The Application of Exogenous $\beta$-Glucanase in Barley Based Diet and its Effects on Some Hematological Parameters of Common Carp (Cyprinus carpio)

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Abstract: Carbohydrates are the cheap energy sources in animal nutrition that will be used in vast ranges. $\beta$-glucan is the main nonstarch polysaccharides in barley and some other cereals. This study was conducted to evaluate the effects of purified $\beta$-glucanase on blood parameters of common carp (Cyprinus carpio). 90 fish in three treatments (30 fish/treatment) and each treatment in three replicates (each replicate contains 10 fish/aquarium) fed by enzyme treated diets in 3 treatments of 0% as control, 0.1% and 0.5%. Enzyme addition affected some hematological blood parameters such as WBC, RBC, hemoglobin, hematocrit, MCV and MCH and some biochemical blood parameters such as glucose, creatine, uric acid, cholesterol, total protein and albumin significantly (p<0.05).

Key words: Non-starch polysaccharides %Glucose %Cholesterol %Triglycerides %Hematocrit

INTRODUCTION

Common carp (Cyprinus carpio) is one of fashionable cultural species in all around the world, so there should be some reaserchers about its fattening course. In some cases reaserchers replaced some diets with others or added some biological agent into diet to acheive better food conversion ratio (FCR) and better growth either, but some of them succeed [1-2] and some did not [3-4]. The water-soluble non-starch polysaccharides (NSP) of the endosperm cell walls of wheat, barley, rye and oats have anti-nutritive properties. It has been clearly demonstrated that the the primary mechanism of the anti-nutritional effects of the soluble NSP activity is related to their viscous properties and hence the presence of soluble $\beta$-glucans in barley is one of the major cause of growth depression and poor feed conversion in monogastric animals [5].

Fishes, like other non-ruminant animals, do not produce enzymes that are able to degrade the cell wall and storage non-starch polysaccharides found in various concentrations of the plant materials used for animal feeding [6]. The NSP’s and lignin are commonly referred to as dietary fibre (DF) and will, because of its indigestibility in the small intestine, be an index for the fraction of the plant material potentially available for fermentation in the large intestine. In the large intestine a variable fraction of DF will be fermented to short-chain fatty acids and thereby provide energy for the host. In addition DF components can interfere with the digestion and absorption processes in the small intestine and the production of digestive enzymes [7]. The chemical composition of barley varies considerably with variety, growing conditions and year [8] which has implications for the nutritive value [9].

A single or elementary plant fibre is a single cell typically of a length from 1 to 50 mm and a diameter of around 10-50 mm. Plant fibres are like microscopic tubes, i.e., cell walls surrounding the central lumen. The lumen contributes to the water uptake behaviour of plant fibres. The fibre consists of several cell walls. These cell walls are formed from oriented reinforcing semi-crystalline cellulose microfibrils embedded in a hemicellulose-lignin matrix of varying composition [10]. The main DF components of the cell wall of barley are cellulose, arabinoxylans and mixed-linked $\beta$ (1-3, 1-4) D-glucan ($\beta$-glucan) [11]. $\beta$-glucan is a linear homopolymer of D-glucose residues which are linked through $\beta$-1,3 glycosidic bonds in the main chain. This polysaccharide, comprising the highest percentage of the fungal cell walls,
has a major role in providing the cell wall with rigidity and protection. This is generally achieved through the assistance of other cell wall components, such as chitin and different proteins [12].

As we know, non-starch polysaccharides are the most anti-nutritive components that found in the cell wall of cereals. Monogastric animals like pigs, poultry and fishes have no digestive enzyme to reduce these anti-nutrients but ruminants breaks them by their microbial colonies in their intestine. There are a plenty ways to reduce this materials and improve diet value such as pelleting [13], extution [14], soaking [15], Gamma irradiation treatment [16], enzyme treatment [17] and so many other ways. Enzymes are biological products that catalyze the biochemical reactions involved in cell life. Enzymes are proteins of high molecular weight (between 10,000 and 500,000 daltons), precipitated by alcohol, acetone and ammonium sulphate. Like all proteins, they are sensitive to the physicochemical environment, variations in which may modify their activity [18]. With enzyme supplementation the effect of varying $\beta$-glucan level is minimal. A series of experiments was conducted to study the effects of enzyme supplementation of diets containing high levels of a local variety of barley on the performance of broiler chickens.

The results of these experiments showed a relatively low effect of beta glucanase on live weight gain (6-10%) at 3 weeks of age in comparison with the results (25-43%) obtained by others with chickens of the same age [19]. There are some differences between resulted data in some cases that would be due to the variation in soluble beta-glucan content of the different barley cultivars grown under different environmental conditions [20]. The purpose of the present experiment was to study the effects of enzyme addition to diets based on locally grown barley and fed to common carp (Cyprinus carpio) in the form of pellets.

**MATERIALS AND METHODS**

**Fishes and Experimental Conditions:** This experiment carried out at a 3x3 factorial as a complete randomized design in the fisheries research center of Gorgan University of Agriculture Sciences and Natural Resource, Gorgan, Iran. 90 pieces of common carp (Cyprinus carpio) with average weight of $13.45 \pm 15$ gram distributed into 9 glass aquaria groups containing 10 fish per group which divided in 3 groups itself. Aquaria filled up to 50 L and temperature was $25 \pm 1^\circ$C and water aerated as well as possible. Dieta prepared and fishes fed by $3.5\%$ of body weight twice a day at 0080 and 2000. The method for food preparation and enzyme addition are described in next sections. Fishes brought from Institute of Aquaculture of the Marjani and transfered to research center and exposed in 2 ppt salt bath before introducing to aquariaums. Initial diet (experimental diet without enzyme) used for one week for adaptation fishes to the new situation and after that, fishes fed by experimental diet (Table 1) for 8 weeks of trial. No fish died during this period. Biometry fulfilled once per two weeks and new amount of food calculated afterward.

**Enzyme Preparation:** $\beta$-glucanase (Endo $\beta$-1-3(4) D-glucanase, EC 3.2.1.6) is in $^{16}$th glucanohydrolase family which degrades the carbohydrate polymers into its component residues by breaking the $\beta$-glycosidic bonds [12]. For making a Citrate-Phosphate buffer (pH=4.8), we mixed 252 ml citric acid 0.1N (Merck, Germany) and 248 ml dibasic sodium phosphate 0.2N (Merck, Germany) in 1000 ml distilled water according to our manual [21]. As resulted in some researches, optimum conditions of this enzyme related to pH, temperature and even some other factors such as wetness [22]. We dissolved $\beta$-glucanase (Sigma, USA) in 20 ml Citrate-Phosphate buffer and then sprayed on dried food by water sprayer.

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<table>
<thead>
<tr>
<th>Feed stuff</th>
<th>Quantity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley flour</td>
<td>50.1</td>
</tr>
<tr>
<td>Fish meal</td>
<td>34.1</td>
</tr>
<tr>
<td>Fish oil</td>
<td>11</td>
</tr>
<tr>
<td>Vitamin and mineral supplementary</td>
<td>2</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 1: Barley based diet formulation

**Proximate composition of ingredients**

- Dry matter (%) 93
- Crude protein (%) 31
- Gross energy (Mcal/kg) 4.091
- Ash (%) 12
- Crude fiber (%) 14

$^*$ Vitaminet water soluble multivitamin plus trace elements manufactured by Damloran Pharma Co. Ingredients per each gram: Vitamin A: 10000 IU; Vitamin D$_3$: 3000 IU; Vitamin E: 3 mg; Vitamin B$_6$: 2 mg; Vitamin B$_2$: 2 mg; Vitamin B$_1$: 1 mg; Vitamin K$_2$: 2 mg; Nicotinamide: 15 mg; Calcium pantothenate: 5 mg; Cu$: 3$ mg; Fe$: 12$ mg; Zn$: 15$ mg; Mn$: 25$ mg.
Feeding Trial: feedstuffs assayed for protein and gross energy content Before mixing, thus formulated and stabilized by winfeed nutrition software (version 2.8). Although requirements of carp are studied by many scientists, but we preferred to use the most reliable reference [23]. The food formulation is given in table 1.

Blood Sampling: At the end of 8th week of trial, 5 fish per replicate selected and blood samples gathered from caudal vein by cutting caudal fins and collecting into heparinized tubes for C.B.C and biochemical analyses. As in mammalian samples, glucose determinations are most useful when performed on serum separated from cellular elements shortly after sampling. If more than 30 minutes to an hour is required to get whole blood sample back to the laboratory and processed, fluoride anticoagulant needs to be used to slow glycolysis, but also other enzymatic processes in the serum and in test reagents used in automatic serum analyzer [24].

Analitical Procedure: Data analyzed by SPSS18 software. All data from experiments were subjected to one way ANOVA and Significant differences among the means were determined by using Duncan’s multiple-range test at p < 0.05.

RESULTS

In this study we found that enzyme application in diet affected the biochemical (Table 2) and blood hematological parameters (Table 3). As seen in table 2, enzyme application affected biochemical characteristics such as glucose, creatine, uric acid, triglycerides and total protein significantly (p<0.05) total protein was higher in control group than enzyme levels but the difference between enzyme treatments was not significant (p>0.05). Albomin and urea had no significant difference. In hematological parameters, enzyme addition in 0.5% level almost affected all parameters significantly (p<0.05) and then 0.1% level was in second place in balance with control group. There were no significant difference between groups in M.C.H.C (p>0.05). In biochemical parameters glucose, creatine and uric acid, total protein in control group was higher than enzyme treats and even in urea significantly but not in latest one (p<0.05). cholesterol and triglycerides were higher in 0.1% enzyme level (p<0.05).

DISCUSSION

Enzyme addition in monogastric animals like fishes would be very useful due to improving nutrient digestibility in at least 2 ways: [1] by supplying enzymes that animal cannot produce in sufficient quantity by itself, or [2] animal may produce enzyme itself but this exogenous enzyme would reduce the secretion of endogenous enzyme [25]. According to deficiency of studies focused on enzyme applying in fishes, we compared our results with other monogastric animals like pigs and poultry. There is no difference between these animals except the energy content of diets that is high in terrestrial animals because of keeping blood warm and consequence reactions that need more energy to fulfill; For example energy requirement for chicks are 5 time than fishes and shrimps [26]. Our results are discussed as bellow.

Table 2: blood biochemical characteristics of common carp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (mg/dl)</th>
<th>Creatine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Albomin (g/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>100</td>
<td>0.525</td>
<td>0.525</td>
<td>162</td>
<td>305</td>
<td>3.5</td>
<td>2</td>
<td>6.76</td>
</tr>
<tr>
<td>0.1%</td>
<td>82.33</td>
<td>0.396</td>
<td>0.36</td>
<td>180</td>
<td>350</td>
<td>3.36</td>
<td>2.03</td>
<td>7</td>
</tr>
<tr>
<td>0.5%</td>
<td>91.66</td>
<td>0.416</td>
<td>0.4</td>
<td>169</td>
<td>338</td>
<td>3.26</td>
<td>1.8</td>
<td>7</td>
</tr>
</tbody>
</table>

Means in the same row with superscripts of different letters differ significantly at p<0.05.

Table 3: blood hematological characteristics of common carp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WBC (/µl)</th>
<th>RBC (mil/µl)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>M.C.V (fl)</th>
<th>M.C.H (pg)</th>
<th>M.C.H.C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>6050</td>
<td>1.39</td>
<td>9.75</td>
<td>29.28</td>
<td>209.3</td>
<td>69.76</td>
<td>33.33</td>
</tr>
<tr>
<td>0.1%</td>
<td>6533</td>
<td>1.41</td>
<td>10.1</td>
<td>30.13</td>
<td>213.65</td>
<td>71.61</td>
<td>33.51</td>
</tr>
<tr>
<td>0.5%</td>
<td>6900</td>
<td>1.43</td>
<td>10.43</td>
<td>31.3</td>
<td>209.3</td>
<td>72.65</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Means in the same row with superscripts of different letters differ significantly at p<0.05.
Glucose: Glucose is probably the most studied of the nonenzymatic and nonprotein components of fish serum. Glucose values tend to increase with increased age in fish [24]. We expected to see higher value in 0.5% enzyme level than other treatments because enzyme breaks down the NSPs to small residues (glucose) but in this study we did not see that, because the glucose was higher in control group than enzyme groups. Our results were not compatible with OÔUZ and GÖNCÜOÔLU (2011) who showed that enzyme application did not affect glucose and Yuan (2008) who reported that enzyme inclusion in levels of 180 and 360 mg/kg due to breaking the NSPs to small residues of glucose so the sugar increases and Ao et al. (2010) who reported that glucose improved due to Endopower (contain 300 unit/g xylanase activity and 220 unit/g $-glucanase activity) and NSPase (0.2 level) applying in piglets diet.

Cholesterol: Cholesterol levels can indicate disorders of lipid and lipoprotein metabolism and liver function which contribute to the decreased cholesterol levels [27]. Cholesterol showed the same values between 0.5% enzyme treatment and control group but cholestrol level in 0.1% enzyme treatment was higher than other groups (p<0.05). Our results was compatible with studies of Mancini and parillo (1991) and hajati (2010) who reported that enzyme application increased the cholesterol level and was incompatible with kermanshahi (2006) and OÔUZ et al. (2011) who reported that enzyme application did not affected the cholesterol level.

Creatine: In fish, creatine predominates over crenatinine. Creatine is excreted through the kidneys, not the gills and forms more than half of the urinary nitrogen of most fish. Creatinin is found in fish, formed by spontaneous, nonenzymatic cyclization of creatine. This levels found in muscle do not appear to correlate with levels of creatine and once formed, creatinin is not metabolized further, but excreted unchanged. Blood levels of creatine in teleost fishes usually are on the order of 0.5 to 2 mg/dl, being higher in marine species [24]. In our study creatine levels in enzyme treatments were lower than control treatment (p>0.05).

Uric Acid: In most monogastric animals, we should take care of nucleic acid supplies. For example Hajati reported that enzyme addition in broiler chicks decreased the uric acids due to nutrient digestibility improvement. Uric acid formed by fish from exogenous and endogenous purine nucleotides and by catabolism of protein via purines. It is converted in the liver and to lesser extent in the kidney, to urea for excretion by the gills [24]. As seen in this study enzyme applying in 0.1% level could decrease the uric acid significantly than other treatments (p<0.05). Hajati (2010) found the same results like ours. He found that enzyme exclusion decreased the uric acid value significantly.

Urea: Most urea in fishes is produced by the liver, but urea passes rapidly though most internal membranes and is consequently found in all fish tissues. It’s excreted in small quantity in relation to total nitrogen excretion, primarily by the gills [24]. Borg et al. (1987) mentioned that blood urea can reflect the state of protein metabolism and amino acid balance and said that when blood urea is low, the balance of amino acids balance is good. There are no significance between groups in urea parameter but the control treatment’s urea value reflects better balance in amino acids than two other groups and therefore we see from here that total protein in control group is higher than two other groups [28]. Yuan et al. (2008) found that enzyme in control and 720 mg/kg treatments was so high and in 360 mg/kg was lower values (p<0.05). Ao et al. (2010) reported that inclusion of multi enzyme (Endopower) containing 300 unit/g xylanase activity and 220 unit/g $-glucanase activity in 0.1% level increased the amount of blood urea in piglets.

Total Protein: Alternate cause of low total proteins include decreased intake through starvation, decreased synthesis due to hepatic dysfuction, increased capillary permeability for plasma proteins, or degradation of protein by proteolytic enzymes released from endothelial cells destroyed by viruses or bacteria [24]. As we described total protein was higher in control treatment significantly (p<0.05).

Triglycerides: OÔUZ et al. (2011) reported that enzyme application decreased the triglycerides. Kermanshahi (2006 a,b) reported that enzyme applying itself in laying hens diet couldn’t affect the triglyceride but the interaction between enzyme and dried berberry fruit and turmeric rhizome powder affected both hematocrit value and triglyceride significantly. Enzyme addition couldn’t affect total cholesterol either.

Albomin: Serum albumin levels fall with liver disease. Due to this phenomenon, calcium falls down too because albumin acts as a ligand carrier for serum calcium [24]. We did not assayed the calcium value but best performance seen in 0.1% level (p<0.05).
As we know, CBC parameters dependent on age of fish [24]; but we saw in this study that enzyme could affect the hematocrit value due to WBC and RBC, hemoglobin, hematocrit, MCV, MCH values due to metabolism improvement that occurred by enzyme addition. One of most powerful hypothesis about RBC increase is due to $\beta$-glucan existence. We know that $\beta$-glucan is a immunity stimulant for fishes [29-30] and shrimps [31] so glucan residues would cause the WBC and maybe RBC increase in this experiment.

At the end we would suggest that even though enzyme inclusion would be useful for situations where we need resistant fishes but considering the costs of this materials shows that enzyme can not help to achieve this goal and traditional ways like vitamins or other immunity stimulants would be better and cheaper than enzymes.

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REFERENCES