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Original article

Clinical effects of combined *Lactobacillus paracasei* and kestose on canine atopic dermatitis

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Abstract

Probiotics and prebiotics are viable bacteria with beneficial effects on the host and components that selectively act on the beneficial commensal bacteria, respectively. The combined use of probiotics and prebiotics is termed synbiotics. Probiotic intake improves dysbiosis in the intestinal microbiota and can positively affect canine atopic dermatitis (CAD). However, clinical studies on improvements in CAD using synbiotics remain limited.

In this study, 15 dogs with CAD who received prednisolone, a synthetic glucocorticoid (GC) used in the treatment of CAD, for more than 90 days were continuously treated with *Lactobacillus paracasei* M-1 from fermented food as a probiotic, and trisaccharide kestose as a prebiotic, for 90 days to determine their synbiotic effects on CAD. The CAD symptoms were evaluated using the canine atopic dermatitis lesion index (CADLI) and pruritus visual analog scores (PVAS) at 30, 60 and 90 days after synbiotic administration. The total prednisolone use for 90 days pre- and post-administration was also evaluated.

Synbiotic administration significantly reduced the CADLI (pre: median, 28.0 [22.0-32.0]; 30 days: median, 20.0 [20.0−28.0]; 60 days: median, 20.0 [10.0−21.0]; 90 days: median, 12.0 [10.0-19.0]) and PVAS (pre: median, 6.0 [5.0-7.0]; 30 days: median, 3.0 [3.0-3.5]; 60 days: median, 3.0 [3.0-3.5]; 90 days: median, 2.0 [2.0-3.5]) scores, and reduced the total prednisone use over 90 days (pre: 112.0 [25-450] mg; post: 80.0 [18.-300.0] mg; $p<0.001$) in the 15 dogs. Thus, the synbiotic activity of *L. paracasei* M-1 and trisaccharide kestose can improve CAD.

Key words: canine atopic dermatitis, kestose, lactobacillus, prebiotics, probiotics, synbiotics

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Introduction

Canine atopic dermatitis (CAD) is a skin disease with various etiological factors and is associated with various offending agents, including environmental and food allergens (Favrot et al. 2010). The clinical characteristics of CAD include prutitus, redness, scales, and other signs observed in pronounced skin diseases that can dramatically diminish the patient's quality of life. According to specific guidelines, the treatment of CAD involves the administration of glucocorticoids (GC), and calcineurin inhibitors such as lokivetmab, oclacitinib, oral ciclosporin and topical tacrolimus are recommended and, in some cases, immunosuppressants; however, long-term use of these agents can increase the risk of liver failure, diabetes, thinning of the skin, and various other adverse events (Olivry 2010, Saridomichelakis et al. 2016, Szczepanik et al. 2020). Therefore, the development of novel treatments for CAD is warranted.

Recent studies have suggested that CAD is linked to disruption (dysbiosis) of the intestinal microbiota. Similar to humans, allergic symptoms in dogs are affected by gut dysbiosis and changes in intestinal permeability (Craig 2016). Furthermore, Guidi et al. (2021) reported that diet therapy with nutraceuticals reduced the dysbiotic index and improved the subjective severity of cutaneous lesions in dogs with CAD (Guidi et al. 2021). Thus, these studies support the idea that CAD is closely correlated with healthier intestinal microbiota, and improving dysbiosis will lead to an improvement in CAD symptoms.

Probiotics and prebiotics are defined as viable bacteria that exert beneficial effects on the host and components that selectively act on such bacteria, respectively (Hill et al. 2014, Gibson et al. 2017). The combined use of probiotics and prebiotics is referred to as synbiotics (Swanson et al. 2020). Synbiotics have been used to improve atopic dermatitis (AD), and Chang et al. (2016) demonstrated the efficacy of synbiotics for the primary prevention and treatment of AD in a meta-analysis. However, while probiotics such as *Lactobacillus paracasei* (*L. paracasei*) have been reported to be effective in the improvement of CAD (Kim et al. 2015, Ohshima-Terada et al. 2015), there are few clinical studies examining the use of synbiotics for the treatment of CAD. Therefore, we product clinically available products with synbiotic effect on CAD, using *L. paracasei* M-1 isolated from fermented food and kestose, an oligosaccharide with reported prebiotic effects in dogs (Ide et al. 2020), and conducted this preliminary study for investigating the synbiotic effect of *L. paracasei* M-1 and kestose on CAD.

Materials and Methods

Ethical Considerations

This study was conducted in accordance with good clinical practice guidelines and the Japanese National Guidelines for the Humane Treatment of Animals (the Ordinance of Ministry of Health, Labour and Welfare, 2022). Written informed consent was obtained from all participating dog owners. Vet Derm Tokyo provided ethical approval for this study as a clinical research project.

Clinical study

Dogs with CAD that met at least 5 of the 10 items in Set 1 according to the diagnostic criteria described by Favrot et al. (2010) were selected for this study. The following dogs were excluded: [1] those with a history of external parasitic diseases (scabies and mange) based on deep/superficial skin scraping and trichography; [2] those with pyoderma, fungal infection, *Malassezia* dermatitis, and flea allergic dermatitis; [3] those who had received other prebiotics or probiotics during the study period; and [4] those who had received drugs that affect the digestive system, such as psychotropic drugs, stomach acid blockers and antibiotics, during the study period. The study was conducted on 15 dogs (mean age: 3.87±0.52 years; sex: five males, five females, three castrated males, and two spayed females; mean body wight 6.51±0.63; breed: four toy poodles, two Yorkshire terriers, two Shih Tzus, two miniature dachshunds, and one beagle, pug, Chihuahua, Shiba, mix) with CAD symptoms that did not improve after treatment with prednisolone, a synthetic GC (NIPRO CORPORATION, Osaka, Japan), alone for more than 90 consecutive days. Prednisolone dosing intervals during the study period were set at daily, every other day, or every third day, depending on symptoms. Symptoms were evaluated every two weeks, and prednisolone dosing intervals were increased if the patient showed an improvement in clinical findings (e.g., from daily to every other day). If the symptoms subsequently worsened, the original dosing interval was resumed. If improvement was observed with dosing every third day, prednisolone use was discontinued. The single dose of prednisolone (1-2 kg, 0.63 mg/day, 2-4 kg, 1.25 mg/day, 3-7 kg, 2.5 mg/day, 7-16 kg, 5 mg/day) was not changed during the study period. None of the patients in this study had previously received other drugs used to treat CAD, such as molecularly targeted drugs, antibody drugs, or calcineurin inhibitors. *L. paracasei* M-1 (Aspac Kigyo, Tokyo, Japan) and kestose (B food science, Aichi, Japan) were administered as capsules (*L. paracasei* M-1: 2×10^{10}

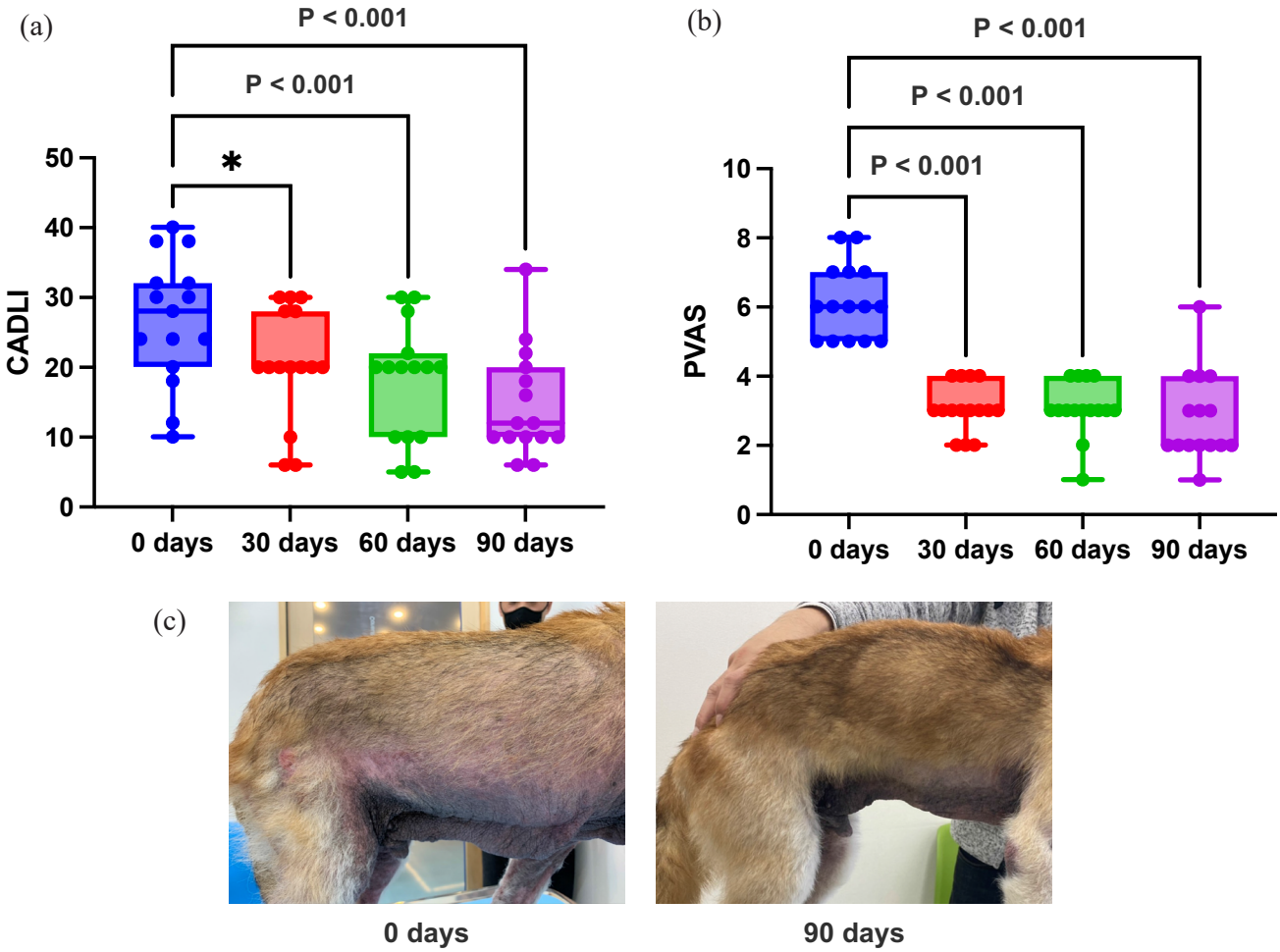

Fig. 1. Changes in atopic dermatitis-related scores and clinical findings.
Changes in (a) canine atopic dermatitis lesion index (CADLI) and (b) pruritus visual analog scale (PVAS) before (0 days), 30, 60, 90 days after synbiotics administration. (c) clinical findings before (0 days) and 90 days after administration (3-year-old male, species: mixed). Statistical analysis was performed using the Friedman test followed by Dunn's multiple comparison test, *p<0.05

colony-forming units/capsule) and tablets (kestose compound 400 mg/tablet), respectively; the dosage was one tablet and one capsule per day at the same time. Symptoms of AD were assessed using the canine atopic dermatitis lesion index canine atopic dermatitis lesion index (CADLI) (Plant et al. 2012). The dogs' pruritus scores were assessed using the pruritus visual analog scale pruritus visual analog scores (PVAS) with a 10-point scale (0, no pruritus; 3-4, mild pruritus; 6-7, moderate pruritus; and 10, severe pruritus that interferes with sleep) (Rybcinek et al. 2009). CADLI and PVAS scores were evaluated by the same veterinarian as before, 30 days after, 60 days after, and 90 days after administration of synbiotics. We also compared the total use of prednisolone during the 90-day period before synbiotic administration to the total use during the 90-day administration period. None of the dogs in this study experienced any adverse drug reactions.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 9.5.0, GraphPad Software, Inc., San Diego, USA). The Friedman test, followed by Dunn's multiple comparison test, was used for statistical analysis in Fig. 1, 2. The Wilcoxon matched-pairs signed rank test was used for statistical analysis in Fig. 2. Statistical significance was set at $p<0.05$.

Results

We examined the effect of synbiotic administration on the CADLI and PVAS scores of dogs with CAD. Both scores significantly decreased 30, 60, 90 days after administration of synbiotics: the median CADLI score decreased from 28.0 (22.0-32.0) pre-administration to 20.0 (20.0-28.0) 30 days ($p<0.05$ compared to pre-administration), 20.0 (10.0-21.0) 60 days, 12.0 (10.0-19.0) 90 days after administration ($p<0.001$

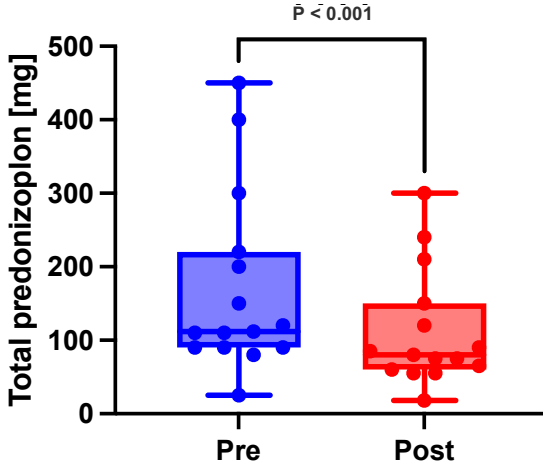

Fig. 2. Change in prednisolone dosage.
Change in total prednisolone dosage pre and post 90 days of synbiotics administration.
Statistical analysis was performed using by the Wilcoxon matched-pairs signed rank test.

compared to pre-administration, respectively), while the median PVAS score decreased from 6.0 (5.0-7.0 pre-administration to 3.0 (3.0-3.5) 30 days, 3.0 (3.0-3.5) 60 days, 2.0 (2.0-3.5) 90 days after administration ($p<0.001$, respectively) (Fig. 1 a, b) The clinical findings also showed marked improvement in the symptoms of CAD after 90 days of synbiotic administration (Fig. 1c). Comparisons of total prednisolone use for 90 days pre- and post-administration of synbiotics revealed that the total prednisolone use significantly decreased from 112.0 (90.0-210.0) mg pre-administration to 80.0 (62.5-135.0) mg post-administration ($p<0.001$) (Fig. 2).

Discussion

Several studies have reported the effects of probiotics and prebiotics on AD and, as previously mentioned, synbiotics, a combination of probiotics and prebiotics, may be the primary prevention and treatment means for AD (Chang et al. 2016). While a few studies have shown the improvement of CAD symptoms with probiotics (Kim et al. 2015, Ohshima-Terada 2015), studies on this topic remain limited. In the present study, the intake of synbiotics combining *L. paracasei* M-1 and kestose dramatically reduced the dogs' CADLI and PVAS scores (indicators of CAD symptoms) and led to a significant reduction in total prednisolone use over 90 days (Figs. 1, 2). These findings indicate that the synbiotics used in this study improved CAD symptoms. However, this was a small-scale, preliminary, and open study. Therefore, these results must be verified in a large-scale double-blind study.

L. paracasei, a gram-positive facultative heterofermentative bacterial species, is widely present in the gastrointestinal tract of mammals and fermented foods (Orlando et al. 2012). The *L. paracasei* M-1 used in the present study is a food-derived *Lactobacillus* isolated from fermented food. Although it has been confirmed to be safe to consume, its effects on physiological functions in dogs remain unclear. However, previous studies have reported that various strains of *L. paracasei* improve AD and CAD (Kim et al. 2015, Ohshima-Terada et al. 2015, Kim et al. 2020, D'Auria 2021). The results of these studies support the findings of the present study.

Trisaccharide kestose has been shown to be more effective than other prebiotics in promoting the growth of bifidobacteria and butyrate-producing bacteria, which are related to AD (Tochio et al. 2016, Tochio 2018), and has been shown to improve AD in humans (Shibata et al. 2009). Butyrate is a short-chain fatty acid in the intestine that contributes to the amelioration of allergic symptoms by regulating the differentiation of regulatory T-cells in the intestine (Furusawa et al. 2013). Kestose is reported to increase the levels of intestinal butyrate in dogs (Ide et al. 2020). According to Endo et al. (2016), kestose is utilized by several strains of *L. paracasei*.

Based on previous studies, we surmised that *L. paracasei* M-1 and kestose exert synergistic synbiotic effects, thereby improving CAD and reducing prednisolone use. However, the present study did not compare the synbiotic effects with those of *L. paracasei* or kestose alone. Further clinical studies with mutually exclusive administration of *L. paracasei* and kestose are required to explore this aspect.

Our study demonstrated that the administration of synbiotics combining *L. paracasei* M-1 and kestose improved CAD. However, the present study was a single-center open study, and a multicenter randomized study is necessary to verify our results.

From a clinical perspective, the synbiotics used in the present study were effective against CAD and are surmised to be highly versatile, highlighting their promise for expanded clinical application in the future.

Acknowledgements

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