

## 学位論文の要旨

### Abstract of Thesis

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学位論文題目 Title of Thesis (学位論文題目が英語の場合は和訳を付記)

Cecum Fermentation and Microbiota in Rats Fed Various Dietary Proteins and Prebiotic Oligosaccharides

(種々のタンパク質及びプレバイオティクスオリゴ糖を摂取したラットの盲腸発酵と腸内細菌叢)

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Evidence has shown that composition of gut microbiota has a profound impact on nutrition, immunity, disease, and health. In addition to host genetics, the environment including aging, stress, medication, and diet can determine the composition of gut microbiota. Although diet is regarded as a key factor modulating gut microbiota, except for dietary fiber and non-digestible carbohydrate polymers, it is still unknown which food constituents specifically promote growth and functionality of desirable gut bacteria. *Bifidobacterium* spp. and *Lactobacillus* spp. are often referred to as beneficial bacteria; supplements and dairy products containing these species are accepted as health-promoting items.

Substrates for gut fermentation are non-digested dietary components and host secretions mainly composing of mucin. Although resistant starch (8–40 g/day) and non-starch polysaccharides (8–18 g/day) may serve as major non-digested components in human diet, a significant amount (4–10 g/day) of non-digested protein may also enter the large intestine and is subjected to bacterial metabolism. If dietary protein enters the large intestine, then the effect of non-digestible but fermentable oligosaccharides (prebiotics) can be modulated by the source of dietary protein.

A number of studies have reported effects of dietary protein on composition of gut microbiota, and differences between protein sources, e.g. non-meat (casein and soybean), red meat (pork and beef), and white meat (chicken and fish) proteins, were demonstrated. Thus, effect of prebiotic oligosaccharides may be augmented or attenuated by the type of the dietary proteins. A large number of rat and mouse studies have been performed on the effect of prebiotics on composition and functionality of gut microbiota; however, most of them used casein as the sole protein source because the AIN 93 diet recommends casein for animal model studies. Because few experiments used other proteins, the function of prebiotics could be evaluated differently if plant proteins were fed.

In this study, two experiments were conducted to examine the influence of dietary protein sources on the effects of prebiotic oligosaccharides on the composition and metabolism of gut microbiota. Casein, soy protein isolate, meat (beef and pork), and fish were fed to rats with cellulose,

raffinose, and fructooligosaccharide as non-digestible but fermentable carbohydrate polymers.

In the first experiment, 30 female rats were fed casein and soy protein isolate with cellulose, raffinose, and fructooligosaccharides for 28 days. Cecum microbiota composition was determined by real-time qPCR and denaturing gradient gel electrophoresis. Cecum acetic acid concentration and *Lactobacillus* spp. population were greater with soy protein than with casein. Raffinose increased the acetic acid concentration, and fructooligosaccharides increased the butyric acid concentration regardless of dietary protein source. Likewise, *Bifidobacterium* sp., *Collinsella* sp., and *Lactobacillus* sp. were detected in microbiota of the rats fed raffinose, and *Bacteroides* sp., *Roseburia* sp., and *Blautia* sp. were seen in microbiota of the rats fed fructooligosaccharides. Neither protein source nor oligosaccharides altered total bacteria, *Bacteroides-Prevotella-Porphyromonas*, or *Blautia coccoides-Eubacterium rectale* populations, whereas raffinose increased the *Bifidobacterium* population regardless of dietary protein. Interactions between dietary proteins and prebiotic oligosaccharides were observed with *Clostridium perfringens* group populations and cecum IgA concentration. Raffinose and fructooligosaccharides decreased *C. perfringens* group populations in casein-fed rats, and the combination of soy protein and raffinose substantially increased cecum IgA concentration.

In the second experiment, 30 female rats were fed soy protein isolate, meat, and fish with cellulose and raffinose for 28 days. Meat and fish were purchased at supermarket and their fats were removed by diethyl ether extraction. Cecum propionic acid concentration was higher with meat than with soy protein, whereas *Bifidobacterium* spp. population were greater with soy protein than with meat. Raffinose increased the concentrations of acetic acid and propionic acid in the cecum. Similar to experiment 1, *Bifidobacterium* sp., *Collinsella* sp., and *Lactobacillus* sp. were detected in microbiota of the rats fed raffinose. Neither protein source nor raffinose affected *Bacteroides-Prevotella-Porphyromonas*, or *Blautia coccoides-Eubacterium rectale* populations, whereas small but significant decrease was seen by raffinose feeding in total bacterial population in the cecum. Although raffinose decreased *C. perfringens* group populations in meat-fed and fish-fed rats, the reduction was not seen in soy protein-fed rats. Likewise, *Lactobacillus* spp. population was increased by raffinose when rats were fed with soy protein but not with meat or fish. Cecum IgA concentration was higher with soy protein than with fish and further increase was seen when soy protein and raffinose were fed in combination.

These results indicate that dietary proteins can differentially modulate the effects of prebiotic oligosaccharides on gut fermentation and microbiota, depending on the type of carbohydrate polymers involved. The findings that a combination of soy protein and raffinose greatly increased cecum IgA concentration and raffinose decreased cecum *C. perfringens* group populations in rats fed animal proteins are of particular interest.