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Differential effects of probiotics, prebiotics, and synbiotics on gut microbiota and gene expression in rats

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ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form 11 December 2014
Accepted 19 December 2014
Available online 21 January 2015

Keywords:
Weight gain
β-defensins
Bifidobacteria
Gut microbiota
Lachnospiraceae
Mucin

ABSTRACT

The effects of probiotics (*Bifidobacterium animalis* subsp. *lactis* HN019 and *Lactobacillus rhamnosus* HN001) and prebiotics (fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and inulin) individually and in synbiotic combinations (*B. lactis* HN019+FOS, *B. lactis* HN019+GOS, *B. lactis* HN019+inulin, *L. rhamnosus* HN001+FOS, *L. rhamnosus* HN001+GOS, and *L. rhamnosus* HN001+inulin) on large bowel health were investigated in rats fed the respective diets for 21 days. All experimental treatments led to significantly lower body weight gains and decreased caecal acetic acid concentrations compared to the control diet (no pro-, pre-, and synbiotics). Caecal *Bifidobacterium* spp. or *Lachnospiraceae* were increased in *L. rhamnosus* HN001, FOS or inulin treatments. Rats fed *L. rhamnosus* HN001 had enhanced colonic β-defensin 1 and mucin (MUC)-4 gene expression. All synbiotic combinations increased the MUC4 gene expression. The pro-, pre-, and synbiotics had beneficial effects on the biomarkers of large bowel health in rats. A selective inclusion of pro-, pre-, and synbiotics in the diet will be required to achieve desired health benefits.

1. Introduction

The gastrointestinal (GI) tract contributes to host health in numerous indirect ways, in addition to the digestion of food and absorption of nutrients. Resident microbial communities in the GI tract are large and complex, and play important roles in the maintenance of intestinal homeostasis and immune system regulation, as well as influencing host development and physiology (Sommer & Backhed, 2013). The human GI tract harbours approximately 10^{14} microbial cells, which outnumber the human cells by a factor of ten. Dominated by anaerobic bacteria, microbial densities vary across different regions of the GI tract: proximal regions (e.g. stomach) contain 10^{1}–10^{3} bacteria per gram of content, whereas more distal regions (e.g. colon) contain 10^{11}–10^{12} bacteria per gram of content (O'Hara & Shanahan, 2006). Diet can have a substantial impact on these microbial communities by eliciting changes in population

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http://dx.doi.org/10.1016/j.jff.2014.12.034
1756-4646/

densities, which in turn affect the production of metabolites (Scott, Gratz, Sheridan, Flint, & Duncan, 2013). Dietary interventions such as probiotics and prebiotics can alter the balance of gut microbiota composition by increasing the growth of beneficial bacteria associated with health-promoting effects (De Preter, Hamer, Windey, & Verbeke, 2011), and may even help to manage metabolic disorders associated with obesity (Delzenne, Neyrinck, Backhed, & Cani, 2011).

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). The most familiar probiotic bacteria belong mainly to the genera *Lactobacillus* and *Bifidobacterium*, which are widely used in fermented foods (Heller, 2001). Probiotics have been shown to modulate intestinal epithelial signalling pathways, influence the secretion of cytokines and immunoglobulin (Ig)-A antibodies, and enhance intestinal epithelial barrier functioning by increasing mucin production (Thomas & Versalovic, 2010). The health-promoting effects of probiotic bacteria are typically strain-specific, and should not be generalised to other bacterial strains even in the same species.

Prebiotics are defined as "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (ISAPP, 2008). Prebiotics escape digestion in the upper GI tract, reaching the lower GI tract where they become available for the resident microbiota to use as substrates. The amounts and types of prebiotics entering the large bowel can influence the growth of microbial populations and the production of short-chain fatty acids (SCFAs), thereby altering gut functionality (Macfarlane, Macfarlane, & Cummings, 2006). The most widely studied prebiotics are fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and inulin, which are selectively used by bifidobacteria and lactobacilli (Watson et al., 2013).

Synbiotics are being developed where probiotics and prebiotics are combined in a food to provide additional (synergistic) health benefits beyond those provided by probiotics and prebiotics alone (Fujimori et al., 2009). Whilst probiotic bacterial strains, e.g. *Bifidobacterium animalis* subsp. *lactis* HN019 and *Lactobacillus rhamnosus* HN001, have been extensively studied from *in vitro* studies through to human clinical trials (Dekker et al., 2009; Gopal, Prasad, Smart, & Gill, 2001; Waller et al., 2011; Wickens et al., 2013), few studies have examined these probiotic strains in synbiotic combinations. In a large study conducted in children, synbiotic combination of *B. lactis* HN019 with GOS reduced early childhood morbidity and risk of anaemia and iron deficiency (Sazawal et al., 2010a, 2010b); however, it was not clear whether the demonstrated benefits were attributable to *B. lactis* HN019 or GOS alone, or dependent upon the synbiotic combination.

In the present study, we investigated the influence of two probiotics (*B. lactis* HN019 and *L. rhamnosus* HN001) and three prebiotics (FOS, GOS, and inulin) both independently and in synbiotic combinations (*B. lactis* HN019+FOS, *B. lactis* HN019+GOS, *B. lactis* HN019+inulin, *L. rhamnosus* HN001+FOS, *L. rhamnosus* HN001+GOS, and *L. rhamnosus* HN001+inulin) on large bowel health in rats by quantifying changes in caecal microbiota composition and SCFAs, and the resulting effects on the expression of genes involved in colonic barrier function and faecal IgA.

2. Materials and methods

2.1. Animals

Experimental procedures used in this study were approved by AgResearch Grasslands Animal Ethics Committee (Palmerston North, New Zealand) according to the Animal Welfare Act 1999, New Zealand. Three-week-old male Sprague-Dawley rats ($n = 144$) were housed individually in hanging cages containing pressed wood chips as bedding. The room housing the rats was maintained at a temperature of 22 ± 1 °C, humidity of 60 ± 5%, air exchanged 12 times/hour, and a 12 h light/dark cycle.

2.2. Experimental design

The experimental design comprised two probiotics, three prebiotics, and six synbiotic combinations, with $n = 12$ rats per treatment (Table 1). Probiotic strains were *B. lactis* HN019 (DR10™) and *L. rhamnosus* HN001 (DR20™) (Fonterra Co-operative Group Ltd, Palmerston North, New Zealand). Prebiotics were FOS (Orafti P95, Beneo-Orafti, Tienen, Belgium), GOS (Vivinal GOS powder Maltodextrin, FrieslandCampina Domo, Amersfoort, The Netherlands), and inulin (Orafti Synergy 1, Beneo-Orafti). Prebiotics were included in the experimental diets at 5%. Probiotic strains *B. lactis* HN019 or *L. rhamnosus* HN001 were given to each rat once a day for 21 days by oral gavage at a dose of 10^8 colony forming units in 50 µl of 10% (w/v) skim milk. Control group rats received a diet with no pro-, pre-, and synbiotics. The components in the experimental diets are shown in Supplementary Table S1.

Before commencing the experimental treatments, all rats were fed the control diet for 7 days. After allocation to experimental treatments, rats were fed the respective diets for 21 days. Rats were given *ad libitum* access to food and water during the experiment. After 21 days of feeding, rats were euthanised by CO_2 asphyxiation. The caecum was removed from each rat and

Table 1 – Dietary treatments and probiotic bacteria.

Treatment	Diet[a]
Control	Control
B. lactis HN019	Control
L. rhamnosus HN001	Control
FOS	FOS
GOS	GOS
Inulin	Inulin
B. lactis HN019+FOS	FOS
B. lactis HN019+GOS	GOS
B. lactis HN019+inulin	Inulin
L. rhamnosus HN001+FOS	FOS
L. rhamnosus HN001+GOS	GOS
L. rhamnosus HN001+inulin	Inulin

[a] Ingredient compositions of the experimental diets are shown in Supplementary Table S1.

Probiotic bacteria administered once a day to rats by oral gavage at a dose of 10^8 colony forming units in 50 µl of 10% (w/v) skim milk. *B. lactis* HN019 – *Bifidobacterium animalis* subsp. *lactis* HN019, *L. rhamnosus* HN001 – *Lactobacillus rhamnosus* HN001, FOS – Fructo-oligosaccharide, and GOS – Galacto-oligosaccharide.