Physiological Shampoo
with Ceramide A2

Technical profile

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Introduction

Over the last twenty years, Virbac has developed a special relationship with veterinary surgeons by providing them with a range of dermo-therapeutic products of high quality, specifically for each type of complaint encountered in dogs and cats.

With the experience acquired in the development of new topical therapeutic technologies, Virbac's ambition today is to put its skills at the forefront of animal dermo-cosmetics and suggest that veterinary surgeons develop the concept of a regular proactive hygiene, for the well-being of pet animals and the pleasure of their owners.

To fulfil its vocation, totally devoted to animals, Virbac wants to enhance the animal hygiene market with care products with innovative properties. These cleansing and restructuring products for frequent use are designed to eliminate skin and coat contaminants, strengthen natural defences, particularly the barrier function of the epidermis and thus prevent the imbalances which can form the starting point for various dermatological conditions.

In this completely new cosmetic range, the Physiological Shampoo with Ceramide A2 has been specifically formulated for regular use on all types of skin and hair, even when fragile and sensitive. This non-detergent shampoo cleanses the skin and coat while maintaining the integrity of the cutaneous barrier and the ecological equilibrium of the skin. Its nutritive and restructuring properties as well as the presence of a patented aldehyde anti-odour complex make it a complete high quality health care product.
I. THE NECESSITY FOR REGULAR PROACTIVE HYGIENE

1. The skin, the frontier between the organism and the external environment

1.1. Structure and functions of the skin

The skin is the largest and most obvious organ in the body. It forms an anatomical and physiological barrier between the internal environment of the animal and its surroundings. It allows the body to maintain internal homeostasis (heat, water, electrolyte or macromolecular exchange) and protects it from external attack of a physical, chemical and microbial nature. It plays a role in numerous metabolic, sensory or recognition functions [23].

The skin of the dog is covered with hair, a characteristic structure of mammals, which plays an important role in protection, thermal insulation and sensory perception.

The skin consists of a thin layer of epithelial tissue, the epidermis, overlying a much thicker supporting connective tissue, the dermis (fig. 1).

The epidermis, the outermost layer, renews itself constantly and rapidly (in 22 days in the dog, [23]). It consists of a superficial cornified layer of lipids and dead cells, one on top of another, and two or three subjacent layers of cells showing intense metabolic activity, principally keratinocytes (85%), with which are associated cells of the immune system, (Langerhans cells, 8%), pigment cells (melanocytes, about 5%) and cells involved in sensory perception (Merkel cells, about 2%). This tissue which is on average 0.1 to 0.5 mm thick in dogs and cats assumes the greater part of the function of protecting the organism [23]. The epidermis does not contain blood vessels and therefore receives its nutrients by diffusion from the subjacent dermis.

Epidermal structures are represented by the hair follicles (the structure where the hair is attached and grows, which descends deeply into the dermis), within which the sebaceous glands and sweat glands release their secretions to form a superficial lipid film covering the visible part of the hair (the hair-shaft) and the surrounding epidermis.

At the dermo-epidermal junction, the epidermis is supported by a basal membrane, which is important for its selective filtration capability, its role in wound healing and maintenance of epidermal proliferation.
The dermis is a vascular permeable tissue which renews itself much more slowly. It is composed of a loose network of cells (fibroblasts, dendrocytes and various cells of the immune system) and protein fibres (collagen and elastin) embedded in an amorphous interstitial substance (a viscoelastic gel of glycosaminoglycans and proteoglycans). This system allows movement and maintains the shape of the body envelope. It provides suppleness and elasticity to the skin. The dermis also contains the vascular, lymphatic and nervous systems.

The hypodermis or sub-cutis represents the greatest thickness and is formed from septa of fibrous connective tissue separating lobules of adipose tissue. The hypodermis has a role as an energy reserve and is involved in thermogenesis and thermal insulation as well as acting as a means of attachment of the skin to the subjacent fibro-skeletal components. It allows movement of the skin by the subcutaneous muscles.

![Structure of the skin](image-url)

*Fig. 1. Structure of the skin*
1.2. The epidermis, an elaborate protective barrier

The barrier function of the epidermis

The epidermis is a site of intense cellular renewal. In its basal layer, stem cells continually produce new keratinocytes which migrate, in successively ordered layers, towards the superficial areas. During this migration the keratinocytes progressively differentiate producing proteins and lipids characteristic of the epidermis. The keratinocytes synthesise particularly large quantities of filaments of cytokeratins, structural proteins of the cytoskeleton. They also produce lamellar bodies, piles of disks formed from lipid bi-layers. In the most superficial layer of the epidermis, the differentiation process is completed: the lipids are extruded into the intercellular spaces and the keratinocytes become flattened dead cells (corneocytes) which contain practically nothing but a dense network of keratin filaments [10].

The most superficial part of the epidermis, or stratum corneum, is therefore composed of an accumulation of superimposed layers of corneocytes (about 50 in the dog, [23]) suspended in an extracellular lipid matrix [7, 1]. The whole structure is often considered as being like "bricks and cement", in which the cornified dead cells represent the bricks and the extracellular lipids the cement holding it all together [2] (fig.2).

The lipids present in the intercellular spaces of the stratum corneum are totally different from the conventional phospholipids that make up cell membranes. About half of them are composed (40 to 50%) of particular sphingolipids, the ceramides, with fatty acids (15 to 25%), and cholesterol (20 to 25%) [1, 10, 3, 6]. The process of extrusion of lipid material from the differentiated keratinocytes in the upper part of the epidermis is dynamic and complex. From it results an arrangement of lipid layers in flattened disks which fuse on being released into the intercellular spaces to form multiple lamellae composed of lipid bi-layers. The ceramides, partly composed of long chain fatty acids, play a primordial role in the formation of these lipid bi-layers. This lamellar organisation is fundamental for an effective barrier function, particularly as regards the exchange of water between the organism and the external environment [1, 2, 4, 11, 12].
Fig. 2. Diagram of the structural and functional organisation of the stratum corneum.

The most superficial corneocytes are continuously exfoliated from the surface of the skin, the speed of desquamation being proportional to the subjacent synthesis, so that the epidermal thickness is maintained constant. The healthy epidermis of domestic carnivores is therefore a site of dynamic equilibrium between the processes of cellular proliferation, differentiation and desquamation. The epidermal lipids and particularly the ceramides and their metabolites directly or indirectly influence all these processes by modulating the interactions between the stratum corneum and the subjacent living cellular layers [1, 23].

The main purpose of epidermal differentiation is to produce an impermeable barrier insulating the organism from the external environment. The specific lipids of the cornified layer of the epidermis, in the forefront of which are the fatty acids and ceramides, play a
physiological role in forming this barrier function. This importance is illustrated by their capacity to regulate the flow of water, to ensure cohesion and correct corneocyte desquamation as well as to control epidermal proliferation and differentiation [10].

Regulation of water exchange

Subcutaneous tissue and the healthy dermis contain 70 to 80% of water. Conversely, the skin surface is relatively "dry". There is therefore a water concentration gradient in the cutaneous tissue. The stratum corneum opposes the passage of water by simple diffusion across the skin, thus limiting losses by evaporation (fig.3). The intercellular lipids, and in particular the long chain ceramides, play an essential role in this reducing of "transepidermal water loss" [1, 4, 21].

Water does not only exist in a free form in the stratum corneum: about 30% of water is bound. Hydration of the upper layers of the epidermis depends directly on the bound water. The lipids present in the intercellular spaces of the stratum corneum form lamellar bi-layers with alternating hydrophilic and hydrophobic layers, which imprison the water within polar layers. Certain ceramides, particularly those with short or medium length chains, play a fundamental role in ensuring correct hydration of the epidermis essential for suppleness, elasticity and flexibility of the skin [1, 4].

One of the essential characteristics of healthy skin is the relationship which exists between the limited transepidermal water loss and correct hydration of the epidermis. Any alteration in the barrier function of the epidermis, in particular of its lipid components, will be expressed as an inversion of this relationship and subsequently by problems of keratinisation and development of the cutaneous microflora [23].
Regulation of cutaneous ecology

The surface of the skin forms a micro-environment populated with natural resident flora (bacteria and a small number of yeasts in the dog) [13]. The populations present are characterised by their capacity to adhere to keratinocytes and to use available nutrients while tolerating the ambient physico-chemical conditions. Factors such as humidity, salinity or skin pH will exert a major influence on the qualitative and quantitative composition of this flora. These factors are controlled by the water retention capacity of the epidermis and the presence of a surface impermeable film covering the stratum corneum. It is formed by an emulsion, produced by secretions from sebaceous and sweat glands opening into the hair follicles. This emulsion contains sebum, inorganic salts and proteins likely to inhibit the growth of pathogenic contaminating micro-organisms and because of this it forms a chemical barrier.

In its turn the symbiotic flora of the surface contributes to the defence of the healthy skin by occupying microbial niches to oppose any colonisation by pathogenic organisms [23].

In addition to its role as a water barrier, the compact stratum corneum forms a physical defence barrier against bacterial colonisation. The continual exfoliation of corneocytes from
the surface (desquamation) eliminates transitory micro-organisms which have a tendency to adhere [1, 15].

Through their capacity to regulate transepidermal water flow and their metabolic influence on the turn-over of keratinocytes, the stratum corneum's fatty acids and ceramides play a major part in maintaining microbiological equilibria on the surface of the epidermis. Maintenance of these microbial ecosystems in its turn guarantees the structural and functional integrity of the skin.

1.3. The fur, a protective and aesthetic coat

One of the essential characteristics of the skin of our pets is that they are covered by a dense coat of fur. In addition to its thermal insulation role, this coat acts as a first physical defence barrier to ultraviolet rays, large foreign bodies or ambient humidity. It also acts to a certain extent as a filter preventing the contact of pathogens with the skin [23]. More important still for the owners of dogs and cats is the aesthetic role of the coat, the appearance of which can be considered as reflecting the general state of health of the animal.

The hair begins life in an invagination of the epidermis into the dermis. At the deepest point of the hair follicle, germ cells divide to produce epithelial cells which become keratinised and progress towards the skin surface forming the sheath and shaft of the hair [23]. The hair is finally composed of epithelial cells in which the nucleus has disappeared and which are organised into three concentric zones (fig. 4):

- the inner medulla, composed of cuboidal non-keratinised cells containing air and glycogen vacuoles,

- the cortex, an intermediate zone, in which the keratinised cells contain melanins, the pigments which give the colour to the coat,

- the external cuticle, formed by flattened cornified cells which are partly superimposed one over another like the tiles of a roof [23].
Bearing in mind the structure of a hair, keratins represent a major constituent of the hair shaft. In addition, the internal structural lipids of the hair are localised in the spaces between the cells of the cuticle. They are associated with proteins, and ensure cohesion between the cells. Analysis of these lipids in the dog has shown that they are composed of ceramides, free fatty acids and cholesterol sulphate [6, 17, 5, 8]. The composition of these structural lipids of the hair recalls the complex lipids of the stratum corneum, which is not surprising bearing in mind the common origin of the two structures and their similar mode of differentiation/keratinisation. The cohesion of cuticular cells with the keratinised sheath of the hair, caused by the presence of ceramides, is thus analogous to the cement function of the ceramides within the stratum corneum. The presence of these ceramides is therefore essential to maintain the beauty, vigour and health of the hair.

Hairs are also coated with external lipids, products of the secretion of the sebaceous glands of the hair follicle [8]. The sebum, rich in waxes, lubricates the hair to produce a closed structure protected from physical and chemical attack and gives it its smooth and silky appearance.

Like the epidermis, the coat is continually being replaced, but the activity of the hair follicles is cyclic and asynchronous in dogs and cats. The hair follicles are assembled into follicular groups with a single opening, composed of a primary hair which is longer and thicker,
Physiological Shampoo with Ceramide A2: technical profile

associated with 5 to 20 secondary hairs. The variation in the respective size and number of these two types of hair determine the different types of coat among races of dogs and cats. The hairs are progressively replaced small area by small area without any particular order (mosaic type of moulting) with an increase in the speed of renewal in the spring and autumn. The hairs therefore grow in a genetically determined cyclic fashion influenced by the animal's age, its nutritional state, state of health and external factors such as the ambient temperature and photoperiodism.

The epidermal lipids, foremost of which are the ceramides, play a major structural and functional role, as much in the stratum corneum as in the fur, by maintaining the epidermal barrier function which is effective against external physical and chemical attack and the invasion of exogenous microorganisms. A dynamic equilibrium is created between the integrity of this cutaneous barrier, its secretions and micro-environment and numerous altering factors present within the organism and in the external environment. This equilibrium is fragile, unstable and can frequently be upset, particularly in canine species.

2. The processes altering cutaneous equilibrium

2.1. The altering factors

Electron microscope studies have revealed that the stratum corneum of the dog is thinner and more compact than in other mammalian species (fig.5). It has an average thickness of only 13µm and it also contains less intercellular lipidic material [1, 2, 20]. Certain authors interpret this data as an inherent fragility of dog skin, partly responsible for a greater incidence of cutaneous bacterial infections in this species [1, 14, 23].

Another characteristic of canine skin is its neutral to alkaline character (pH 7 to 7.4 on average on the surface). It is thus characterised by the absence of an "acid mantle", known in other species and particularly in Man, to act as a protective mechanism against bacterial proliferation [20]. That also implies that shampoos for human use, suited to the acidic pH of human skin, constitute a potential physico-chemical attack in the dog. These shampoos, capable of affecting the cutaneous ecosystem, also modify the electrostatic charges in the lipid bi-layers of the surface and thus can alter the barrier function of the epidermis [22].
Another type of attack linked to the use of unsuitable topical treatments is in the use of irritant shampoos or detergents which alter the composition of the lipids in the cornified layer. From this an increase in transepidermal water loss results together with drying of the epidermis [10, 12, 22]. Often repeated aggressive washing thus leads to disturbances in the water barrier function of the epidermis.

Other potential sources of breakdown of the cutaneous equilibrium are the absence of regular care for the animal (brushing, washing), the type of activity, inappropriate nutrition, age (reduction in keratinocyte and sebaceous gland activity) and upsetting pathological factors (infections, parasites, allergies, endocrine disturbances).

The accumulation on the surface of the skin, in excessive quantities, of secretory products of the epidermal glands, clumps of corneocytes, dirt, contaminants, bacteria and products of their metabolism, pollen and spores can in the long term result in the epidermal barrier becoming fragile favouring the development of pathological conditions [22].

In addition, life in town can sometimes disturb the natural regulatory phenomena of hair renewal: heated buildings, artificial light, reduced activity, etc.

*Fig. 5. Diagram illustrating the characteristic differences between the skin of Man and that of the dog*
2.2. Characteristics of an altered skin

A defect in the protective barrier of the epidermis leads notably to **dryness and a loss of suppleness in the skin.** Alteration in the processes of keratinocyte proliferation/differentiation is responsible for the appearance of **scales** (*photo 1*). The characteristics of adhesion and proliferation of the cutaneous flora are modified and may predispose to the development of infections. The risk of transcutaneous penetration of antigens is increased and can lead to the hypersensitivities being expressed.

![Photo 1. Dry skin and dull coat in a dog](image)

The metabolism of surface lipids by the microbes which proliferate leads to the formation of **unpleasant smelling derivatives.** An unpleasant smell persists, decreasing the pleasure of contact between the owner and his or her pet animal.

In addition, the alteration of the internal (cuticular ceramides) and external lipids (sebum sheath) of the hair no longer guarantees effective protection: the hair of the coat breaks easily and becomes dull. This **dull and tangled coat** gives the appearance of an "old animal" to a still playful pet whose well-being, as well as cosmetic appearance, is threatened.
Physiological Shampoo with Ceramide A2: technical profile

3. The Virbac dermo-cosmetic solution

Dogs and cats then, whatever their type of skin and hair and whatever their age, should receive regular hygienic care treatment specifically designed for them, to maintain and strengthen the essential cutaneous barrier which effectively protects them from dehydration and external attack and strengthens the structure of their coat.

This necessity for preventive hygiene to keep pet animals at their best in terms of beauty and health has led Virbac dermatological research to develop a special shampoo with nutritive and restructuring properties for maintaining the ecological equilibria of the skin of carnivores.

To achieve this objective, it was essential in the composition of these health care products, to use active ingredients similar to those found in animal skin (the idea of cutaneous biomimetics), in order to target their action effectively with perfect local tolerance. Realising the primordial importance of the role of the lipids of the cornified layer of the epidermis and identifying the most active constituents for maintaining the epidermal barrier in the dog clearly underlined the importance of providing these microlipidic nutrients, ceramides and unsaturated fatty acids via the topical route. The benefit of using these constituents is twofold:

- for the skin: maintenance of the hydration and integrity of the epidermis, guaranteeing a supple restructured skin,
- for the coat: strengthening the external envelope of the hair, guaranteeing shiny smooth hair which does not break.

To deal with the problem of disagreeable body odours, sometimes associated with poorly looked after skin or related to epidermal secretions which harm the relationship between the animal and its master, Virbac has become associated with a specialised company that has exclusive patented technology for neutralising unpleasant smells. This innovative technology is based on the concept of long term modification of unpleasantly smelling molecules and not simply of masking them temporarily. The animal is protected in the long term against unpleasant smells and the quantity of perfume in the product is reduced, thus optimising its local tolerance and limiting the risks of allergy.
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Designed to clean the skin and the coat of animals effectively, this liquid physiological shampoo, with abundant foam and at a suitable pH gently eliminates contaminants and dirt from the surface of the epidermis, while maintaining the natural cutaneous ecosystem, and leaves the hair supple, shiny and easy to brush.

By developing this hydrating restructuring active shampoo that neutralises odours Virbac has added a preventive dynamic care aspect to its range of specific dermatological products designed for veterinary use.

II. RESTRUCTURING COMPONENTS WHICH PROTECT AND STRENGTHEN THE EPIDERMAL BARRIER AND THE STRUCTURE OF THE HAIR

1. Ceramide A2

1.1. Ceramides, molecules which regulate epidermal hydration

Ceramides are complex lipids formed from a sphingoid base (sphinganine, sphingosine or phytosphingosine) linked to a saturated fatty acid (fig.6.). They represent 40 to 50% of the lipids in the stratum corneum. Six classes of ceramides can be distinguished, differing in the nature of their sphingoid base as well as in the length and hydroxylation (addition of -OH groups) of the carbon chains of their fatty acids. Fine self-regulating mechanisms control the synthesis of ceramides by the keratinocytes depending on the needs of the epidermis at any given time.

The ceramides have remarkable structural properties which allow the stratum corneum to play its role fully as cutaneous barrier.
In general, lipids are not hygroscopic molecules, capable of binding water like proteins or sugars. But ceramides have a bipolar character: the long aliphatic carbon chains of the molecule are hydrophobic, while the "head" which links them is on the contrary hydrophilic (fig. 7).

In combination with other lipidic molecules of the stratum corneum (fatty acids and cholesterol), ceramides are assembled in micelles, successions of layers hydrophilic on contact with water surrounded by hydrophobic layers [4]. Because of the -OH groups in their hydrophilic heads, the ceramides are capable of forming hydrogen bonds with water molecules which they retain between two hardly permeable layers. About 30% of the water in the stratum corneum is found in this bound form, providing the hydration of the superficial layers of the epidermis.

It is therefore their ability to be oriented, to be structured in multiple lamellar bi-layers, which allows the ceramides to "capture" the water within the cornified layer of the epidermis [1, 10, 3] (fig.8).
Physiological Shampoo with Ceramide A2: technical profile

The ceramides do not all play an identical role in hydrating the stratum corneum. Certain types of ceramides with medium length or short chains, and in particular type 2 ceramides, are more actively involved in holding the water bound within the bi-layers of the stratum corneum. They act as traps for water in transit by fixing the transepidermal water with their polar heads and can thus considerably increase the water holding capacity of the upper layers of the epidermis. These type 2 ceramides are thus true water regulatory units of the "cohesive cement" [1].

As found in humans, recent thin-layer chromatography studies have evidenced the presence of the 6 types of ceramides in dog skin, among with the type 2 ceramides. Interestingly, these studies also demonstrated that the total amount of ceramides in dog skin decreases with age [25].

1.2. The double target of Ceramide A2

The type 2 ceramides are one of the most important functional classes of ceramides in the stratum corneum. The Ceramide A2 incorporated into the Virbac physiological shampoo is a pure molecule obtained by a synthetic route and is an analogue of cutaneous type 2 ceramides (fig.9). It is associated with a polymer which increases the presence of the active substance on the surface of the hair and the skin. Because of its hydrophobic character Ceramide A2 is easily integrated into the intercellular lipidic spaces of the cornified layer of
the epidermis and the cuticle of the hair (fig.10). Supplied topically it acts directly on the sites where it is physiologically localised:
- to trap and hold water needed by the epidermis to remain supple, smooth and hydrated,
- to strengthen the structure of the hair and produce improvement in the vigour, form and shine of the coat.

Fig.9. Structural relationship between type 2 and A2 ceramides

Used regularly as proactive hygiene care, the shampoo with Ceramide A2 maintains and strengthens the hydration and structural integrity of the cutaneous barrier. Through its nutritive properties for the upper layers of the epidermis, it contributes to restoring the cutaneous lipid film, to normalising and rehydrating dry skins. By strengthening the cohesion between the cuticular cells of the hair it also allows shiny smooth non-breaking hair to be obtained (photo 2).
Physiological Shampoo with Ceramide A2: technical profile

Before applying a shampoo with Ceramide A2
An altered hair: loosened cuticular cells, numerous contaminants

3 weeks after applying a shampoo with Ceramide A2
A healthy hair: regular cohesive cuticular cells, absence of loosening, few contaminants

Photo 2. Photos of hairs under a scanning electron microscope
Physiological Shampoo with Ceramide A2: technical profile

Fig. 10. The double target of Ceramide A2

2. The Essential Fatty Acids (EFA)

Fatty acids are formed from a hydrocarbon chain terminating at one end by an acid group (-COOH) and at the other by a methyl group (-CH₃). Linoleic and γ-linolenic acid, which have 18 carbon atoms, have 2 and 3 double bonds (polyunsaturated) respectively, the first of which is situated on the 6th carbon atom from the methyl end (fig.11.). For this reason they are part of the omega 6 family [22]. These fatty acids are essential for skin metabolism but cannot be synthesised by the organism, so external supply is thus essential (in the food or via the topical route) [9].

These two types of EFA are components of the Virbac physiological shampoo:
- **linoleic acid**, the parent molecule of the omega 6 series, as the **major component of the cement between the corneocytes**, which contributes to the formation of certain ceramides,
- \(\gamma\)-linolenic acid, derived from the above, the metabolism of which leads to the compound forming the building blocks of cell membrane phospholipids.

They are thus both cellular and intercellular components with the role of strengthening the tissue structure of the epidermis [1].

**Linoleic acid and the barrier function of the epidermis**

Type 1 and 6 ceramides have very long fatty acid chains (C\textsubscript{30}) which extend across the hydrophobic regions of the intercellular lipidic leaflets thus stabilising the lamellar layers. Type 1 ceramides (O-acyl ceramides) in particular incorporate a molecule of linoleic acid joined by an ester bond to the terminal extremity of the aliphatic fatty chain (fig.12.). These "linoleic tails" are inserted into the regions adjacent to the lipid bi-layers and act as molecular rivets joining two leaflets together [3, 10].

These long chain ceramides, and through them the linoleic acid molecule which they contain, therefore play a major role in the organisation and stabilisation of the intercellular lipid lamellae of the stratum corneum. This property allows them particularly to regulate the passive transepidermal water flow (loss of water by evaporation).

Thus it is as a component of acylceramides that linoleic acid plays its essential role in maintenance of the cutaneous barrier function [3, 10]. Research workers have been able to show in the dog that dry skins contain reduced quantities of linoleic acid [18]. Administered topically, linoleic acid participates to the synthesis of acylceramides in the lamellar bodies of the keratinocytes [1] and produces significant reduction in transepidermal water loss [24].
Virbac physiological shampoo therefore includes constituents which act in a complimentary way on the regulation of water flow in the skin:

- **Linoleic acid**, via the class 1 ceramides, reduces transepidermal water loss from the epidermis by strengthening the barrier which opposes passive diffusion of water;

- **Ceramide A2** fixes the water bound within the stratum corneum and thus ensures good hydration of the epidermis.

**γ-linolenic acid and membrane fluidity**

The skin is overall deficient in the enzyme (delta-6 desaturase) which converts linoleic acid into γ-linolenic acid. It is thus also necessary to provide the latter topically in addition to the former. In the epidermis, γ-linolenic acid is rapidly converted into dihomogamma linolenic acid (DGLA) with 20 carbon atoms. The latter is integrated into the composition of phospholipids in the keratinocyte cell membranes in the living part of the epidermis. The presence of double bonds is in particular responsible for membrane fluidity, essential for the functional integrity of skin cells and hair [9].

*The association of Ceramide A2 and EFAs amplifies the strengthening and restructuring action on the coat and consolidates the cutaneous protective barrier (fig.13). The synergistic properties of its active components make Virbac physiological shampoo a hydrating and lipid replacing care product for pets.*
III. AN INNOVATIVE TECHNOLOGY FOR NEUTRALISING ODOURS

The disagreeable odours coming from animals may be linked to oxidation of the lipids in an altered epidermis and/or to the transformation of these lipids into unpleasant smelling compounds by the bacterial flora. Other osmophoric compounds also add to the "bouquet" such as sulphur and nitrogen derivatives and alcohols and aldehydes. The unpleasant smells detected by the human nose are therefore complex mixtures of volatile compounds, the concentrations of which vary with time and are particularly influenced by the contact of the coat with water [16].
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The classic methods for neutralising these unpleasant smells rely on compensation mechanisms or masking based on the use of potentially irritating doses of perfumed compounds. After shampooing the length of action of these perfumes is often very short. The innovative process used by Virbac relies on a patented method of neutralising unpleasant smells based on the reactivity of aldehyde compounds with the unpleasantly smelling volatile molecules of the skin of carnivores. These compounds, naturally present in certain fruits or plants, interact with the unpleasant smelling molecules, on the one hand neutralising them and on the other, modifying their olfactory perception.

The deodorising composition contains two aldehydes, one of which differs from the other by being unsaturated on the carbon in the alpha position of the aldehyde group. Olfactory tests have demonstrated that a pair of aldehydes belonging to these two different classes produces a greater reduction of the smell than each of the aldehydes taken separately.

The two aldehyde technique thus provides a double advantage compared with traditional methods of reducing smells:

- **the immediate effective disappearance of the unpleasant smells,**

- **a longer period of deodorant action because of the high reactivity of the aldehydes.**

Moreover, the pair selected gives the physiological shampoo a natural perfume similar to coconut which makes its use very pleasant for the owner.

Consequently, the quantities of perfume decrease in a spectacular fashion in the formula of the physiological shampoo compared with other shampoos on the market, which increases its cutaneous tolerance and limits the risks of allergy (fig.14.).
IV. ACTIVE CARE PRESERVING THE SKIN'S ECOLOGY

1. High skin tolerance

Virbac, by its commitment to its ethical charter, guarantees that only useful ingredients are present in its dermatological compositions. The new cosmetic range is subject to the same demands for quality and safety.

The formulation of Physiological Shampoo with Ceramide A2 is optimal, without unnecessary additives, colorants or texturing agents and without addition of traditional perfumes. It is produced from innovative and suitable components and from bio-mimetic active molecules:
Physiological Shampoo with Ceramide A2: technical profile

• gentle in-depth washing components make it a shampoo with a high skin tolerance, non-detergent and non-irritant formula,

• Ceramide A2 and unsaturated fatty acids, nutrient and restructuring agents for the skin and coat,

• a patented anti-odour aldehyde complex with sustained action, without any masking perfume, to respect the skin and coat better,

• a physiological pH conserves the integrity of the skin's ecosystem.

The specially adapted composition of this skin and coat cleaner means it can be used regularly on all types of skin and hair, even if fragile and sensitive without risk of irritation or imbalance in the cutaneous ecosystem.

2. Excellent cleansing power

Physiological Shampoo with Ceramide A2 has been specifically formulated to cleanse the skin and coat of domestic carnivores effectively. Its gel texture produces a generous foam to gently clean, while respecting the integrity of the epidermal barrier.

This abundant foam encourages the elimination of dead hairs and dirt incrusted in the coat and skin. It also removes certain allergens from the skin surface and limits their penetration across the cornified layer. This shampoo rinses away easily without leaving a residue on the surface of the skin and hair.
V. A COMFORT PRODUCT WHICH IS EASY TO USE

1. An ergonomic presentation

The innovative packaging of Physiological Shampoo with Ceramide A2 meets the requirements for ease of use for frequent care.

AN OVAL CONTAINER OPENING UNDERNEATH

A SPECIAL DESIGN FOR EASY HANDLING

A CAP FITTED WITH A VALVE MAKING APPLICATION SIMPLER:
NO SPILLING DURING APPLICATION GUARANTEEING CLEANLINESS AND ECONOMY
2. A wide field of use

_Circumstances for use_

The external appearance of a pet animal is often of great importance for the owner. The object of his attention, a sign of his pride and even of his social ambition, it is above all perceived as a reflection of the good general health of the animal and because of this, of the quality of care given to it.

The veterinary surgeon is often the person to whom his clients come for advice concerning how best to maintain the health and beauty of the skin and coat of their animals. The appearance of "dandruff", a dry skin, dull sparse or breaking hair, prolonged periods of moulting, unpleasant body odour are all grounds for questioning by the owner and require an appropriate dermatological answer.

Physiological Shampoo with Ceramide A2 is a comfort care product which meets these needs: while effectively cleansing the skin and coat of small carnivores, it maintains and strengthens the upper layers of the epidermis and the external covering of the hair. It is thus suited for the cosmetic care of healthy animals where the coats are dirty, dull or lack vigour. It aims to protect the barrier function of the epidermis and prevent possible alterations or imbalances in it which could lead, in the end, to the development of a dermatological condition.

This regular hygiene shampoo is also recommended in the following physiological situations:
- Animals where the _epidermis shows signs of dehydration_: beginnings of apparent desquamation, lack of suppleness.
- Animals where the _coat is dull and tangled_ giving them an "old dog" appearance.
- _Older animals_ where the epidermis is thinner, less hydrated, the surface hydrolipidic film is less abundant and where renewal of hair is slower [19] (the hair is becoming dry and breaks, it is going grey particularly around the muzzle).
- Animals needing _shampooing very frequently_, owing to their activity.
- Animals with _marked body odour_ (or where the owner has a very sensitive nose),
- _Exhibition animals._
The shampoo with Ceramide A2 is therefore a (cosmetic optimising) physiological complement to Virbac's therapeutic range, the latter being used for recognised dermatological conditions.

If allergic phenomena or keratinisation problems occur, there are profound alterations in the barrier function of the epidermis often combined with cutaneous lesions, dermal inflammation and secondary microbial infection. These problems necessitate specific restorative treatments with medicated shampoos, such as Allermyl® or Sebomild P®, associated if necessary with treatment administered by the general route.

Method of use

Physiological Shampoo with Ceramide A2 must be applied on a coat previously wetted with tepid water and the product should be evenly spread over the surface of the coat by circular movements. Massaging all over the body of the animal will produce the foam. After leaving the product for 1 or 2 minutes in contact with the animal's skin, it is carefully rinsed off with clean water. The rinsing time must always be longer than the washing time. For animals who have fur which is initially very dirty, two applications of shampoo are recommended in order to obtain best results.

3. Satisfactory sensory assessment studies

Aim of the cosmetic assessment studies

To test the ability of the new hygiene shampoo Virbac undertook sensory assessment tests by dog grooming parlours, where there is frequent use of the various current care shampoos and where there is particularly awareness of the cosmetic impact of products. The objective was to collect the comments and assessments concerning the shampoo's cleansing and restructuring effectiveness, as well as to confirm the excellent tolerance of the product in normal conditions of use in the field.

Method

This test took place over a period of four weeks. Twenty-one dogs of all ages and both sexes were included in the study. The subjects were not suffering from any overt dermatological condition. Nevertheless certain dogs had a rather dry or greasy coat and a skin with a squamous tendency. The majority of the races represented were small to medium sized dogs
Physiological Shampoo with Ceramide A2: technical profile

(Lhassa Apso, Bichon Frisé, Coton de Tulear, West Highland White Terrier, Shih Tzu, Yorkshire Terrier, Cocker Spaniel, Poodle) the main customers of grooming parlours. Two thirds of these dogs had long hair and nearly one third medium length hair, with only one dog having short hair.

In the grooming parlour all the dogs were washed twice successively with the Physiological Shampoo with Ceramide A2. The quantities of shampoo used as depending on body weight of the animals are summarised in the table below:

<table>
<thead>
<tr>
<th>Size</th>
<th>5-10 kg</th>
<th>11-15 kg</th>
<th>16-20 kg</th>
<th>21-25 kg</th>
<th>26-45 kg</th>
<th>&gt; 46 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short hair</td>
<td>10 ml</td>
<td>12.5 ml</td>
<td>15 ml</td>
<td>17.5 ml</td>
<td>25 ml</td>
<td>30 ml</td>
</tr>
<tr>
<td>Long thick hair</td>
<td>20 ml</td>
<td>25 ml</td>
<td>30 ml</td>
<td>35 ml</td>
<td>50 ml</td>
<td>60 ml</td>
</tr>
</tbody>
</table>

Two assessment criteria were chosen:
- the pleasure and ease of use of the product for the groomer,
- the impact of the use of the shampoo on the skin and coat of the animal as well as on its odour.

Results

Perception of the pleasure of using the Physiological Shampoo with Ceramide A2 by the groomers:

- Foaming power of the product:
  - Foams correctly: 1
  - Foams too much: 18
  - Does not foam enough: 7
  - Does not foam: 20

- Ease of rinsing the product:
  - Very practical and very easy: 1
  - Practical and easy: 3
  - Acceptable: 17
  - Not practical or difficult: 13

- Perception of the smell of the product:
  - Very pleasant: 1
  - Pleasant: 7
  - Acceptable: 13
  - Disagreeable: 1
A large majority of groomers appreciated the use of this shampoo, underlining its agreeable smell, good foaming power and easy rinsing, because of its fluidity.

**Impact of the use of the Physiological Shampoo with Ceramide A2 on the odour of the skin and coat of the dogs:**

**To the question: would you advise your clients to use this shampoo to wash their dogs at home?**

The groomers replied:

- **Yes:** 4
- **No:** 17

General appreciation of its use

**Skin and coat condition before application of the product**

- **Clean:** 4
- **Dirty:** 1
- **Greasy:** 2
- **Dry:** 14

**Skin and coat condition after application of the product**

- **Cleaner:** 1
- **Identical:** 7
- **More greasy:** 1
- **More pleasant to touch:** 3

- **Shinier:** 14
- **Identical:** 20
- **Less greasy:** 18
- **Less pleasant:** 3

**Skin and coat condition after application of the product**

- **Cleaner:** 1
- **Identical:** 7
- **More greasy:** 1
- **More pleasant to touch:** 3

- **Shinier:** 14
- **Identical:** 20
- **Less greasy:** 18
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- **Shinier:** 14
- **Identical:** 20
- **Less greasy:** 18
- **Less pleasant:** 3
The cleansing efficacy of the product satisfied almost all of the groomers, whatever the state of the skin and coat before application. The improvement in the texture of the hair is perceptible: the coat is softer, silky, pleasant to touch, has no electrical charge and is easy to brush. When the contact time of several minutes is kept to before rinsing away the shampoo with Ceramide A2, certain groomers noted that it was unnecessary to use conditioners after the bath.

The shampoo also makes the coat radiant again, the hair is seen as clearly more shiny in the majority of cases. According to one groomer, having taken part over a number of years in canine beauty competitions, the cosmetic impact of the product is even sufficient to recommend its use for exhibitions.

The animal's smell is perceived as agreeable to very agreeable. No itching, skin irritation or abnormal reaction of the animals appeared during or after the bath, which bears witness to the excellent tolerance of the shampoo with Ceramide A2.
Conclusion

Physiological Shampoo with Ceramide A2 from Virbac laboratories represents a new step in the search for better regular care for the skin and coats of pet animals. It is a real health care formula because of its cleansing qualities yet it also has a protective role and prevents imbalance by strengthening the barrier function of the epidermis while maintaining the cutaneous ecosystem. Its nutritive and restructuring qualities confer on it an immediately appreciable cosmetic impact, with perfect cutaneous tolerance.

The skin is rehydrated, the hairs are smooth, shiny and do not break, an odour of well-being emanates from the animal for the greater joy of its owner, who thus rediscovers all the pleasure of contact with his companion.

Skin care – it's their health!
References


Physiological Shampoo with Ceramide A2: technical profile


