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4. Dutta, Tanusree; Acharya, Subhendu and Das, M. K. 2002. Some physiological changes in juvenile *L. rohita* (Ham.-Buch.) subjected to handling, crowding and transportation stress. *In: Proceedings of the National Seminar on Recent Advances in Molecular Physiology held on 4-6 Feb, 2002 at the University of Kalyani, Kalyani 741 235, West Bengal, India. CP-2. 116-120.*

5. Dutta, Tanusree; Acharya, Subhendu and Das, M. K. Physiological effect of cold shock in juvenile *L. rohita* (Hamilton-Buchanan) by in *Indian J. fish.*, 49(2) (in press).
Sublethal temperature stress in juvenile *Labeo rohita* (Ham-Buch.) and *Rita rita* (Ham.): Some physiological changes

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Juveniles of fish *L. rohita* and *R. rita* subjected to a rapid (5 min) sublethal temperature increase from 28 to 35°C showed significant increase in cortisol and decrease in interrenal ascorbic acid. Hypercholesterolemia, hyperglycemia and hyperlactemia were also evident accompanied by increased blood haemoglobin and haematocrit and stable protein levels. Compensatory responses were initiated within 72 hr in both the fishes. *R. rita* recovered more quickly indicating it to be more resistant to the heat stress than *L. rohita*. Hence fishes subjected to sublethal temperature stress should be given a metabolic recovery period of 72 hr prior to further stress being applied.

Fishes are often subjected to the hazards of rapid temperature changes in tropical waters either due to daily variations in water temperature in shallow waters or thermocline in deeper water bodies, due to thermal effluents or simply due to stocking of fishes into warmer receiving water. These effects often become additive or synergistic with those of other adverse stimuli (e.g., low water pH, algae, oxygen shortage). Knowledge of such temperature changes has relevance for fish in natural water bodies and in aquaculture. These temperature changes though sublethal, can place a stress of considerable magnitude on the homeostatic mechanism of fishes. The lethal effect of extreme temperature changes (thermal pollution) are fairly well recognised but the metabolic consequences of sublethal heat stress (thermal additions) have received less attention. The severity of sublethal heat stress and the period needed for recovery are not clear in fishes.

In the present study a comparative assessment of the changes occurring in the levels of haematocrit, haemoglobin, plasma glucose, chloride, cholesterol, protein, lactic acid, cortisol and kidney ascorbic acid and their recovery period has been made in fingerlings of two tropical fishes viz., *Labeo rohita* and *Rita rita* acclimatised to 28°C and subjected to a rapid, sublethal increase to 35°C and then maintained at this enhanced temperature.

### Materials and Methods

Juveniles of *Labeo rohita* and *Rita rita* of average weight and length (14.5 g, 130 mm) and (30 g, 89 mm) respectively were acclimatised in the laboratory in fibre glass tanks at a stocking density of 0.4 g l⁻¹ at 28°C. The water quality conditions were pH (7.8-8.0), alkalinity (85-190 mg l⁻¹), hardness (285-295 mg l⁻¹), dissolved oxygen (5.5-6 mg l⁻¹) with 12 hr light and 12 hr darkness. All the fishes were fed Tubifex *ad libitum* daily and one fourth water was exchanged daily. For initiation of the experiments the water temperature of the aquaria stocked with juveniles of *L. rohita* or *R. rita* @ 0.4 g l⁻¹ was increased within 5 mm to 35°C from the initial 28°C and held there until the experiment was terminated. The fishes were fasted 24 hr before blood and tissue samples were taken. Sub samples of 10 fishes of *L. rohita* or *R. rita* were taken at 0, 1, 3, 24, 48 and 72 hr (or longer as needed).

### Blood and tissue sampling and analysis

To obtain blood, fishes were netted gently and were rapidly anesthetized using MSS 222 (ethyl m-amino benzoate methanesulphonate) at the dose of 60 mg l⁻¹ for *L. rohita* and 100 mg l⁻¹ for *R. rita*. The fishes were immobilized within 1 min. of application. Blood was collected from the caudal artery using 1 ml syringes fitted with 24G needle and in some fishes by caudal peduncle cut. Heparin was used as the anticoagulant. Immediately after collection blood was centrifuged for 5 min. at 3000 rpm and the plasma was separated out and either used for analysis immediately or stored at −20°C for analysis later. Sampling procedure of
netting, anaesthesia and plasma storing was completed within 10 min to avoid influence of netting combined with anaesthesia on the basal cortisol levels.

The methods employed for estimation of various blood parameters were haematocrit, haemoglobin (Sahli's method), plasma glucose, plasma chloride, plasma cholesterol, plasma protein, plasma lactic acid and kidney ascorbic acid. Plasma cortisol was measured by a direct immunoenzymatic determination of cortisol kit manufactured by Equipar srl via 3 Ferrari, 21/N-21047, SARONNO, ITALY using a 96 well micro litre plate read by ELISA micro plate reader (Model EL 31/SX, Bio Tek Instruments INC). The final solution was read at a wavelength of 450 nm. The plasma cortisol concentration was calculated based on a series of standards. Water quality parameters were measured as per standard methods. Statistical analysis of data for all physiological parameters were expressed as mean ± SE. Statistical comparisons between experimental and control fishes were made by Student t test.

Results

Activation of the pituitary-interrenal axis is indicated in both L. rohita and R. rita by the significant increase in plasma cortisol levels and significant decrease in adrenal ascorbic acid from the first hour of exposure to a 7°C rise in temperature. However, within 72 hr regulation of the cortisol and ascorbic acid was attained in both the fishes (Figs 1, 2).

The acid base equilibrium was altered in L. rohita the plasma chloride level decreased significantly in contrast to R. rita where it was maintained (Figs 1, 2). Recovery of chloride levels was achieved within 24 hours in L. rohita. The lactic acid levels increased significantly within 3 hr in both the fishes however regulation was attained within 48 hr for R. rita and 72 hr for L. rohita (Figs 1, 2).

The sterol metabolism was affected in both the fishes. There was a maximum significant increase in both fishes within 24 hr and by 72 hr it showed a declining trend (Figs 1, 2). The carbohydrate metabolism was affected as a rapid hyperglycaemic response was recorded being more pronounced in L. rohita than R. rita. However, the blood sugar regulation was achieved within 72 hr in both the fishes but at a faster rate in R. rita (Figs 1, 2).

The blood haemoglobin level increased in both the fishes attaining significant levels within 24 hr (Figs 1, 2). However, plasma protein levels did not fluctuate appreciably (Figs 1, 2). The haematocrit values also showed an increase in both the fishes (Figs 1, 2).

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<td>Haemoglobin (g/dl)</td>
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<td>Glucose (mg/dl)</td>
<td>Cholesterol (mg/dl)</td>
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Values of physiological parameters of L. rohita at 28°C and following a rapid temperature increase and maintenance at 35°C, values given as x±SE (n = 10)*, indicates significantly different from control (hour 0) values at (P < 0.05), ** (P < 0.01)
The results indicate that in response to a stress of 7°C rise in ambient temperature a fright reaction occurs in both the fishes mediated through the pituitary interrenal axis. This is physiologically manifested by the depletion in the kidney ascorbic acid and an increase in plasma cortisol level. Activation of the pituitary interrenal axis by heat stress elicited similar response in coho salmon and rainbow trout. The metabolic fate of the depleted ascorbic acid is however not clear. In the stressed rats, it was observed that decrease in adrenal ascorbic acid was accompanied by a corresponding increase in plasma ascorbate levels. However, appropriate experiments by Wedemeyer showed that blood ascorbate levels did not change in salmonid fishes when adrenal ascorbic acid depleted. This suggests that ascorbic acid may be participating in steroid biosynthesis. In the present experiment also the plasma cortisol measurements during the 72-hour stress indirectly supports the above contention because the cortisol levels increased as adrenal ascorbate levels decreased. The recovery of plasma cortisol and ascorbic acid levels occurred within 72 hr in both L. rohita and R. rita corroborating the event occurring in coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri) within 24 hr when subjected to 10°C rise in temperature.

The alteration in acid base equilibrium as indicated by hypochloremia in L. rohita following a rapid 10°C temperature increase has also been observed in rainbow trout.

The accumulation of lactic acid in the blood of L. rohita and R. rita could be due to high temperature related hyperactivity. Studies in channel catfish revealed that the stressors viz. anoxia or hyperactivity can cause accumulation of lactic acid in blood. It was also observed that blood pH decreased as blood lactic acid increased in channel catfish under anoxia. In the present experiment the enhanced levels of lactic acid may have decreased the blood pH-causing disturbance in the acid base equilibrium.

The sterol metabolism has been disturbed as indicated by rise in plasma cholesterol in both the fishes. However in contrast to the observations in coho

![Graph](image-url)
salmon and steelhead\textsuperscript{13} where progressive hypocholesterolemia developed when subjected to a 10°C increase to 20°C holding temperature. The rapid hyperglycaemic response elicited in \textit{L. rohita} and \textit{R. rita} is similar to the response in coho salmon and steelhead\textsuperscript{13} subjected to rapid 10°C rise in temperature. The rise in haemoglobin level in both the species indicated disturbed water balance but since protein levels did not increase haemoconcentration can probably be ruled out and the temporary haemoglobin increase may be functional. This was also observed in salmoid fishes\textsuperscript{13} subjected to high temperature. The rise in hematocrit indicated a presumptive evidence of polycythaemia.

It is thus evident that the rise in temperature by 7°C from the ambient temperature (28°C) to a holding temperature of 35°C resulted in significant physiological changes in \textit{L. rohita} and \textit{R. rita}. Simultaneously it was also observed that compensatory responses were initiated in the fishes within 72 hr. This has also been observed in the coho salmon and steelhead fishes\textsuperscript{13} Obviously adaptation to the stress of elevated temperature occurs in both \textit{L. rohita} and \textit{R. rita}.

Although both the fishes suffer some metabolic dysfunction \textit{L. rohita} (an actively swimming carp) is evidently less resistant to sublethal heat stress than \textit{r. rita} (a sedentary benthic catfish). This is consistent with the observations that there is a difference between actively swimming species and more sedentary species of fish\textsuperscript{31} The latter are refractory and appear reluctant to move, and do so in response to aversive stimuli such as extreme temperatures\textsuperscript{32}, while active species such as centrarchids are characterised by a more positive preference response\textsuperscript{33}.

In conclusion a rapid but sublethal 7°C temperature evation and holding temperature of 35°C places a greater stress on the homeostatic mechanism of juvenile \textit{L. rohita} than \textit{R. rita}. The impaired sterol metabolism as exhibited by hypercholesterolemia was evident in both the fishes. Hyperglycaemia and deasecd blood sugar regulatory mechanism occurred both in the fishes but to a lesser extent in \textit{R. rita} than \textit{rohita}. The pituitary activation as evidenced by the renal ascorbic acid depletion and cortisol eleva
tion is more pronounced in \textit{L. rohita} than \textit{R. rita}. The d base balance was not significantly altered in either species. Hence the adaptation was more rapid. The ygen consumption in both the fishes increased and temporary functional polycythemia occurred as ged by increased haemoglobin but stable protein levels. It is apparent that \textit{L. rohita} are less resistant to sublethal heat stress than \textit{R. rita} which is endowed with general superior vigour. It is suggested that for proper fish health management fishes subjected to sublethal temperature stress should not be further stressed within 72 hr for metabolic recovery.

Acknowledgement

The authors are grateful to Dr M Sinha, Director, CIFRI for assistance and advice. The work was supported by grant from ICAR, New Delhi.

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Plasma cortisol levels of *Labeo rohita* fingerlings at rest and subjected to confinement, handling and hypoxia

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Fingerlings of *Labeo rohita* were acclimatised under laboratory conditions. These fishes were sampled for estimation of plasma cortisol by a direct immunoenzymatic cortisol determination kit using ELISA technique. The resting levels ranged from 42-128 ng ml⁻¹. In fingerlings subjected to stress due to confinement and crowding in aquaria for 1 hr and in hapas in pond for 12 hrs there was significant elevation of the cortisol levels indicating activation of the hypothalamus-hypophyseal axis (HPA) as a primary stress response.

Introduction

Secretion of corticosteroid hormones in fish is a characteristic response to a wide range of external stressors. The fish respond physiologically to different stressors by secretion of corticosteroids in the blood. This response is so characteristic that blood corticosteroid level has been used to assess the magnitude of the stress response in fish (Strange and Schreck, 1987, Strange *et al.,* 1981). It is assumed that this represents part of a general adaptive response mediated through the hypothalamus-hypophyseal axis in which cortisol mobilizes, by virtue of its catabolic properties, the stored food reserves thereby enabling the fish to cope with the increased energy demands (Dave *et al.,* 1979; Lindman *et al.,* 1997; Leach and Taylor, 1982 and Paxton *et al.,* 1984). However, excessive or prolonged elevation of plasma cortisol levels may have deleterious effect on the fish defense systems, with a resultant increase in the susceptibility of the fish to disease (Robertson *et al.,* 1963, Pickering and Dutson 1983, and Pickering and Pottinger 1985). In the present investigation an attempt has been made to determine the resting values of cortisol in the teleost *Labeo rohita* reared under optimum laboratory conditions and to evaluate the changes in the cortisol levels in response to the stress of handling, confinement and hypoxia.

Materials and Methods

**Experiment 1. Determination of the resting levels of cortisol**

For determining the normal levels of cortisol, hatchery bred *L. rohita* fingerlings (Avg. wt. 18 gm, Avg. Length 120 mm) were acclimatised and reared in laboratory tanks at temperature range of 29 to 30 °C with 12 hr light and 12 hr darkness and at a stocking density of 0.4 g l⁻¹. All fishes were fed Tubifex *ad lib* and water exchanged periodically. Blood sampling for cortisol analysis was done.
**Experiment 2 A)**  
*Effect of crowding on the water chemistry and on cortisol levels during confinement stress in tank*

Twenty *L. rohita* fingerlings were dip netted from the laboratory tanks, and stocked in an aquarium at a stocking density of 0.1 kg l⁻¹ and kept without aeration. After one hour blood sampling was done for cortisol analysis.

**B)** *Effect of crowding on cortisol levels during confinement stress in happa*

Twenty fingerlings of *L. rohita* were captured by cast net from a 0.5 ha static water pond and kept in a happa overnight in the pond of capture. After 12 hrs blood sampling for cortisol was done.

**Blood sampling**: To obtain blood, sampled fishes were caught with minimum stress and rapidly anesthetized using 60 mg l⁻¹ MS 222. Blood was collected by caudal peduncle cut. Heparin was used as anticoagulant. Fishes were weighed and fork length measured.

**Measurement of plasma cortisol**: It was measured by a direct immuno enzymatic cortisol determination kit manufactured by equipar srl, via G. Ferrari, 21/N-21047 SARONNO, ITALY. It is a solid phase competitive ELISA. The wells are coated with anticortisol IgG. The samples, control and standards are incubated simultaneously with cortisol conjugated to enzyme horse radish peroxidase. Cortisol (antigen) in the sample competes with horse radish peroxidase cortisol (enzyme-labelled antigen) for binding on to the limited number of anticortisol (antibody) sites on the microplates (solid phase). After incubation, the unbound materials are removed by washing. Then the enzyme substrate (H₂O₂) and the chromogen substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development the enzyme reaction is stopped and the absorbance are determined. Cortisol concentration in the sample is calculated from the standard curve. The colour intensity is inversely proportional to the cortisol concentration in the sample. The amount of cortisol present in the sample is given in ng ml⁻¹.

Water quality parameters were measured as per standard methods (APHA, 1989) or by HACH kit.

**Results and Discussion**

It is observed from the results that the mean resting value of plasma cortisol in *L. rohita* is 90 ng ml⁻¹ (Table -1). The effect of handling, confinement and crowding at a high density in aquaria tank had significantly elevated the cortisol level to 150 ng ml⁻¹ (Table 2). The changes in the water quality parameters during the experimental period (1 hr) are shown in Figs. 1,2,3. While there has been a decrease in dissolved oxygen level leading to a hypoxic condition (1.2 mg l⁻¹), there has been an increase in the level of carbondioxide (12 mg l⁻¹) and unionised ammonia (1 mg l⁻¹) creating stress for the fishes. This resulted in the increased cortisol levels. Pickering and Pottinger (1987) also obtained an increase in the cortisol levels in brown trout subjected to confinement at a density of 0.05 kg l⁻¹. Here also the water chemistry changes were similar to the present experiment. Davies et al. (1981) also recorded increased corticoid concentration in channel catfish exposed to high ammonia and low oxygen levels.
Similarly when *L. rohita* fingerlings were confined and crowded in hoppa the cortisol level rose to 226 ngml⁻¹ indicating activation of the hypothalamo hypophyseal (HPI) axis (Table 3)

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<th>Table 1 Resting level of plasma cortisol in <em>Labeo rohita</em></th>
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<th>Table 2 Cortisol level in <em>L. rohita</em> subjected to handing, crowding and confinement (1 hr)</th>
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*Significant (1% level)*

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<th>Table 3 Cortisol level in <em>L. rohita</em> subjected to handling, confinement and crowding in hoppa (12 hrs.)</th>
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*Significant (1% level)*

It is evident from the results that the fish *L. rohita* subjected to handling, confinement and crowding resulted in elevated cortisol levels in blood as compared to the resting values. It is well established that maintenance of fish at high stocking densities for prolonged period results in chronic stimulation of the HPI axis, with acclimation of the system to this form of stress requiring a time period ranging from days to weeks (Schreck, 1981; Pickering and Stewart, 1984). In the present experiment the water quality in the static water seems to have a synergistic effect on the elevation of cortisol though Pickering and Stewart, 1984, opined that effect of high stocking density is independent of water quality. However, it needs to be noted, as opined by Davis *et al.*, 1984, that many of the factors that influence plasma corticoids dynamics (temperature, photoperiod, feeding time, ammonia, nitrite, pH, dissolved oxygen) vary seasonally under natural conditions. All these biotic and abiotic characteristics should be considered when corticosteroid concentration in fish held in ponds are studied.
Changes in water chemistry during 1 hr. confinement of *L. rohita* in aquarium tank (25 l).
Acknowledgement

The authors are grateful to the Director, CIFRI for his keen interest and help. They are also thankful to the Indian Council of Agricultural Research for financial support

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Impact of stress on blood parameters and interrenals of *Labeo rohita*

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*Labeo rohita* fingerlings acclimatised in laboratory tanks and from natural pond with optimum water qualities were sampled for determining the normal ranges viz. haemoglobin (6.6 - 7.5 gdl⁻¹), haematocrit (32.0 - 34.3%), leucocrit (0.66 - 0.68%), plasma chloride (89.0 - 92 meql⁻¹) and glucose (52.4 - 79.8 mgl⁻¹). The normal interrenal cell size and nuclear diameter ranged from 8.68 - 78.5 μm and 1.25 - 3.75 μm respectively. Fingerlings subjected to stress due to confinement and crowding in hundies (1hr) and in hapas (12 hrs) showed significant rise in haematocrit and decline in leucocrit values. There was significant rise in blood glucose and decrease in blood chloride. The interrenal cells of stressed fishes showed an increase in nuclear diameter indicating hyperactivity.

Introduction

Physiological indicators of stress are becoming increasingly popular among fishery workers for stress diagnosis. Measurement of haematocrit, leucocrit, clotting time, plasma glucose, chloride have been useful for fishery biologists in assessing the health of fish and monitoring stress responses (Blaxhall 1972, Hickey 1976, Sorvio and Oikari 1976, Wedemeyer et al., 1990). Since many of the biochemical changes that occur in response to stress are the end result of cellular pathology the histological examination of the interrenal which produce cortisol the stress hormone also forms a useful tool to measure the effect of stress. Keeping this aspect in view experiments were conducted to know the normal values of some stress sensitive haematological parameters of normal *L. rohita* viz., haemoglobin, haematocrit, clotting time, plasma glucose, chloride and their interrenal cell size and nuclear diameter and the changes that occur in these due to stress exerted during cultural practices.

Materials and Methods

Experiment 1. Determination of the normal levels

For determining the normal levels of the physiological parameters two stocks of hatchery bred *L. rohita* fingerlings were sampled.

A) Fingerlings of *L. rohita* were acclimatised and reared in laboratory tanks at a stocking density of 0.4 gl⁻¹ with temperature of 29-30 °C and with 12 hr. light and 12 hr. darkness. All the fishes were fed Tubifex *ad lib* and water exchanged periodically. Fish blood and anterior kidney sampling was done after one month.
B) Fingerlings of *L. rohita* were reared in a 0.5 ha static water pond at Naihati, West Bengal at a stocking density of 6,000 ha⁻¹. Fishes were fed on rice bran/groundnut oil cake and natural food. Fish blood and interrenal sampling was done.

**Experiment 2 Determination of haematological changes due to stress during cultural practices**

Two experiments were designed in a 0.5 ha static water pond.

A) Twenty fingerlings of *L. rohita* were captured by cast net from the pond and put in aluminium containers (hundies) at a stocking density of 0.1 kg l⁻¹ and kept for 1 hr without aeration. After 1 hr blood sampling was done.

B) Fifty fingerlings of *L. rohita* were captured by cast net and kept in hundies overnight in the pond of capture. After 12 hrs, sampling for blood and interrenals was done.

**Blood sampling and analysis** To obtain blood sampled fishes were rapidly anesthetized using 60 mg l⁻¹ MS222. Blood was collected by caudal peduncle cut. Heparin was used as anticoagulant. The methods employed for determination of various haematological parameters were haemoglobin (Sahli's method), clotting time (Casillas and Smith, 1977), haematocrit (Blaxhall and Daisley, 1973), plasma chloride (Schales and Schales 1941), glucose (Hyvannen and Nikkila, 1962).

**Histology** Histological sections of anterior kidney was stained by normal eosin haematoxylin stain and measurement of interrenal cells done through camera lucida. For establishing the normal standard of interrenal cell size and its nuclear diameter 300 interrenal cells were measured in normal as well as stressed fish samples.

Water quality parameters of laboratory tank, pond and hundie water were measured as per standard methods (APHA, 1989) or by HACH kit.

**Results and Discussion**

The normal ranges of the haematological parameters of *L. rohita* obtained in the laboratory tank and in the pond and water quality are given in Table 1. The measurements of cell size and the nuclear diameter of the normal interrenal cells and stressed fishes are given in Table 2.

In the second experiment where fishes were crowded and confined in aluminium container water for 1 hour a perceptible change in the water quality after 1 hour was observed. There has been a gradual depletion of dissolved oxygen from 9 mg l⁻¹ to 1 mg l⁻¹ (Fig. 1). Simultaneously the unionized ammonia levels increased to 1.4 mg l⁻¹ from nil. (Fig. 2). Thus it was seen that a hypoxic condition was created coupled with toxicity generated by rise in unionised ammonia levels. This has resulted in significant alteration in some of the blood parameters (Table 3) when compared to their normal values (Table 1). Though the rise in the haemoglobin values due to stress is marginal but rise in haematocrit values were significant. Since the study of Hall *et al.*, (1962), Holeton and Randall (1967) and Sorvio *et al.*, (1973) it is generally accepted that the haematocrit value...
rise under hypoxic condition. This is because the hypoxic condition created by crowding and confinement cause swelling of the erythrocytes and gives elevated haematocrit values. The leucocrit values declined in the present experiment. Leucocrit is regarded as a sensitive indicator of physiological stress resulting from crowding, handling and other forms of stresses. The stress response of salmonids and other fishes normally includes a moderate to severe leucopenia as a result of corticosteroid lymphocytolytic properties. (Srivastava and Agarwal, 1977, Mc Leay and Gordon 1977). There has been a significant increase in blood glucose level (hyperglycemia) and significant decrease in the blood chloride levels (hypochlorerma) (Table 3) in response to the stress of capture and crowding. This is in agreement with the observation of Chavin et al. (1979), who observed that increase in ammonium ion and decrease in oxygen concentration in water produce increase in blood glucose and decrease in blood chloride levels.

Table 1 Normal ranges of the haematological parameters in L. rohita and the water quality parameters

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<tbody>
<tr>
<td>Haemoglobin (gm dl⁻¹)</td>
<td>7.5 ± 0.22869</td>
<td>6.65 ± 0.268</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>32 ± 0.7096</td>
<td>34.35 ± 1.948</td>
</tr>
<tr>
<td>Leucocrit (%)</td>
<td>0.66 ± 0.1288</td>
<td>0.68 ± 0.316</td>
</tr>
<tr>
<td>Clotting time (Sec.)</td>
<td>41 ± 1.468</td>
<td>33.2 ± 3.583</td>
</tr>
<tr>
<td>Plasma chloride (mEq l⁻¹)</td>
<td>92.1 ± 2.394</td>
<td>89 ± 5.7684</td>
</tr>
<tr>
<td>Plasma glucose (mgl⁻¹)</td>
<td>52.4 ± 2.8087</td>
<td>79.81 ± 8.240</td>
</tr>
</tbody>
</table>

Biological

Fish length (mm) 115 - 210 120 - 186
Fish weight (gm) 36 - 170 30 - 90

Environmental

Air temp (°C) 32.2 29.5
Water temp (°C) 28.0 28.0
Transparency (cm) 36.5 Nil
Alkalinity (mgl⁻¹) 198 182.7
Hardness (mgl⁻¹) 192 171
DO (mgl⁻¹) 7.6 7.0
Unionised ammonia (mgl⁻¹) 0.1 —

pH 8.5 8.1

n = 10

Table 2 Measurement of interrenals of normal and stressed L. rohita

<table>
<thead>
<tr>
<th>Status</th>
<th>Interrenal cell</th>
<th>Mean mm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Cell area</td>
<td>36.88</td>
<td>8.68-78.5</td>
</tr>
<tr>
<td></td>
<td>Nuclear diameter</td>
<td>2.15</td>
<td>1.25-3.75</td>
</tr>
<tr>
<td>Stressed</td>
<td>Cell area</td>
<td>98.56</td>
<td>72.06-139.4</td>
</tr>
<tr>
<td></td>
<td>Nuclear diameter</td>
<td>6.25</td>
<td>4.00-8.33</td>
</tr>
</tbody>
</table>

n = 300
Changes in water chemistry during 1 hr confinement of L. rohita in 30l aluminium container

Table 3 Influence of capture, crowding and confinement on the haematological parameters in L. rohita

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Haemoglobin (gm/dl)</th>
<th>Haematocrit (%)</th>
<th>Leucocrit (%)</th>
<th>Clotting time (sec)</th>
<th>Plasma glucose (mg/100ml)</th>
<th>Plasma chloride (mEq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal</td>
<td>7.5 ± 0.22869</td>
<td>32 ± 0.7096</td>
<td>0.66 ± 0.1288</td>
<td>41 ± 1.468</td>
<td>52.7 ± 2.808</td>
<td>95 ± 2.394</td>
</tr>
<tr>
<td>2 Cast net capture &amp; crowding in hundie for 1 hour</td>
<td>7.6 ± 0.24</td>
<td>39 ± 0.8995**</td>
<td>0.6 ± 0.1194*</td>
<td>40 ± 0.7349*</td>
<td>67.0 ± 1.892</td>
<td>82 ± 1.220*</td>
</tr>
<tr>
<td>3 Cast net capture &amp; confinement in happa for 12 hours</td>
<td>7.7 ± 0.075</td>
<td>44 ± 0.8124**</td>
<td>0.5 ± 0.0377*</td>
<td>38 ± 0.6033</td>
<td>64.0 ± 0.6899</td>
<td>86 ± 1.470*</td>
</tr>
</tbody>
</table>

n = 10, The significance of the difference of experimental fishes to those of normal fishes are shown as * 5% level and ** 1% level

Acknowledgement

The authors are grateful to the Director, CIFTI for his constant encouragement in the work. Financial assistance of India Council of Agricultural Research in gratefully acknowledged.
References


Some physiological changes in juvenile *L. rohita* (Ham.-Buch.) subjected to handling, crowding and transportation stress

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Central Inland Capture Fisheries Research Institute, Barrackpore-743 101 West Bengal, India

Introduction

The fishes are frequently subjected to the stress of aquacultural practices adopted by farmers viz., handling, crowding or transportation or a combination of the three procedures. These effects often become additive or synergistic with those of other stimuli (e.g., low unionised ammonia and dissolved oxygen). These practices can place a stress of considerable magnitude on the homeostatic mechanism of fishes (Wedemeyer, 1984) and may be partly responsible for the observed seasonal post stocking mortality of fishes (Ayles *et al*., 1976). The severity of these stresses and period needed for recovery are not clear in fishes.

In the present study a comparative assessment of the changes occurring in the levels of hematocrit, haemoglobin, leucocrit, plasma glucose, cortisol, chloride, protein, lactic acid and cholesterol and liver/muscle glycogen has been made in fingerlings of *L. rohita* (Ham.-Buch.) subjected to the stress of handling, crowding and transportation for one hour and their recovery under optimum conditions.

Materials and methods

Juveniles of *L. rohita* of average weight and length (14.5 g, 130 mm) were acclimatized in the laboratory for one month in fibre glass tanks at a stocking density of 0.4gm/l. The water quality conditions were pH (7.7-7.9), water temperature (28-28°C), dissolved oxygen (5.5-6.8 mg/l), alkalinity (120-150 mg/l), hardness (220-275 mg/l) with 12 hr light and 12 hr darkness.

All the fishes were fed Tubifex *ad libitum* daily and 1/4th water was exchanged daily. The fishes were fasted 24 hrs before blood and tissue samples were taken. For initiation of the experiment the fishes were gently netted out of the fibre glass tank and put in an aluminium bundle at a stocking density of 75 g/l and transported for 1 hr in a vehicle with occasional handling by net or by hand. The water quality parameters of the hundie water viz., dissolved oxygen, unionised ammonia and carbon-dioxide of water were measured before and after 1 hr. After 1 hr the fishes were again stocked back in the fibre glass tank with continuous aeration at a stocking density of 0.4g/l for recovery. Subsamples of blood and tissue parameters of 7 experimental fishes was done at 0 hr, 1 hr of the experiment and during 1 hr, 3 hr, 24 hr and 48 hr of the recovery period.

Blood and tissue sampling and analysis

To obtain blood *L. rohita* fingerlings were netted out gently and anaesthetized using MS 222 (ethyl-m-aminobenzoate methanesulphonate) at the dose of 60 mg/l. The fishes were immobilized with 1 min of application. Blood was collected from the caudal artery by caudal peduncle cut. Heparin was used as anticoagulant. Immediately after collection, blood was centrifuged for 5 mm at 3000 rpm in cold and the plasma separated out and either used for analysis immediately or stored at -20°C for analysis later. Fishes were dissected and liver and muscle tissues were collected, blotted and then stored at -20°C for analysis. Sampling procedure of netting, anaesthesia and plasma storing was completed within 10 min to avoid influence of netting combined with anaesthesia on the basal cortisol levels (Franklin *et al*., 1990, Tanck, *et al*., 2000).

*Corresponding Author
The methods employed for estimation of various blood parameters were haematocrit (Blaxhall et al., 1973), haemoglobin (Sahhs method), leucocrit, plasma glucose (Hyvannen et al., 1962), chloride (Schales and Schales, 1941), cholesterol (Zak, 1957), protein (Kilow, 1967), lactic acid (Harrower et al., 1972), liver/muscle glycogen (Oser, 1965)

Plasma cortisol was measured by a direct immunoenzymatic determination of cortisol kit manufactured by Equipar srl via G Ferrari, 21’N-21047, SARONNO, ITALY using a 96 well micro litre plate read by ELISA microplate reader (Model EL 31/SX Bio Tek Instruments INC) The final solution was read at a wave length of 450 nm The plasma cortisol concentration was calculated based on a series of standards Water quality parameters were measured as per standard methods (APHA, 1989)

Statistical analysis of data for all physiological parameters expressed as mean ± SE Student t test (Snedecor and Cochran, 1971) was used to find out significant difference between pre-stress and post-stress mean values

Results and discussion
The measurements of the various physiological parameters during stress and subsequent recovery period are given in Table 1

Fishes subjected to crowding, handling and transportation stress in the bundles for 1 hr are also subject to the additional stress of perceptible water quality changes in the bundles There is a gradual depletion of dissolved oxygen from the initial 68 mg l\(^{-1}\) to 18 mg l\(^{-1}\), UItA from nil to 0.13 mg l\(^{-1}\) after and CO\(_2\) from 2.0 mg l\(^{-1}\) to 6.6 mg l\(^{-1}\) 1 hr Thus a hypoxic condition has been created along with toxicity produced by rise in unionised ammonia levels These stressors have significantly altered some of the blood and tissue parameters when compared to the normal values (Table 1)

Table 1: Measurement of various physiological parameters of \(L\) \(r\)ohita subjected to handling crowding and transportation stress and recovery

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stress</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control/0 hr</td>
<td>1 hr</td>
</tr>
<tr>
<td>Haemoglobin (gm/dl)</td>
<td>6.9 ± 0.117</td>
<td>7.5 ± 0.165*</td>
</tr>
<tr>
<td>Hæmatocrit (%)</td>
<td>34.2 ± 0.634</td>
<td>38.6 ± 0.998*</td>
</tr>
<tr>
<td>Leucocrit (%)</td>
<td>1.3 ± 0.063</td>
<td>1.14 ± 0.084</td>
</tr>
<tr>
<td>Plasma cortisol (ng/ml)</td>
<td>96 ± 3.17</td>
<td>180±9.08**</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>95 ± 4.48</td>
<td>269.5±4.75*</td>
</tr>
<tr>
<td>Plasma chlorme (mEq/l)</td>
<td>117.6±1.022</td>
<td>102±0.634**</td>
</tr>
<tr>
<td>Plasma protein (gm/dl)</td>
<td>2.3±0.2156</td>
<td>2.7±0.063</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>274±3.054</td>
<td>2.97±4.634</td>
</tr>
<tr>
<td>Plasma lactic acid (mg/dl)</td>
<td>102.4±2.338</td>
<td>122.7±2.159</td>
</tr>
<tr>
<td>Glycogen (mg/gm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>8.0±0.288</td>
<td>7.0±0.31**</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.2±0.442</td>
<td>3.2±0.213</td>
</tr>
</tbody>
</table>

n = 7
*P (<0.05)
**P (<0.01)
Plasma cortisol levels increased significantly (P<0.01) after 1 hr of stress and continued to be so up to the 24 hr of recovery period in relation to control values. However after 48 hrs, regulation of the cortisol values were obtained. It indicates an activation of the pituitary interrenal axis. This is in agreement to findings of Barton et al., 1980, Swift 1981, Tomasso et al., 1981 a and Pickering et al., 1982 who recorded that handling, confinement and water quality deterioration can stimulate the HPI axis result in increase in cortisol levels in teleost fish. Dutta et al., 1999, also obtained increase in cortisol levels from the resting values of 90±5 to 150±36 ng ml⁻¹ in L. rohita subjected to handling, and crowding in glass aquaria. The regulation of cortisol values in the present experiment was obtained after 48 hr.

Plasma glucose levels also increased significantly (P<0.01) during 1 hr of stress and continued up to 24 hrs of recovery period compared to control. McCormick & Mcleod (1925) reported as physisation hyperglycemia due to the cultural procedure of capture, handling and transportation. Similarly a significant (P<0.01) decrease in plasma chloride were observed after 1 hr of stress and continued up to 24 hrs (P<0.05) of recovery period. This is in agreement with the observation of Chavm et al., 1979 and Sinha et al., 1999 who observed that increase in ammonium ion and decrease in oxygen concentration in water produce increase in blood glucose and decrease in blood chloride levels. Wedemeyer (1972) also reported osmoregulatory dysfunction due to transportation in juvenile coho salmon and steel head trout where the plasma chloride fell to 13-20 mM for 3-5 hr. Both plasma glucose and chloride recovered its normal values within 48 hrs of recovery period.

Plasma lactic acid significantly increased (P<0.01) after 1 hr of stress but recovered after 3 hrs. Because of hyperactivity and hypoxic condition prevailing lactic acid levels increased in blood. Betty & Wardle 1979 reported a peak of lactate in brown trout at 2 hr post handling and as in the present case also observed the rapid removal of lactate from the blood. This may be the result of complete oxidation by the tissues, i.e., gluconeogenic conversion of lactate to glycogen. In most fish, blood lactate levels normally recover within 24 hr of an acute stress (Black 1957 a,b, Wendt and Saunders 1973, Soivio and Oikari, 1976 and Perrier et al., 1978).

Plasma protein levels did not change significantly during stressor post stress in comparison to control value. It may be due to protein sparing action by carbohydrates and lipids which are used as energy fuels during stress. Plasma cholesterol level did not vary significantly except after 1 hr of recovery compared to control. The increase may be due to mobilization of cholesterol from liver to blood due to stress.

Significant decrease in liver glycogen was recorded after 3 hrs of recovery (P<0.01) as compared to control. The depletion of liver glycogen could be due to increase in demand of energy by fish and also due to the fact that feeding was withheld during the experiment. Muscle glycogen levels also decreased after 1 hr of stress. The fall in muscle glycogen indicates availability of readily available energy as evidenced by Black et al., 1962. Regulation of muscle glycogen occurred after 48 hrs.

The haemoglobin value rises significantly after 1 hr of stress (P<0.05) and also during 1 hr of recovery (P<0.05). It returned to the control levels after 3 hr of recovery. Haematocrit levels also rose significantly after 1 hr of stress (P<0.05) and also during 1 hr of recovery (P<0.05) as compared to control. Since the study of Hall et al., 1962; Holeton and Randall (1967) and Soivio et al., 1973 and Sinha et al., 1999 it is generally accepted that the haemoglobin and haematocrit value of fishes rise under hypoxic conditions. The observations in the present case corroborates the findings. This is because the hypoxic condition created by crowding and confinement cause swelling of the erythrocytes and give elevated haematocrit values. A gradual decrease in leucocrit level is seen during stress and recovery period but significantly after 24 hrs of recovery (P<0.05) with the observation of Sinha et al. (1999). Leucocrit is regarded as a sensitive indicator of physiological stress. It may be due to a marked reduction in numbers of circulating lymphocytes by the corticosteroids lymphocytolytic properties (Srivastava and Agarwal, 1977, McLeay and Gordon 1977).

It is evident from the above results the cultural practices of handling, crowding and transportation in hundies frequently adopted by fish farmers, significantly stress the fishes, L. rohita. It is suggested that for proper fish health management fishes, subjected to such stress should not be further stressed within 48 hrs for metabolic recovery.
Acknowledgements
The authors are grateful to the Director, CICFRI, Barrackpore for assistance and advice. The work was supported by grant from ICAR, New Delhi.

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Dated: 14-06-2001

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Project Monitoring & Documentation Section,  
Central Indian Capture Fisheries Res Inst,  
Barrackpore - 743 101, West Bengal.

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Editor  
For Managing Editor.

cks/1512001.
Physiological effect of cold shock in juvenile *L. rohita* (Hamilton-Buchanan)

TANUSREE DATTA, SUBHENDU ACHARYA AND MANAS KR. DAS

Central Inland Capture Fisheries Research Institute
Barrackpore-743 101, West Bengal

Temperature is often referred to as the master factor in the environment. Rapid changes in temperature, either cold or hot shocks are among the stressors with high physiological impact on fish (Crawshaw, 1979). The ability of fishes to survive low temperature has been studied by Regnard (1895), Maurel & Lagriffe (1899), Gift (1977) and Tanck et al. (2000). The changes in the blood and tissue constituents in fishes during low temperature shock and subsequent recovery period are of interest to determine the physiological condition of fishes. Investigations in this aspect on tropical fishes are meagre except the work of Pandey et al., (1977) on *R. rita*. In the present communication the physiological changes in some of the blood and tissue constituents of the carp *L. rohita* juveniles during cold shock and the subsequent recovery period are assessed.

Materials and methods

Juveniles of *L. rohita* of average weight and length (14.5 g and 130 mm) respectively were acclimatized in the laboratory in fibre glass tank at a stocking density of 0.4 g/l and at a temperature of 28°C. The water quality conditions were pH 7.8-8.0, alkalinity 185-190 mg/l, hardness 285-290 mg/l, dissolved oxygen 5.5-6 mg/l with 12 hr light and 12 hr darkness. All fishes were fed Tubifex ad libitum and one fourth water exchanged daily. The low temperature shock was given by immersing 60 juvenile *L. rohita* in water at 5°C for 5 min. After the shock they were transferred to the aquarium water at 28°C for recovery. Subsamples of 10 fishes of *L. rohita* were taken at 0, 20 min, 1 hr, 2 hrs, 5 hrs, 19 hrs and 24 hours.

Blood and tissue sampling and analysis

To obtain blood *L. rohita* were netted gently and were rapidly anesthetized using MSS-222 (ethyl m-aminobenzoate methanesulphonate) at the dose of 60 mg/l. The fishes were immobilized within 1 min of application. Blood was collected from the caudal artery using 1 ml syringes fitted with 24 G needle and in some fishes by caudal peduncle cut. Hepalin was used as the anticoagulant. Immediately after collection blood was centrifuged for 5 min at 3000 rpm and the plasma separated out and either used for analysis immediately or stored at −20°C for analysis later. Sampling procedure of netting, anaesthesia and plasma storing was completed within 10 min to avoid influence of netting combined with anesthesia on the basal cortisol levels (Franklin et al., 1990, Weyts et al, 1997 & Tanck et al., 2000).
The methods employed for estimation of various blood parameters were haematocrit (Blaxhall *et al.*, 1973), plasma glucose (Hyvannen *et al.*, 1962), cholesterol (Zak 1952), lactic acid (Harrower *et al.*, 1972) and kidney ascorbic acid (Mosingr *et al.*, 1959), plasma cortisol was measured by a direct immunoenzymatic determination of cortisol kit manufactured by Equipar srl via G Ferran, 21/N-21047, SARONNO, ITALY using a 96 well microplate plate read by ELISA microplate reader (Model EL31/SX, Bio Tek Instruments INC) The final solution was read at a wavelength of 450 nm The plasma cortisol concentration was calculated based on a series of standards Water quality parameters were measured as per standard methods (APHA, 1989) Statistical analysis of data for all physiological parameters are expressed as mean ±SE Statistical comparisons between experimental and control fishes were made by student t-test (Snedecor *et al.*, 1971)

Results

The changes that occur in the various physiological parameters are given in Table-1

Activation of the pituitary interrenal axis is evident in *L. rohita* subjected to cold shock by the significant increase in cortisol value (Fig 1) A significant decrease in anterior kidney ascorbic acid which was maintained upto 2 hours post shock period was observed (Fig. 2). However, cortisol regulation was achieved after 2 hours Plasma chloride levels decreased significantly within 20 min but recovered within 24 hours Plasma lactic acid showed significant increase even upto 24 hours of recovery period (Fig 3) Hyperglycemia was recorded within 20 min and persisted upto 24 hrs post shock period (Fig 4) There was a significant depletion of muscle glycogen within this 1 hour period However, liver glycogen levels did not show any significant fluctuation The haematocrit, leucocrit, plasma protein and cholesterol values did not show any significant alteration

Discussion

Juveniles of *L. rohita* subjected to cold shock induced a significant rise in plasma cortisol values within 20 minutes after the shock It indicates an activation of pituitary interrenal axis. Similar activation in response to cold shock was observed in *C. carpio* (Tank *et al.*, 2000) The depletion of ascorbic acid in *L. rohita* in response to acute stress of cold shock is because of the demand of ascorbic acid for steroid biosynthesis, as reflected by the increased levels of cortisol (Burns 1961)

The hyperglycemia response in *L. rohita* was also observed in *R. nta* at 3°C (Pandey *et al.*, 1977) Plasma glucose level increased as a result of glycogenolysis in muscle and liver Elevated cortisol levels observed in the plasma by virtue of its catabolic properties mobilizes carbohydrate reserves which are depleted due to increased energy demand (Lindman *et al.*, 1979) Hyperlactemia observed in *L. rohita* even upto 24 hours recovery period is in agreement to the findings in *R. nta* (Pandey *et al.*, 1977) where hyperlactemia did not subside even upto 26 hr of recovery This increase in plasma lactic acid is due to the anaerobic breakdown of glucose At low temperature the respiratory process and activity of *L. rohita* ceases and as a result the oxidation process shows down. This observation has also been recorded in *R. nta* by Pandey *et al.*, (1977). Thus, the aerobic utilization of carbohydrate is slowed down resulting in an increase in glucose and lactic acid.
Table-1. Levels of blood & tissue parameters in *L.rohita* subjected to cold shock

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>20 min.</th>
<th>1 hr.</th>
<th>2 hrs.</th>
<th>5 hrs.</th>
<th>19 hrs.</th>
<th>24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/ml)</td>
<td>100</td>
<td>140**</td>
<td>160**</td>
<td>131.4**</td>
<td>107</td>
<td>109</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>± 3.8733</td>
<td>± 6.8196</td>
<td>± 4.0623</td>
<td>± 4.2244</td>
<td>± 5.2593</td>
<td>± 7.2463</td>
<td>± 5.6119</td>
</tr>
<tr>
<td>Protein (gm/dl)</td>
<td>2.88</td>
<td>3.183</td>
<td>2.87</td>
<td>3.2</td>
<td>3.39</td>
<td>2.972</td>
<td>2.588</td>
</tr>
<tr>
<td></td>
<td>± 0.1347</td>
<td>± 0.1790</td>
<td>± 0.113</td>
<td>± 0.5313</td>
<td>± 0.4179</td>
<td>± 0.1143</td>
<td>± 0.234</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>138.47</td>
<td>241.73**</td>
<td>311.11**</td>
<td>209.29**</td>
<td>175.37</td>
<td>184.88</td>
<td>186.97</td>
</tr>
<tr>
<td></td>
<td>± 4.733</td>
<td>± 7.063</td>
<td>± 8.716</td>
<td>± 5.78</td>
<td>± 23.16</td>
<td>± 23.2</td>
<td>± 23.1</td>
</tr>
<tr>
<td>Chloride (mEq/l)</td>
<td>124.5</td>
<td>114.4**</td>
<td>109.5**</td>
<td>109.1**</td>
<td>128.7</td>
<td>120</td>
<td>115.4</td>
</tr>
<tr>
<td></td>
<td>± 1.797</td>
<td>± 1.9688</td>
<td>± 3.238</td>
<td>± 3.238</td>
<td>± 4.938</td>
<td>± 5.584</td>
<td>± 5.108</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>446.7</td>
<td>435.2</td>
<td>474.9</td>
<td>513.0</td>
<td>491.7</td>
<td>405.6</td>
<td>387.6</td>
</tr>
<tr>
<td></td>
<td>± 3.88</td>
<td>± 2.7041</td>
<td>± 4.048</td>
<td>± 2.3792</td>
<td>± 3.752</td>
<td>± 10.039</td>
<td>± 2.953</td>
</tr>
<tr>
<td>Lactic acid (mg/100 ml)</td>
<td>181.3</td>
<td>191.0</td>
<td>207.82**</td>
<td>200**</td>
<td>212.6**</td>
<td>213.5**</td>
<td>231.2**</td>
</tr>
<tr>
<td></td>
<td>± 5.46</td>
<td>± 3.5</td>
<td>± 4.549</td>
<td>± 5.945</td>
<td>± 10.838</td>
<td>± 7.395</td>
<td>± 10.38</td>
</tr>
<tr>
<td>Ascorbic acid (mg/gm)</td>
<td>2.27</td>
<td>1.88</td>
<td>1.209*</td>
<td>1.12*</td>
<td>1.103*</td>
<td>1.19</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>± 0.12178</td>
<td>± 0.22188</td>
<td>± 0.053</td>
<td>± 0.0954</td>
<td>± 0.0537</td>
<td>± 0.0760</td>
<td>± 0.0718</td>
</tr>
<tr>
<td>Haematocnt (%)</td>
<td>47.6</td>
<td>43.6</td>
<td>38.77</td>
<td>44.47</td>
<td>44.38</td>
<td>35.0</td>
<td>38.67</td>
</tr>
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<td>± 0.961</td>
<td>± 0.721</td>
<td>± 0.9569</td>
<td>± 0.90816</td>
<td>± 0.8532</td>
<td>± 0.9107</td>
<td>± 0.688</td>
</tr>
<tr>
<td>Leucocnt (%)</td>
<td>1.9</td>
<td>1.52</td>
<td>1.033</td>
<td>1.136</td>
<td>1.6</td>
<td>1.23</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>± 0.1529</td>
<td>± 0.0936</td>
<td>± 0.1087</td>
<td>± 0.10746</td>
<td>± 0.1158</td>
<td>± 0.1216</td>
<td>± 0.0853</td>
</tr>
<tr>
<td>Glycogen (mg/gm) Liver</td>
<td>8.36</td>
<td>8.18</td>
<td>7.99</td>
<td>8.0</td>
<td>8.16</td>
<td>8.24</td>
<td>8.2</td>
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<tr>
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<td>± 0.137</td>
<td>± 0.171</td>
<td>± 0.1526</td>
<td>± 0.1311</td>
<td>± 0.147</td>
<td>± 0.124</td>
<td>± 0.099</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.09</td>
<td>3.76</td>
<td>3.45*</td>
<td>3.25*</td>
<td>3.19</td>
<td>3.49</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>± 0.0823</td>
<td>± 0.0827</td>
<td>± 0.0906</td>
<td>± 0.0950</td>
<td>± 0.0655</td>
<td>± 0.122</td>
<td>± 0.0941</td>
</tr>
</tbody>
</table>

Values are mean ± SE, ** Significant at 1% level, * Significant at 5% level.
Fig 1 Plasma cortisol levels in cold shocked *L. rohita* values given as mean ± SE (n=10) ** indicates significantly different from control at 5% level.
Fig. 3. Plasma Lactic acid levels in cold shocked *L. rohita*. Values given as $X \pm SE$ (n=10). ** indicates significantly different from control at 5% level.
Fig. 2. Ascorbic acid levels in cold shocked *L. rohita*. Values given as X ± SE (n=10) * indicates significantly different from control at 1% level.
Fig 4. Plasma glucose levels in cold shocked *L. rohita*. Values given as $\bar{X} \pm SE$ (n=10). ** indicates significantly different from control at 5% level.
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References:


