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INULIN AND OLIGOFRUCTOSE INFLUENCE ON THIAMINE LEVEL IN RAT BLOOD SERUM AND URINE

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The aim of the study was to estimate the effect of 5% and/or 10% of inulin and/or oligofructose on thiamine level in blood serum and urine. The vitamin was administered in doses of: 20 or 40 μg/day/rat. With respect to the control (fructan-free), the diet containing 5% of inulin decreased ($p<0.05$) serum thiamine level when the lower daily vitamin dose was used, and oligofructose had no influence. It was observed that all experimental diets decreased urine thiamine level ($p<0.05$), which remained stable until the 9th day of the experiment. Then, after next 3 days, the urine thiamine increased, except for the I-5 group with 20 μg daily thiamine dose. The diet with 5% of oligofructose increased ($p<0.05$) average daily thiamine excretion when rats consumed 20 μg of vitamin per day. Oligofructose was demonstrated to be capable of improving thiamine nutritional status in thiamine-malnourished rats.

INTRODUCTION

Inulin and oligofructose as prebiotics induce changes in colonic epithelium stimulating proliferation in the crypts, increasing the concentration of polyamines, changing the profile of mucins, modulating endocrine and immune functions [Roberfroid, 2005]. Simultaneously they stimulate the growth of bacteria synthesizing thiamine [Nishizawa, 1960], that can be absorbed to colonocytes [Said *et al*., 2001; Said, 2003; Said & Zainab, 2006]. In microbes, thiamine is involved in the regulation of the expression of the genes required for its own synthesis and import. In *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Salmonella typhimurium* and *Aspergillus parasiticus,* the absence of thiamine in the growth medium results in thiamine biosynthetic genes being expressed, thereby allowing the vitamin to be synthesized during deficient conditions [Maundrell, 1990; Nishimura *et al.,* 1992; Cary & Bhatnagar, 1995; Webb *et al*., 1996; Serganov *et al.,* 2006]. Thus it is likely that the thiamine deficient-diet may induce an intense vitamin production by colon microflora. Inulin and oligofructose have been reported to improve thiamine status, expressed by its serum and urine concentrations, in thiamine deficient rats [Drywień & Koźlicka, 2003; Drywień, 2005]. The importance of thiamine colon synthesis, in the presence of dietary vitamin, is unknown.

The aim of our study was to examine inulin and oligofructose influence on thiamine level in urine and blood serum, as nutritional status markers, after dietary thiamine administartion.

MATERIALS AND METHODS

The experiment was conducted for 15 days (3 days – adaptation period, 12 days – main period) on 60 male Wistar rats weighing 100±10 g. The animals were allocated to two blocks depending on daily thiamine dose (20, 40 μg). In each block, the rats were subdivided into 5 groups (6 rats each) – FF – control group, fructan-free diet; experimental groups, diets containing 5% (I-5), 10% (I-10) of inulin or 5% (OF-5), 10% (OF-10) of oligofructose.

The rats were fed a casein diet according to AIN-93M [Reeves, 1997]. Experimental diets were supplemented with 5% or 10% inulin (RAFTILINE GR; Orafti) or 5% or 10% oligofructose (RAFTILOSE P95; Orafti). The supplements were added instead of wheat starch. The diets were isocaloric and thiamine-free, and a water solution of thiamine hydrochloride (2 drops) was administered to rats *per os*.

The concentrations of total thiamine in the blood serum and free thiamine in urine were determined by modified fluorimetric method [Leveille, 1972] with the use of a photofluorimeter (KONTRON INSTRUMENTS) equipped with a computer software SFM 25.

The results were analysed using multifactor (dose of fructan/vitamin) and one-way ANOVA, and significant differences between groups were determined by Tukey multiple range test at $p\leq0.05$ (SPSS 12.0 PL for Windows software).

RESULTS AND DISCUSSION

The effect of prebiotics on serum thiamine concentration was observed when 20 μg of vitamin per day was applied

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(Figure 1). The inulin diets decreased serum thiamine level and for diet containing 5% of inulin the drop was significant ($p<0.05$). The oligofructose diets were favourable to the maintenance of a higher serum thiamine level as compared to inulin ($p<0.05$). The serum thiamine concentrations were not influenced by prebiotics when 40 μg of vitamin/day/rat was served.

During the experiment, the daily thiamine doses were

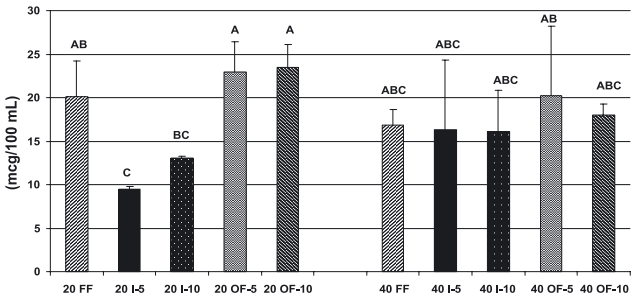

FIGURE 1. The prebiotic influence on serum thiamine level.

stable – 20 or 40 μg/rat and the rate of urine excretion was at a similar level until the 9th day (Figure 2). Then, after next 3 days the urine thiamine increased ($p>0.05$), except for the I-5 group with 20 μg daily vitamin dose. Results are similar to those obtained in an earlier investigation, when thiamine was not administered [Drywień, 2005]. It could prove that 9-day application of prebiotics stimulates the growth of gut microflora, thus the thiamine synthesis could be significant for rats.

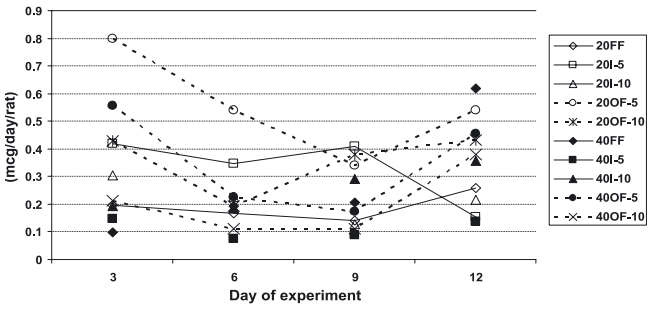

FIGURE 2. Rate of thiamine excretion.

The average daily thiamine excretion was highly influenced by the type and dose of prebiotic ($p<0.01$) when rats were administered with 20 μg of thiamine/day (Figure 3). The diet with 5% of oligofructose increased urine thiamine significantly, as compared to the control diet. Similar differences, but not so strong, were noticed against inulin diets. The effect of prebiotic was not significant when 40 μg of thiamine was administered but the 5% oligofructose diet influence was observed. At high thiamine intakes most of the excess is rapidly excreted with urine [Davis *et al.,* 1984] and is a good indicator of thiamine nutritional status. The higher average urine excretion of thiamine was noticed when the deficient vitamin dose (20 μg /day) was applied to rats ($p<0.05$), (Figure 4). It is known that thiamine deficiency increases its absorption [Laforenza *et al*., 1997] and prob-

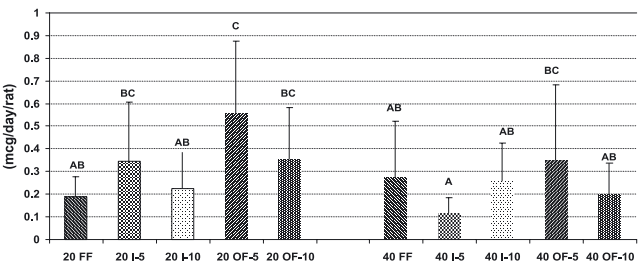

FIGURE 3. The prebiotic influence on average daily urine thiamine excretion.

Means marked with different letters differ significantly ($p<0.05$)

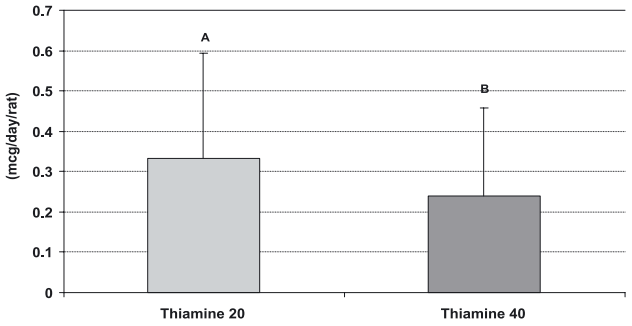

FIGURE 4. The daily thiamine dose influence on average urine thiamine excretion.

Means marked with different letters differ significantly ($p<0.05$)

ably might enhance the utilization of thiamine produced by colon microflora. A similar dependence was observed when thiamine was not administered at all [Drywień, 2005].

The high urine thiamine excretion corresponds to unchanged serum level of this vitamin when 20 μg thiamine and oligofructose diets were administered. It could be a proof that oligofructose enhances thiamine status in rats during vitamin deficiency. Inulin decreased both of these markers, as compared to oligofructose. These variations between prebiotics are likely to result from their different physical properties. Inulin, as a long-chain fructan, is less soluble than oligofructose and could inhibit the thiamine absorption from the diet. This effect is evident when the daily thiamine dose is lower than the recommended level (20 μg/day/rat). Besides, the oligofructose, as better fermentable in the colon, could increase microbial synthesis of thiamine, which is absorbed to the organism.

CONCLUSIONS

Oligofructose, especially its 5% supplement, was demonstrated to be capable of enhancing the nutritional status of thiamine in thiamine-malnourished rats.

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WPŁYW INULINY I OLIGOFRUKTOZY NA ZAWARTOŚĆ TIAMINY W MOCZU I OSOCZU KRWI U SZCZURA

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Przeprowadzono 15-to dniowe doświadczenie z wykorzystaniem szczurów (samców) szczepu Wistar. Badano wpływ diet (pozbawionych tiaminy) zawierających 5% i/lub 10% inuliny i/lub oligofruktozy na zawartość tiaminy w osoczu krwi i moczu dobowym. Tiaminę podawano *per os* w dawkach dziennych 20 lub 40 µg/szczura. W wyniku przeprowadzonych badań stwierdzono istotne obniżenie koncentracji tiaminy w osoczu krwi szczurów karmionych dietą z 5% zawartością inuliny i otrzymujących 20 µg witaminy, przy nie zmienionym poziomie wydalania witaminy z moczem. Dieta z dodatkiem 5% oligofruktozy istotnie zwiększała średnią zawartości tiaminy w moczu dobowym, bez wpływu na jej zawartość w osoczu, gdy zwierzęta otrzymywały niższą dawkę witaminy. Wyniki doświadczenia wskazują, że 5% dodatek oligofruktozy do diety może poprawiać stan odżywienia tiaminą w warunkach jej niedoboru.