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REGULAR ARTICLE

STUDY OF THE ADAPTATION PROCESS IN COMMON CARP (*CYPRINUS CARPIO* L.) AFTER HARVESTING

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ABSTRACT

Fish is sensitive to exogenous and endogenous ammonia. Ammonia formed in fish as a product of metabolism of proteins may be under certain circumstances life-threatening. Ammonia autointoxication is a serious problem and can cause mass mortalities in fish farms. This study focused on the common carp *Cyprinus carpio* L. in large-capacity breeding farms. It was focused on monitoring the blood ammonia levels in fish blood in the period of metabolic attenuation and the influence of harvesting and handling of fish on the fish's ability to withstand such changes. The study results confirmed the effect of sudden changes in water temperature to values of ammonia in the blood of fish. On the contrary, there were no dramatically increased concentrations of ammonia in the blood of fish nor symptoms of autointoxication. The measured ammonia concentrations ranged between 98.3 ± 56µmol/L and 141.4 ± 31 µmol/L in the monitored period, which corresponds with the study results of other authors. This study has confirmed good technological conditions in the market production of carp after harvesting and a good level of adaptation process of the common carp *Cyprinus carpio* L. to these changes.

Keywords: ammonia, autointoxication, ureotelic, ammonotelic animals, storage ponds

INTRODUCTION

Ammonia is for vertebrate animals a highly toxic substance even in low concentrations. It is formed in the organism as the final product of catabolism of proteins and amino acids. Due to its toxicity and especially its neurotoxic properties it cannot be accumulated in the organism and must be eliminated in order to protect the brain and neurons. In case of mammals, the major detoxification means for ammonia is the ureo-synthetic cycle. Ammonia emerging from the intestinal microflora activity is transported to the liver by portal blood. By the ureo-synthetic cycle the ammonia is converted to urea, which is then secreted via the kidneys. In mammals is in this way detoxified approximately 80% of nitrogen formed by catabolism of proteins and amino acids (ureotellic). A small amount of nitrogen is excreted also in the form of uric acid, creatinine and ammonium ion **(Baynes and Dominiczak, 2005).** One way to protect mainly the brain and neurons from the toxic effects of ammonia is the synthesis of glutamine.

Fish are ammonotellic animals and unlike mammals they secrete the endogenous ammonia directly into the aquatic environment in which they live. In case of the common carp *Cyprinus carpio*, the absence of ureo-synthetic cycle results in the excretion of up to 92% of the nitrogen in the form of ammonia and approximately 8% of urea of the total nitrogen compounds. Approximately 88% of the total quantity of ammonia is excreted by gills and the remaining 4% by kidneys **(Wood, 1993).**

Balance between production and utilization of free ammonia is crucial for maintaining good health of fish. In case of teleosts, normally when exogenous ammonia concentration in the environment is low, the ammonia passes from the fish blood through the gills by passive diffusion into the water. If the fish moves in an environment with a high concentration of ammonia, the excretion of endogenous nitrogenous digestion waste may be disrupted or even stopped **(Cameron and Heisler, 1983).**

The balance between production and excretion of ammonia can be disrupted by various factors. Fish that is exhausted physically or by stress or starving fish increase the production of ammonia and is much more sensitive to exogenous ammonia in the environment **(Randall and Tsui, 2002)**.

Some fish species can tolerate even high concentrations of ammonia in the environment. Such fish have in their body a system which protects them from fluctuations of ammonia concentration in blood after feeding. The **Randal and Tsui** study **(2002)** shows that fed fish use a significant detoxification strategy of brain defense by formation of glutamine. In the

post-larval stage of rainbow trout, when cannot be applied detoxification through ornithine ureo-synthetic cycle, has been demonstrated a detoxifying mechanism through converse of ammonia and glutamate to glutamine by the enzyme glutamine synthetase **(Ip *et al*, 2001., Randall and Tsui, 2002).** In fish and mammals is the activity of glutamine synthetase much higher in the brain than in other tissues **(Wright *et al*., 2007).** Also other authors **(Cooper and Plum, 1987)** assume that the primary function of glutamine synthetase in the brain of mammals is a protective effect against toxic effects of ammonia. These assumptions confirm in fish also authors **Ip *et al*. (2001).** By formation of 1 mole of glutamine are detoxified 2 moles of ammonia. Inventories of glutamine in tissues may be in carnivorous fish according to these authors used as an oxidative substrate for return to its normal state.

Formation of glutamine is an important way of fish detoxification. Under certain circumstances, the fish organism may be threatened by products of its own metabolism. A serious condition is autointoxication by ammonia, which can even lead to massive mortalities occurring in fish farms.

High plasmatic concentrations of ammonia in this situation cause symptomatic changes that are similar to the symptoms of high external concentrations of ammonia. Concentration of ammonia may be increased in the blood plasma more than 5-10 times **(Svobodová *et al.*, 1997).** The autointoxication may occur after a sudden significant drop in water temperature, especially after feeding the fish **(Svobodová, 1970).**

Excretion of ammonia by gills may be considerably reduced due to their damage. Stress disrupts the osmo-regulatory functions in fish. Prolonged stress can lead to damage to gills at the cellular level and thus contributes significantly to autointoxication **(Mazeaud *et al*., 1977).**

Harvesting, any manipulation and transportation of the fish, sudden temperature decline and changes in the content of dissolved oxygen are among the main factors that can lead to the state of fish ammonia intoxication.

The aim of our study was to determine the objective conditions of common carp immediately after the harvesting and assessment of its adaptation to the change of environment after transfer to storage ponds.

MATERIAL AND METHODS

For the study has been selected the common carp (*Cyprinus carpio* L.) the Pohořelický lysec line of the market size, coming from the standard breeding conditions in the pond with a natural food source and additional feeding. For the study was chosen market size fish weighing 2.0 ± 0.3 kg. The fish was killed after previous deep stunning and the blood was collected by a heparinized needle. The blood sampling was situated in the autumn period, when due to the climatic conditions occurs the attenuation of fish metabolism and the fish gradually stops ingesting feed.

The blood sampling was scheduled so that we could record the period immediately before harvesting, the harvest and at regular intervals after the harvest. After harvesting the fish was transported in a standard way, maintaining the transport conditions for live fish and welfare conditions, and then moved to the storage ponds, where it remains until delivery to the consumer. The distance from the harvest location to the ponds is within 10 km. Water in hatcheries was intensively aerated by inflow of clean water.

The number of fish in the study was approximately the same in each sampling term. Blood was always collected from 12 - 13 pieces of common carp. In rare cases of blood clotting the sample was excluded from the analysis. The total numbers of analysed blood samples are shown (Tab 1) in the Results section.

The first blood collection of fish was carried out two weeks before the harvest, the second sampling took place after harvesting and transport on the first day of fish stay in storage ponds, the third and fourth blood taking was carried out at regular 2-week intervals after the previous sampling. All fish for the blood collection always came from the same hatcheries and were selected at random with regard to approximately comparable size.

To measure the concentrations of ammonia in the blood was selected and used the method of measuring device Blood Ammonia Meter, PocketChem BA (Japan) after calibration. The concentration of ammonia was measured in whole blood immediately after collection. The results of the concentration of ammonia in the blood in µmol/L were deducted with accuracy to 1.0 µmol/L. The fish blood analysis was carried out on all samples within 10 minutes after collection.

To evaluate the physico-chemical parameters of water the temperature data were recorded in °C and dissolved oxygen in mg/L.

RESULTS

The results of fish blood sampling were for the individual intervals averaged and the standard deviation was calculated. The numbers of fish included in the study and the number of analysed blood samples are given (Tab. 1).

Table 1 The number of blood samples taken on individual dates

Date	Quantity of fish	Blood samples
1.	12	11
2.	12	11
3.	13	12
4.	12	11

The results of the measured average concentration of ammonia in the fish blood from the individual sampling dates were within the range from 98.3 ± 56 µmol/L to 141.4 ± 31 µmol/L. On the first sampling date was the average concentration of ammonia in the fish blood 115.8 ± µmol/L (two weeks before harvesting), in the second term 104.3± 53.6 µmol/L (first day after harvesting), in the third term 141.4 ± 30.9 µmol/L (two weeks after harvesting) and in the fourth term 98.3 ± 56.2 µmol/L (four weeks after the harvest). The overview of ammonia concentrations in the fish blood on the individual sampling dates of is shown (Fig. 1)

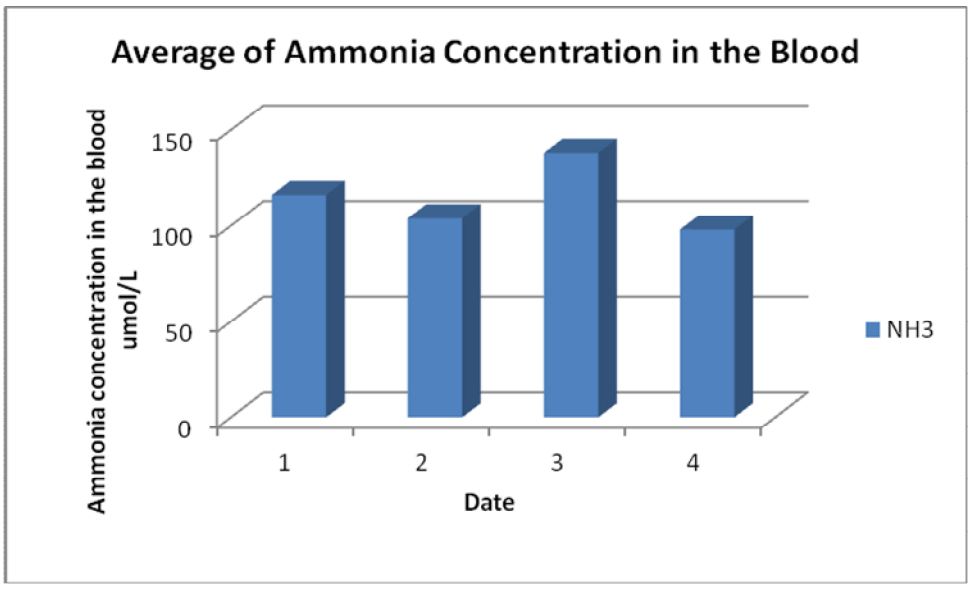

Figure 1 Concentration of ammonia in the fish blood measured on different date

During the sampling period were recorded the water temperature conditions in the storage ponds and dissolved oxygen in water. Figure (Fig 2) shows the overview of the water temperature parameters during the monitored period and at the same time the average values of the measured concentrations of ammonia in the fish blood.

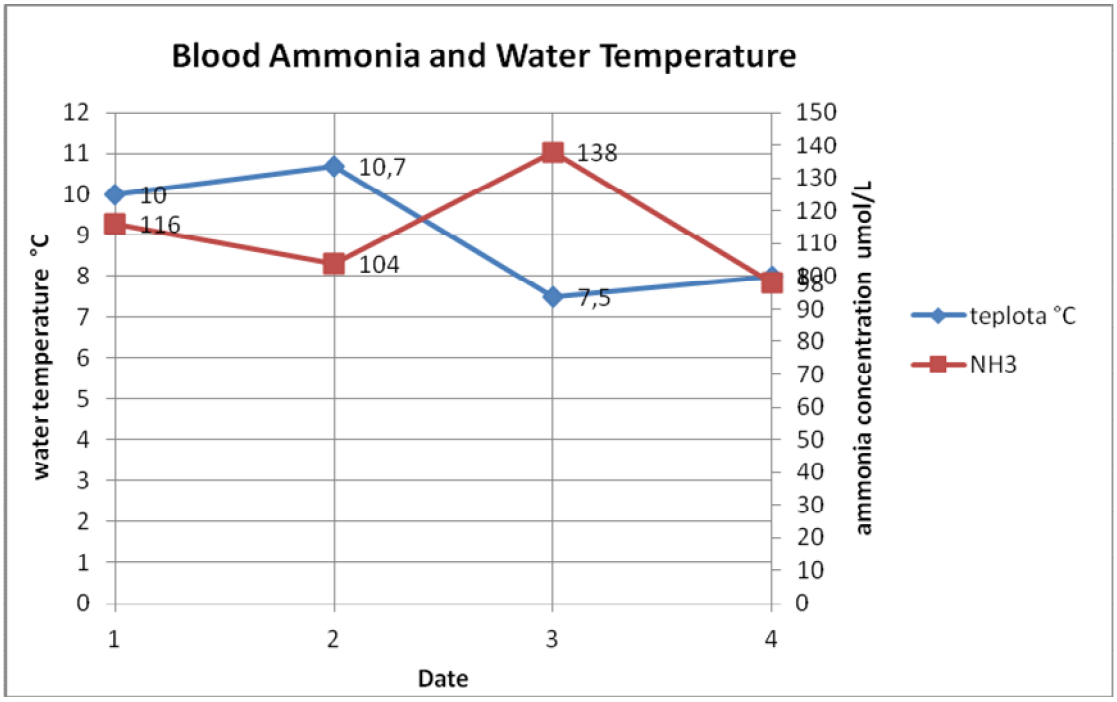

Figure 2 The progression of temperatures and concentrations of ammonia in the fish blood

Together with the thermal parameters was monitored also the dissolved oxygen in water. The concentration of dissolved oxygen in all sampling periods in water from ponds was within the range from 5.0 mg/L to 6.6 mg/l. Water from the breeding pond had the amount of dissolved oxygen at the time of harvest 3.7 mg/L.

The overview of values of dissolved oxygen in water is shown in table (Tab. 2).

Table 2 Concentration of dissolved oxygen

Date	Dissolved oxygen (mg/L)
1.	6,0
2.	6,6
3.	5,0
4.	5,0

Note: Pond at the time of harvest: **3,7 mg/L O_2**

To demonstrate the statistical significance of the increase in the concentration of ammonia in the fish blood depending on the sudden drop in water temperature was used the Mann-Whitney test. The temperature drop occurred in the period between the second and third term of blood collection. The increase in the concentration of ammonia in the blood of fish is statistically significant (Fig 3), ($P < 0.05$).

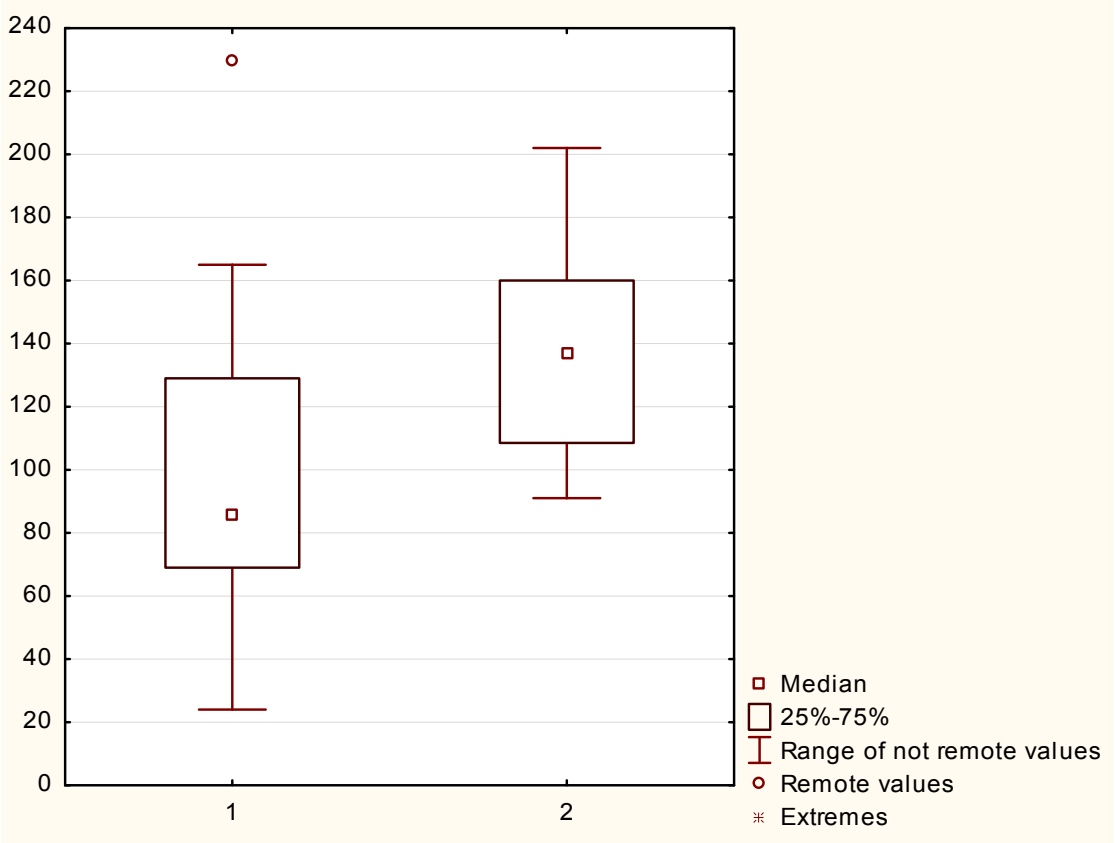

Figure 3 Ammonia concentration change in relation of temperature drop

Point 1 – ammonia concentration in 2-nd date with the temperature of water 10,7 °C. Point 2 – ammonia concentration in 3-th date with the temperature of water 7,5 °C. Significant differences are indicated (P< 0,05).

DISCUSSION

Our study was situated in a time period when fish is in metabolic attenuation with an empty or minimally filled digestive tract.

To measure the concentrations of ammonia in the fish blood was chosen a method that allows immediate determination of ammonia in whole blood with an accuracy of determination to 1 µmol/L. The accuracy of determination is sufficient, with regard to the optimal values of ammonia in the fish blood. Unlike other methods that are available (enzymatic

spectrophotometric determination of ammonia concentration in plasma - BioVendor), it is not necessary in our case, to conduct within 30 minutes after the blood collection the plasma separation, storage on ice, transport and analysis of samples in the laboratory within 3 hours since the blood collection. Such conditions would be hardly feasible in the field when taking multiple samples. Extension of this period or neglecting the conditions before analysis might result in inaccurate and distorted values of ammonia in the blood.

The concentration of ammonia in the blood of the common carp *Cyprinus carpio* L. from breeding to market production was low in the monitored period and ranged from 98.3 do µmol/L do 141.4 µmol/L. These values are in accordance with **Spurný (1998)**, where in the winter season, the concentration of ammonia in the fish blood occur in values 50 – 100 µmol/L. Fish in the winter season have subdued metabolism and do not ingest feed. Fish, especially stored in a pond, are kept after harvesting for approximately two months in storage ponds, where they also do not ingest feed. In this period they reduce weight and occur considerable hematologic changes as well as significant hypoproteinemia **(Svobodová *et al.*, 1997b)**. With the hypoproteinemia is also related decreased concentration of blood ammonia. This situation is also demonstrated by several other authors. **Wick and Randall (2002)** in their study demonstrate the effects of starvation and feeding on the concentration of ammonia in the blood of rainbow trout. Fish that is fed reports in experimental conditions an increase in plasma ammonia concentration 30 minutes after feeding. The increased levels of ammonia according to the study return to the normal state two hours after feeding, but after 8 hours since feeding is in the plasma noticeable the second maximum of these values. It can be assumed that the second increase in ammonia concentration 8 hours after feeding is caused by the higher physical activity during feeding. Authors **Wick and Randall (2002)** explain the second increase in ammonia concentration at the 8-hour interval after feeding in experimental conditions by a sudden change in lighting conditions, fright and stress responses of fish to removal of the covers of the experimental tank with water. The increased ammonia values thus correspond with an increase in metabolism necessary to obtain energy for swimming and start the stress response.

Authors **Wick and Randall (2002)** deal in their study with the question why the fed fish is more resistant to the effects of exogenous and endogenous ammonia than starving fish.

Wick and Randall (2002) write that the pathway, in which is the ammonia utilized, is the formation of amino acids of alanine, aspartate, glutamate and glutamine. All of these amino acids can thus serve as temporary storage of ammonia. In this way, the fish can be protected from the effects of ambient ammonia and autointoxication. These authors state that

the endogenous concentration ammonia in the blood of rainbow trout varies with food intake. However, increasing the endogenous ammonia does not increase its toxicity, provided the fish is at the same time exposed to increased exogenous ammonia in water. This protective effect is observed in fish only for the first 24 hours.

These conclusions are also confirmed by other authors **(Kolarevic *et al.*, 2012)**, who deal with the principle of molecular detoxification of fed fish and fish with restricted food intake. Their study has shown an increased transcription of transport proteins genes in fish exposed to sublethal concentrations of ammonia, and thereby increasing the transport and detoxification of ammonia by gills.

The results of these studies indicate how diet affects the increased production of endogenous ammonia on the one hand, and on the other hand the onset of processes that prevent the toxic effects of elevated levels of ammonia in the brain of fish. And confirmed are therefore also the results of our study on fish without food intake.

Water temperature and its sharp decrease is a threatening factor for fish autointoxication. According to **Svobodová (1970)** and **Spurný (1998)**, is a sudden change in water temperature of 2-5°C dangerous. It may occur after the harvest by the transport to storage ponds, which are supplied from a source of water of lower temperature **(Svobodová, 1997b)**.

Our study was carried out in the period when fish has empty digestive tract and their endogenous ammonia production is therefore not affected by food. Our results confirmed a significant decrease in water temperature in the storage pond in the period between the second and third sampling date, which resulted in a statistically significant increase in the concentration of ammonia in the blood of fish. This increase was within the range of normal values of ammonia in freshwater fish in the winter season (**Spurný, 1998, Svobodová, 1997b)** and the fish was not endangered. These conclusions are also confirmed by other studies **(Lemly, 1996)** in fish during the winter months.

A part of our study was also the collected data on the concentration of dissolved oxygen in the water. The content of dissolved oxygen is according to the authors **Svobodová (1970)** and **Svobodová *et al.* (1997b)** in case of cyprinid fish optimally within the range of 6 - 8 mg O_2/L. After a decrease under 2 mg/L are in fish already noticeable signs of suffocation. The content of dissolved oxygen in the water in the storage ponds was during the monitored period on the lower limit of the range recommended by experts.

The interval from the beginning of storing the fish in ponds and the period of carp starvation did not confirm a significant effect on the change in ammonia concentration in

blood. The only increase in the concentration of ammonia in the blood of fish in the monitored period was observed in connection with a greater decrease in water temperature in the storage pond.

CONCLUSION

Our study did not demonstrate any significantly increased concentrations of ammonia after harvesting and removal of fish into the storage ponds. The increase in the concentration of ammonia in the blood in the middle of the monitored period was associated with a significant change in climate conditions by cooling and reduction of water temperature in storage ponds. The increase in ammonia concentration was not harmful to fish and did not endanger its health.

This study has confirmed a good level of adaptation of fish to harvesting and transport to storage ponds without a significant increase in concentrations of ammonia in the blood. The study thus confirmed also the correct procedures and careful treatment of fish during manipulation and transport to storage ponds in accordance with the welfare rules.

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REFERENCES

BAYNES, W. J. – DOMINICZAK, M. H. 2005. Medical Biochemistry. Elsevier-Mosby, 2nd edition. 693 pp.

CAMERON, J. N. – HEISLER, N. 1983. Studies of Ammonia in the Rainbow Trout:Physico-chemical Parameters, Acid –Base Behaviours and Respiratory Clearance. In *Journal of Experimental Biology,* vol. 105, p. 107-125.

COOPER, A. J. – PLUM, F. 1987. Biochemistry and physiology of brain ammonia. In P*hysiology Review*, vol. 67, p. 440-519.

IP, Y. K. – CHEW, S. F. – RANDALL, D. J. 2001. Ammonia toxicity, tolerance, and excretion. In *Fish Physiology*, vol. 20, pp. 109-148.

KOLAREVIC, J. – TAKLE, H. – FELIP, O. – YTTEBORG, E. – SELSET, R. – GOOD, CH., BAEVERFJORD, G. – ASGARD, T. – TERJESEN,B. 2012. Molecular and physiological

responses to long sublethal amonia exposure in Atlantic salmo (*Salmo salar*). In *Aquatic Toxicology*, vol. 124-125, p. 48-57.

LEMLY, A.D. 1996. Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. In *Ecotoxicology and Environmental Safety,* vol. 34, 223-227.

MAZEAUD, MM. – MAZEAUD, F. – DONALDSON, EM. 1977. Primary and secondary effects of stress in fish: some new data with a general review. In *Transactions of the American Fisheries Society,* vol. 106, p. 201-212

RANDALL,D. J. – TSUI, T. K. N. 2002. Ammonia toxicity in fish. In *Marine Pollution Bulletin,* vol. 45, 2002, Issues 1-12, p. 17-23.

SANDERSON, L. A. – WRIGHT, P. A. – ROBINSON, J. W. – BALLANTYNE, J. S. – BERNIER, N. J. 2010: Inhibition of glutamine synthetase during ammonia exposure in rainbow trout indicates a high reserve capacity to prevent brain ammonia toxicity. In *Journal of Experimental Biology,* 2010, vol. 213, p. 2343-2353.

SPURNÝ, P. 1998. Ichthyology. MUAF, Brno, p. 138 (in Czech)

SPURNÝ, P. – MAREŠ, J. 1997. Adverse effects of environmental factors on fis organism. In *Bulletin of RIFCH,* USB Vodňany, p. 159-165. (in Czech*)*

SVOBODOVÁ, Z. 1970. The level of N-ammonia and urea in the blood serum and brain of healthy and ammonia –intoxicated carp (*Cyprinus carpio* L.). In *Bulletin,* RIFCH Vodňany, p. 113-118. (in Czech)

SVOBODOVÁ, Z. – GROCH, L. – MÁCHOVÁ, J. – FAINA, R. – VYKUSOVÁ, B. – SMOLÍKOVÁ, B. 1997a. Accidental kills of common carp due to ammonium autointoxication. In Kolářová et al. *Health protection of fish. Bulletin RI Vodňany*, p. 185-193 (in Czech).

SVOBODOVÁ, Z. – KOLÁŘOVÁ, J. – VYKUSOVÁ, B. 1997b. Stress at fish generated by conventional breeding technologies. In *Animal Protection and Welfare,* VFU Brno, p. 60-64.

WOOD, C. M. 1993. Ammonia and urea metabolism and excretion. In *Physiology of fish.* CRC Press, p. 379 – 425.

WICKS, B. J. – RANDALL, D. J. 2002. The effect of feeding and fasting on amonia toxicity in juvenile rainbow trout, *Oncorhynchus mykiss.* In *Aquatic Toxicology,* vol. 59, p. 71-82.

WRIGHT, P. A. – STEELE, S. L. – HUITEMA, A. – BERNIER, N. J. 2007. Induction of four glutamine synthetase genes in brain of rainbow trout in response to elevated environmental ammonia. In *J. Exp. Biol*. vol. 210, p. 2905-2911.