24-hour evaluation of dental plaque bacteria and halitosis after consumption of a single placebo or dental treat by dogs

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OBJECTIVE
To determine whether consumption of a single dental treat with specific mechanical properties and active ingredients would provide a 24-hour effect on dental plaque bacteria and halitosis in dogs.

ANIMALS
10 dogs of various breeds from a privately owned colony that had received routine dental scaling and polishing 4 weeks before the study began.

PROCEDURES
Dogs were randomly assigned to receive 1 placebo or dental treat first. A 4-week washout period was provided, and then dogs received the opposite treatment. Oral plaque and breath samples were collected before and 0.5, 3, 12, and 24 hours after treat consumption. Volatile sulfur compounds (VSCs) concentration was measured in breath samples. Total aerobic, total anaerobic, *Porphyromonas gulae*, *Prevotella intermedia*–like, *Tannerella forsythia*, and *Fusobacterium nucleatum* bacterial counts (measured via bacterial culture) and total live bacterial counts, total live and dead bacterial counts, and bacterial vitality (measured via quantitative real-time PCR assay) were assessed in plaque samples.

RESULTS
Compared with placebo treat consumption, dental treat consumption resulted in a significant decrease in breath VSCs concentration and all plaque bacterial counts, without an effect on bacterial vitality. Effects of the dental treat versus the placebo treat persisted for 12 hours for several bacterial counts and for 24 hours for breath VSCs concentration.

CONCLUSIONS AND CLINICAL RELEVANCE
Although clinical benefits should be investigated in larger scale, longer-term studies, results of this study suggested that feeding the evaluated dental treat may help to decrease oral bacterial growth in dogs for 12 hours and oral malodor for 24 hours. A feeding interval of 12 hours is therefore recommended. (*Am J Vet Res* 2016;77:613–619)

Periodontal disease is the most widespread oral disease of dogs, with between 44% and 80% of dogs affected, depending on age, breed, or criteria used to establish the diagnosis.[1–4] The disease is also the second most prevalent disorder of dogs evaluated at primary-care veterinary practices in England.[5]

Colonization of the oral cavity with specific bacteria is believed to be the primary factor contributing to periodontal disease in humans.[6,7] *Porphyromonas* spp (*Porphyromonas salivosa*, *Porphyromonas denticanis*, *Porphyromonas gulae*, and *Porphyromonas gingivalis*), *Tannerella forsythia*, *Prevotella intermedia*, and *Fusobacterium nucleatum* are associated with gingivitis or periodontitis in dogs.[8–13] *Actinomyces* spp, Peptostreptococcaceae, and *Porphyromonas* spp have also been identified as being the most abundant in the mouths of dogs with mild periodontitis.[14] In addition, periodontal disease has been associated with diseases of the cardiovascular system and kidneys in dogs,[15,16] suggesting that canine health may be improved by prevention of periodontal disease.

Regular tooth brushing is considered important for removal of dental plaque, thereby preventing gingivitis and, eventually, periodontitis in dogs. However, in the authors' experience, owner compliance with recommendations to brush their dogs' teeth is generally poor. As a result, various preventive approaches have been proposed as an alternative, including dietary treats or chew products with or without chemical additives to enhance their effectiveness. Beneficial effects of chewing specifically designed dental treats with certain mechanical properties or containing certain ingredients (ie, zinc salts, polyphosphate salts, essential oils, or enzymes) on dental plaque, calculus, and gingivitis have been identified in several medium-

ABBREVIATIONS

qPCR	Quantitative real-time PCR
VSC	Volatile sulfur compound

and long-term studies (with follow-up periods ranging from 7 days to 21 months).[17–25]

In vitro tests have revealed that a patented form of vitamin C[a] has growth-inhibition properties against various oral bacteria (eg, *P gulae*). Unpublished results of an in vivo study[b] involving dogs also suggest that feeding this form of vitamin C for 28 days results in a significant decrease in mean dental plaque and calculus scores and improvement in gingivitis score.

Zinc is an essential trace element that exists naturally in dental plaque, saliva, and dental enamel. This element has good oral substantivity, and high concentrations can persist for many hours in plaque and saliva after oral administration. Zinc salts are used in human oral hygiene products to control plaque, dental calculus, and oral malodor. Zinc ions inhibit bacterial growth in dental plaque.[26–28] The ions are also able to reduce oral malodor by reacting chemically with hydrogen sulfide to remove this component of halitosis, as was confirmed in a complex biofilm model by which > 90% of hydrogen sulfide was removed from perfusate gas.[29] This effect was also confirmed in several in vivo trials.[27,30–32]

To the authors' knowledge, no study has been conducted to examine the 24-hour effect of consumption of a single chew treat to better identify the frequency of treat feeding required to maintain the effect provided. The purpose of the study reported here was to determine, over a 24-hour period, the effects of consumption of a single dental treat, with a shape designed to provide specific mechanical effects (long chewing time and high degree of contact surface with teeth during chewing) and containing the patented vitamin C formulation, on dental bacterial plaque and oral malodor in dogs.

Materials and Methods

Animals

Ten dogs were selected from a colony housed at the Affinity Nutrition Center in Masqueffa, Spain, for inclusion in the study. All dogs had received routine dental scaling and polishing 4 weeks prior to the start of the study, allowing for sufficient plaque accumulation before the effects of dental treat consumption were evaluated. To be included in the study, dogs were also required to allow the study manipulations, which included mouth explorations, without sedation and to accept and eat the dental or placebo treats. Dogs were excluded when they had evidence of any tooth mobility or missing teeth, severe (stage 3 or 4) periodontal disease, or any disease that might have interfered with the results of the study (eg, kidney, liver, gastrointestinal, or endocrine disease); were receiving any medications; or refused to eat the dental or placebo treat. Selected dogs included 2 each of Standard Poodle, Miniature Schnauzer, and Andalusian Bodegero and 1 each of Labrador Retriever cross, Beagle, Pomeranian, and Pomeranian cross. Mean ± SD age was 5.2 ± 4.7 years, and mean body weight was 11.3 ± 9.2 kg.

Study design

Dogs were strategically assigned to 1 of 2 groups to provide as equal as possible distributions of age, breed, and body weight between groups. Within each group, dogs were randomly (via random number generator) and successively allocated to receive 1 of 2 treatments (dental or placebo treat) first in a crossover study design. After a 4-week washout period, dogs received the opposite treatment.

Treatments consisted of 1 dental treat[c] with specific mechanical properties (special shape to increase contact area with teeth during chewing and to increase mastication time) and active ingredients against dental plaque (vitamin C[a] [1,200 mg/kg] as is and zinc sulfate [500 mg/kg] as is) or 1 placebo biscuit[d] (semimost biscuit without active ingredient or the shape specifically designed for mechanical effect). During the washout period, dogs received their regular dry food only and were allowed access to the garden in groups for activity and socialization. The study protocol was approved by the Affinity Petcare Ethics Committee.

Sample collection and monitoring

The veterinary dentist responsible for the trial and the laboratory team responsible for the bacterial analysis were blinded to the treatments received by the dogs. Before each treatment was administered, food was withheld from dogs overnight and baseline samples were collected. Treatments were then administered, and samples were collected 30 minutes and 3, 12, and 24 hours after consumption. Water intake was not restricted during the trial; however, access to water was prevented for 30 minutes prior to sample collection. Food was withheld for the duration of the trial.

Dental and supragingival plaque samples were collected from left and right maxillary and mandibular canine teeth, left and right maxillary fourth premolars, and left and right mandibular first molars by passing the same sterile cotton swab over the buccal surfaces of the selected teeth and the surrounding gingiva 10 times. This collection method was chosen instead of subgingival scraping with a curette or paper point because it allowed a safe and noninvasive means to acquire samples from dogs without the need for sedation. The tip of each cotton swab was cut off with sterile scissors and gently placed into a vial containing 1 mL of reduced transport fluid[33] and stored at 4°C until analysis a maximum of 24 hours later.

Breath samples for measurement of VSCs concentration were collected by application of a portable sulfide monitor[e] to the right side of the buccal surface of the right maxillary fourth premolar.[34] Three successive samples were collected and analyzed by the monitor, which provided the mean VSCs concentration. If a dog moved during sample collection, the result was disregarded and a new set of 3 measurements was obtained.

Bacterial analysis

Swab specimens were used for evaluation of

the following variables: total aerobic, total anaerobic, *P gulae, P intermedia*–like, *T forsythia* and *F nucleatum* bacterial counts (via bacterial culture) and live bacterial counts, live and dead bacterial counts, and bacterial vitality (via qPCR assay). The particular bacterial species evaluated were selected because their prevalence in the mouths of elderly dogs is high,[35] and these species are associated with gingivitis or periodontitis in dogs.[8–13]

Each cotton swab, placed in 1 mL of reduced transport fluid, was homogenized at the laboratory by mixing for 30 seconds with a vortex device. Five serial dilutions of the contents were prepared with PBS solution. Aliquots (0.1 mL) of each dilution were applied to nonselective blood agar plates[f] supplemented with hemin[g] (5 mg/L), menadione[h] (1 mg/L), and 5% sterile horse blood.[i] After 7 to 14 days of anaerobic incubation (80% N_2, 10% CO_2, and 10% H_2), plates containing between 30 and 300 colonies were selected for performance of bacterial counts. Plates containing colonies that were morphologically similar to those of the specific bacterial species of interest were used for specific bacterial counts. Selected colonies were further identified by microscopy, Gram staining, and analysis of enzyme activities (specifically, *N*-acetyl-β-D-glucosaminidase, α-glucosidase, α-galactosidase, α-fucosidase, esculin, indole, and trypsin-like activities; **Appendix**).

After bacterial cultures were performed, undiluted samples were used for microbiological analysis by use of a qPCR assay. For this purpose, two 250-µL aliquots were obtained from each sample and transferred to test tubes, one of which was then treated with propidium monoazide[j] added to achieve a final concentrations of 100µM. Tubes were incubated for 10 minutes at 4°C in the dark and then exposed to a 550-W halogen light source placed 20 cm above the samples to induce cross-linking of propidium monoazide. Tubes were laid horizontally on ice during this period to avoid excessive heating. After cross-linking was induced, tubes were centrifuged at 9,000 × *g* for 3 minutes prior to DNA isolation.

A commercial kit[k] was used to isolate DNA from samples prepared for qPCR analysis in accordance with the manufacturer's instructions. The hydrolysis probes 5′ nuclease method of PCR analysis was used for detection and quantification of bacterial DNA. Primer[l] and probe[m] sequences were targeted against positions 331 and 797 on the *Escherichia coli* 16S rRNA gene, amplicon of 466 bp (forward primer sequence, 5′-TCCTACGGGAGGCAGCAGT-3′; reverse primer sequence, 5′-GGACTACCAGGGTATCTAATCCTGTT-3′; and probe sequence, 5′-[6FAM]-CGTATTACCGCGGCTGCTGGCAC-[TAMRA]-3′). The qPCR amplification was performed by use of a total reaction mixture volume of 20 µL, containing 10 µL of 2× master mixture,[n] optimal concentrations of primers and probe (350, 350, and 100nM for total bacteria), and 5 µL of DNA from samples. The negative control (ie, no-template control) sample consisted of 5 µL of sterile water.[o]

Samples were processed in an initial amplification cycle at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Analyses were made with a thermocycler,[p] and each DNA sample was analyzed in duplicate. Natural-frame, white-welled PCR plates[q] were used for sample analysis and sealed with adhesive material.[r] Quantification cycle values (previously referred to as cycle threshold values), representing the number of the PCR cycle at which the degree of fluorescence increased above the initial value, were determined by use of the software package provided by the thermocycler manufacturer.

Counts of viable bacterial cells by use of qPCR assay were estimated on the basis of standard curves. For this procedure, 1 mL of viable-cell suspension containing 10^{10} CFUs of bacteria/mL with or without propidium monoazide was used for DNA extraction by use of a commercial kit[k] in accordance with the manufacturer's instructions. Serial dilutions of each DNA sample with sterile water[o] were performed to achieve concentrations ranging from 10 to 10^{10} CFUs/mL, and resulting samples were evaluated by qPCR assay as previously described. Standard curves were constructed by plotting quantification cycle values generated from the qPCR process against bacterial concentrations. Correlations between quantification cycle values and bacterial concentrations were automatically generated by the cycler software.[s] All assays were developed with a linear quantitative detection range established by the slope range of 3.3 to 3.7 cycles/logarithmic decade ($r^2 > 0.9$) and an efficiency range of 1.9 to 2.0. Values lower than this linear quantitative detection range may have been detectable but not quantifiable because lower limits of quantification were poorly defined.

Processes were implemented to avoid carryover DNA during qPCR analysis, such as establishment of barrier methods and physical separation of pre- and post-PCR procedures. In addition to these efforts, when DNA was detected in the negative control sample, the limit of detection for the assay was established as the last value of the standard curve that held 5 cycles of difference with the negative control sample.[36,37]

Statistical analysis

All statistical analyses were performed by use of statistical software.[t] For analysis, variable values were logarithmically transformed to achieve a normal distribution, which was confirmed by evaluation of the degree of skewness and kurtosis. Data are reported as mean ± SD untransformed values to facilitate interpretation. No carryover effect was detected.

To evaluate treatment effect, a mixed linear model was constructed, with treatment, time, sequence, and period and treatment-by-time interaction as fixed effects and dog as a random effect. To evaluate the time effect within each treatment, a mixed linear model was constructed, with treatment by time, sequence, and period as fixed effects and dog as a random effect. To evaluate the time effect within each treatment

and to evaluate treatment effect at each sample collection point, preplanned pairwise comparisons between respective values were performed by use of the protected Fisher least squares difference test. Values of $P < 0.05$ were considered significant for all analyses. To facilitate the data interpretation, only main effects of interest (treatment and treatment-by-time interaction) were reported.

Results

Animals

The 5 dogs that received the single dental treat first had a mean ± SD age of 6.3 ± 4.9 years and mean body weight of 13.7 ± 13.0 kg. The 5 dogs that received the single placebo treat first had a mean age of 4.2 ± 4.9 years and a mean body weight of 8.9 ± 2.9 kg. All 10 dogs completed both treatment sessions.

Bacterial counts

With respect to the overall treatment effect, dental treat consumption resulted in significantly lower total aerobic ($P = 0.002$), total anaerobic ($P = 0.04$), *P gulae* ($P = 0.004$), *P intermedia*–like ($P = 0.001$), *T forsythia* ($P = 0.048$), and *F nucleatum* ($P = 0.01$) bacterial counts in dental and supragingival plaque samples, compared with values for placebo consumption **(Table 1)**. Results of qPCR assay indicated overall lower counts of live bacteria ($P = 0.001$) and live and dead bacteria ($P < 0.001$) following dental treat consumption than were achieved after placebo treat consumption. In addition, overall VSCs concentration in breath samples were significantly ($P < 0.001$) lower after dental treat consumption than after placebo treat consumption. No treatment effect was identified on overall bacterial vitality ($P = 0.96$).

A significant treatment by time effect was identified for total aerobic ($P = 0.001$), total anaerobic ($P = 0.001$), *P gulae* ($P = 0.002$), *P intermedia*–like ($P < 0.001$), total live ($P < 0.001$), and total live and dead ($P < 0.001$) bacterial counts in dental and supragingival plaque samples and for VSCs concentration in breath samples ($P < 0.001$; **Table 2**). Compared with baseline values, the antibacterial effects of dental treat consumption on total aerobic, total anaerobic, *P gulae*, and *P intermedia*–like bacterial counts persisted for up to 12 hours. The effect of dental treat consumption on breath VSCs concentration persisted for up to 24 hours. On the other hand, antibacterial effects associated with placebo treat consumption generally persisted for only 30 minutes.

A significant decrease in total live bacterial and total live and dead bacterial counts with time was detected for both treatments (Table 2). With respect to specific sample collection points, significant differences were identified between treatments at 30 minutes after consumption for *P intermedia*–like and total live and dead bacterial counts; at 3 hours after consumption for total aerobic, total anaerobic, *P intermedia*–like, and live and dead bacterial counts; at 12 hours after consumption for *P gulae* and live bacterial counts; and at all points after consumption for breath VSCs concentration.

Discussion

Results of the present study suggested that consumption of a single specifically designed dental treat containing vitamin C and zinc sulfate had antibacterial effects, compared with the effects of a placebo treat. The reported impact on *P gulae* counts,[9–12,38] and to a lesser extent on *P intermedia*,[8] *T forsythia*,[13] and *F nucleatum*[12] counts, is of particular interest because these bacterial species have been associated with gingivitis and periodontal disease in dogs. *Actinomyces* sp, Peptostreptococcaceae (*Peptostreptococcus* sp, *Peptostreptococcaceae* XI sp, and *P* XIII sp), and *Porphyromonas cangingivalis* have more recently been identified as being most abundant in the mouths of dogs with mild periodontitis, and *P cangingivalis* is also one of the most abundant species in the mouths

Table 1—Mean ± SD overall values for various bacterial counts (made by bacterial culture) and other measurements (made by qPCR assay) of dental and supragingival plaque samples as well as breath VSCs concentration for 10 dogs after consumption of a single placebo treat or dental treat in a crossover study design.

Variable	Placebo treat	Dental treat	*P* value
Bacterial count via culture (X 10^6 CFUs)			
Total aerobic	27.1 ± 38.2	13.2 ± 12.7	0.002
Total anaerobic	22.9 ± 27.7	20.2 ± 29.4	0.04
Porphyromonas gulae	4.4 ± 9.6	3.4 ± 8.9	0.004
Prevotella intermedia–like	663.7 ± 2,049.5	601.9 ± 991.5	0.001
Tannerella forsythia	467.5 ± 879.6	412.8 ± 546.6	0.048
Fusobacterium nucleatum	468.3 ± 607.6	309.1 ± 530.6	0.01
Bacterial count via qPCR assay (X 10^6 CFUs/mL)			
Total live	122.6 ± 205.4	70.5 ± 118.1	0.001
Total live and dead	418.5 ± 628.8	191.9 ± 378.9	< 0.001
Proportion that were live (vitality)	0.24 ± 0.08	0.25 ± 0.10	0.96
VSCs (ppb)	63 ± 26	47 ± 28	< 0.001

Data were logarithmically transformed for analysis to achieve a normal distribution; values reported here have been restored to their original (nonnormally distributed) form for ease of interpretation. A 4-week washout period separated treatments. Values of $P < 0.05$ were considered significant.

Table 2—Mean ± SD values for various bacterial counts (made by bacterial culture) and other measurements (made by qPCR assay) of dental and supragingival plaque samples as well as breath VSCs concentration for the dogs in Table 1 before (baseline) and at various points after consumption of a dental treat or placebo treat.

		Measurement point				
Variable	**Treat type**	**Baseline**	**30 minutes**	**3 hours**	**12 hours**	**24 hours**
Bacterial count via culture (X 10^6 CFUs/mL)						
Total aerobic	Placebo	31.9 ± 32.4	14.3 ± 15.5*	20.1 ± 13.6	20.6 ± 17.8	51.4 ± 72.1
	Dental	27.1 ± 17.4	7.1 ± 9.5*	7.7 ± 6.8*†	8.9 ± 4.8*	15.2 ± 9.8
Total anaerobic	Placebo	45.9 ± 48.4	15.3 ± 15.1*	18.2 ± 17.3	13.1 ± 8.0	22.1 ± 21.1
	Dental	49.9 ± 52.7	16.2 ± 17.6*	8.5 ± 10.0*†	7.4 ± 6.2*	19.2 ± 11.8
P gulae	Placebo	12.7 ± 19.1	2.6 ± 3.7*	2.1 ± 3.0	1.5 ± 1.3	3.2 ± 4.3
	Dental	12.8 ± 17.1	0.7 ± 1.5*	0.3 ± 0.3*	0.8 ± 0.9*†	2.4 ± 2.8
P intermedia–like	Placebo	1.3 ± 1.5	0.25 ± 0.37*	0.54 ±1.09	0.15 ± 0.15	0.75 ± 0.89
	Dental	2.7 ± 4.1	0.10 ± 0.22*†	0.04 ± 0.05*†	0.11 ± 0.18*	0.36 ± 0.48
Bacterial count via qPCR assay (X 10^6 CFUs/mL)						
Total live	Placebo	342.4 ± 343.7	56.8 ± 76.7*	36.7 ± 26.8*	62.3 ± 66.3*	114.9 ± 172.4*
	Dental	233.9 ± 179.3	18.9 ± 23.2*	13.5 ±17.5*	26.2 ± 28.8*†	59.9 ± 59.1*
Total live and dead	Placebo	1,549.2 ± 1,206.7	193.7 ± 168.3*	247.5 ± 295.1*	269.2 ± 221.4*	446.0 ± 564.1*
	Dental	812.9 ± 811.6	67.9 ± 69.2*†	70.2 ± 46.0*†	127.6 ± 76.9*	233.2 ± 160.3*
Proportion that were live (vitality)	Placebo	0.24 ± 0.20	0.23 ± 0.14	0.22 ± 0.14	0.28 ± 0.20	0.20 ± 0.09
	Dental	0.31 ± 0.21	0.26 ± 0.19	0.21 ± 0.27	0.21 ± 0.23	0.26 ± 0.20
VSCs (ppb)	Placebo	57 ± 26	62 ± 24	67 ± 26	69 ± 28	59 ± 28
	Dental	77 ± 43	37 ± 12*†	35 ± 16*†	46 ± 14*†	40 ± 22*†

*Value differs significantly ($P < 0.05$) from corresponding baseline value. †Value differs significantly ($P < 0.05$) from corresponding value for the placebo treat.

See Table 1 for remainder of key.

of healthy dogs.[14] These bacterial species should therefore be evaluated in future studies to better estimate the potential effect of antimicrobial compounds in the prevention of periodontal disease. A significant treatment effect was also identified on breath VSCs concentration, which suggested a potential benefit for the dental treat in the control of oral malodor in dogs.

Although these results were promising, the present study had several limitations that require consideration. Because the number of dogs used (10) was small and the variability among them was high, the possibility of type II errors existed, despite the use of a crossover study design. Ideally, the study would be repeated with a larger cohort, with more dogs within each breed and with dogs of similar ages. Bacterial samples were obtained with cotton swabs to facilitate collection of dental and supragingival plaque samples from unsedated dogs. However, it is possible that sample collection by scraping with paper points under the gingiva would have yielded different results.

The dental treat used in the present study was compared with a semimoist biscuit with no special mechanical properties or specific ingredients to control dental plaque. Because the 2 treats had different compositions, we were unable to identify the specific properties of the dental treat that were responsible for the differences identified between the treatments in antibacterial effects. We were also unable to exclude the possibility that use of a different placebo treat or no placebo at all could have yielded different results. However, in preliminary research conducted by our laboratory group in which the effects of the dental treat were compared with that those of no placebo (unpublished data), less bacterial growth was also observed after consumption of the dental treat. In the present study, use of a placebo treat was intended to stimulate saliva flow, as would have occurred during chewing of the dental treat.

Antibacterial effects identified in comparisons of posttreatment values with respective baseline values in the present study persisted for up to 12 hours (bacterial counts) or 24 hours (VSCs concentration) for the dental treat, whereas effects were evident only at 30 minutes after placebo treat consumption, probably owing to mechanical effects and saliva flow stimulated by chewing. Indeed, in humans, rinsing of the mouth once with saline (0.9% NaCl) solution can result in a decrease in salivary bacterial counts by 50%.[39] It should be considered that dogs in the present study were prevented from consuming other foods while treatment effects were measured, and consumption of other foods might have led to different results. However, the methods used were intentionally selected to evaluate the effects of the dental treat alone on oral plaque and malodor, controlling for other factors such as food consumption, which might also affect those outcomes.

When bacterial counts at the various sample collection points were compared between treatments in the present study, the most important differences were identified at 3 hours (total aerobic, total anaerobic, and *P intermedia*–like bacterial counts) and 12 hours (*P gulae* and live bacterial counts), although numerical differences were still identified at 24 hours after consumption. Effects of the dental treat on VSCs concentration were significant at all sample collection

points. Practically, these results suggested that feeding of 1 treat every 24 hours should be efficient to control oral malodor, but growth of bacteria would be better prevented if the treat were fed every 3 to 12 hours. Nonetheless, the antibacterial effects identified in the present study should be confirmed in a study involving more dogs and additional bacterial species such as those identified in dogs with periodontitis.[14] A longer-term study should also be conducted to confirm that the short-term antibacterial effect that we identified might be effective and sufficient to control plaque and periodontal disease in the long-term, improving the oral health of dogs. It would also be interesting to compare our results with those achieved with tooth brushing, as a reference standard, to determine whether the easier to administered dental treat could provide similar effects.

Compared with placebo treat consumption, dental treat consumption resulted in a significant decrease in total live and in total live and dead bacterial counts in the present study. However, no significant differences were identified with respect to bacterial vitality in plaque samples, although the decrease in vitality was numerically more important at 12 hours after dental treat consumption (10.3% decrease for the dental treat vs 3.8% increase for the placebo treat). One explanation could be that the limited sample size did not allow detection of significant differences between groups for this particular variable. It is also possible that the mechanical effect of the dental treat was greater than the chemical effect, which could explain why both live and dead bacterial counts were affected but not vitality. That possibility could be investigated in studies of the effects on oral health of chlorhexidine (a well-known antimicrobial), which reportedly helps prevent plaque but not gingivitis,[17] thereby supporting the notion that the mechanical or chewing effect is more important than the chemical effect. In contrast, cetylpyridinium chloride, a quaternary ammonium compound, controls dental plaque and halitosis when applied to the mouths of dogs in gel or spray form twice a day,[40] demonstrating that a chemical effect is possible without a mechanical or chewing effect.

In humans, a decrease in bacterial vitality in dental plaque after application of dental care products (eg, chlorhexidine mouth rinses) has been identified through culture-based methods.[41] However, a qPCR-assay approach has been proposed and specifically validated for detection and quantification of periodontal bacteria[36,37] and could offer more precise results. To the best of our knowledge, the present study was the first in which bacterial vitality in dental plaque samples was evaluated in dogs, so comparison of our findings with those of other studies involving dogs is precluded. Compared with findings in humans, bacterial vitality in plaque samples from the study dogs was low at baseline and after treat consumption, which could be explained by the maturity of the biofilms from which samples had been collected, given that proportions of viable bacteria in mature biofilms are typically < 40%.[42] The purpose of the present study was to compare treatment effects by evaluation of plaque and breath samples that had been collected in similar conditions, and the low vitality values obtained did not preclude these comparisons, although they did make it more challenging to detect significant differences between treatments. Quantities of both live bacteria and live and dead bacteria decreased with time after dogs consumed either treat. Again, this effect of the placebo treat could have been associated with the mechanical effect of chewing and the stimulation of saliva production.[39]

Despite the preliminary nature of the study reported here, results suggested that feeding a dental treat with a shape designed to increase chewing time and teeth contact and containing vitamin C and zinc may help to decrease amounts of dental bacterial plaque and oral malodor in dogs, potentially improving oral health. On the basis of the results, we recommend that at least 1 such dental treat be fed every 12 hours to control bacterial counts. Clinical benefits should be confirmed in larger, longer-term studies with tooth brushing as a comparison treatment to establish whether the dental treat provides similar effects while facilitating dog-owner compliance.

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Footnotes

a. Vitamin C Stay-clean, DSM, Heerlen, The Netherlands.
b. M. Karutz, global business manager petfood, DSM nutritional products, Basel, Switzerland: Personal communication, 2014.
c. Ultima Inter Dental+, Affinity Petcare SA, Barcelona, Spain.
d. Advance hypoallergenic snack, Affinity Petcare SA, Barcelona, Spain.
e. Halimeter, Interscan Co, Chatsworth, Calif.
f. Blood agar base II, Oxoid, Basingstoke, Hampshire, England.
g. Hemin from bovine (≥ 90%), Sigma-Aldrich Corp, St Louis, Mo.
h. Menadione crystalline, Sigma-Aldrich Corp, St Louis, Mo.
i. Defibrinated horse blood, Oxoid, Basingstoke, Hampshire. England.
j. PMA Biotium Inc, Hayword, Calif.
k. ATP genomic DNA mini kit, ATP biotech, Taipei, Taiwan.
l. Invitrogen Corp, Carlsbad, Calif.
m. Applied Biosystems, Carlsbad, Calif.
n. LC480 probes master, Roche Diagnostic GmbH, Mannheim, Germany.
o. Water (PCR grade), Roche Diagnostic GmbH, Mannheim, Germany.
p. LightCycler 480 II thermocycler, Roche Diagnostic GmbH, Mannheim, Germany.
q. Framstar480, 4titude Ltd, Wotton, Surrey, England.
r. QPCR adhesive clear seals, 4titude Ltd, Wotton, Surrey, England.
s. LC480 software, version 1.5, Roche Diagnostic GmbH, Mannheim, Germany.
t. SPSS, version 19, SPSS Inc, Chicago, Ill.

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Appendix

Biochemical and phenotypic criteria used to identify bacterial species cultured from dental and supragingival plaque samples from dogs.

Species	Cell morphology	Gram staining	*N*-acetyl-β-D-glucosaminidase	α-Glucosidase	α-Galactosidase	α-Fucosidase	Esculin	Indole	Trypsin-like activity
Porphyromonas gulae	Coccobacilli	Negative	Positive	Not done	Negative	Negative	Not done	Positive	Positive
Prevotella intermedia–like	Rods	Negative	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Tannerella forsythia	Coccobacilli	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Fusobacterium nucleatum	Rods	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Not done