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EFFECT OF INULIN AND OLIGOFRUCTOSE ON CAECAL pH DURING VARIOUS THIAMINE DOSES ADMINISTRATION IN RATS

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Key words: inulin, oligofructose, caecal pH, thiamine

A 15-day experiment was performed on male Wistar rats allocated into three blocks diversified by a daily thiamine dose: 0, 20, 40 μg/day/rat. In each block the rats were divided into five groups depending on the type of diet: control group (FF) – fructan-free diet; experimental groups (I-5, I-10, OF-5, OF-10) – diets containing: 5%, 10% of inulin, as well as 5%, 10% of oligofructose respectively. Thiamine-free experimental diets were prepared according to AIN-93M recommendations, where inulin and oligofructose were added instead of wheat starch. The thiamine was administered *per os* as a water solution. Compared to the initial value, caecal pH decreased in all groups of rats. The daily thiamine dose as well as the kind of fructan influenced caecal pH. There could be observed the synergistic action of inulin and thiamine in decreasing pH. Inulin and oligofructose, due to prebiotic properties, can cause fluctuations in the caecum pH but the direction of changes is closely dependent on the presence of dietary thiamine. The most suitable pH values for endogenous thiamine uptake are reported during dietary deficit of this vitamin, independently on the type and dose of fructan.

INTRODUCTION

Inulin and oligofructose, as prebiotics, selectively stimulate the growth and/or activity of specific strains of bacteria in the colon. Mainly an increase in the number of *Bifidobacterium* and *Lactobacillus* is observed upon their action [Buddington *et al.,* 2002]. Inulin and oligofructose are not digested by small intestine enzymes and in unchanged form pass to the colon [Molis *et al.,* 1996].

The colon is a principal site of fructans fermentation by microbes. Short Chain Fatty Acids (SCFA) are fermentation products responsible for colonic pH [Campbell *et al.,* 1997; Cummings *et al.,* 2001]. Bacteria growing in the presence of inulin and oligofructose produce thiamine that can be absorbed by the host [Cummings *et al.,* 2001; Drywień & Koźlicka, 2003]. In microbes, thiamine is involved in the regulation of the expression of genes required for its own synthesis. 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]. Thus the thiamine-deficient diet may induce an intense vitamin production by colonic microflora.

Thiamine plays an important role in carbohydrate metabolism. Nutritional deficiencies of thiamine can lead to cardiovascular (peripheral vasolidation, biventricular myocardial failure, edema, potentially acute fulminant cardiovascular collapse) and neurological disorders (confusion, disordered ocular motility, neuropathy, ataxia of gait). Humans and other mammals have lost their ability to synthesize thiamine, and thus, must obtain the vitamin from exogenous sources [Reidling *et al.,* 2002]. Absorption of dietary thiamine takes place primarily in the proximal part of the small intestine [Rindi & Laforenza, 2000].

Recent investigations have shown that endogenous thiamine, microbially synthesized in the colon, can be utilized by organism as an additional source of that vitamin, apart from the dietary thiamine.

Said *et al.* [2001] demonstrated in an *in vitro* study the existence of a specialized carrier-mediated mechanism for thiamine uptake by human colonocytes. This system appears to involve a thiamine/H^+ exchange mechanism and appears to be under the regulation of intracellular Ca^{2+}/calmodulin-mediated pathway. They suggested that extracellular and intracellular pH of colonocytes may play a important role in this process. Thus, the pH of colonic digesta could be one of important factors affecting thiamine uptake in this part of the alimentary tract.

The reported study was aimed at determining inulin and oligofructose influence on rat caecum pH during deficit and administration of various thiamine doses in the context of thiamine uptake possibility.

MATERIALS AND METHODS

The experiment was conducted for 15 days (3 days – adaptation period, 12 days – main period) on 90 male Wistar rats weighing 100±10 g. The animals were allocated to three blocks depending on daily thiamine dose: 0, 20, 40 μg (40 μg

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thiamine/day was found as the lowest recommended dose [Rains *et al.,* 1997]). In each block, the rats were subdivided into 5 groups (each of 5 rats in "0" block; of 6 ones in "20" and "40" blocks): a control group fed a fructan-free diet (FF); and four experimental groups fed diets containing 5% (I-5) and 10% (I-10) of inulin (RAFTILINE GR; Orafti) or 5% (OF-5) and 10% (OF-10) of oligofructose (RAFTILOSE P95; Orafti). Before the experiment, the opening caecal pH for 5 rats was measured (opening group O).

The rats were fed a casein diet according to AIN-93M [Reeves, 1997]. The supplements were added instead of wheat starch. The diets were isocaloric and thiamine-free. In the 20- and 40-experimental blocks an aqueous solution of thiamine hydrochloride (2 drops) was administered to rats *per os*.

The caecal pH was measured *post mortem* directly in the caecum using a pH-meter (ELMETRON CP-401) with a gel electrode (METRON OSH 12-00).

The results were analysed using multifactor (dose of fructan/vitamin) and one-way ANOVA, and significant differences between the groups were determined with the Tukey multiple range test at $p \leq 0.05$ (SPSS 12.0 PL for Windows software).

RESULTS AND DISCUSSION

Two-way analysis of variance revealed a significant interaction between the factors studied: fructans and thiamine. A decrease of caecal pH value was observed in all animals compared to the opening group ($p<0.05$), (Figures 1 and 2). However, the daily thiamine dose and the kind of prebiotic significantly influenced the rat caecal pH value, there was no difference between 20 and 40 μg doses of thiamine. It seems that the presence of this vitamin itself was more important for pH value than its amount (Figure 1).

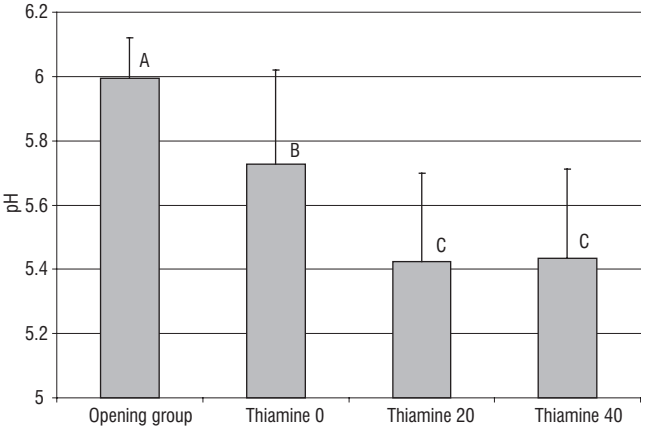

FIGURE 1. Daily thiamine dose influence on caecal pH values.
Means denoted with different letters differ significantly ($p \leq 0.05$)

The caecal pH in FF, I-5 and I-10 groups of thiamine-deficient animals was higher ($p>0.05$) than in animals receiving thiamine (Figure 3). The daily thiamine dose had no effect when oligofructose was added to the diets. Probably the inulin along with thiamine contributed to an increase in the number of caecal microflora, thus the digesta environment was modified, which resulted in pH decrease. Compared to inulin, oligofructose enhanced the caecal pH when thiamine was administered.

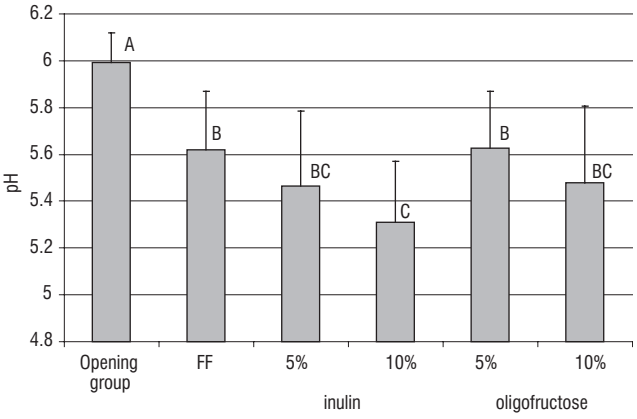

FIGURE 2. Prebiotics influence on caecal pH values.
Means denoted with different letters differ significantly ($p \leq 0.05$)

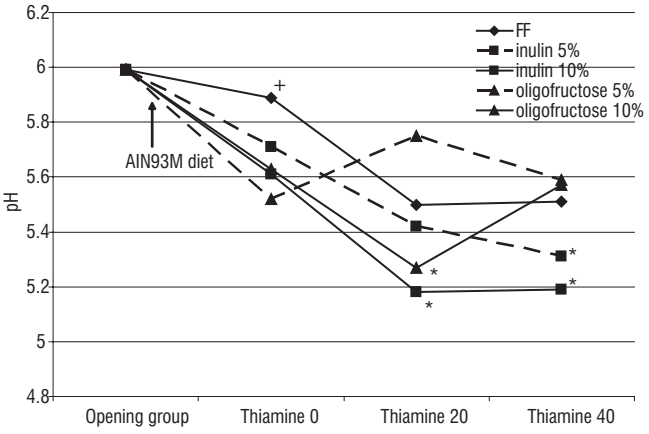

FIGURE 3. Caecal pH values in rats fed inulin and oligofructose diets and different thiamine doses.
Means denoted with „*" differ significantly ($p \leq 0.05$) from means denoted with "+".

The fructan-free diet as well as experimental ones decreased the caecal pH in comparison to the value recorded for the opening group. The pH drop may result from a change of diets and the 5% potato starch addition as a non-digestible rest. In a similar experiment with control diets containing 2% or 4% of cellulose, the values of pH reached 7.5 and 7.2, respectively [Le Blay *et al.,* 1999; Juśkiewicz & Zduńczyk, 2004].

Compared to the fructan-free diet, inulin and oligofructose decreased, but not significantly, the caecal pH once thiamine was absent. However the obtained pH values of 5.52–5.71 were lower than those reported by other authors [Kleessen *et al.,* 2001; Juśkiewicz & Zduńczyk, 2004; Zduńczyk *et al.,* 2004]. According to Le Blay *et al.* [1999], the diet containing 9% of fructooligosaccharides (FOS) decreased caecal pH to 5.6 (against 7.5 in the control), after 2-week administration. Probably, it was an effect of a high lactate concentration in the caecum after 2 weeks of FOS ingestion. This concentration decreased over time, and after 8 weeks of adaptation the pH rose to 6.8. This could be an effect of lactate metabolizing into Short Chain Fatty Acids (SCFA) by intestinal bacteria, such as *Propionibacterium* sp.*, Veillonella* sp.*, Clostridium*

sp. and sulfate-reducers [Macfarlane *et al*., 1994; Durand *et al.,* 1996]. In this study, it can be stated that inulin and oligofructose, after 2-week administration, influenced the environment of rat caecum, similarly to FOS.

The addition of 10% of inulin significantly decreased the caecal pH, irrespectively of a daily dose of thiamine, and 5% of oligofructose increased pH at thiamine dose of 20 μg. Compared to the fructan-free diet, changes in the other groups were not significant. Zduńczyk *et al.* [2004] obtained similar caecal pH values (5.47–5.81) upon administration of diets containing inulin and lactulose. According to other authors, the oligofructose decreased pH to a greater extent than inulin, which depends on SCFA composition as a consequence of microorganisms variations [Roberfroid *et al.,* 1998; Gibson, 1999].

On the basis of this study it can be stated that thiamine dose as well as the type of fructan separately had a negligible effect on caecal pH, but synergistic action of inulin and thiamine in pH decreasing could be observed.

Dietary inulin and oligofructose increase thiamine production in the colon through stimulation of bacteria growth and modification of colon environment [Nishizawa, 1960; Liescher, 1961; Drywień, 2005]. Said *et al.* [2001] found a direct relationship between extracellular and intracellular pH of colonocytes as well as between amount and rate of thiamine absorption. The SCFA produced by intestinal bacteria, first acidify the digesta but their uptake to colonocytes causes alkalization of digesta and decrease of the intracellular pH. The rise of extracellular pH value from 5.0 to 6.5 resulted in a twofold increase of thiamine uptake into colonocytes. Therefore, every change of pH values could affect the amount of absorbed thiamine. This relationship, confirmed by Chu & Montrose [1997], generates beneficial conditions for thiamine uptake in the colon.

Taking into consideration the results of this study as well as findings of Said *et al,* [2001], the most favourable conditions for thiamine uptake in the colon were during dietary vitamin deficiency, irrespective of the type and dose of prebiotic. My previous investigations demonstrated that the supplementation of thiamine-deficient diets with fructans enhanced the excretion of endogenous thiamine in urine and decreased its excretion in faeces in rats [Drywień, 2005].

CONCLUSIONS

Inulin and oligofructose, due to prebiotic potential, can evoke fluctuations in the caecum pH but the direction of changes is closely dependent on the presence of dietary thiamine.

In the context of findings reported by Said *et al.* [2001], the most suitable pH values for endogenous thiamine uptake are reported during dietary deficit of this vitamin, independently on the type and dose of fructan.

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WPŁYW INULINY I OLIGOFRUKTOZY NA pH TREŚCI JELITA ŚLEPEGO SZCZURÓW W WARUNKACH DEFICYTU ORAZ PODAWANIA ZRÓŻNICOWANYCH DAWEK TIAMINY

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Przeprowadzono 15-to dniowe doświadczenie z wykorzystaniem szczurów (samców) szczepu Wistar. Obejmowało ono trzy bloki zróżnicowane pod względem dawki tiaminy: 0, 20, 40 μg/dzień/szczura. W każdym z nich zwierzęta podzielono na grupy zależnie od rodzaju podawanej diety: grupa kontrolna (FF) – dieta bez prebiotyku; grupy badane (I-5, I-10, OF-5, OF-10) – diety zawierające odpowiednio: 5%, 10% inuliny lub 5%, 10% oligofruktozy. Diety doświadczalne, pozbawione tiaminy, przygotowano zgodnie z zaleceniami AIN-93M, w których część skrobi pszennej zastąpiono odpowiednimi dodatkami w/w prebiotyków. Tiaminę podawano *per os* w postaci roztworu wodnego.

Na podstawie uzyskanych wyników stwierdzono, że pH treści jelita ślepego obniżyło się we wszystkich badanych grupach w porównaniu do grupy wyjściowej, która otrzymywała paszę bytową granulowaną (rys. 1 i 2). Zarówno dawka jak i rodzaj prebiotyku wpłynęły na pH treści jelita ślepego szczura. Zaobserwowano również synergistyczne działanie inuliny i tiaminy w obniżaniu pH (rys. 3). Inulina i oligofruktoza, dzięki prebiotycznym właściwościom, mogą zmieniać pH treści jelita ślepego szczurów ale kierunek zmian jest ściśle uzależniony od obecności tiaminy pokarmowej. Najodpowiedniejsze wartości pH dla wchłaniania tiaminy endogennej stwierdzono w warunkach deficytu tej witaminy w diecie, niezależnie od rodzaju i ilości fruktanu.