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Effects of an *Ascophyllum Nodosum* Formulation

On Oral Health Index in Dogs and Cats

Effects of an *Ascophyllum Nodosum* Formulation on Oral Health Index in Dogs and Cats

Abstract

Over 66% of dogs and cats aged 2 and older show symptoms of periodontal disease and this number increases with the age of the affected individuals. Despite the existence of numerous ideas and methods of treating periodontal disease, dental plaque control still remains the most critical means of prevention and therapy. The purpose of the study was to evaluate the clinical effect of a formulation containing *Ascophyllum nodosum* in groups of dogs and cats.

number increases with age of the affected individuals (1). Despite the existence of numerous ideas and methods of treating periodontal disease, dental plaque control still remains the critical means of prevention and therapy (1). The goal of the most common dental procedure in veterinary practice, i.e. sanitation of the oral cavity, is to achieve the best possible conditions for the control of bacterial plaque build-up (2, 3).

Bacterial plaque has been found to be the main contributing factor to periodontal diseases (4). In itself, the tartar visible on the teeth, is not a disease but only a sign of a lack of hygiene and the final mineralisation of a dental plaque build-up. The presence of tartar contributes to dental plaque build-up and may cause mucous ulcerations resulting from mechanical irritation (3).

Salivary glycoproteins start to accumulate on the teeth surface within a few hours following a correctly performed preventive procedure. Pellicle, or a thin layer of these glycoproteins, becomes rapidly colonised (over a few subsequent hours) by bacteria residing in the oral cavity. Thus, dental bacterial plaque may become deposited on the surface of the recently cleaned teeth even as early as on the next day after the procedure (5). The bacteria colonising the pellicle are accompanied by food remains, exfoliated epithelial cells of the oral cavity and microbes. At the initial stage, the so-called "immature" dental plaque forms a thin dental deposit. When left on the surface of the teeth, such form of dental plaque continues to adhere even

stronger to the tooth enamel and it gains a more organised structure, growing into a kind of a colony of bacteria and protozoa - thus turning into a biofilm. The surface of this biofilm is colonised by aerobic bacteria, while the deeper layers which are closer to the tooth surface, become inhabited by anaerobic bacteria (6).

Matrix, which constitutes 75% of the mature plaque, starts to play an increasingly important role in the maturing bacterial plaque. Matrix is a substance which consists of secretions and metabolism by-products of dental plaque microbes. It facilitates the plaque build-up and protects it from chemical and cellular bactericidal substances (6).

Once a mature plaque has been formed (this stage lasts up to several hours), and over the period of the following two days of a non-disturbed existence, the biofilm becomes slowly mineralised and tartar develops.

Over 66% of dogs and cats aged 2 years and older show symptoms of periodontal diseases and this

This formation itself is not pathogenic; however, it creates conditions which contribute to colonisation by pathogens inducing periodontal disease (7). These pathogens secrete toxins which cause inflammation of the gum tissue (*gingivitis*). When left untreated, *gingivitis* progresses into an irreversible form of periodontal inflammation (*periodontitis*) which is characterised by permanent damage of the periodontal ligament system and atrophy of the alveolar process, gums and cementum (4).

Prevention of Periodontal Diseases – Studies

A conclusion arising therefrom is that in order to prevent periodontal diseases, formation and mineralisation of the mature dental plaque must be counteracted. Besides, if no hygienic recommendations helping to control a dental plaque deposition are given after a preventive procedure or an owner of the patient does not comply with them, the first symptoms of deteriorating periodontal conditions can be spotted right during the follow-up visit.

Numerous studies have shown that everyday brushing is the most effective way of periodontal disease prevention (8). Apart from brushing, a dry diet (9) and in particular the many dental diets, play a crucial preventative role as well (10). New means and methods which could potentially boost the effectiveness of a periodontal disease prevention continue to be sought after. It is also expected that the methods meet the requirements of the pet owners who would most often wish for the pet dental hygiene issue to be solved in a simple, cost-effective and effortless way which would not require a great deal of involvement on their part, while fulfilling (preferably completely)

the conditions of efficient prevention as described above.

The studies on compliance with recommendations for oral hygiene conducted in the USA have shown that 24% of the pet owners still brush the teeth of their pets every day over the period of 6 months following the procedure, with the additional 29% of them performing this practice a few times a week. All in all, it may be concluded that over half of the patients (53%) is provided with a thorough and effective hygiene regime over a relatively long period of time. Authors of these studies concede that the findings were based on phone call interviews that did not allow for any means of verification whether at all and how thoroughly the affected teeth were brushed and whether such dental care had indeed any positive impact on the condition of the oral cavity (11). Besides, these studies included only those owners who previously decided to have the dental intervention performed and paid for the treatment of periodontal diseases in their pets. The author's research experience paints a much less optimistic picture, but one has to bear in mind that this study is based on a significantly sized group comprising all the patients, regardless of whether they were at all aware and informed about the importance of dental hygiene in their pets (9). Moreover, the results obtained as part of the studies conducted in 2003-2004 and 2010-2011 respectively were juxtaposed 2 years ago. Those results were gathered using the same methodology in both periods. The most important observations were published during the 21 European Congress of Veterinary Dentistry and they have been compiled in the Table No. 1 (12). For many years now, veterinary dentistry specialists have been continuously studying the issues pertaining to periodontal diseases. The most distinct research trends which

have recently arisen in the periodontology are associated with the effective prevention of periodontal diseases and their contribution to general health. A formulation containing *Ascophyllum nodosum* – a seaweeds extract – is one of the preventive formulas which trigger a great interest as a method of passive oral hygiene. This product has been shown to yield positive effects in the oral hygiene of humans as well as in veterinary dentistry (13, 14).

The aim of the studies was to evaluate the clinical effect of the formulation containing *Ascophyllum nodosum* in dogs and cats according to the methodology developed and implemented as part of the *Uśmiech Pupila (Pet's Smile)* social campaign, which was additionally supplemented by an evaluation of bacterial plaque quantity. Unlike in the case of hitherto conducted studies, this research applied the oral health index in the clinical evaluation of the product, instead of two selected clinical parameters.

Materials and methods

Study group

The study group included dogs and cats who were patients of veterinarians and technicians that had completed their educational program in dentistry in "Diagnostics and Oral Hygiene in Dogs and Cats" and agreed to sit the respective examination.

The studies were conducted in individuals exposed to preventive procedures, except for the application of protective wax. The animals were not on any dietary regimen; however, they were administered the same type of feed at a constant frequency throughout the study. None of the patients was diagnosed with hyperthyroidism.

| Parameter | 2003–2004 | | 2010–2011 | |
|---|----------------|--------------|------------|------------|
| | Dogs | Cats | Dogs | Cats |
| Number of examined animals | 29 702 [76.6%] | 9074 [23.4%] | 3250 [72%] | 1265 [28%] |
| Fed on dry food [%] | 22.5 | 33.7 | 45.0 | 44.5 |
| Everyday oral cavity hygiene [%] | 5.2 | 4.4 | 10.3 | 6.1 |
| Complete lack of hygiene [%] | 60.1 | 71.4 | 33.9 | 70.4 |
| Impalpable lymph nodes [%] | 70.0 | 71.0 | 78.4 | 84.2 |
| Enlarged lymph nodes [%] | 4.0 | 3.3 | 3.2 | 1.6 |
| No visible dental deposits (clean teeth) [%] | 28.3 | 33 | 32.5 | 33.2 |
| Dental deposit covering over 50% of a tooth surface [%] | 20.1 | 17.4 | 12.3 | 6.1 |
| Healthy gums [%] | 63.2 | 58 | 66.7 | 59.9 |
| Signs of periodontitis [%] | 10.7 | 10.3 | 7.6 | 7.7 |

Tab. 1 Comparison of oral health evaluation results in dogs and cats in Poland from 2003-2004 and 2010-2011

Each patient in the study group received doses of the formulation containing *Ascophyllum nodosum* (PlaqueOff, VetExpert, Poland) in compliance with the manufacturer's and distributor's recommendations i.e. cats and dogs up to 10 kg b.w. - ½ - 1 cup of the product, dogs over 10 to 25 kg b.w. - 1-2 cups daily, dogs over 25 kg - 3 cups daily (15). Each time, the formula was added to the feed which was administered (in compliance with the recommended doses) according to individual preferences of the patients.

The control group

The control group comprised dental patients that were treated in the Veterinary Clinic "Arka" in 2012-2013 and subjected to oral sanitation but could not be provided with active oral hygiene or did not receive any other hygienic product for various reasons. Besides, no protective wax was applied after a preventive procedure in these patients (3). All the animals were examined in the periods which were corresponding to the study group protocol and the parameters described in Table No.2 remained under particular control. Follow-up examinations were performed by one person. In total, the control group comprised 6 dogs and 6 cats with an age and physical constitution which was similar to the study group.

Study and its criteria

Oral health examination was based on a simplified scheme which was developed during the *Uśmiech Pupila (Pet's Smile)* preventive action. This scheme was presented in detail during a 2-day course, both in theoretical and practical terms. Additionally, the evaluation included deposit of dental plaque on dental crown surfaces. This assessment was possible thanks to the implementation of one of the dental plaque detection methods: a preparation containing *Ascophyllum nodosum* (PlaqueOff, VetExpert, Poland) or a 380 nm UV light (fig. 1-4). The framework of the study entailed a clinical examination of the mandibular lymph nodes, followed by a visual inspection of teeth with estimation of the size of surface covered with deposit and plaque, as well as an evaluation of the global periodontal condition. Periodontal changes were qualified as *periodontitis* on the basis of clinical implications: tooth mobility, atrophy of gums and a change of teeth position. It was decided that the evaluation would cover the strategic teeth and their buccal surfaces. That included the canine teeth, maxillary third incisors and fourth premolars. The surfaces of these teeth are the most accessible during clinical evaluation without the need to

induce anaesthesia. The Oral Health Index (OHI) was based on a clinical evaluation including the values presented in Table 2. The recorded score represented oral health in terms of a potential disease and reflected the global OHI. The lowest possible score, i.e. 0, meant a total lack of signs suggesting current disease and the highest score - 8 meant the most severe condition.

The findings were recorded on day 0, 2 weeks, 4 weeks and 6 weeks after the administration of the product. In total, it was planned to subject each patient to clinical examination four times, every 2 weeks. Statistical analysis of oral health index was performed using Prism 5.0 (GraphPad Software). Changes in the index value in the individual groups were subjected to a statistical analysis with one-variable analysis of variance, while a direct comparison of the study results obtained on examination days from the control and study groups was performed using the student t-test.

Study results

The control group included cats of 3-4.8 kg body weight (mean: 3.95) at the age of 3.5-6.5 years (mean: 4.5) and dogs of 6-35 kg body weight, (mean: 18.5) aged 4-8 years (mean: 5.67).

| Parameter | Result | | |
|----------------------------|--------|------------------------------------|---------------------------------------|
| | 0 | 1 | 2 |
| Lymph nodes condition | normal | slightly enlarged | moderately and significantly enlarged |
| Presence of dental lesions | no | <i>gingivitis</i> | <i>periodontitis</i> |
| Presence of tartar | no | up to 25% of dental crown surfaces | over 25% of dental crown surfaces |
| Presence of dental plaque | no | up to 25% of dental crown surface | over 25% of dental crown surfaces |

Table No. 2. Oral Health Index (OHI) Evaluation criteria in the clinical study including the control and study group

| Species | Day 0 | 14 days | 28 days | 42 days |
|------------------------|--------------------|---------------------|---------------------|---------------------|
| Dogs - OHI | 0.333 ^a | 1.333 ^{ac} | 2.333 ^{bc} | 3.500 ^b |
| Dogs - increase of OHI | 0 | 1.00 | 1.0 | 1.167 |
| Cats - OHI | 0.333 ^a | 1.500 ^{ac} | 2.500 ^{ac} | 4.000 ^{bc} |
| Cats - increase of OHI | 0 | 1.667 | 1.000 | 1.500 |

Tab. 3. Changes of oral health index in animals from the control group within 42 days of the study. ^{a,b,c} Values in the same lines which are marked with different letters show statistically significant difference at p<0.05

| Species | Day 0 | 14 days | 28 days | 42 days |
|------------------------|--------------------|---------------------|---------------------|---------------------|
| Dogs - OHI | 0.500 ^a | 0.916 ^{ac} | 1.500 ^{bc} | 1.917 ^c |
| Dogs - increase of OHI | 0 | 0.416 | 0.584 | 0.417 |
| Cats - OHI | 0.583 ^a | 1.083 ^{ac} | 1.583 ^{ac} | 2.000 ^{bc} |
| Cats - increase of OHI | 0 | 0.500 | 0.500 | 0.417 |

Table No. 4. Changes in oral health index (OHI) in animals in the study group receiving PlaqueOff formula within 42 days of the study. ^{a,b,c} Values in the same lines which are marked with different letters show statistically significant difference at p<0.05

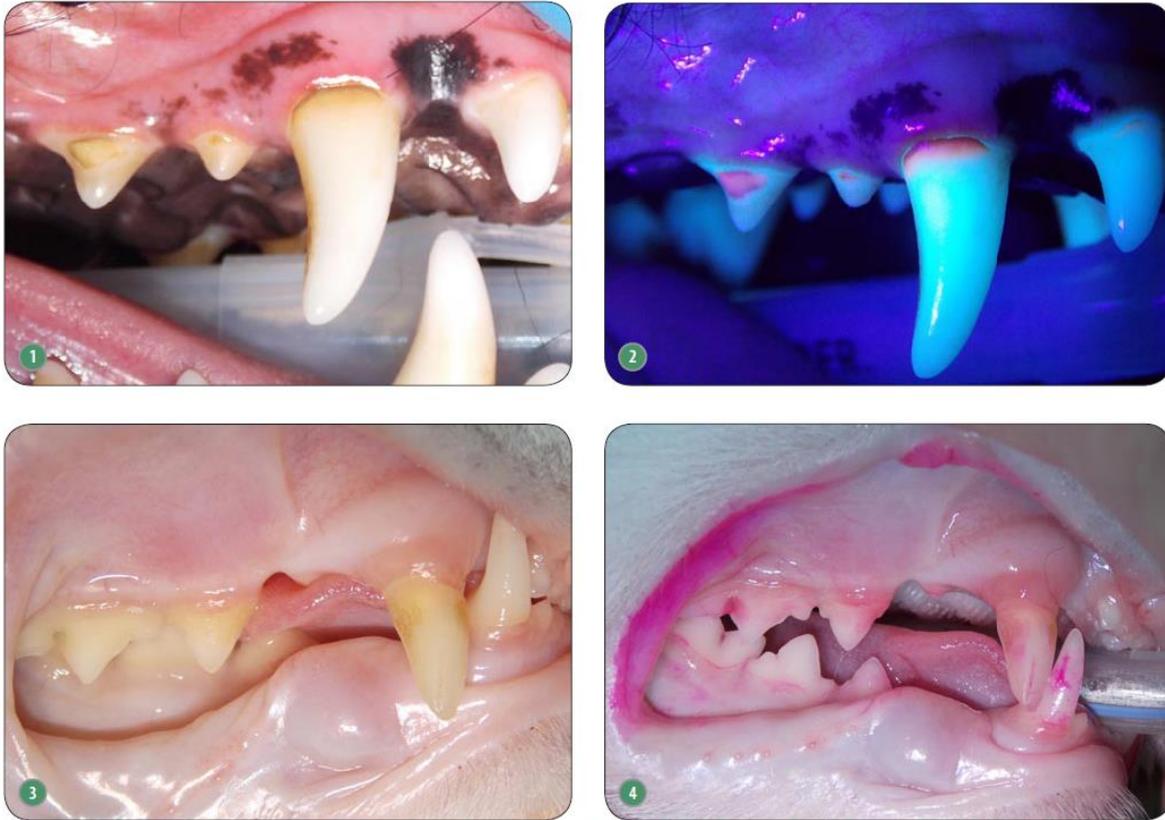


Fig. 1 Dog – a patient from the control group, in which OHI was evaluated. This pet's OHI was scored with 2 (lymph nodes – unchanged, gums – unchanged, plaque – up to 25% of teeth surface, tartar – up to 25% of teeth surface); **Fig. 2** Dog from the figure 1 that was subjected to UV in order to visualise the presence of dental bacterial plaque. This pet's OHI was scored with 2 (lymph nodes – unchanged, gums – unchanged, plaque – up to 25% of teeth surface, tartar – up to 25% of teeth surface); **Fig. 3** A cat from a study group, whose OHI was scored after 6 weeks of using the product; **Fig. 4** A cat depicted in Fig. 3, who was administered *Ascophyllum nodosum* product in order to visualise bacterial dental plaque. This pet's OHI was scored with 2 (lymph nodes – unchanged, gingivitis, bacterial plaque – up to 25%, no dental deposits).

Changes in the oral health index in animals from the control group have been compiled in Table No.3. A systematic time-related progression of oral health effects was observed and it was reflected by statistically significant differences in OHI in dogs and cats between day 0 and 42, following an oral sanitation procedure. In individual feline patients, the range of OHI scores on day 0 oscillated between 0-1 and 2-5 on day 42. In dogs, the results amounted respectively to: 0-1 and 1-6.

The study group included 24 animals: 12 cats of 3.25-8 kg body weight (mean: 4.45) at the age of 4-10 years (mean: 4.96) and 12 dogs of 7-40 kg of body weight (mean: 19.0) aged 3.5-8 years (mean: 5.58). Fourteen persons, including 10 veterinarians and 4 technicians conducted studies in the experimental group. Changes in the oral health index in animals from the study group have been compiled in a tabl.4. Also the study

group showed a progression of health effects in the oral cavity within the period of 6 weeks; however, it was significantly less advanced than in the control group. In individual feline patients, the range of OHI values recorded on day 0 oscillated between 0-2 and 0-3 on day 42. In dogs, the results amounted respectively to: 0-2 and 1-3.

Discussion

The selection of subjects in the control and study groups consisted in excluding the pre-selected patients without complete results and patients whose observation might have been affected by previous treatment or administered medications. The control group is modestly sized, but the results obtained, and specifically the observed phenomenon of oral health deterioration and its intensity in animals deprived of

oral hygiene are very similar to those described in literature (16-20). Baseline clinical evaluation was similar in both the control and study group. The baseline condition was not ideal due to the presence of periodontal lesions and the changes affecting the lymph nodes. However, since the evaluation concerned the formulation's influence on the progression of oral changes, and not the baseline condition, it did not affect the results of the study. The examination of the control group aimed to show the deterioration rate of oral cavity health in individuals who were not subjected to teeth brushing. The results obtained show that oral condition deteriorated significantly after 6 weeks in both analysed species (cats and dogs) in the control group, and the deterioration rate was similar in both species.

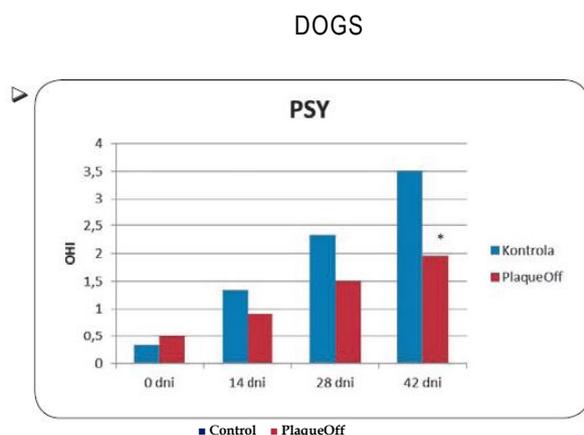


Fig. 5 Oral health index in dogs from the control group and the group receiving the product with *Ascophyllum Nodosum*. Application of the product for 6 weeks resulted in a lower increase of OHI which was statistically significant ($p=0.0166$)

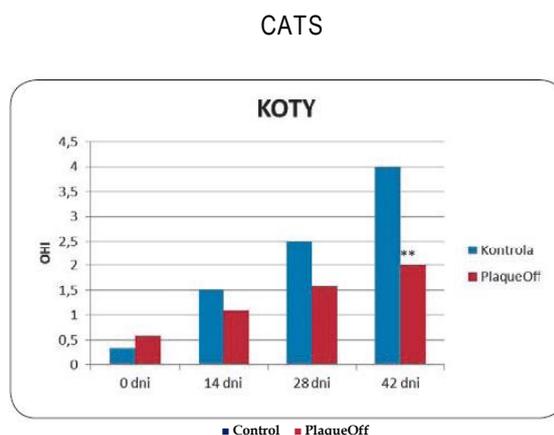


Fig. 6 Oral health index in cats from a control group and a group of cats receiving the product with *Ascophyllum Nodosum*. Application of the product for 6 weeks resulted in a lower increase of OHI which was statistically significant ($p=0.0064$)

The goal of the evaluation of the study group was to answer the question on how administration of the formulation may have affected the condition of the oral cavity in dogs and cats. The obtained results show that the rate of OHI deterioration in subjects receiving the product with *Ascophyllum nodosum* was significantly slower and that the general condition of their oral cavities was better than in the control group over the corresponding periods of time. This resulted primarily from the slower bacterial plaque build-up and its consequent reduced negative impact on periodontal tissues and the lymphatic system (fig. 5, fig.6). Deterioration rate of OHI in both groups was proportional during all evaluation periods and it did not show any critical moment for the condition of the oral cavity. It amounted to 1.0-1.33 (the control group) and 0.42-0.5 (the study group) respectively in cats. In dogs, those values were as follows: 1-1.34 and 0.42-0.68.

Differences in oral health index between the control and study group became statistically significant after 42 days of using the product in dogs and cats (fig. 1, fig.2). It was due to the fact that the overall increase of OHI in dogs receiving *Ascophyllum nodosum* was that of 1.417 (in dogs from the control group it amounted to 3.167) and in cats - 1.417 (in cats from the control group - 4.117).

The described studies were performed by over a dozen persons. In case of field studies, a reservation is usually made that the observations should be carried out by one person only. This requirement, however, applies to studies which involve more precise

methods of clinical parameters evaluation (for example gum bleeding index). The requirements for studies for Veterinary Oral Health Council (VOHC) are more strict and specific, delineated on www.vohc.org (21). The credibility and reproducibility of the method which was employed in the studies is based, first of all, on the fact that all the participants were trained in the same way by the same trainers, and the method itself is simple and relatively insensitive to the human factor.

The studied patients were kept under different conditions and might have been fed with feeds from various manufacturers; however they were not treated with any additional hygiene care products.

On the basis of the findings of the study, the formulation containing *Ascophyllum nodosum* can be considered to be a method of a passive hygiene which results in specific health changes in the patient study group.

It is worth adding that an observation of bigger populations of subjects for a longer time would be recommended, especially since the differences between the control and study group became statistically significant only after 6 weeks, although they were initially reported as early as after 2 weeks of using the product..

An important point in the discussion is the comparative analysis of oral hygiene results which have been provided in Table 1. These results point to a certain civilisation progress which can be observed among dog and cat owners in Poland, which is corroborated by a higher percentage of pets receiving oral

hygiene care. These figures nearly doubled in dogs while in cats they increased by 2%. Interestingly, as a result, health index improved significantly in these pets, with a 3% reduction in the number of animals with periodontal diseases constituting the most important finding.

What is notable in cats is a very similar number of animals which are not provided with oral care (71.4% and 70.4% respectively for both study periods). This situation looks much better in dogs, but still 33.9% of dogs did not receive a proper dental care. The following reservations should be made in terms of the results: first of all, the studies included pet owners who visit veterinarians, thus the percentage may be erroneously heightened, secondly - even if the number of pets subjected to hygienic procedures was observed to significantly increase, the percentage of the pathological lesions was not so significantly reduced.

Summary of the study results

Overall, daily teeth brushing is regarded as a golden standard of oral hygiene, constituting an element of active hygiene (22). In addition to the fact that many owners seek easier methods, teaching pet owners to sustain the teeth-brush routine calls for systematic practice and not all patients (in particular among cats) are willing to participate in such a hygienic regime.

The formulation containing *Ascophyllum nodosum* does not have a specific and proved acting mechanism. Three ingredients are

considered to be potentially active. First of all – presence of three types of ingredients, which are known to affect the deposition of dental plaque: phenols, iodine and phosphates. Phenol derivatives have not been noted as yielding any adverse effects in cats.

Secondly, the high content of fucoidan (10-15%) which is composed of sulphur. It prevents adhesion of bacteria to the pellicle, thus inhibiting deposition of bacterial plaque.

Thirdly, the product contains tartar-inhibiting elements: zinc, silicates and sulphates. Increase of salivary sulphur content results in calcium binding capacity which plays a similar role to that of polyphosphates.

Up until now, the effectiveness of *Ascophyllum nodosum* had been analysed on the basis of the following dental criteria: evaluation of halitosis (on a 3-point scale), dental plaque index, gum bleeding index and salivary pH. Additionally, urinary pH and blood chemistry before and after the administration of this product were evaluated. Differences observed between the study and control groups showed statistical significance which was confirmed by the divergence between the measured parameters in favour of

the studied group. As far as dental plaque is concerned, its increase was significant in the control group ($z=3.13$) but not in the group receiving the *Ascophyllum nodosum* ($z = 1.25$ n.s) preparation (14).

The increase of gum bleeding index was significant in the control group ($z=1.99$), but not in the group receiving the above mentioned formulation ($z=1.65$).

Intensification of halitosis was noticeable in the control group ($z=2.57$), however it was not the case in the group receiving the preparation ($z=0.42$). However, it should be noticed that the studies spanned 88 days i.e. twice as long. Beside the reduction of salivary pH and the increase of urinary pH, no significant changes in serum and haematological parameters were observed (14).

As in the case of the previous studies, the vast majority of the owners reported a significant reduction of halitosis in their pets; nonetheless, this parameter was considered to be subjective and difficult to compare. Additional findings which were observed during administration of the product showed the following: a relatively good tolerance by the pets, no intolerance symptoms such as indigestion or diarrhoea. However, there were a few animals

(4 cats) that were clearly reluctant to consume the food supplement. In dogs who were served feed sprinkled with *Ascophyllum nodosum*, no eating problems were observed.

Conclusions

OHI deterioration was observed in animals which were not subjected to teeth brushing by their owners within 6 weeks following a preventive procedure. Oral health deterioration rate, measured in 2-week intervals, in subjects receiving the product with *Ascophyllum nodosum* was, on average, lower by half than in the group where hygienic procedures were not performed.

Administration of the formulation containing *Ascophyllum nodosum* in dogs and cats reduces the rate of oral health index deterioration, as measured quantitatively with dental plaque and tartar, with the consideration of the general condition of the peridontium and mandibular lymph nodes. A 6-week observation showed significant improvement of the oral health in dogs and cats receiving the above specified product.

