To determination levels of Vitamin, A in *Labeo rohita* & *Clarias batrachus* of Bisalpur reservoir

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Abstract

The objective of this study was to provide some valuable information to the field of freshwater fish determination levels of biochemical parameters of *Labeo rohita* & *Clarius batrachus* which living in natural environment. Present study was conducted on Bisalpur reservoir. . Four areas related to Bisalpur reservoir selected were Bisalpur, Nasirda, Thadoli and negadiya for collection of fishes as well as water samples.Total 80 fishes collected during extreme cold condition when water bodies were having peak environmental contamination in reservoir due to fall in water level and feeble current. From each area 20 fishes were collected which constituted *Clarias batrachus* (10) and *Labeo rohita*(10). The mean values of vitamin A in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of vitamin A in fishes of Thadoli area indicated the presence of oxidative stress.

Key words :- *Vitamin A, Oxidative stress, Clarias batrachus and Labeo rohita.*

Introduction

Fishes are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may however, be evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance. Fishes are located at the top of the food chain, are a highly visible resource, and are known to accumulate toxicants (Rowan and Rasmussen, 1992 and Strait, 1998).

Fish, apart of being a good source of digestible protein, vitamins, minerals and polyunsaturated fatty acids, are also an important source of essential heavy metals (Irwandi and Farida, 2009)

Qing-song *et al*. (2007) determined vitamin A and E in fish tissues. All the vitamins were detected under the wave length of 292 nm. The linearity range between peak area and vitamin content was 0-15μg/ml for vitamin A, 0-10μg/ml for vitamin D_3 and 0-20μg/ml for vitamin E. The lower limits were 0.8×10^{-3} ng for vitamin A, 0.06×10^{-3} ng for vitamin D_3 and 9.0×10^{-3} ng for vitamin E, respectively. The fat-soluble vitamins

A, D_3 and E in fish tissues could be effectively separated under the described conditions and simultaneously determined by HPLC at 292 nm after mineral ether extraction.

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Material & Method

Present study was conducted on Bisalpur reservoir. Bisalpur dam is constructed on the river Banas which originates from Gongunda area of Southern Rajasthan. Four areas related to Bisalpur reservoir selected were Bisalpur, Nasirda, Thadoli and negadiya for collection of fishes as well as water samples from all seasons that is moderate, extreme cold, extreme hot and moist warm ambiences. Total 80 fishes collected during extreme cold condition when water bodies were having peak environmental contamination in reservoir due to fall in water level and feeble current. From each area 20 fishes were collected which constituted *Clarias batrachus* (10) and *Labeo rohita*(10).

Vitamin A were determined by the methods as described by Varley (1988).Proteins are precipitated with alcohol and the retinol and carotenes extracted into light petroleum. After reading the intensity of yellow colour due to carotenes, the light petroleum is evaporated, and the residue is dissolved in chloroform before carrying out colour reaction. Allowance is made for the carotene contribution to the reaction.

Into a 15ml glass–stoppered centrifuge tube, 1 ml serum and 2 ml ethanol were pipetted. It was stoppered, and the contents were mixed with a vortex mixer. Then 3ml light petroleum was added and the tube was placed in a mechanical shaker for 10min to extract the retinol and carotenes into the petroleum phase. Then the tube was centrifuged for 10min at 2500rpm.Then into a cuvette, 2ml of upper layer was taken and optical density was recorded at 450 mμ against a petroleum blank. Simultaneously, 2ml of each carotene working standard was taken into a dry cuvette and optical density was recorded as for serum. It was done without any delay.

Then the content of each cuvette was evaporated to dryness in a water bath (50^{o}C). Then 100µl chloroform was added to each cuvette, mixing briefly with a vortex mixer. Simultaneously the standards were prepared by taking 100µl of each retinol working standard. A blank was prepared by taking 100µl chloroform. Then in each sample, standard and blank tube, 1 ml of TFA was added with automatic pipette. After two seconds, the optical density of each tube was recorded at 620 mμ.

To obtain the sample values, standard curve for vitamin A was prepared by taking the standard tubes of 1.396, 2.792, 4.188 and 5.584 µmol L^{-1} concentrations with the respective optical densities of 0.22, 0.4, 0.6 and 0.82, recorded after their processing in the way like that of samples. Then the values were converted to µg/g**.**

Result & Discussion

Mean ±SEM values of vitamin A in tissues of *Clarias batrachus* and *Labeo rohita* are presented in table 1 and depicted in figures 1 and 2, respectively.

The mean values of vitamin A in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of vitamin A in fishes of Thadoli area indicated the presence of oxidative stress.

In each area, the vitamin A activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the acticity of vitamin A was highest in liver for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the vitamin A activity was significantly higher in *Clarias batrachus* than in *Labeo rohita.*

The mean values of vitamin A in fish tissues collected from Bisalpur and Nasirda areas were more or less similar to the available control values (Qing-song *et al*.,2007). However, the values obtained from the Thadoli and Negdiya areas showed lower concentrations.

Reference

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Table 1. Effect of varying ambiences on vitamin A concentration in tissues of fishes collected from different areas /villages of Bisalpur reservoir (n=10)

Vitamin A, µg/g	Areas							
	Bisalpur		Nasirda		Thadoli		Negdiya	
	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*
Heart	21.30 h ± 0.40	20.0 h ± 0.22	19.0 h ± 0.34	18.0 h ± 0.30	15.0 h ± 0.19	13.1 h ± 0.21	17.2 h ± 0.14	16.2 h ± 0.19
Kidney	22.00 h ± 0.30	21.0 h ± 0.23	20.0 h ± 0.34	19.0 h ± 0.30	16.0 h ± 0.19	14.1 h ± 0.12	18.1 h ± 0.12	17.2 h ± 0.16
Liver	25.90 h ± 0.40	24.0 h ± 0.31	23.0 h ± 0.34	22.0 h ± 0.30	19.0 h ± 0.19	17.1 h ± 0.11	21.9 h ± 0.13	20.2 h ± 0.15
Gills	24.00 h ± 0.20	23.0 h ± 0.22	22.0 h ± 0.34	21.0 h ± 0.30	18.0 h ± 0.19	16.1 h ± 0.11	20.2 h ± 0.11	19.2 h ± 0.16

n= Number of fishes

All the means values of a parameter super scribed by same letter denotes significant ($p \leq 0.05$) differences among different areas .

C *b* = *Clarias batrachus*

L *r* =*Labeo rohita*

Fig. 1: Mean changes in tissue vitamin A in *Clarias batrachus* of different areas

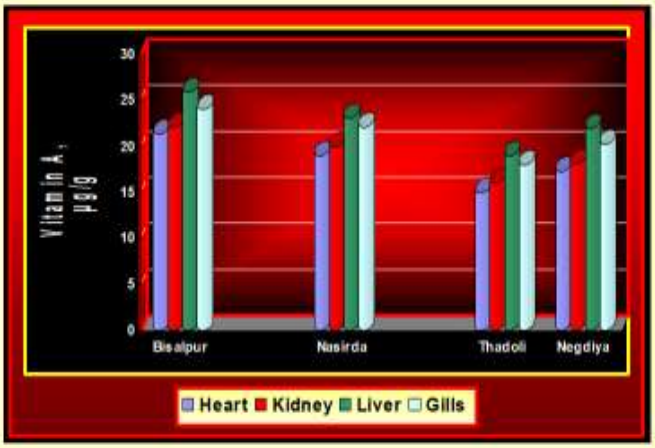

Fig. 2: Mean changes in tissue vitamin A in *Labeo rohita* of different areas