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Effect of sodium hexametaphosphate on dental calculus formation in dogs

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Summary

A series of studies was conducted to identify a practical measure for preventing dental calculus formation in dogs. The studies involved a colony of 27 Beagles that received an initial dental prophylaxis. The dogs were then stratified on the basis of their normal rate of calculus formation and randomly assigned to parallel groups within each strata. During 4-week test periods, a variety of experimental regimens were instituted, followed by clinical assessments of calculus. Major observations were that a crystal growth inhibitor, soluble pyrophosphate, incorporated into a dry dog food modestly reduced calculus formation when used at high concentrations; anticalculus effects attributable to this agent were significant ($P < 0.05$) only when it was used as a surface coating; the coating of dry dog chow or plain biscuits with a calcium sequestrant, sodium hexametaphosphate (HMP), provided the greatest benefit and resulted in significant ($P < 0.05$) reductions in calculus formation of about 60 to 80%, depending on the dosage regimen; and the feeding of a single daily snack of 2 HMP-coated plain biscuits (0.6% HMP) decreased calculus formation by nearly 80%. We concluded that the coating of dry dog chow or plain dog biscuits with HMP is an effective means of reducing calculus formation in dogs.

Dental calculus, commonly called tartar, is known to develop in nearly all domestic dogs and cats. Dental plaque, which forms continuously on tooth surfaces, consists predominantly (70 to 75%) of oral microorganisms with variable amounts of food debris and microbial remnants. In essence, dental calculus is calcified dental plaque covered by an unmineralized bacterial layer[1] and comprises about 75 to 80% inorganic material and 20 to 25% organic matter consisting of remnants of microbial cells, desquamated epithelial cells, and oral debris. It has been suggested that calcification begins intracellularly, in gram-negative microorganisms contained within the dental plaque,[2] and these foci serve as a nidus for continued calcification. In dogs, the composition of the inorganic phase of calculus consists of a predominance of calcium carbonate (calcite) with a small amount of calcium phosphate (apatite), which is considered to reflect the high concentrations of calcium and carbonate, the high pH, and the low concentration of phosphate in the saliva.[3]

Although it is not a primary etiologic factor, the presence of dental calculus is generally associated with existence of periodontal disease.[4-7] Although bacteria present in dental plaque have been repeatedly documented to initiate the inflammatory process (ie, gingivitis) and facilitate its progression to periodontal disease, calculus clearly has an important, albeit indirect, role in development of this disease.[8,9] This indirect role involves the inevitable presence of a layer of plaque covering calculus deposits; presence of tissue irritants (eg, microbial enzymes), which seep from the porosities in calculus; mechanical irritation caused by the physical presence of calculus; and physical interference with normal plaque removal during mastication or performance of oral hygiene. Thus, regular removal or prevention of dental calculus is an important factor in maintenance of oral health.

Although results of some recent studies have documented the partial removal of existing calculus deposits in dogs through the mechanical action of chewing rawhide or combinations thereof,[10,a,b] the only effective means for removing dental calculus is through oral prophylaxis. However, mechanical and chemical approaches have been explored for prevention of calculus formation. The mechanical approach typically involves measures to remove dental plaque before it becomes calcified. Previous research has indicated that regular, meticulous oral hygiene markedly reduces calculus formation,[4-6,11,12] although few dog owners are willing to accept this responsibility. Other mechanical approaches rely on the cleansing action of chewing and mastication to help remove plaque prior to calcification. In this regard, feeding dogs a hard chow regimen resulted in less plaque accumulation than did feeding of a similar regimen that was pulverized and soaked in water.[13] Similarly, a hard diet of bovine tissue (raw trachea, esophagus, muscles, and fat) resulted in less plaque accumulation

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than did a minced diet.[14] More recently, it was reported that consumption of a dry dog chow resulted in significantly less plaque and calculus accumulation than consumption of a canned dog ration in the presence and absence of mechanical oral hygiene.[15] Similarly, decreased accumulations of calculus have been associated with twice daily feeding of oxtails[16,17] and use of a nylon chew device.[18]

Few reports have appeared regarding use of chemical approaches for prevention of dental calculus in companion animals. Although daily use of a zinc ascorbate cysteine rinse/spray[19] and twice daily use of a chlorhexidine solution[20] reduced plaque formation, effect on calculus formation was not apparent or reported; those findings are consistent with studies in human beings in which use of antimicrobial agents either increased or had no significant effect on calculus formation.[21] In 1985, the first reports of the successful use of a chemical crystal growth inhibitor, soluble pyrophosphate, in a dentifrice to reduce calculus formation in human beings appeared[22,23]; since then, results of more than 25 successful clinical trials in adults have been reported.[20]

Recognizing the need to prevent calculus formation to facilitate oral health in companion animals, the desirability for identifying practical measures for achieving this goal, and the appreciable impact on calculus observed in human beings in response to use of chemical agents provided in dentifrices, we initiated a program to identify specific measures for preventing calculus formation in domestic dogs and cats. Because we believed that most pet owners are unwilling to accept the responsibility of providing daily oral hygiene for their pets, the objectives of the study reported here were to use a pet snack food as a vehicle, identify the most efficacious agent for preventing calculus formation that was nutritionally safe and acceptable, and identify a means of incorporating the agent into the pet snack food in an efficacious manner recognizing the poor masticatory habits of dogs.

Materials and Methods

Dogs—For this study conducted over a 5-year period, we used a colony of Beagles, numbers of which ranged in this study from 24 to 27 at various times. Dogs were housed in stainless steel cages with exercise opportunities or runs in an American Association for Accreditation of Laboratory Animal Care-approved facility in the School of Dentistry. The dogs were approximately 2 years old initially and were maintained exclusively for this study. Unless otherwise noted, dogs were fed a commercially available dog chow and tap water. Each study began with each dog receiving a thorough dental prophylaxis; for this procedure anesthesia was induced and maintained with ketamine hydrochloride (5.5 mg/kg of body weight) and xylazine (2 mg/kg), and all supra- and subgingival deposits were meticulously removed with the aid of an ultrasonic scaler and conventional hand instruments. All prophylaxes were completed in the morning and studies were initiated with the afternoon feeding.

Study design—To obtain information to stratify the dogs into the desired number of groups, it was necessary to determine the normal rate of calculus formation for each animal. After a prophylaxis, each dog was provided 150 g of a commercial dog chow moistened with an equivalent amount of tap water 15 minutes prior to feeding twice daily; tap water was provided ad libitum and replaced twice daily. After 28 days, dogs were again anesthetized and teeth were thoroughly dried with compressed air and examined clinically for the presence of calculus on the buccal surfaces of the bilateral maxillary C, P2, P3, P4, and M1 and mandibular C, P3, P4, and M1 teeth, using modifications of the Volpe and Manhold[24,25] and Greene and Vermillion[26] clinical indices. The examination assessed the amount of each buccal tooth surface covered with calculus, using the following numerical scale: 0 = no observable calculus; 1 = calculus covering less than 10% of the surface; 2 = between 10 and 33% coverage; 3 = between 33 and 66% coverage; and 4 = more than 66% coverage. The thickness of the deposit also was assessed with the aid of a graduated periodontal probe according to the following numerical scale: 1 = less than 0.5 mm maximal thickness (light); 2 = 0.5 to 1.0 mm thickness (moderate); and 3 = greater than 1.0 mm thickness (heavy). The numerical score for each surface was obtained by multiplying the coverage score by the thickness value; the total of these scores divided by the number of surfaces graded provided the score for each dog. The foregoing procedures were repeated at 12 to 18 month intervals to consider any influence of increasing animal age on the rate of calculus formation.

Each of the various experiments was initiated by administering a thorough prophylaxis to each dog. Using the information from the most recent calculus formation rate experiment, the dogs were arrayed according to decreasing calculus scores, stratified into the desired number of blocks (ie, blocks of 3 dogs for a 3-group experiment), and the dogs within each block were then randomly assigned to 1 of the predetermined groups. Use of this randomized block stratification procedure, thus, resulted in experimental groups of dogs having similar composite control calculus formation rates.

Concerted measures were taken to maintain the desired elements of blindness to avoid any examiner bias. All examinations were performed by a single experienced examiner (JMW), who had no knowledge of group/treatment assignments and was not otherwise directly involved in conduct of the experiment. To minimize the possible effect of examiner fatigue, the dogs were examined in a different randomized order in each experiment. Finally, all data tabulations were verified and study procedures were monitored by the quality-assurance officer to assure compliance with FDA regulations governing the conduct of such investigations (ie, "Good Laboratory Practice For Nonclinical Laboratory Studies").

Randomized parallel group study designs were routinely used, although in experiment 3, only the first 3 periods of a 4-period crossover design were used. In experiments 1 and 2, the dietary regimen consisted of twice daily feedings of 125 g (adjusted between 100 and 150 g in the early experiments to maintain a constant body weight) of commercial chow moistened with tap water (1:1 [w:w]) about 15 minutes prior to each feeding. In experiments 3 and 4, the regimen consisted of twice daily feedings (125 g each) of dry dog chow containing the various additives. For experiments 5 and 6, the additives or agents were dissolved in 10 ml of deionized water and slowly mixed onto each 125-g portion of dry dog chow with continual stirring; the control regimen was similarly coated with water only. Thus, the surface of the chow was dampened prior to each twice daily feeding. Experiment 7 involved twice daily feeding of 70 g of dog chow moistened with an equivalent amount of tap water followed by the feeding of 2 commercially prepared biscuits (18 g each) 1 hour after each meal. Experiments 8, 9, and 10 were designed to feed only 1 meal/d and the dogs were given a single daily meal of variable amounts (164 to 212 g) of dog chow similarly moistened with water, followed by a single daily snack of 2 commercially prepared biscuits (18 g each) 4 hours after the meal; 1 test group in experiment 10 received only 1 biscuit as a snack. In every experiment, the test period was 4 weeks followed by a 1-week washout period during which the dogs received a standard chow and tap water. In every experiment, food consumption was monitored and recorded for each feeding to ensure that experimental differences were not confounded by differences in food consumption. Similarly, the dogs were weighed weekly or biweekly to ensure maintenance of constant body weight.

Analysis of data—All data were analyzed, using conventional biostatistical procedures comprising a statistical package. Analysis of variance models[27] were used to test for differences among groups. When a significant F value was found, the Duncan's multiple range test was used to identify significant (at $\alpha = 0.05$) differences between individual group means.[c]

Results

In developing this model for studying calculus prevention in dogs, it was recognized that calculus accumulates at a generally linear rate throughout periods up to 4 years.[5,6] Results of a 4-week study in which clinical assessments were performed at weekly intervals were evaluated (Table 1). As expected, the calculus scores increased linearly with time and the amount of calculus present after 4 weeks was deemed adequate to permit future evaluation of preventive measures. This rationale for the selection of the required test period was consistent with prior precedent in human clinical trials of calculus-preventive measures.

Because of findings in dogs[28] and human beings,[29] in which prevalence of dental calculus increases with increasing age, the calculus formation rates of the colony were determined periodically during the course of these studies (Table 2). There was a numerical increase in the average calculus scores with increasing age of the dogs, suggesting the need for definitive clarification of this relation in future studies.

Table 1—Rate of calculus formation in dogs (experiment 1)

Study period	No. of dogs	Calculus score
1 week	27	0.24 ± 0.04
2 weeks	27	0.47 ± 0.20
3 weeks	27	0.79 ± 0.11
4 weeks	27	0.92 ± 0.11

Data are expressed as mean ± SEM.

Table 2—Calculus formation vs age of dogs (experiment 2)

Mean age of dogs	No. of dogs	Calculus score
4.4 years	24	0.92 ± 0.11
5.1 years	27	1.22 ± 0.12
6.1 years	27	1.62 ± 0.13
8.0 years	26	1.57 ± 0.16

Data are expressed as mean ± SEM.

Because results of a series of experiments (not presented) had indicated that topical applications of soluble pyrophosphate solutions with neutral pH decrease calculus formation in our model, and this system had been documented to reduce calculus formation in human clinical trials,[21,22] we investigated the efficacy of various concentrations of this agent incorporated in a conventional dog chow. Results indicated that a concentration of 1.5% pyrophosphate was required to achieve a significant reduction of 31.8% (Table 3). Unfortunately, this concentration of pyrophosphate was considered to be impractical for a variety of reasons (leavening properties, nutrition, cost).

Considering the nature of the masticatory habits of dogs that involve minimal chewing of the food, it was reasoned that a more efficient means of delivering the anticalculus agent was required. Thus, a series of 5 experiments was conducted in which the agent, 1.5% pyrophosphate, was added to the dry dog chow either as a surface coating or incorporated within the dough mixture prior to baking. Results of these experiments were pooled (Table 4) and clearly indicated that a reduction in calculus formation was large only when the agent was added to the dog chow as a surface coating.

Experiment 5 was then initiated to evaluate an alternative agent that might be even more effective than pyrophosphate for preventing calculus in dogs and also have the necessary requirements of safety, nutritional acceptability, palatability, and cost. Pronounced reductions in calculus formation were observed when sodium hexametaphosphate (HMP) was added as a coating to dry dog chow. When sodium HMP was directly compared with an equivalent amount of soluble pyrophosphate, P_2O_7, the HMP was significantly more effective (Table 5). In view of the pronounced efficacy observed with use of HMP, subsequent research focused on practical approaches to use this agent as a coating for dog food products.

Results of a dose response study in which concentrations of 0.59, 1.76, and 2.93% sodium HMP (phosphorus content equivalent to 0.5, 1.5, and 2.5% pyrophosphate tested previously) were added as a surface coating to dry dog chow in our laboratories were compared (Table 6). All 3 concentrations of HMP

Table 3—Incorporation of soluble pyrophosphate (P_2O_7) into dry food vs calculus formation (experiment 3)

Added P_2O_7 (%)	No. of dogs	Calculus score	Percentage reduction
0.0	20	1.29 ± 0.17]*	...
0.5	21	1.17 ± 0.17]	9.3
1.5	20	0.88 ± 0.19]	31.8
2.5	20	0.64 ± 0.12]	50.4

* Values with brackets do not differ significantly.
Data are expressed as mean ± SEM where applicable.

Table 4—Manner of agent incorporation vs calculus formation (experiment 4)

Dietary regimen	No. of trials	Pooled mean calculus score*	Percentage reduction
Dry chow (control)	28	1.27	...
1.5% P_2O_7 added to dough†	16	1.08	15.0
1.5% P_2O_7 added as coating‡	29	0.81	36.2

* Pooled data from 5 studies. † Added to dough mixture prior to baking. ‡ Added as surface coating after baking.

Table 5—Comparison of various agents in coating on dry dog food vs calculus formation (experiment 5)

Agent added	No. of dogs	Calculus score	Percentage reduction
None	9	1.42 ± 0.23*	...
P_2O_7	9	0.75 ± 0.12	47.2
HMP	9	0.33 ± 0.17	76.8

* All means differ significantly ($P < 0.05$).
HMP = sodium hexametaphosphate; P_2O_7 = mixture of sodium dihydrogen pyrophosphate and tetrasodium pyrophosphate.
Data are expressed as mean ± SEM where applicable.

caused pronounced reductions in calculus, with no evidence of a dose response in this model. Quite surprisingly, even the lowest concentration of 0.59% resulted in a near maximal reduction of 81%, suggesting that a benefit may be obtained with even lower concentrations of HMP.

Because of our lack of suitable instrumentation and technology for applying an HMP coating to conventional dog food, we enlisted a pet food manufacturer to prepare plain biscuits resembling traditional dog snack foods to which 3 concentrations of HMP were added as a surface coating (experiment 7). Two biscuits (18 g each) were provided to the dogs twice daily 1 hour after each feeding of moistened dog chow. Results (Table 7) indicated that again, all concentrations of HMP exerted significant reductions in calculus formation of 61% or greater, with no apparent dose response.

Experiment 8 was designed to compare the efficacy of HMP and pyrophosphate (P_2O_7) applied at equivalent concentrations as a coating to plain biscuits, using a modified test design involving a single daily meal of moistened chow followed 4 hours later by a 2-biscuit snack. Although feeding of the P_2O_7-coated biscuit resulted in a numerical calculus reduction of 30.1%, a statistically significant reduction of 62.8% was observed in the dogs fed the HMP-coated biscuits (Table 8).

Experiment 9 was conducted to determine the impact of the plain biscuits with and without the HMP (1.8%) coating, using a regimen similar to that used for experiment 8. The results (Table 9) indicated that

Table 6—Dose response of HMP-coated dog chow vs calculus formation (experiment 6)

Amount of HMP added (%)	No. of dogs	Calculus score	Percentage reduction
0.00	6	1.44 ± 0.31	...
0.59	7	0.27 ± 0.06]*	81.3
1.76	7	0.43 ± 0.11]	70.1
2.93	7	0.15 ± 0.02]	89.6

* Values with brackets do not differ significantly.
Data are expressed as mean ± SEM where applicable.

Table 7—Dose response of HMP-coated plain dog biscuit vs calculus formation (experiment 7)

HMP (%)* added	No. of dogs	Calculus score	Percentage reduction
0.00	6	1.46 ± 0.49	...
0.66	5	0.47 ± 0.12]†	67.8
1.04	7	0.57 ± 0.17]	61.0
1.70	7	0.35 ± 0.06]	76.0

* Based on chemical analysis of test products. † Values within brackets do not differ significantly.
Data are expressed as mean ± SEM where applicable.

Table 8—Plain biscuits coated with HMP or P_2O_7 vs calculus formation (experiment 8)

Snack regimen	No. of dogs	Calculus score	Percentage reduction
Plain biscuit (control)	8	1.13 ± 0.17†	...
P_2O_7-coated biscuit*	9	0.79 ± 0.22	30.1
HMP-coated biscuit*	7	0.42 ± 0.16	62.8

* Added at 0.53% phosphorus (equivalent to 1.5% P_2O_7 and 1.8% HMP), † All means differ significantly ($P < 0.05$).
Data are expressed as mean ± SEM where applicable.

Table 9—Plain HMP-coated biscuit vs calculus formation in dogs (experiment 9)

Snack regimen	No. of dogs	Calculus score	Percentage reduction
None	8	2.12 ± 0.56]*	...
Plain biscuit (control)	8	1.24 ± 0.16]	41.5
HMP-coated biscuit†	9	0.60 ± 0.24	71.7

* Values with brackets do not differ significantly. † 1.8% HMP.
Data are expressed as mean ± SEM where applicable.

feeding 2 non-HMP-coated plain biscuits 4 hours after each meal resulted in a numerical decrease of 41.5% in calculus formation. Similar feeding of 2 plain biscuits coated with HMP decreased calculus formation by 71.7%; this effect was significantly greater than that observed for the plain, non-HMP-coated biscuits.

To investigate the impact of feeding either 1 or 2 HMP-coated biscuits and different concentrations of HMP in the coating, experiment 10 was conducted. Similar to experiments 8 and 9, the test design involved a single daily meal of moistened chow followed by the feeding of various biscuits as a snack 4 hours later. The results (Table 10) indicate that feeding of either 1 or 2 HMP-coated (1.8% HMP) biscuits resulted in statistically significant decreases of 56.5 and 70.8%, respectively, in calculus formation. The similar feeding of 2 HMP-coated biscuits, with variable concentrations of HMP ranging from 0.6 to 1.8% in the coating, resulted in statistically significant reductions in calculus formation ranging in magnitude from 59.3 to 79.6%. Similar to experiments 6 and 7, a dose response was not evident; in fact, the greatest numerical decrease of 79.6% in calculus formation

Table 10—The HMP-coated plain biscuit regimens vs calculus formation in dogs (experiment 10)

Snack biscuit regimen Type	No.	No. of dogs	Calculus score	Percentage reduction
None	...	5	2.16±0.57	...
HMP (1.8%)	1	5	0.94±0.29 *	56.5
HMP (1.2%)	2	5	0.88±0.36	59.3
HMP (1.8%)	2	5	0.63±0.06	70.8
HMP (0.6%)	2	5	0.44±0.11	79.6

* Values with brackets do not differ significantly.
Data are expressed as mean ± SEM where applicable.

was observed for the biscuits coated with the lowest amount of HMP (0.6%).

Discussion

The need for the identification of practical measures to prevent accumulation of dental calculus as a means of improving the oral health of companion animals is widely recognized. As a result of this recognized need, a number of measures have been explored. Quite clearly, the meticulous mechanical removal of dental plaque on a daily or at least an alternate daily basis will virtually eliminate accumulation of dental calculus as well as attendant development of periodontal disease.[4-6] Unfortunately, few pet owners are willing to perform the necessary oral hygiene procedure[5] and alternative measures including use of hard biscuits and rawhide,[10,a] oxtails,[16,17] and a nylon chew device[18] have been evaluated with modest success.

Because of the successful use of chemical agents for calculus prevention in animal models (rats and dogs) and subsequent clinical trials, this program was initiated to identify practical measures, using these types of materials for calculus prevention in dogs and cats. The strategy was to identify a system that could be incorporated into a conventional food item in some manner. Thus, possible agents were required to be nutritionally acceptable, be generally recognized as safe by the FDA, not alter palatability, and be commercially available as a food grade material with reasonable cost. These criteria served to eliminate some agents (eg, zinc chloride, zinc citrate, various diphosphonates) that had been clinically proven for use in human dentifrices.

Results of this study clearly indicate the ability of chemical additives to dog chow or dog biscuits to reduce formation of dental calculus. Furthermore, it was found that the agent must be added as a surface coating to dry dog food to impart significant anticalculus properties (experiment 4). Of the agents studied, sodium HMP was decidedly the most effective; regardless of the use of HMP concentrations between 0.6 and 1.8% in surface coatings, the choice of vehicle (chow or biscuits), or the biscuit snack regimen, reductions in the formation of dental calculus between 57 and 90% were observed. Even though a dose response was not apparent for the range of 0.6 to 1.8% HMP, concentrations of HMP below 0.6% applied as a coating may exert substantial benefits and near maximal anticalculus benefits of HMP can be achieved with concentrations as low as 0.6% applied as a surface coating. The uniqueness of these observations provided the basis for a patent application.[30]

Sodium HMP is a widely recognized sequestrant that forms soluble complexes with a variety of cations. In contrast to the mechanism of action of crystal growth inhibitors, such as soluble pyrophosphate, and zinc compounds used to reduce the rate of calculus in dentifrices and rinses intended for human use, it is postulated that HMP reduces the rate of formation of dental calculus by sequestering or complexing calcium within dental plaque. Thus, we propose that HMP becomes incorporated in dental plaque and forms soluble calcium complexes that diffuse into saliva and, thereby, prevent calcification of the plaque.

The nutritional safety of HMP rests with the fact that this compound is known to be converted to orthophosphate in the presence of any strong acid, such as hydrochloric acid in the stomach. Thus, ingested HMP is simply converted to orthophosphate, which is metabolically utilized by the host.

This series of experiments repeatedly documents the ability of HMP to significantly reduce the formation of dental calculus in dogs when used at concentrations of 0.6% or more applied as a coating to either dry dog chow or plain biscuits provided as a snack. Despite the substantial progress achieved by this study, several questions remain to be answered. For example, although calculus accumulates in a linear manner[5,6] and HMP would be expected to exert a continuous impact of a decreasing rate of calculus formation, longer-term studies are needed to document this effect. Similarly, although the observed decrease in calculus formation would be expected to exert a corresponding improvement in prevention of gingivitis and periodontal disease, longer-term studies are needed to study this relation. Nevertheless, this study has identified a practical means of significantly reducing formation of dental calculus in dogs through use of HMP as a surface coating on dry food preparations.

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