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Effect of Water Hardness on Growth, Survival, Hematocrit and Some Blood Biochemical Indices of Kutum (*Rutilus frisii kutum*) Fingerlings

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Abstract: The responses to water hardness augmentvariesfrom speciesto species. The aim of this research was to verify the growth, survival, hematocrit and some biochemical indices change in Kutum (*Rutilus frisii kutum*) fingerlingsin water with differentlevels of hardness. Fingerling fish (2±0.1 g) were randomlydistributed to trial units (three replicates per treatment) and kept in 15aquaria (20 fingerlings/aquarium). Fingerlingswere exposed to five water hardness amount (30, 70, 150, 300 and 600 $mgL^{-1}CaCO_3$) at pH=8.45. At the end of trial, FCR (feed conversion ratio), specific growth rate (SGR), final biomass, hematocrit, calcium, potassium, sodium, magnesium, glucose, cholesterol and total protein were concluded for each collection. Higher fingerlingsgrowth, survival, weight gain and final biomass were obtained 70 and 150 mgL^{-1} $CaCO_3$. Hence, this is the suggested hardness range for growth and survival of Kutumfingerlings.

Key words: Blood Biochemistry · Survival · Growth · Water Hardness · Kutum

INTRODUCTION

Kutum (*Rutilus frisii kutum*) is one of the economicall yimportant fishes of the Caspian Sea. They are mainly distributedin the southern part of the Caspian Sea, particularly in the region from Astara to Gorgan River and migrate into rivers for spawning. Kutum have two life histories; there are spring and autumn migratory populations. The fish spawn on aquatic plants and river sands [1].

Totalhardness is the concentration of all divalent cations in water and Ca^{2+} & Mg^{2+} are the most common cations in nearly all freshwater systems. The suggested valuefor water hardness for fish culture in ponds is above 20 mgL^{-1} $CaCO_3$ [2], principally because of itseffect on phytoplankton growth and water pH control. However, Ca^{2+} is necessary for fish for some biological processessuch as bone construction, blood coagulation, and multiple other cellular functions [3]. The internalsourceof Ca^{2+} in fish is not easily accessible. Hence, plasma Ca^{2+} wascontrol by its ingestionwith food or by branchial absorption [4].

Skeletalgrowth in vertebrates is frequentlylimited the obtainability of fundamental nutrients and minerals [5]. In particular, calcium, the primeval componentof the classified collagen matrix formingthe bonyskeleton of morevertebrates, is necessary to suitable growth and evolvement. Differencein the attainability of calciumin the aquatic environmentplace constraintson the speed and scope of larval evolvement in fishes [3], can stunt growth and can place extra stresses on fish transplanted from a calcium-rich to a low-calcium environment.

MATERIALS AND METHODS

300 fingerling fish of Kutum produced at the Sijovalarea, Golestan, Iran were acclimated to laboratory conditions for 30 days before being randomly distributed into 15 experimental 70-L aquaria which supplied with freshwater from an urban system. The aquaria were individually aerated and water temperature was controlled at 23±1°C. Dissolved oxygen, pH and water temperature were continuously monitored. Photoperiod was maintained at 12-h light: 12-h dark cycle.

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Length, weight of fish and water salinity were calculated by biometry board (±1mm), balance (±0.01g) and water checker (HORIBA, U-10, Japan) respectively. Water K^+ and Na^+ ions were calculated by flame photometer (Jenwaypfp 7, England), water Mg^{2+} and Ca^{2+} were measured by spectrophotometer (S2000-U V/IS England) [6].

pH was measured by pH meter; EC (electrical conductivity) was measured by water checker (HORIBA, U-10, Japan) and total hardness was measured by titration method in Central Laboratory of Gorgan University of Agricultural Sciences and Natural Resources.

Whole-body specific growth rates (SGR), expressed as a percentage of the body weight was calculated using the growth rate equation of Turker *et al*. [6]:

$$SGR(\%/day)=\{[\ln(Wf)-\ln(Wi)].100\}/t$$

where (*Wi)* and (*Wf)* are the initial and final wet weights (g) of the experimental Kutum, respectively and (*t)* is the length of the experimental period (in days).

The feed conversion ratio (FCR) was calculated in terms of wet weight as:

FCR= wet weight of feed consumed/change in wet weight

Survival (%)=Total live fish (No.) after t/ Total fish at 0 day (No.).100

The quantitative determination of plasma glucose were carried out using commercially available diagnostic experimental protocols kits Pars Azmoon, Iran, at 546 nm and 37 ºC by the glucose oxidase method [7]. Glucose was measured photo metrically according to a method modified from Lovson [5]. 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 Hedayati *et al*. (2010). Calcium (Ca) values were measured by cresophthaleincomplexone [8]. Phosphor values were measured by a colorimetric reaction as a combination of molybdate with phosphate in the presence of acid that forms a final product called molybdenum blue [9].

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

RESULTS

Plasma glucose, cholesterol, calcium, sodium, magnesium, potassium and total protein values are shown in Table 1. No statistically differences were observed in hematocrit and biochemical parameters includingplasma cholesterol, calcium, sodium, magnesium, potassium and total protein values ($p>0.05$) but Plasma glucose concentrations increased in 1, 2 and 5 treatment.

During the period of 56 days, fingerling fish maintained at water hardness of 70 and 150 $mgL^{-1}CaCO_3$ showed greater weight gain, final biomass, SGR and survival also showed lowest FCRthan the other treatments (Fig 1-5).

DISCUSSION

This research was guided to estimate the effects of changing water hardness on growth, survival, hematocrit and biochemical indices change of Kutum (*Rutilus frisii kutum*). Acquire sufficiently and appropriate food is the mainly pompous factor in aquaculture. Deviation in water quality, counteraction correlations of physicochemical factors with each other and fish high density may create popular ranges of physiological variations in fish [10].

Table 1: Hematocrit and Biochemical indices change of Kutum fingerlings fish exposed to different levels of water hardness

	Experimental treatment				
	Treatment1(30)	Treatment2 (70)	Treatment3 (150)	Treatment4 (300)	treatment 5(600)
Hematocrit	45.00±1.00[a]	45.83±1.50[a]	43.00±1.00[a]	43.00±1.00[a]	45.66±1.52[a]
Sodium	121.67±10.40[a]	126.67±7.63[a]	129.00±14.93[a]	131.67±12.58[a]	109.67±10.50[a]
Potassium	3.83±0.76[a]	4.33±0.76[a]	4.50±1.32[a]	4.26±0.68[a]	4.30±0.72[a]
Calcium	4.40±0.52[a]	4.86±0.70[a]	5.16±1.04[a]	5.43±0.92[a]	5.56±1.20[a]
Magnesium	8.23±0.25[a]	8.40±0.10[a]	8.63±0.32[a]	8.76±0.40[a]	8.90±0.65[a]
Glucose	95.66±13.65[a]	90.00±5.00[ab]	80.00±5.00[b]	77.33±6.80[b]	104.33±6.02[a]
Cholesterol	0.55±0.05[a]	0.49±0.02[a]	0.48±0.03[a]	0.49±0.05[a]	0.49±0.08[a]
Total protein	20.66±2.51[a]	18.66±1.52[a]	20.33±2.08[a]	20.33±3.05[a]	21.33±2.08[a]

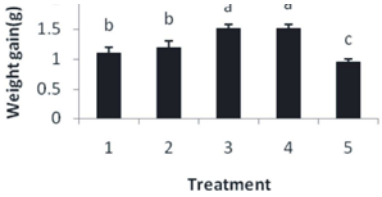

Fig. 1: Weight gain of Kutum after exposure to different levels of water hardness

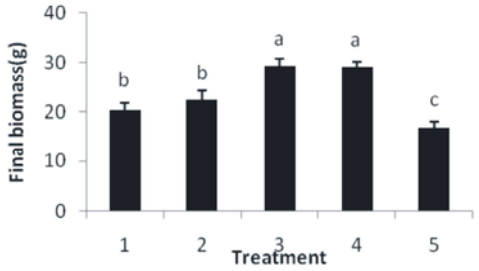

Fig. 2: Final biomass of Kutum after exposure to different levels of water hardness

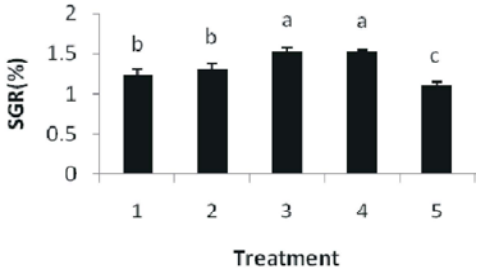

Fig. 3: SGR of Kutum after exposure to different levels of water hardness

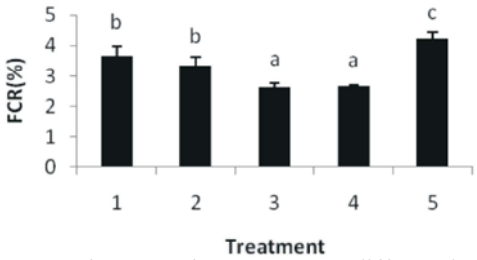

Fig. 4: FCR of Kutum after exposure to different levels of water hardness

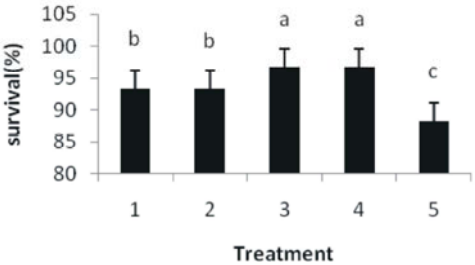

Fig. 5: Survival of Kutum after exposure to different levels of water hardness

Buttner *et al*. [2] informed that physicochemical factor effect on fish growth and they too effect on each other. In this study, significant and positive correlation was observed between hardness (70 and 150) with final weight, growth rate, FCR, hematocrit and biochemical indices. Loveson [5] propose that in hardness permissible domain, anything the water be harder it is appropriate for fish but out of this range fish indicatereduce in growth with enlarge in water hardness.

Loveson [5] established that an increase in water hardness decrease or removed of silver toxicity in early life levels of rainbow trout, while improvement fish survival. Hence in our research the highest survival rate was found at moderately hard water (70 and 150 mgl^{-1} $CaCO_3$).

Plasma glucose levels had decreased inhardness 70 and 150 mgL^{-1}. Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy [11]. Glucose is then released toward blood circulation and enters into cells through the insulin action [12]. Plasma glucose concentration depends on intestinal absorption, hepatic production and tissue uptake of glucose. Several hormones affect homeostasis of glucose, including insulin, glucagons, corticosteroids, adrenocorticotropic hormone (ACTH), growth hormone and catecholamines. The maintenance of blood glucose concentrations occurs via hepatic glycogenolysis, glycolysis and gluconeogenesis [13].

Liming is suggested to stabilize low water pH, because calcium carbonate enlarges water buffering capacity [13]. For silver catfish, liming must not enlarge water hardness beyond 70 mg L^{-1} $CaCO_3$, because this quantity is sufficiently to decrease the effect of acidic and alkaline pH on fingerlings [14].

In this research we found the highest survival rate in 70 and 150 mg/l $CaCO_3$, but Nelson & Cox (2005). studied the effects of water hardness on skeletal development and growth in juvenile fathead minnows and pointed that fathead minnows reared in calcium-abundant water (Ca=65±1.5 mgL^{-1}as $CaCO_3$; hardness 175 mg/l) had significantly multiplied survival but lower whole body mass when compared to their conspecifics in low calcium water.

These results infer that hardness effects on water conditions for use in Kutum rearing and the best hardness range for survival and growth of Kutum fingerling fish is 70–150 mg L^{-1} $CaCO_3$.

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