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Diet supplementation with flaxseed stimulates gut metabolism in mice

Introduction

The close relationship between intestinal microbiota and health and diseases has aroused a great interest in the use of probiotics and prebiotics in the nutrition of humans and animals. The main reason is the modulation of digestive processes via increased counts of beneficial bacteria and their enzymatic activity (Axling et al., 2012; Borovská et al., 2013). One of the possibilities of increasing the effect of beneficial bacteria is combining them with polyunsaturated fatty acids (PUFAs) that are an integral component of cellular membranes and are able to modify the adherence of bacteria to intestinal epithelium (Borovská et al., 2013). Under our conditions, one of the richest plant sources of ω-3 PUFAs is the flaxseed (*Linum usitatissimum* L.). Of total fatty acids (FA) found in flaxseed oil, 9% are saturated, 18% monounsaturated, and 73% polyunsaturated FA containing 50–61% of ω-3 PUFA α-linolenic acid. At the same time, flaxseed is a rich source of fibres and the soluble (slimy) fibrous component is considered functional (Borovská et al., 2013; Martinez et al., 2013). In the digestive tract, it acts as a prebiotic and creates a suitable environment for bacteria beneficial to health.

The aim of the study was to investigate the effects of the fortification of the diet with flaxseed in a model experiment on mice with a focus on intestinal metabolism.

Material and methods

Plant material

The experiment was approved by the State Veterinary and Food Administration of the Slovak Republic (No 1177/14-22) and carried out on 24 mice of BALB/c line, 5 weeks old, allocated to two groups: control group (K^-; n = 12) fed a standard diet for mice

ST–1 (Altromin 1311; Velaz; Czechia) at a dose of 1.75 g/head/day; and the experimental group (MK; n = 12) fed diet supplemented with crushed flaxseed variety Flanders for the period of 35 days, at 5% concentration in feed. Faeces of animals were collected and examined during the experiment and before euthanasia. The animals were killed by cervical dislocation and caecum samples were collected for the determination of the concentration of organic acids using the method of capillary isotachophoresis (ITP).

Statistical analysis

The results obtained were evaluated statistically using software GraphPad Prism 3.0 for Windows (GraphPad Software, San Diego, California, USA). The dynamics of changes in individual parameters were evaluated by Repeated Measures ANOVA and Tukey post hoc tests and the differences between the groups by means of t-tests. The significance level was set to $p < 0.05$.

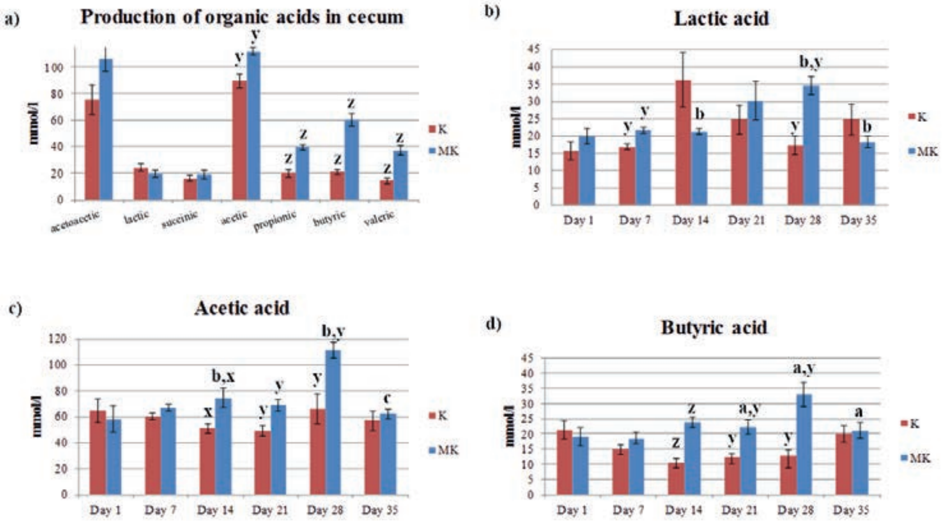

Fig. 1. Level of organic acids in the caecum and faeces of groups K and MK; the level of organic acids in mice caecum after 35 days of supplementation of flaxseed and the dynamics of concentration of acetoacetic, lactic, succinic, acetic, propionic, butyric, and valeric acids in mice faeces; K – group of mice fed exclusively the standard diet; MK – group of mice fed standard diet supplemented with crushed flaxseed (5% added to the feed); mean values (Mean ± SEM) are presented in $mmol.l^{-1}$; ^{a,b,c,x,y,z} differences between columns with the same superscript are significant (^{a,x} = $p < 0.05$; ^{b,y} = $p < 0.01$; ^{c,z} = $p < 0.001$); the superscripts ^{a,b,c} indicate intergroup differences while superscripts ^{x,y,z} indicate differences between groups

Results

Supplementation of diet with flaxseed (FAs) caused a significant increase in the level of organic acids in mice caecum (acetic acid, $p < 0.01$; propionic, butyric and valeric acids $p < 0.001$) in comparison with group K (Fig. 1a).

Examination of faeces of flaxseed-fed mice showed increased concentrations of lactic acid on days 7 and 28 ($p < 0.01$), acetic acid on days 14 ($p < 0.05$), 21 and 28 ($p < 0.01$) and butyric acid on days 14 ($p < 0.001$), 21 and 28 ($p < 0.01$) of supplementation in comparison with control group K (Fig. 1b-d).

The most pronounced effect of the supplementation of flaxseed on the level of all investigated organic acids was observed on day 28 of the feeding experiment (lactic, acetic, and butyric acids $p < 0.01$). On day 35 of the experiment, we observed a pronounced decrease in the concentration of acetic ($p < 0.001$), lactic ($p < 0.01$), and butyric ($p < 0.05$) acids in the fortified group.

Discussion

A positive influence on microbiocenosis of the intestinal tract and thus also on the resistance of the macro-organism has been ascribed *inter alia* also to the prebiotic component of the additive, the flaxseed (Smith et al., 2006; Hekmatdoost et al., 2008). Polyunsaturated fatty acids found in flaxseed are capable of affecting the adherence of bacteria by the modification of the lipid composition of the intestinal wall or the bacterial cell wall. For example, (Yu et al., 2014) reported the stimulation of growth and the adherence of lactobacilli in the digestive tract of mice and (Nemcová et al., 2012) found similar results in pigs. The ω-3 fatty acids reduce intestinal permeability by bacteria and the number of apoptotic cells in ileal mucosa (Generoso et al., 2015). The slimy fibrous material in flaxseed can serve as a specific growth substrate for beneficial bacteria that slow down the production or absorption of toxic products of metabolism, and additionally, by their action, the fibrous material decomposes to volatile FA that fulfil important protective role against infections and maintain the integrity of intestinal mucosa (Smith et al., 2006; Wong, Jenkins, 2007).

One of the mechanisms of the inhibition of pathogenic microorganisms by beneficial microflora involves the production of antibacterial substances, such as organic acids. The concentrations of these acids were significantly increased in the caecum (acetoacetic, acetic, lactic, propionic, and butyric) and faeces (lactic, acetic, butyric) of experimental animals in our study. The beneficial bacteria are able to reduce the production of harmful nitrogenous compounds that cause damage to intestinal mucosa; and, in this way, they exert a positive effect on intestinal function, improve digestive processes, and subsequently increase the weight gain of animals (Serban, 2014). Adhesion of probiotics to intestinal mucosa is important for the promotion of health and the action of bacteria beneficial to intestinal health can be positively affected by ω-3 PUFAs.

The 35-day fortification of mice diet with flaxseed stimulated intestinal metabolism and fermentation activity of beneficial autochtonous intestinal bacteria in mice caecum, which indicates its prospective use for pronounced improvement and protection of animal health.

References

Axling, U., Olsson, C., Xu, J., Fernandez, C., Larsson, S., Ström, Ahrné, S., Holm, C., Molin, G., Berger, K. (2012). Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. *Nutrition & Metabolism*, *9*, 105. DOI: 10.1186/1743-7075-9-105

Borovská, D., Nemcová, R., Gancarčíková, S., Koščová, J. (2013). The synbiotic effect of Lactobacilli and flaxseed on selected intestinal microflora and organic acid levels in weaned piglets. *Microbiology*, *2(10)*, 82–86.

Generoso de Vasconcelos, S., Rodrigues, N.M., Trindale, L.M., Paiva, N.C., Cardoso, V.N., Carneiro, C.M., Ferreira, De Matos, A.D., Faria, A.M.C., Maioli, T.U. (2015). Dietary supplementation with omega-3 fatty acid attenuates 5-fluorouracil induced mucositis in mice. *Lipids in Health and Disease*, *14(54)*, 1–10. DOI: 10.1186/s12944-015-0052-z

Hekmatdoost, A., Feizabadi, M.M., Djazayery, A., Mirshafiey, A., Eshraghian, M.R., Yeganeh, S.M., Sedaghat, R., Jacobson, K. (2008). The effect of dietary oils on caecal microflora in experimental colitis in mice. *Indian Journal of Gastroenterology*, *27*, 186–189

Kiarie, E., Nyachoti, C.M., Slominski, B.A., Blank, G. (2007). Growth performance, gastrointestinal microbial activity and nutrient digestibility in early-weaned pigs fed diets containing flaxseed and carbohydrase enzyme. *Journal of Animal Science*, *85*, 2982–2993. DOI: 10.2527/jas.2006-481

Martinez, F.A.C., Balciunas, E.M., Converti, A., Cotter, P.D., Oliveira, R.P.S. (2013). Bacteriocin production by *Bifidobacterium* spp.: A review. *Biotechnology Advances*, *31*, 482–488. DOI: 10.1016/j.biotechadv. 2013.01.010

Nemcová, R., Borovská, D., Koščová, J., Gancarčíková, S., Mudroňová, D., Buleca, V., Pistl, J. (2012). The effect of supplementation of flax-seed oil on interaction of *Lactobacillus plantarum* – Biocenol™ LP96 and *Escherichia coli* O8:K88ab:H9 in the gut of germ-free piglets. *Research in Veterinary Science*, *93*, 39–41. DOI: 10.1016/j.rvsc.2011.07.031

Serban, D.E. (2014). Gastro-intestinal cancers: influence of gut microbiota, probiotics and prebiotics. *Cancer Letters*, *345(2)*, 258–270. DOI: 10.1016/j.canlet.2013.08.013

Wong, J.M., Jenkins, D.J. (2007). Carbohydrate digestibility and metabolic effects. *Journal of Nutrition*, *137*, 2539–2546. DOI: 10.1093/jn/137.11.2539S

Yu, H-N., Zhu, J., Wen-Sheng, Pan, W-S., Shen, S-R., Shan, W-G., Das, U.N. (2014). Effects of fish oil with a high content of n-3 polyunsaturated fatty acids on mouse gut microbiota. *Archives of Medicinal Research*, *45(3)*, 195–202. DOI: 10.1016/j.arcmed.2014.03.008

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Abstract

Essential polyunsaturated fatty acids (PUFAs) in the feed may affect the gastrointestinal microbiota. The present study investigated the effect of 35-day supplementation of mice diet with 5% concentration of high-ω-3 PUFAs in flaxseed with a focus on the intestinal metabolism of mice. The capillary isotachophoresis method was used for the assessment of the level of organic acids in the gut material and faeces. Supplementation of flaxseed increased the level of organic acids in the caecum (acetic, propionic, butyric, and valeric acids) and faeces (lactic, acetic, butyric acids). The most significant effect was observed on day 28 of flaxseed supplementation. The investigated additive had a stimulatory effect on the intestinal metabolism and fermentation activity of beneficial bacteria.

Key words: flax seed, intestine, mice, microbiota, organic acids

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Suplementacja diety siemieniem lnianym stymuluje metabolizm jelit u myszy

Streszczenie

Niezbędne w pożywieniu długołańcuchowe wielonienasycone kwasy tłuszczowe (ang. PUFAs) wpływają na mikroflorę jelitową. Celem niniejszej pracy było zbadanie wpływu 35-cio dniowej suplementacji diety siemieniem lnianym o 5% stężeniu ω-3 PUFAs na metabolizm jelitowy myszy. Przy użyciu techniki izotachoforezy kapilarnej (ang. CITP) oznaczono stężenie kwasów organicznych w jelicie i kale. Suplementacja siemieniem lnianym powodowała wzrost poziomu kwasów organicznych w kątnicy (kwas octowy, kwas propionowy, kwas masłowy, kwas walerianowy) i kale (kwas mlekowy, kwas octowy, kwas masłowy). Największy istotny wpływ obserwowano w 28 dniu suplementacji siemieniem lnianym. Badany dodatek siemienia miał stymulujące działanie na metabolizm jelitowy i aktywność procesów fermentacyjnych prowadzonych przez pożyteczne bakterie jelitowe.

Słowa kluczowe: siemię lniane, jelita, myszy, mikroflora, kwasy

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