Clinical Evaluation of a Nutraceutical Diet as an Adjuvant to Pharmacological Treatment in Dogs Affected by Epiphora

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ABSTRACT

Epiphora is defined as tear overflow due to impaired tear drainage by way of the nasolacrimal duct. We evaluated a combined therapeutic approach for epiphora based on drug and a commercially available nutraceutical diet administration over a 30 days period in dogs poorly responsive or unresponsive to the immune-suppressive treatment alone.

Forty-five client-owned household dogs of different breeds (19 females and 26 males) and aged 6.5 years were enrolled. Schirmer tear test-1, conjunctival inflammation, corneal keratinization and blepahritis were evaluated before and at the end of the evaluation.

Schirmer's test value significantly decreased from 22.96 ± 0.37 mm, at T0 to 18.86 ± 0.24 mm, at T30 (***$p < 0.001$), conjunctival inflammation significantly decreased from 1.30 ± 0.11, at T0 to 0.14 ± 0.04 at T30 (***$p < 0.001$), corneal keratinization significantly decreased from a T0 value of 0.17 ± 0.05 to a T30 value of 0.07 ± 0.03 (*$p < 0.05$) and blepharitis significantly decreased from 0.64 ± 0.1, at T0 to 0.03 ± 0.02 at T30 (***$p < 0.001$).

This clinical evaluation represents the first evidence of the usefulness of a specific nutraceutical diet as a reliable tool to improve pharmacological treatment of epiphora.

INTRODUCTION

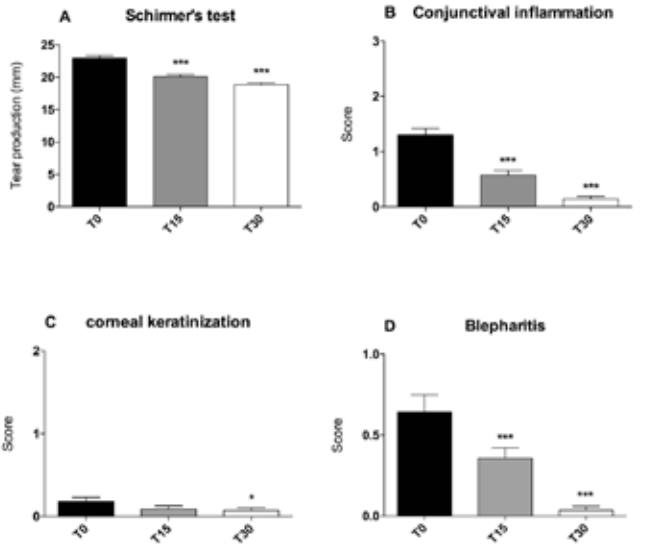
Epiphora is defined as tear overflow because of impaired tear drainage by way of the nasolacrimal duct.[1] It is a fairly common problem in some of the smaller breeds, such

as Bichon Frisé, Maltese Terrier, Miniature and Toy Poodle, and Tibetan Spaniel, where it's particularly evident due to their pale coats, since tears stain them a rusty brown. The onset of this kind of condition is generally observed from 2 – 3 months of age onwards, and it is usually bilateral.[2] Predisposing features are:

- shallow orbit with a prominent globe
- close apposition of the eyelids to the globe, resulting in a shallow lacrimal lake that limits lacrimal drainage
- medial lower eyelid entropion
- misplacement of the lower punctum without medial lower eyelid entropion
- hair at the medial canthus, of both the eyelids and the lacrimal caruncle, tends to impinge on the ptf (pre-ocular tear film), thus keeping tears from flowing in the lacrimal duct.

We aimed to evaluate a combined therapeutic approach for epiphora based on the classical drug administration and the use of a commercially available nutraceutical diet in dogs where the only immune-suppressive treatment alone resulted poorly responsive or ineffective to control the ocular symptoms. The nutraceutical diet consisted in a mixed formula of fish meal and rice, as a single source of carbohydrates, and botanical extracts including Aloe vera, Astaxanthin (from *Hematococcus pluvialis*), bioflavonoids, *Curcuma longa*, *Ribes nigrum*, *Vitis vinifera*, and Omega3-Omega6 fatty acids ratio of 1:3.

Figure 1. *Schematic representation of clinical signs trend during the 30 days evaluation period (*p < 0.05, **p < 0.01, ***p < 0.001)*



MATERIALS AND METHODS

Forty-five client-owned household dogs (19 females and 26 males) aged 6.5 (average) of different breeds were enrolled in this evaluation. Dogs were fed the nutraceutical diet for a 30-day period. Both eyes of each dog were photographed at each visit, and clinical signs were classified according to the scores proposed by Moore et al.[3] Conjunctival inflammation (0-3):

- 0 = normal conjunctiva
- 1 = mild Chanhe hyperemia in hyperemia without chemosis
- 2 = moderate Chanhe hyperemia in hyperemiaa with mild chemosis
- 3 = intense Chanhe hyperemia in hyperemia with moderate to severe chemosis;Corneal keratinization (0-2): 0 = none, 1 = mild opacity, 2 = moderate opacity. Ophthalmic examinations included also slit-lamp biomicroscopy (Kowa Optimed Inc SL-14 Slit Lamp, Kowa Optimed, Europe Ltd, Berkshire, UK), funduscopic examination (Heine Omega 180 Binocular Indirect Ophthalmoscope, HEINE Optotechnik, Herrsching, Ger-

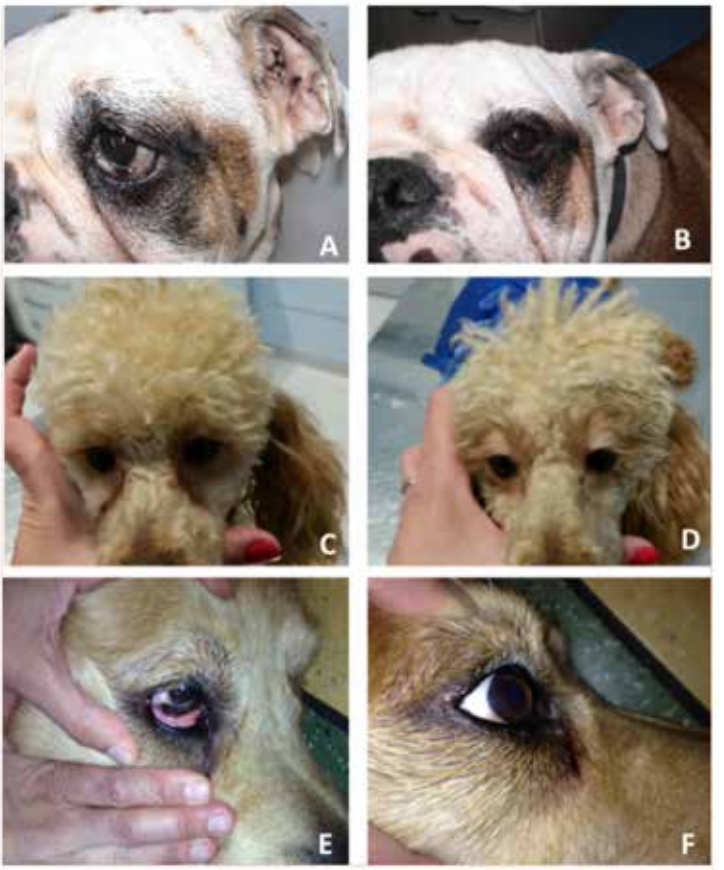
many), fluorescein dye staining (fluorescein 0.5% collyre unidose TVM, Laboratoires TVM, Lempdes, France), and Schirmer tear test-1 (STT-1) (Dina Strip Schirmer-Plus Dina-Hitex) on 90 eyes (two for each dog).[4]

The recommendations of the ARRIVE guidelines in animal research were consulted and considered.[5]

Statistical Analysis

Data were analysed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are presented as the means ± standard error of the mean and were first checked for normality test using the D'Agostino-Pearson normality test. Differences in Schirmer's test, conjunctival inflammation, corneal keratinization, and blepharitis before and at the end of evaluation period were analyzed using a one-way analysis of variance (ANOVA) followed by Turkey multiple comparisons test. A *p < 0.05 was considered significant.

Figure 2. *Graphical representation of eyes improvement before (A, C, E) and after (B, D, F) 1 month of the nutraceutical diet supplementation*



RESULTS

Schirmer's test value significantly decreased from 22.96 ± 0.37 mm, at T0, to 20.09 ± 0.33 mm (***p < 0.001) at T15, to 18.86 ± 0.24 mm, at T30 (***p < 0.001). The same trend was observed for the clinical signs. More in details, conjunctival inflammation significantly decreased from 1.30 ± 0.11, at T0, to 0.57 ± 0.08 (***p < 0.001), at T15, to 0.14 ± 0.04 at T30 (***p < 0.001). Corneal keratinization significantly decreased from a T0 value of 0.17 ± 0.05 to a T30 value of 0.07 ± 0.03 (*p < 0.05). As to the blepharitis score, a significant decrease from 0.64 ± 0.1, at T0, to 0.35 ± 0.06 (***p < 0.001), at T15, to 0.03 ± 0.02 at T30 (***p < 0.001) was observed.

DISCUSSION

To the best of our knowledge, this clinical evaluation represents a first study that proposed the use of a specific nutraceutical diet as a reliable tool to improve pharmacological treatment of epiphora. The nutraceutical approach significantly decreased the eye's tear production, and clinically ameliorated the conjunctival inflammation status, the corneal keratinization and blepharitis in dogs poorly responsive or unresponsive to gold standard pharmacological therapy of

epiphora.

The decreased STT-1 level in response to the nutraceutical diet was in agreement with what previously reported by Saito et al, who compared mean tear production in dogs with and without epiphora.[1] Although we are unaware of the possible action mechanism exerted by botanicals present in the nutraceutical diet, we hypothesize a synergic action with raw materials in modulating functional and mechanical disorders, which are among the causes of epiphora.[1] According to literature reports, *Aloe vera* was observed to exert a positive effect on corneal epithelial cells speeding epithelization decreasing fibrosis *in vitro*.[6] Moreover, it represents an alternative treatment of ocular surface of squamous dysplasia in humans.[7]

Several studies highlighted the effect of Astaxanthin from *Haematococcus pluvialis* and carotenoids in reducing retinal oxidative stress in different animal models.[8-10] In particular, Astaxanthin and carotenoids can attenuate the apoptosis of retinal ganglion cells in rats affected by diabetic retinopathy, representing an alternative drug to treat this pathology, and can limit UVB damages.[11-13] Curcumin, the major curcuminoid contained in *Curcuma longa*, has been largely used for preventing or treating several diseases including ocular ones. Various studies investigate multiple positive effects of this active substance, such as anti-inflammatory, antioxidant and anti-angiogenetic.[14,15] Moreover, Curcumin seems to affect the expression of genes inhibiting the expression of oncogenes in tumor cells in human models.[16,17] *Ribes nigrum* is a perennial shrub rich in anthocyans, flavonols, phenolic acids, and polyunsaturated fatty acids.

A plethora of studies have linked the use of *Ribes nigrum* with the reduction of hypertension and other cardiovascular-associated illnesses and neurodegenerative and ocular diseases.[18,19] *Vitis vinifera* is known worldwide for its medicinal values including ocular promotion. It contains polyphenols that can attenuate ocular inflammation in humans and animal models.[20,21]

We also hypothesize that the inflammatory status that typically characterizes such conditions could be also fostered by the presence of toxic compounds, including antibiotics, ever more present and concentrated within the pet and human food chains.[22-29] In fact, pet food production mainly relies on poultry meal,[30] with a bone inclusion rate of 20-30%, and dragging all those compounds with a high affinity for calcium, in particular oxytetracycline,[24] which has been demonstrated to be cytotoxic, genotoxic, and pro-inflammatory *in vitro*.[23,25,27]

It is quite puzzling how, although Food and Drug Administration[31] and World Health Organization[32] established maximum residue limits in foods, antibiotic residues in foods are present.[33] Unfortunately, this is due to the lack of detailed regulations concerning antibiotic concentration in bones (34): thus, antibiotic residues manage to enter into the human and pet food chains within products such as wurstels. This might explain the onset of ocular manifestations in our cohort of dogs as well as their rapid disappearance after a simple diet supplementation. Moreover, the significant and rapid improvement of symptoms of the dogs involved in this clinical evaluation after diet supplementation is in agreement with that observed by Mazzeranghi et al in cats affected by cutaneous adverse food reactions.[24]

Although we are aware that this work would benefit of further *in vitro* evaluations including immunological profile and serum oxytetracycline evaluation to clearly correlate symptoms onset and disappearance, it introduces a serious warning concerning the presence of toxic compounds in pet and human food and their contribution to the etiopathogenesis of certain clinical conditions.

STATEMENT OF AUTHORSHIP

The authors hereby certify that all work contained in this article is original. The authors claim full responsibility for the contents of the article.

CONFLICT OF INTEREST

The authors confirm that they do not have any conflict of interest

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