J. Elementol. 2010, 15(2): 239–250

ORIGINAL PAPERS

EFFECT OF CULTURE CONDITIONS ON MAGNESIUM AND ZINC CONCENTRATIONS IN MUSCLES OF FRESHWATER FISH*

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Abstract

The aim of the study was to estimate the effect of culture conditions and culture site on magnesium (Mg) and zinc (Zn) concentrations in freshwater fish. The study encompassed dorsal muscles in five fish species: common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walbaum) and Siberian sturgeon (*Acipenser baeri* Brandt), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes).

A total of 125 fish comprised 25 individuals of each species, aged from 6, 9, and 12 months. The fish were cultured in privately owned fish breeding ponds (Western Pomerania, Poland). For chemical and biochemical assays, samples of dorsal muscles were taken from each fish. Tissue samples were wet mineralised in concentrated HNO_3 in a CEM MDS 2000 microwave oven. Magnesium and zinc concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS) in a Jobin Yvon type JY-24 apparatus. The pursuit of the research we had an approval of the Polish Local Ethics Committee nr 9/05. The magnesium concentration in the dorsal muscles ranged from 95.3÷347.6 mg kg^{-1} w.w. The highest Mg concentration was found in rainbow trout

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*The study has been financially supported by the State Committee for Scientific Research under grant no. N 304 026 31/0814.

(347.6±32.2 mg kg^{-1} w.w.), and the lowest in grass carp (95.3±11.3 mg kg^{-1} w.w.). Zinc concentration varied from 6.7÷98.8 mg kg^{-1} w.w. The highest was found in the muscles of Siberian sturgeon (98.8±0.4 mg kg^{-1} w.w.), and the lowest in rainbow trout (6.7±0.7 mg kg^{-1} w.w.). It was found that the breeding place significantly affected the Zn and Mg concentrations in the muscle tissue among individual freshwater fish species. The magnesium and zinc concentrations were also significantly affected by the type of feed.

Key words: common carp, rainbow trout, Siberian sturgeon, northern, grass carp, zinc, magnesium.

WPŁYW WARUNKÓW HODOWLI NA POZIOM MAGNEZU I CYNKU W MIĘŚNIACH RYB SŁODKOWODNYCH

Abstrakt

Celem pracy była ocena wpływu warunków i miejsca hodowli na zawartość magnezu (Mg) i cynku (Zn) w organizmach ryb słodkowodnych. Ocenie poddano mięśnie grzbietowe pobrane od pięciu wybranych gatunków ryb słodkowodnych: pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum), karpia (*Cyprinus carpio* L.), jesiotra syberyjskiego (*Acipenser baeri* Brandt), szczupaka pospolitego (*Esox lucius* L.) i amura białego (*Ctenopharyngodon idella* Valenciennes).

Badaniami objęto ogółem 125 ryb słodkowodnych, po 25 osobników z każdego gatunku. Materiał do badań pobrano w 6., 9. i 12. miesiącu życia ryb. Z każdej ryby do analiz chemicznych pobrano próbki mięśni grzbietowych. Pobrany materiał zmineralizowano na mokro w stężonym HNO_3 w piecu mikrofalowym CEM MDS 2000. Próbki poddano analizie na zawartość Mg i Zn z użyciem emisyjnej spektrometrii atomowej w plazmie indukcyjnie sprzężonej (ICP-AES) w aparacie Jobin Yvon typu JY-24. Na powyższe badania uzyskano zgodę Lokalnej Komisji Etycznej nr 9/05.

Zawartość Mg w mięśniach mieściła się w przedziale 95.3÷347.6 mg kg^{-1} m.m. Najwyższy poziom Mg odnotowano u pstrąga (347.6±32.2 mg kg^{-1} m.m.), a najniższy u amura (95.3±11.3 mg kg^{-1} m.m.). Zawartość Zn mieściła się w przedziale 6.7÷98.8 mg kg^{-1} m.m. Najwyższy poziom Zn odnotowano w mięśniach jesiotra syberyjskiego (98.8±0.4 mg kg^{-1} m.m.), a najniższy u pstrąga (6.7±0.7 mg kg^{-1} m.m.). Wykazano, że miejsce hodowli ma statystycznie istotny wpływ na zawartości magnezu i cynku w tkance mięśniowej poszczególnych gatunków ryb słodkowodnych. Na poziom magnezu i cynku w tkance mięśniowej istotne znaczenie miał również wpływ rodzaj spożywanego pokarmu.

Słowa kluczowe: karp, pstrąg tęczowy, jesiotr syberyjski, szczupak, amur biały, cynk, magnez.

INTRODUCTION

Breeding conditions, including feeding regime, have a significant effect on the development of fish. In the growth and development of both terrestrial and aquatic animals, culture conditions play one of the keys roles. In the natural environment, there are many various chemicals but most of them, despite being in direct contact with live organisms do not penetrate inside them in significant amounts. Due to human actions, we can observe an increasing man-made impact on the environment. It distrubs the homeostasis of aquatic environments, which may lead to some disturbance of the balance between fish.

During the evolution, countless relationships have been developed between organisms and their environment. When those relationships are disrupted by altered environmental conditions, diseases or even death of an organism may occur. Elements of the environment such as water, air and food provide organisms with essential components, but at the same time they may be sources of xenobiotics and harmful substances which are capable of causing impairment impair of life functions in organisms. Human activity exerts increasing pressure on the environment, which results in elevated pollution levels in aquatic and terrestrial ecosystems. By living in aquatic environment, fish are particularly exposed to anthropogenic pressure.

Monitoring micro and macro-elements levels in fish is a significant diagnostic research as it shows the physiological condition of organisms. Concentration of some mineral elements in fish depends mainly on the culture and water type in which the fish are bred, as well as the season of the year and the pastures the fish are fed. All the elements affect the homeostatic behavior of fish, which may vary due to some excess or deficiency of any of the factors. Excessive or deficient value of a factor may lead to serious disorders in biochemical processes, which may result in many diseases.

The aim of this study was to evaluate the effect of culture conditions and culture site on levels of a microelement (Zn) and macroelement (Mg) in five species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), Siberian sturgeon (*Acipenser baeri* Brandt), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes). The study encompassed dorsal muscles in fish.

MATERIAL AND METHODS

The study involved 125 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 25 individuals of each of the five species: rainbow trout, common carp, Siberian sturgeon, northern pike and grass carp. The the research had been approved by the Polish Local Ethics Committee nr 9/05.

The fish aged from 6 to 12 months, weighed from 176.5 to 615.4 g and measured from 20.2 to 35.7 cm. The fish were collected three times: in December, April and August.

The fish were fed Aller Aqua pelleted feeds (Table 1), each species with an appropriate feed type, and a mixture of oats and rape. All the fish feed products are produced by extrusion. Fish feed must cover the basic metabo-

Table 1

Composition of Aller Aqua pasture for individual freshwater fish

Researched parameters of feed	Examined fish		
	common carp	rainbow trout	Siberian sturgeon
Name of feed	Aller classic	Aller 576	Aller M/L
Largeness of feed (mm)	5.0	M/L	M/L
Protein (%)	35.0	42.0	45.0
Fat (%)	9.0	30.0	15.0
Carbohydrates (%)	43.0	14.0	21.0
Ash (%)	7.0	7.5	8.0
Fiber (%)	5.0	1.0	2.5
All-out energy (Gross energy) (Kcal/MJ)	4325/18.1	5823/2.3	4924/20.5
Energy digestible (Kcal/MJ)	3353/14.0	4833/20.2	38887/16.2
Nitrogen (d.m. %)	5.2	7.1	7.9
Phosphorus (d.m. %)	1.3	7.0	1.2
Energy in dry matter (Kcal/MJ)	4701/19.6	6162/25.7	5381/022.5

lism of fish and ensure healthy growth. In order to meet these requirements, the fish feed composition must meet all needs for nutrients, vitamins (A, E and D_3) and minerals. The daily food ration was 3.4±0.8 g per fish. The fish were fed twice a day.

Table 2 presents chemical and biochemical parameters of water in which the fish were kept.

The fish behaviour and appearance were recorded. Intravital examination involved observation of fish behaviour, assessment of rearing conditions, as well as evaluation of the quality and general appearance of fish skin, fins, eyes and gills. *Post mortem* examination involved autopsy to verify if there were any anatomical or pathological changes in internal organs.

For chemical analysis, samples of dorsal muscles were collected from each fish. The collected material was stored at -20 °C. Prior to analysis, 1-g subsamples of organs, weighed to the nearest 0.001 g, were wet mineralized in 3 cm^3 HNO_3 in a CEM MDS 2000 microwave oven. The solutions were quantitatively transferred to polyethylene vials and brought up to 25 g with deionised water. Magnesium (Mg) and zinc (Zn) were determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals were reported as mg kg^{-1} wet weight (mg kg^{-1} w.w.).

Table 2

Hydrochemical parameters from a culture site 20 km off Szczecin

Water parameters	December	April	August	Statistically significant $P \leq 0.05$
	month of fish life			
	6	9	12	
	mean ± SD	mean ± SD	mean ± SD	
Temperature (°C)	3.20 ± 2.50	6.80 ± 3.40	13.80 ± 3.40	*A*
pH	7.78 ± 0.55	7.38 ± 0.95	7.48 ± 0.95	-
Dissolved oxygen (mg dm^{-3})	7.81 ± 0.35	7.98 ± 0.63	7.94 ± 0.55	-
Oxygen saturation (%)	*78.21 ± 2.50*	79.51 ± 3.48	78.79 ± 4.28	-
Alkalinity (mmol dm^{-3})	1.75 ± 0.80	1.69 ± 0.88	1.65 ± 0.68	-
Water hardness (mg dm^{-3})	8.25 ± 1.08	7.25 ± 1.18	7.75 ± 1.58	*A*
ChZT/ChOD (mg dm^{-3})	1.67 ± 1.32	1.71 ± 1.09	1.66 ± 1.12	-
BZTBOD (mg dm^{-3})	4.34 ± 1.22	4.61 ± 1.33	4.81 ± 1.32	*A*
N-NH_4 (mg dm^{-3})	1.18 ± 0.75	1.34 ± 0.48	1.26 ± 0.29	*A*
N-NO_3 (mg dm^{-3})	7.41± 1.05	6.11± 1.15	6.78± 0.55	*A*
N-NO_2 (mg dm^{-3})	0.68 ± 0.16	0.48 ± 0.36	0.56 ± 0.26	*A*
P-PO_4^3 (mg dm^{-3})	0.15 ± 0.07	0.14 ± 0.05	0.13 ± 0.03	-
Ca (mg dm^{-3})	66.51 ± 4.25	59.51 ± 3.75	69.31 ± 2.79	*A*
Cd (mg dm^{-3})	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	-
Pb (mg dm^{-3})	0.03 ± 0.05	0.03 ± 0.07	0.03 ± 0.07	-
Mg (mg dm^{-3})	*16.33 ± 4.05*	15.23 ± 3.33	17.44 ± 4.47	*A*

*A – statistically significant differences (ANOVA, test Duncan) in the water parameter among the three culture sites ($P \leq 0.05$)

The results were subjected to statistical treatment with the Statistica 6.0 software. Analyses of variance (ANOVA) were performed at the significance level of $P = 0.05$.

RESULTS AND DISCUSSION

Increasing industrial and agricultural production has resulted in a rising number of systems affected by contaminants present in discharged wastewater. For example, heavy metals (e.g. Cu, Zn) are known to accumulate in organs of fish (BÁLINT et al. 1997). These metals pollute aquatic and terrestrial ecosystems, adversely affecting the environment and inhabiting organisms. High concentration of metals in fish tissues can lead to redox reaction, generating free radicals, especially reactive oxygen species (DAUTREMEPUITS et al. 2002). These highly reactive compounds may induce tissue alternations and change some physiological responses of fish (PARIS-PALACIOS et al. 2000, VARANKA et al. 2001). Aquatic organisms are more sensitive to exposure and toxicity compared to terrestrial organisms, including mammals, and in this respect they may provide experimental data for evaluation of subtle effects of oxidative stress, mutagenicity and other adverse effects of pollutants (VALAVANIDIS et al. 2006). The influence of high temperature on aquatic biocenoses manifests in an increase in the biological production rate and also in the shortening of lifecycles of aquatic organisms, which die in large numbers due to lack of synchronization with climate rhythms. This results in accumulation of organic matter and increased biological oxygen demand, along with a decrease in oxygen solubility and availability.

Nutritional studies have shown that minerals may play a crucial role in preventing oxidative stress. Increases or decreases in their concentrations may disrupt internal homeostasis and produce various pathological conditions. Toxic effects of metals on different tissues and organs involve structural damage and functional disorders, which may be reflected in changes in blood composition and levels of ions, proteins, hormones or glucose and its metabolites, as well as in changed enzyme activities.

Intravital and *post mortem* examination showed no changes in fish behaviour, as well as in their external and internal appearance. Comparison of water parameters (Table 2) revealed only slight differences among the five fish culture sites.

The research has shown that Mg and Zn concentrations have changed during the growth of fish. An average magnesium content ranged from 95.3 to 347.6 mg kg^{-1} w.w. (Table 3). The highest magnesium levels were detect-

Table 3

Mg level in dorsal muscles of five freshwater fish species in 6, 9, and 12 months

Fish research	Mg (mg kg^{-1} w.w.)			Statistically significant differences $P \leq 0.05$
	month of fish life			
	6	9	12	
	mean ± SD	mean ± SD	mean ± SD	
Common carp	103.6 ± 13.6	168.6 ± 35.7	147.6 ± 18.2	*A*
Rainbow trout	287.6 ±42.5	321.6 ± 28.9	347.6 ± 32.2	*A*
Siberian sturgeon	127.6 ± 27.2	134.6 ± 16.8	143.8 ± 21.2	ns
Northern pike	95.3 ± 11.3	98.7 ± 5.6	103.3 ± 7.3	ns
Grass carp	95.3 ± 11.3	96.7 ± 5.6	98.3 ± 13.3	ns

*w.w. – wet weight, SD – standard deviation; *A* – statistically significant differences $P \leq 0.05$; ns – no significant differences ed in dorsal muscles of rainbow trout (347.6±32.2 mg kg^{-1} w.w.). The lowest magnesium levels were found in dorsal muscles of grass carp (95.3±11.3 mg kg^{-1} w.w.). An average zinc content ranged from 6.7÷98.8 mg kg^{-1} w.w. (Table 4). The highest zinc levels were detected in dorsal muscles of Siberian sturgeon (98.8±0.4 mg kg^{-1} w.w.). The lowest zinc levels were found in dorsal muscles of rainbow trout (6.7±0.7 mg kg^{-1} w.w.). It was found that the breeding site significantly affected the Zn and Mg concentrations in muscle tissue among individual freshwater fish species. The magnesium and zinc concentrations were also significantly influenced by the type of feed. It was noticed that in the fish (common carp, Siberian sturgeon and rainbow trout) fed Aller Aqua pellet pasture, the Zn and Mg concentrations were higher compared to the fish (northern pike and grass carp) fed oat and oilseed rape blend. We have also found that the Mg concentration in all the examined fish was higher than the Zn concentration. The results allow us to

Table 4

Zn level in dorsal muscles of five freshwater fish species in 6, 9, and 12 months

Fish research	Zn (mg kg^{-1} w.w.)			Statistically significant differences $P \leq 0.05$
	month of fish life			
	6	9	12	
	mean ± SD	mean ± SD	mean ± SD	
Common carp	25.3 ± 5.6	26.7 ±3.9	35.3 ± 3.9	*A*
Rainbow trout	6.7 ± 0.7	19.7 ± 5.3	23.1 ± 3.6	*A*
Siberian sturgeon	93.7 ± 5.6	98.8 ± 0.4	98.6± 4.6	ns
Northern pike	37.1 ± 5.6	41.2 ± 5.6	45.8 ± 5.6	A
Grass carp	20.7 ± 3.9	20.1 ± 2.6	21.1 ± 4.6	ns

*w.w. – wet weight, SD – standard deviation; *A* – statistically significant differences $P \leq 0.05$; ns – no significant differences

state that the breeding site, breeding conditions and the feeding type have a significant effect on the Mg and Zn levels in muscle tissue of the examined fish.

Magnesium was distributed in fish bodies according to the following pattern of decreasing concentrations: grass carp > northern pike > common carp > Siberian sturgeon > rainbow trout. Zinc was distributed in fish bodies according to the following pattern of decreasing concentrations: rainbow trout > grass carp > common carp > northern pike > Siberian sturgeon. Many authors have reported considerably higher levels of these elements in muscles of freshwater and marine fish (PUJIN et al. 1990, KARGIN 1996, GROSHEVA et al. 2000, JURKIEWICZ-KARNAKOWSKA 2001).

Zinc is weakly accumulated in fish tissues, as it is retained in the gills, where the metal is deposited in large amounts (WITESKA 2003). This may be explained by the fact that it penetrates into blood less easily than other metals (cadmium, nickel). In turn, changes in zinc levels in the examined tissues resulted from its affinity to erythrocyte membranes (BARRON, ADELMAN 1984) and serum proteins (BETTGER et al. 1987) that participate in its transport. Zinc is transported mainly as zinc-albumin and zinc-macroglobulin complexes, and is excreted mainly in the faeces (70-80%). Zinc displays low toxicity to freshwater fish. Its adverse influence is mainly connected with secondary deficit of copper and does not produce any specific symptoms. Zinc absorption by animals is influenced by food quality and interactions among zinc and other elements. A metabolically significant antagonism occurs between zinc and cadmium, as well as between Zn and Cu. Additionally, calcium and magnesium may reduce zinc absorption.

Levels of some bioelements in fish bodies depend on culture methods, water chemistry, and season of the year and feed quality. All these factors together influence the physiological condition of fish, which can be disturbed by excess or deficiency of minerals. Excess or deficiency of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading, in consequence, to various diseases. TACON (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of micro- and macroelements which were caused by improper nutrition, avitaminosis or poisoning. It is therefore important to monitor levels of macro- and microelements in fish organisms.

Among the examined freshwater fish species, statistically significant differences in the levels of macro- and microelements were observed. The analyzed bioelements (Zn, Mg), which are regarded as some of the most important macro- and microelements, were reported to accumulate in excess in disease conditions caused by bacterial and viral infections, as well as during an increased activity of hepatocytes (POURAMAHAD, O'BRIEN 2000, LUSHCHAK et al. 2005). Levels of microelements recorded in this study were not high (Tables 3, 4) and remained within the normal range for salmonids (*Salmonidae*) and cyprinids (*Cyprinidae*). For the sturgeon family (*Acipenseridae*), an accurate normal range of macro- and microelements has not been determined.

Magnesium is the 11th most abundant element by mass in the vertebrata body. Its ions are essential to all living cells, where they play a major role in manipulating important biological polyphosphate compounds like ATP, DNA, and RNA. Over 300 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing ATP, or those which use other nucleotides to synthesize DNA and RNA. Normally, ATP exists in cells as a chelate of ATP and a magnesium ion. Magnesium plays a regulatory role in prooxidant and antioxidant processes (LOPEZ-TORES et al. 1993, OZMEN et al. 2004). In our research, we have observed that magnesium concentration in fish muscles increased with the age of fish. We have also found statistically significant differences between dorsal muscles during the growth of rainbow trout and Siberian sturgeon. OIKARI et al. (1985) have shown that infusion of magnesium salt into the body cavity of a freshwater-adapted fish (rainbow trout) affects the magnesium concentration in the plasma. Magnesium could either be reabsorbed or secreted in control freshwater-adapted trout, apparently as a function of nutritional status. Fish could switch from reabsorption to secretion in response to magnesium loading. It is suggested that freshwater fish eliminate excess dietary magnesium renally (OKARI et al. 1985). Variability of metal concentrations in freshwater fish must be seen in the wide perspective of other variables such as habitat, seasonal variations, age of fish, Fulton's condition factor and individual ability for metal uptake (ALLEN 1993, CANLI, ATLI 2003, LOPEZ-TORES et al. 1993, MARTINEZ-ALVAREZ et al. 2005, RITOLA et al. 2002, SVOBODOVA et al. 1997).

Zinc has both catalytic and structural roles in enzymes and its antioxidant properties have been widely recognized (POWELL 2000). However, zinc deficiency was reported not to induce hepatic degeneration in trout liver (OGINO, YANG 1978). Anyway, HIDALGO et al. (2002) have demonstrated that dietary Zn deficiency induced oxidative stress in rainbow trout liver, with changes in the SOD band pattern. In the fish examined hereby, zinc levels remained within the physiological reference range reported by PROTASOWICKI and CHODYNIECKI (1988). Zinc is accumulated in fish tissues to a small extent only, as most of the element is absorbed by gills, where it accumulates in considerable amounts. Zinc levels similar to the ones recorded in this study were reported by YOSHITOMI et al. (1998) and AREECHON and PLUMB (1990). Statistically significant differences were found in the zinc concentration during the growth of northern pike, common carp and rainbow trout

Fish are characterized by species and seasonal changeability of micro- and macroelements. SOPIŃSKA (1985) and STOSIK and DEPTUŁA (2000) found changes in zinc and magnesium concentrations due to the season of the year and the change of lymphocytes level in the examined fish. According to these authors, such changes resulted form a close relationship of the season and the sun exposure. In many other studies, it has been shown that Mg and Zn levels were different depending on the temperature, season, sex, feeding type and culture type. (BLAXHALL 1972, THOMAS et al. 1999).

We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected concentration of the analyzed elements.

The results have shown that the content of the analyzed elements was within the physiological reference ranges for fish. The differences found in the levels of the bio-elements resulted from individual and seasonal variability typical of the fish. Owing to their environmental requirements, fish may be regarded as indicators that supply information on the degree of pollution of aquatic environments.

CONCLUSIONS

1. The culture site was found to have statistically significant influence on the magnesium and zinc concentrations in muscle tissue in the examined freshwater fish species.

2. We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected concentrations of the analyzed elements.

3. The differences in concentrations of the elements are a result of individual differences between species.

4. Zn and Mg concentrations in muscle tissue of the examined freshwater fish were significantly influenced by the experimental factors.

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