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LEVELS OF MICROELEMENTS (Cu, Zn, Fe) AND MACROELEMENTS (Mg, Ca) IN FRESHWATER FISH*

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Abstract

The paper evaluates the effect of culture conditions and culture site on levels of certain microelements (Zn, Cu, Fe) and macroelements (Mg, Ca) in three species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), and Siberian sturgeon (*Acipenser baeri* Brandt).

The study involved 90 individuals of freshwater fish aged from 6 to 12 months. Samples of blood, liver, kidney, gills, skin and muscles were collected from each fish and subjected to chemical assay of Mg, Ca, Zn, Cu, Fe with inductively coupled plasma-atomic emission spectrometry in a JY-24 Jobin Yvon apparatus.

The study revealed that culture site had statistically significant impact on levels of the examined elements among the three fish species. Mg content in kidney and skin was significantly higher in carp than in sturgeon. Similar regularities were observed for Ca content in skin and Zn content in kidney. Liver and kidney levels of Fe and Cu were significantly lower in carp than in rainbow trout. Of all the three examined fish species, rainbow trout had the highest skin levels of Ca and Mg, and the highest blood level of Fe.

The results indicate that culture site and culture conditions exerted significant influence on levels of macro- and microelements in freshwater fish.

Key words: freshwater fish, *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, *Acipenser baeri* Brandt, macroelements, microelements.

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OCENA ZAWARTOŚCI MIKROELEMENTÓW (Cu, Zn, Fe) I MAKROELEMENTÓW (Mg, Ca) U RYB SŁODKOWODNYCH

Abstrakt

Celem pracy była ocena wpływu warunków i miejsca prowadzenia hodowli na zawartość mikroelementów (Zn, Cu, Fe) i makroelementów (Mg, Ca) w organizmach ryb słodkowodnych. Ocenie poddano trzy wybrane gatunki ryb: pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum), karpia (*Cyprinus carpio* L.) i jesiotra syberyjskiego (*Acipenser baeri* Brandt).

Badaniu poddano 90 ryb słodkowodnych w okresie od 6. do 12. miesiąca życia. Z każdej ryby do analiz chemicznych pobrano próbki: krwi, wątroby, nerek, skrzeli, skóry i mięśni. Próbki poddano analizie na zawartość Mg, Ca, Zn, Cu, Fe, z użyciem emisyjnej spektrometrii atomowej w plazmie indukcyjnie sprzężonej (ICP-AES), w aparacie Jobin Yvon typ JY-24.

Wykazano, że miejsce hodowli ma statystycznie istotny wpływ na zawartości badanych pierwiastków u poszczególnych gatunków ryb słodkowodnych. Zawartość Mg w nerce i skórze karpi była istotnie wyższa niż u jesiotra. Podobne spostrzeżenia dotyczyły poziomu Ca w skórze i Zn w nerce. W przypadku Fe i Cu obserwowano niższą zawartość w wątrobie i nerkach karpi niż w tych samych narządach u pstrąga. Badania wykazały, że poziom Zn i Cu we wszystkich narządach przebadanych gatunków kształtował się na najniższym poziomie. Spośród badanych gatunków u pstrąga stwierdzono najwyższą zawartość Ca i Mg w skórze, a Fe we krwi.

Otrzymane wyniki pozwalają stwierdzić, że miejsce i warunki prowadzenia hodowli mają istotny wpływ na zawartość badanych makro- i mikroelementów w organizmach ryb słodkowodnych.

Słowa kluczowe: ryby słodkowodne, *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, *Acipenser baeri* Brandt, makroelementy, mikroelementy.

INTRODUCTION

In the growth and development of both terrestrial and aquatic animals, culture conditions play one of key roles. In the natural environment, many various chemicals occur. Most of them, however, do not penetrate in significant amounts into organisms despite being in direct contact with them. Organisms are dependent on the environment in which they live. During the evolution, countless relationships have been developed between organisms and their environment. When those relationships are disrupted by changed environmental conditions, diseases or even death of an organism may occur (JARA, CHODYNIECKI 1999).

Elements of the environment such as water, air and food deliver essential components for organisms, but at the same time they may be sources of xenobiotic and harmful substances that are able to impair life functions of organisms. Human activity exerts increasing pressure on the environment, which results in elevated pollution levels in aquatic and terrestrial ecosystems. Fish, living in the aquatic environment, are particularly exposed to anthropogenic impacts.

The aim of this study was to evaluate the effect of culture conditions and culture site on levels of certain microelements (Zn, Cu, Fe) and macroelements (Mg, Ca) in three species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.) and Siberian sturgeon (*Acipenser baeri* Brandt).

MATERIALS AND METHODS

The study involved 90 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 30 individuals of each of the three species: rainbow trout, common carp and Siberian sturgeon, aged from 6 to 12 months, weighing from 189.5 to 315.4 g and measuring from 21.7 to 31.5 cm. The fish were fed Aller Aqua pelleted feeds (each species with an appropriate feed type). Table 1 presents chemical and biochemical parameters of water in which the fish were kept. 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 blood, liver, kidney, gills, skin and 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 mineralized wet in 3 cm^3 HNO_3 in a CEM MDS 2000 microwave oven. The solutions obtained were quantitatively transferred to polyethylene vials and brought up to 30 g with deionised water. Mg, Ca, Zn, Cu, Fe were determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals have been reported as mg kg^{-1} wet weight (mg kg^{-1} w.w.).

The results obtained were subjected to statistical treatment with the Statistica 6.0 software. Analyses of variance (ANOVA) was performed at the significance levels of P = 0.05 and P = 0.01.

RESULTS AND DISCUSSION

Intravital and *post mortem* examination showed no changes in fish behaviour or in their external and internal appearance. Comparison of water parameters (Table 1) revealed only slight differences among the three fish culture sites.

Table 1

Hydrochemical parameters from groups I and II culture sites in the direct vicinity of the Dolna Odra Power Station and from group III culture site 60-km distant from the power station

Water parameters	I	II	III	Statistically significant differences $P \leq 0.05$
	mean ± SD	mean ± SD	mean ± SD	
Temperature (°C)	14.80 ± 4.50	13.80 ± 3.40	13.20 ± 3.10	A
pH	7.88 ± 0.55	7.48 ± 0.95	7.61 ± 0.55	-
Dissolved oxygen (mg l^{-1})	7.81 ± 0.35	7.94 ± 0.55	7.44 ± 0.85	-
Oxygen saturation (%)	78.21 ± 2.50	79.51 ± 3.48	77.51 ± 3.148	-
Alkalinity (mmol l^{-1})	1.78 ± 0.84	1.68 ± 0.88	1.88 ± 0.58	-
Water hardness (mg l^{-1})	8.25 ± 1.08	7.19 ± 1.78	7.15 ± 1.18	A
ChOD (mg l^{-1})	1.67 ± 1.32	1.56 ± 1.12	1.66 ± 1.52	-
BOD_5 (mg l^{-1})	4.34 ± 1.22	4.61 ± 1.33	4.81 ± 1.72	A
NH_4-N (mg l^{-1})	1.18 ± 0.75	1.34 ± 0.48	1.37 ± 0.28	A
NO_3-N (mg l^{-1})	7.41± 1.05	6.11± 1.15	6.61± 1.45	A
NO_2-N (mg l^{-1})	0.68 ± 0.16	0.48 ± 0.36	0.48 ± 0.36	A
PO_4^{3}-P (mg l^{-1})	0.15 ± 0.07	0.14 ± 0.11	0.12 ± 0.11	-
Ca (mg l^{-1})	66.51 ± 4.25	59.51 ± 3.75	76.41 ± 3.77	A
Cd (mg l^{-1})	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	-
Pb (mg l^{-1})	0.03 ± 0.05	0.03 ± 0.07	0.02 ± 0.05	-
Mg (mg l^{-1})	16.33 ± 4.05	15.23 ± 3.33	15.44 ± 4.17	A

A – statistically significant differences (ANOVA, test Duncan) in the water parameter among the three culture sites ($P \leq 0.05$);
common carp – I group; Siberian sturgeon – II group; rainbow trout – III group

The study revealed that average levels of microelements differed significantly among tissues and organs of the three examined fish species. Average iron content ranged from 3.1 to 54.7 mg kg^{-1} w.w. (Table 2). The highest iron levels were detected in the gills (54.7±5.7 mg kg^{-1} w.w.) and kidney (49.6±6.8 mg kg^{-1} w.w.) of Siberian sturgeon and in the kidney of rainbow trout (45.8±9.7 mg kg^{-1} w.w.). The lowest iron levels were found in the skin and muscles of all the examined fish (Table 2). Iron was distributed in fish bodies according to the following patterns of decreasing concentrations:

– common carp: gills> kidney> blood> liver> muscles> skin,
– rainbow trout: kidney> liver> gills> blood> muscles> skin,
– Siberian sturgeon: gills> kidney> liver> blood> skin> muscles.

Table 2

Microelement (Zn, Fe, Cu) levels in organs of three freshwater fish species

Bioelement	Tissue	Common carp *Cyprinus carpio* L.	Rainbow trout *Oncorhynchus mykiss* Rich	Siberian sturgeon *Acipenser baeri* Brandt
		mean ± SD	mean ± SD	mean ± SD
Fe (mg kg^{-1} w.w.)	blood	27.1 ± 5.6	16.7 ±3.9	35.3 ± 3.9
	liver	23.6 ± 3.7	34.8 ± 4.1	37.3 ± 2.9
	kidney	33.6 ± 6.8	45.8 ± 9.7	49.6 ± 6.8
	gills	36.6 ± 6.4	28.9 ± 3.6	54.7 ± 5.7
	muscles	4.6 ± 1.1	6.2 ± 0.9	9.1 ± 1.2
	skin	3.1 ± 0.6	6.1 ± 0.7	11.5 ± 1.1
Cu (mg kg^{-1} w.w.)	blood	0.8 ± 04	1.1 ± 0.9	3.8 ± 0.4
	liver	15.7 ± 17.6	19.2 ± 8.4	12.7 ± 5.6
	kidney	1.8 ± 0.2	3.3 ± 0.8	1.9 ± 0.3
	gills	3.1 ± 0.4	3.6 ± 0.9	3.4 ± 0.4
	muscles	0.4 ± 0.6	0.9 ± 0.3	0.7 ± 0.2
	skin	1.1 ± 0.4	1.1 ± 0.3	1.2 ± 0.6
Zn (mg kg^{-1} w.w.)	blood	8.8 ± 04	4.1 ± 0.9	3.8 ± 0.4
	liver	50.7 ± 17.6	87.2 ± 8.4	98.7 ± 5.6
	kidney	19.3 ± 0.5	3.3 ± 0.8	3.3 ± 0.3
	gills	3.1 ± 0.4	3.6 ± 0.9	3.4 ± 0.4
	muscles	6.1 ± 17.6	8.1 ± 12.6	7.4 ± 11.6
	skin	52.1 ± 0.7	34.6 ± 0.6	51.9 ± 0.7

w.w. – wet weight, SD – standard deviation

Many authors reported considerably higher levels of this element in the muscles of freshwater and marine fish (Pujin 1990, Kargin 1996, Grosheva et al. 2000, Jurkiewicz-Karnakowska 2001).

Iron and its compounds are not toxic to fish, but disturbances in the mechanism that regulates its absorption often occur due to diseases or long-term exposure to high dietary iron levels. In such cases, iron is bound to proteins or as iron phosphate (haemosiderin), which is deposited in various tissues, mainly in liver. Both absorption and metabolic function of iron are dependent on influences of other elements. Especially Cd, Mn, Pb and Zn act antagonistically to iron. In the case of Cu, the relationship is very complex and often has a synergetic character, as Cu and Fe are involved in reduction-oxidation processes. Phosphorus reduces iron bioavailability because iron phosphates precipitate easily in various conditions (Kabata-Pendias, Pendias 1999).

Tissue levels of copper in the three fish species were comparable. Average tissue levels of copper ranged from 0.4 to 19.2 mg kg^{-1} w.w. The highest copper levels were found in the liver, while the lowest – in the muscles (Table 2). Copper was distributed in fish bodies according to the following patterns of decreasing concentrations:

– common carp and rainbow trout: liver> gills> kidney> skin> blood> muscles,
– Siberian sturgeon: liver> blood> gills> kidney> skin> muscles.

Copper participates in haematopoiesis, but high concentrations of this metal in combination with some other metals such as zinc, mercury, cadmium or lead, may produce anaemia in fish (DICK, DIXON 1985, EL-DOMIATY 1987, BANERJEE, HOMECHAUDHURI 1990, SINGH, REDDY 1990, VAN VUREN et al. 1994, KAZLAUSKIENĖ, VOSYLIENĖ 1995). SINGH and REDDY (1990) imply that long term exposure of fish to copper may produce anaemia caused by a disorder of kidney haematopoietic function. Worth noticing is that copper forms a synergetic system with iron (Cu-Fe), which advantageously influences the course of enzymatic processes (KABATA-PENDIAS, PENDIAS 1999). Copper also accompanies iron in all stages of cellular respiration. The metal forms a reductive-oxidative system, e.g., it conditions activity of oxidases (uricase, ascorbase, lysine oxidase, monoamine oxidases, and also cytochrome c oxidase, tyrosinase and porphobilinogen synthase), protecting the organism from adverse impact of reactive oxygen species (RFT/ROS).

Zinc levels in the examined fish varied from 3.1 to 98.7 mg kg^{-1} w.w. (Table 2). The highest zinc content (50.7 to 98.7 mg kg^{-1} w.w.) was detected in the liver, while the lowest in the gills (3.1 to 3.6 mg kg^{-1} w.w.). Zinc distribution in fish bodies followed the decreasing sequences:

– common carp: skin> liver> kidney> blood> muscles> gills,
– rainbow trout and Siberian sturgeon: liver> skin> muscles> blood> gills> kidney.

Zinc is weakly accumulated in fish tissues, as it is retained by the gills, where the metal is deposited in large amounts (JEZIERSKA, WITESKA 2001, WITESKA 2003). This may be explained by the fact that zinc penetrates to blood less easily than other metals (cadmium, nickel). 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 mostly 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. Metabolically significant antagonism occurs between Zn and Cd, as well as between Zn and Cu. Additionally, calcium and magnesium may reduce zinc absorption (KABATA-PENDIAS, PENDIAS 1999).

This study revealed that average levels of macroelements differed significantly among the three fish species examined. Average magnesium levels varied from 38.1 to 453.3 mg kg^{-1} w.w. Most magnesium was detected in the gills of Siberian sturgeon (453.3±14.5 mg kg^{-1} w.w.) and in the gills and muscles of common carp (346.1±17.6 and 256.1±15.2 mg kg^{-1} w.w., respectively; Table 3). On the contrary, the lowest magnesium level was found in the blood of rainbow trout (38.1±18.1 mg kg^{-1} w.w.). In all the three fish species, Mg distribution followed the same pattern of decreasing levels: gills> muscles> liver> kidney> skin> blood.

Calcium levels in the examined fish ranged from 72.2 to 4954.6 mg kg^{-1} w.w. In all the three species, the lowest calcium levels were recorded in the blood (72.2 to 96.8 mg kg^{-1} w.w.) and the highest in the gills (2214.6 to 4954.6 mg kg^{-1} w.w.; Table 3). Similarly as in the case of magnesium, calcium distribution followed the same pattern for all the three species, in decreasing order: gills> muscles> skin> liver> kidney> blood.

Table 3

Macroelement (Mg, Ca) levels in organs of three freshwater fish species

Bioelement	Tissue	Common carp *Cyprinus carpio* L.	Rainbow trout *Oncorhynchus mykiss* Rich	Siberian sturgeon *Acipenser baeri* Brandt
		mean ± SD	mean ± SD	mean ± SD
Mg (mg kg^{-1} w.w.)	blood	76.6 ± 16.1	38.1 ± 18.1	85.4 ± 11.4
	liver	139.0 ± 14.1	78.0 ± 13.1	157.6 ± 15.3
	kidney	96.3 ± 19.2	63.2 ± 13.6	117.3 ± 14.6
	gills	346.1 ± 17.6	158.1 ± 13.6	453.3 ± 14.5
	muscles	256.1 ± 15.2	122.1 ± 17.6	166.1 ± 17.6
	skin	96.1 ± 17.6	56.1 ± 10.6	113.1 ± 13.3
Ca (mg kg^{-1} w.w.)	blood	76.5 ± 8.1	72.2 ± 11.1	96.8 ± 12.8
	liver	118.2 ± 18.2	122.2 ± 13.3	197.1 ± 15.2
	kidney	103.9 ± 5.9	112.3 ± 25.4	153.2 ± 22.9
	gills	2214.6 ± 62.1	2989.6 ± 88.3	4954.6 ± 92.1
	muscles	277.3 ± 18.2	199.1 ± 14.4	246.8 ± 21.6
	skin	156.1 ± 13.5	186.5 ± 14.6	176.4 ±167.6

w.w. – wet weight, SD – standard deviation

This study focused on assessment of physiological condition of freshwater fish exemplified by common carp, rainbow trout and Siberian sturgeon, based on levels of certain microelements (iron, zinc and copper) and macroelements (magnesium and calcium) in their bodies. 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 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 (Cu, Zn, Fe, Mg and Ca), which are regarded 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 at increased activity of hepatocytes (POURAMAHAD 2000, LUSHCHAK et al. 2005). Levels of microelements recorded in this study were not high (Table 2, 3) and remained within the normal range for salmonids (*Salmonidae*) and cyprinids (*Cyprinidae*). For sturgeon family (*Acipenseridae*), an accurate normal range of macro- and microelements has not been determined.

In the Oder river basin within the West Pomeranian Province, cyprinid and salmonid fish are often reared in cooling water from a power plant, which are collected in a discharge canal and then disposed of to the Oder. Cooling water has nearly constant temperature all year round, which creates favourable conditions for all year round fish farming. Such activities have been undertaken for many years in cooling water from the Dolna Odra Power Station, West Pomerania. Cooling water discharged from the power plant contains various substances essential for proper functioning of ecosystems and also trace amounts of toxic chemicals, concentrations of which stay within the permissible limits (Rozporządzenie Ministerstwa OŚZNiL 1991, Raport WIOŚ 2003, JEZIERSKA, WITESKA 2001).

In all monitored rivers in the West Pomeranian Province, heavy metal concentrations, including cadmium, lead, mercury and nickel, do not exceed the limits (PROTASOWICKI, CHODYNIECKI 1988, Raport WIOŚ 2003). There are numerous reports on levels of macro- and microelements in organs and tissues of common carp of various age (DOBRZAŃSKI et al. 1996, KOŁACZ et al. 1996, MOORE, RAMAMOOTHY 1984, VIRK, KAUR 1999). On the contrary, there are no data on bioelement content in tissues of salmonids and sturgeons cultured in both cooling waters and fish ponds not supported by cooling waters.

Biological effect of a chemical depends on the following processes: absorption, biotransformation, accumulation and elimination of a compound. Biotransformation of xenobiotics may result in formation of metabolites that are less or more toxic, while accumulation means deposition of toxic substances or their metabolites in tissues. Elements present in water are regarded to be bound on the gill surface, which disturbs function of this organ. The amount of bioelements retained in fish body depends on many factors that condition the sorption efficiency. Some of them are: species, age, body weight and length, gender, season of the year and fishing ground (PROTASOWICKI, CHODYNIECKI 1988, LIANG, WONG 2000). Also the amount of bioelements accumulated in different tissues is varied. Interspecies differences derive mainly from different feeding habits (PROTASOWICKI 1991, LIANG, WONG 2000).

Within several minutes from absorption, most of bioelements find their way to heart, liver, kidney and brain. The second phase, when bioelements penetrate into muscles, skin and adipose tissue, is considerably slower, and balance in tissues is established within some half an hour to several hours. In distribution of various substances all over the organism, the circulatory system plays a key role. Substances are carried by blood to particular organs and tissues, and next removed from the body in the processes of excretion, which depends largely on the blood flow rate through the tissues. Blood physiological values in fish are considerably varied as they depend on individual variability, age, culture method, diet and season of the year. Blood parameters typical for healthy fish may vary in a wide range, therefore determination of adequate physiological reference values is much more difficult than for warm-blooded animals (ALLAN 1993, THOMAS et al. 1999). Literature reports indicate that the degree of metal accumulation and excretion in different organs is varied. According to SREEDEVI et al. (1992) common carp, exposed for 4 days to nickel dissolved in water in concentrations ranging from 20 to 70 mg dm^{-3}, accumulated most of the metal in the gills, and less in the liver, muscles and kidney. In contrast, a four-day-long experiment of RAY et al. (1990) on *Clarias batrachus* exposed to nickel revealed that accumulation of the metal in fish organs decreased in order: kidney > liver > gills > gut. Mercury concentrations in organs of bream caught in the Vistula River were the highest in the liver, gut, heart and gills (JEZIERSKA, WITESKA 2001).

This study revealed that culture site exerted statistically significant influence on bioelement levels in the examined fish species. Kidney and skin levels of magnesium were higher in common carp then in Siberian sturgeon. Similar regularities were observed for skin levels of calcium and kidney levels of zinc. Iron and copper levels in the liver and kidney were higher in common carp than rainbow trout. In all organs and tissues of common carp and rainbow trout, similar qualitative relationships were observed among the examined minerals. In all the three fish species, tissue levels of copper and zinc were the lowest. Rainbow trout had the highest skin levels of Ca and Mg, as well as blood level of Fe.

Concentrations of microelements (Zn, Fe, Cu) and macroelements (Mg, Ca) detected in this study in organs and tissues of common carp, rainbow trout and Siberian sturgeon seem to be within the normal physiological range reported for these fish species by other authors (BRUCKA-JASTRZĘBSKA, PROTASOWICKI 2004a, BRUCKA-JASTRZĘBSKA, PROTASOWICKI 2004b, VIRK, KAUR 1999).

CONCLUSIONS

1. The results indicate that culture site and culture conditions exerted significant influence on levels of macro- and microelements in freshwater fish.

2. Monitoring of bioelement levels in tissues of freshwater fish enables early observation of pathological changes in fish bodies. Disorders in the levels of bioelements appear very quickly and precede changes in fish behaviour and visible lesions.

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