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VARIATION IN SERUM BIOCHEMICAL CHANGES DURING THE TRANSPORTATION OF IN CATLA CATLA (HAMILTON, 1822) FINGERLING

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Abstract

Catla fingerlings' weighing average weight (31.36±1.26 g) was 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 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 blood for determining the stress parameter i.e. serum biochemical parametel level 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. It was observed that serum biochemical parameters level got drastically changed and are 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 decreasing trend of protein and globulin content while albumin and Albumin Globulin ratio shows increasing trend in both packing densities as the duration of time increased. 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 serum biochemical parameters. 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. serum biochemical parameters got drastically changed.

Key words: *Catla catla, fingerling, transportation, stress, serum biochemical parameter.*

Introduction

In aquaculture system transportation of live fish is an inevitable procedure. Transportation process includes series of procedure which is broadly categorize into pre transportation (such as collection, grading, netting, air exposure, and packing) and other is actual transportation processes (such as water movement, vibrations, and water condition change)which are

stressful to fishes(Paterson etal.,2003 ; Dhanasiri etal.,2013; Pakhira etal.,2015). Akinrotimi,2006 revealed that this stress depresses the fish growth and high mortality and also easily susceptible to disease(Maule etal.,1989) and there is increase in rearing cost(Gomes etal.,2006). Dobsikova etal.,2009 revealed that transportation procedure should be designed to minimize stress.

Numerious fish species respond to transportation stress by secreting cortisol hormone which is considered as primary stress response (Barton and Iwama,1991) Elevated level of cortisol in fishes results in elevated blood glucose content and altered electrolyte homeostasis (Barton and Iwama,1991; Mc Donald Milligan,1997) and alternation in metabolic activity as hyperglycaemia, hyperlactaemia, and hypercholesterolaemia(Mommsen etal.,1999). Oxidative stress are due stressors like hypoxia, hyperoxia, increased ammonia, and high stocking density.(Lushchak,2011;Sahin etal.,2014; Sun etal.,2014)

Catla catla is one of the most preferred fish of Indian major carp species due to its high commercial value, growth rate ,most preferred by the consumers, compatible with other carps, complimentary food habits (Laxmappa, 2014). Catla seed are regularly transported from hatchery to the fish culture sites, in oxygen inflated plastic bags. It is essential to reduce stress during transporation of catla seed. Stress in fishes are sequential i.e. primary, secondary and tertiary.Most important tertiary stress response is lowered immune competence.Tertiary stress reponse affects survival of fish in early life stage of fish i.e. fish seed of catla.Thus objective of the study was to quantify the effect of transportation stress by the help of serum biochemical parameters. This study will assist people in understanding the physiological responses of channel catfish suffering from transport stress and improving fish welfare.

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. Stress indicator like Protein, Albumin, Globulin and Albumin Globulin ratio (A:G ratio) were estimated from 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 serum biochemical parameter like protein, albumin a,globulin and A:G ratio, which was determined from serum of *Catla catla* fingerlings.

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.,* Protein, Albumin, Globulin and Albumin Globulin (A:G ratio).

Total serum protein

Plasma protein was estimated by biuret method (Reinhold, 1953). Proteins present in the serum bound with copper ions in an alkaline medium of the biuret reagent and produce a purple coloured complex whose absorbance is proportional to the protein concentration.Three test tubes labelled as Blank (B), Standard (S) and Test (T) were taken and in all tubes 1 ml of biuret reagent and 2 ml of distilled water were added. 50 µl of protein standard was taken in the test tube labelled as standard and 50 µl of plasma was added in the test tube labelled as test. It was mixed well and incubated at 37°c for 10 minutes. The absorbance of Standard (S) and Test (T) were measured against Blank (K) in a spectrophotometer at 630 nm. (Genesys 10S UV-VIS, Model No. GIOSUV-VIS, Thermo Scientific) at 630 nm. The sensitivity of the instrument was 0.001ppm.

Total serum protein in g%= Absorbance of Test (T) x 6/Absorbance of Standard (S)

Albumin

Albumin was estimated by bromocresol green binding method (Doumas *et al*.,1971). Albumin in a buffered medium binds with bromocresol green (BCG) and produces a green colour whose absorbance is proportional to the albumin concentration. Three test tubes, labelled as Blank (B), Standard (S), and Test (T) were taken with 1 ml of buffered dye

reagent and 2 ml of distilled water was added to all the test tubes. Lastly, 0.01 ml of albumin standard was taken in the test tube labelled as standard and 0.01 ml of plasma was added into the test tube labelled as test. It was mixed well and incubated at 37°C for 10 minutes. The absorbance of Standard (S) and Test (T) were measured immediately against Blank (B) in a spectrophotometer (Genesys 10S UV-VIS, Model No. GIOSUV-VIS, Thermo Scientific) at 630 nm. The sensitivity of the instrument was 0.001ppm.

The calculation was done as follows:

Albumin in g % = Absorbance of test (T) × 4 / Absorbance of standard (S)

Globulin

Globulin content was calculated by subtracting albumin values from total serum protein.

Globulin in g % = Total protein in g % − Albumin in g%

Albumin – globulin ratio

A/G ratio was calculated by dividing albumin values by globulin values.

$$\text{A/G ratio} = \frac{\text{Albumin in g\%}}{\text{Globulin in g\%}}$$

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

Serum Biochemical parameters of *Catla catla* fingerlings subjected to different transportation duration in two packing densities are given in Table 13.

Protein

There was an decreasing trend of protein content of serum in both packaging densities (*i.e.*,25g/l and 50g/l) as the duration of time increased. The packing density of 25 g/l had high protein content of serum as compared to 50 g/l . Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g/l and 50 g/l) and transportation time,*i.e.,* T_1(0h), T_2,(6h),T_3(12h),T_4(18h) and T_5 (24h) on protein content of serum. There was a significant difference between packing density and in transportation time. Protein content varied significantly ($p<0.05$) for different transportation durations T_1 and T_2,T_3,T_4,T_5; but there was no significant difference between T_2,T_3,T_4,T_5 undertaken in the experiment when the fishes were packed at a density of 25 g/l. However, protein content was significantly different ($p<0.05$) for the transportation durations T_1 and T_2,T_3,T_4,T_5 ; but there was no significant difference between T_2 and T_3 when they were packed at a density of 50 g/l.

Albumin

There was an increasing trend of a Albumin content of serum 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 Albumin content in serum as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g/l and 50 g/l) and transportation time, *i.e.,* T_1(0h) T_2,(6h),T_3(12h),T_4(18h) and T_5(24h) on Albumin content in serum. There was significant difference between transportation time but no significant difference in packing density. Albumin content varied significantly ($p<0.05$) for different transportation durations T_1 and T_3,T_4,T_5 but there was no significant difference between T_2,T_3 ,T_4, T_5 undertaken in the experiment when the fishes were packed at a density of 25 g/l. However, Albumin content was significantly different ($p<0.05$) for the transportation durations T_1 and T_3,T_4,T_5: but there was no significant difference between T_2 ,T_3 ; T_4,T_5 when they were packed at a density of 50 g/l.

Globulin

There was an decreasing trend of Globulin content of serum in both packaging densities (*i.e.*, 25g/l and 50g/l) as the duration of time increased. The packing density of 25 g/l had high Globulin content in serum as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g and 50 g/l) and transportation time, *i.e.*, T_1(0h) T_2,(6h),T_3(12h),T_4(18h) and T_5(24h) on Globulin content in serum. There was a significant difference between transportation time but no significant difference in packing density.

Globulin content of serum varied significantly ($p<0.05$) for different transportation durations T_1 and T_2,T_3 ,T_4,T_5; but there was no significant difference between T_2,T_3 ,T_4, T_5 when the fishes were packed at a density of 25g/l. However, Globulin content of serum was significantly different ($p<0.05$) for the transportation duration T_1 and T_2, T_3 ,T_4, T_5; but there was no significant difference between T_2,T_3 ; T_4,T_5 when they were packed at a density of 50 g/l.

A:G ratio

There was an increasing trend in Albumin Globulin ratio of serum in both packing densities (*i.e.*,25g/l and 50g/l) as the duration of time increased. The packing density of 25 g/l had low Albumin Globulin ratio of serum as compared to 50g/l .Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g/l and 50 g/l) and transportation time ,*i.e.*, T_1(0h),T_2,(6h) ,T_3(12h), T_4(18h) and T_5(24h) on Albumin Globulin ratio of serum. There was a significant difference between transportation time but no significant difference in packing density.

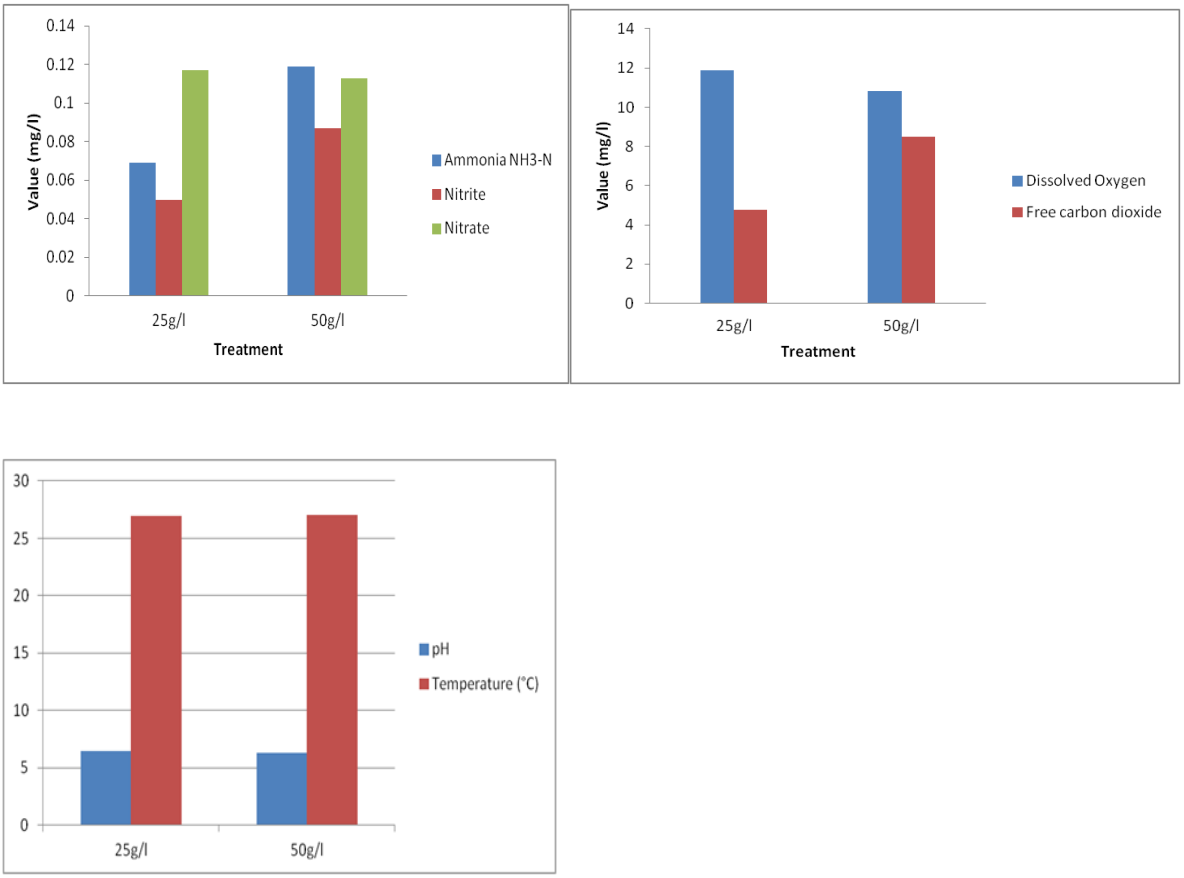
Albumin Globulin ratio of serum varied non-significantly different ($p>0.05$) for transportation durations T_1 T_2, T_3,T_4,T_5 when the fishes were packed at a density of 25 g/l. However, Albumin Globulin ratio of serum was non-significantly different ($p>0.05$) for the transportation durations T_1,T_2,T_3; 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 1. Serum biochemical parameters of *Catla catla* fingerlings transported in oxygen inflated plastic bags for varying time period.

Treatment		Protein (g%)	Albumin (g%)	Globulin (g%)	Albumin: Globulin ratio (A/G)ratio
Packaging density					
25g/l		5.17^{a}	1.79^{a}	3.38^{a}	0.543^{a}
50g/l		4.76^{b}	2.14^{b}	2.618^{b}	1.09^{b}
SEM		0.038	0.027	0.044	0.029
P-value		S(0.00)	S(0.00)	S(0.00)	S(0.00)
Duration					
T_1(0h)		5.70^{c}	1.54^{a}	4.157^{c}	0.372^{a}
T_2 (6h)		5.06^{b}	1.81^{b}	3.250^{b}	0.562^{ab}
T_3 (12h)		4.97^{b}	1.86^{b}	3.11^{b}	0.601^{b}
T4(18h)		4.64^{a}	2.27^{c}	2.37^{a}	1.10^{c}
T_5 (24h)		4.64^{a}	2.33^{c}	2.10^{a}	1.44^{d}
SEM		0.060	0.042	0.069	0.047
P-value		S(0.00)	S(0.00)	S(0.00)	S(0.00)
Packaging Density*Duration					
25g	**T_1(0h)**	5.70^{a}	1.54^{a}	4.18^{c}	0.369^{c}
	T_2 (6h)	5.24^{b}	1.80^{ab}	3.44^{b}	0.525^{c}
	T_3 (12h)	5.12^{b}	1.85^{b}	3.27^{b}	0.567^{c}
	T4(18h)	4.90^{b}	1.86^{b}	3.03^{b}	0.618^{c}
	T_5 (24h)	4.86^{b}	1.89^{b}	2.97^{b}	0.638^{c}
50g	**T_1(0h)**	5.68^{a}	1.54^{a}	4.13^{c}	0.375^{c}
	T_2 (6h)	4.88^{b}	1.82^{ab}	3.05^{b}	0.600^{c}
	T_3 (12h)	4.82^{b}	1.87^{b}	2.95^{b}	0.635^{c}
	T4(18h)	4.39^{a}	2.68^{c}	1.70^{a}	1.596^{b}
	T_5 (24h)	4.017^{a}	2.78^{c}	1.23^{a}	2.259^{a}
SEM		0.085	0.060	0.098	0.066
P-value		S(0.00)	S(0.041)	S(0.006)	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, NS-Nonsignificant.

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



Discussion

In the present experiment, significant interaction between packing density and duration on Plasma Protein ,Albumin, Globulin and Albumin: Globulin ratio in catla fingerlings was observed. Plasma biochemistry of fingerlings exposed to transportation and handling stress resulted in decrease in protein and globulin value coupled with increase in the value of albumin and albumin globulin ratio which is in conformity to the findings of Gbore *et al.* (2006).

Pakhira etal., 2015 showed that the mean total serum protein level in unstressed rohu fish was found to be higher and decreased as the transportation procedure proceeded. The serum protein value was higher−1 before transportation and after transportation it further declined .Significant differences existed in the serum total protein levels between before

transportation and each of the packing densities. Raune et al.,1999 and Biwas et al.,2006 revealed that handling resulted in reduced plasma proteins and immunoglobulin levels in the carp and red sea bream fishes respectively.

Wedemeyer *et al.* (1983) reported that stress due to capture, handling and sampling affected plasma protein in fish and got linked with increased secretion of catecholamine, increased concentration of adrenaline and noradrenaline in the blood of rainbow trout (*Salmo gairdneri*) in response to physical disturbance. Reduction in plasma value has implication on physiological activity and may be vital in immune-suppression of the fingerlings which may have strong negative impact on subsequent performance of the fish. It is concluded that due to transportation there is decrease in plama protein, globulin value and increase in albumin and albumin globulin ratio. Hence serum biochemical parameters are good physiological responses in catla fingerlings undergoing transportation stress.

Conclusion

As per above results, it was concluded that the serum protein, albumin , globulin and albumin globulin ratio are stress indicators during transportation. 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, serum protein, albumin, globulin and albumin globulin ratio. There was a decreasing trend of protein and globulin content while albumin and Albumin Globulin ratio shows increasing trend in both packing densities as the duration of time increased. In case of double the optimum density results, it was found that up to 12hrs, this density was optimum and after that the stress parameters got drastically changed.

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