 mg thiamin; 25 mg riboflavin; 50 mg pyridoxine HCl; 75 mg pantothenic acid; 40 mg niacin; 1 mg biotin; 400 mg inositol; 0.2 mg cyanocobalamin; 10 mg folic acid.

### Growth parameters

All crayfish were deprived of food for 24 hours before weighing and sampling. Growth parameters, such as survival percentage, weight gain percentage, specific growth rate, feed conversion ratio, were calculated by following these formulas at the end of a 45 day trial.

$$\text{Survival} = \frac{\text{Final number of crayfish}}{\text{Initial number of crayfish}} \times 100$$

$$\text{Weight gain (\%)} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \\ = \frac{\text{Ln (final body weight)} - \text{Ln (initial body weight)}}{\text{experimental periods}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

### *Biochemical Analysis*

Plasma levels of glucose, total protein, cholesterol and triglycerides were determined in plasma by standard procedures used in clinical biochemistry laboratories based on manual biochemical kits (Pars Co, Iran). Lactate dehydrogenase (LDH) activity determination is based on measuring the conversion of pyruvate to L-lactate by monitoring the oxidation of NADH. Aspartate aminotransferase (AST) was assayed in a coupled reaction with malate dehydrogenase in the presence of NADH. In alanine aminotransferase (ALT) assay, the enzyme reacts with alanine and a-ketoglutarate to form glutamate and pyruvate.  Pyruvate is converted by lactate dehydrogenase to make lactate and $NAD^+$. All these activities were monitored by measuring the change in absorbance at 340 nm. Alkaline phosphatase (ALP) assay is based on the enzyme-mediated conversion of p-nitrophenol phosphate to nitrophenol in an alkaline buffer at 405 nm. All biochemical parameters were measured in duplicate by UV/Vis spectrophotometer in the biology laboratory.

### *Biochemical body composition analysis*

At the termination of the 45 day trial, survived crayfish from each experimental group were sampled and sacrificed for proximate analysis. The gutted bodies of crayfish were minced and weighed, then moisture was determined by drying pre-weighed samples in porcelain cups at 100 °C for 24 h. Ash was obtained by incinerating the dried samples at 500 °C for 12 h. Crude protein was determined according to the Kjeldahl method by measuring the total nitrogen content of the sample multiplied by the empirical factor 6.25. Soxhlet extraction with petroleum ethers was used for crude lipid at 60-80ºC for 12 h. All analyses were performed in triplicate.

### *Statistical analysis*

Statistical analyses were performed using SPSS (Release 15) software (SPSS Inc. 2006). Data are presented as mean ± SD. All the data were tested for normality (Kolmogorov-Smirnov test). Data were analyzed by one-way of variance analysis (ANOVA). The significant means were compared by Tukey's test and $p < 0.05$ was considered statistically significant.

## RESULTS

The crayfish which were fed with diet (A) had bigger affinity for feed. Also, molting frequency and mortality percentage, as well as cannibalism was lower among the crayfish nourished with diet (A) compared with other groups. The final weight, specific growth rate (SGR) of the crayfish treated with diet (A) were significantly higher than of the crayfish fed with diet (B) ($p<0.05$). The results indicate a significant difference in food conversion ratio (FCR) among the crayfish fed with diet (A) and the crayfish nourished with diet (B) ($p<0.05$) (Table 2).

Table 2. Initial weight, final weight, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were calculated for the crayfish fed diets containing different levels of soybean

| Growth Parameters | Diets | | |
|---|---|---|---|
| | Reference diet | A diet | B diet |
| Initial weight (g) | 35.06±6.41$^{a}$ | 36.39±3.54$^{a}$ | 35.05±2.21$^{a}$ |
| Final weight (g) | 47.09±7.32$^{a}$ | 52.54±4.18$^{b}$ | 46.29±4.37$^{a}$ |
| WG (%) | 35.67±11.95$^{ab}$ | 44.04±11.27$^{b}$ | 32.06±8.88$^{a}$ |
| SGR | 0.67±0.19$^{ab}$ | 0.81±0.18$^{b}$ | 0.61±0.15$^{a}$ |
| FCR | 2.43±0.64$^{ab}$ | 1.92±0.53$^{a}$ | 2.68±0.74$^{b}$ |
| SR (%) | 76.67 | 80.00 | 76.67 |

Values in the same row with different letter notation statistically significantly different at $p<0.05$;

Table 3. Moisture, crude protein, lipid and ash content in gutted body were estimated for the crayfish fed diets containing different levels of soybean

| Biochemical parameters | Diets | | |
|---|---|---|---|
| | Reference diet | A diet | B diet |
| Moisture (%) | 83.53±1.78[a] | 85.70±2.16[a] | 85.22±1.86[a] |
| Protein (%) | 74.00±11.88[a] | 78.67±6.77[a] | 75.15±10.32[a] |
| Lipid (%) | 23.95±2.71[b] | 22.64±2.96[b] | 18.93±1.98[a] |
| Ash (%) | 6.44±0.13[a] | 7.63±0.21[b] | 7.52±0.32[b] |

Values in the same row with different letter notation statistically significantly different at p<0.05;

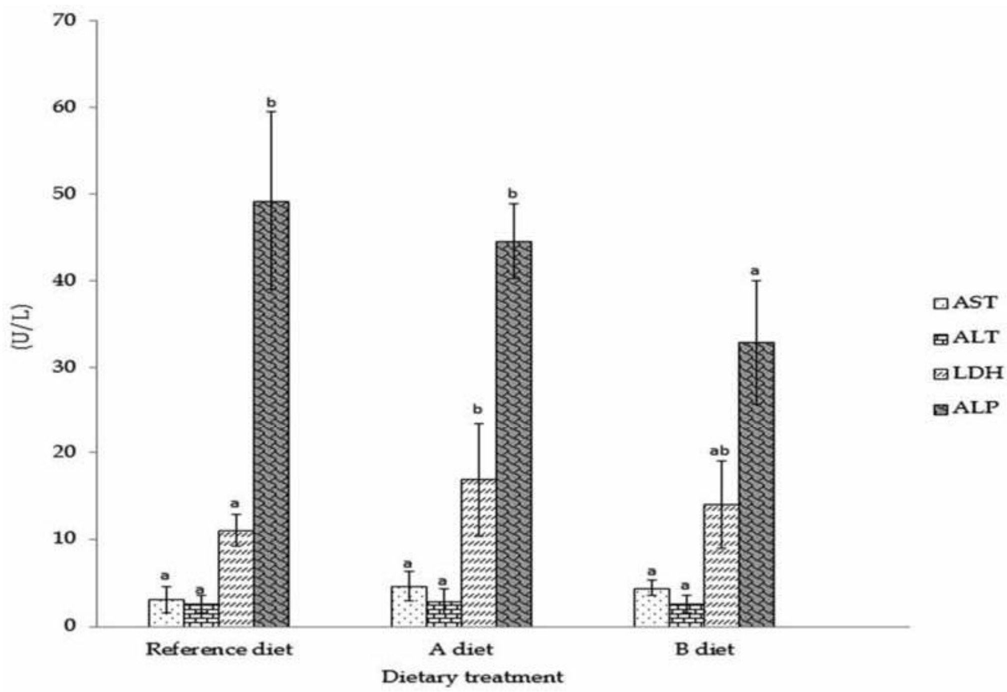


Fig. 1. Enzyme activity levels in the hemolymph of the crayfish fed with diets containing different levels of soybean meal

Carcass analysis of crayfish treated with varied diets shows no significant difference between moisture percentage and carcass protein percentage in different groups (p>0.05), while fat content in the crayfish treated with diet (B) and ash content in the crayfish treated with commercial shrimp diet compared with other groups were significantly lower (p<0.05); (Table 3). Alkaline phosphatase activity level in the hemolymph of the narrow-clawed crayfish treated with diet (B) compared with other experimental groups was reduced significantly (p<0.05). Lactate dehydrogenase activity level in the hemolymph of the crayfish treated with diet (A) was significantly higher than the activity level of this enzyme in the hemolymph of the crayfish treated with commercial shrimp diet (p>0.05). No significant change was observed in the activity level of aspartate aminotransferase and alanine aminotransferase in the hemolymph of the crayfish treated with varied diets (p<0.05); (Figure 1).

There is no significant difference in hemolymph glucose level of narrow-clawed crayfish in varied treatments (p>0.05). The results show that cholesterol level in the hemolymph of the narrow-clawed crayfish fed with diet (B) is significantly lower (p<0.05) compared with other groups. Triglyceride level in the hemolymph of the crayfish fed with diet (A) was significantly higher than the level of this biochemical index in the hemolymph of the crayfish fed with diet (B) (p<0.05); (Figure 2). Although total protein level in the hemolymph of the crayfish fed with diet (A) is significantly higher than that of the crayfish fed with commercial shrimp diet (p<0.05), there is no significant difference between total protein level in the hemolymph of the crayfish fed with diet (B) and that of other groups (p>0.05); (Figure 3).

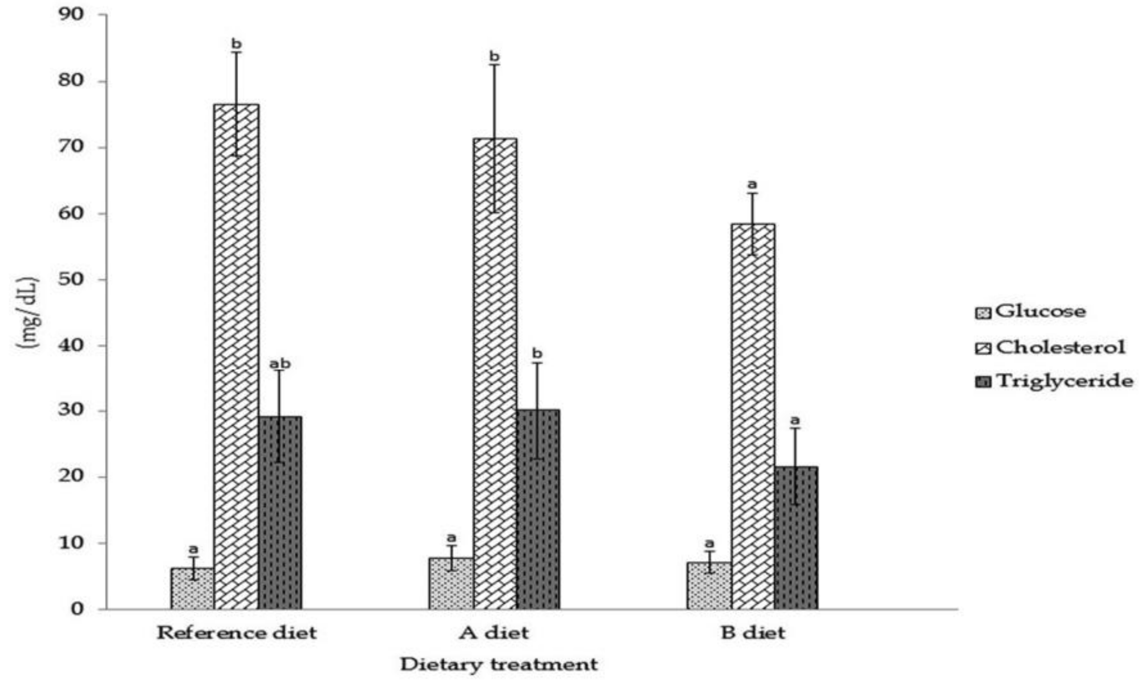


Fig. 2. Hemolymph biochemical parameters of the crayfish fed with diets containing different levels of soybean meal

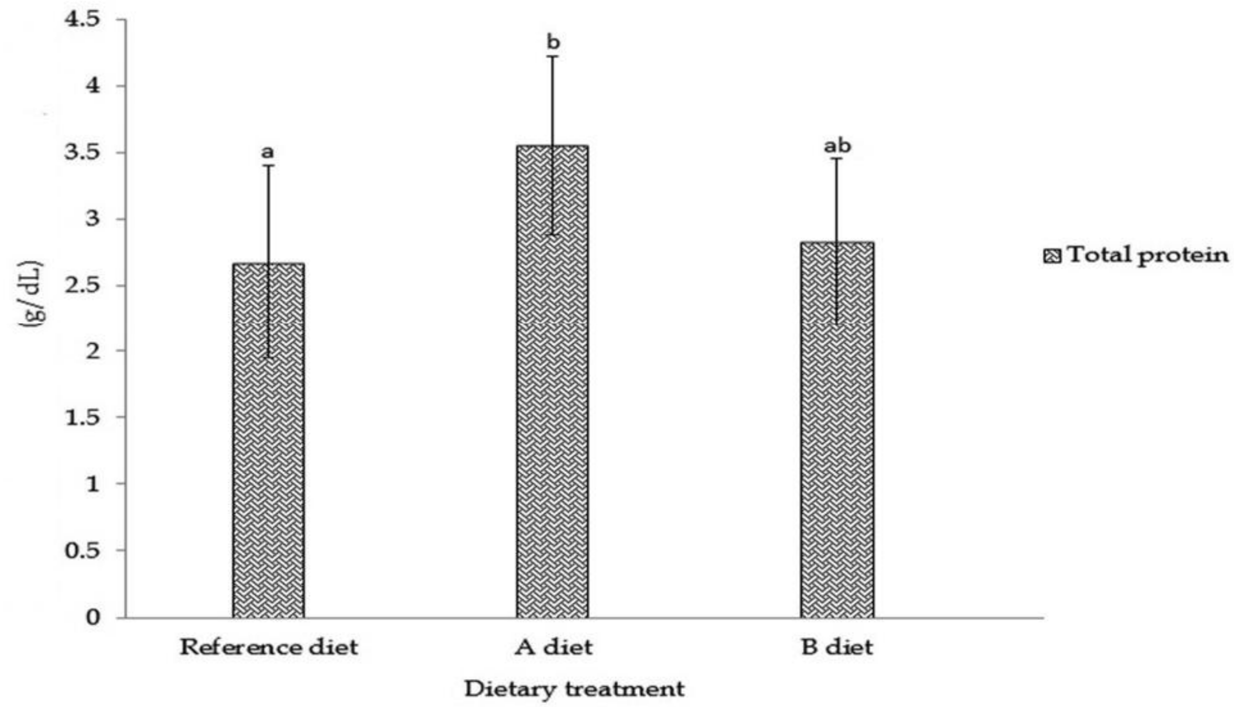


Fig. 3. Hemolymph biochemical parameters of the crayfish fed with diets containing different levels of soybean meal

## DISCUSSION

Narrow-clawed crayfish is the only native freshwater crayfish species in Iran that has been a candidate for breeding species in aquaculture industry of Iran in recent years. Although, many attempts have been made to culture this species, the lack of knowledge about the nutritional needs and favourite foods of this species has led to failure of these efforts. The use of different food sources including animal protein, plant protein source or a combination of both in crayfish diet can help expand our knowledge in order to provide a proper diet for rearing this species. The optimal protein requirements for most cultivated crayfish have shown levels of 25 to 60% in the dry diet (Amaya et al., 2007).

Fish meal is the most important protein ingredient used in diets of farmed fish and crustaceans, and also serves as a food ingredient in human diets. Fish meal contains high levels of essential amino acids and fatty acids. The soybean meal has high potential to be used as a supplement and substitute for fishmeal as a protein source in the diets of aquatic species (Dersjant-Li, 2002). Although the adverse effects of soybean meal in food diet of cultured aquatic species on growth and development, indices and biochemical parameters are verified (Wang et al., 2006; Førde-Skjærvik et al., 2006; Venou et al., 2006) in most studies, there is no report on crustaceans growth reduction or adverse effect of soybean as an alternative to fish meal (García-Ulloa, 2003; Muzinic et al., 2004; Thompson et al., 2005, 2006; Campana-Torres et al., 2005, 2006).

Considering the amount of food remaining in the food trays, the crayfish which were fed with a higher percentage of fish meal were more interested in food, which might be due to balanced amino acid profiles in their diet. Lower molting, mortality and cannibalism were observed in groups fed with diets higher on fishmeal. Lack of some essential animal acids including methionine and cystine compared with fish meal (Peres and Lim, 2008), or the presence of some anti-nutritional materials in plant protein sources (Krogdahl et al., 2010), are accounted for certain deficiencies in species and can affect their appetite and increase tendency to cannibalism. It is worth noting that molting among crayfish is the major driving factor in increasing cannibalism in this species. However, by providing them shelters, cannibalism was resolved to a great extent.

Final weight of the crayfish fed with diet A was significantly higher. Therefore at first it might seem that utilization of a diet having a higher percentage of animal protein is better, compared with a diet having a higher percentage of plant protein. Our results show that increasing the rate of soybean meal in diet can decrease the weight gain percentage, special growth rate and increase the feed conversion ratio (FCR) (Table 2). The results of studies carried out by Changizi et al. (2010) and Ghiasvand et al. (2012) show that using varied levels of protein in diet of *Astacus leptodactylys* could have impressive effects on growth indices such as the increase of final weight growth, average body weight, food conversion ratio, protein efficiency and special growth rate. In contrast to our finding, using soybean as a plant protein source in diet of other crustaceans and cultured crayfish could have similar results to those when fish meal is used as an animal protein in diet (McClain and Romaire, 2009). For example, in the study done on growth indices of Australian red claw crayfish (*Charas quadricarinatus*) no significant difference was observed in a group which was fed with a diet containing 35% of raw protein of plant origin such as soybean and a group which was fed with protein of animal origin (Thompson et al., 2005).

Since carcass quality determines marketability of a product, farmers pay attention to this factor. According to the obtained results, there is a significant difference between animal protein level in the diet and fat content in the carcass; i.e. with the increase of fish meal as an animal protein source in the diet, fat level of the carcass increases. This might be due to the fact that protein level might be more than a physiological need; therefore an extra amount of protein is stored in the form of fat or carbohydrate (Cowey and Sarjent, 1979). However, fat percentage in the carcass of the crayfish fed with a diet containing a higher ratio of soybean was significantly lower compared with that of other groups, which might be due to the influence of soybean compounds on the lipid metabolism process (Sugiyama et al., 1980). The analysis of crayfish carcass shows that by changing the diet, no significant change is observed in the moisture and protein percentage of carcasses (Table 3). Similar results were observed in studies done on *Eriocheir sinesis*; in these crustaceans the change of protein level and source had no impact on the protein level, and source had no impact on the protein level of young crayfish carcasses (Mu et al., 1998). However, in a similar study done on *Eriocheir sinensis*, the increase of protein level and fish meal proportional to soybean in the diet, protein level of carcass increased as well (Chen et al., 1994).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in varied tissues such as hepatopancreas, gill, muscles and the hemolymph of crustaceans (Berges and Ballantyne, 1991; Jiang et al., 2012). So, activity level of these enzymes was used as a biomarker to assess the hepato-pancreas health; because it is expected that using plant protein source which contains anti-nutritional compounds might disturb the performance of hepatopancreas activity and affect the synthesis level and activity of AST and ALT. However, with regard to the absence of significant changes in the activity level of aspartate aminotransferase and alanine aminotransferase of the hemolymph in the crayfish fed with different diets, we can assert that the change of protein source had no effect on the activity level of this enzyme in the hemolymph of narrow-clawed crayfish (Figure 1).

Lactate dehydrogenase is one of the most important active enzymes participating in anaerobic pathway of carbohydrate metabolism which is found in different tissues of crustaceans, such as hemolymph, ovary, gills, hepatopancreas, muscles and spermatheca (Chourpagar and Kulkarni, 2009; Sreenivasan et al., 2010; Banaee and Ahmadi, 2011). Therefore, any kind of change in the activity level of this enzyme illustrates a change in the process of anaerobic metabolism; in such a case the process of converting pyruvate to lactate and the regeneration of glucose is accelerated (Banaee and Ahmadi, 2011). However, there was a significant difference in the activity level of lactate dehydrogenase in the hemolymph of the crayfish fed with a diet containing a higher percentage of fishmeal compared with the activity level of the same enzyme in the hemolymph of the crayfish which were fed with commercial shrimp diet. But the absence of a significant difference between the activity level of LHD enzyme in the hemolymph of the crayfish which were respectively fed with animal and plant protein source indicates that the change of diet in this species had no effect on the activity of this enzyme (Figure 1).

Alkaline phosphatase plays a significant role in phosphate hydrolysis and in membrane transport. It is also a good clinical biomarker of health status of aquatic species which is found in different tissue of crustaceans such as hemolymph, gills, muscle and hepatopancreas (Sreenivasan et al., 2010; Banaee and Ahmadi, 2011). ALP has a significant role in cell membrane and could be used as a proper biological index for assessing the health of cell membrane. Measuring the activity level of this enzyme in investigating the performance of varied tissues especially hepatopancreas is of a great importance (Banaee and Ahmadi, 2011). The present study suggests that we can increase the rate of soybean meal in diet without negative effects on the ALP level in the hemolymph of crayfish (Figure 1).

Based on the obtained results, glucose level in the hemolymph of the crayfish treated with varied diets does not show a significant difference. Glucose is a basal monosaccharide which is found in the hemolymph of crustaceans and has a great role in their physiology, including the synthesis of muco-polysaccharides, synthesis of chitin, ribosome and reduced nicotine amide dinucleotide phosphate (NADPH), the production of pyruvate, glycogen synthesis and is also an energy generation source (Chang and O'Conner, 1983). Stable level of glucose in hemolymph is essential for regulating the performance and activity of neural, muscular and reproductive system of crustaceans. Also, glucose can accumulate as glycogen in hepatopancreas and other tissues such as muscles and gill (Vinagre and Da Silva, 2002; Oliveira et al. 2003). Production and storage cycle of glycogen and glucose level of hemolymph are so varied and can change depending on varied factors: stages of growth, molting, season, diet, nutritional condition, circadian cycle, salinity and dissolved oxygen level in water (Oliveira et al., 2004a,b). Therefore, the absence of glucose level change of hemolymph indicates that the change of plant protein proportion to animal protein proportion of the diet did not have any effects on the level of this biochemical factor (Figure 2).

The absence of fat tissue in crustaceans has made hepatopancreas the most important fat storage place, yet lipids can accumulate in muscle tissue and gonads of females (García et al., 2002). For instance, in burrowing crab *Chasmagnathus granulatus* the total lipid level was more than 20% of hepatopancreas weight (Kucharski and Da'Silva, 1991). Therefore any kind of change in the cholesterol level and triglyceride of hemolymph can act as an index in assessing the health of hepatopancreas. A lower level of cholesterol in the hemolymph of the narrow-clawed crayfish fed with diet B compared with commercial and diet A was found, as well as a significant decrease of triglyceride level in the hemolymph of the crayfish fed with diet B when compared with the triglyceride level in the hemolymph of the crayfish fed with diet A (Figure 2). Since arginine amino acids have higher ratio than lysine amino acids (the same is also true for glycine in comparison to methionine in soybean protein), a decrease of cholesterol in the crayfish which were fed with plant diets is expected. Likewise, a higher proportion of methionine and lysine amino acids in fish meal protein could lead to a higher cholesterol level in the blood (Sugiyama et al., 1980; Morita and Oh-Hashi, 1997). The presence of isoflavones such as genistine, daidzein and glyctin in soybean (Setchell and Raad, 2000) could decrease the fat level of blood in animals under diet, due to having anti-oxidant, anti-bacterial and anti-inflammatory features (Verdrengh et al., 2003).

Although total protein level in the hemolymph of the crayfish fed with a higher proportion of animal protein was significantly higher than that of the crayfish fed with commercial shrimp diet, there was no significant difference in the total protein level in the hemolymph of the crayfish treated with diets based on soybean plant protein, in comparison to other groups (Figure 3). In crustaceans, muscles are apparently the most important place for storing protein and the level of free amino acids in their tissues is several times higher than that of vertebrates (Chen et al., 1994). Studies show that amino acids have a role in osmoregulation and the control of cell volume (Chang and O'Conner, 1983; Schein et al., 2005). Since there is a balance between stored protein content in tissues and total protein level in hemolymph, with regard to the absence of carcass protein level in the crayfish which were fed with plant and animal protein-based diets, it can be concluded that the change of diet has no effect on the total protein level of hemolymph in crayfish.

## CONCLUSION

Although with the increased rate of soybean meal in diet weight gain and specific growth rate of crayfish reduced, no significant changes were observed in biochemical parameters. Crayfish can feed on both animal and plant protein sources, however, according to the results obtained in the present study, we recommend that the effect of soybean meal on growth performance and biochemical parameters of crayfish should be evaluated before empirical use of different ratios of soybean meal as a protein source in their diet.

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*Sažetak*

## UČINCI HRANIDBE SOJINIM BRAŠNOM NA PERFORMANCU RASTA I BIOKEMIJSKA SVOJSTVA HEMOLIMFE DUNAVSKOG RAKA (*Astacus leptodacylus* ESCHSCHOLTZ, 1823)

Poput drugih vrsta rakova, dunavski rak (*Astacus leptodacylus* Eschscholtz, 1823) tijekom različitih životnih ciklusa može promijeniti svoju hranidbu ovisno o hrani koja je dostupna. Promjena hranidbe može utjecati na različite biološke indekse ove vrste, stoga ova studija ima za cilj proučiti promjene koje se mogu pojaviti tijekom promjene hranidbe raka u indeksima rasta, biokemijskim svojstvima hemolimfe i biokemijskim svojstvima njihova mesa. Svrha ovog eksperimenta je procijeniti učinke djelomične zamjene riblje hrane sa sojinim brašnom na performancu rasta, kvalitetu mesa i biokemijska svojstva hemolimfe dunavskog raka. 90 zdravih adultnih dunavskih rakova (W=35,50±4,05 g; TL=16,96±1,92 cm) su raspoređeni nasumce u 9 bazena od fiberglasa (200 L) te su tijekom 45 dana hranjeni s tri različite vrste hrane, uključujući hranidbu komercijalnim škampima, ribljom hranom (A) i sojinim brašnom (B). Rezultati su pokazali da je promjena hranidbe životinjskim proteinom u hranidbu biljnim proteinom uzrokovala značajan pad razine kolestoerola i triglicerida u hemolimfi, kao i masti mesa rakova koji su jeli hranu B, u usporedbi s rakovima koji su jeli hranu A. Nisu zamijećene značajnije promjene razine glukoze hemolimfe, AST i ALT prilikom različitih tretmana tijekom ovog eksperimentalnog razdoblja. Kao zaključak, utvrđeno je da iako se smanjila performanca rasta, povećana hranidba sojinim brašnom od 0,0% do 76% nije imala nikakvih negativnih učinaka na biokemijska svojstva.

**Ključne riječi:** dunavski rak, izvor proteina, indeksi rasta, kvaliteta mesa, biokemijska svojstva

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