The Relationship Between Stocking Density and Sorbitol Dehydrogenase Activity in Rainbow Trout (*Oncorhynchus mykiss*) Tissues

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Dokularında Stok Yoğunluğu ve Sorbitol Dehidrogenaz Aktivitesi Arasındaki İlişki

Research Article

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ABSTRACT

It is well known that almost all the reactions are catalyzed by enzymes in metabolism of living beings. Environmental conditions leads to very significant changes in living metabolism including fish. These changes may be genetic, hormonal, enzymatic and other physiological defects. The aim of this study was to assess the effects of increasing stock density on the activity of sorbitol dehydrogenase enzyme in liver, muscle, gill and kidney tissues of rainbow trout. Fish were reared at different stock densities (15 kg/m^3, 20 kg/m^3, 25 kg/m^3 and 30 kg/m^3), and after adaptation period of 30 days, the experiment was carried out for two months. Stock density of the control group was 15 kg/m^3. Increasing stock density caused significant inhibition of sorbitol dehydrogenase enzyme. Overall results indicate that increasing stock density significantly blocks the SDH activity in rainbow trout tissues and that this might cause undesirable results by disrupting physiological balance as inhibition of this enzyme leads to sorbitol accumulation.

Key Words
Stock density, rainbow trout, inhibition, sorbitol dehydrogenase

ÖZET

Canlı metabolizmasındaki hemen hemen bütün reaksiyonların enzimler tarafından katalizlendiği iyi bilinir. Çevresel şartlar, balıklarıda içine alan canlı metabolizmalarında önemli değişikliklere yol açar. Bu değişiklikler, genetic, hormonal, enzimatik, ve diğer fizyolojik eksiklikler olabilir. Bu çalışmanın amacı, artan stok yoğunluğunun gökkuşağı alabalığının karaciğer, kas, solungaç, böbrek sorbitol dehidrogenaz enzimi üzerine etkilerini belirlemektir. Balıklar farklı stok yoğunluklarında (15 kg/m^3, 20 kg/m^3, 25 kg/m^3 ve 30 kg/m^3) beslendi ve 30 günlük adaptasyon süresi sonunda deney iki ay boyunca uygulandı. Kontrol grubunun stok yoğunluğu 15 kg/m^3'tür. Artan stok yoğunluğu SDH enziminin önemli oranda inhibisyonuna neden oldu. Genel sonuçlar göstermektedir ki artan stok yoğunluğu gökkuşağı alabalık dokularındaki SDH aktivitesini önemli oranda engeller ve bu da enzimin inhibisyonuna bağlı olarak artan sorbitol miktarından dolayı fizyolojik dengede istenmeyen sonuçlara neden olabilir. Böylece, enzimin inhibisyonu sorbitol birikimine öncülük eder ve fizyolojik dengenin bozulmasıyla istenmeyen sonuçlara sebep olur.

Anahtar Kelimeler
Stok yoğunluğu, gökkuşağı alabalığı, inhibisyon, sorbitol dehidrogenaz

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INTRODUCTION

Many factors such as environmental pollutants, drugs, other xenobiotics and living conditions can be cause the oxidative stress in biological systems [1-4]. They play vital roles in the mechanistic aspects of oxidative damage [5]. Living conditions is important for all living things especially density. In the past decade, numerous studies on the effects of oxidative stress were published [6,7]. It is seen that these effects can be in several types as genetic, enzymatic and other physiological changes. These critical changes may reveal some vital results in terms of reproduction, growth, behavior etc. of living things including fish. Now it is known that there is a growing interest in the welfare of intensively farmed fish worldwide. As an area of particular concern, stocking density has been emphasized and the effects of stocking density are known to be complex and appear to comprise of numerous interacting and case specific factors [8]. Studies made on these factors are important to improve welfare. It is known that stocking density, diet, feeding technique and management procedures may affect welfare. Generally, stocking density can be defined as the weight of fish per unit volume or per unit volume in unit time of water flow through the holding environment [8,9]. So, stocking density has a substantial importance in welfare in the fish farming including rainbow trout. Rainbow trout is one of the most cultured fish species in aquacultural industry around the world. Therefore, there are many studies with effects of density on production and physiological parameters of some aquacultures including rainbow trout. For instance, Montero and colleagues (1999) investigated the effect of high stocking density on one of the most important marine fish species for Mediterranean aquaculture, gilthead sea bream (Sparus aurata) [10]. They found that stocking density affected growth, biochemical composition, immune status and hematology. Besides, there are some studies in literature on fish growth and survival associated with effects of stocking density [11-13]. Also, some other articles report about some alterations of fish behaviour, metabolism etc. connected with high stocking density [14,15]. As mentioned above, environmental conditions including stocking density influence genetic components, some stress factors, enzyme activities and oxygen consumption of living beings including fish metabolism. Especially, some factors are important as a target and they have critical function for living metabolism from metal toxicity to density [16,17]. It is well known that almost all reactions are carried out through enzymes in the organism. Because of these, enzymes are seen important target for all external factors.

A Zn-enzyme, sorbitol dehydrogenase (EC 1.1.1.14, SDH) and aldose reductase constitute the sorbitol or polyol pathway, which functions as an important bypass to glycolysis and the pentose phosphate pathway in the metabolism of glucose, via D-sorbitol to D-fructose [18,19]. Sorbitol dehydrogenase (SDH), in concert with NAD^+, oxidizes sorbitol to fructose, and reduces NAD^+ to NADH. Excess flux through SDH, as would be prevailing under diabetic conditions, creates an imbalance in the cytoplasmic NAD+/NADH ratio. It has been reported [20] that inhibition of SDH can restore the altered cytoplasmic red-ox state and can influence the early functional changes observed in experimental diabetes, including alterations in vascular albumin permeation, tissue blood flow, and nerve conduction velocity. Also, Geisen et al. have reported on the effects of inhibiting SDH on renal hyperfiltration in diabetic rats [21]. The abnormal accumulation of intracellular sorbitol has been linked to the onset of a number of diabetic complications. It is thus clear that sorbitol dehydrogenase activity is very important and decreased activity is a great risk in some cases. Therefore, inhibitory effects on SDH must be well characterized.

Because there are not enough investigations on the effects of stocking density on various parameters of living systems and we have not found any relation between stock density and sorbitol dehydrogenase activity, we aimed in this study to examine whether increasing stock density cause negative results on the activity of SDH.

MATERIALS AND METHODS

Materials

Chemicals used for enzyme activity measurements

were purchased from Sigma-Aldrich or Merck. All other chemicals were of analytical grade and obtained from either Sigma-Aldrich or Merck.

Fish Husbandry and Experimental Design

Rainbow trouts were obtained from the Fisheries Department of the Agricultural Faculty at Atatürk University in Erzurum and weighed of 130 ± 20 g. They were fed twice a day with a commercial pelleted trout feed (at 1% body weight). Trout feed was purchased from Pınar Yem Company, İzmir-Turkey. Fish were fed a commercial pellet diet with 49.4% protein, 18.2% fat, 94.3% dry matter, and 9.8% ash at a daily ration of 1% of their wet body mass during the study. Feed was given by hand. Fish treatments were conducted according to Applied Research Ethics National Association.

Prior to the experiment, fish in each group were kept in 1x1.2 m (diameter-deep) fiber-glass tanks for one month. The average water temperature was 10°C during the experiments. Aeration was provided along the experiments. The water quality parameters were measured as O_2 = 8.6 ppm, pH = 7.7, SO_4^{-2} = 0.33 mg/L, PO_4^{-3} = trace, NO_3^- = 3.45 mg/L, NO_2^- = trace, and conductivity = 230 us/cm. Throughout the experiments, one tank was used as control (15 kg/m^3) while other three experimental groups were 20, 25, 30 kg/m^3. After 2 months, five animals from each group were randomly sampled, immediately stunned and sacrificed. Tissue samples were transferred into liquid nitrogen and stored at -80°C until analysis.

Enzyme Assays

Preparation of the Homogenate

Tissue samples were washed three times with 50 mM Tris–HCl+0.1 M Na_2SO_4 (pH 8.0), and each was homogenized by liquid nitrogen, transferred to the same buffer, and centrifuged at 4°C, 15.000 g for 60 min. Supernatant was used in further studies.

Enzyme assay

The initial rate of sorbitol oxidation was determined at 23.5°C by measuring the increase in absorbance at 340 nm for NADH production with spectrophotometer connected to an ABB Goertz SE 120 recorder. The concentration of enzyme stock solutions was calculated as described in the literature [22].

Protein Determination

Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method [23], with bovine serum albumin as a standard.

Statistical Analysis

The statistical analysis was performed using SPSS (version 17.0) software. Data were presented as mean ± standard error of the mean (SEM) and analysed by one-way analysis of variance (ANOVA). The significant means were compared by Duncan's multiple range tests at $p < 0.05$ level ($n = 5$).

RESULTS AND DISCUSSION

With a growing world population, the farm fishery has become more important. It is obvious that this situation brought about by some handicaps. One of the critical handicaps is biological changes in fish metabolism. These changes may be genetic, hormonal, enzymatic and other physiological defects. So, it should not be ignored that people, constitute an important ring in the food chain, will be affected by this situation directly or indirectly. Stocking density is one of many stress factors including chemical compounds, heavy metals, pesticides and other aquatic changes [1,17]. Stress-related changes in biology of organisms usually occur via enzymatic pathways. It is well known that many chemicals, drugs, pesticides and gases affect the living metabolism with enzyme inhibition. Now, it is clear to stoking density is an important stress factor. Recently, many studies in these fields have been performed by scientists on the world [8,24,25]. In the current study we assessed the effects of increasing stock density on the activity of sorbitol dehydrogenase enzyme in liver, muscle, gill and kidney tissues of rainbow trout. Fish were reared at different stock densities (15 kg/m^3, 20 kg/m^3, 25 kg/m^3 and 30 kg/m^3). After adaptation period of 30 days, the experiment was carried out for two months. Stock density of the control group was 15 kg/m^3. Water quality parameters were measured as O_2 = 8.6 ppm, pH = 7.7, SO_4^{-2} = 0.33 mg/L, PO_4^{-3} = trace, NO_3^- = 3.45 mg/L, NO_2^- = trace, and conductivity = 230 us/cm. Tissue samples were homogenized and SDH activity was assayed and compared.

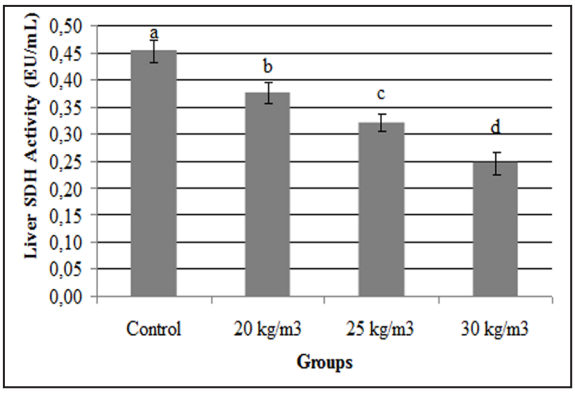

Figure 1. Stock density dependent alterations in liver SDH activity (a, b, c, d shows statistical difference $p < 0.05$).

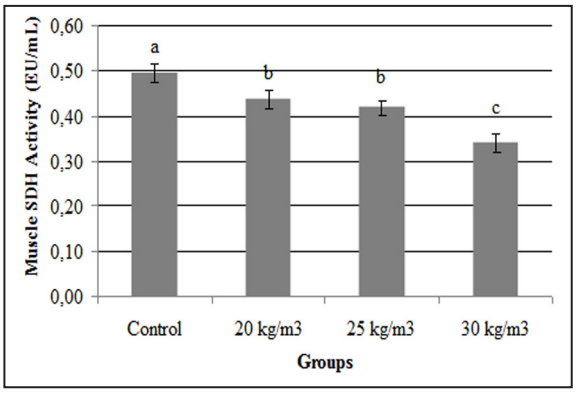

Figure 2. Stock density dependent alterations in muscle SDH activity (a, b, c, d shows statistical difference $p < 0.05$).

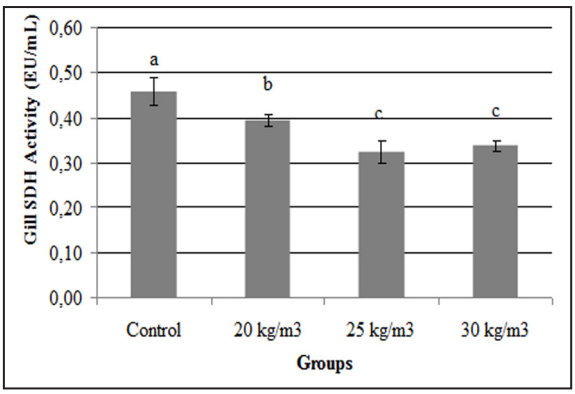

Figure 3. Stock density dependent alterations in gill SDH activity (a, b, c, d shows statistical difference $p < 0.05$).

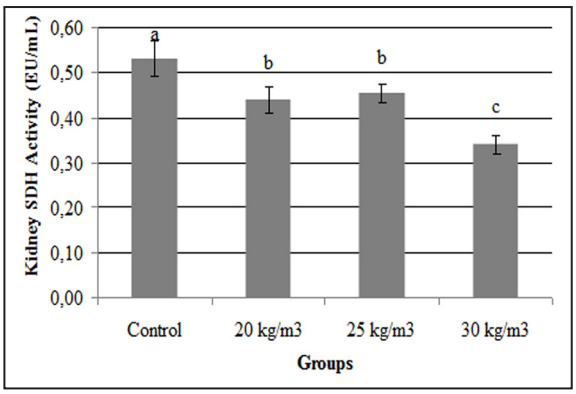

Figure 4. Stock density dependent alterations in kidney SDH activity (a, b, c, d shows statistical difference $p < 0.05$).

Liver SDH activity significantly decreased in 20 kg/m3 density compared to the control group ($p < 0.05$). Similarly, 25 and 30 kg/m^3 stock density decreased the activity significantly ($p < 0.05$).

As can be clearly seen, increasing stock density caused a gradual inhibition in rainbow trout liver sorbitol dehydrogenase enzyme (Figure 1). The muscle enzyme was also significantly inhibited by 20 kg/m^3 stock density ($p < 0.05$). While no significant difference was observed between 20 kg/m^3 and 25 kg/m^3 groups, 30 kg/m^3 stock density lead to significant inhibition ($p < 0.05$) of the enzyme compared to other groups (Figure 2). Gill SDH activity was gradually inhibited ($p < 0.05$) by increasing stock density up to 25 kg/m^3 but no significant attenuation was observed between 20 and 30 kg/m^3 groups (Figure 3). As for the kidney enzyme, 30 kg/m^3 density inhibited the activity significantly ($p < 0.05$), but there was no significant difference between 20 and 25 kg/m^3 groups. However, 30 kg/m^3 density caused a significant ($p < 0.05$) attenuation (Figure 4). Overall results indicate that increasing stock density causes significant inhibition of sorbitol dehydrogenase activity in the liver, muscle, gill and kidney tissues of rainbow trout.

Fish must be provided with optimal environmental conditions in order to achieve potential growth and profit in fish farms. Any deviation from these conditions can result in decreased performance and is clear to affect the profitability of the aquaculture industry. Because high stocking density can contribute to reduced performance due to a number of factors, the effect of stocking density on the welfare is the key factor in fish production. In this context, there are a lot of studies showing that high stocking density produces a wide variety of effects on cultured fish populations, such as alterations in physiological parameters and poor feed utilization, results in mortality and poor growth, and inhibits some vital enzymes.

Li et al. (2006) investigated the effects of dissolved oxygen concentration and stocking density on phenoloxidase, superoxide dismutase, and peroxidase Chinese shrimp (Fenneropenaeus chinensis).

According to their results, the activities of phenoloxidase, superoxide dismutase and peroxidase were significantly affected by dissolved oxygen concentration. Phenoloxidase activity increased with stocking density. On the contrary, superoxide dismutase activity did not show any changes with the stocking density, but the activity was found to be affected by different dissolved oxygen concentrations. No significant alterations were observed in peroxidase activity upon different dissolved oxygen concentration or stocking density [26].

Funasako et al. (1994) measured erythrocyte aldose reductase and sorbitol dehydrogenase activity in erythrocytes in healthy individuals aged from 16 to 91 years to determine the mechanism of age-dependent sorbitol accumulation. They determined that erythrocyte aldose reductase activity increased significantly with age but ageing had no effect on sorbitol dehydrogenase activity. They reported that age and the aldose reductase/ sorbitol dehydrogenase ratio were positively correlated. Findings of the study suggested that an increase in the ratio of aldose reductase to sorbitol dehydrogenase may contribute to the tissue accumulation of sorbitol in the elderly and may be a mechanism of a disease that is common in elderly individuals [27].

Another study examined sorbitol accumulation and transmembrane efflux in JS1 schwannoma cells during osmotic stress in the presence of a sorbitol dehydrogenase inhibitor. SDH inhibition promoted sorbitol accumulation under hyperglycemic and/or hyperosmotic conditions. The study revealed that sorbitol levels in JS1 cells are dependent on SDH activity [28].

There are some similar studies existing in the literature, but effect of stocking density on metabolic enzyme systems is poorly understood. In this context our data have good contributions. We have proved here that increasing stock density inhibits sorbitol dehydogenase which may cause sorbitol accumulation and have unwanted outcomes.

CONCLUSION

Variation of the stocking density can be a source of stress for fish. The stocking density is directly related to animal comfort and to fish culture productivity, and can be a determining factor in the economic return on production. The ideal density is one that does not cause substantial reduction in growth rates or environmental quality. Specially, stress has vital effects both on welfare and productivity in farmed fish and it has been linked to reduction in growth, abnormal behaviour and immuno-depression. Results of our study show that increasing stock density attenuates the activity of a very important enzyme sorbitol dehydrogenase in rainbow trout tissues. Thus, increasing stock density might cause undesirable results for aquaculture and fish welfare by disrupting the physiological balance.

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