The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.)

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ABSTRACT: The effect of 12 h transport on the biochemical and haematological profiles of three-years-old common carp was investigated. There was a significant increase ($P < 0.01$) in ammonia, chloride, phosphorus, lactate dehydrogenase, aspartate aminotransferase and creatine kinase, and a significant decrease ($P < 0.05$) in total proteins. The levels of glucose ($P < 0.01$), lactate ($P < 0.05$), alanine aminotransferase ($P < 0.01$) and calcium ($P < 0.01$) were also significantly changed, but no time-dependent relationships were found. Significant increases in haematocrit ($P < 0.01$) and metamyelocyte count ($P < 0.05$) were found. Mean corpuscular volume ($P < 0.05$) and counts of monocytes ($P < 0.01$), band neutrophils ($P < 0.01$) and segmented neutrophils ($P < 0.05$) were significantly changed independently of the transport duration. Since pre-transport manipulation and transport *per se* were found to be important stressors for three-years-old common carp, peri-transport disturbances should be minimized to ensure optimal fish welfare.

Keywords: erythrocyte profile; common carp; *Cyprinus carpio*; leukocyte profile; plasma biochemical indices; stress response

In aquaculture, fish exposure to external stressors is a commonplace because of management procedures such as weighing, grading, transporting, and the increase in rearing densities. Physiological responses of fish to stressors are adaptive, resulting in mobilization of energy reserves and cardiovascular changes that enable fish to overcome disturbances (Ruane et al., 2002).

Transporting live fish is a multiphase operation that should be designed to minimize stress as well as economic costs. Water quality and fish density must be controlled. Fish are usually starved for about 24 h prior to harvest and transport so that they do not void faeces and foul the transport water (Conte, 2004). Excretory products, mucus and regurgitated food also degrade the water quality. Respiration causes a decrease in the levels of dissolved oxygen and increases the levels of carbon dioxide, and excretion of nitrogenous wastes increases ammonia in the transport water (Paterson et al., 2003).

Fish exposed to stressful stimuli during transport, e.g. handling, netting, loading at high densities, unloading, inadequate water exchange and poor water quality, usually suffer from a fight or flight stress response and it may cause adverse physiological reactions affecting the essential life functions (Barton et al., 1980; Erikson et al., 1997). This General Adaptation Syndrome-like stress response (Selye, 1956) manifests itself in a primary release

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of adrenalin and cortisol, followed by secondary changes in blood and tissues, such as hyperglycaemia, hyperlactaemia, hypercholesterolaemia, changes in blood plasma enzyme activities and ion concentrations, reduced glycogen content of muscle and liver, increased metabolic rate, and shifts in haematological profiles and immunological capacity (Stave and Robertson, 1985; Staurnes et al.1994).

The acute primary physiological response of fish to netting, handling and transport returns to normal levels within 6–24 h. However, physiological recovery may take 10–14 days if the stressors persist and are not lethal (Schreck et al., 1997). Stress and physical activity during transport usually lead to the loss of product quality, such as reducing fish freshness, softening muscle texture, and lowering filet yield. Exposure to stress can have an impact on the economics of fish aquaculture (Nakayama et al., 1994).

The aim of this study was to assess a transport stress response, i.e. the effect of pre-transport manipulation procedures and long-distance transport *per se*, to some biochemical and haematological indices of three-years-old common carp, *Cyprinus carpio* (L). Transport water quality was also monitored.

MATERIAL AND METHODS

The experiment was carried out on common carp transported from storage ponds of the commercial fish farm in Hluboká nad Vltavou (Czech Republic) via Brno (Czech Republic) to a fish pond in Boheľov (Slovakia). Fish were transported in 2.2 m^3 transport tanks (AGK Kronawitter GmbH, Germany), at a density of 364 kg body weight per m^3. Duration of transport was 12.5 h, comprising 7 h from Hluboká nad Vltavou to Brno and 5.5 h from Brno to Boheľov. Tanks were continuously oxygenated during transport. In Boheľov, the carp were released from the tanks through a hose into a fish pond.

Throughout the experiment, water quality parameters were monitored and recorded (Table 1). Oxygen content and temperature were measured *in situ* (WTW pH 235), and pH, NH_4^+, Cl^-, $ANC_{4.5}$ and COD were measured in the laboratory using commercial test kits from Aquamerck (Merck, Germany).

The study was based on a sample of 46 carps. They were caught randomly in a net at each sampling point. Blood samples were taken by cardiocentesis during the loading of fish before transport in Hluboká nad Vltavou (16 specimens), after 7 h of transport in Brno (15 specimens), and after 12.5 h in Boheľov before the unloading of fish (15 specimens). After cardiocentesis, the fish were stunned and killed. Then the total body length, body weight and spleen weight were measured. *In situ*, PCV was measured and a leucogram blood smear was prepared. A small volume of heparinized blood from each of the 46 carps was preserved at 4°C for the determination of erythrocyte count (RBC), leukocyte count (WBC) and haemoglobin concentration (Hb).

The remaining portion of heparinized blood was centrifuged at 855 g for 10 minutes and plasma samples were stored at 4°C in Eppendorf tubes until analyses were performed (till 8 hours after sampling). Plasma biochemical indices (glucose (GLU), lactate (LACT), lactate dehydrogenase (LDH), creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), ammonia (NH_3), chlo-

Table 1. Physical and chemical indices of tank water (controlled oxygenation) during fish transportation

Indices	Hluboká	Brno	Boheľov
Oxygen (%)	36.50	47.50	60.30
Temperature (°C)	16.50	16.70	17.20
pH	6.72	6.73	6.85
NH_4^+ (mg/l)	0.95	1.67	3.34
Cl^- (mg/l)	17.71	24.10	25.09
$ANC_{4.5}$ (mmol/l)	0.90	1.30	1.50
COD (mg/l)	11.68	12.80	16.00

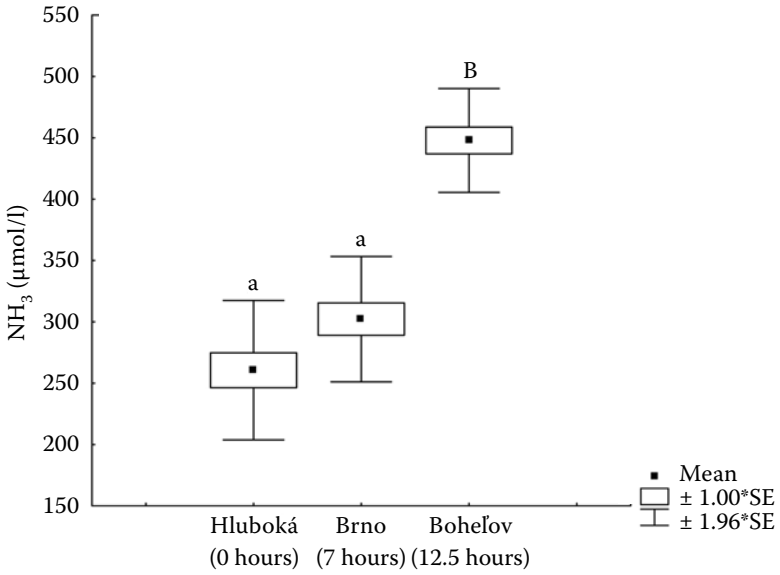

Figure 1. Effect of transportation on plasma NH_3 concentration in common carp; groups with different superscripts differ significantly at $P < 0.01$ (ANOVA)

ride (Cl^-), total proteins (TP), calcium (Ca^{2+}), and inorganic phosphate (P_i)) were measured with a biochemical analyzer Cobas EMira (Hoffman, La Roche, Co.) using commercial test kits (BioVendor). The plasma cortisol level was measured using HPLC/DAD (Waters).

Experimental data were analysed by Kruskal-Wallis ANOVA with the UNISTAT Statistica 6.0 software.

RESULTS

Mean values of fish total body length, body weight and spleen weight (48.96 ± 4.68 cm, 2 403.1 ± 687.4 g and 6.37 ± 2.12 g, 47.53 ± 3.76 cm, 2 256.7 ± 420.0 g and 6.71 ± 1.38 g, and 47.19 ± 3.55 cm, 2 170.0 ± 445.5 g and 6.01 ± 1.63 g in Hluboká, Brno and Boheľov, respectively) did not differ significantly among the tested groups. The values of SSI (relative weight of spleen to body mass) were 0.27 ± 0.03%, 0.30 ± 0.04% and 0.28 ± 0.05% in Hluboká nad Vltavou, Brno and Boheľov, respectively. These values were not significantly different.

The results (mean ± SD) of analyses of the tested biochemical and haematological indices in common carp are presented in Figures 1–6 and Tables 2 and 3.

Plasma biochemical profiles of carp exposed to long-distance transport (Figures 1–5 and Table 2) showed significant increases in concentrations of NH_3, Cl^- and P_i ($P < 0.01$) and in the activity of LDH, AST and CK ($P < 0.01$), and a significant

Table 2. Effect of transportation on biochemical indices of common carp

Indices	Hluboká	Brno	Boheľov
Cortisol (ng/ml)	258.57 ± 99.80^{a}	283.38 ± 204.34^{a}	301.94 ± 120.74^{a}
GLU (mmol/l)	10.80 ± 2.76^{a}	5.86 ± 1.76^{B}	10.14 ± 1.97^{a}
LACT (mmol/l)	7.90 ± 1.78^{a,c}	4.77 ± 4.05^{a}	9.17 ± 2.54^{b,c}
ALT (µkat/l)	1.17 ± 0.30^{a}	1.60 ± 0.48^{a}	0.12 ± 0.05^{B}
ALP (µkat/l)	1.29 ± 1.40^{a}	1.72 ± 2.09^{a}	1.60 ± 2.10^{a}
Cl^- (mmol/l)	96.39 ± 2.17^{a}	98.81 ± 7.53^{a}	107.57 ± 2.45^{B}
Ca^{2+} (mmol/l)	3.78 ± 1.07^{a}	4.45 ± 0.32^{B}	3.23 ± 0.68^{a}
P_i (mmol/l)	3.41 ± 0.40^{a}	3.77 ± 0.38^{b,c}	3.99 ± 0.38^{B,c}

Groups with different superscripts differ significantly at $P < 0.01$ (capitals) and $P < 0.05$ (small letters) (ANOVA)

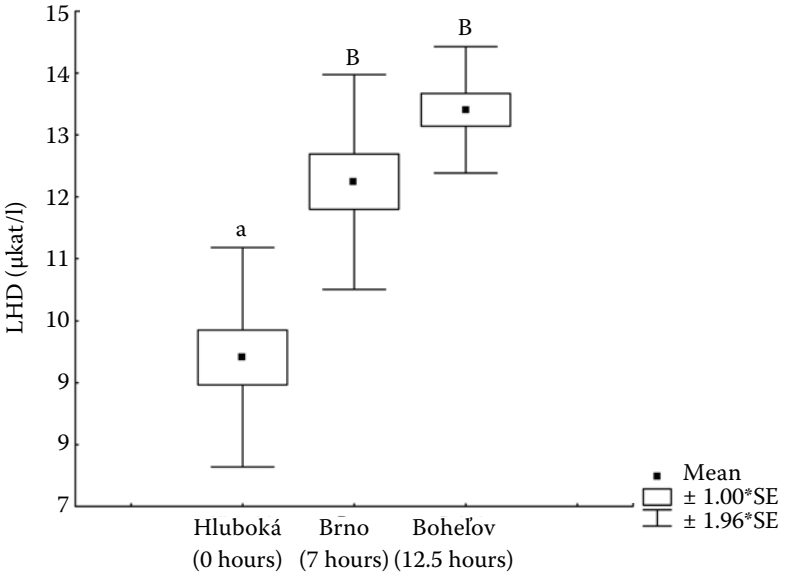

Figure 2. Effect of transportation on plasma LDH activity in common carp; groups with different superscripts differ significantly at $P < 0.01$ (ANOVA)

decrease in the TP level ($P < 0.05$). The levels of GLU ($P < 0.01$), LACT ($P < 0.05$), ALT ($P < 0.01$) and Ca^{2+} ($P < 0.01$) were also significantly influenced by transport, but no time-dependent relationship was found. No changes in plasma cortisol and ALP were recorded.

Regarding the haematological profiles (Figure 6, Table 3), transport caused a significant increase in PCV ($P < 0.01$) and metamyelocyte count ($P < 0.05$). Mean erythrocyte volume ($P < 0.05$) and counts of monocytes ($P < 0.01$), band neutrophils ($P < 0.01$) and segment neutrophils ($P < 0.05$) significantly changed, but independently of transport duration. The other measured haematological indices were not significantly different.

DISCUSSION

Previous studies which focused on fish stress responses to transportation procedures reported that pre-transport manipulation processes and transport *per se* appeared to be severe stressors to fish and that in particular capture and loading are the major cause of the transport stress response (Schreck et al., 1989; Weireich and Tomasso, 1991).

When fish are stressed, their adrenergic response releases adrenaline and noradrenaline into the blood stream and a hypothalamo-pituitary-interrenal response ultimately leads to an increase in the plasma cortisol level (Sumpter, 1997). In all vertebrates, including fish, cortisol plays a key role

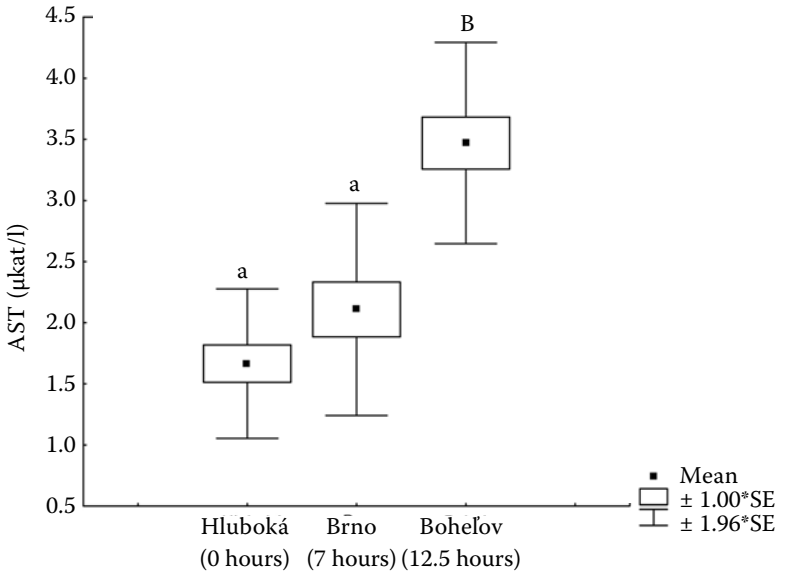

Figure 3. Effect of transportation on plasma AST activity in common carp; groups with different superscripts differ significantly at $P < 0.01$ (ANOVA)

Table 3. Effect of transportation on haematological indices of common carp

Indices	Hluboká	Brno	Boheľov
RBC (T/l)	1.12 ± 0.13^{a}	1.15 ± 0.20^{a}	1.17 ± 0.12^{a}
PCV (l/l)	0.42 ± 0.03^{a}	0.42 ± 0.02^{a}	0.46 ± 0.04^{B}
Hb (g/l)	102.66 ± 14.93^{a}	94.68 ± 18.59^{a}	103.54 ± 13.33^{a}
MCV (fl)	364.36 ± 40.69^{a,c}	347.35 ± 42.32^{a}	406.58 ± 59.06^{b,c}
MCH (pg)	93.05 ± 18.53^{a}	82.52 ± 11.40^{a}	89.17 ± 15.22^{a}
MCHC (l/l)	0.26 ± 0.05^{a}	0.23 ± 0.04^{a}	0.22 ± 0.03^{a}
WBC (G/l)	50.50 ± 14.64^{a}	64.53 ± 26.33^{a}	57.80 ± 22.00^{a}
Lymphocytes (G/l)	42.59 ± 13.80^{a}	50.79 ± 25.26^{a}	46.21 ± 23.15^{a}
Monocytes (G/l)	0.60 ± 0.68^{a,c}	1.22 ± 1.01^{a}	0.22 ± 0.42^{B,c}
Myelocytes (G/l)	2.28 ± 1.09^{a}	3.37 ± 2.66^{a}	3.42 ± 1.94^{a}
Band neutrophils (G/l)	1.26 ± 1.32^{a,c}	2.10 ± 1.71^{a}	0.91 ± 0.78^{B,c}
Segment neutrophils (G/l)	0.34 ± 0.42^{a}	0.96 ± 0.88^{b,c}	0.62 ± 0.63^{a,c}
Basophils (G/l)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.16 ± 0.40^{a}

groups with different superscripts differ significantly at $P < 0.01$ (capitals) and $P < 0.05$ (small letters) (ANOVA)

in the restoration of homeostasis during and/or after stress (Goos and Consten, 2002). However, quantifying stressful conditions proved difficult with many authors reporting a transient increase in plasma cortisol levels during stress, e.g. during crowding (Tort et al., 1996; Ruane et al., 2002) while others reported no effect (Procarione et al., 1999) or reduced cortisol levels (Leatherland and Cho, 1985).

In this study, the plasma cortisol level in fish increased slightly during transport (from 258.57 ng per ml in Hluboká to 301.94 ng/ml in Boheľov), but the increase was not significant. At the beginning of the experiment, carp responded to netting and catching during pre-transport manipulation with a mild cortisol response, similarly to results reported by Ruane et al. (2002). Plasma cortisol also increased slightly during transport, which cor-

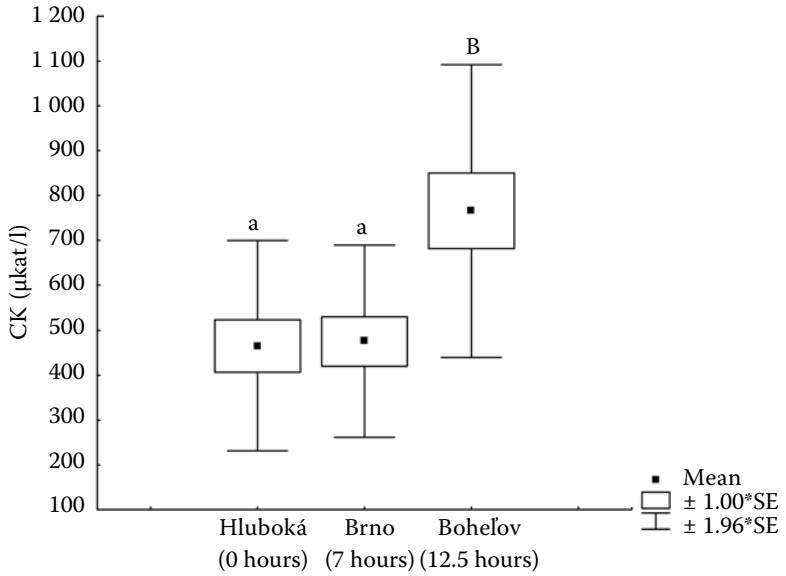

Figure 4. Effect of transportation on plasma CK activity in common carp; groups with different superscripts differ significantly at $P < 0.01$ (ANOVA)

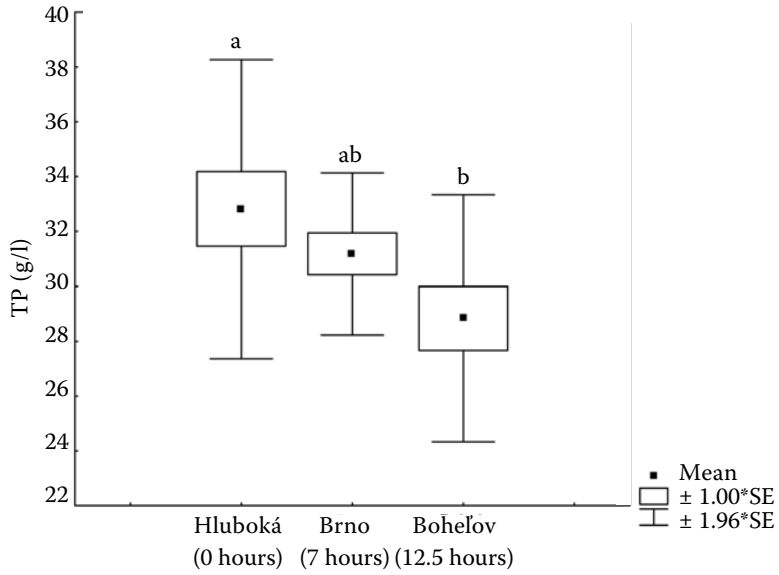

Figure 5. Effect of transportation on plasma TP concentration in common carp; groups with different superscripts differ significantly at $P < 0.05$ (ANOVA)

responded to the cortisol level elevation with the prolongation of transport time reported for a red drum by Robertson et al. (1988).

Plasma glucose is elevated in stressed fish as a consequence of increased blood catecholamine (Wedemeyer et al., 1990). The elevation of plasma lactate follows respiration under anaerobic conditions and extreme exercise (Pottinger, 1998). In our study, glucose and lactate levels were high in fish in Hluboká. This may be attributed to the stress response and physical exertion of carp in relatively hypoxic conditions during loading, when fish were held in nets at a very high density. The glucose level in unstressed carp was reported 2.8–5.6 mmol/l in Hertz et al. (1989). Pottinger (1998) found that the confinement of fish in keepnets caused an elevation in plasma lactate as high as 3.0–13.9 mmol/l.

In our study, long-distance transport led to a significant increase ($P < 0.01$) in the plasma ammonia concentration from 260.62 µmol/l in Hluboká (0 hours) to 447.87 µmol/l in Boheľov (after 12.5 hours). Smutná et al. (2002) stated that exhaustive exercise (escape from predators, locomotion and migration, starvation) increased active energy demand in fish. Energy production (catabolism of glutamine and amino acids provides ATP molecules) through direct deamination of tissue energy sources (i.e. amino acids, especially glutamate, aspartate, histidine, serine, and glutamine) leads to an increase in the tissue ammonia level.

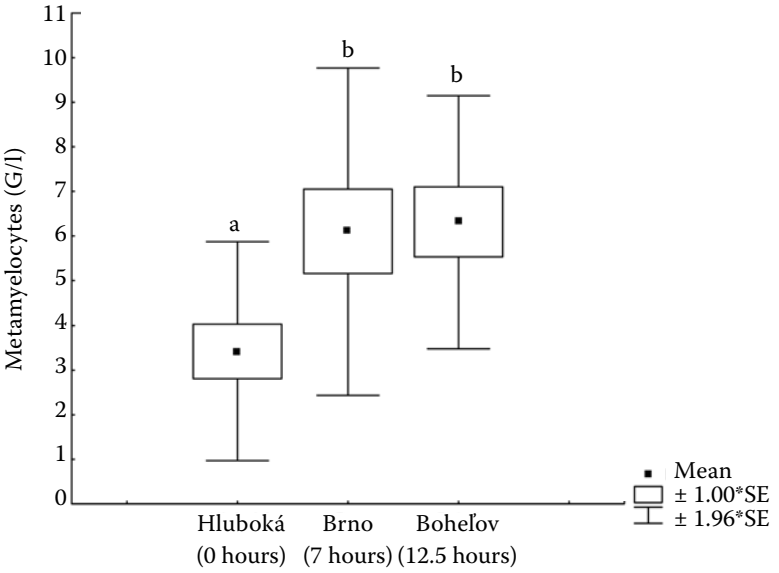

Figure 6. Effect of transportation on metamyelocyte count in common carp; groups with different superscripts differ significantly at $P < 0.05$ (ANOVA)

When the detoxification capabilities of fish are exceeded, ammonia accumulates in tissues and becomes toxic (Philip and Rajasree, 1996; Smutná et al., 2002). Our study revealed an increase in the ammonia level during transport. The results are in agreement with the study by Dobšíková et al. (2006), in which a significant ($P < 0.01$) increase in the ammonia level (from 298.2 to 448.9 μmol/l) was found during 12 h transport.

Haematological profiles have often been used as stress indicators. Major shifts in the haemogram are found in fish exposed to acute or chronic stress. In stressed fish an increase in RBC, haemoglobin concentration and haematocrit is often observed (Svobodová et al., 1994). During stress situations, elevated haemoglobin and haematocrit increase the oxygen carrying capacity of blood, and thus the oxygen supply to major organs, in response to higher metabolic demands (Ruane et al., 1999).

In our study, the fish tested during and after transport showed a significant increase in PCV ($P < 0.01$), which is similar to a significant increase in PCV ($P < 0.05$) in common carp after 12 h transport in Dobšíková et al. (2006). In the present study, RBC values and haemoglobin levels were not significantly altered by transportation. Haemoglobin level and the values of MCV, MCH and MCHC of the present study correspond to the results of Dobšíková et al. (2006) study.

Lymphocytopoenia and neutrophilia are reported secondary effects of stress in fish, as a consequence of stress-related release of catecholamines. The stress-induced elevation of plasma cortisol has a direct cytolytic effect on lymphocytes (Wiik et al., 1989; Engelsma et al., 2003). Lymphocytopoenia in stressed fish may also be due to the extravasation of the cells and their penetration into the epithelium of gills, skin or intestine. The movement of immune cells during stressful periods is influenced by stress hormones, therefore the mobilization of neutrophils and macrophages that form the first line of defence may be important for survival (Ruane et al., 2002). Ortuno et al. (2001) demonstrated that intense acute crowding led to leukocyte migration into the blood from the head kidney. This finding agrees with Wendelaar (1997), who reported that stress caused a rapid increase of neutrophils and a reduction of lymphocytes in peripheral blood. Our results for white blood cells did not confirm these findings. We found an increase ($P < 0.05$) in the count of metamyelocytes, a juvenile form of neutrophil granulocytes, and a non-significant elevation of myelocyte and basophil counts. Counts of monocytes, band and segmented neutrophils were significantly changed, but this change was time-independent. A significant increase ($P < 0.05$) in metamyelocytes, and analogous shifts in lymphocyte and myelocyte counts were found by Dobšíková et al. (2006).

Water quality parameters suggest that the transport media had no adverse effect on fish welfare (Svobodová et al., 1993). Fish are vulnerable to sudden short-term temperature fluctuations, hence necessary care must be taken when transferring fish to new ponds or new culture or transport media (Roberts and Shepherd, 1997). In our experiment, the storage pond and truck tank water temperatures were similar. The critical value for oxygen saturation in water is 15.3% at 15°C for common carp (Svobodová et al., 1993). In the study, the transport water oxygen content increased during transportation due to continual aeration. The pH value increased slightly during transport, but remained within the optimum range 6.4–8.4 (Svobodová et al., 1993). The elevated COD_{Mn} and levels of ammonia and chloride in the transport water may be attributed to the higher fish metabolic rate in a relatively high-density tank volume.

In conclusion, the study showed pre-transport manipulation procedures and transport *per se* to be stress inducers in common carp. Fish can be transported to long distances provided that optimal conditions for their adaptation are ensured and disturbances are minimized.

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