

THE EFFECT OF PREBIOTICS AND SYNBIOTICS ON CLOSTRIDIUM AND ESCHERICHIA COLI COUNTS IN HUMAN INTESTINAL TRACT

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Abstract

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Effect of probiotics and synbiotics consumption based on microbiota of human gut was carried out in our in vivo study. Three groups, P (consuming probiotics), S (consuming synbiotics) and C (control group) of 22 healthy adults were used for this experiment. P and S groups had 10 days long adaptation phase without consuming probiotics and consequently they consumed yoghurt for another 21 days. Control group did not consume yoghurt during the experiment. Faecal samples were collected 10th day of the adaptation phase and then 7, 14 and 21th day of yoghurt consumption phase and finally 26 and 28th day of wash out period.

We registered different effect of probiotics and synbiotics on *Clostridium* sp. and *Escherichia coli* (*E. coli*) counts in human digestive system. Consumption of probiotics decreased of *E. coli* count and consumption of synbiotics increased of both *E. coli* count and *Clostridium* sp. in human digestive system.

probiotics, synbiotics, intestinal microflora

Intestinal microflora plays a key role in function of human digestive system. Bacteria presented in gastrointestinal system have an important function in development human immunity and protection of human health in global. Intestinal microflora is very variable and complex. It consists of more than 400 different bacteria species. Microbial colonisation of gastrointestinal system is very individual, it develops during human life and it is also affected by external conditions. Bacterial colonisation is not equal in the whole intestine. There are lactobacilli, coliform bacteria, streptococci, bifidobacteria and fusobacteria in the small intestine, whereas bacteroides, bifidobacteria, streptococci, eubacteria, fusobacteria, coliform bacteria, clostridia, lactobacilli, staphylococci, yeasts, pseudomonas and *Proteus* are present in the large intestine (Švestka, 2008).

Not only presence but also balance in microbial species distribution at the first place is crucial for normal digestive process. This balance can be easily corrupted by antibiotic treatment, infections,

immunity disorders, diarrhoea or intestinal constipation. Therefore is an effort to re-establish the balance using living microorganisms of human origin called probiotics (Saulnier *et al.*, 2009; Watson, Preedy, 2010; Koning *et al.*, 2010).

Probiotics have positive effect on human health and maintain the balance of intestinal microflora (Rayes *et al.*, 2008). However, not every specimen containing microorganisms can be called probiotic, because it must fulfil fundamental conditions. It must be effective, safe, using living bacteria and it must not be pathogenic. Probiotics must be made from bacteria of human provenance, they have to be resistant against digestive fluids and bile and they also have to be adhesive to intestinal epithelium (Santos *et al.*, 2010).

Principle of probiotics is known nearly 100 years and their importance in medicine is growing lately. Positive effect of probiotics was proven in curing many diseases such as diarrhoea, unspecific intestine inflammation, allergies or cancer genesis (Vrese, Schrezenmeier, 2008). Regular consumption

of probiotics in efficient amount is crucial for reducing or neutralizing the symptoms (Uyeno *et al.*, 2007). 100g of dairy product containing at least 10^6 probiotic bacteria in 1g is considered to be therapeutic minimum (Granato *et al.*, 2010).

Positive health effect of probiotics consumption was observed during treatment of gastrointestinal system diseases including infection caused by viruses (Gill, 2003). On the other hand, there are studies where positive effect of probiotics or synbiotics was not proven in patients suffering from nosocomial infections (Vouloumanou *et al.*, 2009).

Consuming probiotics is not only one way how to re-establish balance of intestinal microflora. Another method is consuming indigestible parts of nutriment called prebiotics stimulating growth of one or more bacteria in the intestine (Krutmann, 2009). Optimal is consuming probiotics and prebiotics that selectively support growth of a particular microorganism. The aim of these so called synbiotics is increasing survival of probiotic microorganisms (bifidobacteria and lactobacilli) and maintaining their viability. Positive effect of synbiotics on growth intestinal microflora was proven using laboratory animals (Quigley, 2010; Quigley, 2011). Yoghurt for human consumption is very suitable synbiotic containing probiotic bifidobacteria and prebiotic oligofructose or inulin.

Consuming probiotics, prebiotics or synbiotics in order to improve condition of intestinal microflora is becoming very popular both in human and veterinary medicine in prevention or curing diseases. That is why probiotics are subject of important clinical research. Effective utilising of probiotics or synbiotics in treating human diseases is dependent on enough studies concerning with influence of particular probiotic microorganism on intestinal microflora. (Li *et al.*, 2007; Ojetto *et al.*, 2009; Maragkoudakis *et al.*, 2010; Wallace *et al.*, 2011). Research should be especially focus on those bacteria, that can negatively affect homeostasis of gastrointestinal system.

Only a few combinations of pre/probiotics have been evaluated as synbiotics, with only a limited number determining effects on the human faecal microbiota using reliable molecular techniques (Saulnier *et al.*, 2009).

The aim of this study was to compare the effect of consumed probiotics and synbiotics on quantitative distribution of selected species of human intestinal microflora. We attempted to prove whether probiotics have greater effect on *E. coli* and *Clostridium* sp. counts in human intestine microflora than synbiotics.

MATERIALS AND METHODS

Three groups of subjects were determined in order to monitor effect of probiotics of synbiotics consumption. Group C (control) did not consume fermented dairy products during the study, group P (probiotic) consumed 200g of white farm yoghurt

containing probiotic BIFI culture and *Lactobacillus acidophilus* and group S (synbiotic) consumed 200g of white farm yoghurt containing probiotic BIFI culture, *Lactobacillus acidophilus* and inulin.

Every group contained 22 persons at average age 22 ± 3 years. The experiment began with 10 day adaptation phase followed by 21 days of consuming yogurt (200g per day) and finished by 7 days of fading phase without consuming fermented dairy products. Faecal samples were collected six times during the experiment: 0 (end of adaptation phase), 7, 14, 21 (eating yoghurt phase), 26 and 28th day (fading phase) of the experiment.

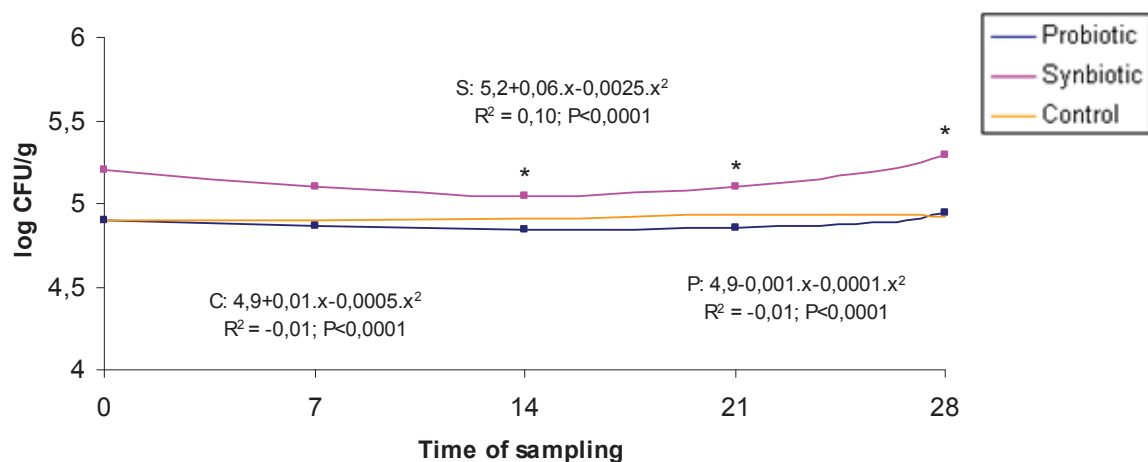
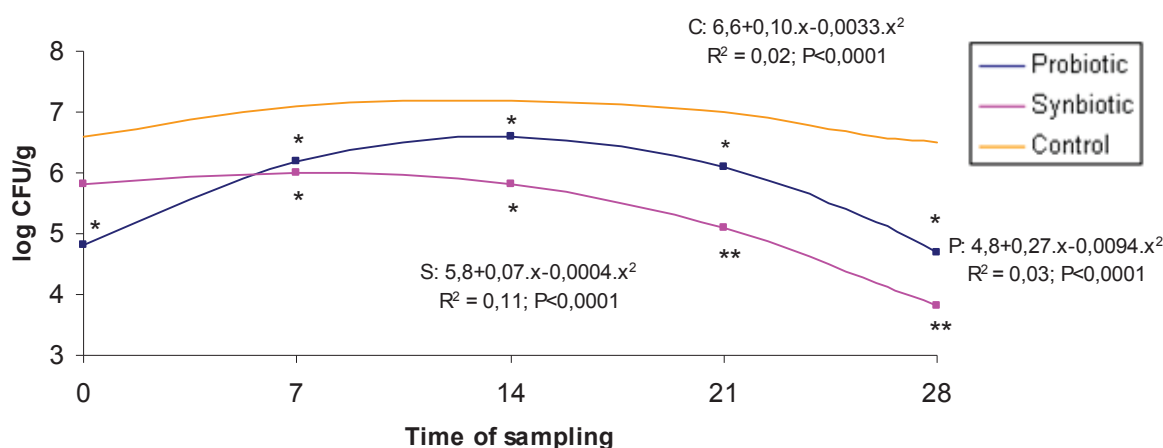
Faecal samples were collected using sterile sampling swabs with activated charcoal (Vitrum, Czech Republic). Nutrient Broth Peptone medium (Himedia, Italy) was used for sample incubation. Cultivation was carried out on agar plates with different nutrient medium in order to determine particular groups of microorganisms. ENDO agar (Biokar Diagnostics, France) was used for aerobic cultivation at 37 °C, 72 hours of *E. coli*. Anaerobic agar (Himedia, Italy) was used for anaerobic cultivation of *Clostridium* sp. Agar plates with *Clostridium* sp. were treated at 85 °C for 10 minutes before microbiological determination and CFU counting in order to inactivate the bacteria. Colonies arose after cultivation was counted on every Petri dish and the amount of CFU in 1g of the sample was calculated. Selected colonies were isolated and purified on selective growth media.

Microbiological parameters were determined twice for every collected sample and average of these two measurements was used for statistical data evaluation. Program Statistica 8 (StatSoft Inc., Tulsa, OK, USA) was used for determining basic statistical characteristics and regression curves (testing of quadratic function), differences between groups of samples in numbers of bacterial colonies (single-classification analysis of variance including post hoc Duncan test). Identification of bacterial species was done using cooperation with the Czech Collection of Microorganisms in Brno.

RESULTS AND DISCUSSION

The aim of this study was comparing the effect of consuming probiotics and synbiotics on quantitative distribution of selected species of human intestinal microflora. The main purpose was to determine if probiotics have greater effect on *E. coli* and *Clostridium* sp. counts in human intestine than synbiotics.

We registered positive effect of both probiotic and synbiotic yoghurt on *E. coli* and *Clostridium* sp. counts during the experiment. Effect of synbiotics was detectable at the end of the adaptation phase when *Clostridium* sp. count was higher than in the group consuming probiotics (Fig. 1). *Clostridium* sp. count decreased till 14th day of synbiotics consumption and then it increased again. On the contrary, consuming probiotics did not significantly affect

Clostridium* $p < 0.05$ 1: The total number of *Clostridium* sp. in fecal samples***E. coli**** $p < 0.05$; ** $p < 0.001$ 2: The total number of *E. coli* strain in fecal samples

numbers of observed bacteria. However, 7, 14 and 21st day after consuming probiotics the numbers of *E. coli* and *Clostridium* sp. were slightly lower.

The increase *Clostridium* sp. count can negatively affects numbers of other important bacteria participating in normal digestive functions of the gastrointestinal system. The increase of *Clostridium* sp. count can be connected with digestion disorders (diarrhoea) or nosocomial infections (Berild *et al.*, 2003; Ben-Horin *et al.*, 2009). Higher count of *Clostridium* sp. in intestine of healthy individuals did not negatively effects the health of the organism. On the other hand, higher count of this bacteria can negatively affect the health in immunodeficient individuals.

We have detected decrease of *E. coli* counts after consuming synbiotics during the whole experiment (Fig. 2). The decrease was statistically significant 7, 14,

21 and 28th day after consuming synbiotics. Effect of synbiotics on of *E. coli* counts was already registered in older studies (Huang *et al.*, 2004; Ferreira *et al.*, 2008; Lee *et al.*, 2009). However, this effect was not studied directly in human digestive system but on biofilm in the in vitro culture (Smith *et al.*, 2011). It is obvious that synbiotics used in our study had double effect. The first, it has a negative effect on the intestinal microflora represented by increasing *Clostridium* sp. count in the intestine. The second, a positive effect demonstrated by decreasing *E. coli* count.

In contrast to synbiotics, probiotics have relatively lower effect on both *E. coli* and *Clostridium* sp. counts (Fig. 1 and 2). Fig. 1 shows that consuming probiotics did not significantly affect *Clostridium* sp. counts in intestine. We can therefore assume that consuming probiotics does not limit both

healthy and immunodeficient individuals, which was already described by Palaria *et al.* (2011) and Shieh *et al.* (2011). It is clear that probiotics significantly decrease *E. coli* counts 14 days after yoghurt consumption ($p < 0.05$). However, the effect of probiotics was lower than the effect of synbiotics from 14th to 28th day after consuming yoghurt.

Fig. 2 shows that the effect of probiotics on *E. coli* counts is higher from 14th day and then the effect was lower than using synbiotics. This situation can be expected because the prebiotics represent the nutrient substrate, which prolongs viability of probiotic contained in synbiotic, and it leads to protracted effect (Collado *et al.*, 2006).

SUMMARY

This experiment showed, that there exist significant difference between effect of probiotics and synbiotics on *Clostridium* sp. and *E. coli* counts in human intestine. Probiotic helps to decrease *E. coli* counts and synbiotic in addition has a positive effect on *Clostridium* sp. counts. We can therefore assume, that synbiotics consumption can be more positive than probiotics, especially for healthy people.

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