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Effect of a Spray Containing Occlusive Agents, Humectants and Physiological Lipids on Skin Hydration of Healthy Dogs When Applied After Bathing With a Chlorhexidine 2%/Miconazole 2% Shampoo

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ABSTRACT

Background: Normal hydration of the canine epidermis is imperative for cutaneous homeostasis. Xerosis may be encountered in canine atopic dermatitis and is aggravated by topical antiseptics.

Hypothesis and Objectives: To evaluate the hydrating properties and the safety of a spray (Sensiderm spray; MP Labo) when applied after shampooing healthy dogs with a chlorhexidine 2%/miconazole 2% product.

Animals: Twelve clinically healthy, privately owned dogs.

Materials and Methods: Dogs were clipped on the top of the head (control site), right and left lateral thorax. They were bathed on the body trunk with the chlorhexidine 2%/miconazole 2% shampoo and subsequently sprayed with Sensiderm on their randomly selected right or left side of the thorax. Skin hydration was measured by electrical capacitance using a corneometer on the three sites before (time point [T]0), and 1–2 h (T1), 6 h (T2), 24 h (T3), 48 h (T4) and 72 h (T5) after interventions.

Results: Two-way repeated-measures ANOVA showed a significant effect of time ($p = 0.015$) and a significant time–treatment interaction ($p = 0.023$) on skin hydration. One-way repeated-measures ANOVA showed that the effect of time was significant only on the Sensiderm spray-treated site, where skin hydration increased over baseline at T1 ($p = 0.009$; 95% confidence interval [CI] = 3.158–17.231), peaked at T2 ($p \leq 0.014$ and 95% CI ≥ 1.680 compared with T0, T3, T4 and T5) and remained increased at T3 ($p \leq 0.037$ and 95% CI ≥ 0.482 compared with T0 and T5). No treatment-related adverse effects were seen.

Conclusions and Clinical Relevance: Sensiderm spray was safe, and it increased hydration of healthy canine skin for ≥ 24 h.

ZUSAMMENFASSUNG

Hintergrund: Eine normale Hydratation der caninen Epidermis ist zwingend nötig, um die kutane Homöostase aufrechtzuerhalten. Eine Xerose kann bei caniner atopischer Dermatitis auftreten und durch topische Antiseptika verstärkt werden.

Hypothese/Ziele: Eine Evaluierung der hydrierenden Eigenschaften und der Sicherheit eines Sprays (Sensiderm Spray; MP Labo), wenn er nach dem Shampoonieren gesunder Hunde mit einem Chlorhexidin 2%/Mikonazol 2% igen Produkt aufgebracht wird.

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Tiere: Zwölf klinisch gesunde Hunde in Privatbesitz.
Materialien und Methoden: Die Hunde wurden am Kopf (Kontrollstelle) sowie rechts und links am lateralen Thorax geschoren. Sie wurden am Rumpf mit dem Chlorhexidin 2%/Mikonazol 2% Shampoo gewaschen und in der Folge mit Sensiderm an einer zufällig ausgewählten rechten oder linken Thoraxseite besprüht. Die Hydratation der Haut wurde mittels elektrischer Kapazitanz mit einem Corneometer an den drei Stellen vor (Zeitpunkt [T]0), und 1-2 h (T1), 6 h (T2), 24 h (T3), 48 h (T4), und 72 h (T5) nach den Behandlungen gemessen.
Ergebnisse: Eine zweifaktorielle Varianzanalyse (ANOVA) zeigte eine signifikante Auswirkung der Zeit ($p = 0{,}015$) und eine signifikante Interaktion von Zeit und Behandlung ($p = 0{,}023$) auf die Hydratation der Haut. Eine einfaktorielle ANOVA zeigte, dass die Auswirkung der Zeit nur an der mit Sensiderm Spray behandelten Körperstelle signifikant war, wo die Hydratation der Haut vom Ausgangswert bei T1 ($p = 0{,}009$; 95%iges Konfidenzintervall [CI] = 3,158-17.231) zunahm, bei T2 ($p \leq 0.014$ und 95% CI ≥ 1.680 im Vergleich zu T0, T3, T4 and T5) den Höchstwert erreichte und bei T3 ($p \leq 0.037$ und 95% CI ≥ 0.482 im Vergleich zu T0 and T5) erhöht blieb. Es wurden keine Behandlungs-abhängigen Nebenwirkungen gesehen.
Schlussfolgerungen und klinische Bedeutung: Sensiderm Spray war sicher und erhöhte die Hydratation der gesunden Hundehaut für ≥24 h.

摘要
背景: 犬表皮的正常水合对于维持皮肤稳态至关重要。干燥症可能出现在犬特应性皮炎中，并可能因外部抗菌剂而加重。
假设与目的: 评估一种喷雾剂(Sensiderm 喷雾；MP Labo)在使用含有 2% 氯己定/2% 咪康唑的洗剂洗浴健康犬后，是否具有保湿作用及安全性。
动物: 12 只临床健康的家养犬。
材料与方法: 犬的头顶部(对照部位)、左右胸外侧被剃毛。躯干使用 2% 氯己定/2% 咪康唑洗剂洗浴后，在随机选择的一侧胸外侧喷洒 Sensiderm。分别在三处部位于干预前(T0)、干预后 1–2 小时(T1)、6 小时(T2)、24 小时(T3)、48 小时(T4)和 72 小时(T5)使用角质层水分仪(corneometer)测量皮肤水合度。
结果: 双向重复测量 ANOVA 显示时间因素(p = 0.015)及时间–处理交互作用(p = 0.023)对皮肤水合有显著影响。单向重复测量 ANOVA 显示时间因素仅在 Sensiderm 喷雾处理部位显著，且皮肤水合度在 T1 时高于基线(p = 0.009；95% CI = 3.158–17.231)，在 T2 达到峰值(p ≤ 0.014 且 95% CI ≥ 1.680，相较 T0、T3、T4 和 T5)，并在 T3 时仍维持较高水平(p ≤ 0.037 且 95% CI ≥ 0.482，相较 T0 和 T5)。未见与治疗相关的不良反应。
结论与临床相关性: Sensiderm 喷雾安全，且可使健康犬皮肤的水合度增加 ≥24 小时。

Résumé
Contexte: Une hydratation normale de l'épiderme canin est indispensable à l'homéostasie cutanée. La xérose peut être observée dans les cas de dermatite atopique canine et être aggravée par les antiseptiques topiques.
Hypothèse et objectifs: Évaluer les propriétés hydratantes et la sécurité d'un spray (Sensiderm spray ; MP Labo) appliqué après le shampooing de chiens en bonne santé avec un produit à base de chlorhexidine 2 %/miconazole 2 %.
Animaux: Douze chiens cliniquement sains appartenant à des particuliers.
Matériel et méthodes: Les chiens ont été tondus sur le sommet de la tête (site témoin), ainsi que sur les côtés droit et gauche du thorax. Ils ont été lavés sur le tronc avec le shampooing à 2 % de chlorhexidine/2 % de miconazole, puis vaporisés avec Sensiderm sur le côté droit ou gauche du thorax, choisi au hasard. L'hydratation cutanée a été mesurée par capacité électrique à l'aide d'un cornéomètre sur les trois sites avant (moment [T]0), puis 1 à 2 heures (T1), 6 heures (T2), 24 heures (T3), 48 heures (T4) et 72 heures (T5) après les interventions.
Résultats: L'ANOVA à mesures répétées à deux facteurs a montré un effet significatif du temps ($p = 0{,}015$) et une interaction significative entre le temps et le traitement ($p = 0{,}023$) sur l'hydratation cutanée. Une ANOVA à mesures répétées à un facteur a montré que l'effet du temps n'était significatif que sur la zone traitée avec le spray Sensiderm, où l'hydratation cutanée a augmenté par rapport à la valeur de référence à T1 ($p = 0{,}009$; intervalle de confiance [IC] à 95 % = 3,158-17,231), a atteint son maximum à T2 ($p \leq 0{,}014$ et IC à 95 % ≥ 1,680 par rapport à T0, T3, T4 et T5) et est restée élevée à T3 ($p \leq 0{,}037$ et IC à 95 % ≥ 0,482 par rapport à T0 et T5). Aucun effet indésirable lié au traitement n'a été observé.
Conclusions et pertinence clinique: Le spray Sensiderm s'est révélé sûr et a augmenté l'hydratation de la peau canine saine pendant ≥24 h.

要約
背景: 犬の表皮の正常な水分保持は皮膚の恒常性に不可欠である。乾皮症は犬のアトピー性皮膚炎において認められることがあり、局所性抗菌薬によって増悪する可能性がある。
仮説/目的: 本研究の目的は、クロルヘキシジン2%/ミコナゾール2%製剤によるシャンプー後に、スプレー(Sensiderm spray; MP Labo)を塗布した際の保湿効果および安全性を評価することであった。

供試動物: 臨床的に健康な、オーナー所有犬12頭。
材料と方法: 犬の頭頂部(対照部位)、右側および左側の胸部外側をクリッピングした。胴体部全体をクロルヘキシジン2%/ミコナゾール2%シャンプーで洗浄し、その後、左右いずれか無作為に選択した胸部外側にSensidermスプレーを噴霧した。皮膚水和度はコルネオメーターを用いた電気容量測定により、3部位において処置前(時点T0)、処置後1–2時間(T1)、6時間(T2)、24時間(T3)、48時間(T4)、72時間(T5)に測定した。
結果: 二元配置反復測定分散分析により、皮膚水和度に対する時間の影響(p = 0.015)、時間および処置の交互作用(p = 0.023)に有意性が示された。一元配置反復測定分散分析では、時間の効果はSensidermスプレー処置部位においてのみ有意であり、この部位において皮膚水和度はT1でベースラインより上昇した(p = 0.009; 95%信頼区間[CI] = 3.158–17.231)。皮膚水和度はT2でピークに達し(p ≤ 0.014かつ95% CI ≥ 1.680[T0, T3, T4, T5との比較])、T3においても上昇が維持された(p ≤ 0.037かつ95% CI ≥ 0.482[T0およびT5との比較])。治療関連の有害事象は認められなかった。
結論と臨床的意義: Sensidermスプレーは安全であり、健常犬の皮膚水和度を24時間以上にわたり増加させる効果を示した。

Resumo
Contexto: A hidratação normal da epiderme canina é fundamental para a homeostase cutânea. A xerose ocorre na dermatite atópica canina e pode ser agravada pelo uso de antissépticos tópicos.
Hipótese e Objetivos: Avaliar as propriedades hidratantes e a segurança de um spray (Sensiderm spray; MP Labo) quando aplicado após a lavagem de cães saudáveis com um produto à base de clorexidina 2%/miconazol 2%.
Animais: Doze cães clinicamente saudáveis, de propriedade particular.
Materiais e Métodos: Os cães foram tosados no topo da cabeça (local controle), tórax lateral direito e esquerdo. Eles foram banhados no tronco com o shampoo à base de clorexidina 2%/miconazol 2% e, posteriormente, Sensiderm foi borrifado no lado direito ou esquerdo do tórax, selecionados aleatoriamente. A hidratação da pele foi medida por capacitância elétrica utilizando um corneômetro nos três locais antes (ponto de tempo [T]0) e 1–2 h (T1), 6 h (T2), 24 h (T3), 48 h (T4) e 72 h (T5) após as intervenções.
Resultados: A ANOVA de medidas repetidas bidirecional mostrou um efeito significativo do tempo ($p = 0{,}015$) e uma interação significativa entre tempo e tratamento ($p = 0{,}023$) na hidratação da pele. A ANOVA unidirecional de medidas repetidas mostrou que o efeito do tempo foi significativo apenas no local tratado com spray Sensiderm, onde a hidratação da pele aumentou em relação ao valor basal em T1 ($p = 0{,}009$; intervalo de confiança [IC] de 95% = 3,158–17,231), atingiu o pico em T2 ($p \leq 0{,}014$ e IC de 95% ≥ 1,680 em comparação com T0, T3, T4 e T5) e permaneceu aumentada em T3 ($p \leq 0{,}037$ e IC de 95% ≥ 0,482 em comparação com T0 e T5). Não foram observados efeitos adversos relacionados ao tratamento.
Conclusões e Relevância Clínica: O spray Sensiderm foi seguro e aumentou a hidratação da pele canina saudável por ≥24 h.

RESUMEN
Introduccion: La hidratación normal de la epidermis canina es fundamental para la homeostasis cutánea. La xerosis puede presentarse en la dermatitis atópica canina y agravarse con antisépticos tópicos.
Hipótesis y objetivos: Evaluar las propiedades hidratantes y la seguridad de un espray (Sensiderm spray; MP Labo) aplicado después de lavar perros sanos con un champú de clorhexidina al 2%/miconazol al 2%.
Animales: Doce perros clínicamente sanos, de propietarios particulares .
Materiales y métodos: Se raparon los perros en la parte superior de la cabeza (zona de control) y en los laterales derecho e izquierdo del tórax. Se les bañó el tronco con el champú de clorhexidina al 2%/miconazol al 2% y posteriormente se les pulverizó Sensiderm en el lado derecho o izquierdo del tórax, elegidos al azar. La hidratación de la piel se midió mediante capacitancia eléctrica con un corneómetro en las tres zonas antes indicadas (punto temporal [T]0) y 1-2 h (T1), 6 h (T2), 24 h (T3), 48 h (T4) y 72 h (T5) después de las intervenciones.
Resultados: El ANOVA de medidas repetidas de dos vías mostró un efecto significativo del tiempo ($p = 0{,}015$) y una interacción significativa entre el tiempo y el tratamiento ($p = 0{,}023$) en la hidratación de la piel. El ANOVA unidireccional de medidas repetidas mostró que el efecto del tiempo solo fue significativo en la zona tratada con Sensiderm en aerosol, donde la hidratación de la piel aumentó con respecto al valor basal en T1 ($p = 0{,}009$; intervalo de confianza del 95 % [IC] = 3,158-17,231), alcanzó su nivel máximo en T2 ($p \leq 0{,}014$ e IC del 95 % ≥ 1,680 en comparación con T0, T3, T4 y T5) y se mantuvo elevada en T3 ($p \leq 0{,}037$ e IC del 95 % ≥ 0,482 en comparación con T0 y T5). No se observaron efectos adversos relacionados con el tratamiento.
Conclusiones y relevancia clínica: Sensiderm en aerosol fue seguro y aumentó la hidratación de la piel sana canina durante ≥24 h.

1 | Introduction

Hydration of the stratum corneum (SC), usually referred to as 'skin hydration', is necessary for cutaneous homeostasis and health. This is exemplified by the macroscopic lesions (roughness, scaling and fissuring) and pruritus that accompany human xerosis resulting from ageing, low environmental temperatures, overuse of detergents and disease states such as ichthyoses, psoriasis and atopic dermatitis (AD) [1, 2]. Likewise, reduced hydration has been reported in the lesional skin of dogs with ichthyosis [3, 4] and AD [5], and is attributed to the structural and biochemical alterations of SC and the ensuing increased trans-epidermal water loss (TEWL) [6]. In these dogs, xerosis may be aggravated by the frequent use of antimicrobial shampoos to control bacterial overgrowth, superficial bacterial infections, and/or *Malassezia* dermatitis [7], that are frequently present and contribute significantly to overall disease severity, skin lesions and pruritus [8].

Proactive application of moisturisers, at least once daily, is indicated for all cases of human AD, irrespective of its severity, and, currently, over-the-counter products are preferred over prescription moisturisers owing to their lower cost, easier application and comparable efficacy [9]. Several moisturisers for veterinary use are commercially available, yet there are only rare reports on the magnitude and duration of their effect on skin hydration [10].

To the best of our knowledge, there is no scientific documentation of the skin hydration effects of an over-the-counter moisturiser containing occlusive agents, humectants and physiological lipids (Sensiderm spray; MP Labo) in dogs. Therefore, the aim of this study was to evaluate the safety and efficacy of this product in increasing skin hydration when applied after shampooing healthy dogs with a product containing 2% chlorhexidine and 2% miconazole (Malaseb; Dechra), that is registered for the treatment of canine staphylococcal and *Malassezia* dermatitis.

2 | Materials and Methods

2.1 | Animals and Study Design

Study protocol was approved by the Animal Ethics Committee of authors' institution (licence no.: 181/17-10-24). Handling of the dogs followed the European Communities Council Directive 2010/63/EU and state laws. An informed signed consent for participation was obtained from all owners.

Within a 7-day period, 12 clinically healthy, privately owned dogs were enrolled. To be included in the study, they were required to: (a) be ≥6 months old; (b) be not pregnant or lactating; (c) have no clinical or historical evidence of cutaneous or systemic disease of any aetiology during the previous 6 months; (d) have not received systemic or topical drugs that can influence the inflammatory response of the skin or pruritus (e.g., glucocorticoids, ciclosporin, oclacitinib, lokivetmab and H1 antihistamines for ≥4 weeks and long-acting parenteral glucocorticoids for ≥8 weeks); and (e) have not been bathed (with or without shampoo) or received topical products (including spot-ons with skin hydration properties) for ≥1 week. No changes in diet or dose/frequency of administration of any medication given on a long-term basis (e.g., ectoparasiticides, endoparasiticides and fatty acid supplements) were permitted during the study. Administration of anti-inflammatory drugs was permitted if considered necessary owing to adverse effects caused by test items or any unrelated reason, with the dog being subsequently disqualified for the rest of the study; however, no such medication interventions had to be made.

At the inclusion visit (1–2 days before the beginning of the trial), three square areas, approximately 3×3 cm, were clipped with a 0.2 mm electric blade on the right and left lateral thorax (approximately in the middle of the 8th rib) and on the top of the head. The latter served as the untreated control site (Site C), whereas the lateral thoracic sides were randomised (https://www.calculator.net/random-number-generator.html) to be treated with only the chlorhexidine 2%/miconazole 2% shampoo (Site M) or with the shampoo followed by the moisturiser spray (Site S). Researchers were blinded to the randomisation until the end of the study.

At T0, a board-certified referral clinician in veterinary dermatology (ESB) examined sites C, M and S for erythema or other macroscopic skin lesions. Scissors were used to atraumatically clip any remaining hair shafts, and skin hydration was measured by a veterinary dermatology resident (AP), starting at the right thorax, followed by the left thorax and finally the head (Site C). Subsequently, owners bathed the body trunk of their dogs with the chlorhexidine 2%/miconazole 2% shampoo: They were instructed to first wet thoroughly the body trunk until the base of the neck, to apply the shampoo at several spots and to massage the coat and skin until lather was raised. After 10 min, the shampoo was rinsed off and the dog was left to dry in a warm environment, with or without using clean towels. Then the moisturiser spray was applied onto the designated side of the trunk from a distance of approximately 10 cm, skin and hair were gently massaged, and they were left to dry. Re-examinations were done after 1–2 h (time point [T]1), 6 ± 1 h (T2), 24 ± 1 h (T3), 48 ± 1 h (T4) and 72 ± 1 h (T5); owners were asked about possible adverse effects, and the same procedures as at T0 were repeated (examination for skin lesions, close clipping of visible hair shafts if present and measurement of skin hydration).

2.2 | Measurement of Skin Hydration

Measurements were performed in a designated room with a stable temperature (20°C ± 1°C) and relative humidity (50% ± 10%), after an acclimatisation period of 30 min and with the dogs in a standing position. An MPA 580 corneometer (Courage-Khazaka) with the SM825 probe was used to measure the electrical capacitance of the skin in arbitrary units set by the manufacturer. The probe was positioned vertically onto the skin surface by applying constant and low pressure until the first measurement was obtained; then, the probe was removed and repositioned after approximately 1 s, and this was repeated until 10 measurements were obtained, and the mean value was calculated for statistical analysis.

2.3 | Statistical Analysis

Based on preliminary observations, 10 dogs had to be included to achieve a statistical power of 80%, at 5% level of significance,

if the mean difference in electrical capacitance between two sites was 5.3 with a standard deviation (SD) of 4.9 (https://statulator.com/SampleSize/ss2PP.html). To account for possible drop-outs or other unexpected events, 12 dogs were finally enrolled.

The distribution of electrical capacitance was examined with the Shapiro–Wilk test. Two-way repeated-measures ANOVA was used to examine the possible effect of time and of time × treatment interaction. The over-time effect of each treatment (sites C, M and S) was examined by one-way repeated-measures ANOVA and, when the result was significant, by the *post hoc* least significant difference test. Statistical analyses were performed with SPSS 29.0.1 for Windows with a 5% level of significance.

TABLE 1 | Mean ± standard deviation of skin electrical capacitance (in arbitrary units) on the head (untreated control site C), on lateral thorax treated with a 2% chlorhexidine/2% miconazole shampoo (site M), and on lateral thorax treated with the shampoo followed by a moisturiser spray (site S), before treatment (Time 0) and after 1 to 2 h (Time 1), 6 ± 1 h (Time 2), 24 ± 1 h (Time 3), 48 ± 1 h (Time 4) and 72 ± 1 h (Time 5).

	Site C	Site M	Site S
Time 0	10.82 ± 7.9	15.64 ± 7.41	15.82 ± 7.94
Time 1	8.63 ± 5.11	16.63 ± 10.27	26.01 ± 16.19[a]
Time 2	10.35 ± 8.01	18.35 ± 8.1	29.16 ± 15.44[a,b,c,d]
Time 3	9.12 ± 3.61	19.96 ± 11.53	22.24 ± 10.74[a,d]
Time 4	8.85 ± 7.95	17.69 ± 12	19.94 ± 10.09
Time 5	6.64 ± 2.88	18.11 ± 15.43	16.15 ± 9.56

[a]Significant difference compared with Time 0 ($p < 0.037$).
[b]Significant difference compared with Time 3 ($p = 0.014$).
[c]Significant difference compared with Time 4 ($p = 0.014$).
[d]Significant difference compared with Time 5 ($p < 0.004$).

3 | Results

Three (25%) dogs were intact males and nine (75%) were females (eight spayed). Their median age was 4 years (range 2–10.5 years), and their median body weight was 9.2 kg (range 4–31.3 kg). Five (41.7%) dogs were pure-bred and seven (58.3%) were cross-bred. The length of their hair coat was classified as short (three of 12; 25%), medium (five of 12; 41.7%) or long (four of 12; 33.3%).

The mean values of electrical capacitance for each treatment site (C, M and S) are shown in Table 1, and the over-time changes for each dog are shown in Figures S1–S3. Two-way repeated-measures ANOVA showed a significant effect of time ($p = 0.015$) and a significant time × treatment interaction ($p = 0.023$). One-way repeated-measures ANOVA showed no effect of time on the electrical capacitance of sites C ($p = 0.43$) and M ($p = 0.562$), whereas the effect of time was significant ($p = 0.014$) for Site S. Electrical capacitance, and thus skin hydration, on Site S increased significantly and reached maximum values at T2, when it was significantly higher than T0 ($p = 0.005$; 95% confidence interval [CI] 5.073–21.613), T3 ($p = 0.014$; 95% CI 1.680–12.152), T4 ($p = 0.014$; 95% CI 2.247–16.191) and T5 ($p = 0.001$; 95% CI 6.220–19.791). The increase was already significant at T1 compared with T0 ($p = 0.009$; 95% CI 3.158–17.231) and remained so at T3 ($p = 0.037$ with 95% CI 482–12.373 compared with T0, and $p = 0.004$ with 95% CI 2.453–9.727 compared with T5) (Table 1; Figure 1).

Two dogs that belonged to the same owner developed moderate-to-severe pruritus on both sides of the body trunk, causing multifocal excoriations that were also evident on sites M and S. Pruritus was noticed soon after T0 and spontaneously disappeared by T3. Both dogs were infested by fleas, despite recent administration of isoxazoline. Most likely, it was a temporal infestation because the dogs were transported to the clinic in a flea-infested vehicle that had been used a few days earlier to transport a stray cat. The Naranjo score for the probability of pruritus being an adverse effect of the treatments was 2 (i.e.,

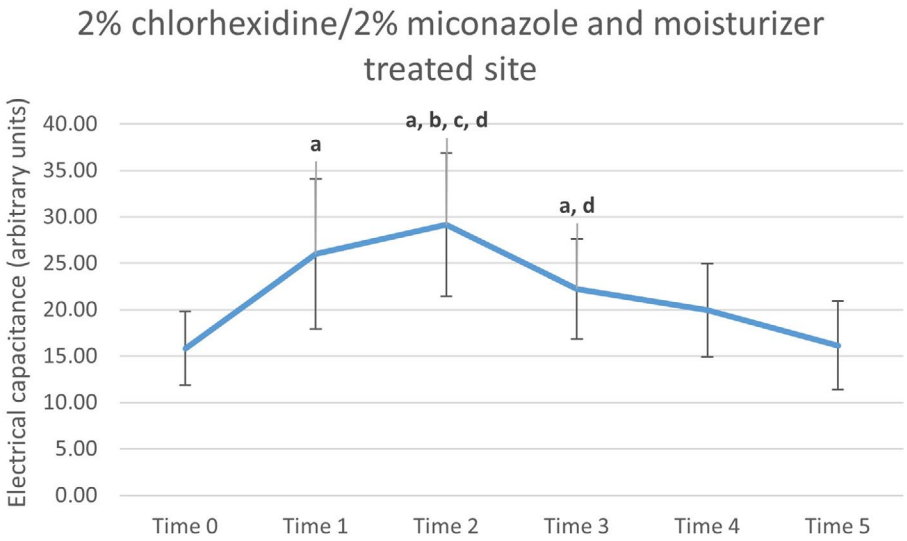

FIGURE 1 | Mean and standard deviation (bars) of skin electrical capacitance (in arbitrary units) on lateral thorax treated with a 2% chlorhexidine/2% miconazole shampoo followed by a moisturiser spray (Site S), before treatment (Time [T]0) and after 1–2 h (T1), 6 ± 1 h (T2), 24 ± 1 h (T3), 48 ± 1 h (T4) and 72 ± 1 h (T5). Significant differences compared with (a) T0 ($p < 0.037$), (b) T3 ($p = 0.014$), (c) T4 ($p = 0.014$) and (d) T5 ($p < 0.004$).

possible adverse drug reaction) [11]. In both dogs, the electrical capacitance was measured on non-excoriated skin and, when statistical analysis was repeated after omission of these dogs, the results did not change.

Clinical evidence of clipper rash of mild severity (erythema, erosions, papules and crusts) was found on one or more electrical capacitance measurement sites of seven dogs, at T0 (three of 12; 25%), T1 (four of 12; 33.3%), T2 (five of 12; 41.6%), T3 (four of 12; 33.3%), T4 (three of 12; 25%) and/or T5 (three of 12; 25%). Prevalence of clipper rash at one or more time points did not differ ($p = 0.539$) among sites C (two of 12; 16.7%), M (four of 12; 33.3%) and S (five of 12; 41.7%). Also, one dog presented mild erythema of unknown aetiology on Site C at T5.

4 | Discussion

The results of this study show that a single bathing of healthy dogs with chlorhexidine 2%/miconazole 2% shampoo does not change lateral thoracic skin hydration. However, when shampooing is followed by the application of an over-the-counter moisturiser spray, skin hydration increases significantly for ≥ 24 h.

Skin hydration can be measured by electrical capacitance, impedance or conductance [12–14]. Of these methods, electrical capacitance (also called corneometry) is the gold standard in human dermatology [12, 14, 15]. The corneometer CM 825 measures hydration to a depth of 10–20 μm [13], which roughly corresponds to the thickness of SC in intact, haired canine skin [16], and the results have been shown to be reliable [13] and highly repeatable [17] in healthy and atopic dogs. In previous studies, the average of three [18], five [5, 13] or 10 [17, 19] successive values, obtained with or without removing and repositioning the probe, was calculated. In our study, 10 values were obtained with probe removal and repositioning, and the high repeatability of the instrument was further confirmed: in none of the 216 measurements (12 dogs × 3 sites × 6 time points) there even a single value deviating > 10% from the average (data not shown). However, there are some important parameters that should be carefully considered to ensure accuracy of the results. (a) Owing to their low water content, hairs can artificially decrease electrical capacitance [13, 14]. This can be avoided by either measuring skin hydration on glabrous skin (e.g., concave aspect of ear pinnae, axillae and inguinal area) or by close clipping of haired skin (e.g., with < 1 mm blade) [13]. In this study, the lateral thorax was preferred to avoid accidental spillover of the moisturiser spray to Site M, which might have happened if axillae or inguinal areas had been selected. Also, in preliminary experiments, we noticed that the repeatability of the measurements was higher on lateral thoracic skin, perhaps because the underlying 8th rib acts as a solid substrate that helps the operator to better control the pressure applied to the probe when in contact with the skin [14]. A 0.2-mm blade was used and any visible, remaining or regrowing hair shafts were clipped with scissors. A drawback of close clipping was the clipper rash. However, it was mild and did not have an impact on the results because it occurred on sites C, M and S with equal frequency, and measurements were always taken from visibly normal skin. (b) Season of the year, environmental temperature and humidity, excitement and stress can affect skin hydration [12, 14, 20]. For this reason, all dogs entered the study almost simultaneously (within 1 week) and they were left quiet in the designated room for 30 min before measurement of skin electrical capacitance. (c) At least in humans, results may be affected by the time of the day [10, 14]. In our study, T0, T3 (24 ± 1 h), T4 (48 ± 1 h) and T5 (72 ± 1 h) corresponded to the same time on four successive days, yet this was not feasible for T1 (1–2 h) and T2 (6 ± 1 h). However, the lack of a significant effect of time on the electrical capacitance of Site C denotes that this did not influence the results. (d) In healthy dogs, skin hydration varies significantly among body sites and it has been shown to be numerically higher on the lateral thorax than on the top of the head [21], as in our study (Table 1). However, these regional differences could not influence our results because skin hydration was compared among sites C, M and S only at T0 and not after treatment.

After a single bath of 11 healthy dogs with the same chlorhexidine 2%/miconazole 2% shampoo that was used in our study and with the same contact time (10 min), followed by towel drying, TEWL values increased significantly on the abdominal skin [22]. However, our results showed no change in skin hydration on Site M. As already explained, this may be a consequence of the lack of an inverse relationship between TEWL and skin hydration, yet it may also be related to the skin area examined (abdominal vs. lateral thoracic skin) and to the time point of the measurements (30 min vs. from 1 to 2 h until 72 ± 1 h after bathing). In any case, our results do not imply that xerosis will not occur after repeated use of the shampoo and/or that xerosis will not deteriorate in dogs with already compromised skin barrier, such as dogs with AD.

A single use of the moisturiser spray, after bathing with the chlorhexidine 2%/miconazole 2% shampoo, increased epidermal hydration. The effect appeared after 1–2h, peaked at 6 ± 1 h and remained significant at 24 ± 1 h, yet was not evident anymore at 48 ± 1 h. According to the label, Sensiderm spray contains multiple ingredients that can increase SC hydration, including occlusive agents (*Butyrospermum parkii* [or *Vitellaria paradoxa* or shea] butter, caprylic/capric acid triglyceride, *Centella asiatica* extract), humectants (glycerine and urea), physiological lipids (ceramide NP, phosphatidylcholine and squalene) and molecules that may promote the production of epidermal lipids by keratinocytes (niacinamide and panthenol) [1, 2, 23–26]. Under the conditions of the present study, it is reasonable to assume that the fast increase of skin hydration was mainly a result of the occlusive and humectant properties of the product. Interestingly, despite a substantial number of controlled trials in healthy dogs [27, 28] and those with AD [10, 29, 30], this is the first time that a commercially available intervention was shown to increase skin hydration. The only other topical treatment that has been found to have a similar effect is a cream for humans, containing physiological lipids (ceramides, free fatty acids and cholesterol), that was modified by the investigators to adjust the pH, and after 2 and 4 weeks of daily application over the entire body there was increased hydration on lateral thorax skin of dogs with AD [10]. Therefore, owing to the immediate hydrating effect and the pharmaceutical form that facilitates frequent application (e.g., once per day), Sensiderm spray has the potential to be useful for the management of dogs with xerosis.

This study has several limitations. First, the chlorhexidine 2%/miconazole 2% shampoo that was applied before the moisturiser may have caused physicochemical alterations to the SC that

affected the hydrating effect of the spray. Second, independently of the shampoo, previous washing with water probably increased the effect of the product, because the moisturising efficacy of all occlusive agents is expected to be higher when they are used after bathing or washing with tap water [2]. Retrospectively, we recognise that it would have been better to include a fourth measurement site where only the spray would have been applied. Third, it is not known whether the results were the same in nonclipped skin, and if they would have been the same after repeated applications, considering that the physiological lipids and the molecules that promote keratinocyte lipid production in Sensiderm spray may increase its efficacy after long-term use. Fourth, and perhaps most importantly, it is unknown whether the same effect will occur in dogs with xerosis, such as dogs with AD, and whether the hydrating effect will result in a meaningful clinical improvement.

5 | Conclusions

A single application of an over-the-counter spray containing occlusive agents, humectants, physiological lipids and molecules that promote keratinocyte lipid production, on the lateral thoracic skin of healthy dogs, after bathing with a chlorhexidine 2%/miconazole 2% shampoo, resulted in an increase in skin hydration lasting for ≥1 day. Further studies are needed to evaluate the therapeutic potential of this product in dogs with xerosis, such as dogs with AD.

Author Contributions

Adamantia Pseftogka: data curation (lead), investigation (lead), methodology (equal), project administration (equal), resources, writing – original draft preparation (equal); writing – review and editing (equal). **Elisabeta Samuel (Badulescu):** data curation (lead), investigation (supporting), methodology (supporting), writing – review and editing (equal). **Manolis K. Chatzis:** data curation (supporting), investigation (supporting), methodology (supporting), writing – review and editing (equal); **Manolis N. Saridomichelakis:** conceptualisation, data curation (supporting), formal analysis, funding acquisition, investigation (supporting), methodology (equal), project administration (equal), supervision, validation, writing – original draft preparation (equal), writing – review and editing (lead).

Conflicts of Interest

During the last 5 years, the authors have received research support, lecture honorarium, and/or consultation fees from the following commercial companies: AP: MP Labo; ESB: MP Labo; MC: MP Labo; MNS: Bayer, Ceva, Elanco, Hellafarm, MP Labo, MSD, Premier Shukuroglou, Provet, Virbac.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Skin electrical capacitance (in arbitrary units) on the head (untreated control site) of 12 healthy dogs at time point [T]0, and after 1–2 h (T1), 6 ± 1 h (T2), 24 ± 1 h (T3), 48 ± 1 h (T4) and 72 ± 1 h (T5). **Figure S2:** Skin electrical capacitance (in arbitrary units) on lateral thorax of 12 healthy dogs treated with a 2% chlorhexidine/2% miconazole shampoo, before treatment (time point [T]0), and after 1–2 h (T1), 6 ± 1 h (T2), 24 ± 1 h (T3), 48 ± 1 h (T4) and 72 ± 1 h (T5). **Figure S3:** Skin electrical capacitance (in arbitrary units) on lateral thorax treated with a 2% chlorhexidine/2% miconazole shampoo followed by a moisturiser spray of 12 healthy dogs, before treatment (time point [T]0), and after 1–2 h (T1), 6 ± 1 h (T2), 24 ± 1 h (T3), 48 ± 1 h (T4) and 72 ± 1 h (T5).