Journal of Fisheries International 2 (2): 130-133, 2007

The Effects of Feeding on Some Enzymes' Activities and Growth Parameters in Rainbow Trout (*Oncorhynchus mykiss*) Fries

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Abstract: Fish were fed with live (*Gammarus pulex*) and wet foods (cattle spleen) per 2 and 4 days in order to determine the effects of feed on the activities of glucose 6 Phosphate Dehydrogenase (G6PD) and Carbonic Anhydrase (CA) enzymes and some growth properties of fry rainbow trout. Although the group fed with *G. pulex* per 2 days had the highest G6PD activity, the group fed with cattle spleen ones at two days showed the lowest enzyme activity. The effect of live feed given per 2 days on G6PD activity was significantly different for treatment groups ($p<0.01$). There was no significant effect of feed on CA activity. On the other hand, the best result for Feed Conversion Ratio (FCR) was found in the control group ($p<0.05$). Specific Growth Rate (SGR) and Survival Rate (SR) values were not different among the groups. At the end of the present study, it was concluded that both *G. pulex* and cattle spleen can be used as alternative food sources for rainbow trout fries. But, similar studies should be carried out for more clearly data about interactions between feeding and enzyme activities in fish.

Key words: Rainbow trout, G6PD, CA, growth parameters, SGR, FCR

INTRODUCTION

Enzymes are mostly protein catalysts which are to speed a chemical reaction up and they are not expended during the reaction (Slenzka *et al*., 1994).

The main role of pentose phosphate pathway is NADPH production. Glucose 6-Phosphate Dehydrogenase (G6PD, EC 1.1.1.49) catalyzes the first step of this pathway (Slenzka *et al*., 1994). NADPHs which are produced by pentose phosphate pathway are generally used in the synthesis of fatty acids, steroids, some amino acids, reduced glutathione and DNA (Bonsignore *et al*., 1966; Bonsignore and De Flora, 1972).

Carbonic Anhydrase Enzyme (CA, EC 4.2.1.1) plays the most important role on CO_2 transport in fish like other mammalians (Kathleen *et al*., 2002) and it can catalyze hydration/dehydration of CO to counter (Henry and Swenson, 2000).

The effects of some factors such as temperature, nutrition regime, feeding composition and frequency on fish, generally have been endeavored understanding of growth rate (Holm *et al*., 1990). However, there are very limited studies showing the effects of these factors on enzyme activities in fish.

Feed cost is about 60% of total input expense in aquaculture (Hew and Fletcher, 2001). Therefore, fish production studies intensify on the decreasing of food costs by using alternative food sources.

The aim of the present study was to test the usefulness of live and wet feed in rainbow trout fries (given per 2 and 4 days) by assessing some growth characteristic and G6PD and CA enzyme activities.

MATERIALS AND METHODS

Fish maintenance and experiment design: Rainbow trout fries (*Oncorhynchus mykiss*) were selected randomly from Research and Extension Center of the Department of Fishery Science at the Agriculture Faculty of Atatürk University. The initial weights were 1.46±0.9 g. In acclimation periods, fish were held in 75 L water volume fiberglass tanks for two weeks. Fish were settled as 20 fish per tank under natural light conditions. Then, the experiment was carried out as below: Tank 1 and 2: Fish were fed *Gammarus pulex* per 2 days for 60 days (GP2 Group). Tank 3 and 4: Fish were fed *Gammarus pulex* per 4 days for 60 days (GP4 Group). Tank 5 and 6: Fish were fed cattle spleen per 2 days for 60 days (CS2 Group).

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Tank 7 and 8: Fish were fed cattle spleen per 4 days for 60 days (CS4 Group). Tank 9 and 10: Fish were fed with commercial rainbow trout feed for 60 days (Control Group). Aerated artesian water with 1 L min^{-1} inflow, 12±1°C temperature, 7.5±0.1 pH and 8.1±0.4 mg L^{-1} dissolved oxygen was used. Commercial pellets and other food which was 4% of body weight per day were given to fish after supplied isocaloric equality (Aras *et al*., 2000).

Enzyme activity assays: To assay G6PD and CA activities, blood samples were taken from the caudal vein with heparinized syringes (Blaxhall and Daisley, 1973). It was immediately transported to laboratory in ice and centrifuged at 2500 g at +4°C for 15 min. Erythrocytes were washed thrice with cold saline 0.16 M Kcl and the pellet was hemolysed with five volumes chilled water (Martinez *et al*., 1994) and Hemoglobin (Hb) concentration in the hemolysate was determined by the cyanmethemoglobin method (Blaxhall and Daisley, 1973).

G6PD activity was determined according to Beutler's method (Beutler, 1983). The activity assay was done by monitoring the increase in absorption at 340 nm due to the reduction of 1 μmol of $NADP^+$/min at pH 8.0.

CA activity was measured using the CO_2-hydration method of Wilbur and Anderson (Wilbur and Anderson, 1976). CO_2-hydratase activity as an Enzyme Unit (EU) was calculated by using the equation $(t_0-t_c)/t_c$. Where t_0 and t_c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively (Hall and Schraer, 1983).

Statistical analysis: The statistical analysis was performed with SAS package program (SAS, 1996). Data were presented as Mean±Standard Deviation (SD) of the mean. Data were analyzed by one-way Analysis of Variance (ANOVA). The significant means were compared by Tukey's multiple range tests at $\alpha = 0.01$ and 0.05 levels.

Growth parameters: Growth parameters and survival rate were calculated according to the below formulas (Aster and Moon, 1981). Food Conversion Ratio (FCR) = Given Food Amount in a Period (g)/Growth Gain in a Period (g), Specific Growth Rate (SGR), % day^{-1} = ln Last Weight (g)-ln Initial Weight (g)/Time (day)×100 and Survival Rate (SR, %) = Number of Living Fish at the End of Trial/Number of Fish at Initial×100.

RESULTS

Enzyme activities: The mean G6PD and CA activities in different groups were given in Table 1 and 2, respectively. As seen from the Table 1, the highest G6PD activity was measured in GP2 group (30.32±3.82 EU gHb^{-1}); the differences among groups were significant ($p<0.01$). Although the highest CA activity was found in GP2 group (10695.2±225.80 (EU gHb^{-1}) like G6PD activity, the differences did not reach to a statistical significance among the groups.

Growth parameters: FCR, SGR and SR in rainbow trout fries were shown in Table 3. As shown in this table, the best FCR was determined in the control group as 1.06±0.09, while the worst in GP4 group (2.52±0.49). The differences between control and other groups were important at $p<0.01$ level. The highest SGR occurred in GP4 and CS4 groups with 2.87±0.094 % day^{-1} and 2.87±0.088, respectively. The control (2.77±0.99 % day^{-1}) and GP2 (2.76±0.045 % day^{-1}) groups followed these two groups. Finally, the maximum SR was found in GP2 group as 97.5% and the lowest value in the control, GP4 and CS2 groups with 90% (SR of three experiment groups were equal). At the end of the study it was not found any significant difference between SGR and SR in rainbow trout fries (Table 3).

Table 1: Effects of different feeds on G6PD enzyme activity in rainbow trout (*Oncorhynchus mykiss*) fries

	GP2 (n = 3)	GP4 (n = 3)	CS2 (n = 4)	CS4 (n = 4)	Control (n = 4)	F
G6PD Activity (EU gHb^{-1})	30.32±3.82^a	22.63±2.91^{ab}	16.20±3.87^b	16.98±2.88^b	24.20±3.05^{ab}	**

(a and b) means in a row with identical letters are not significantly different, values given as mean±s.d., **: ($p<0.01$)

Table 2: Effects of different feeds on CA enzyme activity in rainbow trout (*Oncorhynchus mykiss*) fries

	GP2 (n = 3)	GP4 (n = 3)	CS2 (n = 4)	CS4 (n = 4)	Control (n = 4)	F
CA Activity (EU gHb^{-1})	10695.2±225.8^a	9791.3±184.47^a	8356.6±240.8^a	8290.1±175.84^a	8721.2±197.4^a	NS

A means in a row with identical letter is not significantly different, values given as mean±s.d., NS: Not Significant

Table 3: Some growth parameters and survival rate of rainbow trout (*Oncorhynchus mykiss*) fries fed with different diets

	GP2	GP4	CS2	CS4	Control	F
Food conversion ratio	1.87±0.47^{ab}	2.52±0.49^b	2.38±0.49^b	2.01±0.54^b	1.06±0.09^a	*
Specific growth rate (% day^{-1})	2.76±0.045^a	2.87±0.094^a	2.39±0.000^a	2.87±0.088^a	2.77±0.099^a	NS
Survival rate (%)	97.5±0.00^a	90±0.00^a	90±0.00^a	95±0.00^a	90.00±0.00^a	NS

(a and b) means in a row with identical letter is not significantly different, values given as mean±s.d., *: ($p<0.05$). NS: Not Significant, n = 2

DISCUSSION

As understood from the findings of the present study, GP2 group was fed with live feed had G6PD activities with 25% increase when compared to the control group and G6PD activities were found to be decreased in the remaining groups when compared to the control group. For example, there was 33% decrease in the enzyme activity in CS2 and CS4 groups. On the other hand there was not any change in GP4 group. As well known, the digestion of the commercially available feeds is more difficult due to its low water content in comparison with the wet and live feeds (Gatlin, 2002). Therefore, the administration of CS per 2 and 4 days lead to an increase in FCR but no change in SGR. This finding was not a surprising result because of its high water content. Contrarily, the SR of this group was parallel to the groups. From this finding, it was concluded that this feed could be utilized easily due to its low economical cost and easy digestion as an alternative to commercial feeds. Aras (1991) had reported similar results on this topic.

The fundamental function of G6PD is to catalyze the first reaction of pentose phosphate pathway in which NADPH is produced. So, the production of NADPH of sufficient amounts is used in biosynthesis pathways like the synthesis of fatty acids, steroids and other macromolecular that are essential for growth (Bonsignore *et al*., 1966; Bonsignore and De Flora, 1972). The increases in G6PD activities in the groups fed with commercial feeds and frequently given *Gammarus pulex* indicate that those trout grow better and faster. Indeed, FCR and SGR were found to be higher in commercial diet and GP2 groups (and SR unchanged) when compared to the other groups (Table 3). Additionally, these data imply that growth parameters could be estimated by the measurement of G6PD activity. But, more detailed studies are needed to confirm this speculation.

Another important finding of this study was that CA activity was not significantly affected by the type of feed. Only GP2 group h ad 22% increases in CA activity as in G6PD. CA, having an important role in the transportation of excretion of carbon dioxide (Kathleen *et al*., 2002) was found to be increased with growth and high metabolic activity. That CA activity was low in CS2 and CS4 groups was in agreement with the above findings. But, similar studies should be carried out for more clearly data about interactions between feeding and enzyme activities in fish.

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