Bifidogenic effect of grain larvae extract on serum lipid, glucose and intestinal microflora in rats

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The main objective of this study was to investigate whether orally administered Korean grain larvae ethanol extract (GLE) had a bifidogenic effect in normal rats. Male Sprague–Dawley rats were divided into a negative control group (CO) and GLE orally administered (5.0, 7.0 and 9.0 mg/100 g body weight) groups. Thymus and spleen weights dose-dependently increased by 128.58% and 128.58%, respectively, but abdominal fat decreased by 19.18% after GLE administration compared with that in the CO group ($p<0.05$). Serum triglycerides, total cholesterol, low-density lipoprotein cholesterol, and glucose decreased by 30.26%, 7.33%, 27.20%, and 6.96%, respectively, whereas high-density lipoprotein cholesterol increased by 129.93% in the GLE groups compared with those in the CO group ($p<0.05$). IgG, IgM, IgA in the GLE groups increased 203.68%, 181.41%, and 238.25%, respectively, compared to that in the CO group ($p<0.05$). *Bifidobacteria* and *Lactobacillus* increased by 115.74% and 144.28%, whereas *Bacteroides, Clostridium, Escherichia*, and *Streptococcus* decreased by 17.37%, 17.46%, 21.25%, and 19.16%, respectively, in the GLE groups compared with those in the CO group ($p<0.05$). Total organic acids, acetic acid, and propionic acid increased by151.40%, 188.09%, and 150.17%, whereas butyric acid and valeric acid decreased by 40.65% and 49.24%, respectively, in the GLE groups as compared with those in the CO group ($p < 0.05$). These results suggest that Korean GLE improves the bifidogenic effect by increasing cecal organic acids and modulating gut microflora via a selective increase in *Bifidobacterium* in normal rats.

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1. Introduction

Korean grain larvae, a type of housefly, feed on grain such as corn or soybean in South Korea. Grain larvae are non-toxic and offer various pharmacological efficacies (Ratcliffe *et al.* 2011). Grain larvae are described in the Dictionary of Chinese Medicine, a comprehensive dictionary of Chinese pharmaceutical raw materials, to be effective for blood cleansing and reducing fever and are described in the Sungje Chongrok as a remedy for insatiable appetite. The Boncho Gangmok from the Chosun Dynasty prescribed these larvae when other fever-reducing medicines were ineffective. Boncho Pyeondok is prescribed for malignant lip tumours (Park and Park 2012).

The biotherapeutic technology that uses housefly (*Musca domestica L.*) maggots for patients with chronic wound infections has been used for a long time. Due to the recent emergence of super bacteria, biotherapeutic technology for patients with burns and bedsores has been applied to the medical field (Sherman *et al.* 2000). The 5–22 kDa portion of an ethanol extract from housefly maggot secretions contains antimicrobial peptides that are highly effective against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci strains (Bexfield *et al.* 2004; Chunju *et al.* 2004; Park *et al.* 2007). Previous studies have reported that when different dose levels of housefly maggot and grain larvae ethanol extract (GLE) were orally administered to rats that were fed a chow or a high-fat diet,

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the extracts had bioactive effects such as *in vitro* antimicrobial activity and prevention of alcoholic liver damage (Park *et al.* 2010; Park and Park 2012).

It has been recently found that cerebrovascular and cardiovascular diseases, which are caused by lipid metabolism, take the second place after cancer, the number-one cause of death of Korean people. The disease burden due to metabolic syndrome such as hypercholesterolemia, obesity, cardiovascular disease, and diabetes as a result of rapid aging and a high-fat-meat diet is increasing (Eckel *et al.* 2005). High levels of blood lipids and glucose are factors that lead to obesity, and hyperlipoproteinemia is related to triglycerides, low-density lipoprotein cholesterol (LDL-C), and diabetes (Liu *et al.* 2012). A bifidogenic effect is a bioactive function that promotes growth of the host through a selective increase in bifidobacteria, which are beneficial intestinal bacteria in the colon and cecum of humans and animals. These bacteria improve antimicrobial activity, immunity, and blood lipid metabolism and increase nutrient absorption rates (Modler *et al.* 1990; Gibson and Wang 1994; Veerman-Wauters *et al.* 2011; Lomax *et al.* 2012). Many studies have been conducted on the *in vitro* antitumour and antimicrobial activities of a housefly maggot extract (Jaklic *et al.* 2008; Park *et al.* 2010), whereas only a few reports are available on the bifidogenic effect of a GLE in humans and animals.

The present study was conducted to investigate the bifidogenic effect of orally administered GLE on improving lymphoid weight, blood lipids, immunoglobulin, cecal microflora, and organic acids in normal rats.

2. Materials and methods

2.1 *Preparation of GLE*

500 g of 2- to 3-day-old grain larvae that had been dried in a convection dry oven (70°C) were supplied from Dr. Insectbio Co., Ltd. (Chuncheon South Korea). By pressing the grain larvae for 30 min at 150°C and 1,000 PSI condition, 98% of the lipids were removed. The remaining lipids in the grain larvae were removed completely using hexane, and defatted grain larvae residue was obtained. The defatted grain larvae residue and ethanol (EtOH, 99.5%) were mixed at a ratio of 1:10. Useful material was extracted using a reflux condensing system, and it was then concentrated using a vacuum rotary evaporator (Eyela N-1000, Tokyo Rikakikai Co., Japan) at 40°C under reduced pressure. The total yield of extract of the grain larvae ethanol extract (GLE) containing the 5 kDa antibacterial peptide was 4.30%, and 21.5 g was obtained (Park *et al*. 2007, 2010).

2.2 *Animal and experimental design*

Animal experiments were conducted in accordance with the scientific procedures and ethical principles of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA, 1985) and were approved by the Animal Ethics Committee of Kangwon National University, Republic of Korea. Forty male Sprague–Dawley rats (Daehan Bio Link Co., Ltd. Chungbuk, South Korea), weighing 200 g, were housed individually in plastic-bottom cages under controlled temperature (20–24°C) and a 12 h light/dark cycle. The GLE as an indicator marker used to show the variation across different batches.

In the animal experiment, for the control, 1 mL of saline without GLE was administered, while for three GLE groups, different levels of GLE were dissolved in 1 mL of saline and were administered orally. The purpose of the study was to investigate bifidogenic effects of the oral administration of different levels of GLE, containing the peptide. Thus, an equivalent amount of a non-specific peptide was not used as the control. Ten animals were assigned to each treatment group using a randomized complete block design. The rats were acclimated for 1 week prior to initiating the study and fed AIN-93 purified pellet diet (Reeves *et al.* 1993) for 4 weeks. The purified diet contained 20.0% casein (vitamin free), 13.2% corn starch, 10.0% maltodextrin, 7.0% sugar, 5.0% soybean oil, 5.0% powdered cellulose, 1.00% AIN 93G mineral mixture, 0.30% AIN 93G vitamin mixture, 0.30% L-cystine, 0.25% choline bitartrate, and 0.0014% t-butylhydroquinone. Rats were allowed *ad libitum* access to food and tap water throughout the study.

2.3 *Oral administration of GLE*

GLE was administered orally for 4 weeks using a stomach tube with diameter 1 mm at a specific time every day. For the control group without GLE, 1 mL of saline was administered; for the GLE groups (5.0, 7.0, and 9.0 mg and 9.0 mg), the amount of GLE corresponding to each treatment group was dissolved in 1 ml of saline and was administered orally.

2.4 *Blood and organ collection, lipid profile and glucose analysis*

After 4 weeks of repletion period, the overnight fasted rats were lightly anesthetized with ethyl ether to collect 2 mL of whole blood using a 5 mL syringe and a needle (2.5 cm, 21-gauge) via cardiac puncture. The abdominal fat, kidney, liver, spleen and thymus were harvested and weighed. The relative weight of organs was shown as the percentage of body weight.Whole blood was placed into serum separator

tubes (SST tube, BD Falcon, Franklin Lakes, NJ, USA) and left to sit for 30 min at room temperature for coagulation. Then, the serum was separated by centrifugation at 1500*g* for 15 min at 4°C. The levels of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were analysed with diagnostic kits (Sigma Chemical Co., St, Louis, MO, USA), and the levels of low-density lipoprotein cholesterol (LDL-C) were calculated with the Friedewald formula (Friedwald *et al.* 1972).Serum glucose levels were measured using a Beckman glucose analyser (Beckman Instrument Co., Palo Alto, CA, USA).

2.5 *Determination of serum immunoglobulin*

The determination of serum immunoglobulin, IgG, IgM and IgA levels was conducted with an enzyme-linked immunosorbent assay (Bethyl Laboratories Inc., Montgomery, TX, USA) and a commercial kit (Rat EIA kit, TKR, Shiwa-Cho, Japan). After treating and reacting the organs according to the manufacturer's manual, absorbance in each well was measured using a microplate reader (Molecular Devices, Inc., Sunnyvale, CA, USA) at 450 nm and the levels were calculated by comparison to a concentration curveof standards.

2.6 *Determination of cecal microflora*

To evaluate intestinal microorganisms, rats were sacrificed without stress via diethyl ether anesthesia; the cecum (cecal content of about 1.8 g per animal) was isolated via the anaerobic method and was maintained on the ice. It was kept under anaerobic conditions in sealed anaerobic jars (Oxoid, Basingstoke, UK) equipped with AnaeroGen sachets (Oxoid, Hampshire, UK). All procedures were conducted under anaerobic conditions in an anaerobic chamber (80% N_2, 10% CO_2, and 10% H_2).Cecal contents of about 1.0 g from each animal were placed in tubes containing 10 mL of sterilized saline water for homogenization, and the homogenized contents were diluted consecutively from 10^{-2} to 10^{-8}. A total of 100 µL each of the diluted solutions was divided into sterilized media three times repeatedly, and the plates were cultured for 48 h at 37°C for bacterial counts. *Bacteroides* spp. (Bacteroids bile esculin agar, Difco), *Bifidobacterium* spp. (Modified Columbia agar, Oxoid Ltd., Thermo Fisher Scientific Inc., Rockford, IL, USA), *Clostridium* spp. (egg yolk agar, Difco, Detroit, MI, USA), *Escherichia* spp. (McConkey's agar, BBL, Baltimore, MD, USA), *Lactobacillus* SPP. (MRSagar, Oxoid Ltd) and *Streptococcus* spp. (SF agar, Difco) were used as media. The log of the bacterial counts per g of fresh cecal content ($\log_{10}$ colony forming units/g fresh cecal content) was calculated.

2.7 *Determination of cecal organic acids*

The concentrations of cecal organic acids (standards from Sigma) such as acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid were measured using a gas chromatography (GC) system (model GC-15A, Shimadzu Corp., Kyoto, Japan) and 4-methyl normal valeric acid (Sigma) as the internal standard (Zhang *et al.* 2003).After mixing approximately 0.5 g of cecal content obtained from each animal with 5 mL of distilled water in a screw cap tube and homogenizing the mixture, it was centrifuged at 1500*g* for 10 min at 4°C. After 1 mL of the supernatant liquid was transferred to an ample bottle and left to sit in an icebox for more than 30 min, 0.2 mL of metaphosphoric acid solution was added, and the mixture was centrifuged at 1500*g* for 10 min before the GC analysis. The GC with a flame ionization detector and a polyethylene glycol column (Hewlett Packard, 30 m × 320 µm × 0.50 µm; Dallas, TX, USA) analysed the liquid at 100–150°C using highly purified N_2 (1.8 mL/min) as the carrier gas.

2.8 *Statistical analysis*

For determination of organ weights, serum lipid and glucose, immunoglobulin, cecalmicroflora and organic acidmeasurements, each rat was considered as an experimental unit for statistical analysis. All data were analysed by SAS software (SAS Institute, Cary, NC, USA). The data was analysed by one-way analysis of variance by using general linear model procedure of SAS and mean values and standard deviation were reported. TheP-values less than 0.05 ($P<0.05$) was considered statistically significant (SAS, 2005).

3. Results

3.1 *Lymphoid organs*

Mean diet consumption (23.5±0.21 g/day) and weight gain (65.1±0.35 g/week) of rats during the experimental period did not differ among the treatment groups. Table 1 shows the changes in organ weights in the GLE orally administered groups. Liver and kidney weights were not different among the treatment groups. When 5.0, 7.0, and 9.0 mg/100 g BW GLE was orally administered to rats, the weights of the spleen and thymus increased by 128.58% and 128.58%, respectively, compared with values in the CO group ($p<0.05$) but did not differ among the GLE groups. Abdominal fat decreased by 19.18% in the GLE orally administered groups as compared with values in the CO group ($p<0.05$).In addition, the dose-dependent effect of GLE administration was observed to decrease in the order of 5.0, 7.0, and 9.0 mg and 9.0 mg; however, no significant

Table 1. Effect of the oral administration of GLE on organ weight in rats (g/100 gBW)

	GLE (mg/100 gBW)			
	0	5.0	7.0	9.0
Liver	3.26±0.14	3.34±0.10	3.23±0.13	3.25±0.10
Kidney	0.60±0.02	0.59±0.04	0.57±0.07	0.59±0.04
Abdominal fat	0.73±0.04[a]	0.67±0.01[b]	0.60±0.02[c]	0.59±0.01[c]
Thymus	0.21±0.01[b]	0.26±0.01[a]	0.27±0.01[a]	0.26±0.01[a]
Spleen	0.23±0.01[b]	0.26±0.005[a]	0.27±0.01[a]	0.27±0.005[a]

Means±SD (n=10).

Means with different superscripts in the same row are significantly different ($P<0.05$).

difference was observed in abdominal fat between the 7.0 mg and 9.0 mgGLE groups.

3.2 *Serum lipid and glucose levels*

The changes in serum lipid and glucose levels are shown in table 2. When 5.0, 7.0, and 9.0 mg/100 g BW GLE was orally administered to rats, serum TG, TC, LDL-C and glucose dose-dependently decreased by 30.26%, 7.33%, 27.20%, and 6.96%, respectively, compared with values in the CO group ($p<0.05$).Triglycerides were significantly higher in the group administered 5.0 mg GLE compared with the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was detected between the 7.0 mg and 9.0 mg GLE groups. Total cholesterol and LDL-C were significantly higher in the group administered 5.0 mg GLE compared with the groups administered 7.0 mg and 9.0 mg GLE, but no significant difference was observed between the 7.0 mg and 9.0 mg GLE groups. A significant increase in HDL-Cof 129.92% was observed in the groups administered GLE. The level in the group administered 5 mg GLE was significantly lower than that in the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was detected between the groups administered 7.0 and 9.0 mg GLE.

3.3 *Serum immunoglobulins*

Table 3 shows serum IgG, IgA, and IgM levels in rats after oral administration of GLE. When 5.0, 7.0 and 9.0 mg/100 g BW GLE were orally administered to rats, serum IgG, IgM, and IgA levels dose-dependently increased by 203.68%, 181.41%, and 238.25% ($p<0.05$), respectively, as compared with the values in the CO group.IgG was significantly higher depending on the GLE concentration in the order of 5.0, 7.0, and 9.0 mg. IgM and IgA were significantly lower in the group administered 5.0 mg GLE compared with the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was observed between groups administered 7.0 mg and 9.0 mg GLE.

3.4 *Cecal microflora*

Table 4 shows the bacterial counts based on the colony on specific media in rats after oral administration of GLE. When 5.0, 7.0 and 9.0 mg/100 g BW GLE was orally administered to rats, *Bifidobacteria* and *Lactobacillus* dose-dependently increased by 115.74% and 144.28% ($p<0.05$), respectively, compared with values in the CO group. *Bifidobacteria* was significantly higher in the groups administered 7.0 mg and 9.0 mg GLE compared with the control group and the group

Table 2. Effect of the oral administration of GLE on serum lipid profile and glucose in rats (mg/dL)

	GLE (mg/100 g BW)			
	0	5.0	7.0	9.0
Triglyceride	109.8±2.29[a]	97.93±0.30[b]	76.93±1.43[c]	76.33±1.04[c]
Total cholesterol	78.63±1.33[a]	76.60±0.46[b]	72.87±1.46[c]	73.06±0.60[c]
HDL cholesterol	28.27±1.36[c]	32.73±0.75[b]	36.50±0.50[a]	36.73±0.30[a]
LDL cholesterol	47.43±0.60[a]	41.33±0.60[b]	34.53±0.55[c]	34.63±0.60[c]
Glucose	88.67±1.06[a]	86.53±0.55[b]	83.23±0.87[c]	82.50±0.70[c]

Means±SD (n=10).

Means with different superscripts in the same row are significantly different ($P<0.05$).

Table 3. Effect of the oral administration of GLE on IgG, IgM and IgA in rats (ng/mL)

	GLE (mg/100 g BW)			
	0	5.0	7.0	9.0
IgG	31.02±0.17^{d}	50.71±0.36^{c}	58.16±0.22^{b}	63.18±0.31^{a}
IgM	10.82±0.45^{c}	18.79±0.34^{b}	20.17±0.19^{a}	20.08±0.21^{a}
IgA	3.19±0.33^{c}	6.51±0.26^{b}	7.60±0.35^{a}	7.15±0.24^{a}

Means±SD (n=10).

Means with different superscripts in the same row are significantly different (P<0.05).

administered 5.0 mg GLE. No significant difference was observed between the groups administered 7.0 mg and 9.0 mg GLE. *Bacteroides* and *Clostridium* were dose-dependently reduced by 17.37% and 17.46% (p<0.05) after oral administration of GLE, and *Escherichia* and *Streptococcus* decreased dose-dependently by 21.25% and 19.16%, respectively, compared with values in the CO group (p<0.05).Levels of *Bacteroides, Clostridium, Escherichia,* and *Streptococcus* were significantly higher in the group administered 5.0 mg GLE compared with the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was observed between groups administered 7.0 mg and 9.0 mg GLE.

Table 5 shows the changes in cecal organic acids after oral administration of GLE. When 5.0, 7.0, and 9.0 mg/100 g BW GLE was orally administered to rats, total organic acids, acetic acid, and propionic acid increased by 151.40%, 188.09%, and 150.17%, respectively, compared with the values in the CO group (p<0.05). The GLE 9.0 mg administration group had the highest value, but there was no statistically significant difference between the GLE 5.0 and 7.0 mg groups. Butyric acid, isobutyric acid, valeric acid, and isovaleric acid decreased by 40.65%, 84.58%, 49.24%, and 59.26%, respectively, after administration compared with values in the CO group (p<0.05). Total organic acids were significantly higher in the groups administered GLE than those in the CO group; however, no significant difference was detected between the groups administered GLE. Acetic acid was significantly higher in the group administered 9.0 mg GLE compared with the groups administered 5.0 mg and 7.0 mg GLE; however, no significant difference was observed between the groups administered GLE. Propionic acid was significantly higher in the group administered 5.0 mg GLE compared with the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was observed between the groups administered 7.0 mg and 9.0 mg GLE. Butyric acid and valeric acid were significantly higher in the group administered 9.0 mg GLE compared with the groups administered 5.0 mg and 7.0 mg GLE; however, no significant difference was detected between the group administered 5.0 mg and 7.0 mg GLE. Isobutyric acid and isovaleric acid were significantly higher in the group administered 5.0 mg EM compared with the groups administered 7.0 mg and 9.0 mg GLE; however, no significant difference was observed between the groups administered 7.0 mg and 9.0 mg GLE.

4. Discussion

It is speculated that the bifidogenic effect was due to the *Bifidobacteria* and *Lactobacillus* growth mechanism in the cecum, which was expressed by the antimicrobial peptides in the GLE and increased the weights of lymphoid organs such as the thymus and spleen and also increased the amount of immunoglobulins secreted (Park 2008). There was no change in the liver or kidney weights, suggesting that GLE had no significant effect on organ weight, excluding abdominal fat, thymus, and spleen. Thus, it is important that these are presented although there was no difference. Antibacterial peptides increase the number of *Bifidobacteria* and *Lactobacillus* (Sánchez *et al.* 2010, 2011). The thymus is an important antibody-producing organ, and the thymus

Table 4. Effect of the oral administration of GLE on cecal microflora in rats (log_{10}cfu/g fresh cecal content)

	GLE (mg/100 gBW)			
	0	5.0	7.0	9.0
Bacteroides	7.83±0.15^{a1}	7.20±0.10^{b}	6.53±0.47^{c}	6.47±0.15^{c}
Bifidobactera	6.67±0.15^{c}	7.10±0.10^{b}	7.70±0.10^{a}	7.72±0.12^{a}
Clostridium	6.70±0.10^{a}	6.00±0.10^{b}	5.60±0.10^{c}	5.53±0.05^{c}
Escherichia	5.93±0.11^{a}	5.60±0.10^{b}	4.70±0.10^{c}	4.67±0.15^{c}
Lactobacillus	6.03±0.11^{d}	7.33±0.21^{c}	8.70±0.25^{a}	8.06±0.24^{b}
Streptococcus	7.10±0.10^{a}	6.33±0.15^{b}	5.84±0.05^{c}	5.74±0.13^{c}

[1]Means±SD (n=10).Values show the bacterial counts based on the colony on specific media. Values with different superscripts in the same row are significantly different (P<0.05).

Table 5. Effect of the oral administration of GLE on organicacid in rats (unit: μmoL/g of fresh cecal content)

	GLE (mg/100 gBW)			
	0	5.0	7.0	9.0
Acetic acid	41.55±0.23^{c}	76.61±0.42^{b}	76.27±0.51^{b}	78.15±0.25^{a}
Propionic acid	21.15±0.33^{c}	31.76±0.32^{a}	29.98±0.25^{b}	30.09±0.25^{b}
Butyric acid	8.01±0.20^{a}	4.73±0.37^{b}	4.85±0.23^{b}	5.37±0.28^{c}
Isobutyric acid	2.01±0.33^{a}	1.02±0.11^{b}	0.33±0.10^{c}	0.31±0.09^{c}
Valeric acid	3.96±0.11^{a}	2.01±0.06^{c}	2.08±0.05^{c}	3.07±0.08^{b}
Isoveric acid	0.81±0.05^{a}	0.45±0.02^{b}	0.38±0.05^{c}	0.33±0.06^{c}
Total	77.49±0.23 ^{c}	116.58±0.32^{a}	113.89±0.41^{b}	117.32±0.34^{a}

Means±SD (n=10).

Means with different superscripts in the same row are significantly different (P<0.05).

index data indicated that the antimicrobial peptides in the GLE improved growth of thymus cells in the rats. Immune proteins are important for successful conversion of IgM to IgG as well as for the immune ability of IgA, which mainly depends on the spleen and thymus (Bienenstock *et al.* 1973). Therefore, the increased production of cells containing immune proteins and the elevated concentrations of blood immune proteins might be due to the lymphoid organs in rats fed the GLE. It is concluded that the low level of abdominal fat in the groups that were administered GLE could be attributed not only to decreased lipid levels that was transferred to visceral tissue by decreased blood TG levels (table 2) but also due to the bifidogenic effect of the selective growth of beneficial *Bifidobacteria* and *Lactobacillus* in the cecum (table 4). The reduction in abdominal fat following GLE administration was attributed to a reduction in blood lipids. It is generally recognized that blood lipids migrate to living tissues where they are used to generate energy, and that the remainder are stored in abdominal cavity tissues (Park 2008). Therefore, the reduction in abdominal fat can likely be explained by a reduction in the amount of lipid that moved to the abdominal cavity tissues due to the lowering of blood lipids resulting from administering the GLE antibacterial peptide. *Bifidobacteria* and *Lactobacillus* decrease TG and cholesterol levels by regulating blood lipid metabolism (Patterson and Burkholder 2003).

The decreases in blood glucose and harmful lipid levels in the groups that were administered the grain larvae extract are similar to the results of Park and Park (2012), which is estimated to be attributed to the bifidogenic effect from antimicrobial peptides in grain larvae extract (Patterson and Burkholder 2003; Roberfroid 2000). Hemoglobin A1c (HbA1c) levels of groups were not measured. We found that GLE reduced blood glucose and harmful lipid levels in our study. These results may be attributed to the bifidogenic effect from *Bifidobacteria* and *Lactobacillus* growth by the antimicrobial peptides in a GLE (Gibson and Wang, 1994; Roberfroid 2000; Patterson and Burkholder 2003; Roberfroid 2003; Alejandra *et al.* 2009; Sánchez *et al.* 2010, 2011; Lomax *et al.* 2012).

Antibody titres are increasing but there is requirement of appropriate control, if its relevance has to be correlated with other parameters. The higher concentrations of IgG, IgA, and IgM in the GLE-administered groups may have resulted from the increased lymphoid organ weight in the GLE-administered groups compared to that in the control group, as high levels of IgG, IgA and IgM are secreted from heavier lymphoid organs and are attributed to the bifidogenic effect expressed by the growth of *Bifidobacteria* and *Lactobacillus* from the antibacterial peptide in the GLE (Park 2008).

In the previous study, bifidogenic effect has been reported when broilers were fed experimental diets containing inuloprebiotics, the number of *Bifidobacteria and Lactobacillus* increased significantly while that of *Escherichia coli* decreased significantly in the cecal content of the inuloprebiotics addition group. Bifidogenic effect selectively stimulates the growth of beneficial *Bifidobacteria and Lactobacillus*, thereby inhibiting the growth of harmful bacterial strains (Park 2008). Antibacterial pepetides stimulate bifidogenic effect via increasing the number of *Bifidobacteria* and *Lactobacillus*. The extracellular protein regulates certain signalling pathways and cellular responses, including secretion of different effector molecules such as chemokines, cytokines or antibacterial peptides (Sánchez *et al.* 2010, 2011). Cecal *Bifidobacteria* and *Lactobacillus* are important for increasing circulating concentrations of immunoglobulins and non-specific immune cell activities of granulocytes (Roberfroid2003).The high serum IgG level in rats that were administered the GLE indicates a high efficiency of antimicrobial peptides in the extract for increasing humoral immunity (Park 2008). Immune proteins are produced in B-cells of the bone marrow, and as IgG has

the highest concentration in blood and promotes biological immunity, the blood IgG titer is a marker of humoral immunity (Higgins 1975). If the immunogenic properties are deciding this observed change in population, then a detailed analysis supporting the changes in observation in microbial population with nonspecific immunogenic peptide could only support in little change observed in microbial population. Additional research into this will be needed.

The increased growth of cecal *Bifidobacteria* and *Lactobacillus* in the GLE-administered groups and their elevated immunoglobulin concentrations compared to those in the control group were considered to be a bifidogenic effect from the antimicrobial peptides in the GLE (Modler *et al.* 1990; Park 2008; Sánchez *et al.* 2010, 2011). Microflora in the digestive tract play an important role synthesizing fermentation products that supply the energy necessary for intestinal epithelial cells, stimulating the digestive tract immune system, synthesizing vitamin K, and preventing clustering of extrinsic pathogens (Modler *et al.* 1990). *Bifidobacteria* and *Lactobacillus* compete with potential pathogens for nutrients and attachment sites, resulting in a reduction in the number of intestinal pathogens. *Bifidobacteria* and *Lactobacillus* secrete bacteriocins that inhibit the growth of harmful bacteria such as *Escherichia coli* and produce organic acids such as lactic acid and acetic acid. These substrates inhibit the clustering of intestinal pathogens (Rolfe 2002; Lomax *et al.* 2012). However, in this study we did not measure lactic acid, which is the signature acid produced by *Lactobacillus*. There is a need for further study with related to lactic acid.

The bifidobacteria and lactobacillus numbers of the GLE groups increased significantly, compared with the control. The selective increase in the bifidobacteria numbers may have a bifidogenic effect, enhancing immunity by reducing harmful enteric microorganisms and stimulating the immune system. The increased beneficial *Bifidobacteria* and *Lactobacillus* count in the cecum and significantly decreased bacterial counts of harmful *Bacteroides, Clostridium, Escherichia coli, and Streptococcus* in the GLE-administered groups were considered due to these mechanisms (Park and Park 2012).

It has been suggested that increased organic acids, which are beneficial for the cecal environment, improve lymphoid organ weight and immunoglobulin levels. Thus, the reduced blood lipids in the GLE-administered groups were attributed to the bifidogenic effect that was expressed through the selective growth of *Bifidobacteria* in the rats (Alejandra *et al.* 2009; Lomax *et al.* 2012). *Bifidobacteria* lower intestinal pH by producing acetic and lactic acids, which may suppress growth of pathogenic bacteria (Napoli *et al.* 2003). This study is the first report on the *in vivo* bifidogenic effects of a GLE in animals. In conclusion, the GLE promoted increases in lymphoid weight, blood lipids, IgG, cecal microflora, and organic acids. The *in vivo* bifidogenic effect of the GLE observed in this study can be attributed to the antibacterial peptides present in the GLE.

In conclusion, orally administration of GLE >5 mg/100 g body weight improved the bifidogenic effect and was related to lymphoid weight, blood lipids, IgG, cecal microflora, and organic acids in normal rats. The bifidogenic effect of the GLE observed in this study was attributed to the antibacterial peptides present in the GLE. The GLE may be useful as a biomedical resource from insects to prevent medical disorders.

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