The response of plasma glucose, lactate, protein and hematological parameters to osmotic challenge in common carp *(Cyprinus carpio)*

Salati, A.P.[1]; Baghbanzadeh, A.[1]; Soltani, M.[1]; Peyghan, R.[2] and Riazi, Gh.[3]

[1]Faculty of Veterinary Medicine, The University of Tehran, Tehran-Iran. [2]Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Ahvaz-Iran. [3]Institute of Biophysics and Biochemistry, The University of Tehran, Tehran-Iran.

Key Words:
Common carp; salinity; plasma metabolites; hematology.

Correspondence
Baghbanzadeh, A.,
Department of Physiology, Faculty of Veterinary Medicine, The University of Tehran, P.O. Box: 14155-6453, Tehran-Iran.
Tel: +98(21)61117000,
Fax:+98(21)66933222
E-mail: baghbana@gmail.com

Received 16 May 2009,
Accepted 14 July 2009

Abstract

Fluctuations in salinity impose stress on the physiology of the exposed fish population and can modify their structure. It has been noted that changes in blood characteristics and plasma levels serve as the primary link between environmental change and physiological response (McCormick, 2001). Ninety healthy adult *Cyprinus carpio (C. carpio)* were randomly divided into five groups and kept in salinities of 1 (control group), 3, 6, 9, 12 and 15 g/L The salinity was gradually increased by 3 g/L daily to final their concentrations. Fish were kept for 14 days after transference to the final salinity. Subsequently, plasma glucose, lactate, and protein content, as well as hematological parameters, were evaluated. The highest hematocrit (Hct), hemogloblin level (Hb), red blood cell count (RBC), lactate, and glucose values were found fish kept in the highest salinity. The values of the white blood cell count (WBC) showed an initial increase in fish from the control group until a salinity of 9 g/L, but subsequently decreased at higher salinities. The plasma protein levels among the fish in different groups did not show a significant change. The results demonstrate that changes in blood parameters can be used as indices to monitor the effects of salinity on the physiology of *C. carpio.*

Introduction

Any environmental disturbance can be considered as a potential source of stress as it prompts a number of responses in the animal to deal with the physiological changes triggered by exterior changes. In theory, these responses can be detected in fish and in other vertebrates in the form of changes in hormonal or substrate concentrations in the plasma or alterations in erythrocyte parameters (Donaldson, 1981). Blood parameters have been recognized as valuable tools to monitor fish health and in the interpretation of physiological responses to stresses imposed by the environmental factors (Schutt *et al.,* 1997). It has been noted that changes in blood characteristics and plasma levels of metabolites serve as the primary link between environmental change and physiological response (McCormick, 2001). The physiological responses of freshwater stenohaline species to saline environments have attracted increased interest, particularly with respect to the use of saline water for the optimization of aquaculture practices or the use of these environments for aquaculture. Common carp (*Cyprinus carpio),* a stenohaline freshwater fish, is native to Asia and Eastern Europe, but now common carp has spread worldwide and colonized various environments. This species is one of the earliest species used in aquaculture and currently is one of the most important cultured fish in the world. *Cyprinus carpio* (*C. carpio*) can tolerate a range of different salinities from freshwater up to 12 g/l (Whiterod and Walker, 2006), but the physiological mechanisms that underlie the salinity tolerance of this species are not well understood. The present study examines the possible effects that osmoregulatory processes can have on the blood parameters in *C. carpio* that are subjected to gradually increasing levels of environmental salinity.

Materials and Methods

Ninety healthy adult *C. carpio* (mean weight 986±0.36 g and mean length of 24.25±0.17 cm) were sampled from a fish farm and were stocked in three tanks of 100 L each at 20±1ºC and 12L:12D photoperiod (lights on at 07:00). The tanks were well-aerated and in each aquarium 50% of water volume was renewed daily. The tanks were filled with dechlorinated tap water. After one week of acclimation, fish were randomly divided into five groups (*n*=15 fish at each group) with salinities of 1 (control), 3, 6, 9, 12 and

15g/L The salinity was gradually increased by 3 g/L daily to the final salinity of each group. These salinities were made by the addition of the appropriate amount of sodium chloride to dechlorinated tap water. Fish were kept for 14 days after transference to the final salinity. During this period, water quality was checked daily for ammonia, nitrite, and nitrate to ensure that levels did not exceed 0.1 mg/L, 1 mg/L and 20 mg/L respectively. After 14 days, fish were anesthetized with Tricaine methanesulfonate, MS_{222} (1:10,000) to minimize stress; blood was then sampled via caudal puncture with heparinized syringes. Red and white blood cell counts (RBC and WBC) were determined immediately with a Neubauer hemocytometer through the use of Hendrick's diluting fluid (Houston, 1990). Hematocrit (Hct) was determined in a capillary tube by microhematocrit centrifuge at 12000 rpm for 5 min (Morris and Davey 1996). For the determination of hemoglobin (Hb) levels, Drabkin's solution was added to blood and then the solution was centrifuged (3500 g for 6 min) to remove interferents. Subsequently, light absorbance was measured spectrophotometrically at 450 nm (Stoskopf, 1993). Plasma glucose and lactate were determined with Ziestchem glucose and lactate kits (Ziestchem diagnostics, Tehran). Plasma protein estimation was done in accordance with the method of Lowrey *et al.* (1951). The mean ± standard deviation (SD) was determined for each blood parameter. The differences in the parameters were tested for significance through the use of a one-way analysis of variance (ANOVA) and the least-significant difference (LSD) test ($p<0.05$).

Results

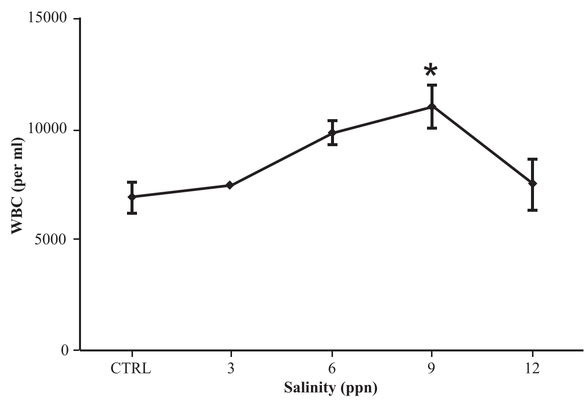
All of the fish in the tank with 15 g/L salinity died two days after their transference. There was no mortality in any other groups. The increase of environmental salinity had significant effects on measured parameters ($p<0.05$). The highest concentration of Hb and RBC values were registered in fish that were exposed to salinity of 12 g/L. These values were significantly higher than those in the control group and salinity of 3 g/L ($p<0.05$), but there were no other statistically significant changes in comparison to any other groups. The highest Hct values were also found in the highest salinity, but there was a statistically significant increase only in comparison to the control group (Table 1). The pattern was different for WBCs: values showed a significant increase in a salinity of 9 g/L, but subsequently returned to normal values in a salinity of 12 g/L (Figure 1). Lactate and glucose values showed a significant increase as salinity increased but protein values did not show a significant change (Table 1).

Table 1: The effect of different levels of salinity on blood parameters. Values are given as mean ± SD. Different letters denote significant differences ($p<0.05$) between treatments.

Salinity (g/L)	RBC (10^6/mm^3)	Hct (%)	Hb (g/dl)	Total protein (mg/dl)	Glucose (mg/dl)	Lactate (mg/dl)
1 (control)	1.420±0.989^a	32.9 ± 0.99^a	10.56 ± 0.23^a	3.98 ± 0.36^a	102.52 ± 3.5^a	36.85 ± 0.5^a
3	1.516±0.149^a	37.1 ± 2.97^a	11.27 ± 0.85^{ab}	3.77 ± 0.37^a	110 ± 7.52^a	42.65 ± 0.88^{ab}
6	1.617±0.140^{ab}	40.6 ± 4.85^a	12.65 ± 0.42^{bc}	3.94 ± 0.18^a	112.15 ± 4.38^a	45.04 ± 1.23^b
9	1.672±0.145^b	42 ± 6.1^a	13.07 ± 1.13^c	4.46 ± 0.44^a	138.91 ± 5.43^b	46.67 ± 3.02^{bc}
12	1.819±0.157^b	45.73 ± 7.18^b	13.2 ± 0.23^c	3.22 ± 0.4^a	165.8 ± 8.06^c	52.63 ± 7.43^c

$^{a, b, c}$ significant difference between treatments

Figure 1: White blood cells in carps exposed to different salinities. Values are given as mean ± SD. * denotes significant differences ($p<0.05$).



Discussion

Environmental salinity fluctuation can act as a stressor and leads to both significant decreases and increases in blood parameters. Therefore, changes in hematological parameters can reflect loss of homeostasis or demonstrate a compensatory response to salinity changes (Schreck, 1990). Almost all of data on this topic have been reported from euryhaline fish that can tolerate a wide range of salinities, but common carp is a stenohaline fish so a small change in environmental salinity could have a large impact on physiological parameters. Increased hematocrit can be an indicator of a hyposmotic stress response (Wu and Woo, 1983) or an increase of red blood cells released from the spleen (Milligan and Wood, 1982). These changes could also be attributed to changes in blood water content caused by changes in environmental salinity. Increased Hct and Hb in response to increased environmental salinity have been described in other stenohaline species, such as the grass carp (Yildiz and Uzbilek, 2001).

WBC values in a salinity of 9 g/L showed a significant increase in comparison to other groups but returned to the levels of the control values in a salinity of 12 g/L. Our results are not in agreement with those of Yildiz and Uzbilek (2001) for the WBC count of grass carp after exposure to saline water. Yildiz and Uzbilek did not demonstrate marked changes in WBC count. The decrease of the WBCs in salinity of 12 g/L may be attributed to suppression of production from the hematopoeitic organs (Matushima and Maiano, 1996).

Blood glucose level is a sensitive indicator of environmental and other stress sources (Hattingh, 1976). Stress-induced hyperglycemia is caused by catecholamines that affect the liver and release glucose from this organ (Van Raaji *et al*., 1995). Cortisol also induces hyperglycemia via increased gluconeogenesis in peripheral tissues (Chan and Woo, 1978). The increased glucose level provides energy for maintenance of plasma osmolarity in the constant range. The increase of plasma lactate indicates an oxygen debt has been incurred, from this it can be inferred that there is an imbalance between oxygen delivery and oxygen requirements. This may be due to an increase of gill epithelial thickness that impairs gas exchange. Lactate can be used in tissues, such as gills, kidney and brain, to supply their energy requirements (Mommsen, 1984). It had previously been suggested that this metabolite becomes more important in osmotic acclimation, presumably due to its metabolic use in those organs. Plasma protein was not changed in the different groups in this study, which suggests proteins are not involved in osmoregulatory responses to increased environmental salinity in common carp.

In this study, the hematological values showed significant changes at levels of salinity above 9 g/L. This means that at this level of salinity and higher, fish could survive but not in a good condition, and they would consume a great deal of energy for compensatory mechanisms. These results show that blood parameters can be used in common carp, which is a stenohaline fish, as in euryhaline fish to evaluate the responses of fish to different levels of environmental salinity.

Acknowledgements

The authors would like to thank the Faculty of Veterinary Medicine, University of Tehran, for their financial support for this research.

References

1. Chan, D.K.O. and Wo, N.Y.S. (1978) Effect of cortisol on the metabolism of the eel, *Anguilla japonica.* Gen. Comp. Endocrinol. 35:205-215.
2. De Boeck, G.; Vlaeminck, A.; Van der Linden, A. and Blust, R. (2000) The energy metabolism of common carp (*Cyprinus carpio*) when exposed to salt stress: an increase in energy expenditure or effects of starvation? Physiol. Biochem. Zool. 73: 102–111.
3. Donaldson, E.M. (1981) The pituitary–interrenal axis as an indicator of stress in fish. In *Stress and Fish* (ed. A. D. Pickering), Academic Press, New York, USA. pp: 11-41.
4. Hattingh, J. (1976) Blood sugar as an indicator of stress in the freshwater fish, *Labeo capensis* (Smith). J. Fish Biol. 10: 191–195.
5. Houston, A.H. (1990) Blood and Circulation. In: Schreck C. B. and Moyle P. B. (eds) Methods for Fish Biology. American Fisheries Society, Bethesda, USA. pp: 273–334.
6. Lowry, H.O.; Rosebrough, N.J.; Farr, A.L. and Randall, R. J. (1952) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
7. Matushima, E. R. and Mariano, M. (1996) Kinetics of the inflammatory reaction induced by carrageen in the swimbladder of Oreochromis niloticus (Nile tilapia). Braz J Vet Res Anim Sci 33:5–10.
8. McCormick, S.D. (2001) Endocrine Control of Osmoregulation in Teleost Fish. Am. Zool. 41: 781-794.
9. Milligan, C. L. and Wood, C. M. (1982) Disturbances in hematology, fluid volume distribution, and circulatory function associated with low environmental pH in the rainbow trout, Salmo gairdneri. J. Exp. Biol. 99: 397–415.
10. Mommsen, T. P. (1984) Metabolism of the fish gill. *Fish Physiology*, Vol XB (ed. W. S. Hoar and D. J. Randall), Academic Press, New York, USA. pp: 203-238.
11. Morris, M.W. and Davey, F.R. (1996) Basic examination of blood. Henry J.B (ed) Clinical Diagnosis and Management by Laboratory Methods. 19th edition., W. B. Saunders Company, New York, USA.
12. Schreck, C.B. (1990) Physiological, behavioral and performance indicators of stress. Adams S. M (ed.) Biological indicators of stress in fish, American Fisheries Society, Bethesda, USA.
13. Schuett, D.A.; Lehmann, J.; Guerlich, R. and Harriers, R. (1997) Hematology of Swordtail Xiphophorus helleri1: Blood parameters and light microscopy of blood cells. J. Appl. Icthyol. 13: 83-89.
14. Stoskopf, M.K. (1993) Clinical pathology. Stoskopf, M. (ed) Fish Medicine. W.B. Saunders, Philadelphia, USA.
15. Van Raaij, M. T.; Van den Thillart, G. E.; Hallemeesch, M.; Balm, P. H. and Steffens, A. B. (1995) Effect of arterially infused catecholamines and insulin on plasma glucose and free fatty acids in carp. Am J Physiol Regul Integr Comp Physiol. 268: R1163-R1170.
16. Whiterod, N.R. and Walker, K.F. (2006) Will rising salinity in the Murray–Darling Basin affect common carp (*Cyprinus carpio* L.)? Marine and Freshwater Research. 57: 817–823.
17. Woo, N.Y.S. and Wu, R.S.S. (1982) Metabolic and osmoregulatory changes in the red grouper, Epinephelus akaara (Temminck and Schlegel), and

the Black Sea bream, Mylio macrocephalus (Basilewsky). J. Exp. Mar. Biol. Ecol. 65: 139–161.

18. Yildiz, H.Y. and Uzbilek, M.K. (2001) The evaluation of secondary stress response of grass carp (Ctnepharyngodon idella; Val. 1844) after exposing to the saline water. Fish Physiol. Biochem. 25: 287–290.