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Effect of *Lactobacillus acidophilus* based probiotic product supplementation on the blood profile, fecal noxious gas emission, and fecal shedding of lactic acid bacteria and coliform bacteria in healthy adult Beagle dogs

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Abstract

The aim of this study was to evaluate the effect of *Lactobacillus acidophilus* probiotic (LAP) product supplementation on the blood profile, fecal noxious gas emission, and fecal shedding of lactic acid bacteria and coliform bacteria in healthy adult Beagle dogs. In total, 14 Beagle dogs with an average initial body weight of 10.19 ± 0.61 kg were randomly assigned into two dietary treatments,with and without LAP supplementation, for a 28-day feeding trial. At the end of the experiment, there was no significant ($p > 0.05$) difference in the concentration of serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), white blood cell (WBC), red blood cell (RBC), blood lymphocyte percentage, fecal hydrogen sulfide (H_2S) and total mercaptans (R.SH) emission, and fecal coliforms counts. However, the serum concentrations of the triglyceride and fecal ammonia (NH_3) emission of the LAP treatment were significantly ($p < 0.05$) decreased in the group compared with the CON dogs. Fecal total lactic acid bacteria counts were significantly ($p < 0.05$) increased in the LAP treatment. In conclusion, the supplementation of LAP in Beagle dog diets could decrease the blood triglyceride level and enhance the gut *Lactobacillus* count which may have positive effects on dogs.

Keywords: adult Beagle dogs, blood profile, fecal bacteria shedding, fecal noxious gas emission, *Lactobacillus acidophilus* probiotic

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Introduction

Probiotics have been widely researched as one of the alternatives to antimicrobial growth promoter following the ban of antibiotics usage as growth promoters in animal feeds throughout Europe in 2006 (Liu et al., 2018a). Probiotics are defined as living microorganisms, which confer beneficial health effect on the host animal by improving the balance of the intestinal microbiota (Lan et al., 2016). *Lactobacillus* is lactic acid-producing bacteria and Gram-positive microbiota which

extensively existed in the gastrointestinal tract of most animals (Fuller et al., 1978). It is the most commonly utilized genus as a probiotic because of its various beneficial properties (McCoy and Gilliland, 2007). Its potential health and nutritional benefits for humans and animals include but not limited to improve nutritional value of food, control of some types of cancer, serum cholesterol levels, and intestinal infection, and maintain the gastrointestinal microbiota structure (Uyeno et al., 2015; Strompfová et al., 2017).

The species of *Lactobacillus acidophilus* (*L. acidophilus*) was first isolated by Moro in 1900 from infant feces and is characterized as homofermentative and microaerophilic (Gonzalez et al., 1989). It was reported that *L. acidophilus* species remains to be the most widely recognized and commercially distributed probiotic culture (Altermann et al., 2005). Previous studies reported that *L. acidophilus* as being probiotic that have positive effects in farm animals (Hossain et al., 2015; Qiao et al., 2015; Lan et al., 2017a). However, researches on the effects of probiotic *L. acidophilus* in dog feed are still limited. Therefore, the objective of this experiment was to investigate the effects of *L. acidophilus* probiotic (LAP) on blood parameters, fecal shedding of lactic acid bacteria and coliform bacteria, and fecal noxious gas emission in adult Beagle dogs.

Materials and Methods

The experimental protocol used in this study was approved by the local animal care and use committee (case no. DK-1-1824), the feeding trials were carried out in Cheonan-si, Chungnam, South Korea.

Experimental design, animals, and diets

A total of 14 Beagle dogs with an initial body weight (BW) of 10.19 ± 0.61 kg were used in a 4-week feeding trail. Dogs based on BW were randomly assigned to two dietary treatments with 7 replications per treatment and one dog per cage. The dietary treatments containing commercial basal diet (CON) and LAP diet (CON + 10 mL of *L. acidophilus* probiotics). Commercial basal diet was formulated in accordance with the Association of American Feed Control Officials (AAFCO, 2009) nutrient guide for dogs and balanced to meet maintenance requirements. The nutrient values of basal diet are shown in Table 1. Dogs were individually fed twice a day (0800 h and 1600 h) in sufficient amount to supply their metabolizable energy (ME) requirements, according to the National Research Council (NRC, 2006). Beagles were housed in cages (100 cm × 210 cm) that were equipped with a feeder, a water bucket, and slatted plastic flooring in an environmentally controlled room. Dogs were allowed *ad libitum* access to drinking water throughout the experiment. Room temperature and relative humidity were maintained at 20 ± 3℃ and 50 ± 10%, respectively.

Product information

In the present study, we used *L. acidophilus* fermentation product derived from *L. acidophilus* in an anaerobic fermentation technology platform to produce beneficial microbial metabolites. *L. acidophilus* fermentation product was kindly provided by a commercial company (B&B Korea CO., LTD., Pyeongtaek, Korea). It was guaranteed to contain at least 3.0×10^8 cfu·mL^{-1} of *L. acidophilus*.

Table 1. Basal diet composition (as-fed basis).

Raw material (%)	Content
Corn	3.00
Wheat	15.73
Rice	8.85
Wheat bran	6.00
Beet pulp	3.63
Soybean meal, 45% crude protein	10.09
Meat bone meal	7.00
Meat meal, 60%, low phosphorus	3.40
Meat meal, 70%, high ash	6.00
Poultry meal	20.00
Tallow	9.20
Poultry fat	5.50
Salt	0.50
Methionine	0.09
Vitamin-mineral premix[z]	0.27
Enzyme	0.03
Dried beer yeast	0.50
Herb	0.12
Yucca	0.02
Antioxidant	0.05
Total	100.00
Calculated composition (%)	
Dry matter	90.59
Crude protein	32.01
Crude fat	19.97
Crude fiber	2.20
Crude ash	8.79
Calcium	1.96
Total phosphorus	1.26

[z] Premix provided the following per kg of complete diet: 14,600 IU·k^{-1} vitamin A, 1,450 IU·kg^{-1} vitamin D3, 130 mg·kg^{-1} vitamin E (alpha tocopherol), 160 mg·kg^{-1} vitamin E (alpha tocopherol), 0.4 mg·kg^{-1} I (potassium idodide), 0.3 mg·kg^{-1} Co (cobalt sulfate heptahydrate), 19.7 mg·kg^{-1} Cu (copper sulfate pentahydrate), 80 mg·kg^{-1} Mn (manganese oxide), 80 mg·kg^{-1} Zn (zinc oxide), and 0.16 mg·kg^{-1} Zn (zinc oxide).

Sampling and measurements

Fecal samples and blood samples were collected at the end of week 4. Blood samples were taken from each dog into vacuum tubes and K_3EDTA tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain serum and whole blood, respectively. White blood cells (WBC), red blood cells (RBC), and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA). Serum was separated by centrifugation for 30 min at 2000 g at 4℃ and stored until further analysis. Serum lipid profiles, including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride were analyzed by using an automatic biochemistry analyzer (Hitachi 747; Hitachi Ltd., Tokyo, Japan) with commercial kits (Sigma Diagnostics, MO, USA) according to the manufacturer's protocol.

For fecal noxious gas emission, fecal samples were collected form each pen. Samples were stored in 2.6-L plastic boxes, in duplicates. Each box had a small hole in the middle of one sidewall, which was sealed with adhesive plaster. The samples were permitted to ferment for a period of 5 at room temperature 25°C. After the fermentation period, a GV-100 gas sampling pump (Gastec Corp., Kanagawa, Japan) was used for the detection of ammonia (NH_3), hydrogen sulfide (H_2S), and total mercaptans (R.SH) using different detection tubes (No. 3L, No. 4LT, and No. 70L; Gastec Corp., Kanagawa, Japan).

For fecal shedding of lactic acid bacteria and coliform bacteria analysis, fecal samples were collected from each dog immediately after defecation and placed on ice for transportation to the laboratory where analysis was immediately carried out. One gram of fecal was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin, Lakes, NJ, USA) and homogenized. Counts of viable bacteria in fecal samples were determined by plating 10-fold serial dilutions (in 1% peptone broth solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and *Lactobacilli* medium agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate coliform bacteria and total lactic acid bacteria, respectively. The *Lactobacilli* medium agar plates were incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

Statistical analysis

The individual Beagle was used as the experimental unit. Data were analyzed as a completely randomized design using the GLM procedure of the SAS software (ASA Institute Inc., Cary, NC, USA). For microbial counts, data were log-transformed prior to statistical analysis. Differences among treatments were separated by the *t*-test. Variability in the data is expressed as the standard error of means (SEM) and a probability level of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

There were no significant ($p > 0.05$) differences in the concentration of total cholesterol, HDL-C, LDL-C, WBC, RBC, and blood lymphocyte percentage; whereas, serum concentration of triglyceride was significantly ($p < 0.05$) changed in LAP group compared with CON dogs (Table 2). As shown in Table 3, fecal ammonia emission was significantly ($p < 0.05$) decreased and fecal total lactic acid bacteria counts was significantly ($p < 0.05$) increased in LAP treatment, respectively. However, the concentrations of fecal H_2S and R.SH emission and fecal coliform bacteria counts were not affected ($p > 0.05$) between treatments.

Table 2. Effects of dietary *Lactobacillus acidophilus* probiotic (LAP) supplementation on blood profiles in adult Beagle dogs.

Items	CON	LAP	SEM	p-value
Cholesterol ($mg \cdot dL^{-1}$)	221.75	217.25	1.79	0.6874
Triglyceride ($mg \cdot dL^{-1}$)	62.25	52.25	1.50	0.0072
HDL-C ($mg \cdot dL^{-1}$)	128.75	134.50	3.69	0.2162
LDL-C ($mg \cdot dL^{-1}$)	5.25	6.00	0.53	0.2782
WBC ($10^3 \cdot \mu L^{-1}$)	14.99	15.14	1.09	0.9511
RBC ($10^6 \cdot \mu L^{-1}$)	6.64	6.75	0.12	0.4852
Lymphocyte[z] (%)	57.55	56.88	2.31	0.8624

CON, basal diet; LAP, CON + 10 mL LAP (3.0×10^8 cfu$\cdot mL^{-1}$); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RBC, red blood cell; SEM, standard error mean; WBC, white blood cell.
[z] Values are presented as a percentage of the total WBC count.

Table 3. Effects of dietary *Lactobacillus acidophilus* probiotic (LAP) supplementation on fecal gas emission and fecal microbial in adult Beagle dogs.

Items	CON	LAP	SEM	p-value
NH_3 (ppm)	29.00	19.50	0.40	0.0373
H_2S (ppm)	1.15	0.90	0.04	0.5876
R.SH (ppm)	5.00	4.60	0.57	0.5528
Lactic acid bacteria (log_{10}cfu·g^{-1})	8.71	8.87	0.04	0.0390
Coliform bacteria (log_{10}cfu·g^{-1})	7.62	7.55	0.06	0.4163

CON, basal diet; LAP, CON + 10 mL of LAP (3.0×10^8 cfu·mL^{-1}); SEM, standard error mean; NH_3, ammonia; H_2S, hydrogen sulfide; R.SH, total mercaptans.

The dog is the first domesticated animal species, which belongs among the most popular companion animals worldwide and the popularity is still increasing (Strompfová et al., 2017). It was reported that the typical longevity of Beagles is 12 - 15 years; thus, maintaining the pet health and seeking optimal nutritional feed for dogs is seen by the owners as an important component of responsible pet ownership (Bontempo, 2005). The concentration of serum triglyceride has been associated with health status of mammals (Maldonado et al., 2002). Furthermore, it was reported that lower serum triglyceride levels could be beneficial for human and animal health (Liu and Kim, 2018). However, the studies about the effect of LAP on serum triglyceride in Beagles are limited. Previously, in a broiler trial conducted by Kalavathy et al. (2003), they suggested that followed ages the serum triglyceride concentration decreased by 16 to 25% in those fed with a probiotic mixture containing *L. acidophilus*. Similar result was observed by Abd El-Gawad et al. (2005) in a rat experiment. But some other studies indicated inconsistent results that serum lipid indexes such as HDL-C, LDL-C, or triglyceride were not affected by *Lactobacillus* species probiotics in pigs and poultry (Zhang et al., 2012; Liu et al., 2015). Although dietary supplementation of LAP had a triglyceride lowering effect, the exact mechanism is still not clear. Tsujii et al. (2008) suggested that during the *Lactobacillus* growth, it gains the ability to deconjugate bile acids. It was also reported that probiotic bacteria may be helpful for fermentation of indigestible carbohydrates and produce short chain fatty acids, which decrease the levels of blood lipids (Ljungh and Wadstrom, 2006). The possible explanation for the decreased serum triglyceride in this experiment may be because these reasons.

The gastrointestinal tract of humans and animals is an essential organ with complex micro-ecosystem consisting of many bacteria. The gut microbiota, with its metabolic, trophic and protective functions plays an important role in host nutrition and health (Munyaka et al., 2015). Previous study indicated that the gut microbiota changes such as increase of pathogenic bacteria and decrease of health-promoting bacteria may influences in promoting and maintaining intestinal inflammation (Andoh and Fujiyama, 2006). The results of the present study revealed that the supplementation of LAP significantly increased the fecal lactic acid bacteria counts. In agreement with this study, Baillon et al. (2004) observed that during supplementation of LAP in dogs, the numbers of fecal *Lactobacilli* was increased. Moreover, many previous studies reported that dietary supplementation of *Lactobacillus*-containing probiotics increase the fecal lactic acid bacteria counts in farm animals like pigs and broilers (Lan et al., 2004; Qiao et al., 2015). Thus, the possible explanation for increased lactic acid bacteria counts in this experiment may be that the LAP may have positive effects on gut balance (Lan et al., 2017a). However, because of different strains may have various functions and survivability and effects host gut in different way, the mode of action of probiotics is still not determined (Ahansan et al., 2015). Further studies are still needed to explain the exact mechanism of LAP in adult Beagle dog gut microbiota.

The fecal noxious gas emission of NH_3, H_2S, and R.SH are a serious environmental concern in farm animals, which may aggravates the N and S deposition in ecosystems. However, no comparisons could be made because there was a scarcity of information on the effects of LAP supplementation in Beagle dogs. Previous studies have shown that dietary supplementation with probiotics could reduce the gases pollutants from manure through the actions of improves feed efficiency, enhanced nutrient utilization, maintenance of beneficial intestinal microbiota (Ferket et al., 2002; Lan et al., 2017b; Liu et al., 2018b). Fuller (2001) indicated that supplementing diets with probiotics could increase the population of beneficial microorganisms and inhibit the proliferation of pathogens in the intestinal microbiota. Therefore, the reduction of fecal NH_3 might be related to the increased fecal Lactobacillus counts.

In summary, Beagle dog diets supplementation with LAP could promoting the health status, enhancing the gut microbiota balance, and controlling the environmental hygiene. The results of this experiment have positive significances in improving the health and welfare of Beagle dogs.

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