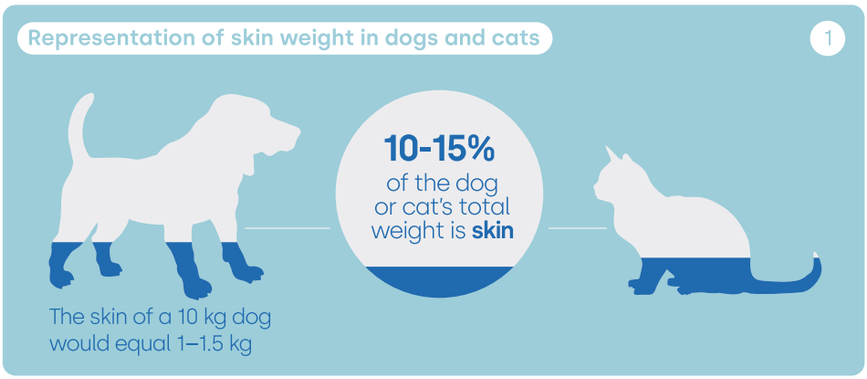
# Nucleoforce Guide to healthy skin for pets

NUCLEOFORCE®
Pets

Bioiberica

## The role skin plays in pet health

We often forget how important skin is to the health of our pets. **Skin is not only the largest organ, 10-15% of the weight of dogs and cats (see figure 1), it is also the main barrier between the body and the outside world,** therefore, the function of both the skin and its ancillary structures fulfil several important functions for the health of pets, such as (1):

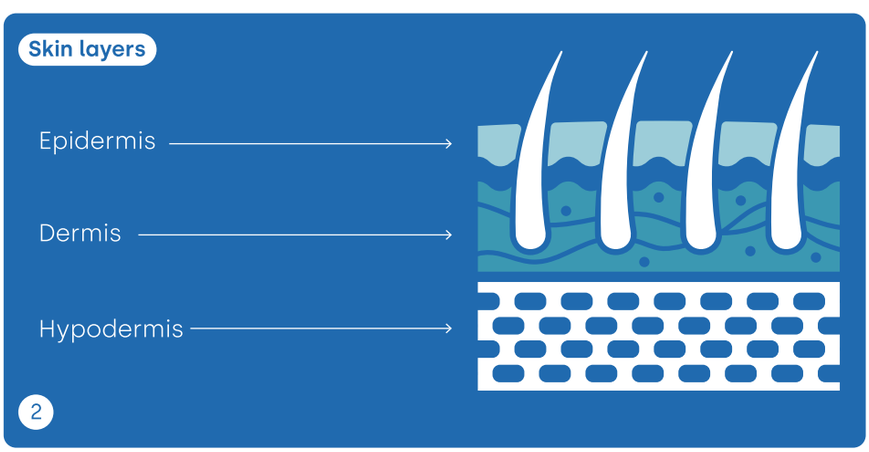



- Protection against the external environment (trauma, pathogens, UV rays)
- Vitamin D synthesis
- Regulating body temperature
- Sensation/ Sensitivity

**The skin can be divided into 3 layers: epidermis, dermis and hypodermis, each with a specific function. See Figure 2.**

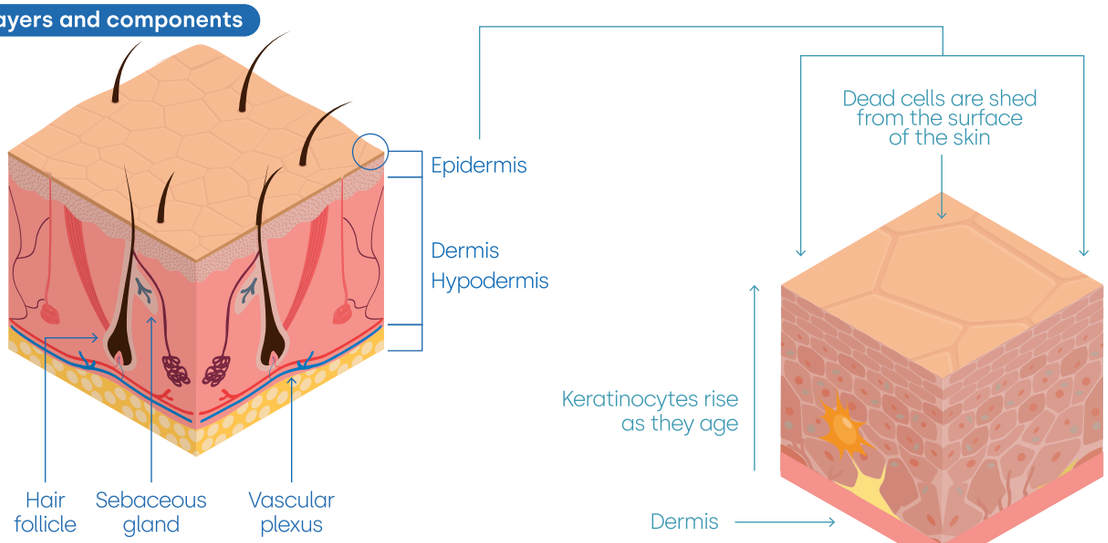
**The hypodermis is the deepest layer** and consists mainly of fatty tissue that can act as an energy store or as an insulating layer to protect underlying tissues.

**The dermis is the middle layer** and gives the skin its strength, elasticity, turgor and resilience. It contains fibroblasts (important for skin healing), blood vessels, nerves, glands and skin hair follicles and is therefore important for regulation and sensation. The dermis also contains sebaceous glands that produce sebum, which lubricates the skin and coat and regulates the skin surface.




**The epidermis is the outermost layer** and consists of four main layers formed by specialized cells called keratinocytes. These cells begin their journey in the deepest part of the epidermis, becoming harder as they migrate through the layers to the outside. At this point, the keratinocytes have hardened and their original state is altered, and they are known as corneocytes. This process is called cornification. Keratinocytes also produce an impermeable fatty substance called the lipid matrix, which fills the spaces between the corneocytes. Together they create a strong, impermeable barrier and act as the first line of defense against harmful pathogens living in our external environment (1). **See Figure 3.**

**Skin layers and components**

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**When this cutaneous homeostasis is lost, various changes may occur that affect the integrity of the skin, compromising its function, as well as the health and well-being of the animal.**

Skin alterations can have a microbiological (parasitic, bacterial and fungal), immune-mediated (allergies and immune-mediated disorders), metabolic-endocrine, acquired or nutritional origins (2).

Metabolic

Inmune

Microbiota changes

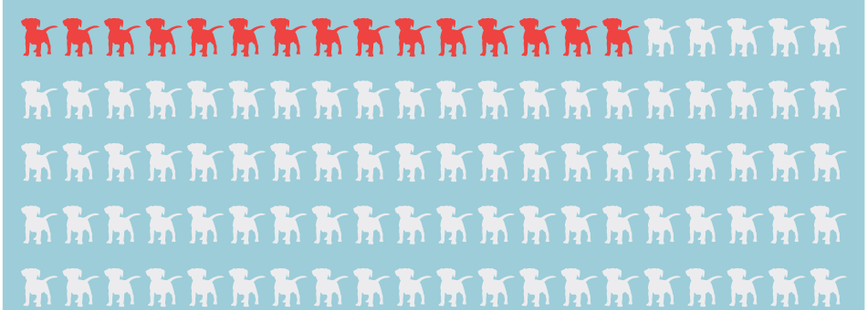
## Canine atopic dermatitis.

Among the diseases associated with immune system disorders, canine atopic dermatitis (CAD) stands out. This is an inflammatory and pruritic skin disease with a genetic, progressive predisposition that causes chronic relapses (3).

**This is a disease that affects 10–15% of the canine population and is the most prevalent dermatological disease in dogs. See Figure 4.**

**Prevalence of atopic dermatitis**

Of every 1**00 dogs, 10–15** suffer from **atopic dermatitis**



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CAD is a genetically predisposed skin disease in which an allergic reaction occurs, causing inflammation and pruritus due to a reaction of immunoglobulin E antibodies against environmental allergens (27). This results in an exacerbated inflammatory response resulting in signs of itching and pruritus, mainly derived from T helper 2 (Th2) lymphocytes such as CD4+. **See Figure 5.** Activated CD4+ cells release Th2 cytokines such as IL-4, IL-5 and IL-13 that contribute to inflammation and the development of lesions commonly found on the face, axillae, inguinal region, abdomen and extremities of affected dogs (3,4,5,6). **See Figure 6.**

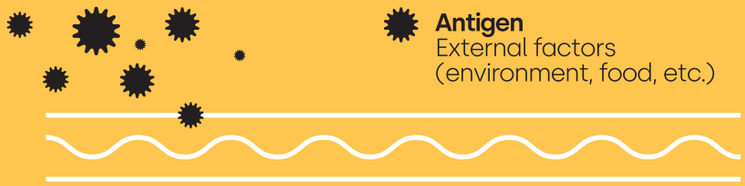
This disease negatively affects the quality of life of the affected dogs, as well as that of their owners. **See Figures 7 y 8.**

**There is currently no curative treatment for CAD. However, there are medications that reduce itching and the severity of dermal lesions.**

**Lokivetmab, oclacitinib, glucocorticoids, cyclosporine, antihistamines and recombinant interferons are commonly used. These treatments can have adverse effects such as vomiting, diarrhoea, weight alterations and immunosuppression, in addition to having very variable efficacy.**

The price of treatment is another factor affecting the accessibility of therapies, especially in middle- and low-income countries (7). Although there is no cure, there are ways of managing the disease that allow dogs to maintain a good quality of life (3,4).

Representation of external antigens that are normally harmless

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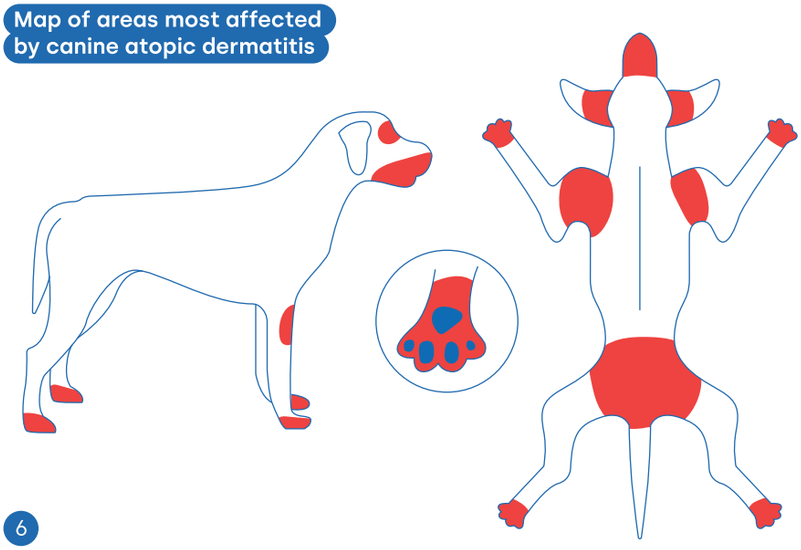
Map of areas most affected by canine atopic dermatitis



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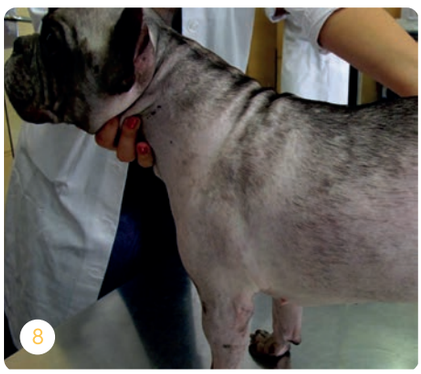

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Actual image of two dogs with atopic dermatitis before treatment with Nucleoforce Pets. In images 11 and 12, the changes after treatment receiving Nucleoforce Pets can be observed.

## Nucleoforce as a complementary treatment against canine atopic dermatitis

**One of the tools that can contribute to improve a dog's response to CAD is Nucleoforce (free nucleotide concentrate).**

There are multiple studies demonstrating the efficacy of **Nucleoforce** in contributing to maintaining immune response against Leishmaniasis in dogs, as well as to improving vaccinal response in puppies (8,9,10,11,12).

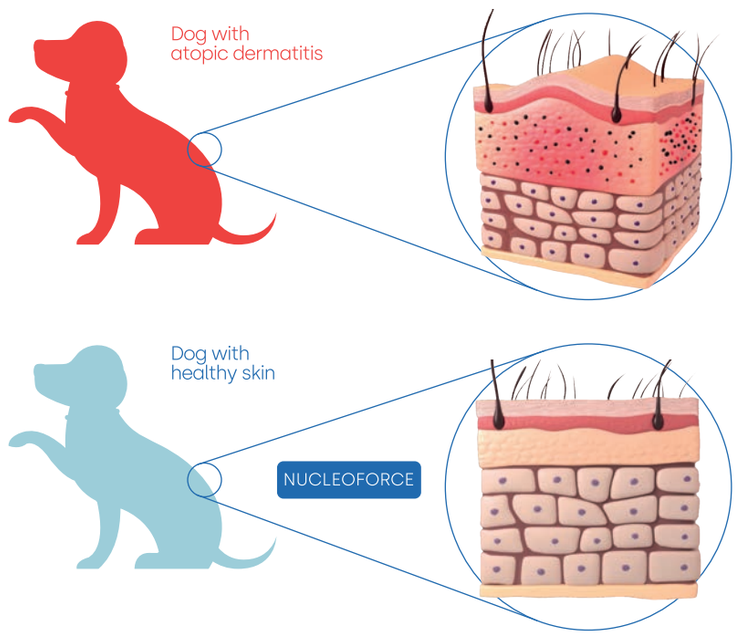
In one 8-week study (13), the nucleotide response was assessed in an induced CAD model in mice and subsequently in dogs that had acquired CAD naturally.

It was observed that **mice that received nucleotides had reduced dermal thickness and contributed to a decrease in inflammation and dermal fibrosis.** In addition, mice that received nucleotides showed a reduction in the intensity of pruritus. **As for the response in dogs, improvements in pruritus and erythema were seen. See Figure 9.** However, alopecia and excoriation were not reversed by the treatment (13).

**The role of nucleotides in contributing to reducing inflammation, pruritus and erythema may be related to immune response modulation (9).**

By reducing the excessive humoral lymphocyte response (Th2), the production of cytokines such as IL-4, IL-5 and IL-13 would be decreased, reducing the excessive production of IgE (14,15,16). This refers to a study by Segarra (9) in which it was observed that Nucleoforce favours cellular response (Th1) to the detriment of the humoral response (Th2). **See Figure 10.**

**Representation of skin in a healthy state with atopic dermatitis.**

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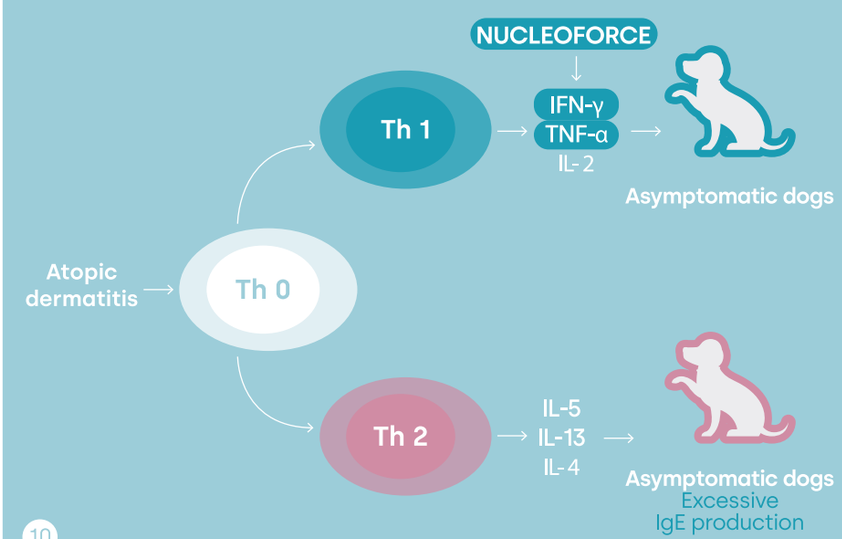
**Nucleoforce-mediated stimulation of cellular response (Th1).**

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Regarding the lack of reversion of the alopecia and excoriation in dogs with atopic dermatitis, it is believed that this may be due to the short duration of the course of treatment (8 weeks), since both alopecia and excoriation are lesions inflicted by the dog itself, so more time is required to notice changes in the animal's behaviour and consequently to observe improvements (17). However, **the observed reduction in pruritus may improve the quality of life of affected dogs and their guardians (17). See Figures 11 and 12.**


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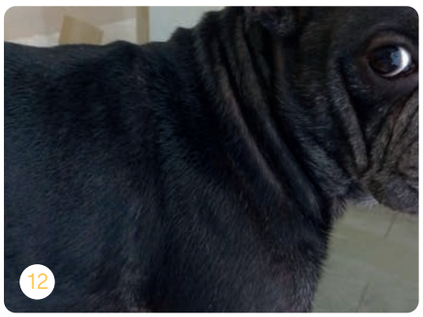
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Actual image of two dogs with atopic dermatitis, after receiving Nucleoforce Pets supplements.

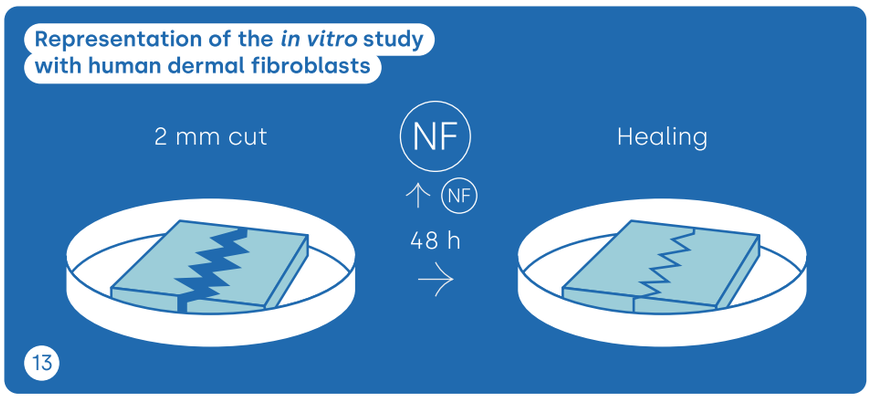
## Nucleoforce to promote fibroblast migration

Recently, in human medicine, nucleotides and polynucleotides have acquired importance as regenerative agents in anti-aging treatments (19,20), this is due to promoting effects of angiogenesis, cellular activity, and collagen synthesis, in addition to having anti-inflammatory activity, among other beneficial effects (19,20). One of the major contributors to improved dermal health in both humans and dogs is the production of collagen, which gives structure and elasticity to the skin. Collagen production in turn depends on fibroblasts, the production of which decreases with age (19,20).

**Nucleoforce has been shown to stimulate fibroblast synthesis and consequently promote collagen and elastin production. This is due to the interaction of receptors on fibroblasts that recognize and bind specifically to nucleotides (18).**

**Nucleotides therefore contribute to maintain the structure and elasticity of the skin, as well as to contribute to promoting its repair (20).** This is especially important in skin pathologies in dogs that affect dermal structure and homeostasis, such as CAD.

To test the effects of Nucleoforce on fibroblast proliferation, Bioiberica performed an in vitro study on human dermal fibroblast cultures (18,21).

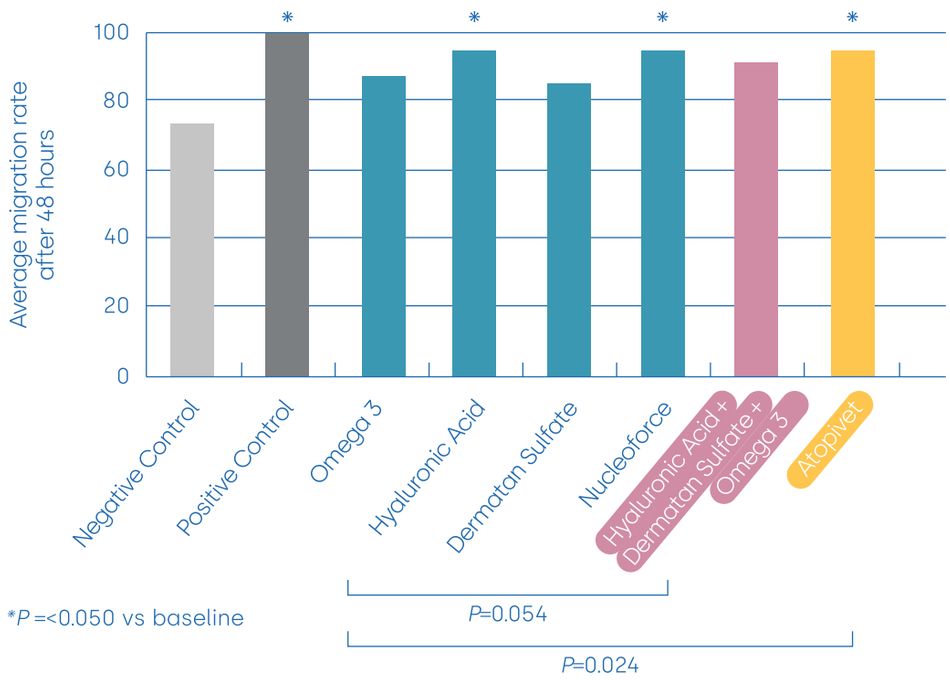



In this study, human dermal fibroblasts were taken and a 2-mm wide cut was made and they had to proliferate towards the other edge to close the cut. **See Figure 13.** The study was carried out over 48 hours and the cultures received different treatments, in addition to the negative and positive control (treated with transforming growth factor β-1, TGF-β1) (18).

**A significantly increased proliferation rate can be observed in the groups supplemented with hyaluronic acid (94.7%) and Nucleoforce (94.1%), as well as with Atopivet (94.7%) and the positive control (99.8%). See figures 14 and 15.**

It can be concluded that Nucleoforce alone or in synergistic combination with Dermial (hyaluronic acid and dermatan sulphate) and Omega 3 contributes to dermal wound repair by promoting fibroblast migration, so it can be a complement to treatment for the healing of dermal lesions.

**Migration rate after 48 hours of treatment**

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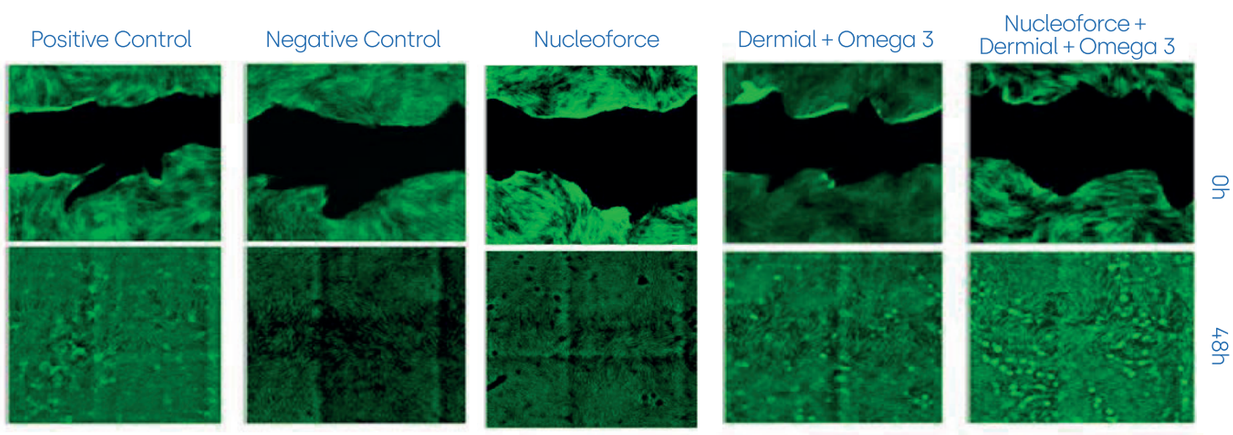
**Digital microscopy showing the proliferation of human dermal fibroblasts using different supplements**

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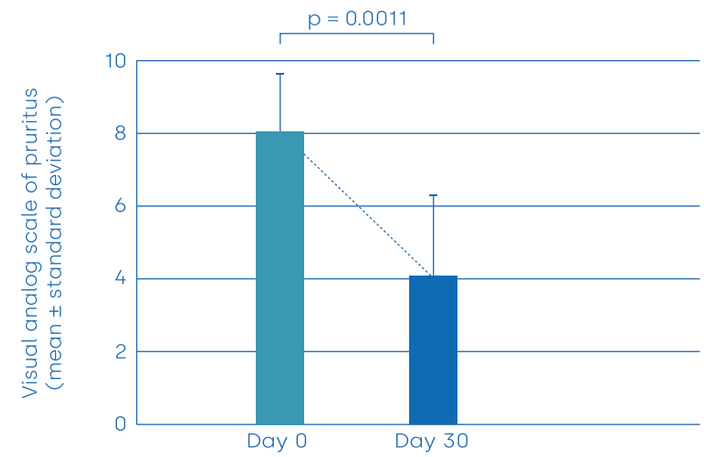
## Nucleoforce as a supplement against canine atopic dermatitis.

Following the good results obtained in the previous *in vitro* study, Bioiberica conducted an *in vivo* study in dogs with Nucleoforce (free nucleotide concentrate) in combination with Dermial and Omega 3.

Eight dogs diagnosed with CAD were used for the study. The dogs received Atopivet, which is a combination of Nucleoforce (nucleotides), Dermial (hyaluronic acid and dermatan sulphate) and omega-3 for 30 days. The veterinarians responsible performed a visual assessment of pruritus before and after treatment (18).

**It can be seen in Figure 16 that after 30 days of treatment with Nucleoforce there is a significant reduction in the visual scale of pruritus, with no adverse effects observed.**

**Changes in the visual scale of pruritus after receiving Nucleoforce**

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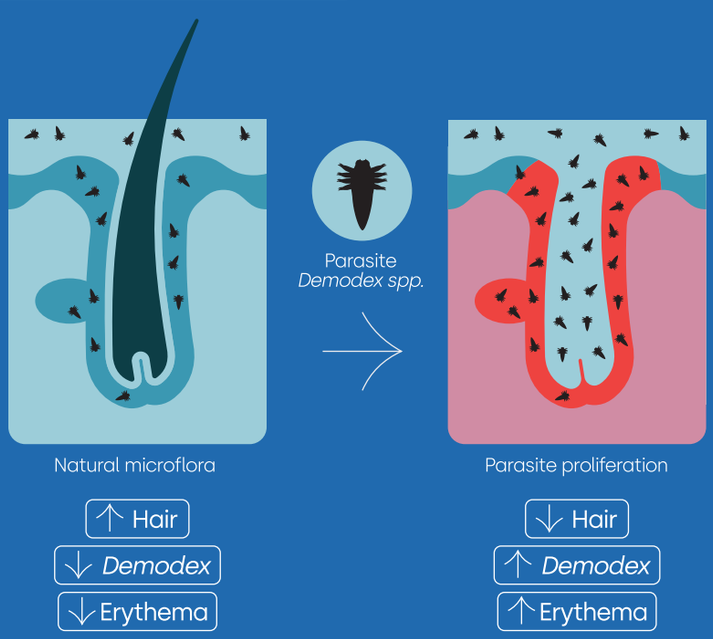
## Canine demodicosis

Another very common dermal pathology in canine clinical practice is demodicosis. Unlike CAD, **demodicosis is defined as an inflammatory skin infection of parasitic origin** (22). The disease is caused by Demodex mites. These mites are organisms that are part of the normal flora of the hair follicles of dogs as well as other mammals (23). The problem occurs when there is a high proliferation of this parasite in the hair follicle, causing erythema, alopecia, pustules, hyperkeratosis and frequent complications such as pyoderma (24). **See Figure 17.**

**One of the causes of this Demodex hyperproliferation is immunosuppression due to a deficient cell-mediated immune response (25).**

**Image of skin under normal conditions and with hyperproliferation of *Demodex spp.***

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## Nucleoforce as a supplement against canine demodicosis

In view of the above, Bioiberica decided to test the effects of Nucleoforce in combination with an active substance for the treatment of non-responsive canine demodicosis (26). For this purpose, 2 Doberman siblings (one female and one male) aged 4 months with recurrent demodicosis and multiple dermal lesions (multifocal alopecia, erythema, folliculitis, furunculosis and pododermatitis) were used. A deep skin scraping and cytology were performed, identifying mites of the genus Demodex canis and secondary pyoderma.

The dogs had been treated with ivermectin and cephalexin for one month, but no improvement was observed. For the study, the dogs received the same treatment and same dose, **but this time Impromune®, which combines Nucleoforce with Immunactive (AHCC), was added orally for 2 months.**

**At 30 days, an improvement in the patients' dermal lesions was observed, and at 60 days all dermal lesions had disappeared. See Figure 18.** However, deep dermal scrapings at day 60 post-supplementation were still positive for Demodex canis. On day 90 post-supplementation, scrapings were negative for D. canis. In addition, serological analyses were performed to measure inflammation markers (C-reactive protein and haptoglobin), antioxidant capacity, albumin and paraoxonase 1 enzyme.

The results shown in **Figure 19** reflect a reduction in inflammatory response and oxidative stress status.

**It can be concluded that supplementation with Nucleoforce and Immunactive (Impromune®) together with standard treatment (ivermectin + cephalexin) improved the clinical signs of both patients with non-responsive demodicosis.**

**Image of a dog before and after supplementation with Nucleoforce**


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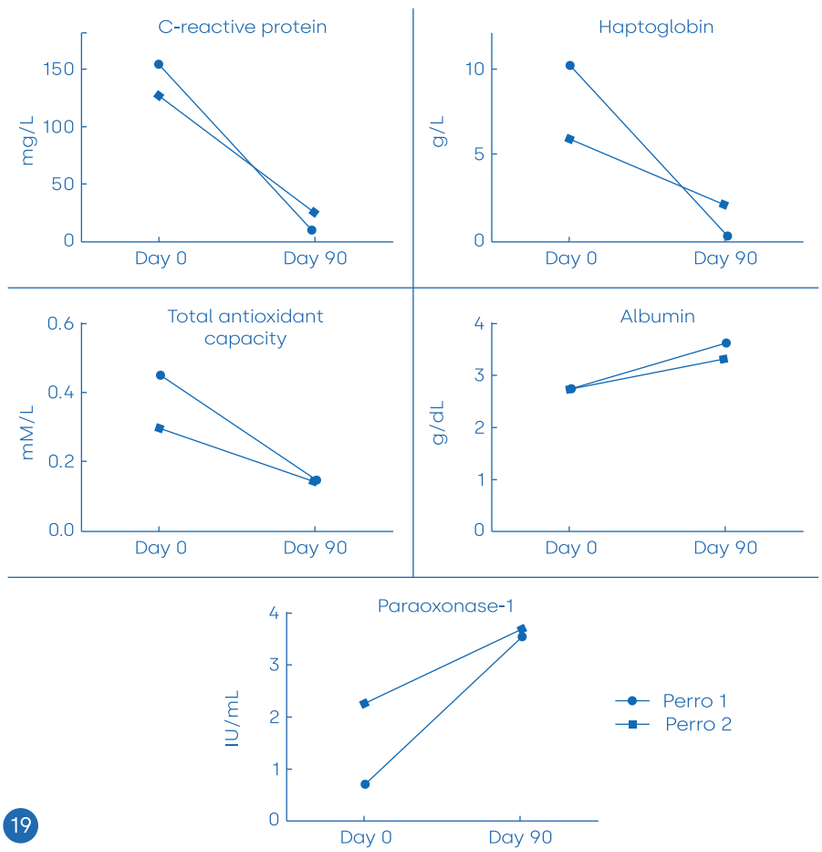
**Serological changes in both dogs supplemented with Nucleoforce**

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## Conclusions

Supplementation with Nucleoforce Pets in the diet of pets can contribute to the maintenance and response of the immune system in situations of immune-mediated disorders or microbiological conditions, but also contributes to the promotion of skin healing by stimulating the proliferation of fibroblasts. This is especially important for the quality of life of pets considering the role the skin plays for our pets.

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