UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

TACKLING CANINE ATOPIC DERMATITIS: A PILOT CLINICAL TRIAL ASSESSING THE EFFICACY OF AN INNOVATIVE TOPICAL SOLUTION

ANTÓNIO SILVA CARVALHO CALDEIRA MENDES

ORIENTADOR(A):

Doutora Ana Mafalda Gonçalves Xavier Félix Lourenço

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"When you arise in the morning think of what a privilege it is to be alive, to think, to enjoy, to love"

Marco Aurélio, Meditações

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The scientific abstract of the study presented in this dissertation was accepted for oral communication at the 20th Montenegro International Veterinary Congress, which will take place in Porto, Portugal, on October 11th and 12th, 2024 (Annexe 1).

The study presented in this dissertation was funded by the European Society of Veterinary Dermatology (ESVD) (Maastricht, The Netherlands) and by the Centre for Interdisciplinary Research in Animal Health (CIISA) (Lisbon, Portugal). Additionally, the present study is integrated within an ongoing PhD project conducted by Beatriz Amaral Pinto Fernandes (FCT fellowship 2021.05985.BD), a PhD student affiliated with CIISA/FMV-Ulisboa, in partnership with ULisboas's Faculty of Pharmacy.

Título – Abordagem à dermatite atópica canina: um estudo clínico piloto para averiguar a eficácia de uma formulação tópica inovadora

Resumo

A dermatite atópica canina (cAD) é uma afeção cutânea crónica com etiologia multifatorial. Na dermatite atópica humana (hAD), o tratamento foca-se na reparação da barreira cutânea com emolientes, enquanto na cAD o tratamento tende a desconsiderar estes tratamentos. Recentemente, foram desenvolvidas novas formulações – os "emolientes *plus*". Estes incluem compostos bioativos não medicamentosos que demostraram aumentar o intervalo entre crises em seres humanos. Em cães, a evidência acerca de emolientes *plus* é escassa. Este estudo piloto teve como objetivo avaliar a eficácia deste novo emoliente *plus* para aplicação tópica, em spray, como um coadjuvante no tratamento da cAD. Vinte e um cães diagnosticados com cAD crónica não sazonal foram recrutados num serviço de dermatologia veterinária. Durante 30 dias, foi aplicado diariamente a todos os cães o emoliente tópico em regiões frequentemente afetadas pela cAD. Foram realizadas avaliações clínicas no dia de recrutamento (D0) e na consulta de reavaliação (D30). Estas utilizaram ferramentas validadas como a extensão de lesões cutâneas (CADESI-04) e a escala visual de prurido (PVAS10). Adicionalmente, foram avaliados parâmetros de integridade da barreira cutânea no D0 e D30, incluindo a perda de água transepidérmica (TEWL) e o pH cutâneo. No D30, os detentores foram questionados acerca da eficácia do tratamento (OGATE) e das propriedades cosméticas do produto. Os resultados obtidos demonstraram uma redução estatisticamente significativa do prurido e da gravidade das lesões cutâneas. O grau de prurido diminuiu de 4,25 ± 1,85 no D0 para 3,38 ± 1,75 no D30 (p = 0,02), e a extensão de lesões cutâneas diminui de 24,6 ± 18,1 para 13,4 ± 7,4 (p = 0,002). No pavilhão auricular, a TEWL e o pH diminuíram significativamente após 30 dias (de 18,63 ± 17,33 para 9,56 ± 10,75 (p = 0,049) e de 6,07 ± 0,97 para 5,41 ± 0,71 (p = 0,01), respetivamente). Na virilha, os valores da TEWL aumentaram de forma não significativa de 13,62 ± 14,59 para 15,32 ± 15,17 (p = 0,75) e o pH diminuiu de forma não significativa de 6,33 ± 1,24 para 6,12 ± 1,13 (p = 0,54). No D30, 90,48% dos detentores classificaram a resposta ao tratamento como "boa" ou "excelente", e o produto foi avaliado positivamente em todas as propriedades cosméticas.

Em conclusão, o novo spray emoliente *plus* demonstrou ser eficaz como adjuvante no tratamento para a cAD, melhorando o quadro clínico dos animais. No entanto, estudos de maior dimensão, controlados e aleatorizados devem confirmar estes resultados e aprofundar o impacto deste produto na reparação da barreira cutânea na cAD.

Palavras-chave: dermatite atópica canina; terapia tópica; emoliente *plus*; barreira cutânea; avaliação cosmética do detentores.

Title - Tackling canine atopic dermatitis: a pilot clinical trial assessing the efficacy of an innovative topical solution

Abstract

Canine atopic dermatitis (cAD) is a chronic inflammatory skin condition with a multifactorial etiology. In human atopic dermatitis (hAD), treatment focuses on repairing the skin barrier with emollients, while in cAD treatments tend to overlook skin barrier care. New emollient formulations have recently been developed - the "emollients plus". These formulations include bioactive, non-medicated substances with treatment-sparing effects and prolonged free-flare periods in humans. In dogs, evidence for their use is scarce.

This pilot study aimed to evaluate the efficacy of a novel biphasic topical emollient plus spray, developed by our research team, as an co-adjuvant therapy for cAD.

Twenty-one client-owned dogs diagnosed with non-seasonal chronic cAD were recruited from a veterinary dermatology service. The dogs underwent a 30-day treatment period involving daily application of the topical emollient to body regions commonly affected cAD. Clinical assessments were performed at baseline (D0) and after the treatment period (D30) using validated tools like the Canine Atopic Dermatitis Extent and Severity Index (CADESI-04) and the Pruritus Visual Analog Scale (PVAS10). Skin barrier function and integrity measurements were collected on D0 and D30, including Transepidermal Water Loss (TEWL) and skin pH. On D30, the owner's global assessment of treatment efficacy (OGATE) and cosmetic evaluation were collected.

Results indicated a significant reduction in both pruritus and skin lesion severity. PVAS10 scores decreased from 4.25 ± 1.85 at baseline to 3.38 ± 1.75 at D30 ($p = 0.02$), and CADESI-04 scores decreased from 24.6 ± 18.1 to 13.4 ± 7.4 ($p = 0.002$). In the pinna, TEWL and pH significantly decreased after 30 days (from 18.63 ± 17.33 to 9.56 ± 10.75 ($p = 0.049$) and from 6.07 ± 0.97 to 5.41 ± 0.71 ($p = 0.01$) respectively). Contrarily, in the inguinal region after 30 days, TEWL values increased non-significantly from 13.62 ± 14.59 to 15.32 ± 15.17 ($p = 0.75$) and pH decreased non-significantly from 6.33 ± 1.24 to 6.12 ± 1.13 ($p = 0.54$). Notably, 90.48% of owners rated the overall treatment response as "good" or "excellent," and the product was positively evaluated across all cosmetic properties.

In conclusion, the novel topical emollient spray showed potential as an effective adjunct therapy for cAD, significantly improving clinical outcomes and yielding high owner satisfaction. However, larger randomized controlled trials must confirm these findings and further explore their impact on skin barrier repair and cAD management.

Keywords: canine atopic dermatitis; topical therapy; emollient plus; skin barrier; owner cosmetic evaluation.

Título – Abordagem à dermatite atópica canina: um estudo clínico piloto para averiguar a eficácia de uma formulação tópica inovadora

Resumo Alargado

A dermatite atópica canina (cAD) é uma afeção crónica de pele que afeta até 30% dos cães. Esta afeção caracteriza-se por prurido intenso, inflamação crónica e disfunção da barreira cutânea, que reduzem significativamente a qualidade de vida dos animais diagnosticados. A etiologia da cAD é multifatorial, com origem em predisposições genéticas, fatores ambientais, disbiose microbiana e disfunção da barreira cutânea. Esta disfunção tem sido identificada como um fator central na fisiopatologia da afeção. A disfunção da barreira cutânea facilita a penetração de alergénios e a desregulação da microbiota, exacerbando a inflamação e o prurido. Nos últimos anos, a reparação da barreira cutânea tornou-se uma das áreas de interesse no maneio terapêutico da cAD. Na dermatite atópica humana (hAD), o tratamento crónico foca-se na reparação da barreira cutânea com emolientes, enquanto na cAD o tratamento tende a desconsiderar esta abordagem. Recentemente, foram desenvolvidas novas formulações tópicas – os "emolientes *plus*". Estes incluem compostos bioativos não medicamentosos que permitem aumentar o intervalo entre crises e que reduzem a necessidade de tratamentos agudos em humanos. Em cães, a evidência acerca de emolientes *plus* é escassa.

Este estudo clínico piloto teve como objetivo geral avaliar a eficácia e segurança de uma nova solução tópica bifásica, desenvolvida pela nossa equipa de investigação, como um tratamento adjuvante para cães com cAD crónica e não sazonal. O objetivo principal deste estudo foi investigar a eficácia da solução tópica bifásica no alívio do prurido e na redução das lesões cutâneas em cães com cAD. Os objetivos secundários foram avaliar as propriedades cosméticas do produto.

Foram recrutados 21 cães diagnosticados com cAD a partir do serviço de referência de dermatologia veterinária do Hospital Escolar Veterinário (HEV) da Faculdade de Medicina Veterinária da Universidade de Lisboa (FMV-Ulisboa). Os critérios de inclusão para o estudo exigiam que os cães tivessem sido diagnosticados com cAD, apresentassem um quadro clínico estável sem infeções secundárias ativas, lhes fosse aplicado um protocolo eficaz de prevenção de ectoparasitas e não tivessem sofrido alterações do seu protocolo terapêutico nas últimas 8 semanas.

A solução emoliente tópica foi aplicada uma vez por dia, durante um período de 30 dias, em áreas tipicamente afetadas pela cAD, tais como a região dos pavilhões auriculares (pinna) e a região inguinal. Foram realizadas avaliações clínicas em dois momentos: no início do estudo (D0) e após 30 dias de tratamento (D30) com recurso a ferramentas validadas. As avaliações clínicas incluíram a gravidade das lesões

cutâneas (CADESI-04), realizada pelo médico veterinário, e o grau de prurido, que foi avaliado através da escala visual de prurido (PVAS10) e fornecido pelos detentores.

Adicionalmente, foram medidos parâmetros fisiológicos indicadores da função e integridade da barreira cutânea, nomeadamente a perda transepidérmica de água (TEWL) e o pH cutâneo.

Para avaliar a perceção dos detentores acerca da eficácia do tratamento, foi utilizado um questionário de eficácia global não validado (OGATE). Por fim, de modo a avaliar as propriedades cosméticas do produto os detentores responderam a um questionário original que avaliava várias características cosméticas do emoliente tópico e da sua utilização.

Os resultados clínicos demonstraram uma melhoria significativa tanto no prurido como nas lesões cutâneas ao longo dos 30 dias de tratamento. A escala PVAS10 mostrou uma redução significativa do prurido, com os valores inicias de 4,25 ± 1,85 a reduzirem para 3,38 ± 1,75 no D30 (p = 0,02). Da mesma forma, o CADESI-04 revelou uma diminuição de 47,7% na gravidade das lesões cutâneas, com uma descida de 24,6 ± 18,1 no D0 para 13,4 ± 7,4 no D30 (p = 0.002). Estes resultados indicam que a solução tópica teve um efeito clinicamente relevante na melhoria do quadro clínico dos cães recrutados.

Em relação à função e integridade da barreira cutânea, os resultados da TEWL e do pH cutâneo foram contraditórios nos dois locais intervencionados. No pavilhão auricular, a TEWL e o pH diminuíram significativamente após 30 dias (de 18,63 ± 17,33 para 9,56 ± 10,75 (p = 0,049) e de 6,07 ± 0,97 para 5,41 ± 0,71 (p = 0,01), respetivamente). Contrariamente, na virilha, os valores da TEWL aumentaram de forma não significativa de 13,62 ± 14,59 para 15,32 ± 15,17 (p = 0,75) e o pH diminuiu de forma não significativa de 6,33 ± 1,24 para 6,12 ± 1,13 (p = 0,54).

A perceção dos detentores em relação à eficácia do tratamento foi, de forma geral, extremamente positiva. Aproximadamente 90% dos detentores classificaram a resposta ao tratamento como "boa" ou "excelente", o que demonstra um elevado grau de satisfação com o tratamento. Em relação às propriedades cosméticas do emoliente tópico, estas também foram avaliadas de forma extremamente positiva. De 0 a 10, os detentores avaliaram as afirmações do questionário cosmético da seguinte forma: "O produto tem boa aparência" – 9,38 ± 1,36; "O produto tem um cheiro agradável" – 9,29 ± 1,28: "A aplicação do produto foi fácil e simples" – 9,00 ± 1,46; "A aplicação do produto foi agradável para mim" – 8,67 ± 1,89; "A aplicação do produto foi tolerada pelo meu cão" – 8,95 ± 1,46; "Não me importaria de incluir este produto no tratamento do meu cão" – 8,95 ± 1,46; "Eu recomendaria este produtor a outros detentores de cães atópicos" – 9,03 ± 1,33"; "Depois da aplicação a pele do meu cão estava hidratada" –

8,62 ± 1,21; “Depois da aplicação a pele do meu cão estava oleosa” – 5,05 ± 3,06; “Depois da aplicação a pele do meu cão estava avermelhada” – 3,05 ± 2,68; “Depois da aplicação a pele do meu cão estava descamativa” – 1,38 ± 1,21.

Este estudo piloto demonstrou que esta nova solução tópica bifásica, com uma formulação original, proprietária e não divulgada, tem um potencial promissor como coadjuvante do tratamento da cAD. As reduções significativas no prurido e nas lesões cutâneas sugerem que a preparação pode desempenhar um papel importante no controlo clínico da cAD, melhorando simultaneamente a qualidade de vida dos cães afetados.

Embora as melhorias observadas nos indicadores de função da barreira cutânea, como a TEWL e o pH, não tenham sido estatisticamente significativas em todos os locais testados, os resultados indicam uma tendência positiva que poderá ser explorada em estudos futuros.

Outro ponto importante a destacar é a elevada aceitação do produto pelos detentores, quer em termos da perceção de eficácia, quer em termos de propriedades cosméticas. A adesão ao tratamento é um fator crucial no maneio de doenças crónicas como a cAD, e o feedback positivo dos detentores sugere que esta solução pode ser uma opção viável a longo prazo, por ser agradável para os detentores, fácil de aplicar e bem tolerada pelos cães.

Em resumo, o emoliente *plus* especialmente desenvolvido para este projeto demonstrou ser cosmeticamente agradável, seguro e eficaz como coadjuvante no tratamento da cAD, proporcionando melhorias significativas no quadro clínico e na barreira cutânea dos animais recrutados. No entanto, são necessários estudos adicionais, preferencialmente aleatorizados, controlados e cegos, para confirmar estes resultados.

Palavras-chave: dermatite atópica canina; terapia tópica; emoliente *plus*; barreira cutânea; avaliação cosmética do tutor.

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AD – atopic dermatitis

AIT – Allergen immunotherapy

cAD – Canine atopic dermatitis

CADESI-04 – Canine Atopic Dermatitis Extent and Severity Index

CC – Closed-chambered

CEBEA – Comissão de Ética para Investigação e o Ensino

CIISA – Centre for Interdisciplinary Research in Animal Health

COSCAD'18 – Core Outcome Set for Canine Atopic Dermatitis

D0 – First appointment

D30 – Reevaluation

ESVD – European Society of Veterinary Dermatology

FCT – Fundação para a Ciência e a Tecnologia

FDA – Food and Drugs Administration

FMV-ULisboa – Faculty of Veterinary Medicine of the University of Lisbon

$g/m^2/h$ - evaporation rate units

hAD – Human atopic dermatitis

HD – Healthy dog

HEV – Hospital Escolar Veterinário

ICADA - International Committee for Allergic Diseases in Animals

OC – Open-chambered

OGATE - Owner Global Assessment of Treatment Efficacy

OTC – Over-the-counter

PVAS10 – 10cm Pruritus Visual Analog Scale

SC – *stratum corneum*

TEWL – Transepidermal water loss

(*) – Undisclosed reference meant to preserve intellectual property and competitive advantage

1. Traineeship Report

The first voluntary traineeship was conducted over a two-week period on Príncipe Island, part of the island nation of São Tomé and Príncipe, located in the Gulf of Guinea. During this period, work was carried out with Veterinários Sem Fronteiras on an awareness and neutering campaign targeting the local small animal population. Awareness efforts were focused on spreading information through local radio stations and engaging with various villages and schools to educate the community. The neutering campaign was done over 12-hour workdays from Monday to Friday and covered eight small towns and the capital city, Santo António. Despite the challenging conditions, this experience provided valuable insights into working in resource-limited settings, enhancing practical veterinary skills, and contributing significantly to animal welfare on the island.

From September 2023 to March 2024, voluntary attendance was pursued in dermatology consultations at the Veterinary Teaching Hospital (HEV) in order to ensure the expected number of animals were recruited for this master's project. The curricular traineeship for the Integrated Master's in Veterinary Medicine was also undertaken at HEV of the Faculty of Veterinary Medicine, University of Lisbon (FMV-ULisboa), from March 11th to August 31st, 2024, spanning 24 weeks. This traineeship encompassed rotating weekday shifts ranging from 8 to 12 hours per day, including two monthly night shifts, totaling approximately 816 hours. The distribution of activities included: 8 weeks in dermatology (280 hours), 4 weeks in general medicine (140 hours), 2 weeks in surgery (70 hours), 2 weeks in internal medicine (70 hours), 2 weeks in oncology (70 hours), 2 weeks in the ultrasound unit (70 hours), 1 week in ophthalmology (35 hours), 1 week in radiology (35 hours), 1 week in exotic animal medicine (35 hours), and 1 week in neurology and cardiology services (35 hours). Under the supervision of the respective service veterinarians, participation in a wide range of clinical activities and medical procedures was possible.

The final voluntary traineeship was conducted from September 2nd to 27th at a dermatology reference service in Hospital Veterinário do Porto under the mentorship of Dr. Diana Ferreira, an European College of Veterinary Dermatology specialist. This traineeship, structured as 8-hour workdays, provided the opportunity to engage with advanced dermatological practices. This experience provided the opportunity to observe and learn cutting-edge, evidence-based procedures and treatments.

2. Literature Review

2.1. Canine Atopic Dermatitis

2.1.1. Etiology and prevalence

Traditionally, canine atopic dermatitis (cAD) used to be 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" (Halliwell 2006, p.1). However, a new definition for cAD has been proposed, describing it as a "hereditary, typically pruritic and predominantly T-cell driven inflammatory skin disease involving the interplay between skin barrier abnormalities, allergen sensitization, and microbial dysbiosis" (Eisenschenk et al. 2024, p.1).

French bouledogs, boxers, Labrador retrievers, pugs, and West Highland white terriers are dog breeds considered predisposed to cAD worldwide (Mazrier et al. 2016). However, cAD's prevalence varies across dog breeds, with breed predispositions also differing depending on geographical location (Jaeger et al. 2010).

Affecting up to 20-30% of dogs, cAD is a prevalent, chronic, and distressing skin condition (Marsella and De Benedetto 2017). cAD's pathogenesis is not yet fully understood, but it is thought to involve complex interactions between genetic and environmental factors, resulting in epidermal barrier dysfunction, immune dysregulation, and cutaneous dysbiosis (Outerbridge and Jordan 2021). Given the abovementioned context, some authors consider cAD to most likely be a clinical syndrome rather than a single disease (Marsella 2012; Santoro et al. 2024).

Since one of the main objectives of this project is to assess skin barrier function and integrity in cAD patients, particular focus will be given to skin barrier dysfunction as a pillar in the physiopathology of the disease.

2.1.2. Clinical signs and diagnosis

Clinical signs of cAD typically develop between six months and three years of age (Griffin and DeBoer 2001). The dominant clinical sign of cAD is moderate to severe pruritus, which, in most cases, precedes other clinical signs (Favrot et al. 2010). Common manifestations of pruritus encompass licking, excessive grooming, scratching, chewing, scooting, and head shaking (Hensel et al. 2015; Outerbridge and Jordan 2021). Pruritus is often accompanied by dermatological lesions, such as erythema, self-induced alopecia, excoriations, hyperpigmentation, and lichenification (Griffin and DeBoer 2001; Favrot et al. 2010; Outerbridge and Jordan 2021). Furthermore, bacterial and yeast infections have been reported as common complications affecting dogs with cAD (Griffin and DeBoer 2001; Outerbridge and Jordan 2021). The most commonly affected body sites include distal limbs, face, ventrum, ears, and flexural areas (Griffin and DeBoer 2001; Favrot et al. 2010). Another frequent clinical sign found in atopic dogs is otitis externa, as up to 50% of dogs can develop this ear condition (Harvey et al. 2019). This observation aligns with reports indicating that cAD is the predominant primary cause of otitis externa in dogs (Saridomichelakis et al. 2007).

In addition to dermatological signs of cAD, non-dermatological signs, such as rhinitis (Hillier and Griffin 2001) and allergic conjunctivitis, can also be observed, with around 60% of atopic dogs suffering concomitantly from the latter (Lourenço-Martins et al. 2011). cAD can also affect the behavior and mood of patients, with undesired behaviors like mounting, chewing, restlessness, coprophagia, and reduced trainability being reported at higher rates in atopic dogs (Linek and Favrot 2010; Harvey et al. 2019).

cAD has no pathognomonic clinical signs; therefore, it is crucial to bear in mind that a conclusive diagnosis based solely on the initial owner interview and clinical examination is challenging (DeBoer and Hillier 2001). Traditionally, the clinical diagnosis involves the ruling out of other skin conditions, like external parasites and food allergy (Drechsler et al. 2024), and a thorough investigation of the historical and clinical features of the patient (Hensel et al. 2015), resourcing to tools like “Favrot’s criteria” (Favrot et al. 2010).

Following what sometimes can be an expensive diagnostic process, it is essential to convey to the owner that cAD is a chronic disease that waxes and wanes (Linek and Favrot 2010) and requires lifelong management and control (Gedon and Mueller 2018).

2.1.3. Treatment options and approaches

The dog’s clinical condition, severity and extension of skin lesions, and pruritus intensity determine the therapeutic approach (Saridomichelakis and Olivry 2016). The chosen treatment must be regularly revised and adapted to each patient (Saridomichelakis and Olivry 2016; Drechsler et al. 2024). Treatment strategies for cAD are usually divided into two different approaches: etiological, which includes allergen immunotherapy (AIT), and symptomatic, which resorts to a variety of topical and systemic medications (Olivry et al. 2003). These approaches can be used individually or simultaneously. However, it is important to note that combining various strategies has been shown to be more effective in controlling cAD (Saridomichelakis and Olivry 2016; Drechsler et al. 2024).

Olivry and Banovic introduced a two-phased treatment approach to cAD in 2019. This approach recommends considering a drug's "inflammation-targeting breadth" as the primary factor for decision-making (Olivry and Banovic 2019), rather than the duration or nature of skin lesions (acute and chronic) (Olivry et al. 2015), given that both types can be found in the same patient (Olivry and Banovic 2019).

2.1.3.1. Phase I – "Reactive Therapy"

Phase I intends to address atopic patients enduring acute flares, that usually present acute and chronic skin lesions, pruritus, and an overall inflammatory clinical presentation (Olivry and Banovic 2019). As such, it is advisable to administer quick-acting and broad-targeting drugs like oral glucocorticoids for their general and systemic actuation, addressing not only observable skin lesions but also the microscopic inflammation present in the seemingly unaffected skin of an atopic dog (Olivry and Banovic 2019). In cases where the patient exhibits mild skin lesions, using oclacitinib as a standalone treatment or in conjunction with a topical glucocorticoid to enhance its anti-inflammatory impact is a viable option (Olivry and Banovic 2019). As cutaneous inflammation diminishes and to minimize the requirement for prolonged courses of oral glucocorticoids, substituting these with medications known for fewer side effects, such as oclacitinib, is advisable (Olivry and Banovic 2019).

2.1.3.2. Phase II – "Proactive Therapy"

After achieving remission of clinical signs for several weeks, Phase II, termed "proactive therapy", aims to prevent the occurrence of new flares or, at the very least, diminish their frequency and severity (Olivry and Banovic 2019). Since complete allergen avoidance is impractical in everyday settings (Olivry et al. 2003), common strategies during this treatment phase include AIT and other pharmacological interventions, such as oclacitinib, cyclosporine, injectable biologicals (lokivetmab) or proactive application of topical glucocorticoids (Olivry and Banovic 2019). The long-term, proactive protocols for applying topical glucocorticoids have been described and proven effective and safe (Lourenço et al. 2016).

If biologicals and topicals do not adequately control flares, it is prudent to initiate an "inflammation reset" through an oral glucocorticoid cycle (Olivry and Banovic 2019).

2.1.3.3. Allergen testing and allergen immunotherapy

Once a clinical diagnosis of cAD is confirmed, allergy testing can be conducted to identify potential causative allergens (Hensel et al. 2015; Marsella and De Benedetto 2017). This information is essential for guiding AIT protocols (Marsella 2012; Olivry et al. 2015; Saridomichelakis and Olivry 2016; Marsella and De Benedetto 2017).

AIT targets the etiological cause of cAD, making it the most effective and proactive management strategy for this condition. This therapeutic approach is generally regarded as safe, and it helps alleviate clinical signs while reducing the overall need for medication (Olivry et al. 2015; Saridomichelakis and Olivry 2016).

Several types of AIT are available, including the traditional subcutaneous injections, sublingual sprays and intralymphatic injections (Drechsler et al. 2024). Recently, epicutaneous AIT for cAD has been investigated as a new noninvasive AIT modality that seems safe, effective, and allows user-friendly at-home administration, which can enhance owner compliance (Pinto et al. 2024).

For more detailed information on allergy testing and allergen-specific immunotherapy please refer to: Pinto MSN 2021, “Epicutaneous immunotherapy as a novel route of allergen administration in dogs with atopic dermatitis: a proof-of-concept study” [master dissertation], Lisboa: FMV-Universidade de Lisboa; and Pinto et al. 2024, “Challenging the norm: Epicutaneous immunotherapy for canine atopic dermatitis” Allergy: European Journal of Allergy and Clinical Immunology. 79(1):255–257. doi:10.1111/ALL.15946.

2.2. Barrier breakdown: understanding skin barrier impairment in cAD

2.2.1. Skin Fundamentals: A brief review

The skin is the dog’s largest organ, accounting for 12% of the adult dog bodyweight (Pavletic 1991; Miller et al. 2012). This organ’s primary function is to act as an anatomic barrier while being an interaction point between animals and their surroundings. Although species-specific anatomic differences occur, the skin retains its essential barrier function across most species (Affolter and Moore 1994; Miller et al. 2012).

As with other mammals, the canine skin is composed of three main layers: an outermost stratified epithelium, the epidermis; an underlying mesh-work of collagen, reticular and elastic fibers, with blood and lymphatic vessels and nerves, the dermis; and a deeper layer of looser collagen and elastic fibers rich in adipose tissue, the hypodermis (Thomsett 1986; Proksch et al. 2008; Baroni et al. 2012; Miller et al. 2012). A schematic representation of the skin and its layers can be found in Figure 1.

The dermis plays a crucial role in cellular and fluid exchange (Bal et al. 2010). This layer is essential for the skin's elasticity and tensile strength, and it is actively involved in wound healing by regulating processes such as cell growth, proliferation, differentiation, adhesion, and migration, which in turn influence the structure and function of the epidermis (Miller et al. 2012).

Beneath the dermis lies the hypodermis, typically the thickest skin layer. This layer primarily functions in thermoregulation and insulation. Due to its adipose tissue interlinked by fibrous bands, it also plays a critical role in protecting and supporting the body and acting as an energy reserve (Miller et al. 2012).

2.2.2. Epidermis: the origin of the skin barrier

While the entire skin structure contributes actively to host defense, the epidermis assumes the pivotal role in reducing water loss to the environment, acting as an inside-outside barrier, and shielding the body against diverse environmental stressors, serving as a reliable outside-inside barrier (Baroni et al. 2012; Miller et al. 2012).

The epidermis is a thin layer of stratified squamous epithelium, composed of four strata of keratinocytes in progressive stages of differentiation and, to a lesser extent, melanocytes, Langerhans cells, and Merkel cells (Nestle et al. 2009; Miller et al. 2012). The deepest, the basal layer, consists of stem cells that continuously multiply and renew the epidermis (Blanpain and Fuchs 2006). Proceeding upwards, the spinous and granular layers are made of keratinocytes united by desmosomes and tight junctions respectively. The last, more superficial layer, the stratum corneum (SC), is formed by differentiated keratinocytes that go through profound structural changes. The resulting cells, the corneocytes, are flat, lack a nucleus, and are surrounded by a lipid matrix containing mainly ceramides, cholesterol, and free fatty acids (Yoon et al. 2011; Baroni et al. 2012; Miller et al. 2012). A schematic representation of the skin and its layers can be found in Figure 1.

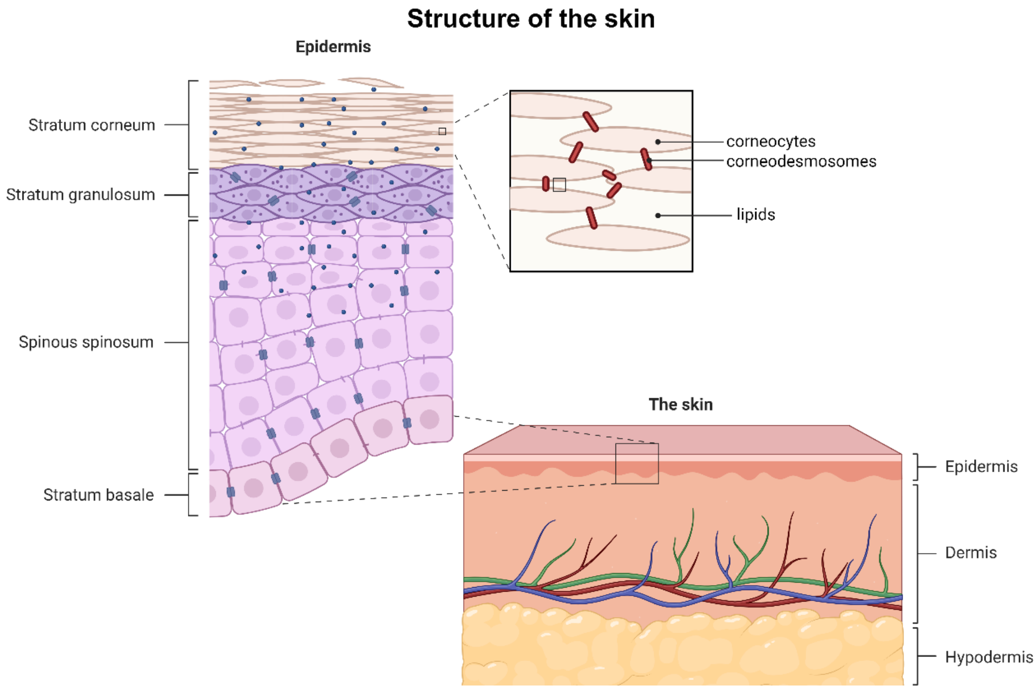


Figure 1. Schematic representation of skin, with special focus on the epidermis and SC (original).

The SC is typically compared to a “brick and mortar model” structure (Elias 1983), where the individual corneocytes represent the “bricks” and the intercorneocyte lipid mantle acts as a space-filling “mortar” (Nemes and Steinert 1999).

The lipid mantle originates in keratinocytes through the accumulation of lipids during their differentiation. These lipids are synthesized by keratinocytes from the intermediate metabolic products of essential fatty acids. Most of these lipids are packaged within tiny organelles referred to as lamellar bodies. After keratinocyte maturation, the lamellar bodies release their contents into intercellular spaces through exocytosis (Baroni et al. 2012).

Subsequently, lipids derived from lamellar bodies undergo modification and reorganization into intercellular lamellae (Baroni et al. 2012). Hydrolysis of glycosphingolipids generates ceramides, and phospholipids are converted into free fatty acids (Bouwstra et al. 2003). Cholesterol, synthesized *in situ* from acetate, contributes to the skin barrier's fluidity and flexibility (Baroni et al. 2012; Boncheva 2014).

Due to this dynamic structure, corneocytes and the unique blend of lipids comprising the lipid mantle act as a barrier to mitigate water and salt loss from the skin while preventing the infiltration of water-soluble substances and large molecules (Yoon et al. 2011; Baroni et al. 2012; Miller et al. 2012).

2.2.3. Skin barrier dysfunction in allergic patients

Skin barrier dysfunction is an established core factor in cAD pathogenesis (Santoro et al. 2015; Santoro et al. 2024). However, it is still up to debate why skin barrier impairment is triggered and if it is a primary defect and/or results secondarily to skin inflammation (Santoro et al. 2024).

A new paradigm, “the epithelial barrier hypothesis”, has emerged to explain the surge and rise of allergic diseases since the 1960s (Akdis 2022; Pat et al. 2023; Ozdemir et al. 2024). Allergies and concomitant leaky epithelial barriers rising rates may be linked to the lifestyle and exposome alterations of modern urban life, namely exposure to cleaning detergents and surfactants, diesel exhausts, ozone, microplastics and highly processed emulsifier-rich foods (Akdis 2022; Pat et al. 2023; Ozdemir et al. 2024).

As the prevalence of atopic dermatitis (AD) is rising across species (Drechsler et al. 2024), dogs have been suggested as sentinels for human health since exposome changes are similar between both species (Sexton and Ruple 2024).

Given these recent insights, understanding the specific modifications in the skin barrier of cAD may now take on greater significance. The following subtopics summarize the current knowledge on this subject.

2.2.3.1. SC lipid alterations in cAD

As previously mentioned, keratinocytes are surrounded by a lipid mantle (Baroni et al. 2012; Boncheva 2014). Within the mantle's lipids, ceramides have been the most studied in the context of cAD for the last decade (Santoro et al. 2024).

In the past, it has been shown, in both humans and dogs, that lesional skin in atopic patients has fewer quantities of ceramides or that the relative proportion of these lipids is altered (Reiter et al. 2009; Yoon et al. 2011; Santoro et al. 2015). Ceramide 1, Ceramide 6, and Ceramide 9 are typically the more altered in cAD (Reiter et al. 2009; Santoro et al. 2015). However, recent research may lead to a reassessment of this information. A small 2018 study concluded that dogs with cAD had less relative free fatty acids and normal relative levels of ceramides and cholesterol when compared to healthy dogs (Chermprapai et al. 2018). Furthermore, the main breakthrough of this study was that the SC of cAD, when compared to the SC of healthy dogs, had a hexagonal lipid packing in place of the healthy and typical orthorhombic lipid organization (Chermprapai et al. 2018).

Standardized ways of collecting and analyzing SC lipids still need to be proposed in veterinary medicine (Fernandes et al. 2023). Nevertheless, the current data suggests that not only quantities and proportions of SC lipids interfere with skin barrier dysfunction, but also that the lipids' spatial organization can play a role in the pathogenesis of cAD (Santoro et al. 2024).

2.2.3.2. SC structural proteins alterations in cAD

Several structural proteins are essential in maintaining skin barrier homeostasis, contributing to the cornified envelope's formation and SC integrity (Drislane and Irvine 2020). Besides their mechanical function, some of these proteins are pivotal in maintaining SC's pH and hydration since their degradation produces natural moisturizing factors like free amino acids (Marsella et al. 2013; Combarros et al. 2020).

In humans, filaggrin gene mutations have been considered one of the most robust predisposing factors contributing to human atopic dermatitis (hAD) (Drislane and Irvine 2020), while in dogs, that connection is not yet clear (Santoro et al. 2024). In some dogs diagnosed with cAD, filaggrin expression can be reduced or even undetectable (Chervet et al. 2010). However, filaggrin gene mutations have not been associated with cAD in most breeds (Wood et al. 2010). This has only been reported in Labrador retrievers from the United Kingdom, which suggests that filaggrin gene mutations might have variable relevance in cAD depending on breed and geographical location (Wood et al. 2010).

Tight junction proteins, like Claudin-1 and Occludin, are a somewhat novel area of interest in cAD being decreased in atopic dog skin (Olivry and Dunston 2015; Roussel et al. 2015; Marsella et al. 2022). More research is needed to evaluate their role in the skin barrier dysfunction of atopic dogs (Santoro et al. 2024).

Recent data suggests structural protein alterations might be mainly secondary to cutaneous allergic inflammation (Olivry et al. 2022). However, the role of structural protein alterations in cAD is still debatable since no data has been able to positively say whether these alterations are primary or secondary to chronic skin inflammation (Santoro et al. 2024).

2.2.3.3. cAD cutaneous microbiome

Mammalian skin is colonized by commensal microorganisms essential in maintaining skin barrier function and integrity (Rodrigues Hoffmann et al. 2014). Dogs with cAD have been shown to have cutaneous dysbiosis, which is a state of imbalance in the number and diversity of beneficial microbes (Weese 2013; Rodrigues Hoffmann et al. 2014; Santoro et al. 2015). The debate over whether skin microbiota dysbiosis is a primary or secondary alteration of cAD is still unclear (Santoro et al. 2024).

Existing evidence shows that the healthy microbiome of the canine skin is highly diverse, while cAD-diagnosed dogs tend to have reduced bacterial diversity in favor of *staphylococcal* organisms, especially S*taphylococcus pseudointermedius* (Santoro et al. 2015; Rodrigues Hoffmann 2017) and reduced fungal diversity in favor of *Malassezia pachydermatis* instead of *Malassezia globosa* (Meason-Smith et al. 2020; Tang et al. 2020).

Dysbiosis has been strongly associated with skin infections in atopic dogs (Weese 2013; Bradley et al. 2016). After a pyoderma episode, it has been reported that dysbiosis persists for two weeks after remission of skin infection lesions (Pierezan et al. 2016). Contrarily, other research has shown that rapid recovery of microbiome diversity occurs after the resolution of a flare and/or skin infection (Bradley et al. 2016), which slowly and gradually reduces to cAD diversity levels again four to six weeks after treatment (Bradley et al. 2016).

Thus far, the exact role of the skin's microbiome and associated dysbiosis in atopic patients has not been fully understood (Bradley et al. 2016; Santoro et al. 2024). Despite that, a lack of bacterial diversity is undeniably correlated with skin barrier dysfunction, a tendency to develop skin infections, and the severity of clinical signs (Pierezan et al. 2016).

2.2.3.4. Modifications in defense peptides in cAD

Besides structural alterations already mentioned in this work, changes in the chemical and immunological barrier have also been investigated in cAD (Santoro et al.

2024). Host defense peptides or antimicrobial peptides, for example, β – defensins, have been thoroughly studied in humans with hAD and, to a lesser degree, in dogs with cAD (Santoro et al. 2015; Santoro et al. 2024). Their primary function, as suggested by their name, is to act as a potent line of defense against microbial colonization of the skin and have immunomodulatory capacities, being a link between innate and adaptive immunity (Santoro et al. 2024).

Evidence shows that host defense peptides are found at different concentrations in lesional versus non-lesional cAD skin (Santoro et al. 2011; Lancto et al. 2013; Santoro, Bunick, et al. 2013). However, likewise, with lipids, structural proteins, and microbiome alteration, it is unclear whether these changes are primary (Santoro et al. 2019; Santoro 2023) or secondary in cAD (Santoro et al. 2024).

Extrapolating from human evidence, it has been proposed that alterations in defense peptides' expression and/or structure could predispose atopic dogs to skin infections (Santoro et al. 2015). Studies have shown that dogs suffering from cAD tend to have higher expression of genes related to β – defensin and cathelicidin (Santoro et al. 2011), especially during periods of flares by active infection (Santoro, Bunick, et al. 2013). Curiously, higher gene expression was not always associated with elevated protein levels (Santoro et al. 2013).

Newer evidence has opened a new paradigm on this topic, proposing new theories on why defense peptides might have lower inhibitory activity against common infection agents in cAD, like *S. pseudointermedius*. It was found that host defense peptides in cAD-diagnosed dogs tend to be retained inside keratinocytes and often remain attached to the the skin surface instead of being dispersed in the epidermis (Santoro et al. 2019; Santoro 2023).

These new findings may suggest that the role of host defense peptides in cAD may not be exclusively related to their lower secretion level in the skin but also due to their reduced functionality and dispersion (Santoro et al. 2024).

2.2.4. Assessing skin barrier function and integrity

In humans, noninvasive tools to assess skin barrier integrity are commonly used in both research and clinical settings (Cobiella et al. 2019). Traditionally, skin barrier function in hAD and cAD is evaluated through indirect measurements, the most common being the measurement of transepidermal water loss (TEWL) (Santoro et al. 2015; Cobiella et al. 2019).

It is important to note that no direct methodologies have been developed or optimized for assessing skin barrier integrity in either humans or dogs (Santoro et al. 2024). However, in an effort to address this, a pilot study measuring skin's hydration, pH, and erythema in conjunction with TEWL has intended to assess the repeatability of the measurement of these parameters in both research and clinical settings of cAD to optimize research in this area (Cobiella et al. 2019; Santoro et al. 2024).

In our pilot study, the available equipment for measuring skin barrier integrity was a pH meter and a TEWL meter. Consequently, these methods will be explored in greater depth.

2.2.4.1. TEWL

As previously stated, TEWL is the most common noninvasive and indirect way of assessing human skin barrier integrity and function (Cobiella et al. 2019). TEWL refers to the outward diffusion of water through the skin, indicating overall cutaneous water loss, including the passive activity of sweat glands. It does not include the water that evaporates due to active sweat gland secretion (Batt and Fairhurst 1986). This measurement uses a humidity captor that obtains an evaporation rate from the skin (Shimada et al. 2008).

Patients with hAD have long been linked to elevated TEWL, which is connected to skin barrier dysfunction, reduced ceramide levels, and decreased water capacitance, ultimately resulting in dry skin (Di Nardo et al. 1998; Cobiella et al. 2019). Additionally, elevated TEWL measured in newborns at high risk for developing AD has been found to predict the onset of clinical signs of AD in early childhood, which indicates that in people, elevated TEWL can be a valid indicator of hAD before clinical signs development (Gupta et al. 2008; Cobiella et al. 2019).

In canines, TEWL has also been the most extensively utilized skin barrier integrity parameter (Santoro et al. 2015). Like in humans, TEWL association with lower ceramide content and diminished skin barrier function in dogs suffering from cAD has been established (Shimada et al. 2008; Shimada et al. 2009; Bradley et al. 2016). However, this measurement is associated with high variability and questionable clinical significance in dogs since it can vary from site to site and day to day (Lau-Gillard et al. 2010; Santoro et al. 2015). Despite this, TEWL remains the gold standard for a noninvasive evaluation of skin barrier integrity in cAD (Santoro et al. 2015).

TEWL measuring devices can be categorized as open chambered (OC) or closed chambered (CC) based on their design (Cobiella et al. 2019). Most studies in veterinary medicine have been conducted using closed-chamber devices; however, the question of which type of device is more suited is still up for debate, with both designs having advantages and disadvantages (Cobiella et al. 2019).

Despite extensive searching, no internationally accepted reference values were found for TEWL in different dog breeds, body sites, and skin conditions, including cAD.

Additionally, different brand TEWL measuring devices were used in the few studies related to this topic, which may further compromise comparison and analysis between reported values. In an effort to summarize the collected data, inguinal and pinna TEWL values have been categorized and organized in the Table 1.

Table 1 – Mean transepidermal water loss (TEWL) values for inguinal or pinna areas on healthy dogs (HD) and canine atopic dermatitis (cAD)-diagnosed dogs.

Year	Author	TEWL device	Inguinal HD	Inguinal cAD	Pina HD	Pina cAD
2020	Marsella et al. *	CC	14.00	-	10.00	-
2019	Cobiella et al. **	CC	7.59 ± 4.24	6.92 ± 2.73	8.40 ± 2.55	9.64 ± 2.63
2019	Szczepanik et al. **	CC	-	91.62 ± 85.11	-	45.23 ± 47.61
2017	Marsella et al. ***	CC	-	11.10 ± 7.99	-	11.34 ± 5.23
2017	Hobi et al. *	CC	-	10.35	-	10.10
2016	Bradley et al. ***	OC	12.70 ± 5.38	20.14 ± 16.73	14.47 ± 11.76	26.3 ± 23.04
2012	Cornegliani et al. *	CC	-	-	8.81	22.47
2010	Hightower et al. **	OC	5.24 ± 3.02	6.58 ± 3.32	10.04 ± 2.67	14.17 ± 3.89
2010	Oh and Oh **	CC	4.6 ± 1.13	-	5.5 ± 0.88	-
2010	Lau-Gillard et al. *	CC	34.38	-	21.19	-
2007	Yoshinara et al. **	OC/CC	18.6 ± 5.4	-	35.5 ± 11.8	-

*- only mean TEWL values without SD were provided;
**- mean TEWL and SD values were provided;
***- individual TEWL values were provided, mean and/or SD values were calculated by the author;
CC – closed-chambered
OC – open-chambered

2.2.4.2. pH

The skin surfaces of haired mammals are generally acidic (Miller et al. 2012). Skin pH plays a vital role in preserving barrier integrity as an acidic environment within the SC contributes for effective epidermal impermeability, antimicrobial defense, and the overall integrity and cohesion of the SC (Miller et al. 2012; Pin et al. 2014).

Many factors contribute to the acidic pH of the skin through different mechanisms (Miller et al. 2012). Some are included in table 2.

Table 2 - Factors that affect skin pH (according to Miller et al. 2012)	
Physiologic Factors	Breed Age Sex Body site Skin moisture Excitement
Exogenous Factors	Skin irritants Topical products
Pathologic Factors	Bacterial Infections Yeasts infections Atopic dermatitis Irritant contact dermatitis Diabetes mellitus Uremia Hepatic disease

In dogs, the pH of healthy skin is quite variable and has been reported to range between 4.84 and 9.95 (Miller et al. 2012; Schlake et al. 2022). This variation is physiological and caused by many factors, including age, sex, spaying, body site, breed, inflammation, and even excitement (Miller et al. 2012).

In humans, the pH of the skin and the factors influencing it have been extensively researched, with numerous articles published on the topic (Schlake et al. 2022). In contrast, veterinary literature on skin pH in dogs is limited, primarily featuring comparative studies or small case series (Schlake et al. 2022).

Dogs suffering from cAD tend to have a more alkaline skin pH (Miller et al. 2012; Pin et al. 2014; Cobiella et al. 2019). As mentioned before, this is related to alterations in the acidic lipid mantle, with a lack of free fatty acids (Chermprapai et al. 2018), reduced secretion of proteins, and consequently reduced production of free amino acids (Marsella 2012; Combarros et al. 2020), and chronic inflammation and dysbiosis of the skin (Santoro et al. 2024).

Skin surface pH is typically measured by a glass electrode, also known as a pH meter (Miller et al. 2012). This measurement has been proposed as an alternative parameter to TEWL (Bradley et al. 2016). Compared to TEWL, pH is more reliable as it presents lower intra and inter-observer variability and higher repeatability (Cobiella et al.

2019). Given this context, pH measuring may be a usefull tool to evaluate skin barrier function and integrity (Bradley et al. 2016; Cobiella et al. 2019).

Once more, no internationally recognized reference values for skin pH in cases of cAD were identified. Moreover, the use of various brands of pH meters in related studies may further hinder the comparison and analysis of reported values. To consolidate the collected data, pH values have been categorized and presented in the Table 3.

Table 3 – Mean pH values for inguinal or pinna areas on healthy dogs (HD) and canine atopic dermatitis (cAD)-diagnosed dogs.

Year	Author	Inguinal HD	Inguinal cAD	Pina HD	Pina cAD
2022	Schlake et al. *	5.36	-	5.79	-
2022	Panzuti et al. **	-	-	6.12 ± 0.36	-
2021	Raquel Reis **	-	6.76 ± 0.57	-	-
2021	Santoro et al. *	-	6.75	-	6.75
2019	Cobiella et al. **	6.67 ± 0.49	7.05 ± 0.61	6.75 ± 0.76	7.22 ± 0.9
2017	Hobi et al. *	-	7.40	-	7.00
2016	Bradley et al. ***	6.39 ± 0.92	6.55 ± 1.01	6.54 ± 1.09	6.76 ± 1.30
2010	Oh and Oh **	7.40 ± 0.99	-	6.80 ± 0.26	-
2010	Diana Ferreira **	6.61 ± 0.81	-	6.41 ± 0.86	-

*- only mean pH values without SD were provided;
**- mean pH and SD values were provided;
***- individual pH values were provided, mean and/or SD values were calculated by the author;

To summarize, the skin's pH is a crucial factor in cAD as it influences an array of mechanisms in the skin physiology, including pruritus (Miller et al. 2012). New approaches that tackle the skin barrier pH values may be of value in dogs suffering from cAD (Santoro et al. 2015; Santoro et al. 2024).

2.3. Topical Emollients: a traditional approach with renewed benefits

Emollients are cosmeceutical formulations designed for topical used to soften, moisturize, and calm the skin (Wollenberg et al. 2018; Drechsler et al. 2024). These formulations predominantly have vehicle-type constituents and lack active pharmacological ingredients (Wollenberg et al. 2018). Traditional emollients have an array of possible presentations and usually contain ingredients that can be categorized into two main groups: Humectants, such as urea, lactic acid, and amino acids, which attract and bind to water molecules, therefore improving the water content of the stratum corneum; Occlusives, such as petrolatum, which act as physical barriers and reduce TEWL (Goh et al. 2022).

2.3.1. Topical emollient use in humans

In human medicine, the evidence supporting the use of emollients is robust. Topical emollients play a crucial role in almost all hAD management plans, being commonly utilized as part of a comprehensive regimen with pharmacologic treatments (Sidbury et al. 2023). Typical formulations have the potential to enhance skin barrier function and diminish the skin's susceptibility to irritants. Products containing excessive ingredients or common allergens like fragrances or undesirable preservatives are commonly avoided (Goh et al. 2022).

Topical emollients are predominantly used between flares, being the mainstay of proactive hAD care (Wollenberg et al. 2018). Nevertheless, exclusive application of topical emollients for one week has been shown to control and improve potentially mild-to-moderate hAD flares (Angelova-Fischer et al. 2014).

Several studies in children have shown that the regular application of topical emollients has both short- and long-term steroid-sparing effects in mild-to-moderate hAD cases. This is evidenced by a reduction in the duration of topical steroid therapy during flares and an increase in intervals between flares, respectively (Grimalt et al. 2007; Szczepanowska et al. 2008).

2.3.2. Revolutionizing topicals – emollients plus

In recent years, several topical emollients designed for addressing hAD have become commercially available, known as “emollient plus” (Wollenberg et al. 2018; Cestari et al. 2023; Zelenkova et al. 2023; Ch’ng 2024). These newer formulations include bioactive ingredients, such as ceramides, plant oils rich in linoleic acid, natural moisturizing factors, vitamins, minerals, and antioxidants (Goh et al. 2022), known for their potential to reduce inflammation and restore barrier lipids. However, these formulations do not meet the criteria for classification as topical drugs and, therefore, do not require a license (Wollenberg et al. 2018). The European guidelines for the treatment of hAD refer to topical emollients as ‘emollients plus’ when these formulations contain vehicle-type substances plus additional bioactive ingredients and non-pharmacological substances able to influence the skin microbiome and inflammatory response (Wollenberg et al. 2018).

2.3.3. Emollient Plus – examples of bioactive ingredients

Evidence for the topical use of emollients plus in dogs is scarce, and most studies address the skin benefits of oral supplementation of this type of ingredients (van Amersfort et al. 2023). Therefore, the information on the following two subtopics is exclusively reported from scientific work on humans and human-like skin models. Additionally, it should be noted that the specific ingredients addressed in the following two subtopics were chosen for their common use in hAD and are not necessarily included in the proprietary formulation developed for this study.

2.3.3.1. Niacinamide

Niacinamide, also known as nicotinamide, is a water-soluble form of vitamin B3 (Forbat et al. 2017). This molecule has a long-standing track record of use in human dermatology (Chen and Damian 2014). However, no published literature on its specific topical or oral use in dogs with cAD was found.

Evidence concerning topical niacinamide use has suggested that it has anti-inflammatory, anti-aging, anti-carcinogenic, and moisturizing properties. In humans, niacinamide can be used to treat an array of skin conditions, including autoimmune blistering disorders, skin cancer, acne, rosacea, aging skin, and hAD (Chen and Damian 2014). However, evidence of its topical use in patients with hAD is still limited (Zhu et al. 2023).

In hAD, niacinamide has been shown to increase the production of ceramides and other stratum corneum lipids, consequently reducing TEWL (Tanno et al. 2000). Complementarily, niacinamide has been shown to prevent the upregulation of aquaporin 3, a gene encoding water-permeable channels that cause water loss from the skin that are upregulated in hAD (Olsson et al. 2006). With these mechanisms, niacinamide can reduce water loss and prevent dry skin (Chen and Damian 2014). Niacinamide is also thought to alleviate pruritus as it can stabilize mast cells via cyclic adenosine monophosphate inhibition and thus reduce histamine release, and via its enhancement in the production of ceramides, deficiencies of which can worsen dry skin and pruritus (Forbat et al. 2017).

A recent single-center, randomized, controlled clinical investigation conducted at Fudan University evaluated the efficacy of niacinamide-containing body emollients combined with cleansing gel in mild AD treatment (Zhu et al. 2023). This study demonstrated that patients utilizing niacinamide-containing body emollients, either independently or in conjunction with a cleansing gel, exhibited superior improvement in clinical symptoms, quality of life impact, and skin barrier function compared to the control group after four weeks (Zhu et al. 2023).

2.3.3.2. Colloidal Oatmeal

Colloidal oatmeal is the powder obtained from grinding and processing whole oat grain. Historically, colloidal oatmeal (Avena sativa) has been used for millennia in the treatment of dry and itchy skin of humans, with records dating back to its use in ancient Egypt and the Roman Empire (Kurtz and Wallo 2007). In the 20th century, commercially available formulations containing colloidal oatmeal first appeared and sparked a new era of scientific investigation on this ingredient and its effects on the skin (Sompayrac and Ross 1959). Colloidal oatmeal has been approved as an over-the-counter (OTC) medication by the United States Food and Drug Administration (FDA) since 1989 (Allais and Friedman 2020), and it is one of only two OTC active ingredients approved for hAD by the FDA (Hebert et al. 2020).

Oats comprise an array of phytochemicals, such as phenolic compounds, β-glucans, avenanthramides, carotenoids, and vitamin E. These phytochemicals are believed to play a role in the oats' diverse functionality and clinical applications (Kurtz and Wallo 2007). Colloidal oat has direct anti-inflammatory, anti-pruritic, anti-oxidant, anti-fungal, prebiotic, barrier repair properties, and beneficial effects on skin pH (Allais and Friedman 2020).

Colloidal oat's anti-inflammatory properties come from its ability to inhibit phospholipase A2 in keratinocytes consequently decreasing arachidonic acid release and pro-inflammatory eicosanoid formation (Makdisi et al. 2013). Avenanthramides have also been found to suppress the secretion of pro-inflammatory cytokines such as IL-6, IL-8, and MCP-1 in endothelial cells (Guo et al. 2008).

Colloidal oatmeal also has anti-pruritic properties via its ability to inhibit neurogenic inflammation, which has been demonstrated both in murine models and in human patients with various pruritic skin diseases, including hAD (Sur et al. 2008; Allais and Friedman 2020; Lisante et al. 2023).

Recent evidence has also shown that colloidal oatmeal has potentially prebiotic properties. *In vitro* studies have shown that colloidal oatmeal is metabolized by and promotes the multiplication of commensal bacteria of the skin, including *Staphylococcus epidermidis, Staphylococcus aureus*, and *Cutibacterium acnes* (Liu-Walsh et al. 2021).

According to *in vitro* and *in vivo* experiments by the same author, the metabolism of colloidal oatmeal results in increased production of lactic acid, a natural moisturizing factor that presents hygroscopic properties (Liu-Walsh et al. 2021). Another study conducted by Capone et al. (2020) in patients with mild to moderate hAD examined the effect of twice daily use of 1% colloidal oatmeal cream on the skin microbiome. Compared to treatment with a standard moisturizer, treatment with 1% colloidal oat cream was associated with higher microbial diversity of lesional skin, approaching the level of diversity on non-lesional skin. In contrast, a similar improvement in microbial diversity was not observed with a standard moisturizer (Capone et al. 2020).

Oats also contain linoleic acid, which can directly reduce TEWL and restore the skin barrier (Ziboh et al. 2000), and lipophilic molecules that have agonist properties towards receptors and genes involved in epidermal differentiation, lipid synthesis, and ceramide processing that potentially result the skin barrier restoration, indirectly reducing TEWL (Chon et al. 2015).

Topically applied oat extract may also benefit the skin's pH since it has been shown to act as an active buffer, reducing the alkaline pH found in inflamed skin to its normal, slightly acidic range (Grais 1953). Although more up-to-date research on the effects of colloidal oatmeal on the skin's pH is needed, to the author's knowledge, this is the only published data concerning the effects of colloidal oatmeal on the skin's pH.

Several studies have been conducted on patients afflicted with hAD employing colloidal oatmeal as an active therapeutic agent adjuvant to traditional treatment (Allais and Friedman 2020; Lisante et al. 2023). In a study by Nebus J et al. (2009), 25 patients exhibiting mild to moderate hAD, were enrolled in an eight-week trial. This regimen involved twice daily application of an oat-based occlusive cream and once daily use of an oat-based body wash. Statistically significant improvements were observed in hAD clinical scores, as well as pruritus severity as soon as week two. In another study, Lisante et al. (2017) conducted a randomized, double-blinded, two-arm trial involving 90 patients diagnosed with mild to moderate hAD. Participants were randomly assigned to utilize either colloidal oatmeal cream or a standard, steroid-free prescription barrier cream twice daily. After three weeks, the results showed that the efficacy of the colloidal oatmeal cream was comparable to that of the prescription barrier cream (Lisante et al. 2017). Similar results were observed more recently, concluding that an OTC 1% oatmeal cream was at least as effective and safe as prescription barrier cream in Black/African American children suffering from mild to moderate hAD (Lisante et al. 2023).

2.3.4. Topical emollient plus use in dogs

In the last decade, the topical application of emollients containing bioactive ingredients has garnered increasing attention in cAD management (Nuttall et al. 2019). To the best of the author's knowledge, only five different topical emollient products have been studied in dogs with cAD. The results found in those studies have been summarized in the next paragraphs and Table 4.

Three small open-label studies evaluated the safety and effectiveness of the same veterinary topical emollient plus spot-on product that contained free fatty acids, ceramides 1, 3, and 6, and cholesterol (Piekutowska et al. 2008; Fujimura et al. 2011; Popa et al. 2012). All studies had small sample sizes, varying from 5 to 10 dogs, and the established protocol involved applying the product twice weekly, from 3 to 6 weeks (Piekutowska et al. 2008; Fujimura et al. 2011; Popa et al. 2012).

Significant improvements were reported in the structure and organization of the SC, including an increase in the number of lipid lamellae, elevated ceramide and free fatty acid levels, and decreased levels of glucosylceramides in the SC (Piekutowska et al. 2008; Fujimura et al. 2011; Popa et al. 2012). After 6 weeks of treatment, significant clinical score improvements were observed in atopic dogs, especially in erythema and alopecia (Fujimura et al. 2011).

In contrast, a randomized, double-blinded, placebo-controlled study conducted using the same topical formulation on 26 privately owned dogs saw no significant differences in clinical scores, pruritus, TEWL, and SC pH levels between the placebo and the treatment group (Hobi et al. 2017). No adverse effects were reported (Piekutowska et al. 2008; Fujimura et al. 2011; Popa et al. 2012; Hobi et al. 2017).

Another veterinary commercially-available formulation, in the form of spot-on and topical spray containing essential oils and unsaturated fatty acids, was tested in three different works: two small open-label studies (Tretter and Mueller 2011; Idée et al. 2022) and a randomized, multicenter, double-blinded, placebo-controlled clinical trial with a sample of 48 privately owned dogs (Blaskovic et al. 2014). Internationally recognized cAD clinical scores and pruritus levels were evaluated (Tretter and Mueller 2011; Blaskovic et al. 2014; Idée et al. 2022) as well as TEWL (Tretter and Mueller 2011; Idée et al. 2022).

In the small open-label clinical trials, healthy dogs used as a control showed no significant changes in cAD clinical scores and TEWL after treatment (Tretter and Mueller 2011; Idée et al. 2022). Atopic dogs had statistically significantly reduced clinical scores with both delivery methods, but only the spray formulation group had significant pruritus relief (Tretter and Mueller 2011). TEWL was significantly reduced in both open-label studies, but its significance varied depending on body site (Tretter and Mueller 2011; Idée et al. 2022). Similarly, in the randomized, multicenter, double-blinded, placebo-controlled study, clinical scores and pruritus were significantly improved after treatment when compared to the placebo group, and the incidence of cAD flares was higher in atopic dogs receiving the placebo formulation (Blaskovic et al. 2014). No adverse effects were reported (Tretter and Mueller 2011; Blaskovic et al. 2014; Idée et al. 2022).

In 2014, South Korean investigators purposed to study the efficacy of a commercially available topical emollient plus in atopic dogs (Jung et al. 2013). This Korean cosmetic included ceramides, cholesterol, free fatty acids in a 3:1:1 proportion, and other bioactive ingredients like niacinamide, allantoin, and glycosaminoglycans (Jung et al. 2013). Daily application all over the body was conducted in 20 atopic dogs for 4 weeks, and TEWL, clinical scores, and pruritus were evaluated on days 1, 14, and 28 (Jung et al. 2013). At the end of the clinical trial, significant decreases were found in all analyzed variables (Jung et al. 2013).

In 2017, a topical emollient plus spray containing essential fatty acids, ceramides, panthenol, and licorice root extract was tested in 28 atopic dogs with twice-daily application in cAD-commonly affected body sites (Marsella et al. 2017). It was found that after 30 days, the spray could provide symptomatic relief from pruritus, although not statistically significant (Marsella et al. 2017). After 90 days, no significant differences were found in cAD clinical scores and TEWL (Marsella et al. 2017). Contact dermatitis was reported as a side effect in 2 dogs, one from the treatment group and one from the placebo group (Marsella et al. 2017).

Recent literature, involving topical emollients plus, has incorporated the topical application of synthetic pseudoceramides, also known as sphingolipids (Nuttall et al. 2019). These may offer a more cost-effective alternative to natural ceramides as they are thought to be converted into ceramides in the SC (Nuttall et al. 2019).

The same topical emollient plus, including sphingolipids and glycosaminoglycans was tested in two different clinical trials using different delivery methods: a spot-on and a collar formulation (Marsella et al. 2020; Segarra et al. 2023).

The spot-on formulation was applied on 6 atopic beagles twice a week for 8 weeks, while 6 atopic beagles served as controls with no spot-on application (Marsella et al. 2020). After allergen-challenges, cAD clinical scores significantly increased in the control group and decreased in the treatment group, although not statistically significantly; pruritus levels were significantly lower in treated dogs; TEWL changes were never significant between and within groups at any time point of the study; SC levels of ceramides did not change significantly between groups, but unsaturated fatty acid levels in the treatment group increased significantly (Marsella et al. 2020).

The collar formulation was applied on 12 dogs for 8 weeks, with cAD clinical scores and pruritus measurements taking place at various time points (Segarra et al. 2023). It was found that clinical signs and pruritus, from the veterinarian's and the owner's perspectives, significantly decreased at the end of the study (Segarra et al. 2023).

Topical emollients plus positive effects on the skin barrier of atopic dogs have been summarized in Table 4.

Table 4 – Reported topical emollient plus benefits on the skin barrier of atopic dogs.

Topical emollient benefits	References
Increased *stratum corneum* lipids	Piekutowska et al. 2008; Popa et al. 2012; Marsella et al. 2020;
Decreased clinical scores	Fujimura et al. 2011; Tretter and Mueller 2011; Jung et al. 2013; Blaskovic et al. 2014; Marsella et al. 2020; Segarra et al. 2023;
Decreased pruritus	Tretter and Mueller 2011; Blaskovic et al. 2014; Marsella et al. 2017; Marsella et al. 2020; Segarra et al. 2023;
Decreased TEWL	Tretter and Mueller 2011; Blaskovic et al. 2014; Idée et al. 2022;

Legend: TEWL – transepidermal water loss

Evidence suggests that spray formulations may be more effective when compared to *spot-on* delivery systems (Tretter and Mueller 2011) but can also lead to lower owner treatment compliance (Marsella et al. 2017). While the positive effects of topical emollients plus on the canine skin barrier are becoming clear, these formulations seem to be ineffective as a monotherapy in managing more severe cases of cAD (Marsella et al. 2017). This is consistent with topical emollient use and effectiveness found in humans (Wollenberg et al. 2018).

In conclusion, there is a significant need to develop effective topical emollient plus products specifically tailored for cAD (Nuttall et al. 2019) that can significantly impact the clinical management of atopic dogs. Additionally, to the author's knowledge, no studies on this subject have evaluated the owner's perspective on treatment efficacy and product cosmetic properties, which strongly influence treatment compliance (Olivry et al. 2018). Topical emollients plus promisingly serve as novel and effective alternatives to traditional treatment approaches, with mounting skin barrier restoration benefits and additional systemic drug-sparing and treatment cost-reducing effects (Marsella et al. 2020; Segarra et al. 2023).

3. TACKLING CANINE ATOPIC DERMATITIS: A PILOT CLINICAL TRIAL ASSESSING THE EFFICACY OF AN INNOVATIVE TOPICAL SOLUTION

3.1. Objectives of the study

The primary objective of the present study was to evaluate the efficacy of a novel topical emollient plus formulation as a co-adjuvant treatment of cAD. The secondary objective was to investigate if atopic dog owners perceive this treatment option as a useful, efficacious, and practical tool in their dog's treatment plans.

3.2. Materials and methods

3.2.1. Overview

The study was designed as a proof-of-concept, bench-to-bedside prospective pilot study comprising the daily application of the emollient plus spray for four weeks. .

The project was submitted to the Comissão de Ética para a Investigação e o Ensino (CEIE) at FMV-ULisboa for ethical standards evaluation and received approval (N/Refª 008/2023). Participants were recruited from HEV at FMV-ULisboa reference dermatology service after being screened at a routine follow-up consultation, and inclusion criteria were met. Owners were contacted on-site or via telephone/e-mail for more detailed information regarding the study, and their willingness to enroll their dogs was assessed. Additionally, promotional flyers and social media posts were created to reach more HEV dermatology service dog owners who could voluntarily schedule a dermatology appointment (Annexe 2). Dogs who met the inclusion criteria on these consultations were also included. The recruitment was conducted between May 2023 and May 2024. Owners provided informed written consent to participate in the study and could withdraw anytime. The product, appointments, and analysis were free to promote owner compliance.

3.2.2. Enrolment criteria

Client-owned dogs with a clinical diagnosis of nonseasonal cAD that were clinically stable were eligible for inclusion. The diagnosis was based on a compatible history and clinical signs strongly indicative of cAD and in accordance with Favrot's criteria (Favrot et al. 2010). Other similar pruritic skin conditions were excluded. All participants must have been on a veterinarian-approved flea control regimen 8 weeks prior to and during the clinical trial (Lourenço et al. 2016; Pinto et al. 2024).

Other concomitant antipruritic medicines were allowed, including systemic/topical steroids and oclacitinib if given for at least four weeks at unchanged doses/regimen, and ciclosporin and lokivetmab if given for at least eight weeks at unchanged doses/regimen, prior to inclusion and during the study period. Concurrent vitamin and essential fatty acid supplements administration was permitted if it had been ongoing for at least 4 weeks before inclusion and during the study. Baths with products usually used by pet owners were accepted, except on the follow-up day. Concurrent use of AIT was allowed if it had been ongoing for at least 12 months. The introduction of any skin therapeutic tools throughout the study was not admitted (Pinto et al. 2024).

Dogs were ineligible for this study if they presented with uncontrolled cAD, had a parasitic infestation, uncontrolled bacterial or yeast infection at the time of inclusion, a concurrent non-CAD-related disease affecting the skin, or if they were pregnant or lactating (Deboer et al. 2016; Timm et al. 2018; Pinto et al 2024).

Criteria for study withdrawal included unacceptable adverse reactions, significant worsening of clinical signs or poor pet-owner adherence to the treatment protocol and/or scheduled clinical appointments (Timm et al. 2018; Pinto et al. 2024).

3.2.3. Study design

3.2.3.1. Topical emollient plus development

The novel topical emollient plus was developed before the execution of the present study, and it was designed and formulated by our team, especially Beatriz Fernandes, as part of Fernandes' PhD research project (FCT fellowship 2021.05985.BD).

This novel formulation was specially tailored to have a supportive and repairing effect on the skin barrier while simultaneously being suitable for application on dogs' fur. At the time of this study, the novel topical emollient has already been submitted to various tests, including: *in vitro* stability testing, proving its stability for at least six months after production and packaging and delivery system testing, to ensure the formula's protection from external factors and simple and correct dispensation.

3.2.3.2. Treatment protocol

At the first visit (D0), all owners received further instructions on the study protocols, and the remaining doubts were clarified. Every dog owner signed an informed written consent form (Annexe 3) and could leave the study anytime. On D0, the owners were also educated on how to prepare and apply the topical emollient plus, with the first application being made in their presence by a team member of HEV’s dermatology service. Mandatory follow-up consultations were scheduled 30 days after D0 (D30).

The topical emollient plus application protocol comprised the daily application of one or two spray pumps after vigorously shaking the spray bottle. The application was ideally made before feeding, playing, or walking and on typical cAD-affected body sites like the concave surface of the pinna, axillae, inguinal area, abdomen, and interdigital spaces. After the application, a quick massage was recommended to further promote the product's absorption (Phuong and Maibach 2015 Jul 15; Li et al. 2019).

3.2.3.3. Clinical assessments

For this pilot study, various relevant clinical assessments and skin barrier function and integrity parameters were recorded on D0 and D30. For the clinical evaluation of atopic dogs, published and validated pruritus scales (PVAS10) and skin lesion scores (CADESI-04) were collected, as well as skin barrier function parameters such as TEWL and pH at D0 and D30. Additionally, on D30, owners completed a global assessment of treatment efficacy and a cosmetic product evaluation questionnaire and reported any adverse effects noted.

These assessments are schematically represented in Figure 2, as well as their time points in the study.

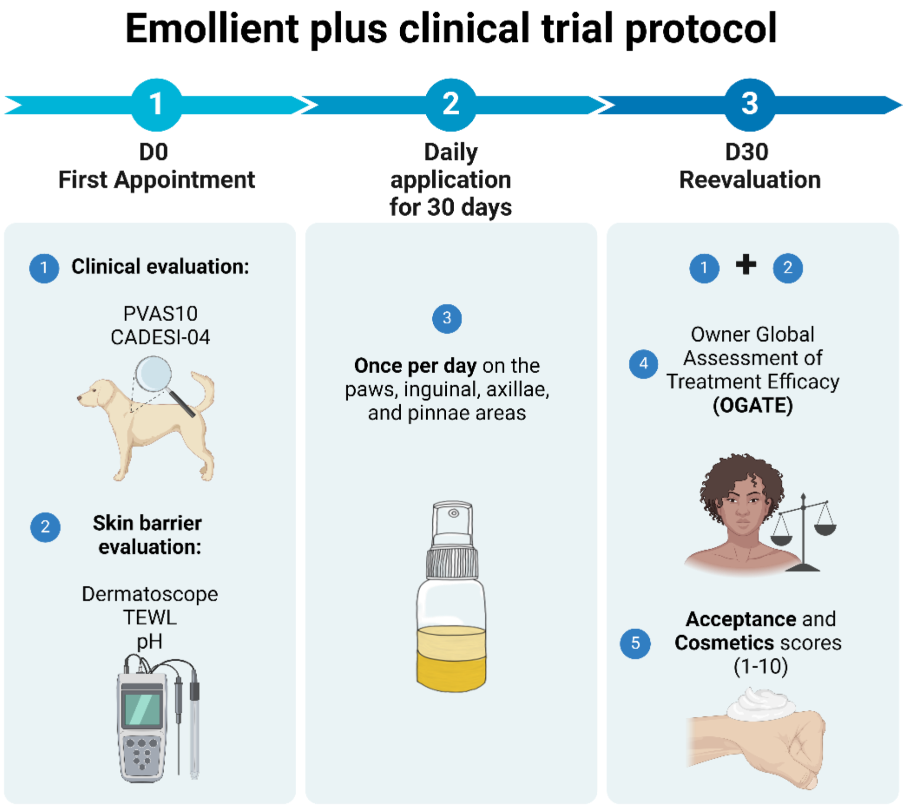


Figure 2. Schematic representation of the study design and timeline (original).

3.2.3.3.1. Pruritus

For pruritus evaluation, a validated owner-assessed 10-cm Visual Analog Scale (PVAS10) (Annexe 4) with various detailed descriptions of pruritus intensities was used

(Hill et al. 2007; Rybníček et al. 2009). The pruritus value was obtained by measuring the distance in cm from the bottom of the line to the owner's mark. The owner's assessment of pruritus was recorded on D0 and D30.

3.2.3.3.2. Extent and severity of skin lesions

In order to evaluate the extent and severity of skin lesions, a validated lesion scale was utilized on D0 and D30 (Annexe 5). The Canine Atopic Dermatitis Extent and Severity Index (CADESI-04) (Olivry et al. 2014) considers the 20 body sites typically affected in atopic dogs and scores three lesion types (erythema, lichenification, and alopecia/excoriation) on a four-point ordinal scale (0 – none; 1 – mild; 2 – moderate; 3 – severe). All assessments were obtained by a veterinarian from the HEV's Dermatology Service who was blinded to previous score results.

3.2.3.3.3. TEWL and pH

All instruments were calibrated before the start of the study and recalibrated as necessary according to the manufacturer's instructions.

Upon arrival, all dogs were allowed to acclimatize to the testing room for 10-15 minutes. After acclimatization, while in lateral recumbency or in stance, measurements were collected in two body sites: the inguinal area and the concave surface of the pinna either on the left or right side of the dog.

The TEWL was assessed using the Tewameter® TM 210 (Courage + Khazaka; Köln – Germany), an OC TEWL device. The probe was lightly rested perpendicularly to the body site for around 60 s until TEWL values stabilized. Ten repeated measurements were collected per body site, and TEWL values were expressed as evaporation rate ($g/m^2/h$).

The skin surface pH was measured using the LAQUA® PH220 (Delfin Technologies; Kuopio, Finland) by lightly placing the probe perpendicular to the specified anatomical sites for around 30 s until pH values stabilized. Three repeated measurements were collected per body site, and pH values were expressed in pH units. TEWL and pH values were registered on original data recording sheets (Annexe 6).

Figure 3 illustrates the assessment of skin pH and TEWL values on an enrolled atopic dog.

Figure 3. Assessing skin surface pH (left) and TEWL (right) in an enrolled atopic dog (original photos).

3.2.3.3.4. Dermatoscopic evaluation

To visually assess skin lesions, a dermatoscopic examination was performed on D0 and D30 on the pinnae and inguinal area skin. The dermatoscope used was DermLite DL5 (3Gen, Inc.; California, USA).

3.2.3.3.5. Owner's overall assessment of efficacy

On D30, the final appointment, dog owners completed a questionnaire to report their views on the topical emollient plus efficacy. The unvalidated questionnaire Owner Global Assessment of Treatment Efficacy (OGATE) (Olivry et al. 2018) (Annexe 7) directly evaluates the treatment response using a single question ("How would you rate the overall response to treatment?") and provides five possible answers: 0= "no response"; 1= "a poor response"; 2= "a fair response"; 3= "a good response"; 4= "an excellent response".

3.2.3.3.6. Adverse effects

Owners were advised to report any behavioral changes and signs of adverse reaction to the topical emollient plus and to send visual records of these situations. For that purpose, telephone and e-mail contacts were provided to streamline communication between owners and the team. The owners registered any behavioral change or adverse reaction in the “Observações” topic in Annexe 7.

3.2.3.4. Cosmetic evaluation

On D30, dog owners were required to complete a questionnaire to provide their views on the cosmetic properties of the topical emollient plus. This original unvalidated questionnaire was divided into two different sections. The first section required owners to classify the affirmations “The product has good appearance”, “The product has a pleasant smell”, “The product application was easy and simple”, “Applying the product was pleasant to me”, “Applying the product was tolerated by my dog”, “I would not mind to include this product in my dog treatment plan”, and “I would recommend this product to other owners of atopic dogs”; from 1 = “strongly disagree” to 10 = “strongly agree” (Annexe 7). The second section questioned owners about the characteristics of their dog’s skin after the application of the topical emollient plus, namely the dog’s skin “Hydration”, “Oiliness”, “Redness”, and “Scaling”. These cosmetic variables were classified from 1 = “not hydrated/oily/red/scaly at all” to 10 = “extremely hydrated/oily/red/scaly” (Annexe 7).

3.2.4. Outcome measurements

Clinical improvement and treatment efficacy were primarily assessed through pruritus manifestations (PVAS10), clinician-assessed skin lesions extent (CADESI-4), and the owner’s perception of treatment efficacy (OGATE). Secondarily, outcomes like TEWL and pH were considered to evaluate barrier function and integrity, and the Cosmetic score was used to evaluate the owner’s perspectives, treatment feasibility, and cosmetic properties of the topical emollient plus.

According to the International Committee on Allergic Diseases of Animals (ICADA)’s COSCAD’18 (Olivry et al. 2018), for dogs that on D0 presented PVAS10 scores in the moderate to severe cAD range (PVAS10 ≥ 3.6), success of the PVAS10 outcome was defined as the percentage of dogs with PVAS10 scores in the range of normal dogs or those with mild cAD at study end (PVAS10 < 3.6). On the contrary, failure was considered when, at the end of the study, PVAS10 scores remained > 3.6. If, at enrollment, participants had reported PVAS10 scores in the mild cAD range (2 ≤ PVAS10 ≤ 3.5), success was defined as the percentage of dogs with PVAS10 scores in the range of normal dogs at study end (PVAS10 < 2) (Olivry et al. 2018). Contrarily, failure was considered when PVAS10 scores were ≥ 2. Finally, some dogs were enrolled with “normal” PVAS10 scores (PVAS10 < 2). In this case, success was defined as remaining within “normal” PVAS10 scores at the end of the study and improving skin barrier assessments (TEWL and pH) on at least one of the body sites tested. Failure was considered when PVAS10 scores were ≥ 2.

The success of the CADESI-04 outcome measure for dogs with moderate to severe cAD at the study start (CADESI0-4 ≥ 35) was defined as the percentage of dogs with skin lesion score in the range of normal dogs or with mild cAD at study end (CADESI-04 < 35) (Olivry et al. 2018). For dogs enrolled whose CADESI-04 score fell into the mild cAD range (10 ≤ CADESI-04 ≤ 34), success was defined as the percentage of dogs with CADESI-04 scores in the range of normal dogs at study end (CADESI-04 <10). Contrarily, failure was considered when CADESI-04 scores were ≥ 10 (Olivry et al. 2018). Finally, some dogs were enrolled with “normal” CADESI-04 scores (CADESI-04 < 10). In this case, success was defined as remaining within “normal” CADESI-04 scores at the end of the study and improving skin barrier assessments (TEWL and pH) on at least one of the body sites tested. Failure was considered when CADESI-04 scores were ≥ 10.

The success of the OGATE outcome measures was defined as the percentage of dogs whose owner rated the overall response to treatment as “good” or “excellent” (OGATE ≥ 3) (Olivry et al. 2018). Failure of these outcome measures was considered as having a final OGATE score < 3.

Lastly, in order to further evaluate barrier repair the success of secondary outcomes measures was defined as the percentage of dogs whose TEWL and pH values significantly decreased at the end of the study.

3.2.5. Statistical tests

First, descriptive statistics were performed, obtaining absolute frequency and relative frequency for categorical variables and means, medians, standard deviation, 25th and 75th percentiles, and minimum and maximum values for each continuous variable. Regarding inferential statistics, a repeated-measures analysis of variance (ANOVA) was used to assess differences in the means of the CADESI-04, PVAS10, TEWL, and skin pH variables across two-time points (D0 - baseline and D30 - post-intervention). This test was chosen because it can account for the correlation between measurements taken from the same subjects at different time points, thereby controlling for within-subject variability. Assumptions of normality and sphericity were tested prior to the analysis. Normality was assessed using Shapiro-Wilk, and sphericity was evaluated using Mauchly's test. In cases where the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied to adjust the degrees of freedom. The statistical significance was set at a p-value of < 0.05. All statistical analyses were conducted using SPSS version 27 (IBM Corp., Armonk, NY).

3.3. Results

3.3.1. Sample characterization

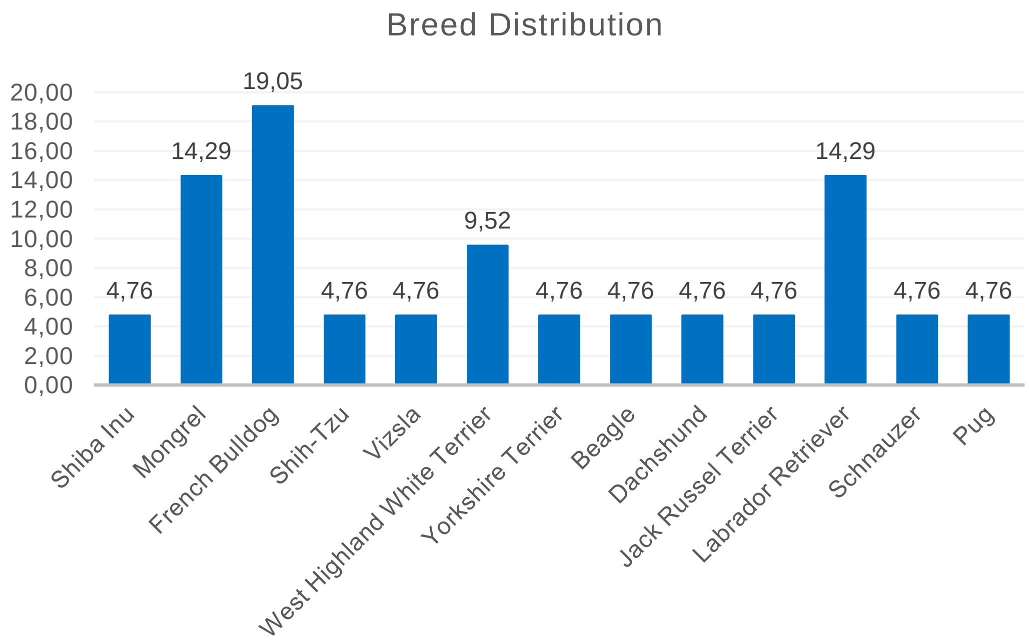
The present study recruited 27 privately owned dogs with controlled, spontaneous, non-seasonal cAD from the Dermatology Service of the HEV, FMV-ULisboa. All dogs met the inclusion criteria.

During the study, six dogs were excluded from the study. One mongrel (dog number 1) was excluded due to the development of an acute flare of cAD. One American Pit Bull Terrier (dog number 5) was excluded due to loss of follow-up. Two Jack Russel Terriers (dogs number 10 and 20) were excluded due to mild potential side effects. Finally, two dogs, a Weimaraner (dog number 14) and a Jack Russell Terrier (dog number 21) were excluded from the study due to lack of owners' compliance. These six dogs were removed from the statistical analysis.

The 21 remaining dogs successfully completed the study's proposed protocol and were included in the statistical analysis. Among the cohort of 21 dogs, 13 were males (61.90%) and 8 were females (31.10%). The mean age of the group was 4 years and 11 months (4.97 ± 2.88) with the minimum age being 1 year, the maximum being 11 years and the median 6 years. The 25th percentile was 2 years and the 75th 7 years of age.

Regarding dog breeds, 4 (9.05%) were French Bouledog, 3 (14.29%) were Labrador retriever, 3 (14.29%) were mongrels, 2 were West Highland White Terriers (9.52%) and the remaining 9 dogs corresponded to one breed including, Beagle, Dachshund, Jack Russell Terrier, Pug, Schnauzer, Shiba Inu, Shih Tzu, Vizsla, and Yorkshire Terrier. The breed distribution is represented in Graph 1.

Graph 1 – Bar graph representing enrolled dogs breed distribution.



3.3.2. Pruritus analysis

Pruritus in dogs was assessed by owners on both D0 and D30 using PVAS10. The recorded PVAS values from D0 and D30 are presented in Table 5.

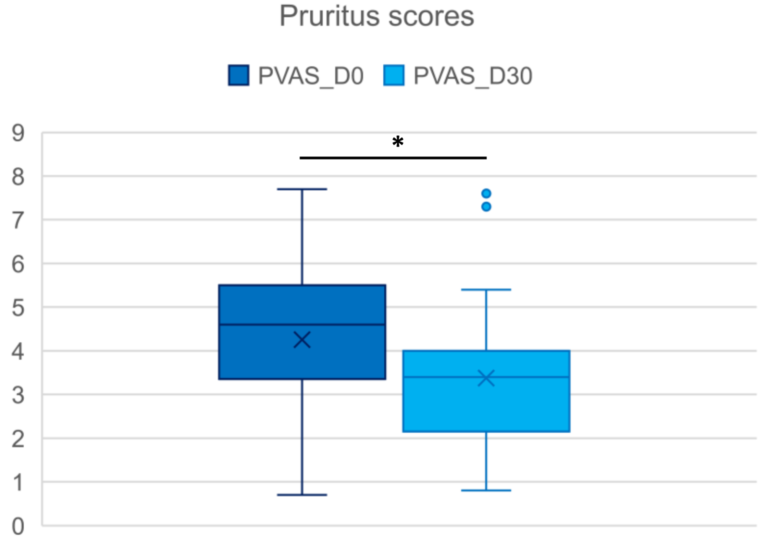
Table 5 - Pruritus values reported by owners on D0 and D30.

Dog ID	Pruritus – D0	Pruritus – D30
2	5,5	2,5
3	0,9	0,8
4	5,8	5,4
6	3,7	3,5
7	4,2	3,4
8	1,8	0,8
9	5,2	1,6
11	5,9	7,6
12	6,4	4,6
13	3,7	2,7
15	5,3	3,4
16	1,6	3,7
17	0,7	1,5
18	4,6	1,9
19	3,4	3,3
22	3,5	4,3
23	7,7	7,3
24	5,5	3,5
25	5,3	2,4
26	5,4	3,5
27	3,3	3,2

The mean PVAS10 value on D0 was 4.25 ± 1.85, with the minimum score being 0.70, the maximum being 7.70, and the median being 4.60. On D30, the mean PVAS10 value was 3.38 ± 1.79, with the minimum score being 0.80, the maximum being 7.60, and the median being 3.40.

A repeated-measures ANOVA was performed to analyze the variation in the mean PVAS10 values' from D0 to D30. A significant difference was found in PVAS10 mean values between the study's start and end ($p = 0.016$). A visual pruritus score evolution throughout the 30-day emollient plus application is represented in Graph 2.

Graph 2 – Boxplot of pruritus (PVAS10) scores evolution from D0 to D30; * - statistically significant; circles represent outliers.



Of the 21 enrolled atopic dogs, 17 (80.95%) improved pruritus scores after 30 days. The four (19.05%) who did not improve PVAS10 scores were dogs number 11, 16, 17, and 22, but only dogs 16 and 22 (9.52%) had worsened sufficiently to increase PVAS10 severity classification. For the dogs that showed improvement in PVAS10, the scores decreased by an average of 27%.

Pruritus scores were analyzed according to ICADA's COSCAD'18 guidelines (Olivry et al., 2018). Table 6 presents the distribution of dogs by severity category based on their PVAS10 scores, before and after the daily application of the topical emollient plus for 30 days. This color-coded table details the number and percentage of dogs within each pruritus severity category.

Table 6 – Distribution of dogs by pruritus (PVAS10) score severity on D0 and D30.

PVAS10

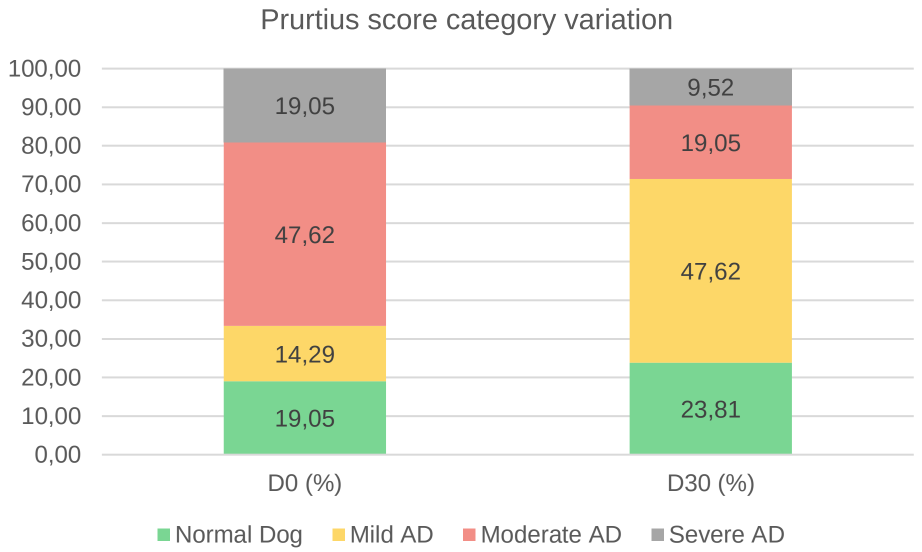
Severity Level	Value Range	D0	D0 (%)	D30	D30 (%)
Normal Dog	0.0 - 1.9	4	19.05	5	23.81
Mild AD	2.0 - 3.5	3	14.29	10	47.62
Moderate AD	3.6 - 5.5	10	47.62	4	19.05
Severe AD	5.6 - 10.0	4	19.05	2	9.52

Before the emollient plus application, 14 out of 21 dogs (66.67%) were included in the moderate-to-severe cAD score; 3 out of 21 dogs (14.29%) were in the mild cAD level; and 4 out of 21 (19.05%) dogs had PVAS10 values that would classify them as "normal dog" status.

After the 30-day emollient plus application, 6 out of 21 dogs (28.57%) were included in the moderate-to-severe cAD score; 10 out of 21 dogs (47.62%) were in the mild cAD level; and 5 out of 21 (23.81%) dogs had PVAS10 values that would classify them as “normal dog” status.

In order to better illustrate the change in PVAS10 scores between D0 and D30 for the cohort of dogs, Graph 3 was generated. Graph 3 presents the changes in sample distribution across each PVAS10 score severity category from D0 to D30.

Graph 3 - Changes in sample distribution across each atopic dermatitis (AD) pruritus severity score category from D0 to D30.



PVAS10 outcome measure success was individually analyzed for each atopic dog, considering the descriptions provided in section "3.2.4. Outcome measurements".

According to the ICADA’s COSCAD’18 (Olivry et al. 2018), the number and percentage of dogs that succeeded and failed the PVAS10 outcome evaluation after topical emollient plus daily application were calculated and are presented in Table 7. A total of 17 out of the 21 dogs (80.95%) qualified for this classification. At the end of the clinical trial, 10 of the 17 dogs (58.82%) successfully reduced their PVAS10 score.

Table 7 – Pruritus (PVAS10) score reduction success rate after treatment.

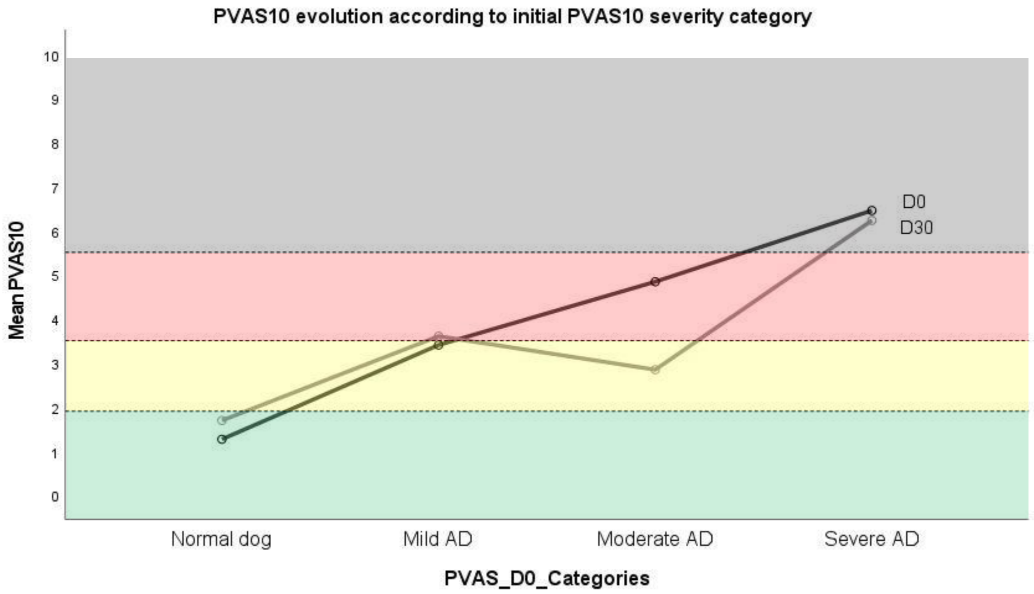
PVAS10	D30	D30 (%)
Success	10	58.82%
Failure	7	41.18%

Additionally, four dogs had "normal" PVAS10 scores at enrollment (dogs number 3, 8, 16, and 17). According to the descriptions provided in section "3.2.4. Outcome measurements", three (dogs number 3, 8, and 17) were classified as having a successful response to treatment, as they maintained their PVAS10 score severity category and improved skin barrier assessments in the pinna (Annexe 8). Dog number 16 was classified as a failure as its PVAS10 score was initially classified as "normal" and on D30, its pruritus increased to a "moderate" level.

A repeated-measures ANOVA was performed to analyze the interaction between the mean PVAS10 scores evolution from D0 to D30 according to the initial PVAS10 severity category. A statistically significant interaction was found ($p = 0.005$) between both variables. This interaction was probably caused by the more significant decrease in the moderate pruritus group. However, concluding that only the moderate group experienced pruritus improvement is unreasonable, as this deviation is likely coincidental. The overall trend suggests that the initial PVAS10 score did not influence the degree of improvement in pruritus at the end of the 30 days.

The graphic representation of PVAS10 evolution according to initial PVAS10 severity category is represented in Graph 4.

Graph 4 – Interaction between the mean atopic dermatitis' (AD) pruritus (PVAS10) scores evolution in time according to the initial PVAS10 severity category.



3.3.3. Extent and severity of skin lesions' analysis

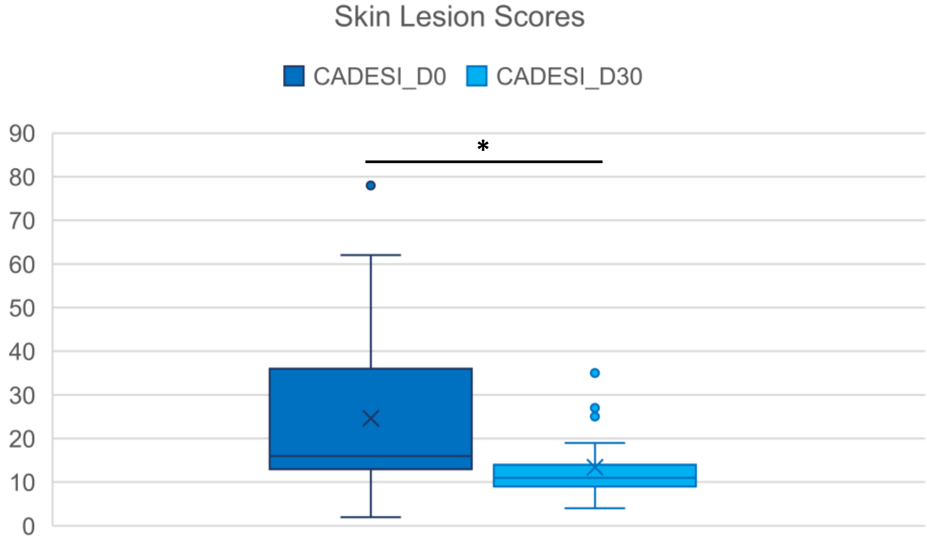
The HEV dermatology service clinicians assessed the extent and severity of skin lesions on both D0 and D30 using the validated CADESI-04.The recorded CADESI-04 values from D0 and D30 are presented in Table 8.

Table 8 – All skin lesion (CADESI-04) values recorded by the dermatology service veterinarians on D0 and D30.		
Dog ID	**CADESI–04 – D0**	**CADESI–04 – D30**
2	36	14
3	14	13
4	14	4
6	78	25
7	15	9
8	2	6
9	23	27
11	38	19
12	24	10
13	27	14
15	39	8
16	12	10
17	12	14
18	14	12
19	7	11
22	16	10
23	36	13
24	62	35
25	11	11
26	15	9
27	22	8

The mean CADESI-04 score on D0 was 24.62 ± 18.48, with the minimum score being 2.00, the maximum being 78.00, and the median being 16.00. On D30, the mean CADESI-04 value was 13.43 ± 7.44, with the minimum score being 4.00, the maximum being 35.00, and the median being 11.00.

A repeated-measures ANOVA was performed to analyze the variation in the mean CADESI-04 values from D0 to D30. A significant difference was found in CADESI-04 mean values between the study's start and end ($p = 0.02$). The evolution of the skin lesion's extension and severity score throughout the 30-day emollient plus application is represented in Graph 5.

Graph 5 – Boxplot of skin lesion scores (CADESI-04) evolution from D0 to D30; * - statistically significant; circles represent outliers.



Of the 21 enrolled atopic dogs, 16 (76.19%) improved skin lesion severity and extension scores after 30 days. The four (19.05%) who did not improve CADESI-4 scores were dogs 8, 9, 17, and 19, with only dog 19 (4.76%) increasing its cAD severity classification. Dog number 25 (4.76%) had the same CADESI-04 value on D0 and D30. For the dogs that showed improvement in CADESI-04, the scores decreased by an average of 47%.

CADESI-04 scores were analyzed according to ICADA's COSCAD'18 guidelines (Olivry et al., 2018). Table 9 presents the distribution of dogs by severity category based on their CADESI-04 scores, before and after the daily application of the topical emollient plus for 30 days. These color-coded tables detail the number and percentage of dogs within each skin lesions severity category.

Table 9 – Distribution of dogs by atopic dermatitis (AD) skin lesion scores (CADESI-04) severity on D0 and D30.

CADESI-04

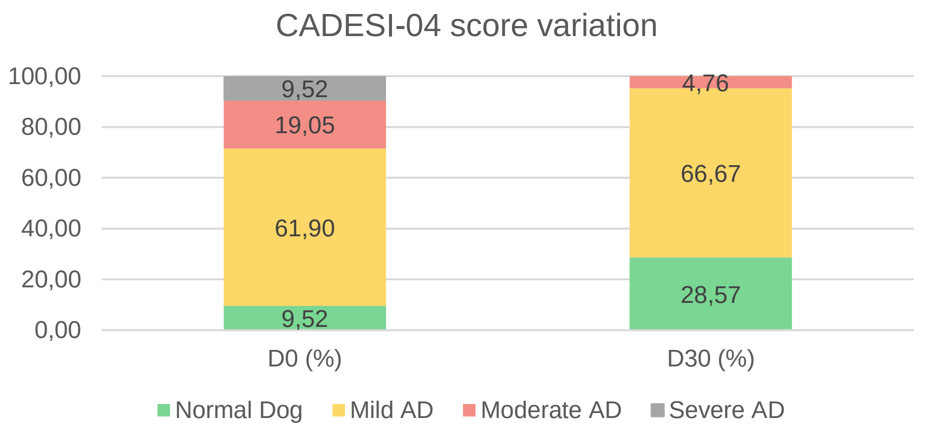
Severity Level	Value Range	D0	D0 (%)	D30	D30 (%)
Normal Dog	0 - 9	2	9.52	6	28.57
Mild AD	10 - 34	13	61.90	14	66.67
Moderate AD	35 - 59	4	19.05	1	4.76
Severe AD	60 - 180	2	9.52	0	0.00

Before the emollient plus application, 6 out of 21 dogs (28.57%) were included in the moderate to severe cAD score; 13 out of 21 dogs (61.90%) were in the mild cAD level; and 2 out of 21 (9.52%) dogs had CADESI-04 values that would classify them as "normal dog" status.

After the 30-day emollient plus application, no dogs were included in the severe cAD score. Only 1 out of the 21 (4.76%) was included in the moderate cAD severity score; 14 out of 21 dogs (66.67%) were in the mild cAD level; and 6 out of 21 (28.57%) dogs had CADESI-04 values that would classify them as "normal dog" status.

Graph 6 was created to effectively illustrate the change in CADESI-04 scores between D0 and D30 for the cohort of dogs. Graph 6 displays the sample distribution within each cAD severity category at both D0 and D30.

Graph 6 – Changes in sample distribution across each atopic dermatitis (AD) skin lesion scores (CADESI-04) severity category from D0 to D30.



CADESI-04 outcome measure success was individually analyzed for each atopic dog, considering the descriptions provided in section "3.2.4. Outcome measurements".

According to the ICADA's COSCAD'18 (Olivry et al. 2018), the number and percentage of dogs that succeeded and failed the CADESI-04 outcome evaluation after topical emollient plus daily application were calculated and are presented in Table 10. A total of 19 out of the 21 dogs (90.48%) qualified for this classification. At the end of the clinical trial, nine out of the 19 dogs (47.37%) successfully reduced their CADESI-04.

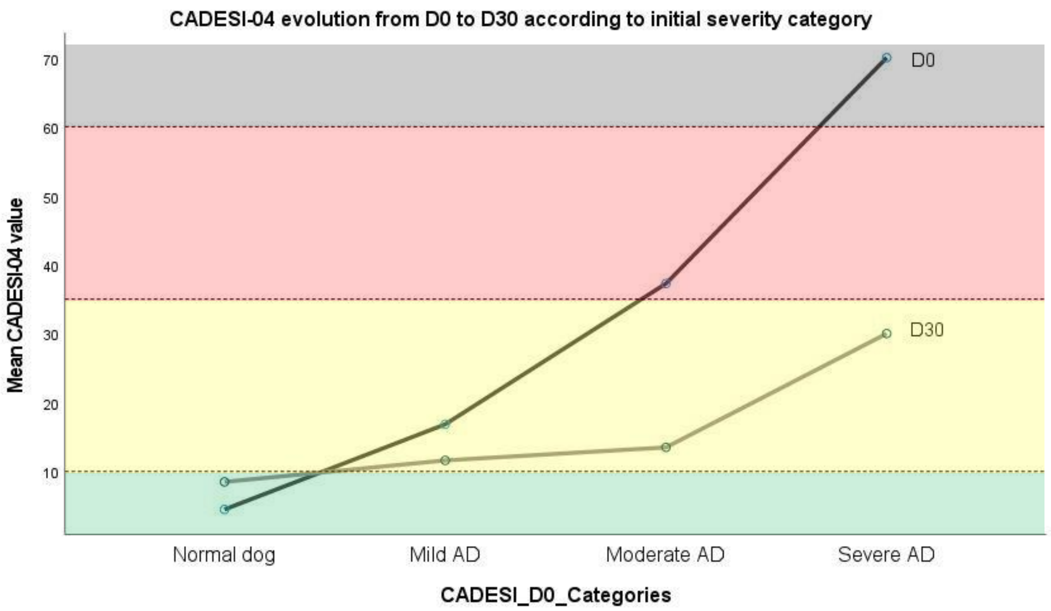
Table 10 – Skin lesion (CADESI-04) score reduction success rate after treatment.

CADESI-04	D30	D30 (%)
Success	9	47.37%
Failure	10	52.63%

Additionally, two dogs had "normal" CADESI-04 scores at enrollment (dogs number 8 and 19). According to the descriptions provided in section "3.2.4. Outcome measurements", one (dog number 8) was classified as having a successful response to treatment, as it maintained its CADESI-04 score severity category and improved skin barrier assessments in the pinna (Annexe 8). Dog number 19 was classified as a failure as its CADESI-04 score was initially classified as "normal" and on D30, its skin lesion severity slightly increased to a "mild" level.

A repeated-measures ANOVA was performed to analyze the interaction between the mean CADESI-04 scores evolution in time according to the initial CADESI-04 severity category. A statistically significant interaction was found ($p = 0.000006$) between both variables, revealing that the greater the severity of the skin lesions on D0, the more significant the improvements observed at D30. The graphic representation of CADESI-04 evolution according to initial CADESI-04 severity category is represented in Graph 7.

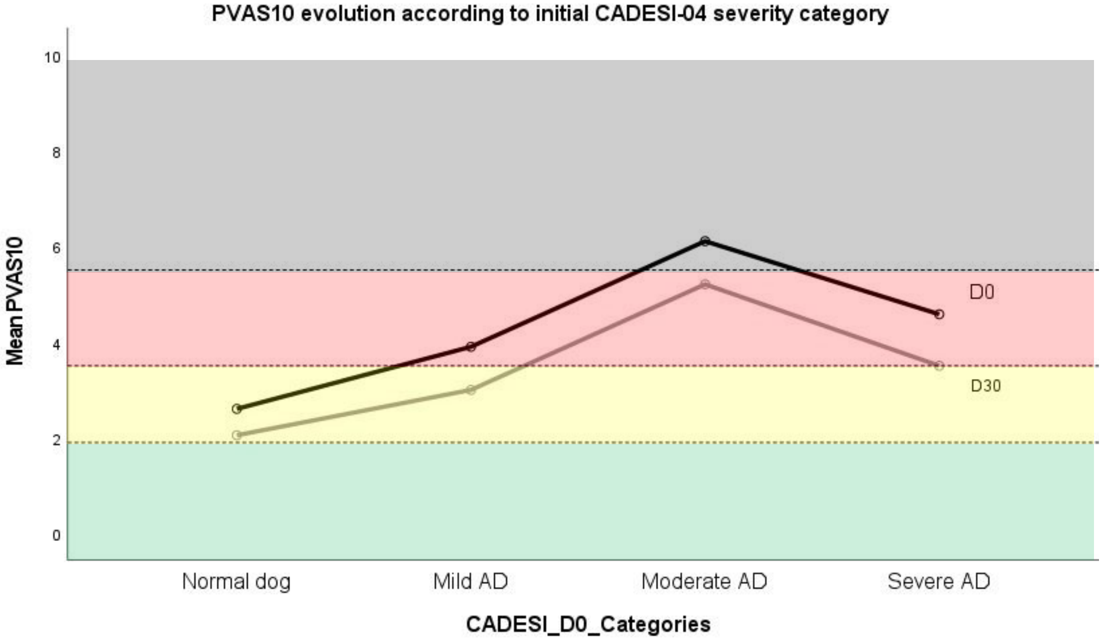
Graph 7 – Interaction between the mean atopic dermatitis (AD) skin lesion (CADESI-04) scores evolution in time according to the initial CADESI-04 severity category.



3.3.4. Pruritus and Skin lesion interaction

A repeated-measures ANOVA was performed to analyze the interaction between the mean PVAS10 scores evolution in time according to the initial CADESI-04 severity category. No interaction was found ($p = 0.99$) between both variables. The graphic representation of PVAS10 evolution according to initial CADESI-04 severity category is represented in Graph 8.

Graph 8 – Interaction between atopic dermatitis (AD) mean pruritus (PVAS10) scores evolution in time according to the initial skin lesion (CADESI-04) severity category.



3.3.5. TEWL analysis

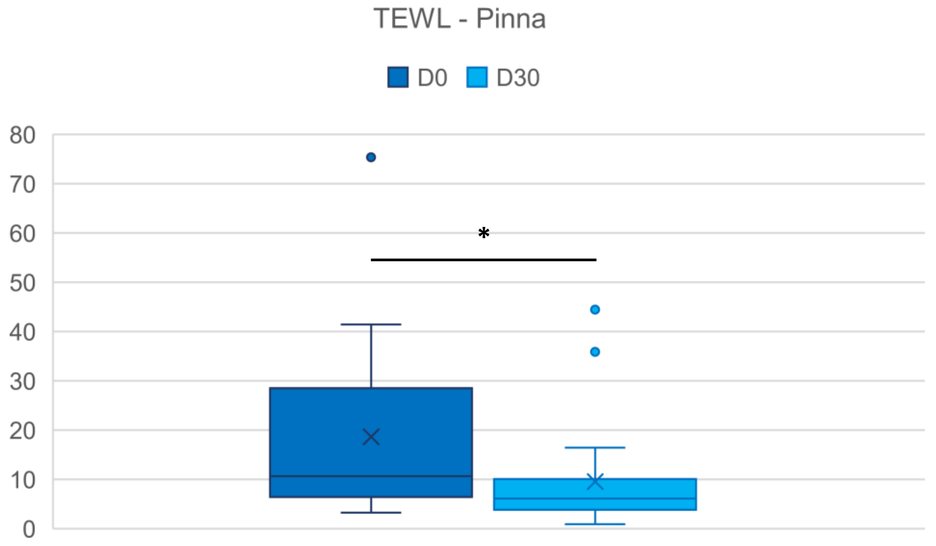
The TEWL was measured on D0 and D30 on the dog's inguinal area and the 3 concave surface of the pinna

3.3.5.1. TEWL analysis – Pinna

The mean TEWL values for all dogs on the pinna were 18.63 ± 17.33 g/m²/h on D0, with a minimum of 3.20 g/m²/h, a median of 10.62 g/m²/h and maximum of 75.34 g/m²/h. On D30, mean TEWL values decreased to 9.56 ± 10.75 g/m²/h, with a minimum of 0.92 g/m²/h, a median of 6.08 g/m²/h, and a maximum of 44.46 g/m²/h.

A repeated-measures ANOVA was performed to analyze the variation in the mean TEWL values' from D0 to D30. A significant difference was found in pinna TEWL mean values between the study start and end ($p = 0.049$). Pinna mean TEWL change between D0 and D30 is represented in Graph 9.

Graph 9 – Boxplot of transepidermal water loss (TEWL) value reduction from D0 to D30 on all the pinna; * - statistically significant; circles represent outliers.

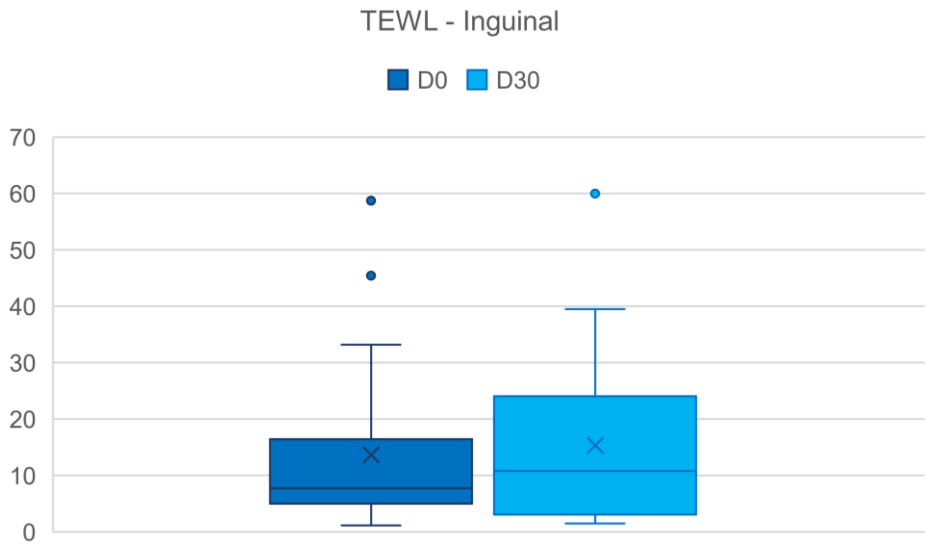


3.3.5.2. TEWL analysis – Inguinal

The mean TEWL values for all dogs on the inguinal region were 13.62 ± 14.59 g/m²/h on D0, with a minimum of 1.14 g/m²/h, a median of 7.74 g/m²/h and maximum of 58.73 g/m²/h. On D30, mean TEWL values increased to 15.32 ± 15.17 g/m²/h, with a minimum of 1.47 g/m²/h, a median of 10.79 g/m²/h, and a maximum of 59.98 g/m²/h.

A repeated-measures ANOVA was performed to analyze the variation in the mean TEWL values' from D0 to D30. The difference found in inguinal TEWL mean values between the study start and end was not significant ($p = 0.75$). Inguinal mean TEWL change between D0 and D30 is represented in Graph 10.

Graph 10 – Boxplot of transepidermal water loss (TEWL) value change from D0 to D30 on the inguinal area; circles represent outliers.



3.3.6. pH analysis

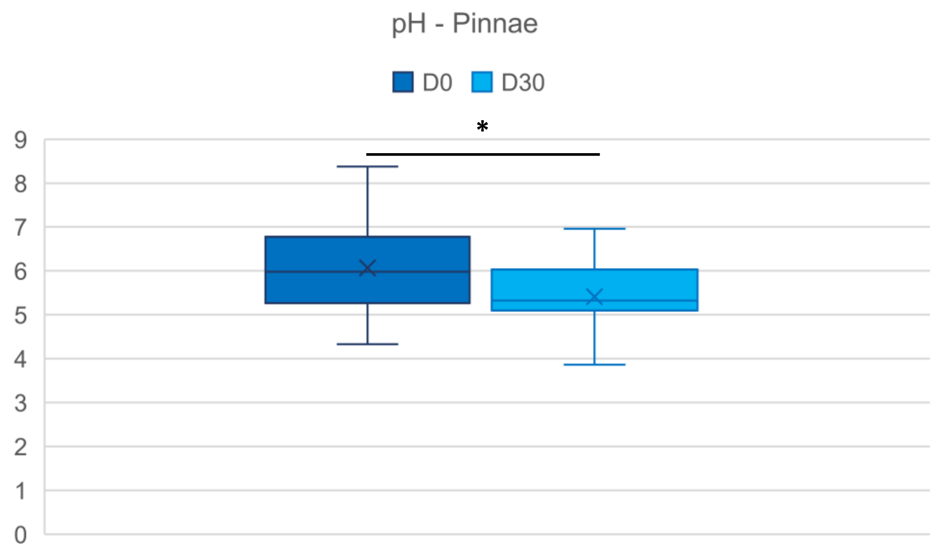
The pH was measured on D0 and D30 on the dog's inguinal area and concave surface of the pinna.

3.3.6.1. pH analysis – Pinna

The mean pH values for all dogs on the pinna were 6.07 ± 0.97 on D0, with a minimum of 4.33, a median of 5.98 and maximum of 8.38. On D30, mean pH values decreased to 5.41 ± 0.71, with a minimum of 3.86, a median of 5.32, and a maximum of 6.96.

A repeated-measures ANOVA was performed to analyze the variation in mean pH values from D0 to D30. A significant statistical difference was found in pinna pH mean values between the study start and end ($p = 0.01$). Pinna mean pH change between D0 and D30 is represented in Graph 11.

Graph 11 – Boxplot of pH value reduction from D0 to D30 on the pinna; * - statistically significant.

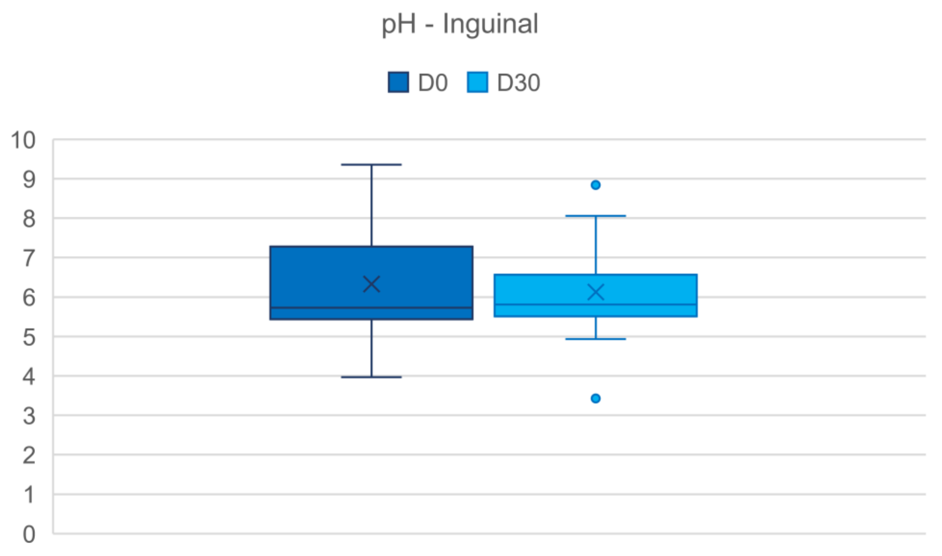


3.3.6.2. pH analysis – Inguinal

The mean pH values for all dogs on the inguinal area were 6.33 ± 1.24 on D0, with a minimum of 3.97, a median of 5.73 and maximum of 9.36. On D30, mean pH values decreased to 6.12 ± 1.13, with a minimum of 3.43, a median of 5.81, and a maximum of 8.84.

A repeated-measures ANOVA was performed to analyze the variation in mean pH values from D0 to D30. The difference found in inguinal pH mean values between the study start and end was not statistically significant ($p = 0.54$). Inguinal mean pH change between D0 and D30 is represented on Graph 12.

Graph 12 – Boxplot of pH value reduction from D0 to D30 on the inguinal area; circles represent outliers.



3.3.7. Dermatoscopic evaluation results

For visual access of skin barrier integrity, a dermatoscopic examination was performed on D0 and D30 on the pinnae and inguinal skin area by one of the clinicians from the HEV's dermatology service.

The following figure, Figure 4, presents dermatoscope images from dog number 11 on D0 and D30. D0 image illustrates rough and scaling skin with erythema, while the D30 image shows a reduction in erythema, with the skin appearing less inflamed, visibly shinier, and more hydrated.

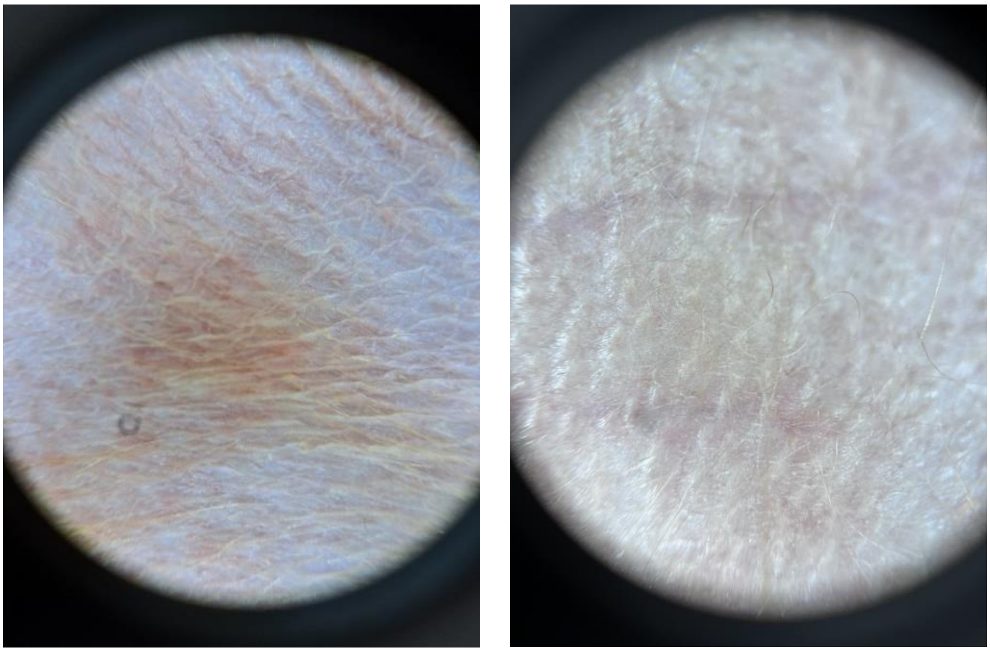


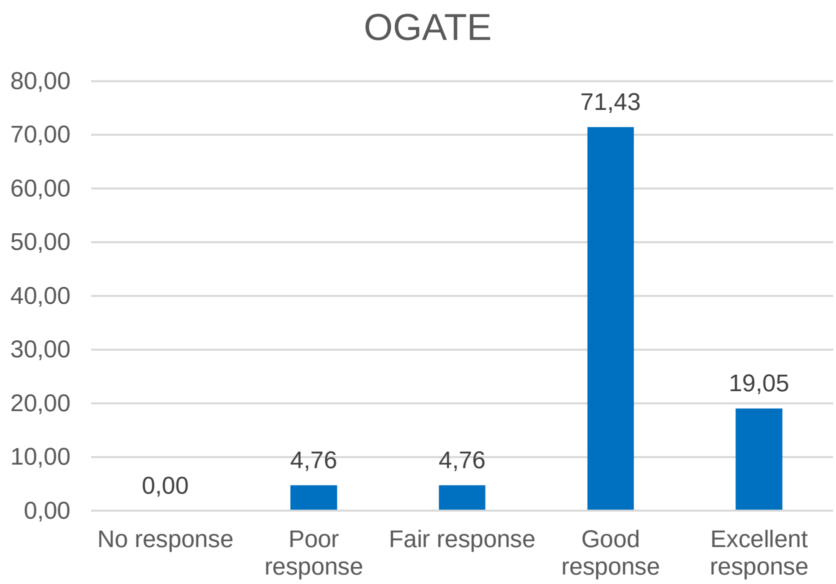
Figure 4. Dermatoscopic pictures of skin on D0 (left) and D30 (right) (original photos).

3.3.8. Owner's overall assessment of efficacy of the topical emollient plus

The efficacy of the topical emollient perceived by owners was assessed on D30 using an unvalidated subjective five-point scale, the OGATE.

After the 30-day daily application of the emollient plus, 19 out of the 21 owners (90.48%) rated the treatment response as ≥ 3 ("good-to-excellent response"). The other two ratings included one owner (4.76%) who classified the topical emollient plus efficacy as OGATE = 2 ("fair response") and, lastly, one owner (4.76%) who classified the treatment response as "poor" (OGATE = 1). Graph 13 illustrates the distribution of the OGATE scores.

Graph 13 – Bar graph representing de distribution of owner's assessment of treatment efficacy (OGATE) scores reported on D30 in relative frequencies.



3.3.9. Cosmetic scores analysis

At the end of the study, dog owners were asked to complete an original two-part questionnaire to provide their views on the cosmetic properties of the topical emollient plus.

The mean scores for the first section of the cosmetic evaluation (refer to section 3.2.3.4 – Cosmetic Evaluation) are compiled in Table 11 and illustrated in Graph 14 for clarity.

Table 11 – Cosmetic score: section 1 mean answers (1-10)	
Affirmations	**Mean score ± SD**
The product has good appearance	9.38 ± 1.36
The product has a pleasant smell	9.29 ± 1.28
The product application was easy and simple	9.00 ± 1.46
Applying the product was pleasant to me	8.67 ± 1.89
Applying the product was tolerated by my dog	8.95 ± 1.46
I would not mind to include this product in my dog treatment plan	9.19 ± 1.10
I would recommend this product to other owners of atopic dogs	9.03 ± 1.33

Graph 14 – Radar chart of the mean cosmetic evaluation (section 1).

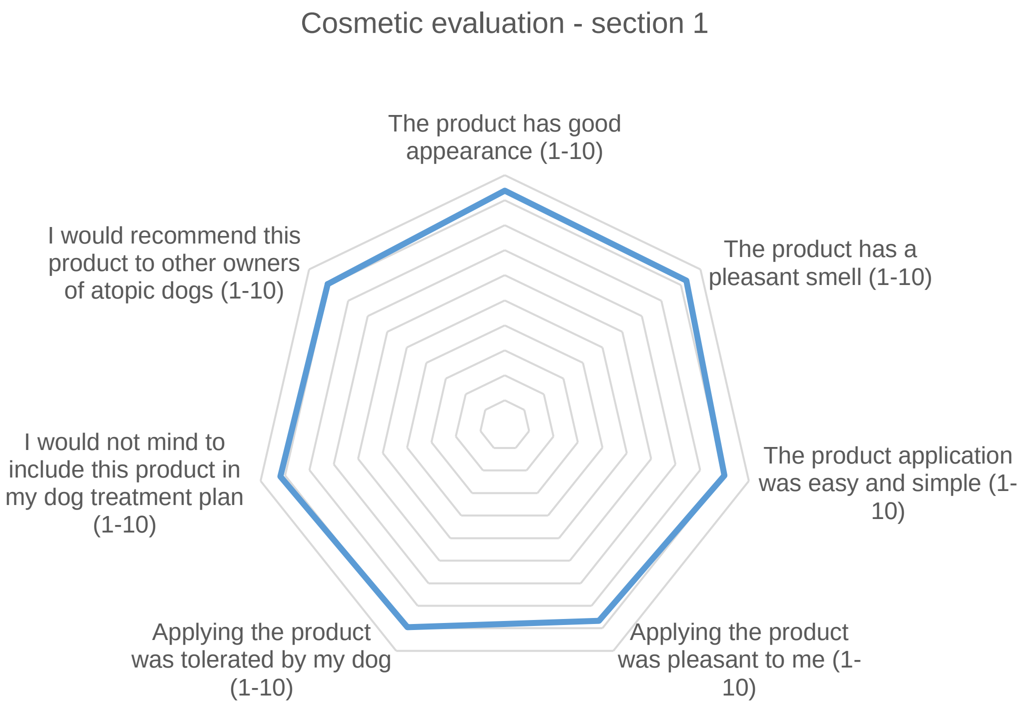
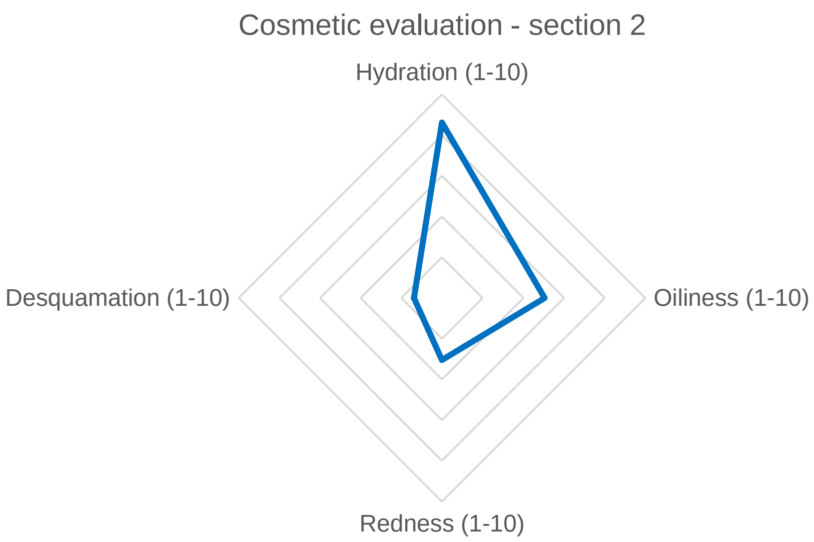


Table 12 shows the average scores for the second part of the cosmetic evaluation (see Section 3.2.3.4), and Graph 15 illustrates these scores for better visual comprehension.

Table 12 – Cosmetic score: section 2 mean answers (1-10)	
Affirmation – After application, classify your dog's skin:	**Mean score ± SD**
Hydration	8.62 ± 1.21
Oiliness	5.05 ± 3.06
Redness	3.05 ± 2.68
Desquamation	1.38 ± 1.21

Graph 15 – Radar chart of the mean cosmetic evaluation (section 2).



3.3.10. Reported adverse effects

Adverse side effects potentially associated with the topical emollient were documented in 2 out of 27 dogs (7.40%), specifically in two Jack Russell Terriers, identified as dogs number 10 and 20.

Dog number 10 exhibited owner-reported hyperpigmentation-like lesions in the inguinal and pinna regions. Due to the loss of follow-up and the owner's unavailability, neither photographic documentation nor in-clinic re-evaluation could be pursued.

Dog number 20 presented with an erythematous rash in the inguinal region that subsided after three days of treatment interruption. Upon re-exposure to the emollient, the symptoms recurred. The owner was subsequently advised to cease the application of the topical emollient. The lesions observed in this subject are illustrated in Figure 5.

Figure 5. Erythematous rash in the inguinal region of dog number 20 after the application of the topical emollient plus (original photo).

4. Discussion

The present proof-of-concept pilot study, integrated within the PhD research conducted by Beatriz Amaral Pinto Fernandes (FCT fellowship 2021.05985.BD), was the first clinical study evaluating the efficacy and safety of this novel and proprietary topical emollient plus formulation as an adjuvant in the treatment of chronic cAD. To the authors' knowledge, this was also the first study in the veterinary medicine field that considered the owner's assessment of the treatment efficacy and cosmetic evaluation of a topical emollient concurrently with the traditionally assessed clinical outcomes.

In our view, these promising preliminary results could represent a first step in moving this treatment option from "bench to bedside". However, due to proprietary considerations and the need to protect potential future business opportunities, this novel product's complete formulation and detailed ingredient list cannot be disclosed. This confidentiality aims to preserve intellectual property and competitive advantage. Considering this, certain citations included in this discussion cannot be disclosed and will be denoted by an asterisk (*) symbol.

cAD's pathogenesis is not yet fully understood; however, skin barrier dysfunction is an established hallmark of this skin condition (Santoro et al. 2015; Outerbridge and Jordan 2021; Santoro et al. 2024). Therefore, restoring skin barrier function and integrity is fundamental in atopic dogs (Olivry et al. 2015).

Evidence pertaining to topical emollient plus use in dogs is scarce, and according to the best of the author's knowledge, only five different emollients have been studied. Of the five different topical emollients evaluated in previous research, only four have been tailor-made for atopic dogs and only three are commercially available. None are available in spray form. Additionally, the efficacy of these topical emollients in supporting clinical outcomes and skin barrier function has been reported to yield mixed results (Piekutowska et al. 2008; Fujimura et al. 2011; Tretter and Mueller 2011; Popa et al. 2012; Jung et al. 2013; Blaskovic et al. 2014; Marsella et al. 2017; Marsella et al. 2020; Idée et al. 2022; Segarra et al. 2023). Consequently, developing our novel topical emollient plus, specially tailored for atopic dogs with evidence-based bioactive ingredients, aimed to fill the knowledge and, potentially, market gaps around topical emollient usage in dogs.

Considering this, it was assumed that strengthening the enrolled atopic dog's skin barrier via the topical emollient plus application could promote better skin barrier health and, consequently, improve cAD clinical signs.

Therefore, 21 dogs underwent daily emollient plus topical application on cAD-affected body sites for 30 days. The only change in treatment protocols in the cohort of atopic dogs was the addition of the topical emollient. This approach is widely used and accepted in the scientific community regarding cAD, with several cAD studies published in high-impact scientific journals following similar treatment restrictions to the ones used on this dissertation (Deboer et al. 2016; Timm et al. 2018; Pinto et al. 2024). Furthermore, as a pilot proof-of-concept study and by the principles of the 3Rs – replacement, reduction, and refinement in ethical experimentation – it would not be justifiable to use additional animals to establish control groups in a preliminary clinical evaluation.

Given that the only treatment plan modification was the addition of the emollient plus, this may suggest that the improvement in analytical and clinical outcomes may be linked to the topical emollient plus.

4.1. Skin barrier function and integrity improvements

TEWL is the most common measurement for objectively assessing skin barrier integrity in humans and dogs (Cobiella et al. 2019). Elevated TEWL values have been described in atopic dogs, indicating increased permeability and barrier dysfunction in their skin (Shimada et al. 2008; Shimada et al. 2009; Bradley et al. 2016). This study obtained TEWL values from the pinna and the inguinal region of atopic dogs, before and after applying an emollient plus, with both body sites having contradictory results.

After the 30-day topical emollient application, the mean TEWL in the pinna region decreased significantly ($p = 0.049$) from 18.63 ± 17.33 $g/m^2/h$ to 9.56 ± 10.75 $g/m^2/h$, a finding consistent with previous literature (Tretter and Mueller 2011; Blaskovic et al. 2014; Idée et al. 2022), suggesting improved skin barrier function and integrity. Since the only treatment change in the enrolled dogs was the addition of the topical emollient, the reduction in pinna's TEWL values may be related to the daily application of this product.

The statistically significant reduction in TEWL values in the pinna may be linked to various hypotheses. First, the topical emollient might have replenished the lipid matrix in the SC (Piekutowska et al. 2008; Popa et al. 2012; Marsella et al. 2020). Second, non-disclosed bioactive ingredients included in this formula can stimulate the endogenous production of SC lipids (*). An increased level of SC lipids can improve skin barrier cohesion, which may have contributed to a reduction in TEWL levels. Third, this novel product included humectants and occlusives that combined can improve the skin barrier's water-holding capacity and consequently reduce TEWL (Goh et al. 2022). These mechanisms, individually or combined, may have impacted TEWL levels.

Contrarily, the mean TEWL values measured in the inguinal area showed a non-statistically significant ($p = 0.75$) increase from D0, 13.62 ± 14.59 g/m^2/h, to D30, 15.32 ± 15.17 g/m^2/h. This reported but unexpected finding is also consistent with previous literature (Tretter and Mueller 2011; Hobi et al. 2017; Marsella et al. 2017; Marsella et al. 2020). Numerous reasons may explain the failure to decrease TEWL in the inguinal region.

Firstly, individual dogs vary in their clinical signs and responses to treatment (Drechsler et al., 2024).

Secondly, the home-application of the topical emollient may have resulted in application errors and inconsistent compliance, especially in a harder-to-reach area like the inguinal region. Additionally, the absence of guidelines for the optimal application frequency suggests that more frequent use may be necessary for some dogs and/or body areas.

Thirdly, compared to the concave surface of the pinna, the inguinal region is more occluded, has higher humidity levels, and is exposed to higher friction. This may contribute to higher levels of skin barrier dysfunction, which might require longer treatment periods to show analytical improvements. Additionally, the inguinal area SC has been shown to be slightly thicker than the pinna's SC (Theerawatanasirikul et al. 2012). Considering that the SC and its dynamic structure are responsible for the skin's barrier function, impermeability, and protective capacities, the slightly thicker SC of the inguinal area might have limited the penetration of the emollient plus bioactive ingredients, consequently reducing its benefits. Another plausible factor could be the increased exposure of the inguinal region to cleaning products used in everyday home settings, for example, when the dogs lie down. These products have been shown, even in small quantities, to disrupt the skin barrier and trigger inflammation in the skin (Rinaldi et al. 2024).

Fourthly, although TEWL is considered the gold standard measurement for skin barrier assessment, it has several limitations. The values obtained from the TEWL meter vary from body site to body site. They are affected by external factors like the weather, the room temperature and humidity, the subject's movement, the amount of hair in the analyzed body area, the device used, and the operator himself (Shimada et al. 2008; Shimada et al. 2009; Cobiella et al. 2019).

Since the recruitment for this project took place from May 2023 to May 2024, the temperature and humidity in the dermatology service's consultation room may have slightly varied throughout the study, possibly influencing the obtained TEWL values. However, these variations are unlikely to affect the inguinal region exclusively.

Additionally, due to the anatomical characteristics of the inguinal region, every time a subject moved, it was harder to maintain the correct position of the TEWL device's probe when compared to measuring TEWL in the dog's pinna. These practical constraints and covering of the TEWL probe may have affected the readings in the inguinal area, particularly because an open-chamber TEWL device was used (Shimada et al. 2008; Shimada et al. 2009).

Furthermore, the dog's inguinal region is typically more densely-haired than the pinna's concave surface. Previous literature has shown that in regions with a thicker hair coat, the hair strains can absorb moisture and alter TEWL values (Shimada et al. 2009).

Lastly, ensuring that the same individual conducted TEWL measurements consistently was only sometimes possible due to scheduling conflicts and personal commitments. This might have impacted TEWL value assessment since TEWL measurement devices have high inter-operator variability and low repeatability (Cobiella et al. 2019).

To the best of the author's knowledge, the mean TEWL values reported for healthy dogs vary from 4.6 to 34.38 $g/m^2/h$ in the inguinal area and 5.5 to 35.5 $g/m^2/h$ in the pinna (see Table 1 in section 2.2.4.1. TEWL). For atopic dogs, the TEWL values are between 6.58 and 91.62 $g/m^2/h$ in the inguinal region and 9.64 and 45.23 $g/m^2/h$ in the pinna. These values were derived from the author's bibliographic research and compilation and are not standardized values recognized by peers, as such standards do not currently exist.

Notably, despite atopic dogs consistently exhibiting higher upper-limit TEWL values, there is considerable overlap between the TEWL values of atopic and normal dogs. This overlap suggests that dogs with identical TEWL values could be categorized as having either normal or elevated TEWL. This observation may imply that TEWL, as a standalone measurement, may only be somewhat reliable for assessing dog skin barrier function.

Although different TEWL devices were used across existing studies, a recent systematic review suggests that TEWL values obtained with different devices show good reliability and frequently correlate across studies (Klotz et al. 2022). However, existing literature mostly reports mean TEWL values and not full ranges of TEWL measurement, limiting direct comparison with the minimum, maximum, and median values obtained in this study.

A preliminary review of the data indicates that the TEWL values obtained in this study generally align with those reported in the literature, particularly for the pinna, where D0 values resembled the atopic range and D30 values approached those of healthy dogs. However, further research and the development of internationally accepted, standardized reference values are needed to ensure more robust conclusions.

Recently, pH has been proposed as a more reliable indicator for assessing skin barrier function and integrity due to its lower intra- and inter-operator variability compared to TEWL (Cobiella et al. 2019). The physiological pH of the dog's skin has been described as being in the range of 4.84 to 9.95, with various factors that can influence it (Miller et al. 2012). Although limited, the literature assessing the dog's skin pH has shown that atopic dogs tend to have slightly more alkaline pH values when compared to healthy dogs (Miller et al. 2012; Pin et al. 2014; Cobiella et al. 2019).

In this study, applying the topical emollient for 30 days resulted in a statistically significant decrease ($p = 0.01$) in pH levels in the pinna, indicating an improvement in the skin's barrier function. On D0, the average pH value for the pinna was 6.07 ± 0.97; on D30, this mean value was significantly reduced to 5.41 ± 0.71. This reduction in pH is consistent with the expected outcome of using emollients in humans (Wollenberg et al. 2018; Goh et al. 2022; Sidbury et al. 2023), which often include pH-adjusting components aimed at restoring a more balanced skin environment.

However, the pH levels in the inguinal region decreased, but not statistically significantly after treatment, suggesting regional variation in the skin's response to the topical emollient. On D0, the average pH value for the inguinal region was 6.33 ± 1.24; on D30, this mean value was reduced to 6.12 ± 1.13.

The lack of significant improvement in this area could be attributed to several factors. First, the inguinal region is anatomically different from the pinna, being more occluded and exposed to friction and moisture, which may hinder the efficacy of the emollient in restoring skin barrier function. The inguinal region may also require more extended treatment periods or a more frequent application to achieve the same therapeutic effect as observed in the pinna. Additionally, application errors, inconsistent compliance, especially in the harder-to-reach inguinal region, and the lack of guidelines for the optimal topical emollient application frequency might have hindered the inguinal results. Although not always significant, the mean pH values decreased in both body sites tested. This reduction may be attributed to several hypotheses.

First, the topical emollient may have contributed to directly restoring the lipid matrix in the SC (Piekutowska et al. 2008; Popa et al. 2012; Marsella et al. 2020) or indirectly, through the action of undisclosed bioactive ingredients (*). Secondly, the formulation's inclusion of prebiotic ingredients might have stimulated the local microbiome's lactic acid production (*).

Indirectly, via its barrier-repairing properties, the topical emollient might have promoted the decrease in pH due to the higher water and lipid content of the SC that, in turn, facilitates the release and spread of free amino acids (Ziboh et al. 2000; Sur et al. 2008; Chon et al. 2015; Allais and Friedman 2020; Capone et al. 2020; Liu-Walsh et al. 2021; Lisante et al. 2023). Consequently, the lower pH likely enhanced the SC lipid synthesis, improving skin barrier integrity and hydration.

Curiously, the mean pinna and inguinal area pH values of the enrolled atopic dogs fell into the reported range of pH values for healthy dogs in the same regions on D0 (see Table 3 in section 2.2.4.2 pH). On D30, the mean inguinal pH values also fell into the healthy reported range, and the mean pinna pH values were lower than the reported healthy range (see Table 3 in section 2.2.4.2 pH). All collected pH values fell into the reported physiologic range on canine skin.

In contrast, human skin pH is much more stable, with skin pH values ranging between 4 and 6 and most of the population being in the lower part of that range (Ali and Yosipovitch 2013). It is also known that the skin pH of humans has been extensively studied, compared to dog skin pH (Cobiella et al. 2019; Schlake et al. 2022) and that the literature reporting values for dog skin pH is limited (Schlake et al. 2022). Since the considered physiologic pH range for the dog's skin is so ample (4.84 to 9.95) (Miller et al. 2012), the comparison between healthy and atopic dogs is difficult since a dog with a healthy and functional skin barrier might have higher SC pH values compared to an atopic dog. This may suggest that pH, likewise TEWL, might be limited in its utility when considered in isolation, without considering other skin barrier parameters and clinical signs. More comprehensive research is needed to address healthy and atopic dog skin pH to create more reliable pH ranges.

The TEWL and pH response variability highlights the complexity of treating cAD and underscores the need for further research on skin barrier integrity and function in dogs. While the reduction in TEWL and pH in the pinna is promising, it is essential to investigate whether this effect can be replicated consistently across other anatomical sites and in different severities of cAD and what the ideal application frequencies are on various body regions.

The reduction in TEWL and pH values and consequent skin barrier improvements may have contributed to the overall enhancements in pruritus and skin lesion scores reported in section 3.3 – Results. Evidence shows that barrier dysfunction, with consequent higher TEWL and pH values, can lead to drier skin, which may exacerbate cAD clinical signs like pruritus and inflammatory responses, predisposing atopic dogs to an exacerbation of their condition (Olivry et al. 2015; Drechsler et al. 2024).

4.2. Clinical improvements

This study assessed two significant clinical signs of cAD to evaluate the clinical impact of the topical emollient plus on pruritus and skin lesions. Pruritus is the hallmark clinical sign of cAD and is one of the primary factors leading to a reduced quality of life in atopic dogs and their owners (Outerbridge and Jordan 2021; Segarra et al. 2023). Skin lesions of atopic dogs are a visible manifestation of these patients' chronic inflammation and barrier dysfunction (Olivry et al. 2015; Eisenschenk et al. 2024).

On D0, the mean pruritus score across the cohort was 4.25 ± 1.85, reflecting moderate to severe pruritus in most dogs. By D30, the mean PVAS10 score decreased to 3.38 ± 1.75, showing an average 27% reduction in overall pruritus in dogs that improved their PVAS10 scores. This decrease was statistically significant ($p = 0.016$), suggesting that the topical emollient might affect pruritus.

Notably, 80.95% of the dogs improved their PVAS10 scores, with 61.9% meeting the success criteria defined by ICADA's COSCAD'18 guidelines.

At the end of the study, the number of dogs classified as having "normal" or "mild" pruritus increased notably, with 71.43% of the cohort falling into these categories at D30, compared to only 33.34% at baseline.

These results are consistent with previous literature showing significant improvement in atopic dog pruritus after treatment with topical emollients (Tretter and Mueller 2011; Blaskovic et al. 2014; Marsella et al. 2017; Marsella et al. 2020).

At enrollment, the mean CADESI-04 score across the cohort was 24.62 ± 18.48, indicating mild skin lesion severity in the enrolled dogs. By the end of the 30-day treatment period, the mean CADESI-04 score had decreased to 13.43 ± 7.44, representing a 47.70% reduction in lesion severity among the dogs that improved their CADESI-04 scores. This decrease was statistically significant ($p = 0.02$), suggesting that the topical spray may have led to a meaningful clinical improvement. By D30, 76.19% of the dogs improved their CADESI-04 scores, highlighting the emollient's effectiveness in managing the dermatological manifestations of cAD.

At the end of the study, the number of dogs classified as having "moderate" or "severe" pruritus decreased notably, with 4.76% of the cohort falling into these categories at D30, compared to 28.57% at baseline – representing approximately a six-fold reduction.

Curiously, the topical emollient plus used in this project did not have pharmaceuticals that specifically and robustly address pruritus and skin inflammation. However, this formulation included bioactive ingredients that may act on these clinical signs. Consequently, the significant change in PVAS10 and CADESI-04 scores in most dogs may be due to direct and/or indirect mechanisms of action in this assessment.

As previously mentioned, non-disclosed bioactive ingredients have been described to have anti-pruritic and anti-inflammatory properties (*). Other bioactives included may also act directly on pruritus due to their ability to diminish neurogenic inflammation (*) and on inflammation due to its anti-inflammatory compounds (*) and through its barrier-repairing, prebiotic, emollient, and buffering properties (*). Other non-disclosed ingredients also present similar capacities. Therefore, the reduction in pruritus might be directly linked to the effects of these bioactive ingredients. However, the evidence supporting these properties in the specified ingredients was not obtained from studies on atopic dogs. Thus, this hypothesis may need to be interpreted cautiously, as it might not fully apply to this population.

Indirectly, the topical emollient plus may have decreased PVAS10 and CADESI-04 scores through various interlinked mechanisms.

As previously shown, topical emollient application can increase SC lipid production and promote increased SC organization (Piekutowska et al. 2008; Popa et al. 2012; Marsella et al. 2020), restoring the skin's barrier. This may reduce pruritus and inflammation due to reduced skin allergen penetration and higher SC hydration levels (Marsella 2013). Reducing skin inflammation has been linked to improving skin barrier function and reducing pruritus (Marsella 2013; Pin et al. 2014; Olivry et al. 2015; Nuttall et al. 2019; Drechsler et al. 2024; Santoro et al. 2024), which in turn, may represent another mechanism of action from which the topical emollient plus might have reduced pruritus levels in enrolled dogs.

Graph 8 (see Graph 8 in 3.3.4 Pruritus and Skin lesion interaction) shows a remarkably similar decrease in pruritus levels ($p = 0.99$) across all initial CADESI-04 score categories, indicating a consistent treatment effect. Interestingly, dogs with higher initial CADESI-04 scores experienced significantly greater reductions in CADESI-04 scores throughout the study than those with milder initial clinical signs as seen in Graph 7 (see Graph 7 in section 3.3.3 Extent and severity of ski lesions' analysis). However, this pattern was not observed in the PVAS10 score reduction on D30, which did not correlate with initial PVAS10 scores (see Graph 4 in section 3.3.2 Pruritus analysis).

Given that inflammation is typically a major driver of pruritus in cAD (Marsella 2013; Pin et al. 2014; Olivry et al. 2015; Nuttall et al. 2019; Drechsler et al. 2024; Santoro et al. 2024), the lack of correlation between the reduction of CADESI-04 and PVAS10 scores may suggest that factors other than the direct anti-inflammatory effects of the emollient plus may influence the recorded reduction in pruritus.

Consequently, the topical emollient's barrier-repairing properties and the above-mentioned positive consequences of increased skin barrier integrity may be more critical in reducing pruritus than the product's anti-inflammatory activity. This hypothesis aligns with the statistical analyses presented in Graph 8 (see Graph 8 in 3.3.4 Pruritus and Skin lesion interaction), which support the idea that skin barrier improvement may be the critical factor in the observed pruritus reduction.

Although pruritus and skin lesion scores significantly decreased from D0 to D30, not all enrolled dogs decreased these assessments with topical emollient use. Four dogs increased their PVAS10 score, but only two increased their PVAS10 severity classification, and four dogs increased their CADESI-04 scores, with only one dog experiencing a worsening of its CADESI-04 score severity category. Notably, this subject, dog number 19, barely increased its CADESI-04 scores. On D0, it had a CADESI-04 score of 7, classified as a "normal" dog, and on D30, its score increased to 11, the second value included in the "mild" cAD range.

Various factors may have contributed to the different responses to treatment.

Firstly, it is recognized that individual dogs exhibit varying clinical signs and may respond differently to the same treatment (Drechsler et al., 2024). Consequently, the dogs that did not improve their pruritus or skin lesion severity may have had a refractory response to the emollient plus based on their characteristics.

Secondly, the topical emollient was always applied at home without supervision. Although the application of the emollient was explained and demonstrated at enrollment, owners were encouraged to rehearse the application in front of the dermatology service veterinarians in order to prevent any compliance errors in the emollient application, either its frequency, quantity, or form, errors in owner emollient application and compliance might have hindered the results.

Thirdly, in the specific case of the PVAS10 scores, there were instances where different owners brought the same dog on D0 and D30. In some cases, it was apparent that certain owners were less familiar with their pet's behavioral routines, and it was clearly stated that the other owners would have been more accurate in their responses. Furthermore, there were situations where couples disagreed significantly on the dog's placement on the PVAS10 scale, highlighting that not all owners have the same sensitivity or perception regarding their pet's pruritus levels. Even though the PVAS10 scale has been validated and demonstrated to be reliable and repeatable, even when scores are obtained from different owners (Hill et al. 2007; Rybníček et al. 2009), this might have impacted the PVAS10 scores obtained in some dogs.

Lastly, no established guidelines currently exist to recommend the optimal frequency for topical emollient application (Hobi et al. 2017), suggesting that more frequent and/or longer application regimens may be required in some dogs.

4.3. Owner's assessment of the treatment efficacy

The OGATE is an unvalidated questionnaire that aims to provide insights into how owners perceived the overall effectiveness of treatment (Olivry et al. 2018). According to the ICADA's COSCAD'18 guidelines, OGATE success was defined as the percentage of owners that rated the response to treatment as "good" or "excellent" (Olivry et al. 2018).

Following the 30-day treatment, 90.47% of the owners rated the emollient as having a "good-to-excellent" effect on their dogs' skin condition, reflecting an extremely high level of satisfaction with the product. This positive feedback is particularly significant in cAD management, where owner compliance and perception of treatment efficacy are critical for long-term success (Olivry et al. 2018).

An overwhelming portion of owners perceived the treatment as effective, suggesting that the emollient produced visible improvements in the dogs' skin condition. This aligns with previous literature that indicates that topical emollients can have a role in managing cAD by restoring skin barrier function and alleviating the clinical signs associated with the disease (Fujimura et al. 2011; Tretter and Mueller 2011; Jung et al. 2013; Blaskovic et al. 2014; Marsella et al. 2020; Segarra et al. 2023).

Despite these overwhelmingly positive results, it is important to consider the two cases where owners reported either a "fair" or "poor" response to treatment. Logically, it is possible that the dogs in question had more severe cases of cAD that required a more extended treatment duration or other treatment options to see substantial improvements. It is also possible that individual differences in the dogs' response to the emollient or variations in the owners' application practices could have contributed to the less favorable outcomes.

However, the owner's expectations regarding treatment outcomes seem to be a critical factor to consider in these sub-optimal responses. If owners expected a more immediate or dramatic improvement in their dog's condition, they might have rated the treatment as less effective even if moderate improvements were observed. This was evident in one case where owner expectations were unmet, and the owner classified the treatment efficacy as "poor." The dog in question improved its CADESI-04 scores and TEWL and pH values after the 30-day emollient plus application. Paying attention to this discrepancy is essential, as it relates closely to the next point of discussion.

While extremely valuable, owner-reported outcomes are inherently subjective and may not always correlate directly with clinical and measurement improvements. Therefore, clear communication with owners regarding the expected timeline for treatment effects and therapy goals could help manage expectations and improve satisfaction with the treatment. To the best of the author's ability, clear communication and management of expectations were attempted; however, these efforts may not have been fully effective with all dog owners.

4.4. Cosmetic scores

To the best of the author's knowledge, this was the first study that evaluated owner's opinions on the cosmetic properties of a topical emollient tailored for dogs. Our original, unvalidated cosmetic questionnaire was divided into two different sections.

In the first section, owners rated the product highly across all cosmetic properties (see Table 13 in section 3.3.8 - Cosmetic scores analysis). The extremely high and stable scores related to the product's cosmetic properties, such as its smell and appearance, reflect its positive reception among owners. Owners also agreed that applying the product was simple and well-tolerated by their dogs, suggesting that the formulation did not cause discomfort or irritation. These high ratings are encouraging as they suggest that the emollient is both practical and pleasant to use, which could increase the likelihood of consistent application.

The second section questioned owners about the characteristics of their dog's skin after the application of the topical emollient plus, namely the dog's skin "Hydration", "Oiliness", "Redness", and "Scaling". The high score for hydration (8.62 ± 1.21) is particularly noteworthy, as it aligns with the reduction in TEWL values observed, especially in the pinna, suggesting that the emollient may have effectively improved the skin's ability to retain moisture. This suspected improvement in skin hydration might have contributed to the reduction in pruritus and lesion severity observed in many dogs. These responses are also supported by the low score attributed to desquamation (1.38 ± 1.21) since this skin finding is often related to low SC's hydration levels (Marsella et al. 2011; Olivry et al. 2015; Saridomichelakis and Olivry 2016; Santoro et al. 2024).

However, the variability in the scores for oiliness (5.05 ± 3.06) and redness (3.05 ± 2.68) warrants further investigation. While the emollient was effective in hydrating the skin, some owners noted increased oiliness or persistent redness in their dogs' skin. These results could indicate that the novel biphasic formulation might need refinement to better balance skin hydration without increasing oiliness. Alternatively, better communication could have been established with owners since oiliness is an expected outcome as topical emollients are inherently oily, a characteristic that contributes to some of their benefits. The persistent redness could also reflect individual variations in skin response, where certain dogs might have more persistent erythema or require additional therapeutic interventions to fully resolve redness and inflammation. However, it is important to note that for most dogs, the CADESI-04 scores decreased during the trial, once again supporting that some owners may not objectively recognize skin appearance improvements and their expectations must be managed through good communication.

The overall cosmetic score results related to the dog's skin condition post-emollient application suggest that, from the owner's perspective, the product was extremely pleasant and highly contributed to improving the dog's skin appearance and overall health.

These findings are particularly relevant because compliance with topical treatments is often a challenge in veterinary dermatology (Marsella et al. 2017; Marsella and De Benedetto 2017). If a product is considered easy and pleasant to use and does not cause distress to the pet, owners may be more likely to purchase the product and follow through with the recommended treatment regimen. Consequently, better disease control and quality of life may be achieved.

4.5. Adverse effects

During the clinical trial, pet owners were encouraged to report any unexpected reactions and to provide visual records for evaluation by the dermatology service team.

Two out of the 27 dogs (7.40%) reported adverse effects. The low prevalence of these reports may indicate that this novel formulation is generally well tolerated. Given the small sample size and the isolated nature of these adverse reactions, it is difficult to draw definitive conclusions regarding breed predisposition.

Dog number 10's exclusion was due to suspected skin hyperpigmentation. After the owner's communication of this reaction, follow-up was lost and no re-evaluation consults were possible. Therefore, due to the lack of information, discussion of this possible adverse reaction is limited.

In contrast, the reaction observed in dog number 20, which appeared to be a case of contact dermatitis, aligns more closely with previous literature on topical emollient use in cAD (Marsella et al. 2017). Atopic dogs have a compromised skin barrier, which allows for increased epidermal penetration of allergens and other topical substances, predisposing them to contact dermatitis (Marsella 2013). Dog number 20 presented with an erythematous rash in the inguinal region that subsided after three days of treatment interruption. The dog's symptoms recurred after re-exposure to the emollient, strengthening the likelihood that the product may have caused the dermatitis. This owner was advised to discontinue the topical emollient application and to initiate a topical hydrocortisone spray to relieve the symptoms. The rapid resolution of symptoms following cessation of the emollient and initiation of corticosteroid therapy further validate the diagnosis of contact dermatitis.

No other potential adverse side effects were reported.

4.6. Study limitations

Several limitations should be acknowledged when interpreting the findings of this pilot proof-of-concept study.

First, the relatively small sample size (n=27, with 21 completing the trial) limits the generalizability of the results. It is crucial to conduct larger clinical studies to confirm the efficacy of the emollient in broader populations, including different breeds, age groups, and severity levels of cAD.

Second, the absence of a control group means that the improvements observed could not be definitively attributed to the topical emollient alone. The study adopted a simplified design as a pilot proof of concept study with strict inclusion and exclusion criteria and a constrained timeframe. This approach, while necessary for the feasibility of the initial investigation, inherently accepted certain limitations, including an increased potential for bias from both owners and clinicians. Randomized, double-blinded, placebo-controlled trials would be necessary to strengthen the evidence for this formulation efficacy.

Third, owner compliance and application technique variability could have affected the results. To ensure more reliable results, future studies need to be conducted under more controlled conditions.

Fouth, there is the inherent variability associated with TEWL measurements. Uncontrolled factors such as room temperature and humidity fluctuations, prolonged recruitment periods with concomitant weather variability, and operator differences may significantly influence TEWL readings. These sources of variability may impact the consistency and reliability of the results, thereby affecting the interpretation of the data.

4.7. Future research

Despite the promising results, this study has highlighted the need for further research. The efficacy of the formulation should be tested in larger, more diverse populations.

Although significant clinical and analytical improvements were observed, the exact mechanisms by which the emollient exerts its therapeutic effects remain to be fully understood. Future studies should explore these mechanisms in more detail, focusing on the role of skin barrier repair. One possible way to achieve this might be resorting to techniques like lipidomics analysis to evaluate if the emollient plus influenced the lipidic constitution of the SC. This technique will be pursued in the continuation of this study, as SC samples were collected on D0 and D30. These results were not included in this dissertation but will be available in Beatriz Fernandes' doctoral thesis.

While improvements in TEWL and pH were significant in the pinna, these effects were not consistent across other body regions, such as the inguinal area. This suggests that the efficacy of the emollient may vary depending on the anatomical location and the severity of the skin barrier dysfunction. Therefore, future research must explore whether region-specific treatment protocols could optimize the therapeutic benefits across different body areas.

In addition to TEWL and pH, other barrier assessments may be pursued in future research, including parameters like SC hydration, objective evaluation of skin absorbance and erythema, and skin microbiome changes, before and after treatment with the topical emollient.

Lastly, although compliance rates were high in this study, implementing stricter protocols for follow-up visits or offering more appealing incentives to owners might improve compliance and reduce data loss.

5. Conclusion

This prove-of-concept, bench-to-bedside pilot study demonstrated our proprietary topical emollient's potential as a safe, cosmetically pleasant, and clinically effective co-treatment for managing cAD. As a result, it was considered that all conditions were met for further product scalability, and commercial venture meetings have been held to further promote the development of this novel product.

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7. Annexes

Annexe 1 - Abstract accepted for oral communication at the 20th Montenegro International Veterinary Congress.

A new emollient plus spray for canine atopic dermatitis: clinical outcomes and user experience

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Introduction: Canine atopic dermatitis (cAD) is a common chronic allergic skin disease similar to human atopic dermatitis (hAD) (1). While hAD treatments focus on repairing the skin barrier (SB) with emollients, cAD treatments mostly target the immune system, neglecting SB care (2,3). Recently, new and improved emollient formulations have been developed - the “emollients plus” (4). This novel generation contains active, non-medicated substances, proven antipruritic and anti-inflammatory treatment-sparing effects, and prolonged free-flare periods in humans (4). In dogs, evidence for their use is lacking.

Aims: To test an innovative, easy-to-apply biphasic emollient plus spray formulation previously developed by this team on SB improvement and chronic cAD management.

Materials and Methods: A clinical trial evaluated this emollient plus as a daily cAD maintenance therapeutic option on 21 client-owned atopic dogs from a referral dermatology service. Dogs were only accepted if medications were unchanged during the study period (30 days) and in the previous 8 weeks. Outcomes assessed included indirect SB integrity parameters such as transepidermal water loss (TEWL) and skin pH, internationally validated clinical scores, such as canine atopic dermatitis extent and severity index (CADESI-04), pruritus score (PVAS10) and owner global assessment of treatment efficacy (OGATE). Additionally, it included sensory and acceptance scores (1-5 and 1-10) given by owners. All outcomes were measured at both the beginning and end of the study, except for the OGATE and owners sensory and acceptance scores, which were only measured at the end.

Results: There was a statistically significant decrease in CADESI-04 and PVAS10 from the beginning to the end of the study (24.6±18.1 to 13.4±7.4; p=0.002; 4.3±1.9 to 3.4±1.8; p=0.02, respectively). A decrease in TEWL and pH values was observed, though it was not statistically significant (TEWL: 16.1±15.6 to 12.4±10.1, p=0.39; pH: 6.2±1.0 to 5.8±0.8, p=0.08). Regarding OGATE 90.48% owners thought the response was good or excellent. Owner acceptance scores (1-5) were: Easy to apply: 4.6±0.5; Well-tolerated by the dog: 4.5±0.8; Would recommend to other owners: 4.5±0.7; Would buy this product: 4.2±0.8. Sensorial characteristics (1-10) were: Product looks good: 9.3±1.5; It has an agreeable smell: 9.2±1.4; Agreeable application for owners: 8.6±1.5; Agreeable application for dogs: 8.4±1.9.

Conclusions: These findings underscore the potential of our innovative biphasic emollient plus spray as an effective treatment option for chronic cAD, having a significant impact on the clinical outcomes. Although the decrease in TEWL and pH were not statistically significant, the trend observed, suggest a potential positive impact on SB. The high owners' acceptance and sensorial scores indicate the practicality and user-friendliness of the emollient plus spray, which is crucial for treatment adherence.

Annexe 2 - Promotional flyers used to promote the study among HEV clients.

Annexe 3 – Informed written consent signed by enrolled dog owner's.

FCT Fundação para a Ciência e a Tecnologia

Consentimento informado para participação em estudo científico/ensaio clínico

Se está a ler este documento é porque o seu cão é elegível para integrar um **estudo clínico** no âmbito de um projeto de investigação de carácter exploratório, **financiado pela Sociedade Europeia de Dermatologia Veterinária (ESVD),** conducente a tese de doutoramento, **que visa arranjar novas formas de ajudar cães com Dermatite Atópica no maneio da sua doença.**

Este estudo tem como principal objetivo avaliar o **potencial terapêutico de uma nova formulação tópica especialmente desenvolvida para reparação da barreira cutânea na Dermatite Atópica Canina.** Esta formulação foi **projetada para ser altamente eficaz e segura**, tendo em conta as propriedades dos seus constituintes e a sua capacidade de melhorar os pilares da doença, nomeadamente a restauração da barreira cutânea. Após este estudo entraremos no próximo passo: testar um protocolo de prevenção primária, ou seja, evitar o aparecimento da doença. Por outras palavras, iremos investigar se a aplicação frequente deste produto poderá ter algum impacto na prevenção da doença ou na diminuição da sua gravidade.

A participação neste estudo é inteiramente voluntária. A sua identidade e a do seu cão permanecerão confidenciais durante toda a duração do estudo, bem como aquando da apresentação pública dos resultados. **Este estudo foi aprovado pela Comissão de Ética para a Investigação e o Ensino (CEIE), garantindo assim que obedece a todos os critérios que garantem o bem-estar animal e boas práticas-clínicas.**

Eu, ____,

portador(a) do CC nº______________, tutor(a) do cão de nome____________________,

de raça______________________________, sexo __________ e idade _________,

declaro que fui informado e que autorizo a participação do meu cão acima referido no projeto "From Itch to Relief: exploring the benefits of a novel topical product for canine atopic dermatitis – a pilot clinical trial" autorizando a colheita, processamento e armazenamento de:

___ Medição não invasiva de perda de água transepidermal e e pH do estrato córneo com uso de sondas;

___Recolha não invasiva de estrato córneo com técnica de tape stripping (fita cola);

___ Dados clínicos e outras informações (sempre que necessário);

___ Fotografias/vídeos (sempre que necessário);

Fui informado sobre o estudo, verbalmente e por escrito, nomeadamente sobre as suas condições e procedimentos práticos inerentes que de mim dependerão, bem como das suas vantagens clínicas e potenciais efeitos secundários ou reações adversas desta modalidade de tratamento.

Declaro estar ciente de que:

- **O protocolo em estudo, as consultas de reavaliação do estudo e todas as análises a ele inerentes serão gratuitos e não incorrerão em qualquer despesa;**
- Serão medidos parâmetros objetivos de barreira cutânea (perda de água transepidermal e pH do estrato córneo), em 2 pontos no tempo (no início e no fim do tratamento), para determinações do estado da barreira cutânea no âmbito do estudo;

As informações estão sujeitas a processamento informático para monitorização do estudo e serão guardadas por um período de 20 anos.
De acordo com os regulamentos relativos a dados pessoais, tem o direito de aceder, retificar e limitar o processamento e portabilidade dos seus dados. Antes do início do estudo poderá anular o consentimento informado e solicitar a eliminação dos seus dados.
Para exercer os seus direitos poderá contactar por e-mail: dermatologia@fmv.ulisboa.pt.

- Serão colhidas amostras de estrato córneo ao meu cão através de técnicas não invasivas e não dolorosas em 2 pontos no tempo (antes e após o tratamento), para determinações do estado da barreira cutânea no âmbito do estudo;
- Serão colhidos dados clínicos e registos fotográficos/audiovisuais do meu cão;
- Posso desistir do estudo a qualquer momento.

Declaro comprometer-me a:

- Cumprir o protocolo de tratamento selecionado para o meu cão, durante todo o período de estudo (30 dias), tal como me explicado pelo médico veterinário responsável pelo estudo;
- Comparecer à consulta de reavaliação do estudo, no término do tratamento, bem como se o meu cão apresentar pioria do seu quadro clínico;
- Informar prontamente o médico veterinário responsável pelo estudo caso verifique alguma alteração do estado de saúde do meu cão durante o período do estudo, mesmo que não esteja relacionado com o problema dermatológico, assim como se verificar algum efeito secundário ao tratamento ou uma pioria da condição clínica do meu cão durante o período do estudo;
- Informar prontamente o médico veterinário responsável pelo estudo caso o meu cão inicie uma nova medicação ou tratamento durante o período do estudo;
- Responder com verdade a todos os questionários clínicos apresentados no âmbito do estudo, sempre que me for solicitado.

Declaro ter compreendido que:

- Os dados e informações recolhidos destinam-se exclusivamente ao estudo e os resultados decorrentes do mesmo serão apresentados publicamente e publicados em sítios científicos próprios, podendo ser utilizados em âmbito académico, educativo, científico ou empresarial;
- Os dados e resultados decorrentes deste estudo poderão ser usados para dar suporte a outras investigações do mesmo cariz no futuro, podendo vir a ser partilhados com outros investigadores.

Deste modo, dou fé de:

- Ter recebido e compreendido a informação que me foi transmitida verbalmente e por escrito sobre o estudo, a qual considero suficiente;
- Ter lido e compreendido a informação que me foi entregue;
- Ter podido fazer as perguntas que entendi por necessárias sobre o estudo;
- Aceitar as implicações deste estudo, pelos quais me comprometo.

Lisboa, ______ de ____________ de 2022 ______________________________

(Assinatura do/a tutor/a)

As informações estão sujeitas a processamento informático para monitorização do estudo e serão guardadas por um período de 20 anos.
De acordo com os regulamentos relativos a dados pessoais, tem o direito de aceder, retificar e limitar o processamento e portabilidade dos seus dados. Antes do início do estudo poderá anular o consentimento informado e solicitar a eliminação dos seus dados.
Para exercer os seus direitos poderá contactar por e-mail: dermatologia@fmv.ulisboa.pt.

Annexe 4 – Validated owner-assessed 10-cm Visual Analog Scale (PVAS10) form.

Escala de gravidade do prurido

Nome: Nº qvet: Nº Estudo:
Nº consulta: Data:

Esta escala foi desenhada para medir a gravidade do prurido em cães. Este pode manifestar-se através de comportamentos tais como arranhar-se, coçar-se, morder-se, lamber-se e/ou esfregar-se.

Leia as descrições abaixo, começando sempre de baixo para cima. Depois use uma caneta para marcar qualquer sítio na linha vertical para indicar onde acha que se encontra o nível de prurido do seu cão.

Prurido muito grave / quase contínuo. O prurido não cessa, independentemente do que esteja a acontecer em seu redor. O animal tem de ser fisicamente impedido de se coçar, por exemplo, com a colocação de um colar isabelino.

Prurido grave / episódios prolongados. Podem ocorrer episódios de prurido durante a noite (ex: acordar para se coçar/morder/lamber e acordar os donos a coçar-se/morder-se/lamber-se) e também enquanto come, brinca, faz exercício ou está distraído.

Prurido moderado / episódios regulares. Podem ocorrer episódios durante a noite (ex: acordar para se coçar/morder/lamber e acordar os donos a coçar-se/morder-se/lamber-se), mas não enquanto come, brinca, faz exercício ou está distraído.

Prurido ligeiro / episódios um pouco mais frequentes. Não há episódios de prurido durante a noite, enquanto come, brinca, faz exercício ou está distraído.

Prurido muito ligeiro / apenas episódios ocasionais. O cão apresenta apenas um pouco mais de comichão do que antes do problema de pele começar.

Cão normal – não considero que o prurido seja um problema.

Annexe 5 – Clinician-assessed Canine Atopic Dermatitis Extent and Severity Index (CADESI-04) form.

Nome: **nº qvet:** **nº estudo:**

Nº consulta: **Data:**

CADESI-04 (ICADA)			Erythema	Lichenification	Excoriations and/or Alopecia	TOTAL
Perilabial Area *(left and right combined)*		1				
Medial Pinnae (concave pinnae)	Left	2				
	Right	3				
Axillae	Left	4				
	Right	5				
Front Paws *(dorsal and palmar sides combined)*	Left	6				
	Right	7				
Hind Paws *(dorsal and plantar sides combined)*	Left	8				
	Right	9				
Cubital Flexor *(elbow folds)*	Left	10				
	Right	11				
Palmar Metacarpal *(from carpal to metacarpal pads)*	Left	12				
	Right	13				
Flanks	Left	14				
	Right	15				
Inguinal Areas *(groin)*	Left	16				
	Right	17				
Abdomen		18				
Perineum *(from vulva/scrotum to anus)*		19				
Ventral Tail *(proximal)*		20				
grade each site and each lesion type: *none: 0; mild: 1; moderate: 2; severe: 3*					**TOTAL Score** (20 x 3 x 3 = 180)	

Annexe 6 – Original data recording sheets for TEWL and pH values.

Nome: Nº qvet: Nº Estudo:

Nº consulta: Data:

☐ Aclimatização durante pelo menos 30 minutos

pH (unidades pH):

A			
	Média ± DP :		

V			
	Média ± DP :		

TEWL (g·m–2·h–1) – (grama por m2 por hora):

A										
	Média ± DP :									
V										
	Média ± DP :									

Annexe 7 – Owner Global Assessment of Treatment Efficacy (OGATE) and original owner-assessed cosmetic score questionnaires.

Nome: Nº qvet: Nº Estudo:

Nº consulta: Data:

Avaliação Global dos Proprietários sobre a eficácia da terapêutica (OGATE):

Como classificaria a resposta geral ao tratamento? (selecione apenas uma das cinco respostas a seguir indicadas):

0. Sem resposta

1. Resposta fraca

2. Resposta moderada

3. Boa resposta

4. Excelente resposta

Score Cosmético – Secção 1

Atribua um único valor, de 1 a 10 (1 - discordo completamente, 10 – concordo completamente), que melhor se adequa à sua opinião relativamente à aplicação deste tratamento no seu animal:

1. O produto tem um bom aspeto. ____
2. O produto tem um cheiro agradável. ____
3. A aplicação do produto é fácil e simples. ____
4. A aplicação deste produto foi agradável para mim. ____
5. A aplicação deste produto foi tolerado pelo meu cão. ____
6. Não me importaria de incluir este produto no maneiro da doença do meu cão. ____
7. Recomendaria este tratamento a outros donos de cães com dermatite atópica. ____

Score Cosmético – Secção 2

Avalie as seguintes características da pele do seu cão após a aplicação do produto, de 1 a 10 (1 – nada hidratada/oelosa/etc ; 10 – extremamente hidratada/oleosa/etc):

1. Hidratação ___
2. Oleosidade ___
3. Vermelhidão ___
4. Descamação ___

Observações:

Annexe 8 – Detailed TEWL and pH values for dogs number 3, 8, and 17 on D0 and D30 on both body sites tested.

	Pinna				Inguinal			
	TEWL		pH		TEWL		pH	
Dog ID	**D0**	**D30**	**D0**	**D30**	**D0**	**D30**	**D0**	**D30**
3	5.36	4.07	4.33	4.07	2.75	2.25	3.97*	6.19*
8	3.20	1.11	5.70	5.22	2.97*	5.22*	5.34	3.43
17	8.36	3.54	5.25	5.08	14.97*	36.59*	5.40*	8.84*

* - Skin barrier assessment values that did not improve from D0 to D30.