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Diets supplemented with *Mytilus galloprovincialis* from polluted and non-polluted waters and their influence on zinc content in liver of rats loaded with cholesterol

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Abstract: *Diets supplemented with Mytilus galloprovincialis from polluted and non-polluted waters and their influence on zinc content in liver of rats loaded with cholesterol.* Mussels may reflect the environmental problems that the ecosystem faces. *Mytilus galloprovincialis* is widely used as an indicator of water pollution in biological monitoring studies. These mussels are filter feeders and may accumulate important ecosystem pollutants what in turn may pose a potential risk to other organisms in the food chain. The aim of the study was to determine zinc content in the diets supplemented with mussels from polluted and non-polluted areas and in the livers of rats fed these diets for 30 days. The zinc content in the diets with mussels from contaminated and non-contaminated waters and in rat livers was examined using a flame atomic absorption spectrophotometry (Perkin-Elmer 1100B). The slight increase in zinc content from 73.2 ±1.2 mg/ /kg in ch diet (control with 1% of cholesterol) to 77.2 ±0.5 mg/kg in the diet with mussels from polluted site was found, but zinc content in all diet was within the normal limits reviewed by McDowell (1992) – 40 to 100 ppm. Zinc content in the diet supplemented with mussels from non-polluted site (73.7 ±3.7 mg/kg) was similar to that found in ch diet. It can be concluded that cholesterol does not affect zinc bioaccumulation. However, prolonged consumption of mussels from contaminated sites may increase accumulation of zinc in the liver of rats.

Key words: mussels, polluted, non-polluted, zinc, rat, liver

INTRODUCTION

The mussels are invertebrates of great importance for the economy and the environment. They have a high nutritional value (Leontowicz et al. 2008). The popularity, availability and consumption of seafood has increased (Gorinstein et al. 2008). Several reports indicate that mussels possess high level of proteins, vitamins, minerals, polyphenols and other antioxidants (Gorinstein et al. 2008, Leontowicz et al. 2008, Namiesnik et al. 2008). However, these marine organisms are filter feeders and may uptake from the water organic pollutants, heavy metals and accumulated them in the body (Potrykus et al. 2003, Spada et al. 2013). This physiological ability makes mussles – *Mytilus galloprovincialis* – biomarker of seawater pollution. Metal accumulation and rate of metal uptake in *Mytilus galloprovincialis* has been presented in many reports (Machado et al. 1999, Adami et al. 2002, Kanduc et al. 2011, Marrone et al. 2011, Ergul and Aksan 2013, Spada et al. 2013). Some authors (Lopez et al. 2001, Mosquera et al. 2003) present genetic variability and differences in protein expression between intertidal and cultured *Mytilus galloprovincialis.* It has been shown that the habitat of mussels may affect changes in heavy metals, antioxidant compounds, free radical scavengers in their tissues (Gorinstein et al. 2006, Leontowicz et al. 2008). It was decided to investigate the concentration of zinc in the diets with mussels from polluted and non-polluted areas and also evaluate the influence of these diets on zinc content in the liver of rats. Zinc is released to the environment from both natural and anthropogenic sources, however, releases from the second sources are greater. Severe zinc pollution tends to be confined to areas near emission sources, like ports, where zinc is deposited primarily in sediments through adsorption and precipitation. Large intake of foods high in zinc content, such as oysters and mussels, may be unsafe and may lead to metal bioaccumulation.

MATERIAL AND METHODS

Samples and sites of collection

Mussels were collected in two regions of Mokpo coast (Republic of Korea):

- non-polluted area: out of the port, 20 mi. North-Westfrom Mokpo bay;
- polluted site: the Mokpo port, in the bay of Halla Ship large scale construction company which belongs to Hyundai group) at sea depth of 3–4 m (Leontowicz et al. 2008).

The collected mussels from both sites length 4.37 ±0.5 cm and it was 75–85 % of the maximum size. This approach allowed to assume that mussels were at the age of the similar metabolism (Gorinstein et al. 2006). Whole soft tissue (n = 30 of each population) were frozen and storage at –80°C. Then the samples were freeze dried in glass flasks on Finn-Aqua, Lyovac GT-2 equipment for 36 h and transported to Department of Physiological Sciences (Warsaw, Poland), where were waiting for further analysis.

Rats, diets and management

The study was conducted in the Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW and lasted 30 days. The results of feed intake, body gains, plasma lipids and antioxidant activity were published previously (Leontowicz et al. 2008). The mean body weight of the male Wistar rats (n = 28) at the beginning of the experiment was 111 ±3.0 g. The animals were randomly divided into 4 groups. The control (C) receiving basal diet (BD) (Leontowicz et al. 2011) only, and the diets of the other three groups were supplemented with 1% of cholesterol (ch). Two cholesterol diets contained 5.6% of mussel dry matter from polluted (chMP) and non-polluted areas (chMNP). All rats were fed *ad libitum* and had free access to water. The diets were offered once a day. Before the section the rats were not fed for 24 h. At the end of the experiment, the rats were anaesthetized using Narcotan® (Zentiva) for inhalation, and from the liver of each experimental rats left lateral lobe were dissected. The Animal Care Committee of the Warsaw University of Life

Sciences – SGGW, Poland approved this study (No. 10/2007).

Zinc determination

Approximately 0.5 g of four diets: control (C), control with cholesterol (ch) and with lyophilised mussels from polluted (chMP) and non-polluted areas (chMNP) and 1.0 g of liver were mineralised in microwave oven (Milestone Ethos 900) with 5 ml 65% HNO_3 (Merck 1.00441) and 1 ml 30% H_2O_2 (Merck 1.07298) and the content of zinc was determined using a flame atomic absorption spectrophotometer (Perkin-Elmer 1100B), at 210 nm. The method provides a linearity in the concentration range of 0–1 mg/l with a detection limit of 1 μg/l. The standards of 0.5 and 1.0 mg/l were prepared using 9953 Titrisol Zinc standard (Merck).

Statistical analysis

One-way ANOVA analysis of variance (Duncan's test) was performed using Statistica 9 software. Differences were considered significant at $P \leq 0.05$. Data are presented in figure as means ±standard deviation.

RESULTS AND DISCUSSION

Zinc is capable to form complexes with a variety of organic and inorganic groups. The environmental parameters like temperature, salinity and turbidity may influence zinc bioaccumulation. Zinc bioconcentrates moderately in aquatic organisms. The crustaceans and mussels species accumulate more heavy metals and other chemical contaminations than fish living in open water (Namiesnik et al. 2008, Ergul and Aksan 2013). The reason for this may be less mobile lifestyle and often limiting to the bottom of the sea. The biota contain relatively little zinc compared to the sediments. In addition, the zinc content in sediment closely correlated with the depth, organic matter content, and clay content of the sediments. Mussels may reflect the environmental problems that an ecosystem faces. *Mytilus galloprovincialis* are widely used as an indicator of water pollution in biological monitoring studies. In young and small-sized individuals trend to accumulate heavy metals is reduced. The concentration of toxic elements is connected to the dry mass of tissue and that is why the adult mussels are preferable. This allows also the measurement of trace elements, even if their contents in environment are low (Ravera et al. 2003). In our experiment, mussels were characterised by a similar maximum length and size reached within each population. This approach makes that physiological differences between two populations would be less pronounced.

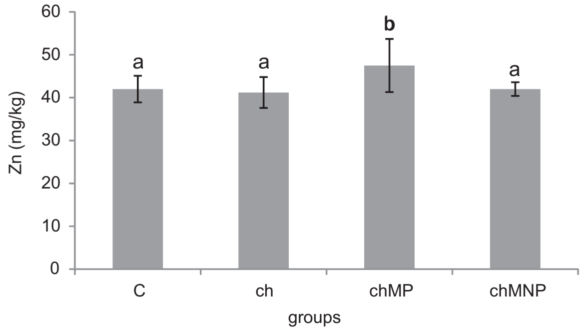
M. galloprovincialis process large volume of the water they live in to obtain food. At the same time they uptake other substances like: polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organotins, heavy metals presented in contaminated waters (Namiesnik et al. 2008). This may lead to concentrate important ecosystem pollutants in the mussel tissues and be a potential risk to other organisms in the food chain. Gorinstein et al. (2006) have found that the amounts of zinc and copper were significantly higher in the mussel proteins from the polluted than from the non-polluted sites of the Bulgarian

Black Sea coast. It could be concluded that the high content of zinc in the water and good physical and chemical environmental conditions for zinc accumulation influence zinc bioaccumulation in marine organisms and may contribute to higher risk of exposure to it. The diets except the mussels collected from areas with different levels of contamination, included wheat starch, casein, soybean oil, vitamin and mineral mixtures (Leontowicz et al. 2011). The inclusion rate of mussels in the diet (chMP and chMNP) was 5.6% and no significant differences were noted in zinc content between all experimental diets. However, slight increase in zinc content from 73.2 ±1.2 in ch diet to 77.2 ±0.5 mg/kg in the diet with mussels from polluted site (chMP) was found, but zinc content in all diet was within the normal limits reviewed by McDowell (1992) – 40 to 100 ppm. Zinc content in the diet supplemented with mussels from non-polluted site (73.7 ±3.7 mg/kg) was similar to that found in ch diet.

Most of *in vivo* studies dedicated to mussels concern their effects on body weight gains of animals fed diets supplemented with different species of mussels and in different forms – raw, dry, as mixed commercial feed (Mao et al. 1999, Jönsson and Holm 2011, Anagnostidis et al. 2013). These authors concluded that mussels are a good source of protein and various biologically active substances. The results showed by Mao et al. (1999) indicate that selenium in mussel extract could be better absorbed in the rats and effectively promote their growth. Jönsson and Holm (2011) conducted in preliminary study on 12 hens, which were fed diets with 15% addition of normal or 15% addition of toxic mussels, that mussel meal could be a novel protein source for laying hens and toxic mussels at determined level may be included in the feed without negative effects on health and egg production.

Conducted research was designed to answer if supplementation with mussels from polluted and non-polluted areas influences liver zinc content in rats loaded with cholesterol. Several reports noted that mussels can be used as nutritional supplement in diet for animals like hens, fish and could improve feed and protein efficiency of commercial feeds (Anagnostidis et al. 2013). However, studies on the toxicity of mussels from contaminated sites on the accumulation of heavy metals in the body of animals that consume them are not so numerous. The content of zinc in most mammalian tissues ranged from 10 to 100 µg/g fresh weight which equals 30–250 µg/g dry weight (McDowell 1992). As shown at Figure 1, zinc content in the rat livers ranged from 41.2 ±3.6 in ch group to 47.5 ±6.2 mg/kg fresh weight in chMP group; was within the normal limits. It can be concluded that cholesterol does not affect zinc accumulation. The zinc content in the liver of rats receiving diet with mussels from polluted waters increase significantly ($P \leq 0.05$) in related to other three groups, although there was no difference ($P > 0.05$) in feed intake between animal groups (Leontowicz et al. 2008) and zinc content in the experimental diets also did not differ ($P > 0.05$).

This small increase in the content of zinc in the diet with mussels from polluted waters could affect its accumulation in the liver of rats. Animals had received



a–b – columns marked with different letter differ at P ≤ 0.05; values are means ±SD (n = 6); C – control group; ch – control with 1% of cholesterol; chMP – group with 5.6% of mussels from polluted site and cholesterol; chMNP – group with 5.6% of mussels from non-polluted site and cholesterol.

FIGURE 1. Zn content in the liver (fresh weight) of rats fed diets with mussels from polluted and non--polluted areas and with cholesterol

this diet for 30 days and it could be the cause of increase liver zinc content. Another cause could be diet components and/or heavy metals from contaminated waters, which could influence the absorption of zinc and its bioavailability. Bat et al. (2012) concluded that the high copper and zinc content in mussels reduces the absorption of toxic metals such as cadmium and lead. However, it should be noted, that zinc and copper in excessive amounts are also harmful. On the other hand supplementation of diets, containing cholesterol, with mussels from both contaminated and non--contaminated areas may improve animal protein metabolism and positively affect plasma lipid profile and plasma antioxidant activity (Gorinstein et al. 2008, Leontowicz et al. 2008).

CONCLUSIONS

Prolonged consumption of mussels from contaminated sites may increase accumulation of zinc in the liver of rats.

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Streszczenie: *Diety z dodatkiem Mytilus galloprovincialis z wód niezanieczyszczonych i zanieczyszczonych oraz ich wpływ na zawartość cynku w wątrobie szczurów obciążonych cholesterolem*. Małże mogą odzwierciedlać problemy środowiskowe, przed którymi stoi ekosystem. *Mytilus galloprovincialis* jest szeroko wykorzystywany w biomonitoringu. Małże te są filtratorami i mogą kumulować w tkankach ważne zanieczyszczenia z ekosystemu, co z kolei może stanowić potencjalne zagrożenie dla innych organizmów w łańcuchu troficznym. Celem badania było określenie zawartości cynku w dietach z dodatkiem małży z obszarów niezanieczyszczonych i zanieczyszczonych oraz w wątrobach szczurów żywionych tymi dietami przez 30 dni. Zawartość cynku w dietach z małżami z zanieczyszczonych i niezanieczyszczonych wód i wątrobach szczurów oznaczono metodą płomieniowej absorpcji atomowej (Perkin-Elmer 1100B). Wykazano nieznaczny wzrost zawartości cynku w diecie z małżami z wód zanieczyszczonych (77,2 ±0,5 mg/kg) względem grupy ch – kontrola z 1% udziałem cholesterolu (73,2 ±1,2 mg/kg), ale zawartość cynku we wszystkich dietach mieściła się w granicach normy podanych przez McDowell (1992) – 40–100 ppm. Zawartość cynku w diecie z małżami z obszarów niezanieczyszczonych (73,7 ±3,7 mg/kg) była zbliżona do zawartości w diecie grupy ch. Podsumowując wyniki, można stwierdzić, że cholesterol nie wpływa na bioakumulację cynku. Jednak, długotrwałe spożywanie małży z wód zanieczyszczonych może zwiększyć akumulację cynku w wątrobie szczurów.

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