

Pilot evaluation of *Enterococcus faecium* SF68 as adjunctive therapy for oclacitinib-responsive adult atopic dermatitis in dogs

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OBJECTIVE: To evaluate the adjunctive effect of supplementation with *Enterococcus faecium* SF68 (FortiFlora; Purina Pro Plan Veterinary Diets) on oclacitinib (Apoquel, Zoetis) dose reduction, while maintaining or reducing the Pruritus Visual Analog Score and Canine Atopic Dermatitis Extent and Severity Index values in client-owned adult dogs with environmental atopic dermatitis.

MATERIALS AND METHODS: Enrolled dogs had exhibited control of atopic dermatitis on oclacitinib for at least 6 months before, and continuing throughout, the study. Dogs with non-seasonal pruritus were blindly randomised to receive either SF68 (1×10^8 colony forming units/g orally twice daily) or placebo for 12 weeks. After 8 weeks of supplementation, oclacitinib dose was decreased by approximately 25%, aiming to maintain and reduce the clinical disease scores.

RESULTS: Supplementation with SF68 revealed no difference in oclacitinib dose reduction versus placebo in 21 client-owned dogs with atopic dermatitis. Clinical disease scores were not different between groups at study completion.

CLINICAL SIGNIFICANCE: Further larger-scale studies are warranted to investigate optimal strain(s), dosing and duration of probiotic supplementation as an adjunctive strategy in management of canine atopic dermatitis.

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INTRODUCTION

Canine atopic dermatitis (AD) is a chronic genetically-predisposed inflammatory and pruritic allergic skin disease associated with, initially, a type-2 helper T cell (Th2)-dominant immune response and complex interactions with other lymphocyte cytokines, chemokines and responses (Hill & Olivry 2001, Inoue *et al.* 2014, Pucheu-Haston *et al.* 2015). Diminished microbial stimulation and subsequent altered gut microflora are proposed to result in decreased Th1 cell development and a dysregulation of Th2-type responsiveness that may result in clinical manifestation of allergic disease (Mogna *et al.* 2008, Okada *et al.* 2010, Rho *et al.* 2017).

Probiotics, defined as live microbial organisms conferring a health benefit on the host when administered in adequate amounts (FAO/WHO 2006), have demonstrated immunomodulatory effects that could impact the clinical manifestation of atopic dermatitis in humans and veterinary patients. Optimal dose to suffice as “adequate” has not been defined, but a range of dosing regimens have met with mixed success (Notay *et al.* 2017). Multiple mechanisms through which probiotics impact the immune system have been proposed, including, but not limited to, induction of regulatory cytokines interleukin (IL)-10, transforming growth factor-beta, IL-12 and interferon-gamma that downregulate Th2 cytokines. As demonstrated in human serum and rodent tissue models, this subsequently induces

oral tolerance, facilitates T cell differentiation, maturation and prevents allergic inflammation (Bermudez-Brito *et al.* 2012, Mccusker & Sidbury 2016, Cross *et al.*, 2001, Kalliomäki *et al.* 2001, Kim *et al.* 2018, Inoue *et al.* 2014). The addition of lactic acid-producing bacterium has been investigated in children, adults and young dogs to prevent and alleviate the symptoms of AD (Inoue *et al.* 2014, Kalliomäki *et al.* 2001, Kalliomäki *et al.* 2003, Isolauri *et al.* 2000., Marsella 2009, Marsella *et al.* 2012).

Limited studies with *Lactobacillus* in adult dogs reported promise for a drug-sparing effect (Bermudez-Brito *et al.* 2012, Kim *et al.* 2015, Ohshima-Terada *et al.* 2015). *Enterococcus faecium* SF68 (FortiFlora; Purina Pro Plan Veterinary Diets) is a hydrolysed commercially-available lactic acid-producing bacterium formulation licensed for veterinary use and has been shown to have immunomodulatory effects in young healthy dogs (Benyacoub *et al.* 2003). After labelled dosing for 4 weeks, flow cytometry identified increased B cell expression of surface-bound IgG and MHCII; after 12 weeks, increased T lymphocyte proliferative responses were identified.

Manufactured at 5×10^8 colony forming units (CFU)/g and guaranteed to contain a minimum of 1×10^8 CFU/g, FortiFlora (SF68) has been employed for the prevention and treatment of stress-induced and antibiotic-associated diarrhoea in affected dogs and cats (Bybee *et al.* 2011, Fenimore *et al.* 2017). There is limited evidence for probiotics decreasing clinical signs in canine AD or evaluating probiotic supplementation as adjunctive therapy for AD dogs treated with oclacitinib.

Oclacitinib (Apoquel; Zoetis) is an approved treatment for canine AD with published effective dose range. (Cosgrove *et al.* 2013) It has also been stated that multimodal therapy is often a preferred method for control of canine AD and may have drug-sparing effects, improved clinical response and decreased client cost (Olivry *et al.* 2015). The objective of this study was to evaluate the effect of SF68 on oclacitinib dose reduction while maintaining clinical efficacy. The hypothesis was that the addition of SF68 would permit decrease of oclacitinib dose in chronically treated canine AD patients while maintaining or reducing the Pruritus Visual Analog Score (PVAS) (Hill *et al.* 2007, Rybníček *et al.* 2008) and Canine Atopic Dermatitis Extent and Severity Index (CADESI-4) (Olivry *et al.* 2014) clinical disease scores.

MATERIALS AND METHODS

Privately owned dogs previously diagnosed, by a veterinary dermatology specialist or resident, with non-food-induced AD according to accepted exclusion criteria (Deboer & Hillier 2001) and responsive to oclacitinib, were screened for enrolment. Since this was a pilot study, a sample size calculation was not performed, but we sought to enroll 40 patients to align with previously published veterinary studies (Marsella 2009, Ohshima-Terada *et al.* 2015). Before screening, all patients had normal routine biochemistry and haematology profiles. Inclusion criteria included non-seasonal mild pruritus (in-clinic owner-reported PVAS ≤ 3.5 out of 10) and a mild to low-moderate lesion score (CADESI-4 ≤ 16 out of 180 total points) controlled with consis-

tent dosing of oclacitinib for the previous 6 months. If applicable, patients must have been on allergen-specific immunotherapy (ASIT) for at least the 12 preceding months. Oral isoxazoline or spinosad at manufacturer-recommended intervals was required for ≥ 6 months before enrolment. All topical therapies (bathing, medicated spray, mousse or pledgette application, otic rinses) were to be used at the same frequency as they had been for ≥ 6 months before enrolment. Exclusion criteria included concurrent endocrinopathy, neoplasia, treatment with systemic or topical glucocorticoids, ciclosporin or lokivetmab within 6 months and systemic antibiotics for treatment of the skin within 4 weeks of enrolment. Metronidazole was permissible if indicated, given SF68's reported resistance to this drug (Fenimore *et al.* 2017). Once enrolled, no changes to diet, supplements or other systemic medications (including antimicrobials, ASIT, ectoparasiticides) were allowed for the 12-week duration of study participation.

Both *E. faecium* SF68 and placebo were provided by the manufacturer as identical-appearing granules contained in identical air tight screw-top jars with one of two identifying treatment group numbers, corresponding to either SF68 or placebo. Investigators, clients and clinic staff were blinded throughout the study. On the day of enrolment (T0), patients were randomised to either SF68 treatment or placebo group using an online randomiser program (<http://jklp.org/html/choose.html>) by a designated hospital employee. Current safety and efficacy studies of SF68 for gastrointestinal benefit support a dose of 1×10^8 CFU/g per dose (1 g) once daily by mouth (Bybee *et al.* 2011, Lucena *et al.*, 2018). A dose of 1×10^8 CFU/g was selected for use in this study and has been shown to be safe according to manufacturer recommendations (Holzapfel *et al.* 2018). The decision was made by both investigators and manufacturer to divide the dose into twice daily doses to facilitate palatability and ingestion, without compromising the total daily dose of 2×10^8 CFU/g. Clients were instructed to administer 1 level scoop of provided granules onto the patient's food twice daily for the 12-week study period. Granules contained either 1×10^8 CFU/g of SF68 or placebo, which comprised only the hydrolysed palatability-enhancer used in the commercial product. All product was stored at 20 to 22°C for the duration of the study according to manufacturer guidelines.

CADESI-4 scores were assessed by the investigators (CY, WR) at all visits: enrolment (T0), days 56 (T1), 70 (T2) and 84 (T3). Clients documented PVAS scores, as previously validated (Hill *et al.* 2007, Rybníček *et al.* 2008), at all in-clinic visits. Skin and otic cytology were evaluated at visits if applicable based on increased PVAS and CADESI-4 scores. If diagnosed, pyoderma, *Malassezia* dermatitis, paronychia and otitis externa were treated with topical antiseptic therapies as previously defined (Morris 1999, Ginel *et al.* 2002, Bensignor 2006, Miller *et al.* 2013, Udenberg *et al.* 2014, Lo & Rosenkrantz 2016).

Clients were directed to document PVAS scores at home three times weekly for the initial 6 weeks, then daily for the remainder of the study (additional 6 weeks). To maintain consistency, the same individual was encouraged to perform all PVAS assessments. A phone call was made to the client on day 42 to evaluate for any changes in PVAS or other concerning signs since initiation of supplementation.

A disease “flare” was defined as an at-home PVAS score measuring twice that of the baseline value and/or increased investigator-reported CADESI-4 scores from baseline. If a flare was noted by the client between study visits, the client was instructed to notify investigators, increase the application of topical therapies (bathing, medicated spray, mousse or pledgette application) and return for in-clinic evaluation. When client-recorded PVAS and investigator-recorded CADESI-4 score were verified to have returned to baseline, the frequency of application was also reduced to that of baseline.

Patients qualified for oclacitinib dose reduction at T1 or T2 if the in-clinic reported PVAS and CADESI-4 scores were at or below baseline. The dose was reduced by whole or half-tablet increments to reach approximately a 25% dose reduction that was maintained if PVAS and CADESI-4 remained at or below baseline. If patients experienced a flare following the dose reduction, clients were instructed to immediately return to the baseline oclacitinib dose. If this alone was insufficient to resolve the flare, the dog was to return for recheck examination. Upon completion of the final enrolled patient, remaining granules from both groups were cultured to verify presence and viability of SF68.

The study protocol was reviewed and approved by research committees at both Nestlé Purina PetCare and the hospitals where enrolment occurred. All clients were informed of potential risks and benefits of participation before screening procedures were performed, and informed written consent was obtained from each client before study enrolment. Screening and enrolment took place at three hospitals in southern California between September 2017 and October 2018.

Statistical analyses were performed using SAS V 9.3. A linear mixed effects model with fixed factors of group and visit, and group-by-visit interaction effect with random intercept for each dog, was used to compare investigator-reported CADESI-4 values, client-reported PVAS scores and oclacitinib dose between SF68 and placebo groups for each visit. Comparisons of each visit to baseline were corrected for using Dunnett's test. A linear mixed effects model with fixed factors of treatment group, continuous covariate of study day, and group-by-study-day interaction effect with random intercept and slope for each dog was used to compare client-reported PVAS between groups. The proportion of dogs that tolerated oclacitinib dose reduction out of all that qualified, as well as the proportion of dogs that qualified for dose reduction out of all dogs enrolled were compared between groups using Fisher's exact tests. Statistical significance was defined as $P < 0.05$ in all analyses.

RESULTS

Results, including breed, age, body weights, oclacitinib dose and concomitant medication information for all enrolled dogs, are summarised in Tables 1–3. Twenty-one dogs were enrolled within the study time frame of 13 months; 11 into the SF68 group and 10 into the placebo group. Nineteen dogs completed the study, with one dog exiting on day 56 for owner-reported reasons irrelevant to study participation (SF68 group), and another dog (also

SF68 group) being lost to follow up on day 76 after moving to a new city. Reported comorbidities diagnosed before enrolment included inflammatory bowel syndrome (IBS) in two patients ($n=1$ SF68, $n=1$ placebo), urinary incontinence ($n=1$, SF68), myxomatous mitral valve disease ($n=1$, placebo) and hypertension ($n=1$, SF68). The dogs with IBS were both on metronidazole. No adverse events were reported for any patient enrolled as a result of participation in the study.

Five of 11 dogs in the SF68 group qualified for oclacitinib dose reduction. Three of these five dogs remained controlled at this lower dose. Eight of 10 dogs in the placebo group qualified for oclacitinib dose reduction, and seven of these dogs also maintained control on the lower dose. The number of patients that qualified for dose reduction was not statistically different between groups ($P=0.064$). Within the placebo group, the oclacitinib dose was significantly lower than baseline at T2, T3. The oclacitinib dose was not significantly different between SF68 and placebo groups at any visit, including enrolment at T0 (Figs 1 and 2).

Flares and clinical lesions were noted, and subsequent cytology was performed (Tables 1 and 2). Seven dogs (four in SF68, three in placebo) flared before dose reduction, with two (one each in SF68 and placebo) qualifying for dose reduction after increasing topical therapies; these two remained controlled at the reduced dose. Five were never eligible for dose reduction despite topical therapy adjustments.

Two (one each in SF68 and placebo) of three dogs flared as a direct result of oclacitinib dose reduction at T1. The remaining dog (SF68) experienced only a mild increase in PVAS by less than half of baseline following dose reduction, and therefore was not considered a true “flare”. All dogs regained control of PVAS and CADESI-4 scores shortly after return to baseline oclacitinib dose.

CADESI-4 scores were significantly lower in the placebo group than in the SF68 group at T1 ($P=0.010$). Within the placebo group, CADESI-4 scores were significantly decreased from baseline at T3 (Table 2). However, at study completion, there was no significant difference in CADESI-4 scores between groups (Table 4). There was no significant change in PVAS from baseline in either group at any time point (Table 3).

Viability of SF68 granules at both beginning and end of clinical study was verified by growth of *E. faecium* in pure culture. Culture of placebo granules yielded no growth of bacteria.

DISCUSSION

The findings of this pilot study suggest that a dose of 1×10^8 CFU/g *E. faecium* SF68 orally q12h is ineffective at reducing oclacitinib dosage while maintaining or reducing CADESI-4 and PVAS scores in dogs with environmental AD. Both groups experienced an overall reduction, albeit not statistically significant, in PVAS and CADESI scores, as compared with baseline (Table 3).

Several possibilities could account for the inability of SF68 to allow for oclacitinib dose reduction in this study. Firstly, the small sample size could preclude detection of any but very large effects. Secondly, no optimum dose has been determined for

Table 1. Clinical features of enrolled patients in SF68 group

SF68 group			Oclacitinib doses by day (mg/kg/day)									
Patient No.	Age	Breed	Sex	BW (kg)	T0	T1	T2	T3	Systemic medications	Topicals	Flare	Cytology
1	8 years 5 months	Golden retriever	CM	39.3	0.41	0.32*	0.32*	0.32*	ASIT (41), diphenhydramine	Hypoallergenic shampoo		
2	4 years 4 months	Manchester terrier	SF	8.2	0.44	0.45	0.46	0.46		Pramoxine [†] shampoo, chlorhexidine [†] pledgettes	d 63	0 to 4 cocci/OIF in left axilla
3	9 years 9 months	Golden retriever	CM	53.6	0.3	0.23*	0.23*	0.23*		Pramoxine [†] shampoo		
4	10 years 5 months	Maltese mix	CM	8.7	0.41	0.44	0.33*	0.33*	Metronidazole	Phytophingosine [‡] shampoo, chlorhexidine/-azole [§]	d 56	2 to 4 cocci and Malassezia/OIF in claw folds
5	11 years 4 months	Wheaten terrier	SF	15	0.27	n/a	n/a	n/a	SLIT (37)	Chlorhexidine/miconazole shampoo		
6	7 years 10 months	Pitbull terrier mix	SF	33.4	0.48	0.49	0.38*	0.49	ASIT (23), EFA	Chlorhexidine ^{††} shampoo and spray	d 75	2 to 5 cocci/OIF on groin, 0-2 cocci/OIF in left ear
7	3 years 7 months	English bulldog	CM	26.7	0.6	0.45*	0.58	0.56	ASIT (19), hydroxyzine	Chlorhexidine/-azole [§] shampoo and pledgettes		
8	4 years 11 months	Doberman Pinscher	CM	43.6	0.55	0.53	0.53	0.53		Chlorhexidine/-azole [‡] shampoo	d 45	Corneocytes
9	4 years 10 months	Greyhound	CM	41.8	0.38	0.39	0.37	0.38	SLIT (29)	Hypoallergenic shampoo		
10	3 years 3 months	French bulldog	CM	15.5	0.51	0.54	0.54	0.54	Diphenhydramine	Phytophingosine [‡] shampoo, chlorhexidine/-azole pledgettes [§]	d 47	Corneocytes
11	6 years 5 months	Shih-Tzu mix	CM	12.8	0.42	0.42	0.42	n/a		Hypoallergenic shampoo		
Average	7.025 years			24.5	0.43	0.43	0.42	0.43	ASIT/SLIT: 29.8 (5/10 patients)			
OIF Oil immersion field, 1000x magnification, ASIT Allergen-specific immunotherapy (injection), SLIT Sublingual immunotherapy												
*Oclacitinib dose reduced												
†VetBiotech Innovative Solutions BioCalm												
‡VetBiotech Innovative Solutions BioHex												
§Douxo Chlorhexidine PS												
Douxo Calm Shampoo												
Bayer Malaseb Shampoo												
††Dechra TrizChLOR4												
‡‡Virbac Sebazole												

OIF Oil immersion field, 1000x magnification. ASIT Allergen-specific immunotherapy (injection), SLIT Sublingual immunotherapy

Duration of therapy denoted in months

*Oclacitinib dose reduced

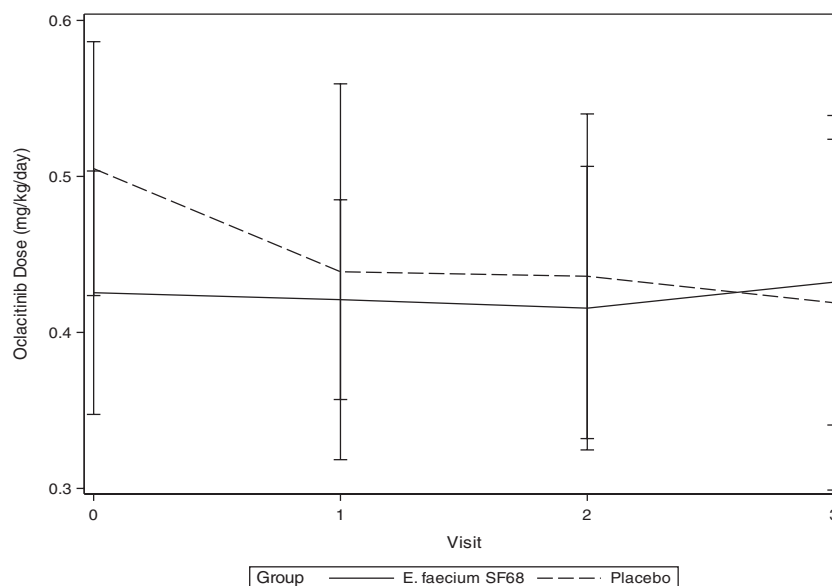
[†]VetBiotech Innovative Solutions BioCalm^{††}VetBiotech Innovative Solutions Biohex[‡]Douxo Chlorhexidine PS[§]Douxo Calm Shampoo^{||}Bayer Malaseb Shampoo ^{‡‡}Bayer Preva Medicated Wipes^{†††}Dechra TrizCHLOR4^{‡‡‡}Virbac Sebazole

Table 2. Clinical features of enrolled patients in placebo group												
Placebo group			Oclacitinib doses by day (mg/kg/day)									
Patient No.	Age	Breed	Sex	BW (kg)	T0	T1	T2	T3	Systemic Medications	Topicals	Flare Day	Cytology
1	3 years 5 months	Mastiff	SF	59.1	0.41	0.3*	0.3*	0.3*	ASIT (30), EFA, hydroxyzine	Phytosphingosine shampoo*, 25 ug/mL nisin wipes**		
2	8 years 7 months	Golden retriever	CM	42.3	0.38	0.3*	0.3*	0.15*		Hypoallergenic shampoo		
3	6 years 4 months	German shepherd dog	SF	32.3	0.5	0.5	0.5	0.5		Hypoallergenic shampoo	d 70	Corneocytes
4	6 years 11 months	Golden retriever	CM	40.6	0.59	0.47*	0.47*	0.49*	ASIT (70)	Hypoallergenic shampoo		
5	8 years 11 months	Cockapoo	SF	7.8	0.46	0.46	0.47	0.47	Metronidazole, cetirizine, phenylpropanolamine	Salicylic acid/sulfur/-azole** shampoo	d 71	10 to 20 cocci/OIF in claw folds
6	11 years 6 months	Havanese	CM	7.7	0.7	0.53*	0.53*	0.49*	ASIT (54), EFA, pimobendan	Hypoallergenic shampoo		
7	2 years 8 months	Labrador retriever	SF	30	0.53	0.41*	0.41*	0.41*	ASIT (12)	Hypoallergenic shampoo, chlorhexidine/-azole* pledgettes		
8	6 years 9 months	Golden retriever	SF	38	0.63	0.66	0.48*	0.49*	ASIT (17)	Chlorhexidine/-azole shampoo	d 56	3 to 5 yeast/OIF in left ear
9	13 years 9 months	Boston terrier	SF	7.6	0.71	0.63*	0.71	0.71	Cetirizine, metoclopramide	Chlorhexidine/-azole* shampoo and pledgettes	d 73	Corneocytes
10	6 years 7 months	Chesapeake Bay retriever	CM	48.2	0.25	0.19*	0.19*	0.19*	ASIT (13)	Chlorhexidine/-azole* shampoo		
Average	7.25 years			31.3	0.52	0.45	0.44	0.42	ASIT: 32.7 (6/10 patients)			
OIF Oil immersion field = 1000x magnification, ASIT Allergen-specific immunotherapy (injection), SLIT Sublingual immunotherapy												
Duration on treatment in months												
*Oclacitinib dose reduced												
†VetBiotek Innovative Solutions BioCalm												
‡VetBiotek Innovative Solutions Biohex¶Douxo Chlorhexidine PS												
Douxo Calm Shampoo												
Bayer Malaseb Shampoo												
**Bayer Preva Medicated Wipes††Dechra TrizChlor4												
‡‡Virbac Sebazole												

Table 3. Assessment of oclacitinib dose, CADESI-4 and PVAS at various time points between SF68 and placebo

SF68 group							
Study day	Average oclacitinib dose (mg/kg/day)	Oclacitinib P-value compared to T0	Average CADESI-4 [†]	CADESI-4 P-value compared to T0	Average PVAS [‡]	PVAS P-value compared to T0	n
T0	0.43 (0.27 to 0.6)	-	10 (2 to 16)	-	2.6 (0.2 to 3.5)	-	11
T1	0.43 (0.23 to 0.54)	0.9317	11 (1 to 18)	0.6976	2.7 (0.3 to 6.7)	1.0	11
T2	0.42 (0.23 to 0.58)	0.6222	6 (2 to 11)	0.0655	2.5 (0.4 to 5.5)	0.9856	10
T3	0.43 (0.23 to 0.56)	0.9566	5.7 (2 to 12)	0.0606	2.2 (0 to 3.5)	0.4794	9
Placebo group							
Study day	Average oclacitinib dose (mg/kg/day)	Oclacitinib P-value compared to T0	Average CADESI-4 [†]	CADESI-4 P-value compared to T0	Average PVAS [‡]	PVAS P-value compared to T0	n
T0	0.52 (0.24 to 0.7)	-	10.6 (7 to 16)	-	2.1 (0 to 3.5)	-	10
T1	0.45 (0.19 to 0.66)	0.0711	7 (3 to 14)	0.0791	1.4 (0 to 5.5)	0.5431	10
T2	0.44 (0.19 to 0.71)	0.0291*	7.1 (2 to 12)	0.1023	1.8 (0.1 to 5)	0.9019	10
T3	0.42 (0.19 to 0.71)	0.0050*	6 (1 to 20)	0.0183*	1.3 (0 to 3.5)	0.1908	10

PVAS Pruritus Visual Analog Score
 *Statistical significance was defined as P<0.05 in all analyses
 †PVAS score range 0 to 10; normal (≤1.9) (Hill *et al.* 2007, Rybníček *et al.* 2008)
 ‡CADESI-4 score range 0 to 180; mild (10), moderate (35), severe (60) (Olivry *et al.* 2014)

**FIG 1.** Oclacitinib dose (mg/kg/day) of treatment (FortiFlora, SF68) and placebo dogs at visits (T0, T1, T2, T3)

altering immune responses with probiotics. Previous veterinary studies evaluating human-directed probiotics dosed between 100 and 200 CFU/d (Marsella 2009, Marsella *et al.* 2012) and 5 mg/kg/d (CFU not reported) (Ohshima-Terada *et al.* 2015) demonstrated preventive and drug-sparing effects. Supplementing puppies with a dose of 5×10^8 CFU/day SF68 after 18 weeks revealed enhanced faecal IgA levels (Benyacoub *et al.* 2003). However, a dose greater than 100 mg/kg/d heat-inactivated *Enterococcus faecalis* FK23p reportedly produced only a small benefit (Osumi *et al.* 2019). Human studies report benefit on doses ranging from 4×10^{10} CFU/d (Kalliomäki *et al.* 2001, Notay *et al.* 2017) to 20.7 mg/kg/day (Inoue *et al.* 2014). Not only does dosing in CFU *versus* mg without reporting CFU complicate comparisons, but the frequency of dosing also varies between once and twice daily administration. The variable dosing in humans makes extrapolation

to our veterinary patients a challenge. The duration of supplementation, too, may have affected results. Previous reports of immunomodulation in dogs after 4 weeks with SF68 at labelled doses (Lappin *et al.* 2017) and meta-analyses of human AD studies found benefit in as little as 8 weeks, although they used higher doses – of between 6×10^9 and 4×10^{10} CFU/day (Inoue *et al.* 2014, Notay *et al.* 2017). In the current study, it was agreed by the investigators and manufacturers to administer a dose of 1×10^8 CFU/g SF68 twice daily for 12 weeks. Although doubled from label dose, even higher dosages, possibly given over a longer duration than reported here, may be required to alleviate clinical signs of AD.

The specific microbial strain could also have influenced our results. Previous veterinary studies were optimistic for improvement of AD with *Lactobacillus* species intended for humans (Marsella 2009, Inoue *et al.* 2014, Kim *et al.* 2015, Ohshima-Terada

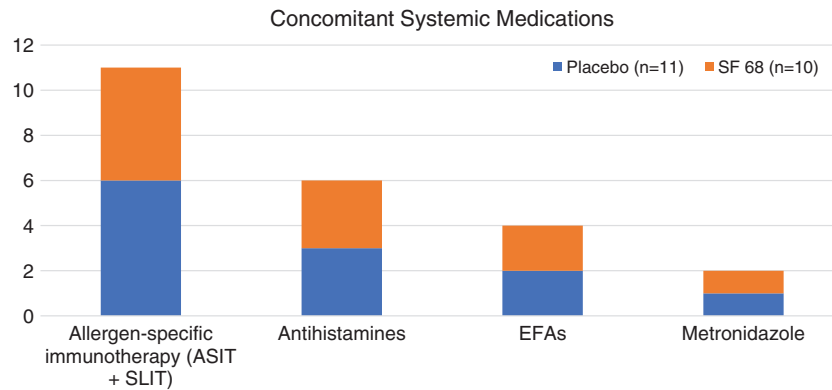


FIG 2. Concomitant medications for 21 dogs with AD enrolled and receiving either SF68 or placebo

Table 4. Comparison of CADESI-4 scores between SF68 and placebo

Study day	P-value
T0	0.8234
T1	0.0101*
T2	0.6524
T3	0.9492

*Statistical significance was defined as $P < 0.05$ in all analyses

et al. 2015, Notay *et al.* 2017). Support exists for altered immune parameters in puppies supplemented with SF68 for more than 18 weeks (Benyacoub *et al.* 2003) as well as *E. faecium* FC-K in laboratory models of Th2-polarised disease (Rho *et al.* 2017), but fewer studies report use of enterococcal species in human and canine adults with AD. This study sought to explore use of SF68, a single-strain veterinary-approved supplement. Controversy remains in human AD patients regarding efficacy using single *versus* multiple strain supplementation (Mccusker & Sidbury 2016, Notay *et al.* 2017). It is possible that multiple strain supplements could have different results in canine AD patients.

The effect of age when starting probiotic supplementation has not been studied. Previous canine studies initiated supplementation with *Lactobacillus* at perinatal, juvenile stages, and adulthood and all suggest preventive and drug-sparing effects (Marsella *et al.* 2012, Ohshima-Terada *et al.* 2015). It has yet to be elucidated if and how best to use probiotics in the management of AD in adult dogs, given the paucity of prospective blinded studies.

The varied frequency and types of systemic and topical therapy employed could have influenced responses in this population of dogs. Topical therapies can be critical to successful management of dogs with AD, employed to decrease allergen load, facilitate epidermal barrier function by hydrating the skin and also decreasing colonisation by pathogenic microbes. It is unknown if orally administered probiotics can influence cutaneous microflora or if different topical therapies may have impacted the cutaneous microflora and subsequent interactions with intestinal microflora immune system effects. The cutaneous and gastrointestinal microbiomes are complex and future studies evaluating their effects on canine health and disease are anticipated for better understanding of the pathomechanisms involved and potential targeted therapeutic intervention.

Finally, client compliance with return visits, PVAS reporting and dosing may have impacted our results. However, incentives (oclacitinib, examinations and diagnostics) were provided to clients to assure return visits, supplement jars were weighed at each visit to verify appropriate dosing, and owner-recorded PVAS scores were transcribed by the investigator.

The combined effect of probiotics and ASIT on cytokine alteration in dogs has not yet been explored. Of 19 dogs that completed the study, nine were concurrently on ASIT, with six placebo and one SF68 tolerating the oclacitinib dose reduction. Statistical analysis was not performed on this inadvertent finding and conclusions within this study cannot be made, but warrant further investigation.

Despite the inability of SF68 to facilitate a significant oclacitinib dose reduction in this study, several dogs flared before dosage reduction, supporting the cyclic nature of AD. Two dogs that flared could be controlled back to their baseline PVAS with a 1-week increase in topical therapies. This stresses the importance of periodic re-evaluation by the clinician not only as pertaining to PVAS and CADESI-4 scores, but also topical and systemic components of multimodal treatment to improve clinical signs.

Further larger-scale studies evaluating the specific probiotic strain, dosage, life-stage, and treatment duration are indicated to elucidate their role in management practices of canine AD.

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Conflict of interest

One of the authors (CG) has lectured for and received research support from Nestlé Purina PetCare.

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