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Complementary Effect of Probiotics on Canine Atopic Dermatitis

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Complementary Effect of Probiotics On Canine Atopic Dermatitis

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ABSTRACT

Canine atopic dermatitis (CAD) is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies that are most commonly directed against environmental allergens. The purpose of this study was to evaluate complementary therapeutic effect of probioticson CAD and identify on-set time and duration of the effects.

Twenty dogs clinically diagnosed as CAD in Veterinary Medical Teaching Hospital, Seoul National University were subjected to the present study and randomly

assigned to two groups, a probiotics administration group (probiotics group, n=10) and a non-probiotics administration group (control group, n=10). All dogs were allowed to maintain medications at the same dose that they had been taking prior to the study. The study was divided into two phases. Phase 1 was toevaluatethe effects of probiotics compared tothe control group, and phase 2 was a follow-up study for monitoring probiotics group after cessation of administration. Canine atopic dermatitis extent and severity index, pruritus visual analog scale and dermatology specific questions were performed for evaluating the effects of the probiotics on CAD.

In the phase 1, both probiotics and control group showed a decreasing tendency of scores in clinical evaluation parameters after treatment. However, scores of probiotics group declined significantly more than those of control group at the end of the phase 1 period. Furthermore, clinical evaluation parameters of probiotics group were gradually exacerbated back in phase 2. Subsidiary effects, such as reduced greasiness and foul smell, were only detected in probiotics group and these effects diminished after halt of probiotics.

In conclusion, probiotics has complementary therapeutic effect on CAD. Probiotics showed its efficacy markedly in 8th week and the effects were lasted less than 8 weeks. Therefore, continuous administration of probiotics to CAD patients is recommended for the treatment and prevention of CAD in dogs.

Keywords: Canine atopic dermatitis, Probiotics, *Bifidobacterium*, *Lactobacillus*, Dog

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INTRODUCTION

Canine atopic dermatitis (CAD) is one of the major dermatologic diseases in veterinary clinics. CAD is defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens (1). Thus, regulating immune system is the most important part to treat CAD. Various therapies for CAD such as glucocorticoids, cyclosporin, oclacitinib and allergen specific immunotherapy have been proposed (2). In 2010, the International Task Force on Canine Atopic Dermatitis (ITFCAD; now International Committee on Allergic Diseases of Animals, ICADA) commented that long-term use of oral or topical glucocorticoids or the concurrent long-term administration of oral cyclosporin and glucocorticoids may cause some adverse effects (3-5). Thus, it requires close monitoring, dose adjustment and well-tolerated additional therapies to support efficacy or reduce the dose of glucocorticoids for CAD (2, 3). Various treatments are reported as useful complementary therapies that have some benefits on CAD (6-10).

Probiotics is one of adjunctive therapies. *Lactobacilli* and *Bifidobacteria* were reported as immunomodulating factors in recent studies (11, 12). In the study using a murine model of inflammatory bowel disease, oral feeding of *Bifidobacterium bifidum* (BGN4) inhibited host's $CD4^+$ T lymphocyte infiltration to large intestine and inflammatory cytokine productions, particularly IFN-γ and MCP-1 (11). One *ex vivo* human skin culture study reported that *Lactobacillus parakacei* CNCM-I 2116 could

modulate inflammatory mechanisms associated with reactive skin (12). Also, recent researches in human medicine reported that probiotics could be helpful to regulate allergic diseases (13-17). Numerous studies in veterinary medicine also reported that oral administration of probiotics has some beneficial effects on CAD (18-21). Preventive effects of probiotics on CAD are also well known (20). Therefore, this study was performed to test the hypothesis that probiotics modulates host's immune system and has favorable effects on CAD.

The present study was conducted to evaluate the effects of probiotic mix on treating CAD, to identify the period when probiotics manifests its efficacy and to determine the duration of the effects.

MATERIALS AND METHODS

1. Study population

The study included twenty dogs that were diagnosed as moderate to severe CAD in Veterinary Medical Teaching Hospital of Seoul National University (Table 1). Dogs were diagnosed as CAD based on the animal's history, clinical findings, the Favrot's criteria, serum allergen-specific IgE test and/or intradermal skin test (22, 23). Dogs with food allergy or seasonal pruritus were excluded and with concurrent diseases, such as ectoparasites infestation, cutaneous bacterial or fungal infection, were controlled using standard diagnostic procedures and therapeutic methods before inclusion in this study. All subjects had been confirmed that there was no symptoms fluctuation of CAD at least for one month before the beginning of the study. All dogs were required to maintain their medications, feeding and management that they had been taking before the study. The purpose and content of this clinical evaluation were explained to dog's owners and consent forms were signed prior to enrollments and dogs were assigned to probiotics group or control group randomly.

2. Probiotics

Probiotic mix (2g, Real Bifidus®; Estien corp., Seoul, Korea) were repackaged in

opaque tin foil sachets for the blind test. Each sachet contained 2.5×10^9cfu/g of *Bifidobacterium bifidum BGN4* and *Bifidobacterium longum BORI*, 5×10^8cfu/g of *Lactobacillus acidophilus AD031* and *Lactobacillus casei IBS041*. A sachet was one dose and probiotics made to bead type dosage form. Probiotics in a sachet was given to the dog once daily at every afternoon without any food. Products were stored at 4°C until use.

3. Study design

This study was designed in two stages, phase 1 was a randomized, double-blind, controlled study for 8 weeks to compare probiotics group with control group. Probiotics group included ten dogs which were given probiotic mix and control group comprised ten dogs which were not administrated probiotic mix. Probiotic mix was given to probiotics group once in a day during the phase 1. Phase 2 was a follow-up monitoring of probiotics group for another 8 weeks after cessation of the probiotics. A dog owner in probiotics group was lost the contact after the phase 1 study thus dogs of probiotics group in phase 2 were nine. Their medications, feeding and management were not changed than phase1 but only halted the administration of probiotics.

4. Intervention

For evaluating effect of the probiotics on CAD, canine atopic dermatitis extent and severity index (CADESI), pruritus visual analog scale (PVAS) and dermatology

specific questions were evaluated. CADESI is validated measuring tools to assess clinical signs of atopic skin lesions and pruritus which are reliably in dogs with AD. This study used CADESI-04 version. CADESI-04 was generated by evaluating 1) erythema, 2) lichenification, 3) excoriations and alopecia at 20 different body areas on a scale of none (score 0), mild (score 1), moderate (score 2) and severe (score 3). Total score can be from zero to 180 and benchmarks for mild, moderate and severe AD skin lesions are 10, 35 and 60, respectively (22, 24). Probiotics group and control group were assessed by skilled veterinarians using this CADESI-04. Also, PVAS was used to estimate the degree of dog's pruritus by the dog owners (25, 26). PVAS provides the line which combined features of the severity, behavior to owners. Owners were asked to check the point that best describes their dog's pruritus level on the scale then the length from bottom to the point which was converted as a score from 0 to 10. Owners were also surveyed by written single choice/scoring format questionnaires (Table 2 and 3). If the written surveys were not able to, owners were surveyed by telephone conversation. This dermatology specific questionnaire aimed to survey the owner global evaluation score (Table 2) and subsidiary changes of dog's skin (Table 3). CADESI and PVAS were performed at day 0 and every four weeks. Dermatology specific questionnaire was carried out at day 0 and end of each period.

5. Statistical analyses

Normality of continuous variables was tested by the Shapiro-wilk test. Repeated-

measures ANOVA was conducted to analyze CADESI, PVAS data and owner survey values. Student's *t*-test was used for comparison of two groups of normally distributed data. Wilcoxon signed rank test or paired *t*-test was chosen for comparison between time points within a group depending on distribution types of data. Multiple regression analysis was employed to identify statistical relationship between a clinical evaluation parameter and probiotics or any otherfactors such as age, sex, breeds and prescribed drugs. All data were presented as mean ± standard deviation (SD). All statistical calculations were carried out by using IBM SPSS statistics software, version 22 (SPSS Inc., Chicago, IL, USA). A *p* value less than 0.05 was considered statistically significant.

RESULTS

1. Phase 1: Main study

1.1 CADESI score

Comparing with day 0, CADESI scores of probiotics group and control group both decreased in Phase 1 (Figure 1). There were remarkable differences of CADESI scores between day 0 and 4th week in both groups ($p< 0.05$) and between day 0 and 8th week in probiotics group ($p< 0.01$).

Mean gap between day 0 and 4th week were 33.7 (SD: ± 25.0) in probiotics group and 15.8 (SD: ± 10.1) in control group. CADESI scores of both groups showed remarkable declines at 4th week than day 0 ($p< 0.05$). Also, comparing two groups, score reduction of probiotics group was greater than control group. All dogs of probiotics group showed decreased CADESI scores at 4th week. Nine of ten dogs in control groups showed decreased CADESI scores and one of control group had same score as day 0 at 4th week. There was no significant differences between the two groups were seen at 4th week.

Mean gap between day 0 and 8th week were 49.7 (SD:± 19.0) in probiotics group and 11.5 (SD:± 19.1) in control group. CADESI score of probiotics group showed remarkable decline at 8th week than day 0 ($p< 0.01$) while CADESI score of control group showed no significant change. Also, comparing two groups, score reduction of probiotics

group was greater than control group like as 4th week. At 8th week, nine of ten dogs in probiotics groups showed decreased CADESI scores than scores at 4th week and one of probiotics group showed increased score than the score at 4th week however it was still lower than the score of the dog at day 0. Whereas six of ten dogs in control groups showed increased, among those six dogs, two dogs showed higher scores than scores at day 0 and four dogs still lower than scores at day 0 but higher than those at 4th week. Four dogs of ten in control group showed decreased CADESI scores than scores at 4th week. Also, significant differences between the two groups were seen at 8th week ($p< 0.05$, Figure 2). In multiple regression analysis, probiotics (32.8) and sex (-20.1) were significant regression coefficients ($p< 0.05$).

1.2 PVAS score

The result ofPVAS showed similar to CADESI. PVAS score of probiotics group showed more declined than control group (Figure 3). Remarkable differences of PVAS score was shown betweenday 0 and 8th week in probiotics group ($p< 0.05$).

Mean gap between day 0 and 4th week were 1.62 (SD: ± 1.38) in probiotics group and 0.74 (SD: ± 0.96) in control group. PVAS scores of both groups showed no remarkable decline at 4th week than day 0. However, comparing two groups, score reduction of probiotics group was greater than control group. Eight dogs of probiotics group showed decreased PVAS scores, one dog showed same score as day 0 at 4th week and one dog showed increased score than PVAS scores at day 0. Seven of ten dogs in

control groups showed decreased scores and three dogs showed increased scores than PVAS scores at day 0. In PVAS, decreasing gap of probiotics group was not significantly different from that of control group at 4th week.

Mean gap between day 0 and 8th week were 2.44 (SD: ± 1.65) in probiotics group and 0.68 (SD: ± 1.46) in control group. PVAS score of probiotics group showed remarkable decline at 8th week than day 0 ($p< 0.01$) while PVAS score of control group showed no significant difference. Also, comparing two groups, score reduction of probiotics group was greater than control group as 4th week. At 8th week, all dogs in probiotics groups showed decreased PVAS scores than the scores at 4th week. Whereas six of ten dogs in control groups showed decreased and four dogs of ten in control group showed increased PVAS scores than the scores at 4th week. Among these four of ten dogs in control groups, three dogs showed higher scores than scores at day 0 and a dog still lower than scores at day 0 but higher than those at 4th week. In PVAS, decreasing gap of probiotics group was significantly different from that of control group at 8th week ($p< 0.05$, Figure 4). Probiotics (1.76) was a significant regression coefficient ($p< 0.05$) in multiple regression analysis.

1.3 Owner global evaluation score

In owner global evaluation score, probiotics group showed more declined than control group (Figure 5). Significant differences of owner global evaluation score was shown between day 0 and 8th week in probiotics group ($p< 0.01$).

Mean gap between day 0 and 8th week were 1.70 (SD: ± 0.95) in probiotics group and 0.50 (SD: ± 1.27) in control group. Owner global evaluation score of probiotics group showed remarkable decline at 8th week than day 0 ($p< 0.01$) while owner global evaluation score of control group showed no significant difference. Also, comparing two groups, score reduction of probiotics group was greater than control group. At 8th week, nine of ten dogs in probiotics groups showed decreased owner global evaluation scores than the scores at day 0 and one dog's score was same as day 0. Whereas four of ten dogs in control groups showed decreased, four dogs showed same score as day 0 and last two dogs in control group showed increased owner global evaluation scores than the scores at day 0. Significantly reduced global evaluation score was shown at 8th week ($p< 0.05$, Figure 6). Probiotics (1.2) was a significant regression coefficient ($p< 0.05$) in multiple regression analysis.

1.4 Subsidiary changes of dog's skin

At 8th week of phase 1, nine of ten dog owners (90%) in probiotics group answered that the time when the dog became greasy, got longer than before the study. In other words, greasiness of dog's skin was improved. One dog owner (10%) in probiotics group answered that there was no change on her dog's skin. While, eight of ten dog owners (80%) in control group answered that there was no change on their dogs' skin and a dog owner (10%) answered that it was improved and last one (10%) answered that the time when the dog became greasy, got shorter than before, in short, it worsened (Figure 7).

In case of malodor, all dog owners (100%) in probiotics group answered that the time when the dog got malodor, got longer than before the study. In other words, the smell of dog's skin was improved. While, six of ten dog owners (60%) in control group answered there was no change on their dogs' skin, three dog owners (30%) answered that it worsened and last one (10%) answered that it was improved (Figure 7).

2. Phase 2: Follow-up study of probiotics group

2.1 CADESI score

The CADESI score increased again slowly after halt of probiotics in phase 2 (Figure 1). Mean gap was 36.1 (SD: ± 30.7) between day 0 and 12th week in probiotics group. Six of nine dogs showed increased CADESI scores at 12th week, among those six dogs, a dog showed higher scores than scores at day 0 and five dogs still lower than scores at day 0 but higher than those at 8th week. Two of nine dogs showed decreased than the scores at 8th week and one dog's score was same as 8th week. Also, there was no remarkable difference between 12th week and any other time point.

Mean gap was 27.2 (SD: ± 23.1) between day 0 and 16th week in probiotics group. Five of nine dogs showed increased CADESI scores at 16th week, all those five dogs still lower than scores at day 0 but higher than those at 12th week. Four of nine dogs showed decreased than the scores at 12th week. CADESI scores of probiotics group showed remarkable elevation at 16th week than 8th week. ($p< 0.05$)

2.2 PVAS score

The PVAS score increased again slowly after end of probiotics in phase 2 (Figure 3). Mean gap was 1.32 (SD: ± 2.75) between day 0 and 12th week in probiotics group. Eight of nine dogs showed increased PVAS scores at 12th week, among those eight dogs, two dogs showed higher scores than scores at day 0 and six dogs still lower than scores at day 0 but higher than those at 8th week. A dog showed decreased than the scores at 8th week. Also, there was no remarkable difference between 12th week and any other time point.

Mean gap was 1.36 (SD: ± 2.79) between day 0 and 16th week in probiotics group. Seven of nine dogs showed increased PVAS scores at 16th week, four of them showed higher scores than scores at day 0 and three dogs still lower than scores at day 0 but higher than those at 12th week. Two of nine dogs showed decreased than the scores at 12th week. PVAS score of probiotics group was significantly elevated at 16th week than 8th week ($p< 0.05$).

2.3 Owner global evaluation score

After ceasing probiotics, owners sensed their dog's skin worse again (from 2.4 to 3.2). Mean gap between day 0 and 16th week were 0.78 (SD: ± 0.97) in probiotics group. At 16th week, six of nine dogs in probiotics groups showed increased owner global evaluation scores than the scores at 8th week among those six dogs, one dog showed

higher scores than scores at day 0 and five dogs still lower than scores at day 0 but higher than those at 8th week. A dog showed decreased than the scores at 8th week and one dog's score was same as 8th week. However, owner global evaluation score showed no significant difference between 16th week and day 0 or 8th week (Figure 5).

2.4 Subsidiary changes of dog's skin

At 16th week, the end of the phase 2, five of nine dog owners (56%) in probiotics group answered that greasiness of their dog's skin worsened. Last four dog owners (44%) answered that there was no change on their dog's skin (Figure 8).

In case of malodor, five of nine dog owners (56%) in probiotics group answered that malodor from their dog's skin worsened. Last four dog owners (44%) answered that there was no change on their dog's skin (Figure 8).

3. Adverse events

The probiotics was well tolerated and no significant adverse effects were reported during the all administration period.

DISCUSSION

CAD is a kind of chronic immune disorder. CAD is hypersensitive immune reaction to specific allergens in dog. Thus, immunomodulating drugs are treated to CAD patients for a long period. Adjunctive therapies for reducing dose or adverse effects of the long-term use of immunomodulating drugs, such as glucocorticoid or cyclosporin, have received attention in CAD treatment. Various treatments including essential fatty acid, supplement, regular bathing, topical ceramide cream, low level laser therapy, natural extracts and probiotics are known as useful adjunctive therapies which have some benefits on CAD (6-10, 18, 27-29). Among them, administration of probiotics is heavily researched recently. Therapeutic effects of probiotics on allergic disease was reported in veterinary and human medicine (6, 15, 19-21, 30, 31). Veterinary researches about probiotics on CAD could be categorized two types by its aims, preventive or therapeutic effect (18-21). Preventive effects were evaluated probiotics on prenatal, postnatal puppies which have high risk of CAD (20, 21) and therapeutic effects were aimed at treating adult dogs which are suffering from atopic dermatitis (18, 19). Therapeutic effects of probiotics on CAD would be clinically more important than preventive effects in practical medicine. The reason is that, many CAD patients visit to clinics when they developed atopic symptoms. While, there are many difficulties for ensuring preventive effects. Above all, dogs with CAD should have been administrated probiotics when they were neonates however, dog owners could not know whether their neonatal puppies will get CAD or not. Moreover, most owners commonly adopt their dogs from others or dog kennels after dogs

passed their neonatal period. Therefore, it is hard to apply probiotics for preventive treatment to potential CAD patients in clinical practice.

Recent studies verified that probiotics have immunomodulating activities and skin reactivity (11, 12, 31-34). However, still, there is no standard protocol or data about onset and duration of probiotic effect. Therefore this study aimed to evaluate complementary therapeutic effect of probiotic mix and determine onset and duration of the effects on CAD.

The result of phase 1 suggests that probiotics have complementary therapeutic effect on CAD. Both probiotics group and control group presented gradually decreased scores of CADESI, PVAS and owner global evaluation in phase 1 (Figure 1, 3 and 5). However, mean scores of probiotics group and control group at day 0 were different in all clinical evaluation parameters. Therefore, comparing two groups had to be performed by using score gaps between day 0 and 4^{th} week or 8^{th} week. Analyzing score gaps of two groups, all clinical evaluation parameters scores of probiotics group had greater degree of decline than those of control group at 4^{th} week and 8^{th} week (Figure 2, 4 and 6). All clinical evaluation parameters of probiotics group showed significant difference from control group at 8^{th} week ($p< 0.05$). Furthermore, probiotics only had positive regression coefficient value in multivariate regression analyses. This result indicated that probiotics was the only factor related to favorable changes in all clinical evaluation parameters in the present study. This study proposed that clinical symptoms of CAD significantly improved in probiotics group at 8^{th} week and therefore, 8 weeks of continuous administration of probiotics is needed for certain therapeutic effect. This result is

consistent with those found in the previous studies (18, 19). One previous study which tested about administrations of *Lactobacillus sakei Probio-65* for the prevention of CAD had analogous tendencies in CADESI score (19). Another study evaluating effects of *Lactobacillus paracasei K71* (18) also showed greater reduction rate of CADESI score in probiotics group than in control group.

All clinical evaluation parameters gradually exacerbated again in phase 2 (Figure 1, 3 and 5). Significant difference in CADESI and PVAS was shown at 16th week from 8th week ($p< 0.05$). This suggested that the effect of the probiotics was disappeared gradually, probiotics would have settled in host's intestine then eliminated slowly spanned less than 8 weeks. This result could be explained by one previous study analyzed fecal microflora of human patients with atopic dermatitis, they were able to isolate administrated probiotics even 1 month after probiotic suspension in some patients, but in lower quantities (60%) than the amounts observed immediately (100%) after the end of one-month treatment (13).

Furthermore, analyzing two test periods together, probiotics group showed 'V' shaped rebound graph in all clinical evaluation parameters (Figure 1, 3 and 5). The group had improved scores of clinical symptoms and owner global evaluations by administrating probiotics and deteriorated again after the halt of probiotics. There were also significant differences were observed between test periods, day 0 and 8th week, 8th week and 16th week in CADESI and PVAS. This rebound pattern of clinical evaluation parameters fortifies that probiotics would contribute to relieve CAD symptoms.

Interestingly, unexpected subsidiary effects like as reduced greasiness and

malodor were observed in the present study. These effects were detected more frequently in probiotics group and diminished after halt of probiotics. The owner survey was only way to evaluate greasiness and malodor of skin in this study, because, so far, there is no objective tools for measuring greasiness and malodor of skin. This result could be inferred that these favorable subsidiary effects were associated with probiotics in spite of limitation of the evaluation method. Further study is warranted to determine the underlying mechanism.

It is convincing that probiotics is affordable as a complementary treatment for CAD. Present study indicated that the probiotics showed significant efficacy at 8th weeks and the effects fade away by 8 weeks after the halt of the probiotics. Thus, continuous administration over 8 weeks is recommended for affected host and probiotics efficacy would be sustained about 8 weeks.Further study, comparing probiotic mix group with *Lactobacillus* or *Bifidobacterium* only group, would be helpful to identify synergic effect between two bacterial strains. Also, this study did not include fecal analysis thus, to assure settlement of probiotics in gut microbiota, analyzing dogs' feces samples should be performed, too.

In conclusion, administration of probiotics showed favorable complementary therapeutic effect on CAD and it showed significant efficacy in 8th week and effects would be sustained about 8 weeks. Therefore, continuous administration of probiotics to CAD patients is recommended to treat and prevent CAD in dogs.

Table 1. Signalment of dogs enrolled in the study.

Patient No.	Age (years)	Sex	Breed	Previously prescribed drugs	Previously Controlled Disease
P1	10	MC	Shitzu	-	-
P2	6	FS	Shitzu	-	-
P3	10	FS	Poodle	Cyclosporin A 5mg/kg, SID	-
P4	8	FS	Poodle	-	-
P5	7	FS	Shitzu	-	-
P6	6	MC	Shitzu	-	Malassezia Dermatitis
P7	8	FS	Cocker Spaniel	-	-
P8	11	MC	Shitzu	-	-
P9	9	MC	Dachshund	-	-
P10	5	FS	Pomeranian	-	-
C1	5	FS	Shitzu	-	-
C2	7	FS	French Bulldog	Cyclosporin A 5mg/kg, SID	Bacterial Folliculitis
C3	10	FS	Poodle	Cyclosporin A 5mg/kg, SID	-
C4	7	FS	Shitzu	-	-
C5	11	MC	Shitzu	-	-
C6	9	FS	Poodle	-	-
C7	14	MC	Shitzu	Cyclosporin A 3.29mg/kg, SID	Malassezia Dermatitis
C8	10	MC	Maltese	-	-
C9	9	MC	Shitzu	Cyclosporin A 3.4mg/kg, SID	-
C10	9	MC	Maltese	Cyclosporin A 7.8mg/kg, SID	-

P = probiotics group, C = control group, MC = male castrated, FS = female spayed

Table 2. Owners' global evaluation score. The owners were asked to score that most closely resembled their dog's skin condition

Score	Description contents
1	He/she smells good or none, has no itch, dog's skin looks pale pink, not greasy, and never purulent. My dog's skin is healthy condition.
2	He/she smells bad, feels mildly itchy sometimes, looks mild red, somewhat greasy, and partially purulent. My dog's skin is mildly bad condition.
3	He/she smells bad, feels moderately itchy often, looks red, greasy, and partially to totally purulent. My dog's skin is bad condition.
4	He/she smells very bad and feels itchy moderately to extremely, looks very red, greasy, and severely purulent on some parts of body. My dog's skin is worse condition.
5	He/she smells very bad, and feels itchy extremely, looks very red, greasy, and severely purulent on whole body. My dog's skin is worst condition.

Table 3. Subsidiary changes of dog's skin. The owners were asked to check that the most closely time of changing dog's skin condition.

No.	Questions
1.	When did your dog become greasy after a shower? (1) right after (2) next day (3) two days later (4) three days later (5) four days later or more (days) (6) none
2.	When did your dog get malodor after a shower? (1) right after (2) next day (3) two days later (4) three days later (5) four days later or more (days) (6) none

Table 4. Raw data of clinical evaluation parameters in probiotics group

	Patient No.	Day 0	Week 4	Week 8	Week 12	Week 16
CADESI (Range: 0 to 180)	**P1**	136	122	68	72	89
	P2	105	97	61	61	100
	P3	108	40	48	47	87
	P4	113	37	25	53	61
	P5	88	39	34	97	82
	P6	89	59	57	72	66
	P7	77	72	42	71	67
	P8	79	63	46	71	67
	P9	108	62	53	34	39
	P10	104	79	76	-	-
PVAS (Range: 0 to 10)	**P1**	8	3.7	3.8	7.3	4.9
	P2	2	0.2	0.14	0.87	0.9
	P3	5.4	5.4	5	5.1	5.3
	P4	7.2	5.3	2.6	1.7	3.2
	P5	8	5.4	5.6	7.6	8.2
	P6	5	5.5	4.5	6.8	6.9
	P7	6	5.4	4.2	8	8.15
	P8	3.5	2.3	2	3.46	4
	P9	6.6	4.6	1.5	3.8	2.8
	P10	6.2	3.9	4.2	-	-
Owner Global Evaluation (Range: 1 to 5)	**P1**	4		2		3
	P2	2		1		1
	P3	3		1		3
	P4	5		2		4
	P5	4		2		3
	P6	5		5		5
	P7	4		2		5
	P8	5		4		3
	P9	4		1		2
	P10	5		4		-

P = probiotics group

Table 5. Raw data of clinical evaluation parameters in control group

	Patient No.	Day 0	Week 4	Week 8
CADESI (Range: 0 to 180)	**C1**	108	97	75
	C2	62	45	56
	C3	87	59	64
	C4	100	92	71
	C5	100	71	110
	C6	47	47	34
	C7	107	97	135
	C8	60	41	55
	C9	63	55	46
	C10	67	39	40
PVAS (Range: 0 to 10)	**C1**	5.7	5.3	3.4
	C2	3.9	3.7	4.6
	C3	5.3	3	2.8
	C4	0.9	0.6	0.38
	C5	4.5	4.8	3.2
	C6	4	4.2	3.5
	C7	5.6	5.8	6.6
	C8	3.8	1.9	5.6
	C9	8.8	7.3	6.8
	C10	2.15	0.7	1
Owner Global Evaluation (Range: 1 to 5)	**C1**	5		3
	C2	4		4
	C3	3		2
	C4	2		2
	C5	4		4
	C6	5		4
	C7	3		4
	C8	2		3
	C9	4		4
	C10	4		1

C = control group

Table 6. Result of subsidiary changes (Number of days after shower)

Patient No.	Greasiness			Malodor		
	Day 0	Week 8	Week 16	Day 0	Week 8	Week 16
P1	1	2	1	1	2	1
P2	3	5	5	3	5	5
P3	2	3	1	2	3	1
P4	1	4	2	1	4	2
P5	1	3	1	1	3	1
P6	2	4	4	2	4	4
P7	5	10	7	3	7	2
P8	1	1	1	0	1	1
P9	1	5	5	0	5	5
P10	3	4		2	4	
C1	2	2		2	2	
C2	2	2		2	2	
C3	1	0		1	0	
C4	3	3		3	3	
C5	1	1		1	1	
C6	2	2		2	2	
C7	1	1		2	1	
C8	none	none		3	2	
C9	2	2		2	2	
C10	4	5		4	5	

P: Probiotics group, C: Control group

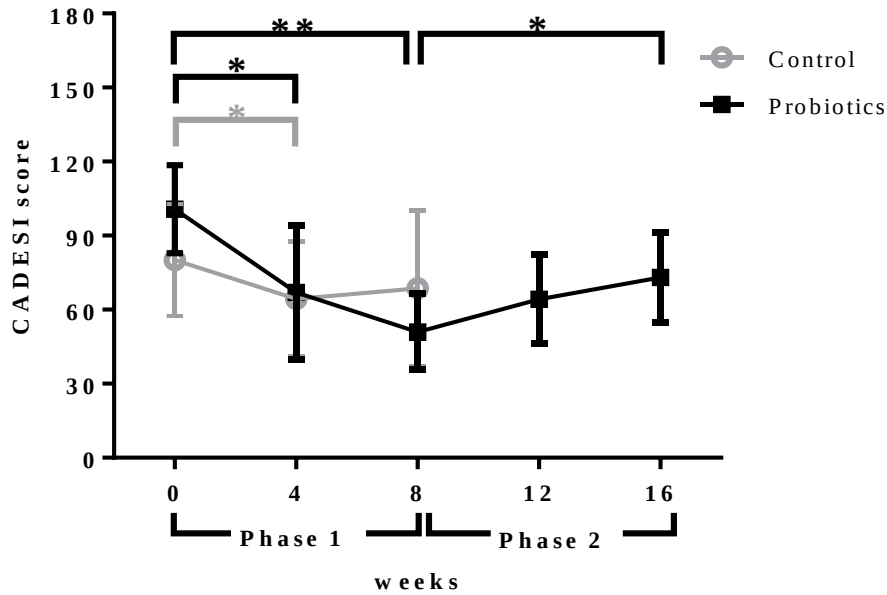

Figure 1. Changes of Canine Atopic Dermatitis Extent and Severity Index (CADESI, ranges from 0 to 180) score in probiotics group (Phase 1: n = 10, Phase 2: n = 9) and control group (n = 10).

Phase 1: Administrated probiotics for 8 weeks, Phase 2: Without probiotics for 8 weeks.

*p< 0.05, **p< 0.01

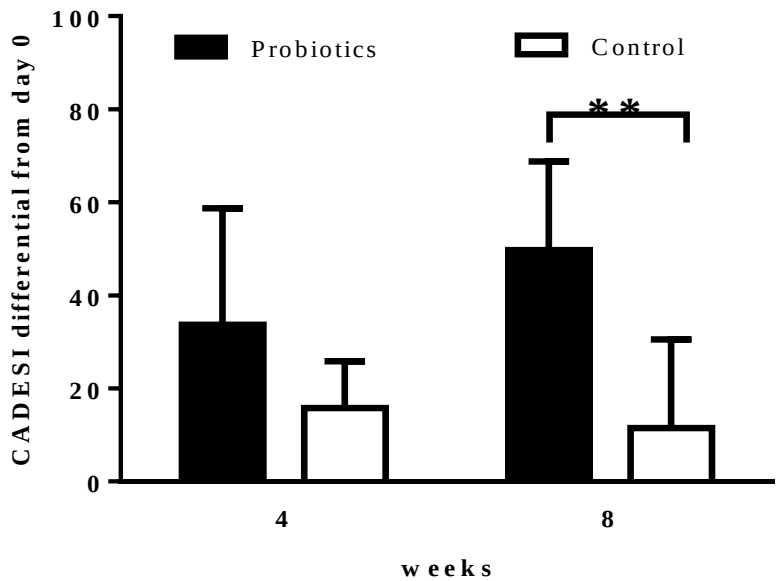

Figure 2. Comparing CADESI score gaps from day 0 and 4th week or 8th week.

Columns = the mean score at day 0 – the mean score at 4th week or 8th week, $**p < 0.01$

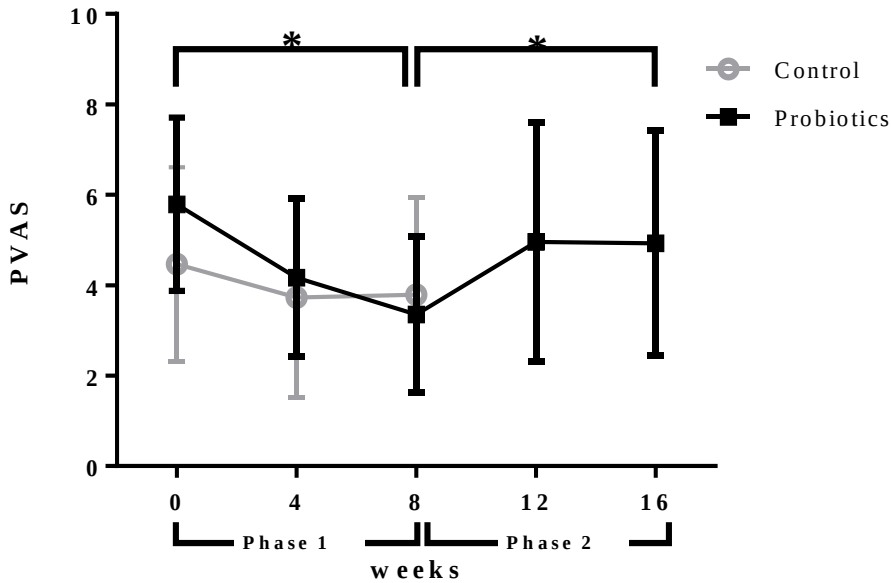

Figure 3. Changes of pruritus visual analog scale (PVAS, ranges from 0 to 10) inprobiotics group (Phase 1: n=10, Phase 2: n=9) and control group (n=10).

Phase 1: Administrated probiotics for 8 weeks, Phase 2: Without probiotics for 8 weeks.

*p< 0.05

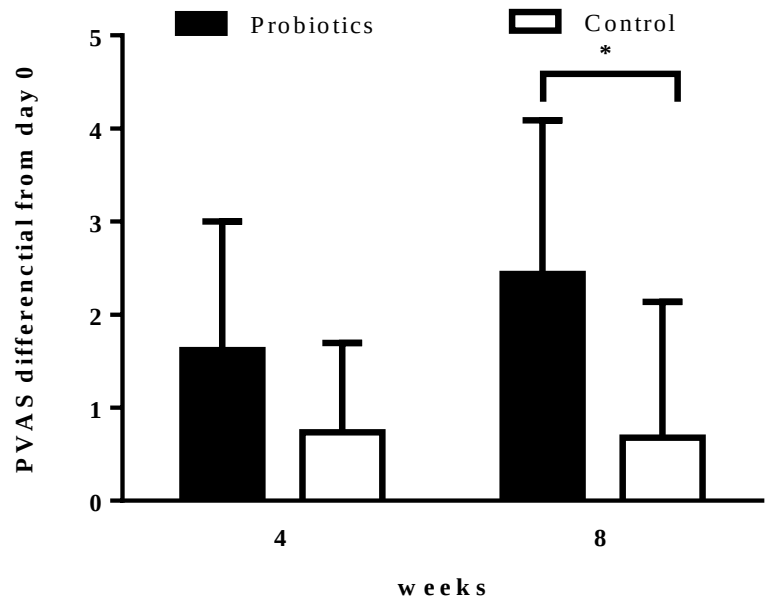

Figure 4.Comparing PVAS score gap from day 0 and 4thweek or 8thweek.

Columns = the mean score at day 0 – the mean score at 4th week or 8th week, *p< 0.05

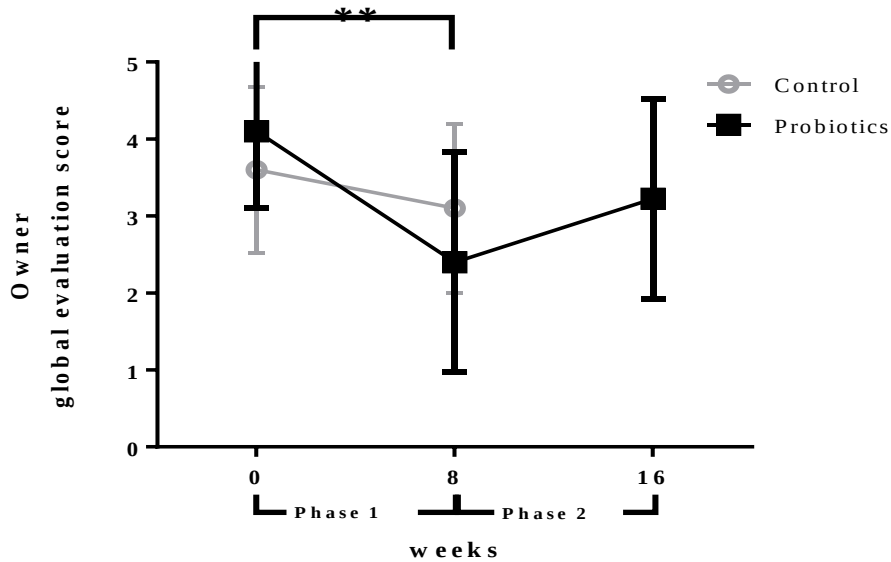

Figure 5. Changes of Owner global evaluation score (ranges from 1 to 5) in probiotics group (Phase 1: n=10, Phase 2: n=9) and control group (n=10).

Phase 1: Administrated probiotics for 8 weeks, Phase 2: Without probiotics for 8 weeks.

$^{**}p < 0.01$

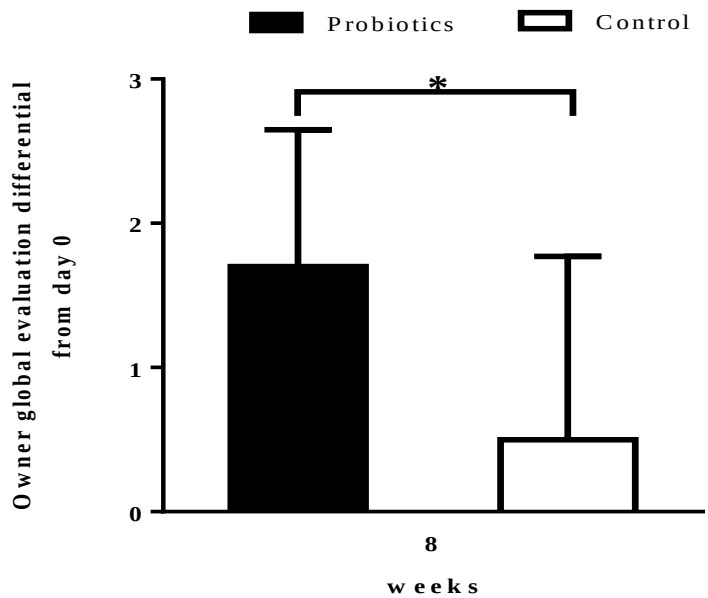

Figure 6. Comparing owner global evaluation score gap from day 0 and 4thweek or 8thweek.

Columns = the mean score at day 0 – the mean score at 8th week, *p< 0.05

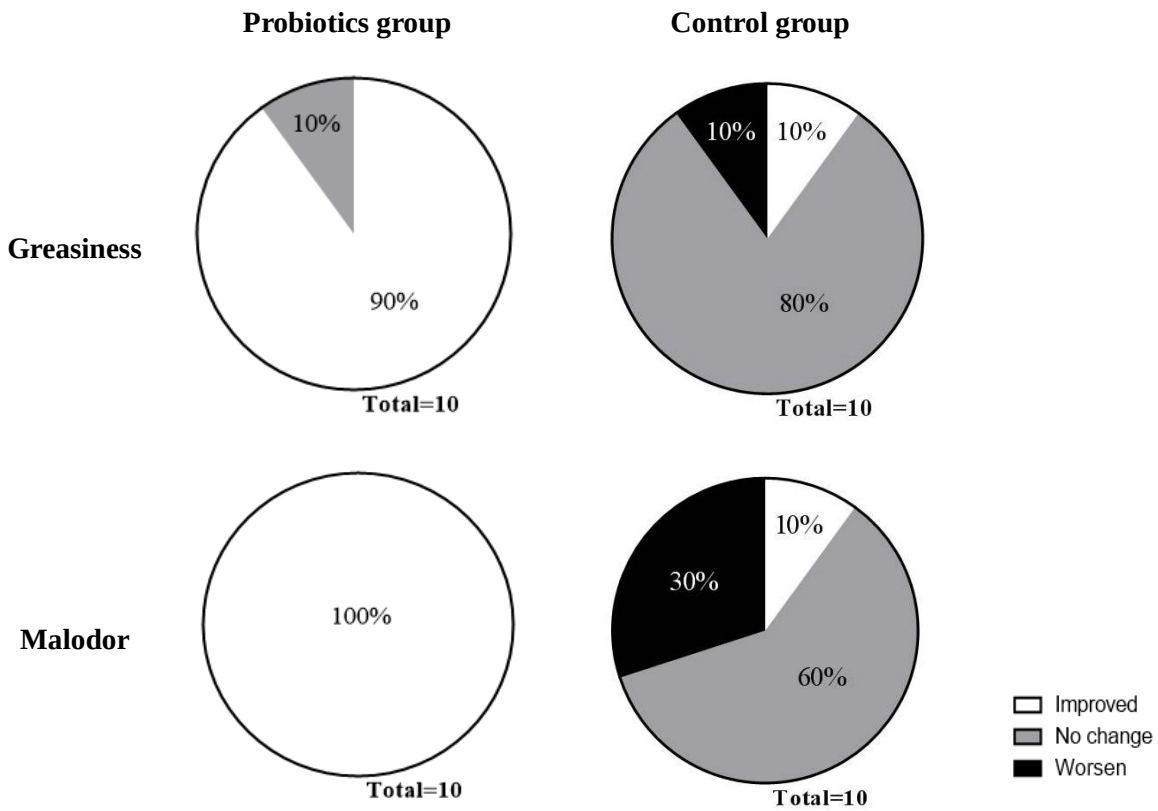

Figure 7. Owner evaluation about subsidiary changes of dog's skin of probiotics group and control group in phase 1.

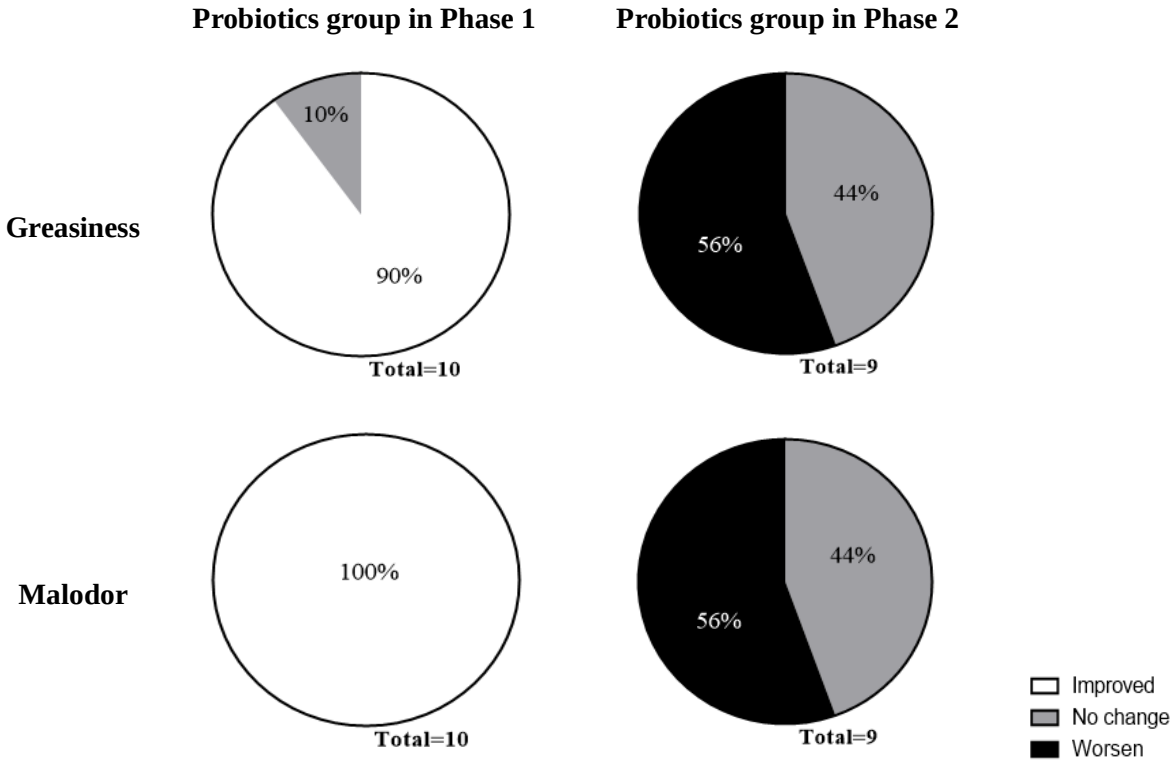

Figure 8.Owner evaluation about subsidiary changes of dog's skin of probiotics group in phase 1 and 2.

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국문초록

개의 아토피성 피부염에 대한 유산균 경구투여의 보완적인 효과

지도교수: 황철용

황선희

서울대학교 대학원

수의학과 임상수의학(피부과학) 전공

본 연구는 아토피성 피부염을 앓고 있는 개에게 유산균 혼합물을 투여할 시, 아토피성 피부염 증상에 대한 개선효과가 있는가를 평가하고, 이 효과가 나타나는 시점과 복용중단 후 그 효과가 얼마나 유지되는가를 확인하고자 실시하였다.

병력과 임상증상, Favrot’s criteria, 혈청항원 특이적 IgE시험과 피내접종

반응검사 등을 종합하여 개 아토피성 피부염으로 진단받은 20마리의 개를 본 연구에 포함시켰다. 연구에 참여한 개들은 기존에 처방 받은 약물복용이나 사양관리는 시험기간에도 동일하게 유지하도록 하였다. 음식 알러지와 계절성소양증이 있는 개들은 배제하였고 외부기생충, 세균, 곰팡이와 같은 이차적인 감염이 확인될 경우에는 완치한 이후에 참여시켰다. 개들은 무작위로 열 마리씩 유산균 복용군과 대조군으로 나누어 무작위 이중맹검법으로 평가하였다. 본 시험의 시험기간은 유산균 투여군과 대조군을 비교하는 Phase 1과 유산균투여 중단 후 기간동안 유산균 투여군의 변화를 추적연구하는 Phase 2로 실시되었다. 개 아토피성 피부염 증상평가를 위해 숙련된 수의사가 Canine Atopic Dermatitis Extent and Severity Index (CADESI) 방법으로 피부병변 평가를 실시하였다. 그리고 보호자 설문을 통해 Pruritus Visual Analog Scale (PVAS)와 피부와 관련한 질문들을 통해 소양감 평가 및 전반적인 피부상태와 부수적인 피부상태 평가를 진행하였다. Phase 1의 유산균 투여군과 대조군을 비교하기 위하여 Student's *t*-test를 적용하였고 Phase 1과 2의 유산균 투여군에 대하여 각 시점간 비교를 위해서는 Repeated-measures ANOVA를 적용하였으며, 각 임상평가에 대한 유산균 및 다른 요인들의 영향을 알아보기 위해서 Multivariate regression 분석을 실시하였다.

그 결과 Phase 1 기간 동안 CADESI, PVAS, 환견의 전반적인 피부상태와 피부 끈적임과 악취 같은 부수적인 피부상태에 있어서 유산균 투여군이 대조군에 비해 더 큰 폭으로 피부상태가 개선된 양상을 보였다. 유산균 투여

8주차에는 모든 임상평가 항목에서 대조군 대비, 통계적으로 유의한 차이도 확인할 수 있었다. 또한, Multivariate regression 분석결과를 통해 본 시험 동안 각 피부염 증상평가에 영향을 주는 요인은 유산균 밖에 없음을 확인하였다.

Phase 2 기간에서 유산균 투여군의 모든 평가항목들이 유산균 복용 중단 후 점차 다시 악화되는 경향을 나타내었으며, 유산균 중단 후 8주차에서는 유의한 증상악화를 확인할 수 있었다.

이러한 결과들을 바탕으로 분석하여 볼 때, 유산균은 개의 아토피성 피부염 환자의 치료에 있어서 유의미한 효과가 있다고 판단된다. 또한, 유산균 투여 시 임상증상 개선과 투여중단 후 임상증상 악화의 경향을 종합하여 분석하여보면 유산균의 효과는 복용 8주차에서 뚜렷이 확인되기 시작하며, 그 효과는 중단 후 8주 미만으로 지속된다는 것을 확인할 수 있다. 이상의 결과를 바탕으로 아토피성 피부염 환자의 치료에는 유산균의 투여가 유효하며 치료 및 재발방지를 위하여 지속적인 투여가 필요하다고 판단된다.

주요어 : Canine atopic dermatitis, Probiotics, *Bifidobacterium*, *Lactobacillus*, Dog

학번 : 2014-22956

Supplement 1. Consent form of the study

아토피 환자에 대한 유산균 효능 평가 임상 시험 참가 동의서

환자 명 : 나이 : 성별 : 품종 :

보호자 명: 연락처 :

아토피 환견(犬)에 대한 유산균 효능 평가 연구를 위해 본 견(犬) _____의 기본 신체검사 및 피부 평가, 유산균 복용을 하는 것에 동의 합니다. 신체검사 및 피부 평가는 총 6회 (시작, 복용 2주 후, 복용 4주 후, 복용 8주 후, 중단 4주 후, 중단 8주 후)에 걸쳐 실시합니다. 전신 피부 증상 평가와 피부표면의 수분 손실도, 환자의 소양감 지수 평가 등을 진행하게 되며, 통증을 유발하거나 침습적인 검사는 없습니다. 환견이 최대한 스트레스 받지 않도록 수의사의 관리하에 진행됩니다. 본 연구 평가를 위해 두 달 동안 복용할 유산균은 이미 널리 사용되고 있는 건강보조제의 성분으로서 부작용이 없는 안전한 건강보조식품 입니다.

2015. . .

보호자 성명 ( ) 서명 ( )