学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士(ｿﾌﾄﾏﾀｰ科学)　氏　名　甘　莉

学 位 論 文 題 名

A Basic Study of the Effects of Mulberry Leaf Administration to Mice on Gut Microbiota and Metabolites
（マウスへの桑の葉投与が腸内細菌叢と代謝物に及ぼす影響に関する基礎研究）

The mulberry tree, which is classified in the genus *Morus*, is extensively cultivated in Japan, China, Korea, Thailand, and several other Asian countries, and its dried leaves are used as tea leaves. Mulberry tea has long been considered a Chinese medicine and a functional food in Asian countries. A suspension of fine powder or liquid extracted with hot water is consumed as tea. Previous studies indicated that mulberry leaves are rich in bioactive compounds, including flavonoids, alkaloids, polysaccharides, polyphenols, and volatile oils, while also containing a wealth of amino acids, various inorganic trace elements, and vitamins. These constituents confer upon mulberry leaves a range of functions contributing to health, including anti-hyperglycemic, anti-hyperlipidemic, anti-obesity, antioxidant, and anti-inflammatory effects. Among these, reports of its strong anti-diabetic effects are increasing and attracting attention.

Among the constituents of mulberry leaves involved in diabetes inhibition, 1-deoxynojirimycin (1-DNJ) is a well-known bioactive compound unique to mulberries. 1-DNJ is an iminosugar, a glucose analog in which the oxygen atom of the pyranose ring is replaced with an NH group. Several studies have reported that 1-DNJ is a potent α-glucosidase inhibitor that controls blood glucose levels and improves insulin sensitivity. Owing to these effects, 1-DNJ and mulberry leaves are considered promising therapeutic approaches for treating type 2 diabetes mellitus (T2DM). Considering the increasing prevalence of T2DM patients worldwide, it is important to investigate the effects of mulberry leaves on animals.

However, to the best of our knowledge, no study has directly examined the inhibition of polysaccharide degradation in fecal samples, which may be important with regard to the primary mechanism of action of 1-DNJ. In particular, the strong α-glucosidase inhibitory activity of 1-DNJ may not only inhibit saccharide absorption in the host small intestine but also provide a source of nutrients to the microbiota in the digestive tract. Furthermore, several studies examined the effects of

the mulberry leaf extract and 1-DNJ on the gut microbiota, and these studies focused on the effects in disease-model mice. Considering that mulberry leaf tea is consumed daily as a luxury or functional food, knowledge of the gut microbiota and metabolites in the normal host intestine is important.

Therefore, this study examined the effects of a mulberry leaf powder (MLP) suspension administered to healthy mice for a relatively long period of 9 weeks on gut microbiota and metabolites. The composition of the gut microbiota was analyzed using 16S-rDNA sequencing. Furthermore, a nuclear magnetic resonance (NMR)-based metabolomics approach was employed to investigate changes in water-soluble metabolites, including saccharides, in the feces of MLP-fed mice.

Daily body weight measurements were performed for all the mice, and no significant differences were observed in weight change between the MLP-treated and control groups. Mice blood glucose levels were measured weekly. After week 3, the blood glucose levels in the MLP group were slightly lower than those in the control group, and by week 9, they were significantly lower. These results suggest that MLP had a slight suppressive effect on blood glucose levels in healthy mice.

To clarify the differences between the control and MLP-treated groups at 0–9 weeks, an OPLS-DA multivariate statistical analysis was performed on the binning values of the NMR spectra. In the OPLS-DA model, the score plot showed further discrimination between the control and MLP-treated groups. In the OPLS-DA coefficient loading plot, significant signals for discrimination between the control and MLP-treated groups were observed. Based on the chemical shifts, compounds including amino acids (alanine and BCAAs), organic acids (acetate and propionate), and carbohydrates (glucose and maltose) were shown to contribute to this separation.

The results of the taxonomic analysis of the mouse intestinal microbiota revealed no significant differences in the diversity and community structure of the gut microbiota in the C57BL/6 mice with or without MLP supplementation. Thirty-nine metabolites were identified via ^{1}H-NMR analysis, and carbohydrates and amino acids were significantly ($p < 0.01$–0.05) altered upon MLP treatment. In the MLP-treated group, there was a marked increase and decrease in maltose and glucose concentrations, respectively, possibly due to the degradation inhibitory activity of oligosaccharides. After 5 weeks, all amino acid concentrations decreased. Furthermore, despite clear fluctuations in fecal saccharide concentrations, short-chain fatty acid production via intestinal bacterial metabolism was not strongly affected. This study provides the knowledge that MLP administration can alter the gut metabolites without affecting the normal gut microbiota, which is useful for considering MLP as a healthy food source.