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 crude 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 trustworthy 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
Table 1: Formulation and chemical composition of the diets

<table>
<thead>
<tr>
<th>Nutrient material</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>50</td>
<td>34</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Corn gluten</td>
<td>0</td>
<td>8</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Sesame oil cake</td>
<td>0</td>
<td>8</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Casein</td>
<td>5.1</td>
<td>8</td>
<td>14</td>
<td>15.5</td>
</tr>
<tr>
<td>Gelatin</td>
<td>7</td>
<td>8</td>
<td>7.3</td>
<td>8</td>
</tr>
<tr>
<td>Starch</td>
<td>31</td>
<td>25.1</td>
<td>18.3</td>
<td>14.6</td>
</tr>
<tr>
<td>Fish oil</td>
<td>4</td>
<td>6</td>
<td>7.5</td>
<td>9</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamins and minerals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Lipid</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>10.5</td>
</tr>
<tr>
<td>Energy crude</td>
<td>3200 Kal</td>
<td>3200 Kal</td>
<td>3200 Kal</td>
<td>3200 Kal</td>
</tr>
</tbody>
</table>

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 spectrophotometrically 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 g kg⁻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

<table>
<thead>
<tr>
<th>Plasma metabolites</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>64.5±1.34</td>
<td>75.5±2.77</td>
<td>77.3±12.2</td>
<td>84.6±2.55</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>3.4±0.11</td>
<td>3.2±0.11</td>
<td>3.14±0.12</td>
<td>3.02±0.12</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>336±17.81</td>
<td>325±12.9</td>
<td>323.4±28.5</td>
<td>281.5±24.7</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>4.1±0.15</td>
<td>3.8±0.18</td>
<td>3.6±0.14</td>
<td>3.7±0.21</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.7±0.13</td>
<td>1.77±0.17</td>
<td>1.73±0.25</td>
<td>1.84±0.15</td>
</tr>
</tbody>
</table>

Means with the same superscript letters at the same row are not significantly different (P>0.05)
Hemoglobin (g/dl) 22.46±0.34 20.94±0.16 20.03±0.14 19.83±0.33
Parameters Treatment 1 Treatment 2 Treatment 3 Treatment 4
(P>0.05)
Means with the same superscript letters at the same row are not significantly different
Red blood cell (×10 /mm ) 10128±52.88 10090±81.72 10063±62.42 10102±39.15
White blood cell (×10 /mm ) 1.63±0.22 1.56±0.12 1.53±0.26 1.46±0.11
Hematocrit (%) 8.63±0.13 8.18±0.14 7.58±0.23 7.53±0.33
Table 3: Hematological profiles of Cyprinus carpio fed with four experimental diets

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, glucagon, 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.

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REFERENCES


