Clinical study of *PCSO-524 (ANTINOL™)* as neutraceutical in canine allergic skin disease

Introduction

PCSO-524 is an extract from New Zealand green lipped mussel (*Perna canaliculus*). It contains several classes of lipid. *PCSO-524* contains two of the long chain omega-3 polyunsaturated fatty acid (PUFAs); eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). *PCSO-524* is compose of 13% EPA, 21% DHA and about 30% cholesterol.[1] In addition, it also contains novel ω-3 PUFAs; 5,9,12,15-octodecatretraenoic acid, 5,9,12,16-nondecatertraenoic acid, 7,11,14,17-eicosatetraenoic acid, and 5,9,12,15,18-heneicsapententaenoic acid.[2] EPA, DHA and the o-3 PUFA 7,11,14,17-eicosatetraenoic acid have similar structure to arachidonic acid which is the precursor of the inflammatory agents, prostaglandins and leukotrienes.[2]

There have been many reports in human study about *P. canaliculus* effects. EPA and DHA have been reported to reduce the levels of inflammatory prostaglandins and leukotrienes as competitive substrates for the cyclooxygenase enzyme (COX) and the lipoxygenase enzyme (LOX). It has been shown that *PCSO-524* can inhibit PGE2 production and abolish leukotriene formation.[3,4] Another study showed anti-histamine activity of *P. canaliculus*.[5]

In animal study, it has been shown that *PCSO-524* has mild anti-inflammatory activity to reduce swelling in induced arthritis.[4] Other study reported that *PCSO-524* or glucocorticoid alone did not have clinical benefit on reducing inflammation but combined treatment of these two substances can reduce paw swelling.[6] However, there have been no reported to compare clinical effects of *PCSO-524* and glucocorticoid in standard model or arthritis.[2] The effects of *P. canaliculus* has aloso been done in the collagen type-II-induced severe polyarthritis in rat and mouse. The results showed that P. canaliculus can reduce incidence of arthritis from 58% from control group to 17% in *P. canaliculus* treated group.[7] Many studies of *PCSO-524* effects in dogs with arthritis have been reported. Bui and Bierer (2001) reported beneficial effects of *P. Canaliculus* that can treatment group showed an improvement of osteoarthritis when compared to untreated dogs.[8] This study showed that *PCSO-524* has beneficial effects on Arthritis scores (mobility, pain, swelling and crepitus). Another report showed effectiveness of green lipped muscle extract in dogs with generative joint disease.[9] This study showed that green lipped mussel extract have beneficial effects to improved musculoskeletal dysfunction by 67% of the 81 lame dogs with no side effects throughout the treatment period. This study also showed clinical response was seen at 56 days after treatment. A comparison between *PCSO-524* and non-steroidal anti-inflammatory drug (carprofen) was done in 45 dogs with osteoarthritis.[10] Dogs in placebo group showed 20-40% improvement in pain, mobility index and locomotion after 8-week treatment period while dogs treated with *PCSO-524* and carprofen showed 67% and 86% improvement in pain VAS. So carprofen was shown to be more effective than *PCSO-524* but its adverse effects, especially with long term use, are of concern. Even though many studies reported anti-inflammatory effects of *PCSO-524* but no clinical study in inflammatory skin diseases have been reported.

Allergic dermatitis refers to any inflammatory skin diseases caused by any types of allergy. It is caused by various antigens inducing immunological reactions. The severity and progression of clinical signs depend on etiologic factors. The clinical presentation of hypersensitivity can either be short-lived or lifelong. In small animal practice, several types of allergic diseases are documented but the most common in dermatology practice includes atopic dermatitis, food hypersensitivity, contact hypersentitivity, and flea allergic dermatitis. [11-13] The characteristics of allergic dermatitis are pruritus and subsequent inflammation. Therefore, allergic dermatitis is a good candidate to study anti-inflammatory effects of PCSO-524 for the treatment of skin diseases.

Objectives

1. To study the beneficial effects of *PCSO-524 (ANTINOL™)* as a neutraceutical to improve skin and coat condition

2. To investigate therapeutic effects of *PCSO-524 (ANTINOL™)* in canine allergic skin diseases

Materials and methods

Animals

The 31 dogs included in this study were selected from dogs with skin diseases presenting to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. All dogs had clinical histories consistent with chronic pruritis, including some degree of erythema, focal or multifocal alopecia, excoriation, and recurrent bacterial or yeast infection. The dogs remained under their owner's care throughout the study period.

Inclusion criteria

The dogs underwent a general and dermatological examination conducted by the same veterinarian, to identify skin lesions. Hematological and biochemical analysis were performed to evaluate health status. Cytology examination and bacterial culture and susceptibility test were also done to identify the presence of yeast and bacterial infection and select appropriate treatment for affected dogs. Allergic skin disease was defined using a criterion of chronic pruritus and inflammation of skin with other skin diseases excluded based on history, clinical signs, skin scraping, cytology examination, hematology, and blood chemistry profiles.[13]

Treatment protocol

Dogs included in this study were classified into 3 treatment groups based on severity of dermatological signs; mild, moderate and severe. The classification was made on the basis of degree of pruritus, distribution and extension of the lesions and skin and coat condition.[14] for ethical reasons, there was no control group without treatment.

Group 1 Mild degree: *PCSO-524*
Group 2 Moderate degree: *PCSO-524* + antihistamine (hydroxyzine hydrochloride)
Group 3 Severe degree: *PCSO-524* + prednisolone

All dogs were treated with appropriate symptomatic therapy to control secondary infection in order to reduce other involving factors apart from allergic reaction. This was prescribed before start of the experiment or during experimental period if secondary infection was diagnosed up on clinical examination and cytology examination. Adverse effects were monitored clinically by veterinarians and noted if any adverse effects were seen by owners or veterinarians.

Dose prescription

- *PCSO-524*: All the dogs received *PCSO-524* of loading dose for 2 weeks followed by maintenance throughout the experiment. Loading dose was prescribed as 1 capsule/ 10 kg BW twice a day and maintenance dose was 1 capsule/ 10 kg BW once a day.
- Antihistamine (hydroxyzine hydrochloride) 2-4 mg/kg bid
- Prednisolone: 0.5-1 mg/kg/d for 7 days then taper down to 0.25 mg/kg/d once a day every other day
- Cephalexin : 25-30 mg/kg/d bid

Monitoring of the dogs

All dogs were scheduled to revisit and be evaluated at 2 weeks, 4 weeks, 8 weeks, 12 weeks and 16 weeks after treatment. Evaluation of clinical response i.e. improvement of clinical signs, response to therapy and skin and coat condition were assessed by owners and veterinarians**.**

Veterinary evaluation

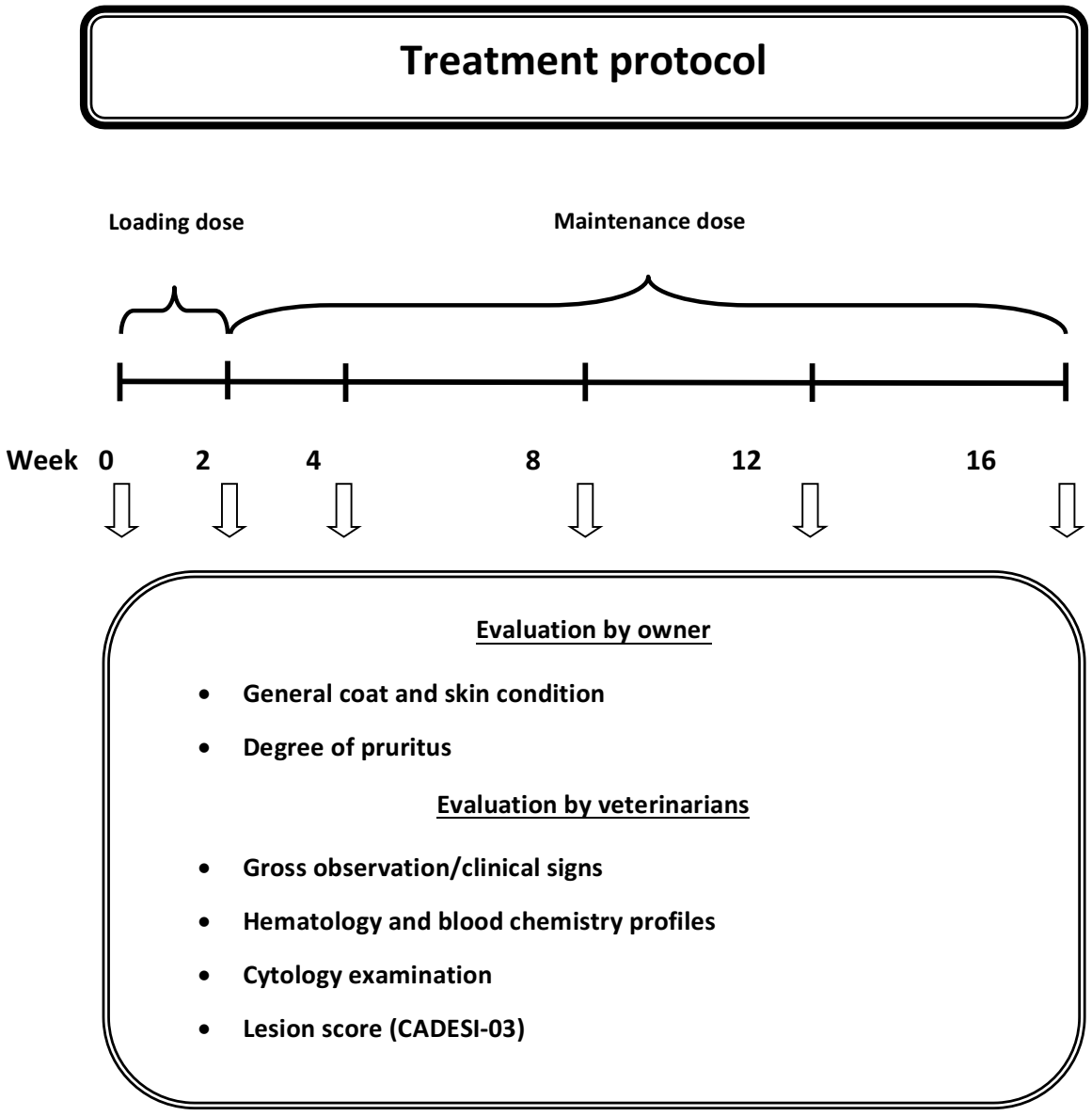
Gross observation and dermatological examination were done by veterinarians to assess effects of the treatment on each visit. Two veterinarian subjectively assessed 4 parameters; erythema, lichenification, excoriation and self-induced alopecia using canine atopic dermatitis extent and severity index 03 (CADESI-03) at week 0, 2, 4, 8, 12 and 16. The scores assigned by veterinarian were summed to form a veterinary-assessed index score.

Owner evaluation

Owners answered a questionnaire on general history of the dogs including gross appearance of the dogs, general skin and coat condition, degree of pruritus, age of onset and previous treatment were recorded on first visit. On each following visit, observation of owners on general skin and coat condition and degree of pruritus (pruritus visual analog scale, PVAS) were noted to form an owner-assed index used a descriptive scale of 0-10. [14]

Major outcome criteria and statistical analysis

The major outcome criterion was the proportion of dogs with improvement, stable and worsening of clinical signs performed at each visit. Statistical analysis were carried out by using the Statistical Analysis System (SAS) software version 9.0 (SAS Inst. Inc.,Cary, NC, USA). Descriptive statistics were obtained using MEANS and FREQ procedures. Analysis of variance was applied using the MIXED and GLM procedures. Traits analyzed were score of improvement in skin and coat condition, degree of pruritus and lesion scores. One-way ANOVAs were used to evaluate changes in CADESI-03 and pruritus scores between the treatment groups from week 0 to 16. The proportions of dogs in each treatment groups that showed improvement at week 0, 2, 4, 8, 12 and 16 were assessed. Chi-square test was used to analyze differences in the proportions of dogs that show improvement. Values with $P < 0.05$ were regarded as statistically significant.



Result

Breed distribution

There were 31 dogs diagnosed as having allergic skin diseases. Several breeds included in the study with the highest number in Shih tzu (n=9). The other breeds also included in this study were Poodle (n=6), Pomeranian (n=6), Mixed breed (n=3), Miniature pincher (n=2), Maltese (n=1), English cocker (n=1), Pug (n=1), Bangkaew (n=1) and Chihuahua (n=1).

Sex

From total of 31 dogs, the numbers of male and female dog were 15 (48%) and 16 (52%). The number of dogs and percentage were not statistically different.

Comparison between 3 treatment groups

Number of dogs with improvement in skin and coat condition in group 1, 2 and 3 are 9 of 12, 3 of 9 and 5 of 10 respectively. Group1 showed higher percentage (75%) compared with group 2 (33.33%) and group3 (50%). Similarly, number of dogs with reduction in pruritic score (PVAS) was also higher in improvement in group1 (50%) with less effects in group2 (44.44%) and 3 (40%). The more prominent effects of lesion score (CADESI-03) improvement were observed with significant higher percentage as compared with the other two groups.

Group 1 showed the shortest time for improvement seen when compared with group 2 and 3. Time at improvement seen in Group 1, 2 and 3 were 5.33 $\pm$ 1.56, 7.33 $\pm$ 2.70 and 12 $\pm$ 2.09, respectively.

Adverse events

One dog in group 3 had diarrhea 2 weeks after treatment. Diarrhea was mild and transient. There was no evidence of serious side effects or moderate or severe gastrointestinal events. In particular, no changes in hematology and blood chemistry profiles in dogs treated with PCSO-524 alone. Some dogs in group 3 (PCSO-524+prednisolone) had increased liver enzyme levels.

Table 1 Subject characteristics

	Subject	Age (year)	Sex	Breed	Age of onset (year)
Group 1	1	7	F	Poodle	4-7
	2	4	M	Shih tzu	1-3
	3	12	M	Shih tzu	1-3
	4	3	M	Chihuahua	1-3
	5	8	M	Shih tzu	4-7
	6	8	Fs	Shih tzu	4-7
	7	9	M	Pomeranian	4-7
	8	10.5	M	Pomeranian	4-7
	9	5	F	Pomeranian	4-7
	10	9	M	Maltese	4-7
	11	5	M	Pomeranian	1-3
	12	10	F	Shih tzu	4-7
Group 2	1	5	F	Shih tzu	1-3
	2	4	F	Pug	1-3
	3	8	Fs	Mixed	<1
	4	8	M	Pomeranian	4-7
	5	7.5	F	Poole	4-7
	6	14	M	Bangkaew	1-3
	7	4	F	Miniature pincher	<1
	8	9	M	Shih tzu	4-7
	9	19	M	Miniature pincher	>7
Group 3	1	8	M	Mixed	4-7
	2	10	F	Poodle	1-3
	3	8	M	Pomeranian	>7
	4	8	F	Mixed	4-7
	5	13	F	Poodle	4-7
	6	12	F	Poodle	4-7
	7	6	F	Shih tzu	4-7
	8	13	M	Poodle	>7
	9	14	F	English cocker	>7
	10	9	F	Shih tzu	4-7

M = male; F = female; Fs = spayed female

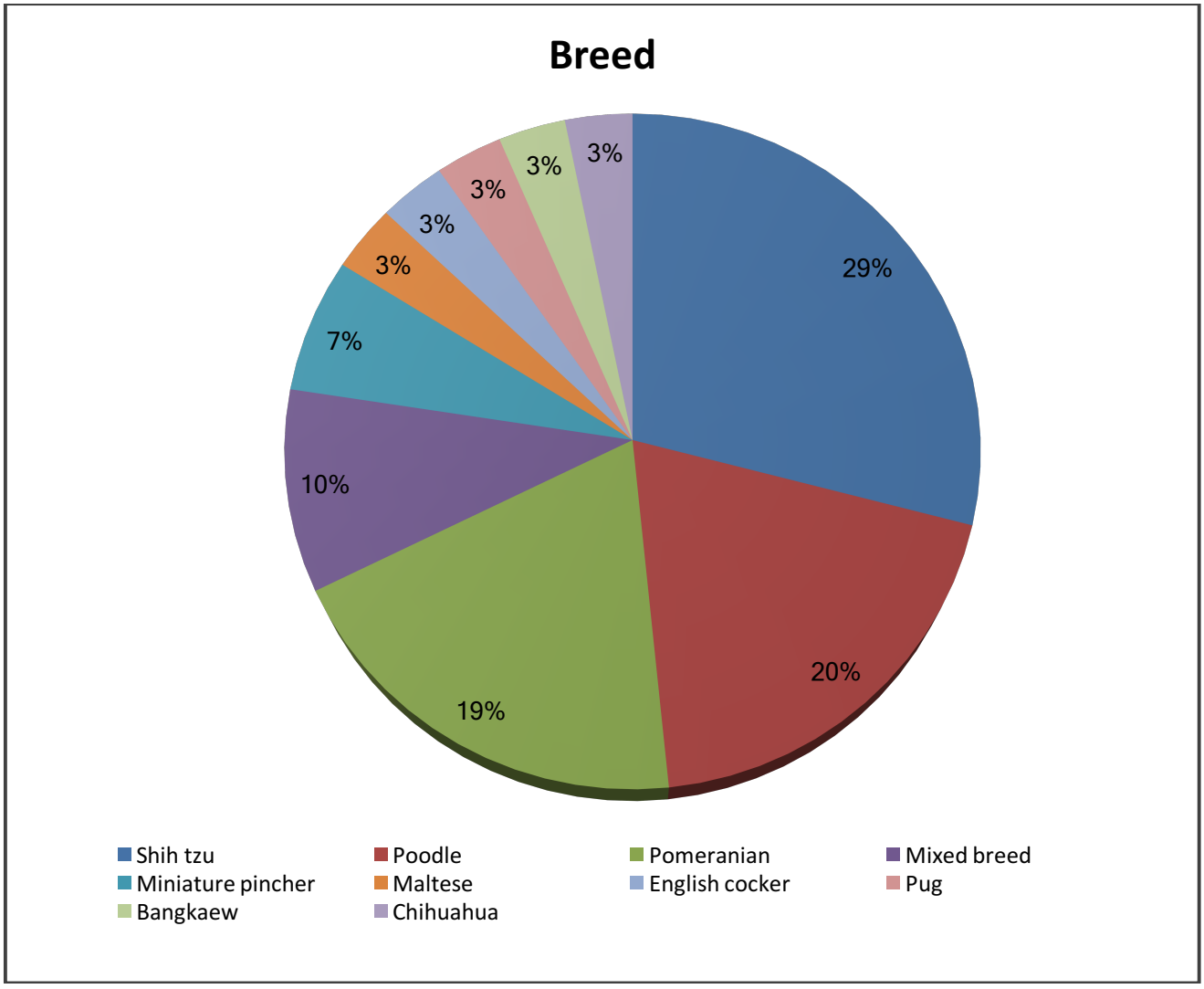


Figure 1 Diagram shows breed distribution of 31 dogs included in the study; Shih tzu (n=9), Poodle (n=6), Pomeranian (n=6), Mixed breed (n=3), Miniature pincher (n=2), Maltese (n=1), English cocker (n=1), Pug (n=1), Bangkaew (n=1) and Chihuahua (n=1).

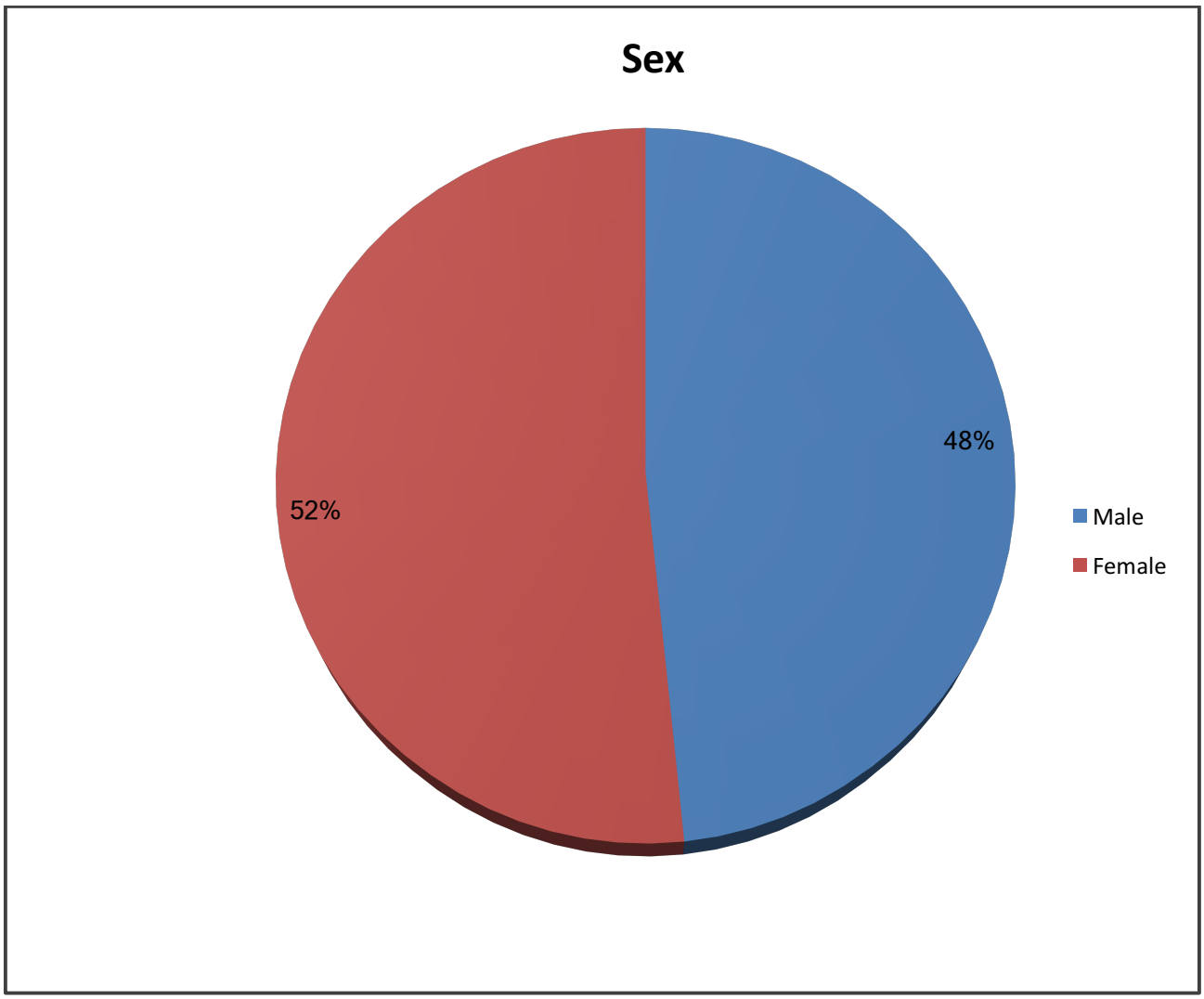


Figure 2 Diagram shows sex of 31 dogs diagnosed as having allergic skin disease in this study; male (n=15) and female (n=16).

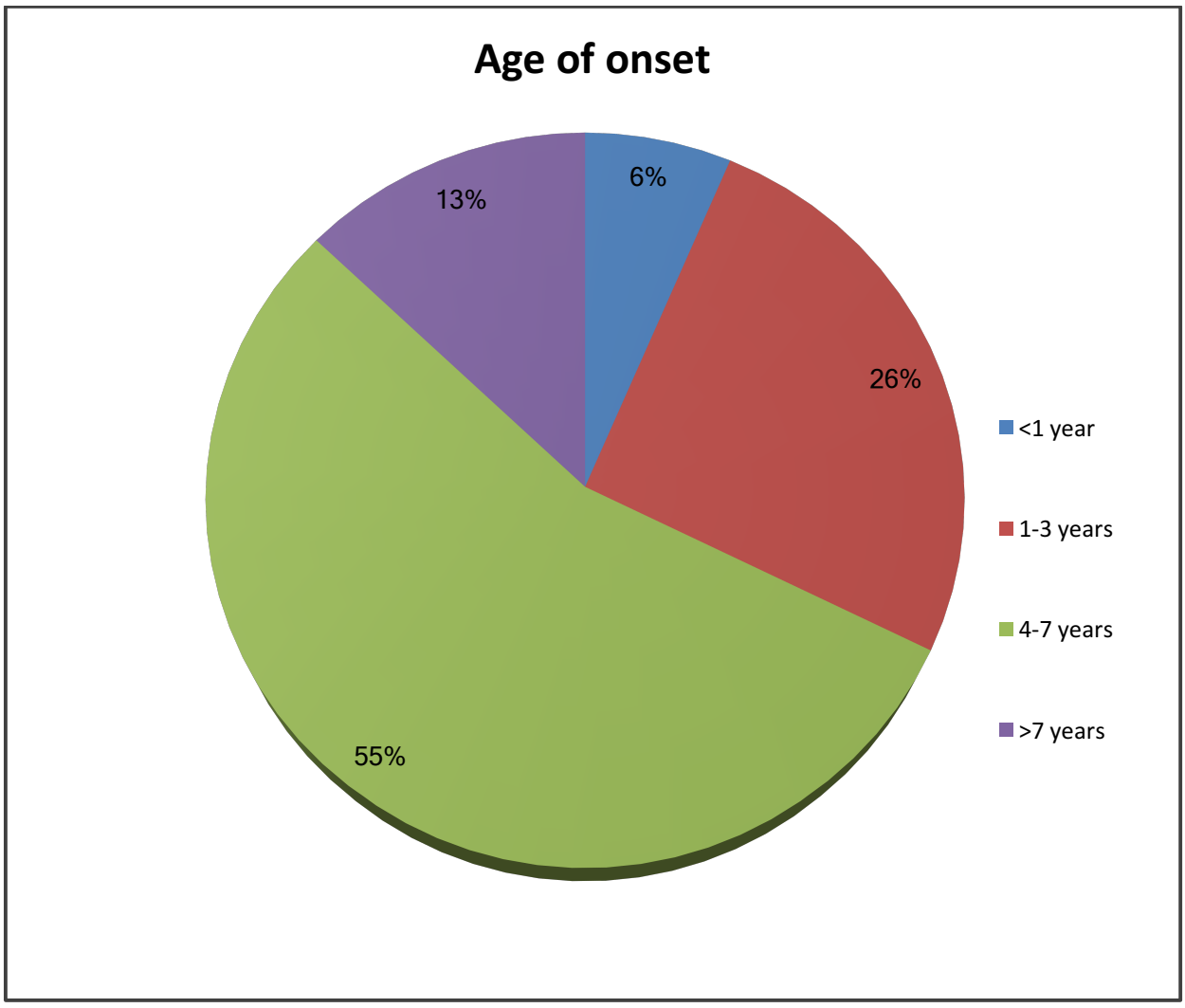


Figure 3 Age of onset noted in all subjects (31 dogs) were classified into 4 age ranges; <1 years (n=2), 1-3 years (n=8), 4-7 years (n=17) and > 7 years (n=4).

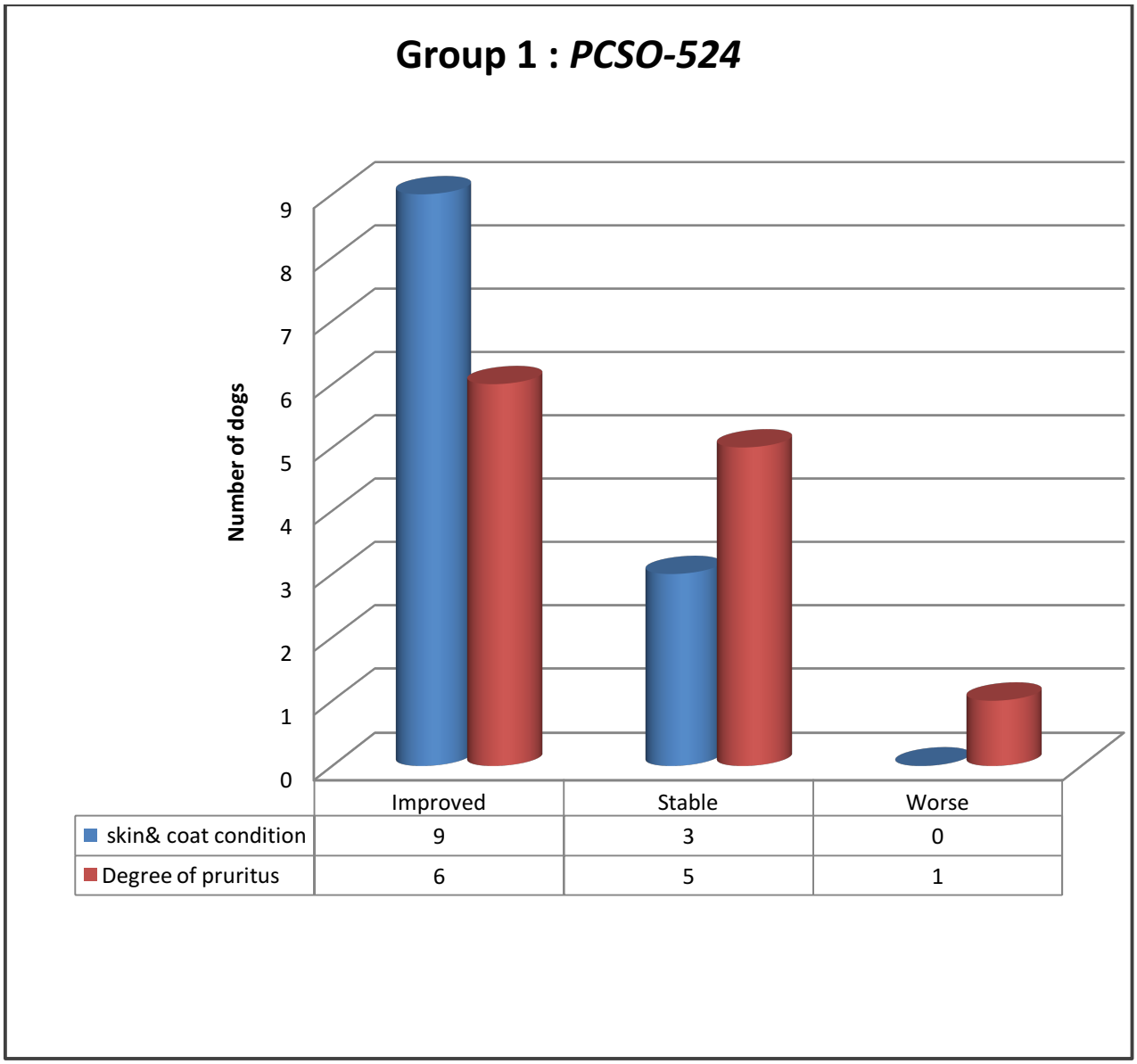


Figure 4 Clinical response (skin & coat condition and degree of pruritus) in allergic dogs treated with PCSO-524 alone (n=12).

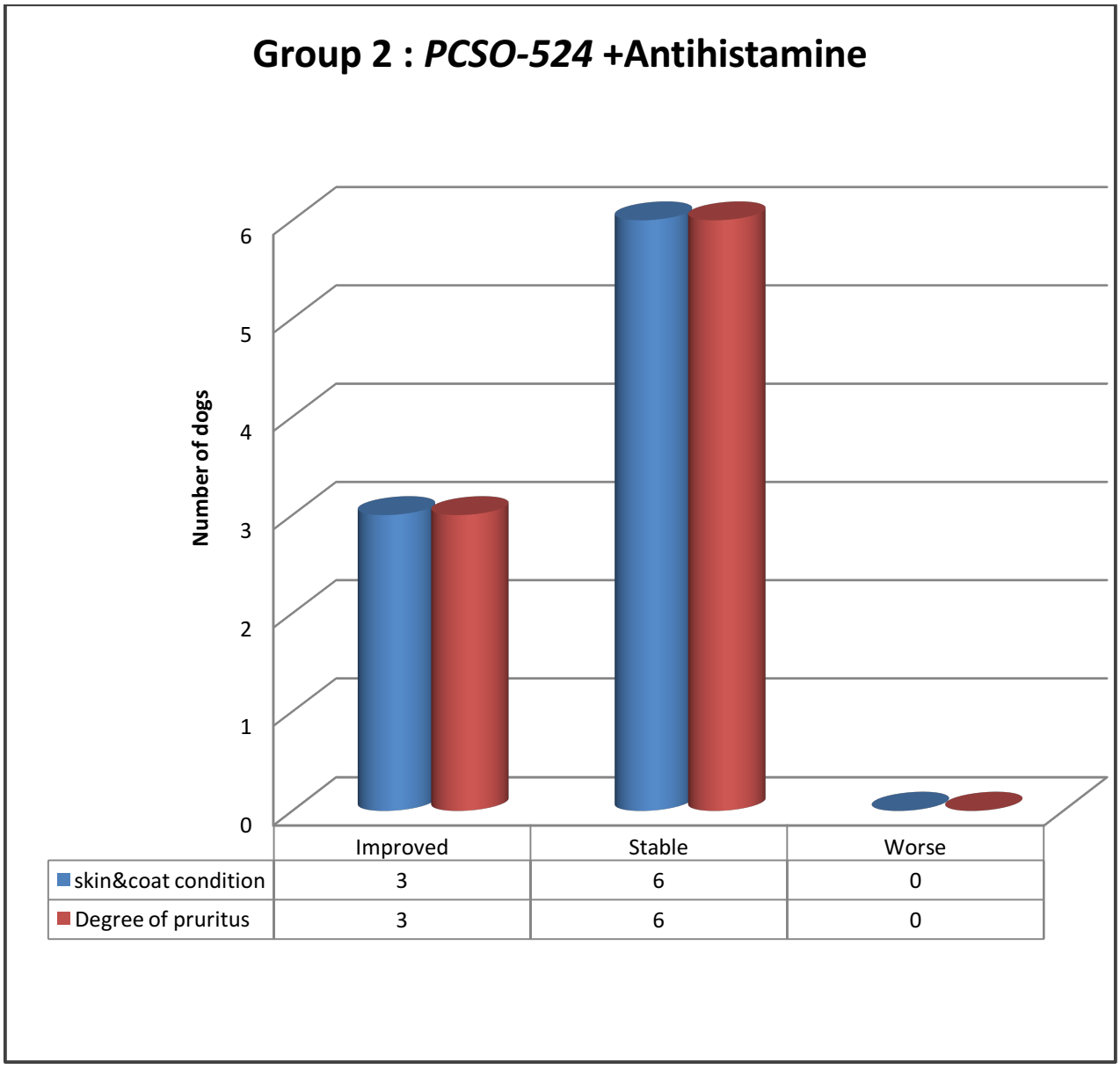


Figure 5 Clinical response (skin & coat condition and degree of pruritus) in allergic dogs treated with combination therapy of PCSO-524 and antihistamine (n=9).

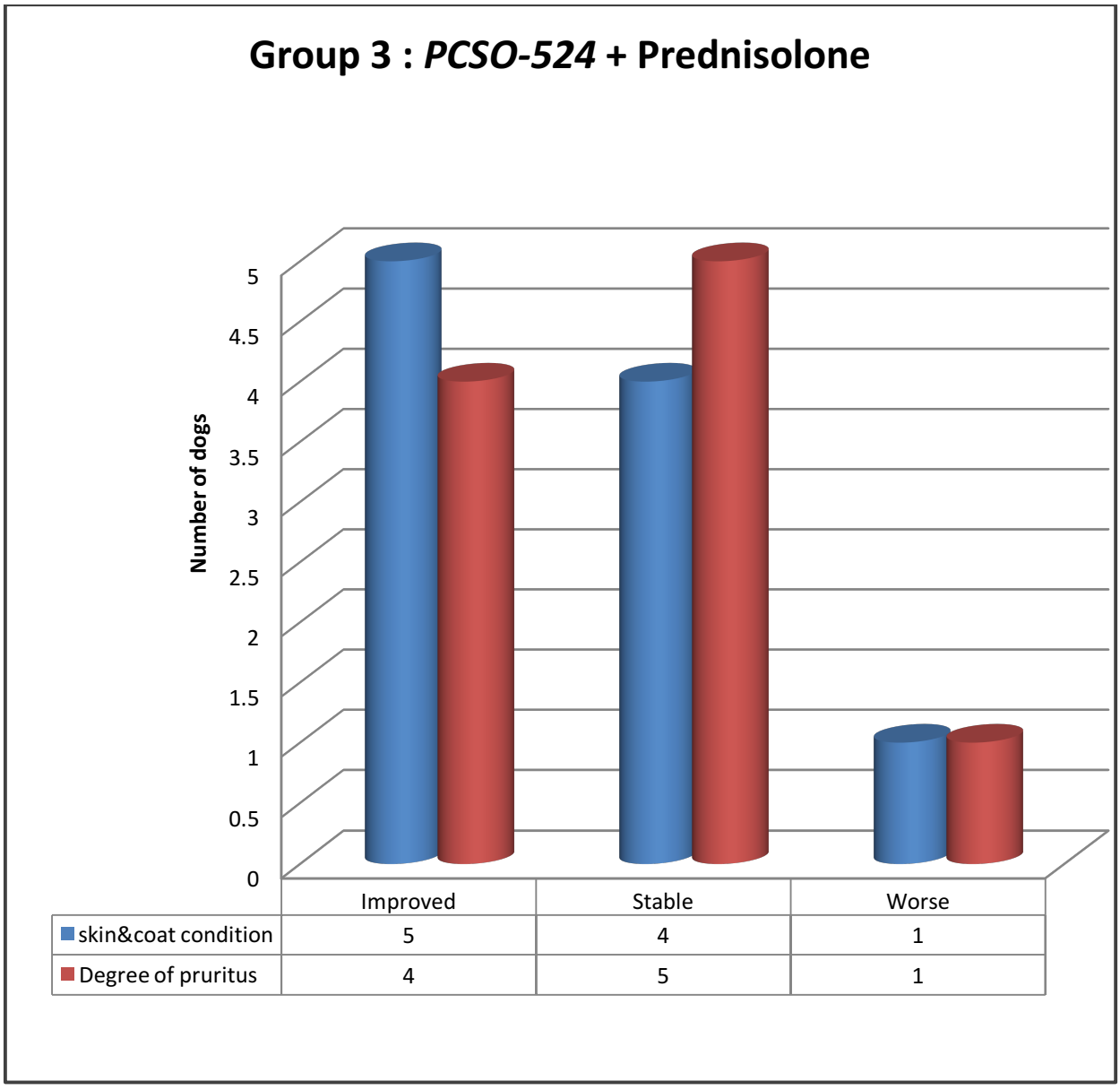


Figure 6 Clinical response (skin & coat condition and degree of pruritus) in allergic dogs treated with combination therapy of PCSO-524 and prednisolone (n=10).

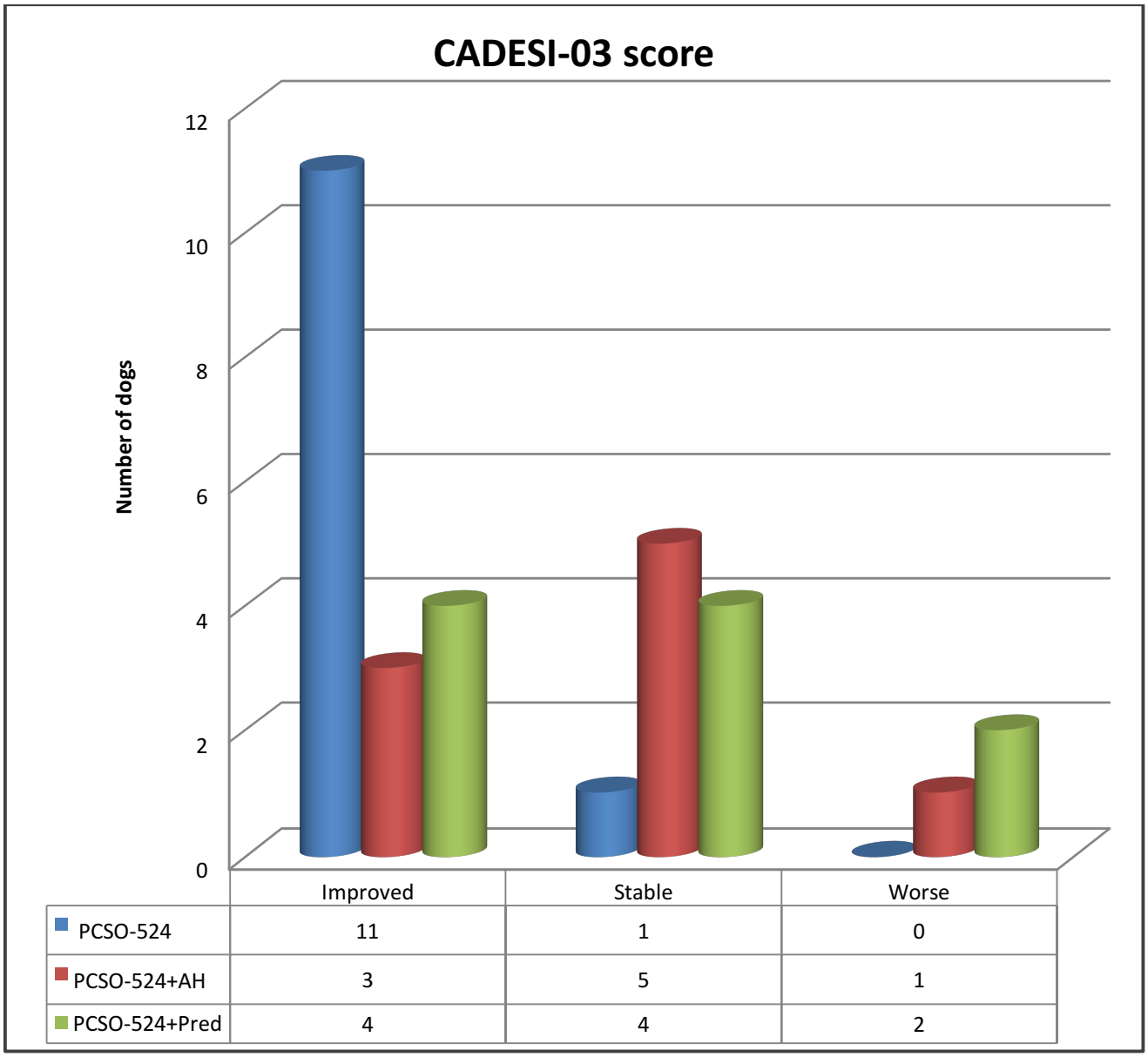


Figure 7 Canine atopic dermatitis extension and severity index (CADESI-03) score of allergic dogs treated with PCSO-524 alone (n=12), combination therapy of PCSO-524 and antihistamine (n=9) and PCSO-524 and prednisolone (n=10).

Table 2 Percentage of improved dogs and time at improvement seen for evaluated variables, per group

	Number of dogs with improvement (%)			Time at improvement seen (weeks)		
	Group 1 PCSO-524 (n=12)	Group 2 PCSO-524+AH (n=9)	Group 3 PCSO-524+Pred (n=10)	Group 1 PCSO-524 (n=12)	Group 2 PCSO-524+AH (n=9)	Group 3 PCSO-524+Pred (n=10)
Skin & coat condition	9/12 (75%)	3/9 (33.33%)	5/10 [b, c] (50%)	5.33 ± 1.56 [c]	7.33 ± 2.70	12 ± 2.09 [a]
Degree of pruritus (PVAS)	6/12 (50%)	4/9 (44.44%)	4/10 (40%)	4 ± 0.95	4.67 ± 1.40	5 ± 1.17
Lesion score (CADESI-03)	11/12 [b,c] (91.67%)	3/9 [a] (33.33%)	4/10 [a] (40%)	4.55 ± 1.28 [c]	4.67 ± 2.45	10.50 ± 2.12 [a]

[a] significant difference between when compare with group 1
[b] significant difference between when compare with group 2
[c] significant difference between when compare with group 3

Table 3 A comparison of 3 parameter (mean ± SEM) ; skin and coat condition, degree of pruritus and lesion score in 3 treatment groups at week 0, 2, 4, 8, 12 and 16

		Week Mean ± SEM					
		0	2	4	8	12	16
Skin & coat condition	Group 1 PCSO-524 (n=12)	6 ± 0.67	5.7 ± 0.61	3.91 ± 0.64	2.67 ± 0.56 [c]	2.89 ± 0.66 [b]	1.88 ± 0.63
	Group 2 PCSO-524+AH (n=9)	7 ± 0.77	6.13 ± 0.68	5.22 ± 0.74	4.29 ± 0.73	5.40 ± 0.88 [a]	5.50 ± 0.72
	Group 3 PCSO-524+Pred (n=10)	6 ± 0.73	6.00 ± 0.68	5.1 ± 0.71	5.30 ± 0.61 [a]	4.50 ± 0.69	4 ± 0.67
Degree of pruritus (PVAS)	Group 1 PCSO-524 (n=12)	5 ± 0.72	4.4 ± 0.59	3.5 ± 0.63	2.33 ± 0.6 [b,c]	2 ± 0.58 [b,c]	2 ± 0.78 [b]
	Group 2 PCSO-524+AH (n=9)	6.89 ± 0.83	6.38 ± 0.67	5.33 ± 0.73	5.00 ± 0.85 [a]	5.6 ± 0.73 [a]	4.67 ± 0.90 [a]
	Group 3 PCSO-524+Pred (n=10)	6.1 ± 0.79	5.75 ± 0.67	5.1 ± 0.69	4.9 ± 0.66 [a]	5.13 ± 0.58 [a]	3.85 ± 0.84
Lesion scores (CADESI-03)	Group 1 PCSO-524 (n=12)	250 ± 52.51	196 ± 53.17	89.75 ± 36.77 [b,c]	94.22 ± 26.75 [b,c]	75.89 ± 32.18 [b,c]	37.25 ± 34.31 [b,c]
	Group 2 PCSO-524+AH (n=9)	254 ± 60.63	326.67 ± 68.64	211.56 ± 42.46 [a]	124.57 ± 30.34 [a]	206.14 ± 36.49 [a]	177.57 ± 36.67 [a]
	Group 3 PCSO-524+Pred (n=10)	359 ± 57.51	255 ± 59.44	211.30 ± 40.28 [a]	231.30 ± 25.38 [a]	232.50 ± 34.13 [a]	240.71 ± 36.67 [a]

[a] significant difference between when compare with group 1
[b] significant difference between when compare with group 2
[c] significant difference between when compare with group 3

Discussion

Allergic dermatitis is defined as any inflammatory skin diseases caused by any types of allergy. It can be caused by a variety of allergen inducing immunological reaction of animals. In this study, allergic dogs refers to dogs with clinical signs of atopic dermatitis and food allergy as other diseases were ruled out by history, physical examination, cytological examination and blood tests. The diagnosis followed 2010 clinical practice guidelines from the international task force on canine atopic dermatitis.[14]

Eicosapentaenoic acid (EPA and docosahexaenoic acid (DHA) are the main active portions of fish oil. their anti-inflammatory effects are possibly due to inhibition of eicosanoids and cytokines production.[15] An extract from New Zealand green lipped mussel (*Perna canaliculus*), *PCSO-524* also contains polyunsaturated fatty acid (PUFAs); eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) including novel ω-3 PUFAs (7, 11, 14, 17-eicosatetraenoic acid).[1,2] Many studies showed anti-inflammatory activity of these PUFAs and also anti-histamine activity.[4] Fish oil has been shown to have higher inhibitory effects on COX-1 and COX-2 production as compared to *PCSO-524*. However, hydrolysed form of *PCSO-524* showed similar inhibitory activity on COX enzyzmes production.[16] *PCSO-524* has beneficial effects on reducing inflammation in animal study. It has been shown to reduce swelling in arthritis.[3] In addition, *PCSO-524* has also been shown to have anti-inflammatory effects when used in combination with glucocorticoid.[6] The anti-inflammatory effects was absent when animals were treated with *PCSO-524* or glucocorticoid alone.[5] Another study showed the effectiveness of combination of *PCSO-524*, EPA and DHA in the treatment of rheumatoid arthritis.[17] However, the study of *PCSO-524* effects on skin diseases has never been investigated.

From total of 31 dogs, the proportion of male and female dogs diagnosed of having allergic dermatitis was not significant. There were 15 male dogs (48%) and 16 female dogs (52%). The results correlated with previous study that allergic dermatitis has no gender predisposition.[14]

Dogs in group 1, treated with PCSO-524 only, showed significant improvement of skin and coat condition compared with group 2 and 3. This was evaluated by the owners, with score range from 0 to 10 based on skin and coat gross appearance. Similarly, number of dogs with reduction in pruritic score (PVAS) was also higher in group1 (50%) with less effects in group2 (44.44%) and 3 (40%). The results indicated that dogs with mild degree of allergic dermatitis seem to have benefit from PCSO-524 by improving skin and coat condition and reducing degree of pruritus. Dogs with moderate degree (group2) and severe degree of allergic dermatitis (group3) did not have beneficial effects by owner evaluation results. The improvement observed in group1 was possibly due to anti-inflammatory and anti-histamine activity of PCSO-524.[2-4] Failure of treatments in group2 and 3 could be a combination of degree of severity, presence of recurrent bacterial and yeast infection and chronicity of the disease. Olivry et al. (2010)[14] suggested that clinical signs of allergic dogs, e.g. urticaria or pruritus, might be present with or without skin lesion. It was reasonable for Dogs in group1 with mild degree of allergy to have improved clinical appearance. Dogs in group 2 and 3 with moderate and severe degree of allergy might have higher degree of inflammation or hypersenstitivity, in which, combination therapy was incapable of treating the dogs. All allergic dogs in this study might represent atopic dermatitis or food allergy

or both. The relationship between atopic dermatitis and food allergy has been controversial but it is suggested food components might cause the flare of atopic dermatitis. This includes urticaria or pruritus with or without skin lesion (9of guideline). Therefore, dietary factors could be one factor of treatment failure. Moreover, dogs in group 2 and 3 with higher degree of severity had more frequent recurrence of bacterial or yeast infection. This is a major flare factors in allergic dogs.[14]

Evaluation by veterinarian also showed consistent results of PCSO-524 benefit on reduction in lesion score (CADESI-03). The improvement of lesions scores was observed with significant higher percentage in group 1 compared with the other two groups.

The time at improvement seen was also observed to investigate proper treatment period for effective results. Average time to have improvement in group 1, 2 and 3 were 5.33 $\pm$ 1.56, 7.33 $\pm$ 2.70 and 12 $\pm$ 2.09 weeks, respectively. The results were consistent with previous study.[18]

Conclusion

PCSO-524 might have beneficial effects for the treatment of canine allergic skin disease. The improvement in skin and coat condition, degree of pruritus and lesion scores was observed in dogs with mild degree of allergic skin disease but not in dogs with moderate or severe allergic dermatitis. Other contributing factors, i.e. secondary bacterial or yeast infection, skin and coat hygiene and causing allergens should be of concern and well-controlled in more severe cases.

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