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GLUCOSE LEVEL IN CATLA CATLA (HAMILTON, 1822) FINGERLING EXPOSED TO TRANSPORTATION STRESS

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Abstract

Catla fingerlings' weighing average weight (31.36±1.26 g) were selected for further transportation experiment. Catla catla fingerlings were packed at two densities, 25 g/l (optimum) and 50 g/l (double of the optimum) of ten replicates each for 6 h ,12 h,18 h and 24 h respectively in two sets in polyethylene bags of dimensions (L-77.8 cm x B-40 cm), which were filled with 5 liters water, i.e., 1/3rd water and 2/3rd oxygen. The samples were regularly collected at CIFE Aquaculture Wet Laboratory at every 6 h (i.e., 6, 12, 18 and 24 h) intervals for taking stress parameter i.e. glucose of catla fingerlings. The vehicle was continuously running for 24 hrs in and around Mumbai from 8.00 A.M to 8.00 A.M. covering a distance of about 640 Km . One set of packing was used for determining stress parameters Glucose, which was determined from serum of Catla catla fingerlings. Another set was used for determining survival percentage after 7 days post-transportation. After transportation fishes were reared in separate tanks with aeration and water exchange. Fingerlings mortality was monitored for 7 days. The seven days post-transportation survival was determined by rearing the fishes in separate tanks for 25 g/l and 50 g/l packing densities with regular water exchange. It was observed that glucose level, is a good indicators of stress during transportation of Catla catla fingerlings packed at high density and increasing transportation duration. Hence, 25 g/l was the optimum packing density of Catla catla fingerlings for 24hrs duration. It was also concluded that increase in packing density resulted in increase of stress. There was a statistical significant interaction between the packing density (i.e., 25g/l and 50g/l) and transportation period (i.e., 0h, 6h, 12h, 18h and 24h) on glucose level. In case of double the optimum density results, it was found that up to 12hrs, this density was optimum and after that the stress parameter i.e. Glucose level got drastically changed.

Key words: *Catla catla, fingerling, transportation, stress, glucose level.*

Introduction

Carps are the mainstay of Indian fish farming contributing over 85% of the total aquaculture production. The three Indian major carps, *viz.* catla, rohu and mrigal represent the bulk of the production in the country, whereas commonly cultivated exotic carps such as silver carp, grass carp and common carp form the second important group. Due to their fast growing nature, mutually compatible and complimentary food habits and taste, Indian major carps enjoy a prime position in the Indian aquaculture scenario (Laxmappa, 2014). Availability of quality seed of cultivable freshwater fish species has been a limiting factor for intensification of fish farming and also for coverage of additional area under aquaculture. While availability of fish seed is satisfactory in certain parts of the country; in other areas, farmers face difficulties for procurement of quality seed in desirable quantities. Further, the seed in such deficit areas has to be transported over long distances, which adds to the cost of inputs (NFDB, 2009).

Requirement of quality fingerlings of 80-100 mm size is a prerequisite for fisheries development in reservoirs and in culture ponds, since realization would be more if large size fingerlings are stocked. Fingerlings (100–150 mm) production involves rearing of fry for about 3 months in rearing ponds. Large (above 100 mm) and healthy fingerlings fetch almost double the price of smaller ones (NFDB, 2009).

Likewise, fingerlings of grass carp, silver carp and catla are sold at about double the price of fingerlings of the same size of species like rohu, mrigal and common carp (Kumar, 1992). The closed system makes use of sealed containers in which all the basic requirements for fish survival are self-contained. It is by far the most ideal method for live fish transport. The container water is oxygenated like in polyethylene bags or tanks. Polyethylene bags should not be used to transport brooder/adult fish or post fingerling with sharp spines, as this will result in bursting of the container. It is essential to maintain adequate oxygen in the water while transporting fish using this method. The technique recommended for oxygenating water during fish transport is the use of pure bottled oxygen. It may be bubbled continuously into an unsealed container during transport or injected into a plastic bag containing water and fish which is then sealed air-tight for transport.

During the entire process of transportation, the seed is exposed to various stressors like netting, handling, crowding and confinement and this often results in high mortality either during or after transportation (Singh et al., 2004).In hatchery operations fish undergoes variety of handling and transport related stress such disturbance leads to detectable

physiological changes which are very useful indicator of degree of stress experienced by fishes in overall aquaculture operations. (Wedemeyer et al. 1990; Iwama et al. 1995, 1997; Wendelaar Bonga 1997). Physiological response experienced by fishes are grouped as primary response(Hormonal changes), secondary response(change in metabolites,blood ions and hematology) and last tertiary response(whole animal performance) (Wedemeyer et al. 1990; Iwama et al. 1995, 1997; Wendelaar Bonga 1997).

Packing density and duration of transport are two parameters that can vary for successful transportation aiming to ensure maximum survival at an optimum packing density for a specified duration (Carmichael,1984). Literature on the transportation of Indian and exotic major carps has specified various packing densities for different durations (Alikunhi, 1957; Ramachandran, 1969); however, on-site farmers and hatchery managers often decide the packing density based upon the size, duration and mode of transport. The stress exists in fish acts through the hypothalamus pituitary chromaffin axis and the hypothalamus pituitary inter renal axis which respectively stimulate the production of catecholamines and cortisol. Catecholamine activates glycogenolysis and cortisol gluconeogenesis resulting in increased production of glucose which is needed to combat stress (Pickering 1993; Schreck 1996). Transportation stress elicits the same responses as other forms of stress (Maule et al.,1988). Therefore, the present study was undertaken to evaluate the secondary response i.e. glucose level in *Catla catla* fingerlings exposed to transportation stress

Methodology

The fingerlings of *Catla catla* were packed at Aquaculture Division Wet Lab of Central Institute of Fisheries Education, Versova, Mumbai and placed in motorized vehicle for transporation in and around Mumbai for 24 hrs. The sampling of the catla fingerlings was done in Aquaculture Biology Lab.

Experimental fish and their maintenance

Before proceeding to the experiment, *Catla catla* fingerlings were procured from Khar Land Research Station, Panvel of Dr. B. S. K. K. V., Dapoli, Maharashtra, India and were acclimatized for 30 days in 2000 L fibreglass tank at the wet laboratory of Aquaculture Division, Central Institute of Fisheries Education, Versova, Mumbai with proper aeration and 25 percent water replenishment on daily basis. During this acclimatization process, they were fed with 2% of their body weight twice daily with formulated diet containing groundnut oilcake, fishmeal, soybean flour, rice powder, carboxymethyl cellulose, cod liver oil, sunflower oil, vitamin and mineral premix. Water parameters were fortnightly

observed and found in optimum range. Feeding was stopped to fingerlings 24 hrs prior to commencement of the transportation experiment.

Experimental design and sampling

Catla fingerlings' body measurements, *i.e.*, average weight and length were taken prior to packaging. Healthy fingerlings weighing average weight (31.36±1.26 g) were selected for further transportation experiment. The fingerlings were packed at five densities, *i.e.*, 25 g/L (T_1), 50 g/L (T_2), 75 g/L (T_3), 100 g/L (T_4) and 125 g/L (T_5) in polyethylene bags of dimensions (L-77.8 cm x B-40 cm), which were filled with 5 liters water, *i.e.*, 1/3rd water and 2/3rd oxygen. These five groups of packing densities were packed in triplicate.

Transportation Protocol

Fishes were packed at different stocking densities, *i.e.*, 25 g, 50 g, 75 g, 100 g and 125 g per litre in triplicate at Aquaculture Wet Laboratory, Central Institute of Fisheries Education, Versova, Mumbai and transported for a period of 24 hrs in a motorized vehicle. The samples were regularly collected at CIFE Aquaculture Wet Laboratory at every 6 h (*i.e.*, 6, 12, 18 and 24 h) intervals for taking various stress parameters of catla fingerlings. The vehicle was continuously running for 24 hrs in and around Mumbai from 8.00 A.M to 8.00 A.M. covering a distance of about 640 Km .

Three fish from each replicate were drawn from these five groups after 6, 12, 18 and 24 h of transportation. Various stress indicators like Cortisol, Glucose, NBT, RBC, WBC, Haematocrit, Protein, Albumin, Globulin and Albumin Globulin ratio (A:G ratio) were estimated from blood and serum of fingerlings. It was observed from these estimations that optimum packing density for 6 h, 12 h, 18 h and 24 h transportation of catla fingerlings was 75 g/l, 75 g/l, 50 g/l and 25 g/l respectively. From the above trials, it was concluded that for 24 h transportation, 25 g/l was optimum packing density for *Catla catla* fingerlings.

Catla catla fingerlings were packed at two densities, 25 g/l (optimum) and 50 g/l (double of the optimum) of ten replicates each for 6 h ,12 h,18 h and 24 h respectively in two sets. Above said transportation protocol was followed. One set of packing was used for determining stress parameters Glucose, which was determined from serum of *Catla catla* fingerlings. Another set was used for determining survival percentage after 7 days post-transportation. After transportation fishes were reared in separate tanks with aeration and water exchange. Fingerlings mortality was monitored for 7 days. The seven days post-transportation survival was determined by rearing the fishes in separate tanks for 25 g/l and 50 g/l packing densities with regular water exchange. Fingerlings were kept according to the

duration of transport in tanks, *i.e.*, 0 h, 6 h, 12 h,18 h and 24 h for 25 g/l and 50 g/l packing densities.

Serum Collection

For collection of serum, the blood was drawn from caudal vasculature of anesthetized fingerlings using 1 ml uncoated syringe. Collected blood was immediately transferred to dried eppendorff tube. These tubes were allowed to stand in tilted position at room temperature for clotting. After some time, due to clotting of blood, the yellow straw colour serum was carefully separated out and collected and transferred to another tube which was kept at -20 ^{o}C with proper labelling for further analysis, *i.e.,* Glucose.

Estimation of Serum Glucose

Glucose level in serum was determined with a commercial kit(Merkotest Glucose) based on GOD/POD. Glucose oxidase (GOD) converts the glucose into gluconate. The Hydrogenperoxide (H_2O_2) produced in the reaction is degraded by peroxidise (POD) and gives a coloured product phenol and 4-aminoantipyrine which is measurable using Trinder indicator reaction at 505nm. The increase in absorbance correlates with glucose concentration of sample.

$$\text{Glucose} + 0_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4H_20$$

10µl of serum /plasma of each treatment was taken in a labelled round bottom eppendoff tube of 1.5 ml capacity and then added 1000 µl reagent supplied with the help of pipette and labelled two eppendoff tubes as blank and standard. In blank tube, 10µl of distilled water was taken and 1000µl of Kit reagent (Phosphate Buffer, Glucose oxide, Peroxidase and 4 Amino Antipyrine with preservative and stabilizer) was added. In standard tube,10 µl of Standard (Glucose standard-100 mg/dl conc.) was added and then 1000 µl of Kit reagent was added. All the tubes with the reaction mixture was incubated for 10 minutes at 37^{o}C and reading was taken at 510nm wavelength on spectrophotometer.

Conc. of unknown sample =

$$\frac{\text{Concentration of Standard} \times \text{Abs. of unknown sample} - \text{Abs.of reagent blan}}{\text{Abs. Standard-Abs. of Reagent Blank}}$$

Physico-chemical parameters of Water

Water quality parameters, *viz,*.temperature, pH (pH meter having the temperature probe), dissolved oxygen by azide modification method (APHA-AWWA-WEF.,1998), free carbon dioxide titrimetric method (APHA-AWWA-WEF,1998), ammonia by spectrophotometerically at 640nm wavelength by phenate method (APHA-AWWA-WEF,1998), nitrite was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF,1998) and nitrate was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF.,1998) were recorded in this experiment.

Statistical Analysis

All data obtained were subjected to Two way ANOVA procedure of IBM SPSS(Ver.16.0) and significant means were separated by Tukey's HSD option of same software and further data obtained were subjected to Independent sample t-Test procedure of IBM SPSS(Ver.16.0) and significant means were separated by Tukey's HSD option of same software.

Results

There was an increasing trend of glucose level in both packaging densities (*i.e.,* 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had low glucose level as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25g and 50g/l) and transportation time (*i.e.,* T_1(0h), T_2, (6h), T_3(12h),T_4(18h) and T_5 (24h) on plasma glucose. There was a significant difference between packing density and transportation time.

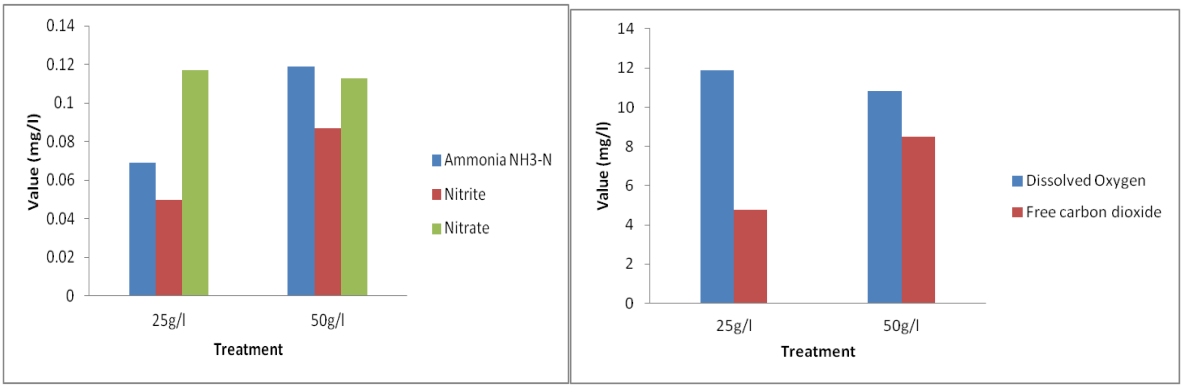
Glucose level did not vary significantly ($p>0.05$) for different transportation durations undertaken in the experiment when the fishes were packed at a density 25 g/l. However, there was a significant difference in glucose level when they were packed at a density of 50 g/l. At this density glucose level was found to be significantly higher when transported for 18 h and more. Glucose level did not differ significantly up to 12 h of transportation when packed at a density of 50 g/l.

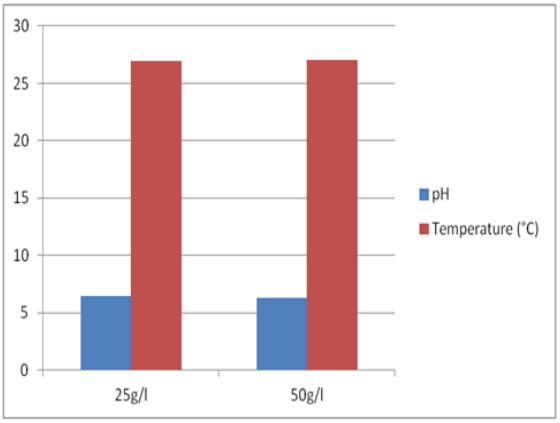
Table 1. Glucose level of *Catla catla* fingerlings transported in oxygen inflated plastic bags for varying time period.

Treatment		**Glucose**
Packaging density		
25g/l		132.41[a]
50g/l		149.33[b]
SEM		1.60
P-value		S(0.00)
T_1(0h)		128.83[a]
T_2 (6h)		132.74[a]
T_3 (12h)		134.42[a]
T4(18h)		153.68[b]
T_5 (24h)		155.34[b]
SEM		2.53
P-value		S(0.00)
Packaging Density*Duration		
25g/l	**T_1(0h)**	128.28[a]
	T_2 (6h)	131.69[a]
	T_3 (12h)	133.03[a]
	T4(18h)	134.76[a]
	T_5 (24h)	135.63[a]
50g/l	**T_1(0h)**	129.38[a]
	T_2 (6h)	133.79[a]
	T_3 (12h)	135.82[a]
	T4(18h)	172.61[b]
	T_5 (24h)	175.04[b]
	SEM	3.59
	P-value	S(0.041)

*Treatment means represent the average values of three plastic tubs per treatment. Tukey HSD range test was conducted for treatment means only if there was a significant interaction (ANOVA, $p < 0.05$). Means value in same column with different superscript differ significantly ($p<0.05$). S-Significant, NS-Nonsignificant.

Fig.1 Water quality parameters after different transportation durations at two packing densities





Survival after 7 days period

Survival percentage of fingerlings after transportation are given in Table 2. Two way ANOVA reveals that there is a significant ($p<0.05$) interaction between packing density and transportation duration on survival of fingerlings. It also reveals that there was a significant ($p<0.05$) effect on the packing and transportation duration on survival of fingerlings. Survival after seven days was non significantly different ($p>0.05$) for transportation durations T_1 ,T_2,T_3 ,T_4,T_5 when the fishes were packed at a density of 25 g/l. However, survival after seven days was non-significantly different ($p>0.05$) for the transportation durations T_1,T_2, T_3; but there was significant difference ($p<0.05$) between T_3 and T_4, T_5 when they were packed at a density of 50g/l.

Table 2. Post survival after transportation for first seven days in two packing densities

Treatment		**Survival (%)**
Packing density		
25g/l		100
50g/l		90.83
SEM		0.589
P-value		S(0.00)
Duration		
T_1 (0h)		100^a
T_2 (6h)		100^a
T_3 (12h)		100^a
T4(18h)		89.58^b
T_5 (24h)		87.50^b
SEM		2.08
P-value		S(0.00)
Packing Density*Duration		
25g/l	T_1 (0h)	100^a
	T_2 (6h)	100^a
	T_3 (12h)	100^a
	T4(18h)	100^a
	T_5 (24h)	100a
50g/l	T_1 (0h)	100^a
	T_2 (6h)	100^a
	T_3 (12h)	100^a
	T4(18h)	79.16^b
	T_5 (24h)	75^b
SEM		**1.31**
P-value		**S (0.00)**

*Treatment means represent the average values of three plastic tubs per treatment. Tukey HSD range test was conducted for treatment means only if there was a significant interaction (ANOVA, $p < 0.05$). Means value in same column with different superscript differ significantly ($p<0.05$). S-significant.

Discussion

The present study reveals that there is a significant interaction ($p<0.05$) between packing density (*i.e.*,25 g/l and 50 g/l) and transportation duration (0h, 6h, 12h, 18h and 24h) on cortisol and glucose levels. During transportation of catla fingerlings, there is a significant difference ($p<0.05$) in glucose levels between the groups in both packing densities 25g/l and 50g/l.

Elevated plasma glucose has been seen to increase after acute and chronic stress as reported by Farbridge and Leatherland,1992; Vijayan and Moon,1992. Gomes *et al.*(2003), reported that there was a significant increase in plasma glucose and cortisol immediately after transportation in juvenile Tambaqui, *Colossoma macropomum.*

The present study results are in conformity with the results obtained by Chatterjee *et al.*, 2010, where glucose level was found to increase both in response to higher packing density and increased in length of confinement, indicating an increased energy demand. Similar results are also reported by other workers (Specker and Schreck,1980; Iverson *et al.*,1998; Perez-Casanova *et al.*, 2008) in response to various stressors like transportation, confinement and handling. Therefore, it can be concluded that glucose parameter is a good marker of stress during transporation of catla fingerlings. Further, it is also concluded that 25 g/l packing density of catla fingerlings can be transported upto 24 hrs as far as glucose level is concerned.

Conclusion

It was observed that glucose level, is a good indicators of stress during transportation of *Catla catla* fingerlings packed at high density and increasing transportation duration. Hence, 25 g/l was the optimum packing density of *Catla catla* fingerlings for 24hrs duration. It was also concluded that increase in packing density resulted in increase of stress. There was a statistical significant interaction between the packing density (*i.e.,* 25g/l and 50g/l) and transportation period (*i.e.*, 0h, 6h, 12h, 18h and 24h) on glucose level. In case of double the optimum density results, it was found that up to 12hrs, this density was optimum and after that the stress parameter i.e. Glucose level got drastically changed.

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