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Some hormones and metabolic activities in blood of the common carp *Cyprinus carpio* after a short distance transport stress

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Abstract:

A total of 500 handling specimens of the common carp *Cyprinus carpio* were transported from a fish farm in Maysan Province in a special truck to Marine Sciences Center of the University of Basrah, in six plastic tanks with water volume of 500 liters per tank, with a fish density of 300 kg/m^3. Three methods were used to reserve the fishes, aerators, ice and without additives (control) in duplicate for each method for four hours. Blood was collected directly from fishes, and serum was extracted to conduct cortisol, thyroid-stimulating hormone (TSH), glucose and total protein. The results indicated a significant rise in the activities of all parameters in transported fishes from those in the wild. The highest values were recorded in the control containers and the lowest were recorded in the ice containers. Survival rates were 100% in ice containers and aerated containers and 85% in control containers. The low values recorded in ice containers 0.54 µg/dl for cortisol, 0.55 uIU/ml for TSH, 3.54 Mmol.L^{-1} for glucose and 2.43 gm/100 ml for total protein indicated that the physiological activities of stressed fishes were at their lowest level, this is necessary to reduce the stress during fish transportation. This confirms the possibility of using ice as a stress-reducing agent when transferring handlings of common carp for short distances.

Keywords: transport stress, ice, cortisol, TSH, glucose, total protein

Introduction

Animal welfare has become a subject of great importance to consumers, research institutions and to authorities in some countries. It is also becoming an important issue in fish industry [1]. In aquaculture, stressors can be numerous and of various sources; but handling and transporting are probably the most severe stressors seen in commercial aquaculture of fishes [2; 3]. Commercially important cultured and wild fishes are actually subjected to numerous stressors in their life progress. Examples of general stressors are reduction of water level [4; 5], fish catching in nets [6], increased stocking density [7], water temperature changes [8], artificial and natural reproduction [9; 10; 11; 12], fish handling [13], transporting [14; 15] etc. From both the commercial and fish welfare points of view, it is important to understand the physiological changes that take place in fish metabolism during transport period by implementing appropriate measures to real understanding of the situation in order to reduce the stress intensity that happen [16; 17].

Numerous metabolical and hematological indicators are used to determine and evaluate the effect of stressors on fish physiological parameters like glucose concentration, total protein, cortisol and TSH. [18; 19; 20; 21]. Cortisol activity also glucose concentration were seem to be the best essential stress indicators in fishes [21].

The aim of this research was to quantify the variations in blood concentration of cortisol, TSH, glucose and total protein as indicators of stress which may occur from short distance transportation of common carp.

Material and Methods

Fish transportation

Fishes were transported in an open transportation system from a fish farm in Maysan Province. A classic transporting container were used: treatment I: the control containers without any additives, treatment II: by using aerator and treatment III by using ice, in a specific car for a short-distance transportation of common carp in duplicates for each container. Water in treatment II was ventilated with air created by the car compressor, while in treatment III ice was added to the water. Total length and total weight for fishes in culture pond were 22.13 ± 0.125 cm and 172.18 ± 2.85 gm, respectively. Fish stocking density in each container was 300 kg/m^3. Blood was taken from heart of selected farmed fishes as time zero samples. Water temperature and dissolved oxygen content at the establishment and the completion of transportation period are shown in Table 1.

Blood sampling and assay procedures

After about four hours transportation of handlings common carps, blood was collected from heart of four fish specimens in each container by cardiopunction, using a 3ml needle and putted in sterile Eppendrof tubes with volume of 1ml. Serum was separated by centrifugation and kept in a freezer at -20°C. The cortisol and TSH activities in serum were assessed by ELISA using commercially available kits, manufactured by Monobined Inc., USA. Glucose concentration (mmol/l) was determined spectrophotometrically by using a commercial kit manufactured by RANDOX laboratories/UK in a colorimetric method [22]. Total protein (g/100ml) was determined with Biuret method using a commercially available kit manufactured by BIOLABO SA, 02160 maizy, France in a colorimetric method, which its reaction principles were described [23]. The water temperature and oxygen content in containers were determined by YSI meter (Germany). Statistical analyses of data were performed by *f*-test, one-way ANOVA [24].

Results

Table (1) showed the environmental factors recorded in containers in the short distance transportation experiment. Salinity ranged from 4.67 psu to 3.4 psu in the three types of containers, dissolved oxygen ranged from 9.00 mg/l to 5.7 mg/l and water temperatures were 19.2. 16.77 and 3.7 ºC in the three types of containers I, II, III, respectively.

Table 1. Environmental factors recorded in containers in short distance transportation experiments (mean ±S.E.).

Environmental factor	I*	II**	III***
Water Temperature (ºC)	19.2 ± 0.01	16.77 ± 0.02	3.7 ± 0.22
Salinity (psu)	4.67 ± 0.02	4.5 ± 0.02	3.4 ± 0.42
D.O. (mg/l)	5.7 ± 0.12	8.2 ± 0.11	9.00 ± 0.16
pH	7.5 ± 0.001	7.8 ± 0.002	7.76 ± 0.004

*Control containers, ** aerated containers, ***Iced containers.

Survival rates were 100% in ice containers and aerated containers and 85% in control containers. Table (2) showed mean lengths (cm) and weights (g) for handlings *C. carpio* in the three treatments in short distance transportation experiments. Means of fish length and weight were 21.84 ± 0.42 cm and 158.25 ± 6.94 g, respectively.

Table 2. Length (cm) and weight (g) for handlings *C. carpio* in short distance transportation experiments (mean ± S.E.).

Factors	I*	II**	III***
Length (cm)	22.56 ± 0.06^{a}	21.84 ± 0.02^{b}	21.11 ± 0.02^{c}
Weight (g)	167.85 ± 0.3^{a}	162.15 ± 0.51^{b}	144.76 ± 0.3^{c}

*Control containers, ** aerated containers, ***Iced containers.

Table (3) showed Cortisol concentration (µg/dl) in the serum of handlings *C. carpio* in short distance transportation experiments. The high value of hormone concentration was obtained in the control container (2.30 ± 0.05 µg/dl), while the lowest one was in ice container (0.54 ± 0.008 µg/dl). Statistical analysis presented significant variances ($P < 0.05$) among all treatments.

Table 3. Cortisol concentration (µg/dl) in the serum of handlings *C. carpio* in short distance transportation experiments (mean ± S.E.).

Time	I*	II**	III***
0 h.	0.65 ± 0.0002^{a}	0.65 ± 0.0002^{a}	0.65 ± 0.0002^{a}
4 h.	2.30 ± 0.05^{a}	1.66 ± 0.002^{b}	0.54 ± 0.008^{c}

*Control containers, ** aerated containers, ***Iced containers.

Table (4) showed thyroid stimulating hormone TSH (uIU/ml) in the serum of handlings *C. carpio* in short distance transportation experiments. The high value of TSH concentration obtained in the control container was 3.45 ± 0.01, while the lowest one in ice container was 0.55 ± 0.005. Statistical analysis presented significant variances ($P < 0.05$) among all treatments.

Table 4. Thyroid stimulating hormone TSH (uIU/ml) in the serum of handlings of *Cyprinus carpio* in short distance transportation experiments (mean ± S.E.).

Time	I*	II**	III***
0 h.	0.46 ± 0.0001^{a}	0.46 ± 0.0001^{a}	0.46 ± 0.0001^{a}
4 h.	3.45 ± 0.01^{a}	2.77 ± 0.05^{b}	0.55 ± 0.005^{c}

*Control containers, ** aerated containers, ***Iced containers.

Table (5) showed Glucose concentration ($Mmol.L^{-1}$) in the serum of handlings of *C. carpio* in short distance transportation experiments. The high value of glucose concentration obtained in the control container was 9.49 ± 0.05 $Mmol.L^{-1}$, while the lowest one in aerator container was 3.54 ± 0.12 $Mmol.L^{-1}$. Statistical analysis presented significant variances ($P < 0.05$) among all treatments.

Table 5. Glucose concentration ($Mmol.L^{-1}$) in the serum of handlings of *C. carpio* in short distance transportation experiments (mean ± S.E.).

time	I*	II**	III***
0 h.	0.66 ± 0.021^{a}	0.66 ± 0.021^{a}	0.66 ± 0.021^{a}
4 h.	9.49 ± 0.05^{a}	5.60 ± 0.06^{b}	3.54 ± 0.12^{c}

*Control containers, ** aerated containers, ***Iced containers.

Table (6) showed the total protein (gm/100 ml) in the serum of handlings *C. carpio* in short distance transportation experiments. The high value of total protein concentration was obtained in the control container (6.60 ± 0.02 gm/100 ml), while the lowest one was in ice container (2.43 ± 0.008 gm/100 ml). Statistical analysis presented significant variances ($P < 0.05$) among all treatments.

Table 6. Total protein (gm/100 ml) in the serum of handlings *Cyprinus carpio* in short distance transportation experiments (mean ± S.E.).

Time	I*	II**	III***
0 h.	1.68 ± 0.04^{a}	1.68 ± 0.04^{a}	1.68 ± 0.04^{a}
4 h.	6.60 ± 0.02^{a}	3.37 ± 0.05^{b}	2.43 ± 0.008^{c}

*Control containers, ** aerated containers, ***Iced containers.

Discussion

Stress in fish is a brief of physiological events which occur when fish endeavor to conserve internal homeostasis [25; 26]. When fishes are stressed, their endocrine response releases adrenaline and noradrenaline into the blood stream and a hypothalamo-pituitary-interrenal response finally leads to an increase in the plasma cortisol level [27; 28]. In all vertebrates, including fishes, cortisol plays a key role in the renovation of homeostasis during and after stress [29; 30]. However, many authors reported a transient increase in plasma cortisol levels during stress, such as in crowding [31; 32], while others reported no effect [33] or reduced cortisol levels [34].

The first phase of fish response to the stressor effects is the endocrine variations, which seem as initial responses, the catechol amines and glucocorticoids are the most increased in this primary response. They control after the metabolic and osmotic changes, as a secondary response to the stressors [35; 36; 37; 38; 39]. Secondary responses in the study were symbolized by variations in concentrations of glucose and total protein in serum. While changes of cortisol concentration in blood serum considered as initial response, the highest value obtained in the control container after four hours of transport, but the ice container gave the lowest value and aerator container occurs in the middle in cortisol activity. In a same way, TSH concentration as a primary reaction like cortisol, gave the highest value in the control container and the lowest value obtained in ice container.

Alterations in the glucose concentration, indicating the secondary response, the glucose concentration in blood serum increased significantly after four hours of transport. The high value of glucose concentration was obtained in the control container, while the lowest one was in ice container. Van-Raaij *et al.* [40] stated that hyperglycemia in stressed fishes is perhaps a consequence of liver glycogenolysis motivated by catecholamine and of stimulation of gluconeogenesis by cortisol during healing.

Results showed that cortisol and TSH concentrations in blood serum of iced fish were significantly the lowest from the control and aerated fish. Also, the glucose and total protein concentrations in blood serum of iced fishes were dropped significantly in comparison with the aerated and control groups. This is essential for lowering the exhaustion of stocked energy of stressed fishes during transport period. Ice normally drops the employment of energy stores, which in situation of not adding ice during transporting period, would decline sharply [41]. Similar findings were presented by Jeny *et al.* [42] by studying the effect of anesthetics Propanidid and MS 222 to reduce stress from artificial reproduction of *C. carpio*. Sutphin *et al.* [43] found a transit increase in cortisol, glucose and lactate concentrations as an adaptive response, with a peak level obtained immediately during transport and back to normal levels within 24 hours after transportation in high densities of three types of fish species.

Conclusions

Results of the present study recommended the use of ice in fish transportation for a short distance as a reducing stress method restraining the harmful effects of stress in order to limit losses of energy and enhancing of fish survival during short distance transport.

Author Contributions Statement

All research stage: laboratory work, collection and analysis of data, research write were done by the author.

Declaration of competing interest

The author confirms that there was no competing interest with others.

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