BIOCELL
2022 46(9): 2101-2109

Tech Science Press

Effect of *Lycopus lucidus* Turcz. supplementation on gut microflora and short chain fatty acid composition in Crj: CD-1 mice

Eun NA[1]; Sun Young LIM[2,*]

[1] Ocean Science and Technology School, Korea Maritime & Ocean University, Busan, Korea
[2] Division of Convergence on Marine Science, Korea Maritime & Ocean University, Busan, Korea

Key words: *Lycopus lucidus* Turcz, Fatty acid composition, Intestinal microflora, 16S rDNA sequencing, Cytokines

Abstract: We investigated the diversity and composition of microflora in feces of *Lycopus lucidus* Turcz.-fed mice. In addition, we evaluated the production of major cytokines (Interleukin-6 and -10) which are related to inflammation and fatty acid composition of several tissues. 16S ribosomal DNA sequencing-based microbiome taxonomic profiling analysis was performed utilizing the EzBioCloud data base. Male mice fed on *L. lucidus* showed a significantly reduced number of lactic acid bacteria and coliform in the feces compared with the control group ($p < 0.05$). 16S rDNA sequencing analysis of fecal samples showed that *L. lucidus* supplementation decreased the community of harmful microflora (*Enterobacteriaceae* including *Escherichia coli* and *Bacteroides* sp.) in feces compared with the control group ($p < 0.05$). There were no significant differences in mRNA expression of cytokine IL-6 and IL-10 between the control and *L. lucidus* fed groups. The fecal fatty acid composition in the *L. lucidus* group had percentages of 4:0, 6:0, 8:0 and 10:0 in the intestine but those short chain fatty acids were not detected in the control group. Our results showed that *L. lucidus* supplementation influenced gut environment by decreasing harmful microflora and increased the percentages of several short fatty acids.

Introduction

Lycopus lucidus Turcz. (*L. lucidus*) is belong to A *Lamiaceae* family herb and belongs a common plant in East Asia including Korea and China. Roots of *L. lucidus* are consumed routinely in salad and fermented foods as a functional food (Lu *et al.*, 2015) and have been traditionally used for healing menstrual disorder and inflammation in Asia (Liu *et al.*, 2019; Fan *et al.*, 2020). Bioactive polysaccharides, terpenes, flavonoids, and phenolic compounds, which are rich in roots of *L. lucidus*, are highly associated with antimicrobial (Yu *et al.*, 2011), antioxidant (Lu *et al.*, 2015; Song *et al.*, 2016; Yang, 2017; Lee and Lim, 2018), anti-inflammation (Park, 2019; Min *et al.*, 2021; Zhang *et al.*, 2021; Kim *et al.*, 2021a) and also anticancer properties (Yu *et al.*, 2011; Park *et al.*, 2013; Kim *et al.*, 2018). Flavonoids are classified as a major class of secondary metabolites and the most-studied group of polyphenols in relation to antioxidant and anti-inflammatory activities (Panche *et al.*, 2017). In the intestine, flavonoids may influence the composition of the microbiota and also may be metabolized by the resident microbiota. The resulting end-products may have a certain bioactivity, which will act differently from those of the initial compounds (Braune and Blaut, 2016). Espley *et al.* (2014) reported that the intake of a high-flavonoid apple decreased some inflammation markers and modulated gut microbiota when fed to healthy mice. Duenas *et al.* (2015) suggested that the intake of polyphenols can increase beneficial strains of *Bifidobacterium* and *Lactobacillus*, in the gut while reducing pathogenic strains such as *Clostridium perfringens* and *C. bistolyticum*. The frequent consumption of polyphenols could modify gastrointestinal environment with beneficial microorganisms. It has been also widely reported that the gastrointestinal microbiota composition is associated with improving human intestinal health (Holscher, 2017). Many studies suggest that disturbed gut microflora composition may affect the function of mucosal immune system, resulting in intestinal inflammation (Tung *et al.*, 2011; Goldsmith and Sartor, 2014; Li *et al.*, 2017).

One of our previous studies analyzed the total flavonoid content of aceton+methylene chloride extract from *L. lucidus* roots and showed a content of 233.2 mg/g (mg rutin equivalent/g) (Lee and Lim, 2018). The extract from *L. lucidus* roots had a scavenging effect towards 1,1-diphenyl-2picryhydrazyl (DPPH) and 2-2'-azino-bis(3-rthlbenz-thiazoline-6-sulfunic acid) (ABTs) radicals and decreased

*Address correspondence to: Sun Young Lim, sylim@kmou.ac.kr
Received: 16 October 2021; Accepted: 31 December 2021

Doi: 10.32604/biocell.2022.019807

cellular reactive oxygen species production induced by H_2O_2. Woo and Piao (2004) isolated two flavonoids, luteolin and luteolin-7-beta-D-glucuronide methyl ester from root of *L. lucidus* and these flavonoids exhibited antioxidative activity. According to the current literature and our previous studies, *L. lucidus* contains polysaccharides and flavonoids as active compounds. However, the effect of *L. lucidus* supplementation on fecal microflora and cytokine expression has not yet been investigated. Thus, in this study, the changes in diversity and composition of microbiota in feces were analyzed in *L. lucidus*-supplemented mice. In addition, we evaluated the production of major cytokines (IL-6 and -10) related with inflammation and fatty acid composition in several tissues.

Materials and Methods

Animals and diets

Dried *L. lucidus* roots were obtained from Misan Inc. (Daegu, Korea). Dried *L. lucidus* roots were extracted with boiled water at 100°C for 2 h. The extract was centrifuged at 1000 g for 20 min at 4°C and filtered with Whatman No. 3 filter paper. The resulting supernatant was lyophilized to produce a powder. Male Crj: CD-1 mice four weeks of age were obtained from Samtako Inc. (Osan, Korea). As soon as mice arrived at the laboratory, they were kept in habituation for one week. Then twenty-six mice were randomly divided into two groups of thirteen: The first group was the control group that was fed on 5% palm oil (Herbnoori Inc., Daegu, Korea) as a fat source (Control) (Table 1). The second group was fed on diet with 20% powder of *L. lucidus* roots. *L. lucidus* root extract contained 5% protein, 0.3% fat and 12.9% carbohydrate. The diet composition was adjusted according to the amounts of nutrients contained in *L. lucidus* root extract. The composition of diets followed the AIN-93M (Reeves *et al.*, 1993). Customized diets were stored at −4°C, and fresh supplies were given to the mice once every two days. Body weights were measured once a week. Mice were maintained at our thermo-hygrostat facility under conventional conditions of controlled temperature (23 ± 1°C), relative humidity (65 ± 5%) and illumination (12-hours light:dark cycle). Two mice per cage were housed and were allowed to free access to food and water and maintained on these diets for 8 weeks. At the end experiments, the mice were sacrificed by decapitation. Intestine and mesenteric lymph node were removed and stored at −70°C.

TABLE 1

The composition of experimental diets

Ingredients	Diet group (g/kg)	
	Control	*L. lucidus* Turcz.
Corn starch	488	462.2
Casein	200	190
Sucrose	150	150
Cellulose	50	50
Mineral	40	40
Vitamin	20	20
Methionine	2	2
Palm oil	50	49.4
L. lucidus Turcz. Root		200

Measurements of microorganism using the 3M petrifilm^{TM} plate method

Feces were collected from three mice per each group before feeding. Microflora analysis of mouse feces followed recommendations from manufacturer 3M Petrifilm^{TM} (Aerobic, Lactic Acid Bacteria, and Coliform Count plates). 1 g of each stool sample was weighed and diluted in 9 ml of phosphate buffer saline (PBS) in a sterile test tube. Subsequently they were homogenized in a Vortex shaker at 10^{-1} dilution, from which the other dilutions were made up to 10^{-3}. Then, 1 ml of the 10^{-3} of each sample was inoculated into a 3M Petrifilm^{TM}, respectively. Counting was performed after 24 and 48 hours of incubation. For total microbial counts, all colonies staining in various shades of red were counted. For ascertaining the number of coliform and lactic acid bacteria colonies, only red colonies with one or more gas-associated bubbles (within 1 colony diameter) were counted (Park *et al.*, 2001). The microbial counts were calculated as colony-forming units (CFU) per milliliter sample according to the equation:

CFU per ml = (number of colonies x dilution factor of plate)/aliquot plated.

The results will be expressed as log (CFU/ml).

DNA extraction and analysis of 16S rDNA gene sequences

To investigate the change of intestinal microbial composition, we extracted DNA from feces (n = 1 per group) that collected right before the sacrifice using DNeasy Blood & Tissue Kit (Qiagen, CA, USA). DNA samples were prepared according to sequencing company's guideline. Next generation sequencing (NGS) microbiome taxonomic profiling analysis was performed by ChunLab Inc. (Seoul, Korea) using EzBioCloud data base (Yoon *et al.*, 2017).

RNA preparation and quantitative polymerase chain reaction (q-PCR)

The total RNA from homogenized mesenteric lymph node tissues was isolated with the use of a Minibest Universal RNA Extraction Kit (Takara, Kusatsu, Japan) and performed according to the manufacturer's protocol. The total RNA concentration has set to 476 ng and then synthesized using Prime Script 1st Strand cDNA Synthesis Kit (Takara, Kusatsu, Japan). qPCR was performed on cDNA samples using the SYBR™ Green PCR Master Mix (Applied Systems, IL, USA) and performed according to the manufacturer's protocol. Primers used were at Table 2. The thermal cycle conditions were as follows: 50°C for 4 min and 95°C for 15 min of initial denaturation followed by 50 cycles at 95°C for 10 sec, 50°C for 20 sec, 72°C for 30 sec. This was then followed by a melt curve analysis, in which the temperature was 95°C for 20 sec, 60°C for 40 sec, 95°C for 15 sec. Analysis used the sequence detection software supplied with the instrument (StepOne Real-Time PCR System, applied biosystems, IL, USA). The relative quantitation value is expressed as 2^{-} DcT, where DcT is the difference between

TABLE 2

Primer sequences used in the study

Name	Sequence (5'-3')	Size
β-actin_F[1]	AAGATCTGGCACCACACCTT	20 mer
β-actin_R[2]	CCTGTGGTACGACCAGAG	18 mer
IL6_F	TCTGGGAAATCGTGGAAATG	20 mer
IL6_R	GGTACTCCAGAAGACCAGA	19 mer
IL10_F	ATAACTGCACCCACTTCCCA	20 mer
IL10_R	GGGCATCACTTCTACCAG	18 mer

Note: [1]Forward primer. [2] Reverse primer.

the mean CT value of duplicates of the sample and of the β-actin control (Kim *et al*., 2010).

Measurement of fatty acid composition

After the experiment, the mice were then decapitated. Intestine and mesenteric lymph node were removed and stored at −80°C. The lipids extracted from the tissues were prepared (Kim *et al*., 2021b). The lipid extracts were then transmethylated with 14% BF_3-methanol at 100°C for 60 min using a modified version of the method employed by Morrison and Smith (Morrison and Smith, 1964) that involved the addition of hexane. Fatty acid methyl esters were then analyzed by gas liquid chromatography (Varian, CA, USA) as previously described (Salem *et al*., 1996).

Statistical analysis

All results were expressed as means ± the standard error of the mean (SEM), with statistical significance determined by *t*-test using the SIGMASTAT statistical program package (Jandel Co., Erkrath, Germany).

Results

Food intake and body weight

At the start of the study, the body weight of the control and *L. lucidus*. supplemented mice groups did not differ significantly. The body weight increased gradually during the 8-week period, and that of the control and *L. lucidus*-supplemented mice was 43.9 ± 1.31 and 42.8 ± 1.40 g, respectively (Fig. 1). The daily food intake of the control and *L. lucidus*-supplemented mice was 7.0 ± 0.5 and 6.8 ± 0.00 g, respectively. The food efficiency rate (FER) of control and *L. lucidus* groups was 5.3 and 5.0, respectively.

Effect of L. lucidus supplementation on fecal microorganism composition

Fig. 2 shows the community of fecal microbiota using 3M petrifilm assay. Supplementation with *L. lucidus* significantly reduced the counts of coliform and lactic acid bacteria in the feces compared with that in the control ($p < 0.05$). 16S rDNA sequencing data showed that supplementation with *L. lucidus* decreased greatly the community of harmful microflora (*Enterobacteriaceae* including *E. coli*, and *Bacteroides* sp.) in feces compared with that in the control (Figs. 3 and 4).

Effect of L. lucidus supplementation on cytokine expression in mesenteric lymph node

The changes in cytokine expression patterns in stimulated mesenteric lymph node are shown in Fig. 5. Mice supplemented with *L. lucidus* had a higher mRNA expression of IL-6 and a lower mRNA expression of IL-10 compared with that in the control but the differences were not significant.

Effect of L. lucidus supplementation on fatty acid composition in intestine and feces

There were marked differences in fatty acid composition of intestine between the control and *L. lucidus* groups (Table 3). The *L. lucidus* group had short chain fatty acids (SCFAs) including 4:0, 6:0, 8:0 and 10:0 but there was no detection of these SCFAs in the control group. In addition, the *L. lucidus* group showed higher total saturated, n-6 and n-3 polyunsaturated fatty acids and lower total monounsaturated ($p < 0.05$). Among n-3 fatty acids, the percentages of 20:3n-3, 20:5n-3 and 22:6n-3 were significantly higher in the *L. lucidus* group than those of the control ($p < 0.05$). In the mesenteric lymph node, the percentage of total saturated was found to be significantly higher in the *L. lucidus* group, while the percentage total monounsaturated was lower compared with that in the control ($p < 0.05$) (Table 4). There was no difference in the percentage of 6:0 between two experimental groups. No differences were uncovered between the two groups in terms of the percentages of total saturated,

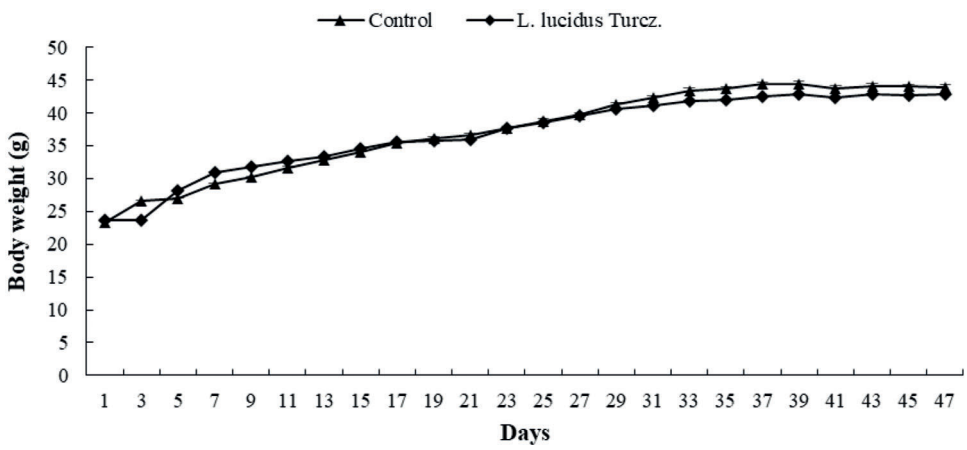

FIGURE 1. Effect of supplementation with *L. lucidus* Turcz. on body weight.

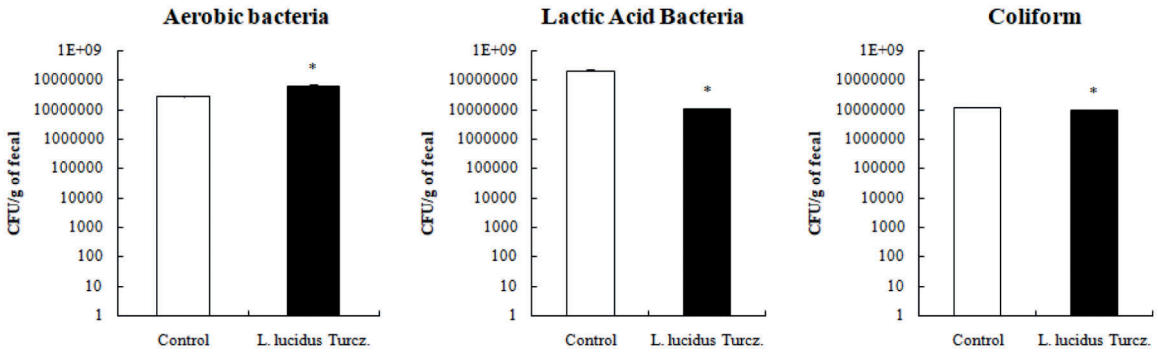

FIGURE 2. Effect of supplementation with *L. lucidus* Turcz. on bacterial composition of fecal samples using 3M petrifilm assay. The values were represented as the mean ± SEM and *significantly different between the control and *L. lucidus* Turcz. groups at $p < 0.05$.

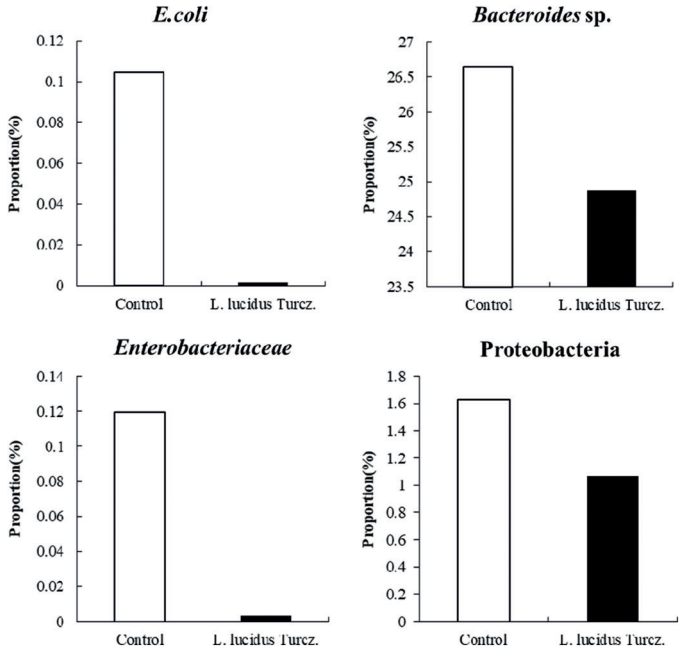

FIGURE 3. Effect of supplementation with *S L. lucidus* Turcz. on harmful bacterial community composition of fecal samples analyzed by 16S rDNA sequencing.

monounsaturated, n-6 and n-3 polyunsaturated fatty acids found in feces (Table 5).

Discussion

Healthy gastrointestinal microflora are characterized by high numbers and diversity of bacteria (Vandeputter *et al.*, 2016), which interact with mucosal epithelium and are responsible for normal substance metabolism, immune response, and intestinal angiogenesis (Candela *et al.*, 2014). In the current study, we found that supplementation with *L. lucidus* decreased the harmful microbiota in feces. Based on sequencing of 16S rDNA gene of gut microbiota, the four major phyla in the feces were identified to be Bacteroidetes, Verrucomicrobia, Firmicutes and Proteobacteria, which are consistent with our current results. Lactic acid bacteria are known probiotics with many health benefits, including improvement of normal microflora, inhibition of infectious diseases, reduction of serum cholesterol, and alleviation of intestinal bowel disease symptoms (Maldonado Galdeano *et al.*, 2007). However, in the present study, supplementation with *L. lucidus* did not increase the population of lactic acid bacteria. There were few studies on relationship between *L. lucidus* intake and gut microorganisms. Xi *et al.* (2020) studied stachyose, a oligosaccharide from Chinese artichoke (*Stachys sieboldii* Miq.) tuber and found that its consumption increased the proliferation of *Bifidobacterium* and *lactobacilli* and decreased *enteric bacilli*.

Overproduction of cytokines are associated with lethal diseases since cytokines can act not only locally to amplify the cellular immune response, but also systemically to change behavior, metabolism, and neuroendocrine secretions (Johnson, 1997). T cells and macrophages secrete IL-6 to stimulate the immune response, particularly in tissue damages leading to numerous types of inflammatory processes (McCurry *et al.*, 1993). IL-10 has potent anti-inflammatory properties and inhibits the Th1 cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ (Mosmann, 1994). Bogert *et al.* (2014) suggested that some pathogenic bacteria including *Streptococcus* and *Veillonella* increased levels of cytokines IL-8, IL-6, IL-10, and TNF-α. Costa *et al.* (2014) reported that treatment of betulinic acid

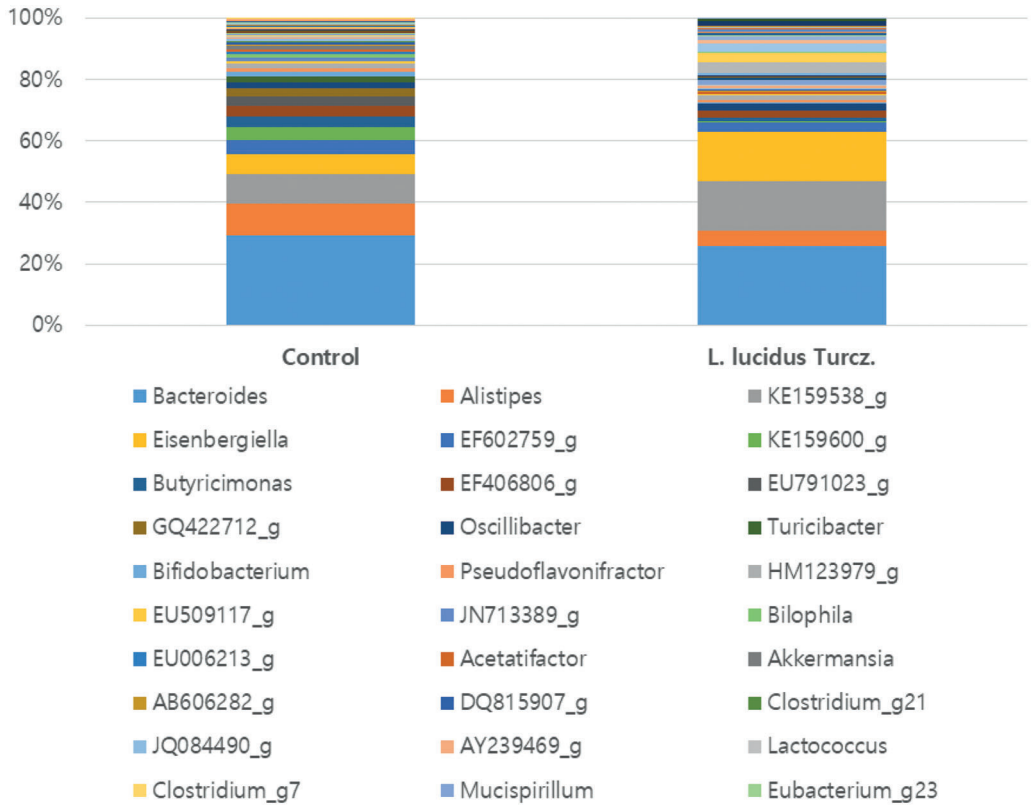

FIGURE 4. The column chart of genus classification of fecal samples analyzed by 16S rDNA sequencing.

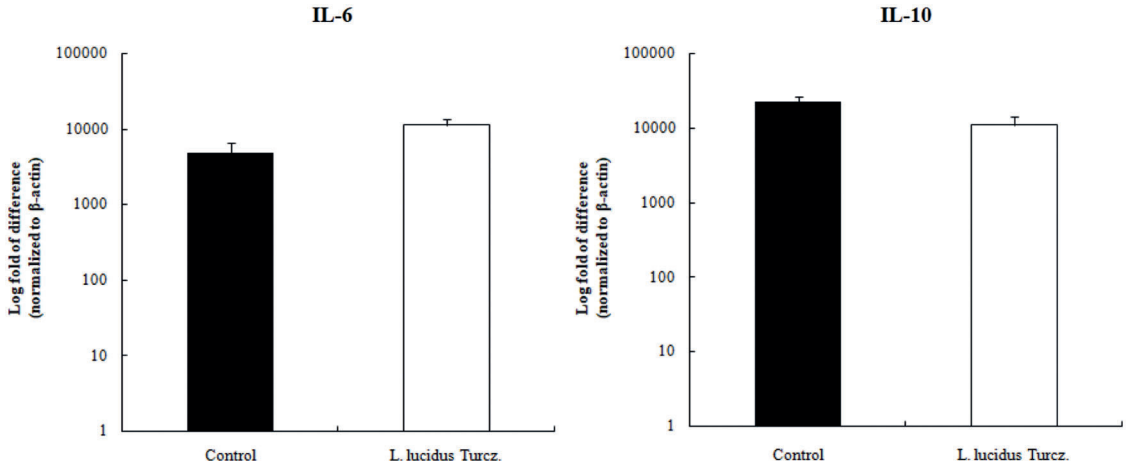

FIGURE 5. Effect of supplementation with *L. lucidus* Turcz. on cytokine expression in mesenteric lymph node analyzed by quantitative RT-PCR.

from *L. lucidus* led to increased IL-10 and reduced TNF-α expression but no alteration in IL-6 production. However, in the present study, we failed to prove the modulating effect of *L. lucidus* on cytokine expression of IL-6 and IL-10. A limitation of this study is that it could not explain the causal factor; therefore, the possibility that the resident microbes influenced IL-6 and IL-10 levels remains. Moreover, IL-6 is a pleiotropic property with pro and anti-inflammatory cytokine (Costa *et al.*, 2014). Soares *et al.* (1998) suggested that vasoactive sand fly peptide inhibited inflammatory action through increased IL-6 levels.

Under the activation by prebiotics including oligosaccharides, bacteria produce a large amount of SCFAs, which reduce intestinal pH value, prevent the growth of harmful bacteria and promote intestinal peristalsis to accelerate the excretion of pathogenic bacteria and toxins (Bogert *et al.*, 2014). Yang *et al.* (2010) isolated water-soluble polysaccharides from *L. lucidus* and identified that its polysaccharides were composed of galactose, followed by galacturonic acid, and nine monosaccharides. Lin *et al.* (2012) also separated *L. lucidus* polysaccharides and reported their anti-oxidation and anti-aging effects in aged mice induced by D-galactose. An increase in the amount of some beneficial gut microbiota can produce high SCFA and low ammonia contents compared to other feed ingredients (Velazquez *et al.*, 2000; Topping and Clifton, 2001). Total SCFAs are produced in large intestine through gut microbiota fermentation of plant-derived complex oligosaccharides that have escaped digestion and absorption in small intestine (Blachier *et al.*, 2007). Acetate, propionate, and butyrate are the most abundant SCFAs in gastrointestinal tract, whereas formate, valerate, caproate, etc.,

TABLE 3

Effect of supplementation with *L. lucidus* Turcz. on selected fatty acid composition in intestine

	Control	*L. lucidus* Turcz.
Fatty acids	*% by weight of total fatty acids*	
4:0	ND[2]	4.14 ± 0.13
6:0	ND	0.79 ± 0.06
8:0	ND	1.80 ± 0.17
10:0	ND	0.86 ± 0.06
12:0	0.02±0.04	1.00 ± 0.06*
14:0	1.15 ± 0.16	1.45 ± 0.07*
16:0	25.9 ± 0.68	19.3 ± 0.46*
18:0	6.40 ± 2.45	9.38 ± 0.50
20:0	9.36 ± 2.88	14.4 ± 0.34
22:0	0.71 ± 0.55	0.66 ± 0.10
24:0	ND	0.44 ± 0.05
Total Sat.[3]	48.2 ± 7.85	63.7 ± 1.02
14:1n-9	0.09 ± 0.08	0.84 ± 0.51
16:1n-9	9.93 ± 2.76	2.81 ± 0.24*
18:1n-9	39.1 ± 6.09	19.5 ± 0.87*
20:1n-9	ND	0.64 ± 0.15
22:1n-9	0.88 ± 0.46	2.21 ± 0.11*
24:1n-9	0.18 ± 0.15	0.42 ± 0.02
Total Mono.[4]	50.5 ± 8.34	29.7 ± 0.84*
18:2n-6	0.34 ± 0.11	0.60 ± 0.04*
18:3n-6	0.34 ± 0.26	1.21 ± 0.23*
20:2n-6	ND	1.28 ± 1.50
20:3n-6	0.02 ± 0.04	0.92 ± 0.07*
20:4n-6	ND	0.12 ± 0.21
22:2n-6	ND	0.40 ± 0.04
Total n-6[5]	0.70 ± 0.33	4.52 ± 1.85*
18:3n-3	ND	0.56 ± 0.03
20:3n-3	0.02 ± 0.04	0.46 ± 0.16*
20:5n-3	0.17 ± 0.04	0.46 ± 0.01*
22:6n-3	0.46 ± 0.16	0.56 ± 0.08*
Total n-3[6]	0.65 ± 0.16	2.60 ± 0.07*

Note: [1]Data were expressed as mean ± the standard error of the mean (SEM), n = 5. [2]ND means not detected; [3]Sat. means saturated fatty acids; [4]Mono. means monounsaturated fatty acids; [5]n-6 means n-6 polyunsaturated fatty acids; [6]n-3 means n-3 polyunsaturated fatty acids. *$p < 0.05$, significant effect between the control and *L. lucidus* Turcz. groups.

TABLE 4

Effect of supplementation with *L. lucidus* Turcz. on selected fatty acid composition in mesenteric lymph node

	Control	*L. lucidus* Turcz.
Fatty Acids	*% by weight of total fatty acids*	
6:0	0.01 ± 0.01	0.02 ± 0.03
12:0	0.06 ± 0.05	0.04 ± 0.06
14:0	1.65 ± 0.03	2.05 ± 0.43
16:0	27.7 ± 0.85	30.7 ± 2.89
18:0	1.73 ± 0.09	1.85 ± 0.23
20:0	5.68 ± 0.59	7.16 ± 0.39*
22:0	2.29 ± 3.15	6.23 ± 0.47
24:0	ND	0.03 ± 0.04
Total Sat.[3]	40.6 ± 3.84	48.9 ± 2.41*
14:1n-9	0.17 ± 0.02	0.04 ± 0.07*
16:1n-9	12.7 ± 1.19	11.2 ± 0.90
18:1n-9	45.2 ± 2.54	38.0 ± 2.58*
22:1n-9	0.06 ± 0.05	0.04 ± 0.07
24:1n-9	ND	0.02 ± 0.03
Total Mono.[4]	58.4 ± 3.77	49.4 ± 3.40*
18:2n-6	0.50 ± 0.61	0.05 ± 0.08
18:3n-6	0.08 ± 0.07	0.06 ± 0.10
20:2n-6	0.06 ± 0.06	0.65 ± 1.08
20:3n-6	0.28 ± 0.06	0.22 ± 0.19
20:4n-6	0.01 ± 0.02	ND
22:2n-6	0.01 ± 0.01	ND
Total n-6[5]	0.94 ± 0.42	0.97 ± 1.13
18:3n-3	0.04 ± 0.06	0.02 ± 0.03
20:5n-3	0.02 ± 0.03	0.02 ± 0.03
22:6n-3	0.01 ± 0.02	0.65 ± 1.05
Total n-3[6]	0.07 ± 0.06	0.69 ± 1.03

Note: [1]Data were expressed as mean ± the standard error of the mean (SEM), n = 5. [2]ND means not detected; [3]Sat. means saturated fatty acids; [4]Mono. means monounsaturated fatty acids; [5]n-6 means n-6 polyunsaturated fatty acids; [6]n-3 means n-3 polyunsaturated fatty acids. *$p < 0.05$, significant effect between the control and *L. lucidus* Turcz. groups.

make up the remaining (Den Besten *et al.*, 2013). Zhou *et al.* (2014) reported that supplementation with soybean oligosaccharides increased the concentrations of propionate, butyrate, and total SCFA in the ileum and colon contents as well as those of acetate and valerate. It has been known that SCFAs play an important role in colon epithelium renewal as a fuel for colon epithelial cells (Cotter and Hill, 2003). Therefore, we evaluated the fatty acid composition in intestine and feces. As SCFAs, fatty acids of 4:0, 6:0, 8:0 and 10:0 were increased in intestine of mice with *L. lucidus*. Thus, administration with *L. lucidus* would affect gut environment producing SCFAs in our system.

The human intestine is a complex ecosystem of more than 1,000 microbial species with a population of up to 10^4, providing a good habit for these microorganisms (Sender *et al.*, 2016). Intestinal microorganisms are an important bridge between diet and human health and plays a vital role in maintaining the homeostasis of the human body diversity of the species, the stability of the flora structure and the balance of micro ecology (Uchiyama *et al.*, 2019). The human body lacks carbohydrate active enzymes, meaning that most polysaccharides cannot be directly digested and absorbed by the stomach and intestine, and thus pass through to the colon (Chen *et al.*, 2018). Polysaccharides are fermented by specific intestinal microorganism to produce SCFAs and other metabolites. SCFAs are easily absorbed and exert beneficial physiological effects on the host such as the prevention of type-2 diabetes, inflammatory bowel diseases (IBD) and colon cancer (Song *et al.*, 2021). Natural

TABLE 5

Effect of supplementation with *L. lucidus* Turcz. on selected fatty acid composition in feces

	Control	*L. lucidus* Turcz.
Fatty Acids	*% by weight of total fatty acids*	
14:0	0.42 ± 0.73	0.95 ± 0.82
16:0	42.5 ± 2.26	43.5 ± 0.80
18:0	2.68 ± 4.64	5.81 ± 0.72
20:0	9.22 ± 2.55	8.74 ± 1.32
22:0	ND	1.57 ± 2.72
Total Sat.[3]	55.4 ± 6.94	62.0 ± 4.32
14:1n-9	ND	1.12 ± 1.26
16:1n-9	2.68 ± 3.91	1.76 ± 1.70
18:1n-9	30.4 ± 11.9	33.8 ± 6.19
20:1n-9	0.59 ± 1.03	0.55 ± 0.49
22:1n-9	0.50 ± 0.86	ND
24:1n-9	0.34 ± 0.58	0.80 ± 0.72
Total Mono.[4]	34.9 ± 15.5	38.0 ± 4.32
18:2n-6	9.70 ± 0.07	ND
Total n-6[5]	0.94 ± 0.42	ND

Note: [1]Data were expressed as mean ± the standard error of the mean (SEM), n = 5. [2]ND means not detected; [3]Sat. means saturated fatty acids; [4]Mono. means monounsaturated fatty acids; [5]n-6 means n-6 polyunsaturated fatty acids; [6]n-3 means n-3 polyunsaturated fatty acids.

polysaccharides can also suppress excessive inflammatory responses by improving the intestinal microbiota composition, strengthening intestinal barrier function, and reducing pro-inflammatory mediators (Tang *et al.*, 2019). Thus, our hypothesis is that supplementation of *L. lucidus*, which contains polysaccharides, would improve gut microbiota composition, which may lead to increased percentages of SCFAs and decreased pro-inflammatory cytokines. We found that supplementation with *L. lucidus* greatly decreased the community of harmful microbiota including *E. coli* and *Bacteroides* sp. in feces. There were no significant differences in mRNA expression of IL-6 and IL-10 in mesenteric lymph node after supplementation with *L. lucidus*. Fatty acid composition analysis showed that supplementation with *L. lucidus* resulted in increased percentages of SCFAs (4:0, 6:0, 8:0 and 10:0). Many studies have reported an increase in the population of potentially harmful Enterobacteriaceae family in IBD patients, which may lead to reduced SCFAs and dysbiosis condition in the gut (Lupp *et al.*, 2007; Morgan *et al.*, 2012; Mukhopadhya *et al.*, 2012; Lavelle *et al.*, 2015). The loss of beneficial microbes that produce anti-inflammatory molecules provides a favorable environment for the expansion of pathogens (Baldelli *et al.*, 2021). Because the associated mechanisms by which *L. lucidus* intake influences gut microbiota composition were not investigated in this study, the next step will be to conduct an in-depth study on the mechanism related to improvement in the gut environment.

The present study is first report to clarify the effect of *L. lucidus* supplementation on 16S rDNA gene sequence of fecal microflora. Public health and dietary guidance recommendations focus on disease prevention and health maintenance. In this context, we suggest that consistent intake of *L. lucidus* might be associated with maintaining healthy gut function.

Ethics Approval: The experimental protocol was approved by Gyeongsang National University (Approval No. GNU-171116-M0051). Ethical approval for this study was obtained from Korea Maritime & Ocean University–Institutional Animal Care and USE Committee (KMOU-IACUC).

Availability of Data and Materials: The datasets generated or analyzed during this current study are available from the corresponding authors on reasonable request.

Authors' Contribution: Study conception, design and manuscript preparation: Sun Young Lim; data collection, analysis and interpretation of results: Eun Na. All authors reviewed the results and approved the final version of the manuscript.

Funding Statement: This research was supported by Basic Science Research Program of the National Research Foundation of Korea (NRF) in the Ministry of Science, ICT and Future Planning (NRF-2017R1A2B4005915) supported this research.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Baldelli V, Scaldaferri F, Putignani L, Del Chierico F (2021). The role of Enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms* **9**. DOI 10.3390/microorganisms9040697.

Blachier F, Mariotti F, Huneau JF, Tome D (2007). Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids* **33**: 547–562.

Bogert BVD, Meijerink M, Zoetendal EG, Wells JM, Kleerebezem M (2014). Immunomodulatory Properties of *Streptococcus* and *Veillonella* Isolates from the human small intestine microbiota. *PLoS One* **9**: e114277. DOI 10.1371/journal.pone.0114277.

Braune A, Blaut M (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes* **7**: 216–234.

Candela M, Turroni S, Biagi E, Carbonero F, Rampelli S et al. (2014). Inflammation and colorectal cancer, when microbiota-host mutualism breaks. *World Journal of Gastroenterology* **20**: 908–922.

Chen G, Xie M, Wan P, Chen D, Ye H et al. (2018). Digestion under saliva, simulated gastric and small intestinal conditions and fermentation *in vitro* by human intestinal microbiota of polysaccharides from *Fushuan brick* tea. *Food Chemistry* **244**: 331–339.

Costa JFO, Barbosa-Filho JM, Maia GLA, Guimaraes ET, Meira CS et al. (2014). Potent anti-inflammatory activity of betulinic acid treatment in a model of lethal endotoxemia. *International Immunopharmacology* **23**: 469–474.

Cotter PD, Hill C (2003). Surviving the acid test: Response of gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews* **67**: 429–453.

Den Besten G, van Eunen K, Groen AK, Venema K, Reijingoud DJ et al. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research* **54**: 2325–2340.

Duenas M, Munoz-Gonzalez I, Cueva C, Jimenez-Giron A, Sanchez-Patan F et al. (2015). A survey of modulation of gut microbiota by dietary polyphenols. *Biomed Research International* **2015**. DOI 10.1155/2015/850902.

Espley RV, Burts CA, Laing WA, Martell S, Smith H et al. (2014). Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. *Journal of Nutrition* **144**: 146–154.

Fan TT, Cheng BL, Fang XM, Chen YC, Su F (2020). Application of Chinese medicine in the management of critical conditions: A review on Sepsis. *American Journal of Clinical Medicine* **48**: 1315–1330.

Goldsmith JR, Sartor B (2014). The role of diet on intestinal microbiota metabolism: Downstream impacts on host immune function and health, and therapeutic implications. *Journal of Gastroenterology* **49**: 7855–7798.

Holscher H (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes* **8**: 172–184.

Johnson RW (1997). Inhibition of growth by pro-inflammatory cytokines: An integrated view. *Journal of Animal Science* **75**: 1244–1255.

Kim H, Hong JY, Jeon WJ, Lee J, Baek SH et al. (2021a). *Lycopus lucidus* Turcz exerts neuroprotective effects against H_2O_2-induced neuroinflammation by inhibiting NLRP3 inflammasome activation in cortical neurons. *Journal of Inflammation Research* **14**: 1759–1773.

Kim H, Jayapala HPS, Jo WH, Nam HS, Lim SY (2021b). A comparative study on the chemical characteristics and antioxidant effects of sea mustards sourced from different areas in Taejongdae. *Journal of Life Science* **31**: 559–567.

Kim JY, Cho MK, Choi SH, Lee KH, Ahn SC et al. (2010). Inhibition of dextran sulfate sodium (DSS)-induced intestinal inflammation via enhanced IL-10 and TGF-β production by galectin-9 homologues isolated from intestinal parasites. *Molecular and Biochemical Parasitology* **174**: 53–61.

Kim KY, Oh TW, Ma JY, Park KI (2018). Ethanol extract of Lycopus lucidus Turcz. ex Benth inhibits metastasis by downregulation of Runx-2 in mouse colon cancer cells. *Evidence-Based Complementary and Alterative Medicine* **2018**. DOI 10.115/2018/9513290.

Lavelle A, Lennon G, O'Sulivan O, Docherty N, Balfe A et al. (2015). Spatial variation of the colonic microbiota in patients with ulcerative colitis control volunteers. *Gut* **64**: 1553–1561.

Lee JW, Lim SY (2018). Comparison of flavonoid content and antioxidant effect of extrcts from *Stachys sieboldii* Miq. and *Lycopus lucidus* Turcz. *Journal of Life Science* **28**: 841–848.

Li HL, Lu L, Wang XS, Qin LY, Wang P et al. (2017). Alteration of gut microbiota and inflammatory cytokine/chemokine profiles in 5-fluorouracil induced intestinal mucositis. *Frontiers Cellular Infection Microbial* **7**: 1490. DOI 10.3389/fcimb.2017.00455.

Lin C, Zuo S, Xiong W, Chen G (2012). Antioxidation effects of *Lycopus lucidus* polysaccharides on aged induced by D-gallactose. *Medicinal Plant* **3**: 53–55.

Liu J, Bhuvanagiri S, Qu X (2019). The protective effects of *Lycopus lucidus* Turcz. in diabetic retinopathy and its possible mechanisms. *Artificial Cells, Nanomedicine, and Biotechnology* **47**: 2900–2908.

Lu Y, Huang J, Li Y, Ma T, Sang P et al. (2015). Variation in nutritional compositions, antioxidant activity and microstructure of *Lycopus lucidus* Turcz. root at different harvest times. *Food Chemistry* **183**: 91–100.

Lupp C, Robertson ML, Wickham ME, Sekinov I, Champion OL et al. (2007). Host-mediated inflammation disrupts the intestinal mmicrobiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host & Microbe* **2**: 119–129.

Maldonado Galdeano C, de Moreno de LeBlianc A, Vinderola G, Bibas Bonet ME, Perdigon G (2007). Proposed model mechanisms of immunomodulation induced by probiotic bacteria. *Clinical and Vaccine Immunology* **14**: 485–492.

McCurry KR, Campbell DA Jr, Scales WE, Warren JS, Remick DG (1993). Tumor necrosis factor, interleukin 6 and the acute phase response following hepatic ischemia/reperfusion. *Journal of Surgical Research* **55**: 49–54. DOI 10.1006/jsre.1993.1107.

Min GY, Kim EY, Hong S, Kim JH, Kim M et al. (2021). *Lycopus lucidus* Turcz ameliorates DNCB-induced atopic dermatitis in BALB/c mice. *Molecular Medicine Reports* **827**. DOI 10.3892/mmr.2021.12467.

Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL et al. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biology* **13**: R79. DOI 10.1186/gb-2012-13-9-r79.

Morrison WR, Smith LM (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* **5**: 600–608. DOI 10.1016/S0022-2275(20)40190-7.

Mosmann TR (1994). Properties and functions of interleukin-10. *Advances in Immunology* **56**: 1–26. DOI 10.1016/S0065-2776(08)60449-6.

Mukhopadhya I, Hansen R, El-Omar EM, Hold GL (2012). IBD-what role do proteobacteia play? *Nature Reviews Gastroenterology Hepatology* **9**: 219–230. DOI 10.1038/nrgastro.2012.14.

Panche AN, Diwan AD, Channdra SR (2017). Flavonoids: An overview. *Journal of Nutritional Science* **8**: 172–184.

Park HJ (2019). Isolation of constituent inhibiting nitric oxide formation from *Lycopus lucidus* in LPS-induced macrophage cells. *Korean Journal of Plant Research* **32**: 264–269.

Park HJ, Jin S, Oh YM, yun SG, Lee JY et al. (2013). Induction of G1 arrest by methanol extract of Lycopus lucidus in human lung adenocarcinoma A549 cell. *Journal of Life Science* **23**: 1109–1117.

Park YH, Seo KS, Ahn JS, Yoo HS, Kim SP (2001). Evaluation of the Petrifilm plate method for the enumeration of aerobic microorganisms and coliforms in retailed meat samples. *Journal of Food Protection* **64**: 1841–1843.

Reeves PC, Nielson FH, Fahey GC Jr (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition* **123**: 1939–1951.

Salem N, Reyzer M, Karanian J (1996). Losses of arachidonic acid in rat liver after alcohol inhalation. *Lipids* **31**: S153–S156.

Sender R, Fuchs S, Milo R (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology* **14**: e1002533. DOI 10.1371/journal.pbio.1002533.

Soares MB, Titus RG, Shoemaker CB, David JR, Bozza M (1998). The vasoactive peptide maxadilan from sand fly sliva inhibits TNF-alpha and induces IL-6 by mouse macrophages through interaction with the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor. *Journal of Immunology* **160**: 811–1816.

Song YJ, Chang JP, Yoo JH (2016). Antioxidant activities of water extracts from different parts of *Lycopus lucidus* Turcz. ex Benth. *Korean Journal of Herbaology* **31**: 21–28.

Song Q, Wang Y, Huang L, Shen M, Yu Y et al. (2021). Review of the relationships among polysaccharides, gut microbiota, and human health. *Food Research International* **140**. DOI 10.1016/j.fodres.2020.109858.

Tang C, Ding R, Sun J, Liu J, Kan J, Jin C (2019). The impacts of natural polysaccharides on intestinal microbiota and immune responses–A review. *Food Function* **10**: 2290–2312.

Topping DL, Clifton PM (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiology Review* **81**: 1031–1064.

Tung D, Cheung PH, Tudor G, Booth C, Saha S (2011). *In vivo* effects of immunomodulators in a murine model of fluorouracil-induced mucositis. *Current Therapeutic Research* **72**: 262–272.

Uchiyama K, Naito Y, Takagi T (2019). Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacol Therapeutics* **199**: 164–172.

Vandeputter D, Falony G, Vieira-Silva S, Tiro RY, Joosens M et al. (2016). Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* **65**: 57–62.

Velazquez M, Davies C, Marett R, Slavin JL, Feirtag JM (2000). Effect of oligosaccharides and fibre substitutes on short-chain fatty acid production by human faecal microflora. *Anaerobe* **6**: 87–92.

Woo ER, Piao MS (2004). Antioxidant constituents from *Lycopus lucidus*. *Archives of Pharmacology Research* **27**: 173–176.

Xi M, Yao Q, Ge W, Chen Y, Cao B et al. (2020). Effects of Stachyose on intestinal microbiota and immunity in mice infected with enterotoxigenic Escherichia coli. *Journal of Functional Foods* **64**: 103689. DOI 10.1016/j.jff.2019.103689.

Yang MO (2017). Antioxidant properties of hot water extract of *Lycopus lucidus* Turcz. tubers. *Korean Journal of Community Living Science* **28**: 103–113.

Yang X, Lv Y, Tian L, Zhao Y (2010). Composition and systemic immune activity of the polysaccharides from an herbal tea (*Lycopus lucidus* Turcz). *Journal of Agriculture and Food Chemistry* **58**: 6075–6080.

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic Evolutionary Microbiology* **67**: 1613–1617.

Yu JQ, Lei JC, Zhang XQ, Yu HD, Tian DZ et al. (2011). Anticancer, antioxidant and antimicrobial activities of the essential oil of *Lycopus lucidus* Turcz. var. hirtus Regel. *Food Chemistry* **126**: 1593–1598.

Zhang W, Hu Y, He J, Guo D, Zhao J et al. (2021). Structural characterization and immunomodulatory activity of a novel polysaccharide from Lycopi herba. *Frontiers Pharmacology* **25**. DOI 10.3389/fphar.2021.69995.

Zhou XL, Kong XF, Lian GQ, Blachier F, Geng MM et al. (2014). Dietary supplementation with soybean oligosaccharides increase short-chain fatty acids but decreases protein-derived catabolites in the intestinal luminal content of weaned Huanjiang mini-piglets. *Nutrition Research* **34**: 780–788.