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# EFFECT OF INULIN-TYPE FRUCTANS ON BODY WEIGHT GAIN AND SELECTED BIOCHEMICAL PARAMETERS AT CALCIUM HYPOALIMENTATION IN RATS

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Key words: calcium deficiency, fructans, biochemical parameters, rat femur

The aim of the present study was to determine changes in body weight gain and the level of selected biochemical blood serum parameters under conditions of diminished calcium content in rat diet and after introducing inulin-type fructans into the diet. The effect of inulin or oligofructose on the content of calcium in femur was studied as well. The 21-day experiments were carried out on albinotic male Wistar rats. The animals were fed with semi-synthetic AIN'93 G diet with various amounts of calcium in a mineral mix ($Ca_{100}$, $Ca_{50}$, $Ca_{25}$) and containing inulin ($Ca_{50}$+I, $Ca_{25}$+I) or oligofructose ($Ca_{50}$+OF, $Ca_{25}$+OF) in conditions of Ca deficiency – 50% and 75%, respectively. Our results showed that the level of total and ionised calcium, magnesium and phosphorus in blood serum as well as Ca content in femur varied with the amount of Ca in rat diet. The intake of inulin-type fructans increased significantly Ca content in rat femur, both at 50% (33% w/w Ca in the $Ca_{50}$ group *vs.* 38% w/w for animals fed diet with inulin, and 37% w/w for oligofructose fed rats) and 25% of the recommended dietary calcium level (25% w/w $Ca_{25}$ group, 31% – after inulin intake, and 30% after oligofructose consumption).

## INTRODUCTION

Adequate calcium intake is critical to achieve optimal peak bone mass, and it influences the rate of bone loss associated with aging. The chance of improvement of calcium status can be seen in the increase of the amount of this element in diet or its bioavailability.

The stimulatory effects of fructans, non-digestible (due to presence of β-2-1 glycoside bonds) carbohydrates on minerals such as calcium, magnesium, zinc and iron absorption have been well documented so far [Scholz-Achrens *et al*., 2001b]. A stimulating effect of fructans on calcium absorption was demonstrated also for rats after ovariectomy [Scholz-Achrens *et al.*, 2001a] and in a conditions of magnesium deficiency [Ohta *et al.*, 1994], iron deficiency [Ohta *et al.*, 1995] and anaemia after gastrectomy [Ohta *et al.*, 1998] and in different ages [Coudray *et al.*, 2005b]. Taguchi *et al.* [1995] showed that short-chain oligofructose (2.5% and 5% in the diet) prevented bone loss in the ovariectomized rats. Lemort & Roberfroid [1997] showed an increased BMD (*bone mineral density*) in the growing rats fed with 5% or 10% inulin. Another studies [Scholz-Ahrens & Schrezenmeier, 2002] indicated that the intake of oligofructose led to an increase of bone trabecula perimeter.

Usually, only about 30% of the dietary calcium is absorbed. Improved calcium absorption in the intestine would have important consequences on the occurrence of osteoporosis and bone fractures. Moreover, the consumption of this mineral, especially among young people in many countries is insufficient. Hence, the assessment of the potential of fructans to prevent adverse effects of calcium deficiency is very important from the public health point of view.

Thus, the main aims of this study were: (1) to investigate in experiments on rats changes in their body weight gain and the level of selected biochemical parameters in blood serum and femoral bone in order to indicate consequences of calcium deficiency as well as (2) to determine the effect of including fructans into calcium-deficient diets on the level of these parameters.

## MATERIAL AND METHODS

### Animals and experimental conditions

Laboratory male Wistar rats with initial body weight of approx. 110 g, aged 5-6 weeks, were purchased from Laboratory Animals Husbandry in Trąbki (southern Poland) and randomised to appropriate feeding groups (6 rats in each group) as shown in Table 1. After a 7-day adaptive period, with *ad libitum* access to standard GLM-1 granulate and drinking water, 21-day experiments were carried out. Animals were housed individually in wire-bottomed stainless steel cages at a temperature (20-23°C), humidity (55--65%) and light (12:12-h day:night cycle) controlled room. Rats were fed with a semi-synthetic AIN'93 G diet according to Reeves [1997], with free access to deionised water. A restricted feeding level, *i.e.* 14±2 g of diet/animal/day was

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TABLE 1. The composition of diets.

| Ingredient (g/kg diet) | | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $Ca_{100}$ | $Ca_{50}$ | $Ca_{50}$+I | $Ca_{50}$ + OF | $Ca_{25}$ | $Ca_{25}$+I | $Ca_{25}$+OF |
| Cornstarch | | 532.486 | 532.486 | 432.486 | 432.486 | 532.486 | 432.486 | 432.486 |
| Inulin | | 0 | 0 | 100 | 0 | 0 | 100 | 0 |
| Oligofructose | | 0 | 0 | 0 | 100 | 0 | 0 | 100 |
| Mineral mix* | | | | | | | | |
| including | $CaCO_3$ (g/kg mix) | 375.00 | 187.50 | 187.50 | 187.50 | 93.75 | 93.75 | 93.75 |
| | starch (g/kg mix) | 0.00 | 187.50 | 187.50 | 187.50 | 281.25 | 281.25 | 281.25 |

*besides ingredients given in Table 1, each diet consisted of casein 200 g/kg, sucrose 100 g/kg, soybean oil 70 g/kg, fiber 50 g/kg, vitamin mix 10 g/kg, choline 2.5 g/kg, tert-butylhydroquinone 0.014 g/kg; ** composition of mineral mix (g/kg mix): potassium phosphate–196.00, potassium citrate–70.78; sodium chloride–74.00; potassium sulfate–46.60; magnesium oxide–24.00; ferric citrate– 6.06; zinc carbonate–1.65; manganous carbonate–0.63; cupric carbonate–0.30; potassium iodate–0.01; sodium selenate–0.01; ammonium molybdate–0.008; powdered sucrose– 204.952.

adopted. The feeding groups had various amounts of calcium in the mineral mix ($Ca_{100}$, $Ca_{50}$, $Ca_{25}$), while Ca deficiency in the mix was supplemented with starch (Table 1). Moreover, to assess the effect of fructans on selected parameters of calcium metabolism at Ca deficiency in diet, animals were fed with diets containing 10% inulin ($Ca_{50}$+I; $Ca_{25}$+I) or oligofructose ($Ca_{50}$+OF; $Ca_{25}$+OF). Fructans ("Raftiline", ORAFTI) or ("Raftilose", ORAFTI), respectively were introduced into diets instead of starch. The composition of experimental diets was listed in Table 1.

**Sampling procedures for analysis**

The experiment was approved by the Local Ethical Commision in Kraków (No. 75/OP/2002).

The rats were anaesthetised with an injection of 75 mg of thiopental/kg body weight, then their blood was withdrawn from the heart and centrifuged (10 min, 3000 rpm) to achieve serum. The right femur was removed and the middle part of each one was taken for analysis.

**Blood serum biochemical parameters**

Levels of total and ionised calcium, magnesium and phosphorus in the serum were determined with the use of an Olympus AU 400 analyser.

Calcium was measured using a BioVendor reagents system (No. 12101) with the colorimetric method. The level of ionised calcium (pH=7.4) was calculated as follows:

$$[\text{the amount of total Ca} + 0.02 \times \times (40 - \text{the amount of albumin}] \times 0.46.$$

Magnesium was determined colorimetrically by using a BioVendor reagents system (No. 12401). Inorganic phosphorus was determined colorimetrically with ammonium molybdate and a BioVendor reagents system (No. 11352).

**Determination of Ca content in rat femur**

*Preparation of bone samples*

After the right bones were dissected, the muscular tissues were removed and the middle part of each bone was taken for analyses. The exposed osseous tissue was obtained by traversely cutting of bony cuff with a razor blade and grinding the resulting surface with abrasive paper. The prepared samples were cleaned for 10 min in an ultrasound scrubber in 100% acetone.

Then, the bones were air dried for one hour and placed on graphite plates (Agar, Scientific, Stansted, Essex, United Kingdom) with carbon glue (Agar Scientific Limited, Stansted, Essex, United Kingdom). After drying, the bone sections were coated with a conducting lawyer (carbon) in a vacuum sublimator JEOL (JEE-4C, JVG-N1, Tokyo, Japan) to remove electric charges.

*Determination of Ca with a scanning electron microscope*

The prepared samples were analysed in a JSM 5410 scanning electron microscope (JEOL, Japan) equipped with the Noran 679A-3SES energy-dispersive spectrometer system (Noran Instruments, Inc. Middletown, WI, USA), with Voyager v 3.3. software. The following working conditions of the detector were applied: accelerating voltage: 20 keV, secondary electron detection system, specimen inclination angle 0°, acquiring angle: 25°, specimen distance from the detector: 40 mm, microscope working distance: 25 mm, and absorption current on pure aluminium: $4 \times 10^{-10}$ A. The spectra were obtained by focusing electron beam at 750x magnification and a live time of 100 s. There were about 2000 of X-ray quanta per second recorded by the detector.

The quantitative analyses based on the peak-to-background ratio were performed by using standard apatite for calcium, where 144.375 counts (*Net Counts*) corresponded to 39.74% w/w Ca.

**Statistical analysis**

All results were presented as mean values±SEM, and they were elaborated by means of one-way ANOVA. The Student's t-test was performed to compare groups. The Pearson correlation coefficients were calculated as well. Differences were considered statistically significant at $p<0.05$.

**RESULTS**

Body weight gain in the control group was significantly higher than in animals fed diet containing 25% of the recommended calcium dose ($Ca_{25}$), (Table 2). The total calcium serum level decreased significantly with diminishing amount of dietary calcium. It was shown that the level of ionised calcium was statistically higher (1.44 mmol/L) in the control group ($Ca_{100}$) than in the other animals – 1.37 mmol/L for $Ca_{50}$ and 1.34 mmol/L

TABLE 2. Body weight gain and the level of selected parameters in rat blood serum and calcium femoral bone depending on dietary calcium level.

| Group | Body weight gain (g) | | Total calcium in serum (mmol/L) | | $Ca^{2+}$ in serum (mmol/L) | | $Mg^{2+}$ in serum (mmol/L) | | P in serum (mmol/L) | | Ca in femur (w/w) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| A – $Ca_{100}$ | $64^a$ | 1.7 | $3.05^b$ | 0.056 | $1.44^b$ | 0.023 | $0.90^a$ | 0.024 | $2.77^a$ | 0.062 | $39^a$ | 0.7 |
| B – $Ca_{50}$ | $62^a$ | 2.7 | $2.73^a$ | 0.049 | $1.37^a$ | 0.009 | $1.48^b$ | 0.072 | $2.80^a$ | 0.091 | $33^b$ | 0.7 |
| C – $Ca_{25}$ | $54^b$ | 1.8 | $2.71^a$ | 0.058 | $1.34^a$ | 0.019 | $1.50^b$ | 0.030 | $3.10^b$ | 0.075 | $25^c$ | 1.0 |
| $F_{emp}$ | 6.35 | | 12.36 | | 8.83 | | 52.23 | | 5.83 | | 2.70 | |
| p | 0.05 | | 0.01 | | 0.01 | | 0.01 | | 0.05 | | 0.01 | |

a, b – the same letters mean the lack of statistically significant differences between groups ($p > 0.05$); $F_{emp.}$ – F empirical; p – level of significance; ns – not significant.

for and $Ca_{25,}$ respectively. The concentration of magnesium in blood serum of the animals from $Ca_{100}$ group fed with a diet of recommended calcium level significantly increased with decreasing calcium content. Moreover, an increase of phosphorus concentration in rats' blood with a decreasing amount of this element in the diet was observed (Table 2).

There were significant differences in calcium content in rat femur with increasing calcium deficiency in the diet. The highest level (39% w/w) of this element in the bone was recorded for the control group ($Ca_{100}$), as expected. Significantly lower values (33% and 25% w/w respectively) were obtained for groups with a lower calcium levels – ($Ca_{50}$) and ($Ca_{25}$), (Table 2).

To establish relationships between selected parameters the Pearson correlation coefficients were calculated. A significant positive correlation was observed between the content of calcium in femur, and the level of total calcium (r=0.63) and ionised calcium (r=0.60) in rat blood serum. In turn, negative correlations were found between the concentration of magnesium in blood and total calcium (r=-0.79) and ionised calcium (r=-0.68) in serum. At the level of 50% of the recommended calcium intake ($Ca_{50}$), body mass gain was lower in the groups fed a diet containing inulin ($Ca_{50}$+I) – 56 g and FOS ($Ca_{50}$+OF) – 57 g than in the animals from the $Ca_{50}$ group (Table 3). However, the differences between groups were not statistically significant. No significant results were observed in the case of total and ionised calcium in rat serum too, despite the highest amounts of this element in the group $Ca_{50}$+I. In contrast, magnesium ions concentration in serum was significantly lower (1.30 mmol/L) in rats fed with inulin than in the others (1.48 mmol/L and 1.40 mmol/L for $Ca_{50}$ and $Ca_{50}$+OF, respectively). There were no statistically significant changes in the level of phosphorus between groups, but only a tendency to gain higher results for $Ca_{50}$+OF group (Table 3). In the diet with 50% of the recommended dietary calcium level, inulin increased significantly the content of calcium in rat femur (33% w/w in the control group *vs.* 38% w/w for animals fed diet with inulin). Calcium content in bone was higher under oligofructose intake than in the control rats (Table 3). Under these conditions of experiment, a positive correlation was observed between the blood serum concentration of ionised calcium and phosphorus (r=0.53).

Under conditions of 75% dietary calcium deficiency ($Ca_{25}$), adding fructans to rat diet did not cause any significant changes in body weight gain or blood serum biochemical parameters (Table 4). The exception was only the content of Mg in serum – it was statistically higher (1.48 mmol/L) in animals fed a diet without fructans ($Ca_{25}$) than in the $Ca_{25}$+I group (1.30 mmol/L). Moreover, a tendency was observed for a decrease in the level of phosphorus in blood in $Ca_{25}$+I group as compared with the others animals, but the differences were not statistically significant (Table 4). It was observed that calcium content in rat femur was dependent on the presence of inulin-type fructans in the $Ca_{25}$ diet. Femoral Ca level in this group was 25% w/w and significantly increased to 31% w/w after inulin intake and to 30% w/w after feeding oligofructose (Table 4).

TABLE 3. Body weight gain and the level of selected parameters in rat blood serum and calcium femoral bone under conditions of 50% calcium deficiency and presence of inulin-type fructans in rat diet.

| Group | Body mass gain (g) | | Total calcium in serum (mmol/L) | | $Ca^{2+}$ in serum (mmol/L) | | $Mg^{2+}$ in serum (mmol/L) | | P in serum (mmol/L) | | Ca in femur (w/w) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| A – $Ca_{50}$ | $62^a$ | 2.7 | $2.73^a$ | 0.049 | $1.37^a$ | 0.009 | $1.48^a$ | 0.072 | $2.80^a$ | 0.091 | $33^a$ | 0.7 |
| B – $Ca_{50}$+I | $56^a$ | 4.0 | $2.84^a$ | 0.054 | $1.39^a$ | 0.022 | $1.30^b$ | 0.065 | $3.11^a$ | 0.171 | $38^b$ | 0.8 |
| C – $Ca_{50}$+OF | $57^a$ | 3.0 | $2.75^a$ | 0.073 | $1.38^a$ | 0.011 | $1.40^a$ | 0.060 | $3.19^a$ | 0.109 | $37^b$ | 0.5 |
| $F_{emp}$ | 0.86 | | 0.92 | | 0.50 | | 1.83 | | 5.83 | | 4.71 | |
| p | ns | | ns | | ns | | ns | | 0.05 | | 0.05 | |

a, b – the same letters mean the lack of statistically significant differences between groups ($p > 0.05$); $F_{emp.}$ – F empirical; p – level of significance; ns – not significant

TABLE 4. Body weight gain and the level of biochemical parameters in blood serum under conditions of 75% calcium deficiency and presence of inulin-type fructans in rat diet.

| Group | Body weight gain (g) | | Total calcium in serum (mmol/L) | | $Ca^{2+}$ in serum (mmol/L) | | $Mg^{2+}$ in serum (mmol/L) | | P in serum (mmol/L) | | Ca in femur (w/w) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| A – $Ca_{25}$ | 54[a] | 1.8 | 2.71[a] | 0.058 | 1.34[a] | 0.019 | 1.48[a] | 0.072 | 1.50[a] | 0.030 | 25[a] | 0.7 |
| B – $Ca_{25}$+I | 49[a] | 1.8 | 2.76[a] | 0.063 | 1.37[a] | 0.016 | 1.30[b] | 0.065 | 1.44[a] | 0.056 | 31[b] | 0.8 |
| C – $Ca_{25}$+OF | 51[a] | 2.6 | 2.72[a] | 0.087 | 1.38[a] | 0.022 | 1.40[a] | 0.060 | 1.48[a] | 0.019 | 30[b] | 0.5 |
| $F_{emp}$ | 1.52 | | 0.14 | | 1.14 | | 1.83 | | 0.73 | | 6.68 | |
| p | ns | | ns | | ns | | ns | | ns | | 0.01 | |

a, b – the same letter means no statistically significant differences between groups ($p > 0.05$); $F_{emp.}$ – F empirical; p – the level of significance; ns – not significant.

A positive correlation was found between serum concentrations of phosphorus and magnesium (r=0.53).

## DISCUSSION

### Body weight gain in rats depending on calcium content and fructans presence in their diet

It was observed that decrease in the amount of dietary calcium level lowered body weight gain, but significantly only on the level of 25% recommended dose of this mineral, probably due to disturbances in homeostasis (Table 2). However, the feeding experiment performed by Creedon & Cashman [2001] indicated that mean body weight gain by young growing rats (with initial weight of 102 g) was unaffected by dietary Ca concentration; lowered, normal or twice higher than the recommended value.

There was no significant difference in body weight gain between groups after inulin-type fructans intake both at 50% and 75% deficiency. There was only a tendency to decrease animal body gain after inulin-type fructans intake, probably because of the lower caloric value of these diets. However, obtained results are consistent with those reported by Takahara *et al.* [2000], who did not observe significant changes in body weight after feeding animals with a diet containing fructans. Similar results were obtained also by Trautwein *et al.* [1998] on hamsters, and Younes *et al.* [2001] after inulin and resistance starch intake. Just the opposite, studies of Roberfroid *et al.* [2002] showed the decrease in body weight gain by 5-7% in rats fed with diet containing 10% of inulin compared to control. Similar results were obtained by Daubioul *et al.* [2000], and Delzenne *et al.* [2000]. However, Daubioul *et al.* [2000] observed such effects after 7 weeks. Possibly, longer time of presence of fructans (especially inulin) in intestinal epithelium could cause statistically significant differences between the conditions of experiments presented in this paper.

### Biochemical parameters in blood serum depending on calcium level and the presence of inulin-type fructans in rat diet

It was shown that total calcium in blood serum lowered with decreasing the calcium content in rat diet, especially at 75% deficiency of this mineral (Table 2). Rummens *et al.* [2000] showed that calcium deficient diet led to hypocalcemia in guinea pigs.

From a biological point of view, the concentration of ionised calcium is the most important indicator of an adequate amount of this mineral in the rats organism. It was found that the concentration of ionised calcium significantly decreased with lowering the amount of dietary Ca in a rat diet, both at 50% and 75% Ca deficiency (Table 2). The studies carried out by Tordoff *et al.* [1998] showed that calcium deficiency led to decrease in concentration of total, bounded and ionised calcium, while the fastest changes were observed for $Ca^{2+}$ in blood serum.

With decreasing amount of calcium in diet, a significant increase of magnesium concentration in blood serum was observed, by 64% and 67% within groups of 50% and 25% recommended calcium intake, respectively (Table 2).

As expected, negative correlation coefficients were found for concentration of magnesium and calcium, due to the fact that both these elements are probably absorbed by the same mechanism (thus relative overabundance of one element inhibits absorption of another). A statistically significant increase of phosphorus concentration in blood serum was observed in animals fed with a diet containing 50 and 25% of calcium (Table 2). Such changes of phosphorus concentration in blood serum may have resulted not only from decreased amount of calcium but also from disproportionate amount of calcium and phosphorus in diet caused by removing calcium carbonate from the mineral mix (while maintaining an unchanged level of potassium phosphate).

There were no significant differences between groups in the total and ionized Ca concentration in serum after inulin-type fructans intake with diets at the level of $Ca_{50}$ as well as $Ca_{25}$. Only a tendency to increase the level of this element in the presence of inulin was observed (Tables 3 and 4) at lowered dietary calcium levels.

### Changes of Ca content in rat femur depending on amount of calcium and the presence of inulin-type fructans in rat diet

Our results showed that lowering the calcium amount in diet led to a statistically significant decrease of this mineral content in rat femur – from 39% w/w within control group of recommended calcium intake through 33% w/w for 50% deficiency to 25% w/w in animals fed with a diet containing

1/4 of the recommended Ca intake (Table 2). It is known that up to 99% calcium occur in bones and other calcified tissues, thus this element is necessary for development and maintenance of the proper condition of bones [Cashman & Flynn, 1999]. Based on animal tests a linear relationship between the mass of femoral bone and calcium concentration in diet was found [Matkovic *et al.*, 1995], while reduced calcium level led to a decrease in Ca concentration in femoral bone [Persson *et al.*, 1993; Takeda *et al.*, 1993; Peterson *et al.*, 1995]. It was observed that Ca deficient diet increases bone resorption [Ginty *et al.*, 1998; Talbott *et al.*, 1999], decreases bone mass [Talbott *et al.*, 1999] and increases the risk of osteoporosis [Heaney, 1996].

Calcium is used in the calcification process, and it is present in bones mainly in the form of hydroxyapatite [$(Ca_{10}(PO_4)_6(OH)_2$] [Heaney, 1996]. Thus, an increase of Ca concentration could be related to the size of hydroxyapatite crystals. One may conclude that reduced amount of calcium in rat diet led to hypocalcemia. Calcium deficiency in diet, affecting intestinal calcium absorption, could be insufficient to inhibit activity of parathormone.

Inulin-type fructans intake at 50% as well as 75% calcium deficiency in diet increased Ca content in the bone (Table 3 and 4). The obtained results are consistent with those of Scholz-Ahrens *et al.* [2002] who showed that oligofructose increases minerals content of the femur. Roberfroid *et al.* [2002] reported also an positive effect of inulin on bone mass content and density in growing rat males at various calcium levels in diet (0.2, 0.5 or 1 g/100 g). Nzeusseu *et al.* [2006] suggested that, although both inulin and oligofructose have a positive effect on bone mass density; the greatest effect of inulin is related to the higher capacity of this fructan to reduce bone resorption. Raschka & Daniel [2005] concluded that inulin-type fructans at dietary levels of 10% do increase mineral absorption, retention and accumulation in bone in the case of Ca, Mg and Zn but when the mineral demand is particularly high as during growth. Scholz-Ahrens & Schrezenmeier [2002] showed that oligofructose can prevent reduction of bone mineral concentrations by lowering pH in large intestine. It is possible that bone resorption is slightly reduced by increasing calcium absorption due to inulin-type fructans intake, although it does not exclude that these sugars enhance osteogenesis, too. Takahara *et al.* [2000] concluded that fructans have an effect on bone local structure. It is also possible that inulin-type fructans increase $K_m$ constant, thus increasing calcium level in blood serum and in turn limiting parathormon activity.

One can conclude that an increasing calcium content in rat femur may result from improved calcium absorption from diet containing inulin-type fructans. Several animal studies showed enhancement of calcium absorption by inulin-type fructans. Several hypothesis have been suggested to explain how inulin-type fructans enhance calcium absorption. First, an increased calcium solubility in the colon due to pH reduction as a consequence of fructans fermentation; second, osmotic effects increasing fluid transfer in the colonic lumen and, an increased permeability between intracellular enterocyte junctions; third, calcium/hydrogen exchange in the colon activated by absorption of short-chain fatty carboxylic acids; fourth an increase of the calbindin D9k protein [Roberfroid *et al.*, 2002]. The last one, calbindin-D9k (CaBP) is a cholecalciferol-induced calcium-binding protein with a high affinity for calcium. The production of CaBP is stimulated by dietary vitamin D or calcium restriction, so CaBP is thought to play an important role in intestinal calcium transport.

In the present study, inulin intake showed a tendency to increase femoral calcium content more than in case of oligofructose. The different antiresorptive capacity of inulin and oligofructose might be related to their different impact on calcium absorption and bioavailability since the increase in the amount of calbindin-9K in the cecum. Nzeusseu *et al.* [2006] confirmed that this protein level was higher in rats fed inulin than in animals fed oligofructose.

Our study was a short-term experiment. Further studies are required to determine whether the effect observed in presented study persists with long-term use. Coudray *et al.* [2005a] determined how the short- and long-term dietary calcium intake modulated the effect inulin on absorption of this mineral in rats. The increasing effect of inulin on Ca absorption depended on dietary Ca level and on experiment duration.

## CONCLUSIONS

Our results showed that lowering the amount of calcium in a diet decreased significantly body weight gain of rats. As a result of disturbances in calcium homeostasis, the total and ionised calcium in blood serum decreased, too. It was observed that deficiency of this mineral in diet led to a decrease of the content of Ca in rat femur.

It was shown that inulin-type fructans intake tended to increase calcium level in serum, both at 50% and 75% deficiency of this element in the diet. It was also observed that fructans, both at 50% and 25% of the recommended calcium intake, increased the calcium content in the femur. When analysing the obtained results one can conclude that increasing calcium content in rat femur indicates an improved absorption of this mineral from a diet containing inulin-type fructans.

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