Effect of genetically modified maize on zinc and copper retention in rat organs

Wpływ kukurydzy transgenicznej na retencję cynku i miedzi w narządach u szczurów

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Wprowadzenie. Uprawa roślin transgenicznych wydaje się być alternatywą dla zaspokojenia potrzeb żywieniowych rosnącej populacji ludzi na świecie.

Cel. Określenie wpływu kukurydzy z genem oporności na herbicydy (RR) na stężenie cynku i miedzi w wątrobie, nerkach i kości udowej szczurów.

Materiały i metody. Doświadczenie przeprowadzono na 48 szczurach Wistar umieszczonych w indywidualnych klatkach bilansowych, w klimatyzowanym pomieszczeniu z możliwością kontroli oświetlenia. Po okresie adaptacji szczury były karmione dietami zawierającymi konwencjonalną lub transgeniczną kukurydzę. Grupa kontrolna składała się z 8 zwierząt karmionych kukurydzą izogeniczną. Po tygodniu, zwierzęta z grupy 1 (n=30) – karmione kukurydzą transgeniczną były uśpione w następujących odstępach czasowych: 0, 2, 4, 8, 12, 24 godziny. Szczury z grupy 2 i 3 były uśpione tydzień (n=5) i dwa tygodnie (n=5) później. W każdym punkcie czasowych usypiano 1 zwierzę losowo wybrane z grupy kontrolnej. Pobrano wątroby, nerki i kości udowe w celu oznaczenia cynku i miedzi metodą płomieniową ASA.

Wyniki. Nie stwierdzono żadnych różnic w stężeniu miedzi w badanych narządach pomiędzy różnymi czasami. Stężenie cynku w kości udowej szczurów karmionych kukurydzą transgeniczną było wyższe w 3 tygodniu niż w tygodniu 1 i 2. Nie było natomiast różnic między grup kontrolną i grupą 1 (kukurydza transgeniczna, 1 tydzień).

Wnioski. Skarmianie szczurów kukurydzą transgeniczną (NK603) nie miało wpływu na retencję cynku i miedzi w narządach szczurów.

Słowa kluczowe: *kukurydza transgeniczna, cynk, miedź, retencja, szczur*

Introduction. Genetically modified plants of first or second generation become one of possible solutions as far as sufficient nutrition of the world population is concerned.

Aim. To evaluate the effect of transgenic maize (RR maize-introduced gene of herbicide resistance) on zinc and copper concentration in rat liver, kidney and femur bone.

Material & Method. The experiment was performed on 48 male Wistar rats. The animals were placed individually in balance cages with controlled light regime and constant temperature. After adaptation to the experimental conditions, the rats were fed ad libitum the diets containing conventional or transgenic RR maize. Forty animals were fed diets with RR maize. The control group consisted of 8 animals fed the diet containing isogenic maize. After seven days of feeding the rats of the group 1 (n=30) were put down at 0, 2, 4, 8, 12 and 24 h. Group 2 and 3 were put down one and two weeks later, respectively. At each time one control rat was also put down. Livers, kidneys and femur bones were collected to determine zinc and copper (flame ASA).

Results. There were no differences in the copper content in tested organs at any time point. The zinc content in femur bone was higher after three weeks than after one and two weeks of feeding with RR maize. No difference was observed between the control group and group 1 (RR maize, one week).

Conclusion. Feeding diets with transgenic Roundap Ready maize (NK603) does not affect the zinc and copper retention in rat organs.

Key words: *Roundap ready maize, zinc, copper, retention, rat*

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Nadesłano: 03.01.2017
Zakwalifikowano do druku: 15.06.2017

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Introduction

With growing number of world population the resources of food per inhabitant decrease, bringing about uncertainty as far as sufficient nutrition is concerned. Introduction of recombinant DNA technologies and foreign genes incorporation into economically important plants genome gives cause for increased interest in safety of plants, modified in this way, in food chains of animals and humans. Genetically modified plants of first or second generation become one of possible solutions. Introduction of recombinant DNA technologies and foreign genes incorporation into economically important plants genome gives cause for increased interest in safety of plants, modified in this way, in food chains of animals and humans. One of the first, if not the first, gene introduced to plants, was the gene of Roundup resistance. Roundup, produced by company Monsanto (St. Louis, Missouri), is a trade name of a broad spectrum of systemic herbicide in which glyphosate in an active substance. It inhibits synthesis of aromatic amino acids in plants, and thus can be used only before sowing of the crops. On the fields of plants with introduced gene of glyphosate resistance (Roundup Ready), farmers can kill weeds through the most of the vegetation period. For many years, RR soybean and RR maize have been the main subject of discussions on GM organisms [1].

The GM maize produced by Monsanto (St. Louis, Missouri) has been developed for tolerance to glyphosate by the introduction, via particle gun acceleration, of gene coding for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). It is currently authorized for commercialization in the EU for food and feed use (http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=61).

The equivalence of isogenic and transgenic maize was confirmed by many previous experiments [1-3]. However five statistically significant differences were identified in composition in grains of maize NK603 as compared to isogenic counterpart (phosphorus, leucine, zinc, crude protein and carbohydrate). The maximum inclusion rate of this maize in rat diet reported by the European Food Safety Authority was 33% [4].

Aim

The objective of this work was to determine the influence of high inclusion rate of transgenic RR maize (NK603) on the retention of zinc and copper in rats.

Material and method

Feed mixture

The experiment was carried out on isogenic (*Zea mays* L.) and genetically modified maize NK603 of the Monsanto company (MON-00603-6) that contains the gene inserted to confer tolerance to glyphosate herbicides (*cp4 epsps*). The tested transgenic and isogenic line of maize created 94% content of feed mixtures (93.8 and 94.1) and it represented the only source of crude protein in diets (approximately 10%). The diets were balanced for energy with soybean oil (3.7 and 3.8%) and supplemented with mineral mixture [1/] (1.3 and 1.4%) as well as with vitamin mixture [2/] (both 0.9%).

[1/] 15 g $CaCO_3$, 14 g $Ca_3(PO_4)_2$, 10 g K_2HPO_4, 8 g NaCl, 7 g Na_2HPO_4, 5 g $MgSO_4 \cdot 7H_2O$, 0.48 g Fe citrate, 0.04 g $ZnCO_3$, 0.0195 g $CuSO_4 \cdot 5H_2O$, 0.45 g $MnSO_4 \cdot 4H_2O$, 0.0005 g KI, 0.01g NaF

[2/] 5000 IU vit. A, 500 IU vit. D_3, 50 mg vit. E, 1 mg vit. K, 20 mg vit. C, 20 mg vit. B_1, 20 mg vit. B_2, 10 mg vit. B_6, 50 mg Ca pantotenate, 50 mg nicotinic acid, 1000 mg choline HCl, 2 mg folic acid, 100 mg inositol, 200 mg p-aminobenzoic acid, 30 µg vit. B_{12} ad 20 g starch

Experimental animals and design

The experiment was performed on 48 male Wistar rats (75 g initial weight) obtained from UEF SAS Dobra Voda. The animals were placed individually in balance cages with controlled light regime and constant temperature. After adaptation to the experimental conditions, rats were fed *ad libitum* for one week with diets containing conventional or transgenic RR maize. The control group consisted of 8 animals fed the diet containing isogenic maize. Forty animals were fed the diets with RR maize. The rats of group 1 (n=30) were put down during one-full day at 0, 2, 4, 8, 12 and 24 h. Group 2 and 3 (five rats each) were put down one and two weeks later, respectively. Each time one control rat was also put down (n=8). Livers, kidneys and femur bones were collected to determine the zinc and copper content (flame ASA).

The feed intake during the whole experimental period was controlled. The presence of individual nutrients in both forms of maize was assessed by chemical analyses [2].

Analysis of zinc and copper

Organs were removed and kept until analysis at 18°C. Samples of tissues (0.5-1.0 g) were mineralized in mixture of 5 ml HNO_3 (Merck 1.00441) and 1 ml H_2O_2 (Merck 107298) in hermetic high-pressure vessels in microwave oven Ethos 900 (Milestone). Mineral elements were estimated by flame (air-acetylene) atomic absorption spectrophotometry (Perkin-Elmer 1100B) using hollow cathode lamps with deuterium background correction (except copper analysis). Accuracy and precision of the analysis were checked routinely using replicate samples and certified reference zinc solution (Titrisol, Merck, Schwalbach, Germany, 9953 – Zn, 9987 – Cu). The method provides a linearity in the concentration range of 0-1 mg/l with a detection limit of 10 µg/l, for both zinc and copper.

Statistical Analysis

For statistical evaluation the one-way analysis of variance (group) was applied. For evaluation of significance among groups the post-hoc test F Ryan-Einot-Gabriel-Welsch was used ($p \leq 0.05$) (SPSS 23.0 pl).

The experiment had an approval of the Slovak Local Commission of Animal Welfare and the principles of animal care were followed.

Results

Total body gain during the experiment was similar in all rats and varied from 59.2 to 65.0 g, and digestibility of the diet ranged from 88.0 to 89.2%. The mean concentration of zinc in all animals was 75.6±17.3 mg/kg, 55.6±14.3 mg/kg and 198±20 mg/kg in liver, kidney and bone, respectively. The zinc content in femur bone was higher after three weeks than after one and two weeks of feeding with RR maize (Fig. 1). No difference was observed between the control group and group 1 (RR maize, 1st day). There was a significant difference in the kidney zinc concentration between group 1 (1st day) and group 2 (1st week). The kidney zinc concentration in group 3 (2nd week) did not significantly differ from values found in groups 1 and 2.

The mean concentration of copper in all groups was 11.1±2.3 mg/kg liver, 16.0±4.5 mg/kg kidney and 6.1±0.4 mg/kg bone. There were no differences in the copper content in tested organs at any time point (Fig. 2).

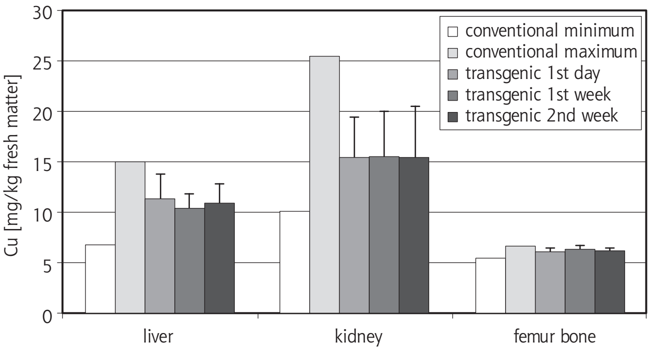

Fig. 1. Zinc concentration in organs of rats fed transgenic maize (M±SD)

Ryc. 1. Stężenie cynku w narządach szczurów karmionych kukurydzą transgeniczną (M±SD)

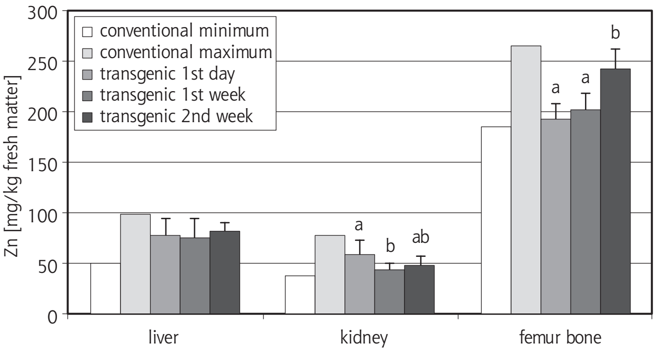

Fig. 2. Copper concentration in organs of rats fed transgenic maize (M±SD)

Ryc. 2. Stężenie miedzi w narządach szczurów karmionych kukurydzą transgeniczną (M±SD)

Discussion

The differences in chemical composition of genetically modified and conventional maize were very small and thus not biologically relevant. On the basis of previous results it can be summarized that genetically modified maize in rat nutrition did not negatively influence the health status of rats [4]. Feeding the genetically modified RR maize (NK603) had no negative influence on growth performance and digestibility of nutrients. Similarly, other authors did not observe any significant changes in balance experiments with other animal species [5, 6].

Although both varieties had the similar crude protein content (9.73 and 9.66% dry matter), the GMO maize has lower content of albumin/globulin fraction (0.213 *vs.* 0.281% total N), and prolamines (0.168 *vs.* 0.213% total N) than isogenic (conventional) maize. Moreover, the GMO maize contains newly expressed enzyme/protein cp4 epsps. The literature shows that quality of protein in the diet can influence mineral absorption [7].

In our study, no differences of the copper concentration in organs were observed. Average values ranged: 10.4-11.4 mg/kg in liver, 15.4-21.0 mg/kg in kidney and 6.1-6.3 mg/kg in tibia. The low liver copper concentration may suggest marginal deficiency of copper in the diets [8, 9]. Unfortunately the feed copper content was not analyzed.

No differences in zinc concentration in organs between animals killed at the same time were observed (control group *vs.* group 1). Liver zinc content ranged from 69.0±20.1 (the control group) to 80.5±9.0 mg/kg (group 3). There was a decrease ($p \leqslant 0.05$) after three weeks of feeding with transgenic maize (group 2) in the zinc concentration in kidneys (42.2±6.6 mg/kg) as compared to the control group (63.1±18.3 mg/kg). However, all values were within the range of values estimated in the control rats (36.4-77.3 mg Zn/kg). In group 3, the kidney zinc concentration was similar to the mean of group 2 (46.2±10.6 mg/kg). After the three-week feeding with RR maize the bone zinc concentration was significantly higher (242±19 mg/kg) than in other groups (192-201 mg/kg). It seemed to be caused by the accumulation of mineral elements (e.g. calcium, phosphorus and zinc) in growing bones, as observed earlier [10, 11]. The concentrations of both trace elements in tested organs were always in the range found in the control rats (Fig. 1 and 2). Generally this study confirms the conclusions of our previous studies [3], that GM maize fed to animals does not affect the metabolism of trace elements.

Conclusion

The results of this study demonstrated that genetically modified RR maize (94% of the diet) used in this experiment (NK603) did not influence the zinc and copper retention in liver, kidney and femur bone in rats.

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