



RESEARCH PAPER

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Evaluation effect of soybean meal and baker's yeast on resistance to anoxia stress and blood biochemical parameters of fingerlings (*Mesopotamichthys sharpeyi* Günther, 1874)

Fatemeh Mohammadinafchi, Hamid Mohammadiazarm*, Vahid Yavari

Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Khozestan, Iran

Key words: Fish meal, soybean meal, baker's yeast, Blood biochemical parameters, Anoxia Stress.

<http://dx.doi.org/10.12692/ijb/5.8.215-222>

Article published on October 29, 2014

Abstract

This study was carried out to investigate the replacement of fish meal with soybean meal and baker's yeast on plasma biochemical parameters and resistance to anoxia stress in fingerlings of *Mesopotamichthys sharpeyi* belongs to the family cyprinidae. Fish with initial average weight of $4/40 \pm 0/29$ gr were fed experimental diets (0, 25, 50, 75 and 100% Replacement of fish meal) for 90 days. At the end of the experiment, biochemical factors such as cholesterol, triglycerides, total protein, albumin and globulin were measured. Based on the results, no significant difference was observed in total protein and globulin in experimental treatments compared with control group ($p > 0.05$). But the amount of albumin in the control group was significantly higher than other treatments ($p < 0.05$). Also, Cholesterol levels were significantly increased while amount of triglycerides were significantly decreased in alternative treatments compared with control group ($p < 0.05$). Results of resistance to anoxia stress showed that used different levels of soybean and baker's yeast in diet, had not negative impact on the survival rate of *Mesopotamichthys sharpeyi*, even fish resistance was more in anoxia stress compared with control group.

*Corresponding Author: Hamid Mohammadiazarm ✉ Azarmhamid@gamil.com

Introduction

Aquaculture industry has the highest potential for fish production and responding to the growing demand for seafood (FAO, 2010). A sustainable aquaculture depends on an affordable diet and nutritionally balanced (Cahu *et al.*, 2003; Kim and Lee, 2009). The cost of diet is one of the main variables in profit rate of industrial production aquaculture.

Fish meal is an important component and the best source of animal protein in fish diets (Peres *et al.*, 2003) that the price has increased with higher demand and decrease of fish stocks. Therefore, it is necessary to reduce the percentage of it in the formulation of diets to the extent that there is no negative impact on the growth and health of farmed species (Ogunkoya *et al.*, 2006). Soybean meal (SBM) is regarded as a nutritious feedstuff with high crude protein content (40-45%), high digestible protein and energy contents and a reasonably balanced amino acid profile compared to other source of plant proteins (Markovic *et al.*, 2011; Yu *et al.*, 2013). Plant proteins such as SBM have anti-nutritional factors that reduce digestibility and growth performance; Therefore, One of practical ways have been suggested to improve utilization of plant proteins is blending various protein sources (Azarm and Sang min lee, 2014) such as yeast in diet of fish. The digestible energy content of yeast is high. Yeast phosphorus is available for common carp (92 %) and rainbow trout (91%) (Burr *et al.*, 2008; He *et al.*, 2011). Used baker's yeast in diet of fish induces ensuring the health of the fish, increase in survival and resistance to environmental stress.

Also, it is reported that physiological condition of fish is influenced by composition of diets (Poordavood and Sajadi, 2010). Measurement of hematologic parameters is one of the important and reliable indicators in checking of health status and physiology of fish that is influenced by nutrition, environmental factors and age of fish (Ghaderi Ramazi *et al.*, 2013). So far, many researchers have been done studies on the effect of dietary composition on blood biochemical parameters and resistance to

environmental stress to adjust the composition of the diet that provided nutrient requirements of fish and increase production.

On the other hand, *Mesopotamichthys sharpeyi* or Binni is an omnivorous fish and one of the commercially valuable species and endemic in Khuzestan which is considered due to high tolerance to environmental conditions and high economic value and as an important source of protein to the residents of these areas (Kahkesh *et al.*, 2010). Nevertheless, the dietary requirements of binni fish is not available. So, this experiment was designed for evaluation effect of soybean meal and baker's yeast on resistance to anoxia stress and blood biochemical parameters of fingerlings.

Material and method

Diet preparation

Five isonitrogenous and isolipidic diets were formulated to contain 0, 250, 500, 750 and 1.000 g kg⁻¹ soybean meal and baker's yeast with the ratio of 1:1 as control, SY25, SY50, SY75 and SY100. Ingredients and nutrient contents of the experimental diets are presented in Table 1. Kilka fish meal was used as the primary protein source and Kilka fish oil and soybean oil were used as lipid sources. All ingredients were thoroughly mixed with 300 g kg⁻¹ distilled water, and pellets were prepared using a moist pelleting machine. The pellets were dried at room temperature for 24 h and ground into desirable particle sizes. All diets were stored at -20 C° until used (Table 1).

Experiment fish and feeding conditions

Fingerlings fish were obtained from a local farm (Maleki Farm, Khozestan, Iran). The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Fingerlings fish (initial mean weight, 4.40±0.29 g) were allocated randomly into 300 L circular plastic tanks with 40 fish per each tank for the feeding trial after being collectively weighed. Three replicate groups of fish were hand-fed to apparent satiation three times a day (9:00, 13:00 and 17:00) for 8 weeks. During the experimental

period, mean water temperature was $26 \pm 1^{\circ}\text{C}$, dissolved oxygen was $6/33 \pm 0/073 \text{ mg L}^{-1}$ and the pH was about 7. The photoperiod was left under natural conditions during the feeding trail.

Chemical analyses

Proximate analyses of the diets were determined according to the method of AOAC (1995). Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System (Kjeltec TM2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105°C for 24 h and ash by a furnace muffler (550°C for 4 h).

Sample collection and analysis

At the end of the feeding experiment, blood samples were collected from ten fish in each treatment after starvation for 24 h. Fish anaesthetized with Carnation powder at a concentration of 30 mg L^{-1} (Velisek *et al.*, 2005). Blood samples were taken from the caudal vein with heparinized syringes and centrifuged for 10 minutes at 3000 rpm (Acerete *et al.*, 2004) and using by isolated plasma of blood were measured serum parameters (total protein, albumin, cholesterol and triglycerides). Amount of cholesterol, triglycerides, albumin (wavelength of 546 nm) and total protein (wavelength of 630 nm) were measured by clinical kit (Pars Azmoon, Tehran, Iran) and mindray BS-200 autoanalyzer.

Resistance to Anoxia Stress

At the end of the feeding experiment, ten fish from each tank (15 tank) were exposed to anoxia stress, so the fish were out of the water for 12 minutes (Simultaneously and separately).

After this period were returned to the water without aeration and controlled for two days and mortality rates were examined (Niromand *et al.*, 2009).

Statistical Analysis

In outline, this study was planned and executed entirely by accident. All data are collected normal distribution using the Shapiro-Wilk test, and

significant differences between treatments at different levels ($p \leq 0/05$) were examined using ANOVA (One-way ANOVA) and post- Duncan test. Analysis of all the data and the operations were performed by SPSS 16.0 software.

Results

The result of different levels of soybean meal and baker's yeast instead of fish meal on blood biochemical parameters and resistance to anoxia stress in fingerlings of *Mesopotamichthys sharpeyi* is shown in Table 2. At the end of the trial period, no significant differences were detected in amount of total protein and globulin between control and other treatments ($p > 0.05$). Amount of triglycerides significantly reduced and amount of cholesterol significantly increased with increase in levels of soybean meal and baker's yeast in diets ($p < 0.05$). The amount of albumin in the control group was significantly higher than other treatments ($p < 0.05$). Results of survival resistance to anoxia stress showed that using of different levels of soybean and baker's yeast in diet, had not negative impact on the survival rate of fingerlings, even fish resistance was more in anoxia stress compared with the control treatment.

Discussion

Changes in blood biochemical parameters are an indicator of fish responses to their diet (Satheeshkumar *et al.*, 2010). Therefore, changes and fluctuations in the amount of total protein, cholesterol and triglyceride is related to their composition of diet that it is used as energy for vital body activities. Cholesterol plays an important role in the function of nerve fibers, the formation of bile salts, maintains cell membrane structure and is as a precursor of steroid hormones. Also, triglycerides are as a reserve source of energy for body metabolism (Hoseini and Ghelichpour, 2012). Triglyceride and cholesterol concentration levels are as main indicators of health status of fish, such that changes in cholesterol and triglyceride concentration levels are indicant metabolism in the liver (Zhou *et al.*, 2005; Gul *et al.*, 2011).

In the present study, amount of cholesterol significantly increased with increase in levels of soybean meal and baker's yeast in diets that was similar to those reported in Japanese flounder (*Paralichthys olivaceus*) (Ye *et al.*, 2011), Cobia (*Rachycentron canadum*) (Zhou *et al.*, 2005), Nile tilapia (*Oreochromis niloticus*) (Metwally and El-Gellal, 2009). Also reports were presented based on decrease blood cholesterol levels in Tiger puffer (*Takifugu rubripes*) (Lim *et al.*, 2011), Seabream (*Diplodus vulgaris*) (Acar *et al.*, 2013), Common carp (*Cyprinus carpio*) (Ghaderi Ramazi *et al.*, 2013). In this study, amount of triglycerides significantly reduced with increase in levels of soybean meal and baker's yeast in diets that was accordance with those reported in Parrot fish (*Oplegnathus fasciatus*) (Lim and Lee, 2008), Common carp (*Cyprinus carpio*) (Moradi *et al.*, 2013), Seabream (*Diplodus vulgaris*)

(Acar *et al.*, 2013), Rainbow trout (*Oncorhynchus mykiss*) (Romarheim *et al.*, 2006) and disagree with results in Nile tilapia (*Oreochromis niloticus*) (Metwally and El-Gellal, 2009), Japanese flounder (*Paralichthys olivaceus*) (Ye *et al.*, 2011), Common carp (*Cyprinus carpio*) (Ghaderi Ramazi *et al.*, 2013). Soybean phytoestrogens (isoflavones, genistein and daidzein) which are known as reduction factors of triglycerides and LDL, causes the difference in the results of various studies (Anderson *et al.*, 1995). Since the most important site of lipid metabolism is liver; so reduction of triglyceride and LDL prevent from fatty liver syndrome in fish (Hosseini *et al.*, 2013). On the other hand, a significant increase in cholesterol levels is due to baker's yeast which is decreasing to LDL and increasing to HDL (Paryad and Mahmoudi, 2008).

Table 1. Ingredient and proximate composition of experimental diets.

Treatment	Control	SY25	SY50	SY75	SY100
Ingredients diets(g/100g)					
Fish meal ¹	22.84	17.25	11.50	5.75	0.0
Soybean meal	0.0	2.90	5.75	8.63	11.50
Baker's yeast	0.0	2.90	5.75	8.63	11.50
Corn gluten meal	10.09	12.80	15.77	18.70	21.66
Wheat meal	33.04	30.12	27.19	24.26	21.28
Wheat bran	14.73	14.58	14.43	14.26	14.10
Fish oil ²	6.00	6.00	6.00	6.00	6.00
Soybean oil	6.00	6.00	6.00	6.00	6.00
Vitamin premix ³	3.00	3.00	3.00	3.00	3.00
Mineral premix ⁴	2.00	2.00	2.00	2.00	2.00
Binder ⁵	2.00	2.00	2.00	2.00	2.00
Antioxidant ⁶	0.02	0.02	0.02	0.02	0.02
Lysine	0.27	0.42	0.58	0.73	0.89
Methionine	0.0	0.0	0.0	0.01	0.05
Proximate composition					
Moisture	10.40	10.20	9.20	8.80	9.40
Crude protein (% DM)	32.37	31.85	31.15	31.85	31.15
Ash (% DM)	8.42	8.67	8.77	8.40	8.10
Lysine (% DM)	2.00	2.00	2.00	2.00	2.00
Methionine (% DM)	0.70	0.70	0.70	0.70	0.70
Gross Energy (MJ/kg) ⁷	18.80	18.70	18.80	19.20	19.20

¹Clopeonella meal, Iran

²Kilka oil, Mazandaran Co, Iran

³Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, B1=6000 mg, B2=8000 mg, B3=12000 mg, B5=40000 mg, B6=4000 mg, B9=2000 mg, B12=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg

⁴Mineral premix (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, CoCl₂:6000 mg

⁵Binder: Amet Binder (Component: Crude Protein: 71.98%, Crude Fiber: 0.9%, Ash: 17.8%, Moisture: 9.55%)

⁶Antioxidant: Butylated hydroxytoluene (BHT)

DM, dry matter

⁷Based on 23.4 KJ/g protein, 39.2 KJ/ g lipid and 17.2 KJ/ g carbohydrate.

Based on the results of this experiment, no significant differences were detected in amount of total protein and globulin between control and other treatments but the amount of albumin in the control group was significantly higher than other treatments that was similar to those reported in Persian sturgeon

(*Acipenser persicus*) (Imanpoor *et al.*, 2010), Parrot fish (*Oplegnathus fasciatus*) (Lim and Lee, 2008), Cobia (*Rachycentron canadum*) (Zhou *et al.*, 2005), European seabass (*Dicentrarchus labrax*) (Adamidou, 2008), Rainbow trout (*Oncorhynchus mykiss*) (Hosseinfard *et al.*, 2013).

Table 2. The result of blood biochemical parameters and resistance to anoxia stress in fingerlings of *Mesopotamichthys sharpeyi* fed different experimental diets for 90 days.

Treatment	Control	SY25	SY50	SY75	SY100
Total Protein(g/dl)	6.85±0.05 ^a	6.69±0.005 ^a	6.59±0.09 ^a	6.69±0.29 ^a	6.56±0.035 ^a
Cholesterol(mg/dl)	261.00±0.57 ^a	265.67±0.33 ^b	270.00±0.57 ^c	276.00±1.00 ^d	283.33±0.88 ^e
Triglyceride(mg/dl)	401.00±1.00 ^c	370.33±1.52 ^d	349.00±0.57 ^c	324.33±0.33 ^b	312.33±0.33 ^a
Albumin (g/dl)	1.75±0.15 ^b	1.10±0.10 ^a	1.41±0.06 ^a	1.37±0.03 ^a	1.32±0.11 ^a
Globulin (g/dl)	5.10±0.10 ^a	5.59±0.09 ^a	5.18±0.03 ^a	4.67±0.57 ^a	5.24±0.04 ^a
Survival (%)	93.33±6.66 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a

(Mean ± SE), n=3 with different letters in each row, indicate the presence of significant differences between the experimental groups (P<0/05).

Approximately 5 to 10% of blood plasma is blood proteins. However, Changes in plasma levels of blood total protein is not a specific indicator, but can be indicant metabolic changes and pathology (Sabri *et al.*, 2009). Albumin, is one of the most important protein in blood that produced in liver and plays an important role in protection of tissues and cells against free radical damage (antioxidant activity), transportation of vitamins, minerals and hormones in blood and maintain osmotic balance as the main body buffer (Burtis and Ashwood.1994; Svetina *et al.* 2002). High protein and globulin levels in blood plasma is indicant high level of safety in fish that is result of stimulation of leukocytes and secretion of immunoglobulins (Nayak *et al.*, 2004). Baker's yeast in aquatic feed is cause activating the anabolic capacity of liver cells in production of blood proteins (Ghodratizadeh *et al.*, 2011) and finally, increase the health of aquatic organisms (Abdel-Tawwab *et al.*, 2008).

Based on this experiment, used baker's yeast as a nutritional supplementation in diet was effective on fish resistance to anoxia stress that can be related to Immune-stimulating compounds in yeast such as mannose polymers attached to peptides (Manu

protein), glucose polymers (Glucans), chitin, nucleic acids and as a result improvement of growth, increase of immune responses, increase of resistance to environmental conditions and aquatic animal health (Gatesoupe, 2007; Medne and Savicka, 2003; Li *et al.*, 2004).

Conclusion

Generally based on the result of blood biochemical parameters, resistance to anoxia stress, we can use soybean meal and baker's yeast as a new protein sources without fish meal in diet of fingerlings binni.

Acknowledgements

This project was supported by Khorramshar University of Marine Science and Technology, Khorramshar, Iran.

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