Global Veterinaria 11 (2): 233-237, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.2.7533

Hematological and Biochemical Changes Induced by Replacing Fish Meal with Plant Protein in the *Cyprinus carpio* Linnaeus (1785)

Negar Moradi, Mohamadreza Imanpoor and Vahid Taghizadeh

Department of Fisheries,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This study was conducted to find the possibility of fish meal replacement in the feeds for Carp with plant sources of protein (Sesame oil cake and Corn gluten). Experimental diets with 33% of crud protein, GE: 10.5 Kal g^{-1} were prepared in 4 replacement levels diet (1) 0, diet (2)160, diet (3) 340 and diet (4) 500 g kg^{-1} were prepared and growth tests in 60 days were carried out. *Cyprinus carpio* have been fed for 8 weeks. Experimental diets showed a significant differences in glucose, Hematocrit, Hemoglobin and Total protein (P<0.05). No significant differences were found in Cholesterol, Albomin, Triglycerides, White blood cell and Red blood cell (P>0.05). The results of the present study revealed that the maximum levels of fish meal replacement by corn gluten and sesame oil cake in diets of *Cyprinus carpio* could be 68 % of total protein sources of diet.

Key words: *Cyprinus carpio* · Hematological · Replacement · Diet

INTRODUCTION

One of the main prerequisites to increase aquaculture extension is the acquisition and cost of feed [1]. Proteins in high modality fish meal are delicious, highly digestible (~90%) and anti-nutritional factors (ANFs) are more or less non existing [2]. Substantial attention has been devoted to the assessment of plant proteins as components in aquatic animals feeds containing soybean meal [3 -5], solvent-extracted cottonseed meal [6], various legumes (cowpea, green mung bean and rice bean), leaf meals [7,8] and papaya or camote leaf meal [9]. Common Carp is considered among the most major economic fishes of Caspian Sea with high nutritional value that has good marketability because of its less value [10]. Many studies have been published on digestibility of corn gluten meal, emblematic in general good results for inclusion levels below 40% of the dietary protein for different fish species [11, 12]. Digestibility of corn gluten meal is universally high, with reported values of 95 and 96 % for carp and trout [13, 14]. Hematological parameters of fish are closely related to the response of fish to environmental and biological factors [15-17]. For example, in response to ecological and physiological conditions, major changes occur in the fish blood constituents, such as fluctuations in the levels of red blood cells, white blood cells, hormones, hematocrit, hemoglobin concentration and other pivotal components. Therefore, the analysis of blood indices is a valuable guidance in assessing the condition of fish, as it provides a trusty index of their physiological condition [18], in another word analysis of blood indices have to be considered an important dataset in carp aquaculture. *Cyprinus carpio* juveniles like other teleosts need more requirements of micronutrients because of fast growth at this phase of their life cycle. The aim of this study is to assess the effects of replacing fish meal with two plant proteins (sesame oil cake and corn gluten) on biochemical and hematological indices as well on reducing of the feed cost of juvenile Cyprinus carpio.

MATERIALS AND METHODS

The experiments conducted on juvenile carp (average weight: 94.65±2.85g) produced at the Institute of Aquaculture of the Sijaval for carp, Golestan, Iran. Fish were acclimated to laboratory conditions for 30 days before being randomly distributed into the aquaria of 50 L capacity. Fish with 94.6 ±2.85 g initial body weights were distributed into 12 experimental aquaria in four groups of 120 fish each (10 fish per aquarium). Aquaria are supplied with urban fresh water. Weight and length of

Corresponding Author: Negar Moradi, Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Table 1: Formulation and chemical composition of the diets

Nutrient material	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Fish meal	50	34	16	0
Corn gluten	0	8	17	25
Sesame oil cake	0	8	17	25
Casein	5.1	8	14	15.5
Gelatin	7	8	7.3	8
Starch	31	25.1	18.3	14.6
Fish oil	4	6	7.5	9
Lysine	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2
Antioxidant	0.25	0.25	0.25	0.25
Vitamins and minerals				
Chemical composition				
(% or cal g^{-1})	2	2	2	2
Antifungal	0.25	0.25	0.25	0.25
Chemical composition				
(% or cal. g^{-1})				
Crude protein	33	33	33	33
Lipid	11	10	11	10.5
Energy crude	3200 Kal	3200 Kal	3200 Kal	3200 Kal

120 fish were individually measured once every 2 weeks. The aquaria were individually aerated and water temperature was controlled at 23°C. Dissolved oxygen, pH and water temperature were continuously monitored. Photoperiod was maintained at 12-h light: 12-h dark cycle. Four groups were fed by hand twice a day to 2% of whole body weight. Each group was weighed every 2 weeks to follow growth and feed utilization.

Experimental Diets Preparation: Four experimental diets were formulated to contain 33% crude protein (Table 1). The four isonitrogenous diets consisting of one control diet based on fish meal and the other three test diets made by substituting fish meal with corn gluten meal and sesame oil cake. After preparing the ingredients of diets, an experimental extruder was used for producing the pellet size of 0.5 mm diameter.

Fish Sampling and Blood Analyze: Nine fish from each treatment were anesthetized (with clove oil at 100 mg L^{-1}) and blood samples were obtained from caudal vein and transferred to unheparinized and heparinized sterile tubes (1-1.5 ml). The quantitative determination of plasma glucose and triglyceride were carried out using commercially available diagnostic kits (Pars Azmoon Co. Iran) at 546 nm and 37 °C by the glucose oxidase method [19]. Glucose and triglyceride were measured photometrically according to a method modified based on the quantification of NADH after a glucose oxidation catalyzed by glucose dehydrogenase. Plasma total protein and cholesterol levels were determined spectro photo metrically using commercial kits Sigma 337-B and Sigma 401-25P by the method of Canli [20]. Freshly collected heparinized whole blood samples were used for estimation of hematological parameters like hemoglobin (Hb) using cyanmethemoglobin method [21] packed cell volume or hematocrit value, were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 5 min at 10,500 rpm in a microhematocrit centrifuge (Hettich, Germany) then measuring the packed cell volume; Hematocrit readings were performed with the aid of a microhematocrit reader. The number of red blood cells (RBCs) and total number of leukocyte (WBCs) were calculated using a Neubauer chamber, according to Ranzani- Paiva *et al*. [22].

Statistical Analysis: All data were analyzed with one-way analysis of variance by using SPSS16.0 for windows. Differences between means were determined using Duncan's multiple test (significance P<0.05).

RESULTS

Plasma glucose, cholesterol, triglyceride and total protein values are shown in Table 2. With decreased fish meal in experimental diets caused that plasma glucose concentrations increased in treatments 2, 3 and 4. Cholesterol, triglyceride and albumin of plasma showed no significant differences in fish fed the four experimental diets.

No statistically difference was observed in hematological parameters including WBCs and RBCs (p>0.05; Table 3). Experimental diets showed a significant differences in glucose, hematocrit, hemoglobin and total protein (*P*<0.05). Fish meal replacement in experimental diets caused elevation in plasma hemoglobin in treatments 3 and 4. In control fish, the highest hemoglobin percentage was observed, whereas in fish fed 500 gkg^{-1} plant protein (sesame oil cake and corn gluten) the lowest hemoglobin percentage was seen (Table 3).

Table 2: Plasma levels of glucose, cholesterol, triglyceride, total protein and albumin in fish fed the experimental diets

Plasma metabolites	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Glucose (mg/dl)	64.5 ± 1.34^{c}	75.52 ± 2.77^{b}	77.3 ± 5.12^{b}	84.66 ± 2.55^{a}
Cholesterol (mg/dl)	3.43 ± 0.41^{a}	3.22 ± 0.11^{a}	3.14 ± 0.12^{a}	3.02 ± 0.12^{a}
Triglycerides (mg/dl)	336 ± 17.81^{a}	325.2 ± 12.9^{a}	323.44 ± 28.5^{a}	281.58 ± 24.7^{b}
Total protein (mg/dl)	4.1 ± 0.13^{a}	3.8 ± 0.18^{ab}	3.6 ± 0.14^{b}	3.7 ± 0.21^{b}
Albumin	1.71 ± 0.13^{a}	1.77 ± 0.17^{a}	1.73 ± 0.25^{a}	1.84 ± 0.15^{a}

Means with the same superscript letters at the same row are not significantly different (P>0.05)

Table 3: Hematological profiles of *Cyprinus carpio* fed with four experimental diets

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Hemoglobin (g/dl)	22.46±0.34^{a}	20.94±0.16^{b}	20.03±.0.14^{c}	19.83±0.33^{c}
Hematocrit (%)	8.63±0.13^{a}	8.18±0.14^{b}	7.58±0.23^{c}	7.53±0.33^{c}
White blood cell (×10^{3}/mm^{3})	1.63±0.22^{a}	1.56±0.12^{a}	1.53±0.26^{a}	1.46±0.11^{a}
Red blood cell (×10^{4}/mm^{3})	10128±52.88^{a}	10090±81.72^{a}	10063±62.42^{a}	10102±39.15^{a}

Means with the same superscript letters at the same row are not significantly different (P>0.05)

DISCUSSION

In the current study, replacing fish meal with plant protein caused a significant (P<0.05) differences in the hematocrit, hemoglobin and total protein while significant differences were not found in WBCs, RBCs, Cholesterol, triglyceride and albumin plasma (p>0.05).

Any environmental perturbation can be considered as potential stressors since it prompts a number of responses in the animal to deal with the physiological changes triggered by outer changes [23]. However, triglyceride showed no significant change; it was decreased along with replacement. The conceivable secondary physiological effects of dietary plant protein intake have also been little studied in fish. The decrease in plasma triglyceride concentrations with increment dietary corn gluten meal (CGM) levels has been observed in the yellowtail fish [24]. The decline in whole body fat quantity along with the decrease in plasma triglyceride concentrations suggest considerable lipid mobilization in these groups exhibiting very poor growth rates. Plant proteins are known to affect cholesterol metabolism [25]. Kaushik *et al.* [26] observed a significant hypocholesterolemic effect of dietary soybean in rainbow trout and also reduction with high dietary CGM levels. Hematocrit concentration decreased parallel to inclusion plant protein in diet; however, in fish meal diet, it was higher. The few data that have been published on the effect of the CGM and sesame oil cake on hematocrit are not always stable. What is more, there are no lucid relationships between the embedment level of fishmeal with another proteins in the diet and deterioration of hematological parameters of the cultured fish [27]. Our Experimental diet treatments resulted in a significant differences in glucose, hematocrit, hemoglobin and total protein (P<0.05). No significant differences were found in Cholesterol, albumin, triglycerides, WBCs, RBCs (P>0.05). No significant change was found between RBCs and WBCs that shows there is no significant stress on the studied fish due to replacing the fish meal with protein from plant sources. Food quality [28] also strongly influences the morphological specifications of blood and the qualitative and quantitative attributes of hemoglobin in juvenile sturgeons. Fluctuations in lymphocyte levels in fish in response to stress and corticosteroid hormones are similar to those in mammals [29]. Levels of glucose were measured as conventional stress symptom to calculate the trustiness of stress response triggered under diet substitution [30]. Plasma glucose levels had increased with increasing protein sources in diet. Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being converted to chemical energy, which in turn can be imparted as mechanical energy [31]. Glucose is then released toward blood circulation and enters into cells through the insulin action [32]. Plasma glucose concentration depends on intestinal sorption, hepatic production and tissue uptake of glucose. Several hormones affect homeostasis of glucose, including insulin, glucagons, corticosteroids, adrenocorticotropic hormone, growth hormone and catecholamines. The keeping of blood glucose concentrations occurs via hepatic glycogenolysis, glycolysis and gluconeogenesis [33]. Total plasma proteins are a major constituent in the metabolism of diet; therefore, it is important to finding of alterations in protein metabolism induced by diet substitution for further information [34]. Changes that may occur are the increased synthesis or breakdown of proteins and the inhibition or activation of certain enzymes [35]. Most biochemical defenses respond to cellular injury by increasing levels of defenses through self-regulating signal transduction mechanisms. These defenses are usually proteins that serve numerous cellular functions, many still unknown. In summary, according to the results obtained in the present experiment, maximum proposed levels of fish meal replacement by CGM and sesame oil cake in diets for *Cyprinus carpio* can be recognized at 68 % of total protein.

ACKNOWLEDGEMENTS

The authors thank the Aquaculture Research Center and Fishery Group for the supply of research material. This work was supported by the Gorgan University of Agricultural Sciences and Natural Resources.

REFERENCES

1. James, D., 1992. Seafood technology in the 1990: The needs of developing countries. in: E. Graham Bligh (Ed.). Seafood Science and Technology. Fishing News Books, pp: 12-23.

2. Gatlin, D.M., F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D.J. Skonberg, E. Souza, D. Stone, R. Wilson and E. Wurtele, 2007. Expanding the utilization of sustainable plant products in aqua feeds: a review. Journal of Aquaculture, 38: 551-579.
3. Lim, C. and W. Dominy, 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp *Penaeus vannamei*. Journal of Aquaculture, 87: 53-64.
4. Tidwell, J.H. C.D. Webster, D.H. Yancey and L.R. D'Abramo, 1993. Partial and total replacement of fish meal with soybean meal and distillers by-products in diets for pond culture of the freshwater prawn (*Macrobrachium rosenbergii*). Journal of Aquaculture, 118: 119-130.
5. Sudaryono, A., M.J. Hoxey, S.G. Kailis and L.H. Evans, 1995. Investigation of alternative protein sources in practical diets for juvenile shrimp *Penaeus monodon*. Journal of Aquaculture, 134: 313-323.
6. Lim, C., 1996. Substitution of cottonseed meal for marine animal protein in diets for *Penaeus vannamei*. Journal of World Aquacult. Soc., 27: 402-409.
7. Eusebio, P., 1991. Effect of dehulling on the nutritive value of some leguminous seeds as protein sources for tiger prawn, *Peaneus monodon*, juveniles. Journal of Aquaculture, 99: 297-308.
8. Eusebio, P. and R.M. Coloso, 1998. Evaluation of leguminous seed meals and leaf meals as plant protein sources in diets for juvenile *Penaeus indicus*. Israeli Journal of Aquacult. Bamidgeh, 50: 47-54.
9. Penaflorida, V.D., 1995. Growth and survival of juvenile tiger shrimp fed diets where fish meal is partially replaced with papaya (*Carica papaya* L.) or camote (*Ipomea batatas* Lam.) leaf meal. Israeli Journal of Aquacult. Bamidgeh, 47: 25-33.
10. Sargent, J.R. and A.G. Tacon, 1999. Development of farmed fish: A nutritionally alternative to meat Proceedings of the Nutritional Society, 58: 377-383.
11. Alexis, M.N., E. Papaparaskeva and V. Theochari, 1985. Formulation of practical diets for rainbow trout (*Salmo gairdneri*) made by partial or complete substitution of fish meal by poultry by-products and certain plant by-products. Journal of Aquaculture, 50: 61-73.
12. Davies, S.J., I. Nengas and M. Alexis, 1991. Partial substitution of fish meal with different meat meal products in diets for seabream (*Sparus aurata*). Fish Nutrition in Practice, June 24-27, 1991, INRA (Ed.), (Les Colloques, No. 6 1). Biarritz, France, pp: 907-911.
13. Pongmaneerat, I. and T. Watanabe, 1991. Nutritive value of protein of feed ingredients for carp, *Cyprinus carpio*. Nippon Suisan Gakk, 57: 503-510.
14. Morales, A.E., 1993. Valoracition de la utilizacition nutritiva de materias primas alternative as a la harina de pescado coma componentes de dietas comerciales para la trucha (*Oncorhynchus mykiss*). Tesis Doctoral, Univ. De Granada.
15. Steinhagen, D., P. Kruse and W. Korting, 1990. Some hematological observations on carp *Cyprinus carpio* L. experimentally infected with *Trypanoplasma borelli* Laveran and Mesnil, 1901 (Protozoa: Kitenoplastida). Journal of Fish Dis., 14: 157-162.
16. Haider, G., 1973. Comparative studies of blood morphology and haemopoiesis of some teleost. Observations on cells of the red series. Journal of Zool., 179: 355-383.
17. Fernandes M.N. and A.F. Mazon, 2003. Environmental pollution and fish gill morphology. In: Val AL, Kapoor BG (eds) Fish adaptation Science, Enfield, pp: 203-231.
18. Alyakrinskyaya, I.O. and S.N. Dolgova, 1984. Hematological features of young sturgeons. Vopr Ikhtiol, 4: 135-139.
19. Hedayati, A., A. Safahieh, A. Savar and J. Ghofleh Marammazi, 2010. Assessment of aminotransferase enzymes in Yellowfin sea bream under experimental condition as biomarkers of mercury pollution. World J. Fish Mar. Sci., 2: 186-192.
20. Canli, M., 1996. Effects of mercury, chromium and nickel on glycogen reserves and protein levels in tissues of *Cyprinus carpio*. Journal of Zool., 20: 161-168.
21. Varley, H., 1991. Text book of clinical practical biochemistry, vol 1, 5th edn. CBS, New Delhi, India, pp: 479-480.
22. Ranzani-Paiva, M.J.T., C.M. Ishikawa, A.C. Das Eiras and V.R. Da Silveira, 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757). Braz. Arch. Biol. Tech., 6: 945-953.
23. Donaldson, E.M., 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering AD (ed) Stress and fish. Academic, London, pp: 11-41.
24. Shimeno, S., T. Masumoto, T. Hujita, T. Mima and S. Uenos, 1993. Alternative protein sources for fish meal in diets of young yellowtail. Nippon Suisan Gakk. 1: 137-143.

25. Forsythe, W.A., 1995. Soy protein, thyroid regulation and cholesterol metabolism. Journal of Nut., 125: 6195-6235.
26. Kaushik, S.J., J.P. Cravedi, J.P. Lalles, J. Sumpter, B. Fauconneau and M. Laroche, 1995. Partial or total replacement of fish meal by soya protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout. Journal of Aquaculture, 133: 257-274.
27. Moreau, R., K. Dabrowski, S. Czesny and F. Chila, 1999a. Vitamin C vitamin E interaction in juvenile lake sturgeon (*Acipenser fulvescens*), a fish able to synthesize ascorbic acid. Journal of Appl. Ichthyol., 15: 250-257.
28. Domezain, A.E., R.M. Martínez-Álvarez, M.C. Hidalgo, A. Morales, M. García Gallego and A. Sanz, 2002. Physiological changes of sturgeon *Acipenser naccarii* caused by increasing environmental salinity. Journal of Exp. Biol., 205: 3699-3706.
29. Ellis, E.M., C.M. Slattery and J.D. Hayes, 2003. Characterization of the rat aflatoxin B1 aldehyde reductase gene, AKR7A1: structure and chromosomal localization of AKR7A1 as well as identification of antioxidant response elements in the gene promoter. Carcinogenesis, 24: 727-737.
30. Wendelaar-Bonga, S.E., 1997. The stress response in fish. Physiol. Rev., 77: 591-625.
31. Lucas, A., 1996. Physical concepts of bioenergetics. In: Lucas A (ed) Bioenergetics of aquatic animals, English th edn. Taylor & Francis, France.
32. Nelson, D.L. and M.M. Cox, 2005. Lehninger principles of biochemistry, 4^{th} edn. WH Freeman and Co, New York, pp: 1013.
33. Haschek, W.M., M.A. Walling and C. Rousseaux, 2010. fundamental of toxicologic pathology. Academic, New York, pp: 686.
34. Reddy, P.S. and A. Bhagyalakshmi, 1994. Changes in oxidative metabolism in selected tissues of the crab (*Scylla serrata*) in response to cadmium toxicity. Ecotoxicol. Environ Saf., 29: 255-264.
35. Canli, M., 1996. Effects of mercury, chromium and nickel on glycogen reserves and protein levels in tissues of *Cyprinus carpio*. Journal of Zool., 20: 161-16.