EFFECTS OF NOVEL DENTAL CHEWS ON ORAL HEALTH OUTCOMES AND HALITOSIS IN ADULT DOGS

BY

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THESIS

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ABSTRACT

Periodontal disease (PD) is the most common clinical condition occurring in adult dogs and cats but is preventable. The objective of this study was to evaluate the benefits of daily dental chew administration on oral health outcomes in adult dogs. Twelve adult (mean age = 5.31 ± 1.08 years; mean BW = 13.12 ± 1.39 kg) female beagles were used in a replicated 4x4 Latin square design consisting of 28-day periods. All animal care and experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. On day 0 of each period, teeth were cleaned by a veterinary dentist blinded to treatments. Teeth were then scored for plaque, calculus, and gingivitis by the same veterinary dentist on day 28 of each period. Breath samples were measured for malodor (volatile sulfur compounds) on days 1, 7, 14, 21, and 27 of each period. All dogs consumed the same commercial dry diet throughout the study. Control dogs were offered the diet only, while treatment groups received the diet plus one of three dental chews. Two novel chews [Bones & Chews Dental Treats (Chewy, Inc., Dania Beach, FL); Dr. Lyon's Grain-Free Dental Treats (Dr. Lyon's, LLC, Dania Beach, FL)] and a leading brand chew [Greenies Dental Treats (Mars Petcare US, Franklin, TN)] were tested. Each day, one chew was provided four hours after mealtime. All tooth scoring data were analyzed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC). Halimeter data were analyzed using repeated measures using the Mixed Models procedure of SAS, testing for differences due to treatment, time, and treatment*time interaction. Data are reported as LS means ± SEM with statistical significance set at $p<0.05$. Dr. Lyon's Dental Treats performed similarly to the leading brand, Greenies, as both resulted in a reduction ($p<0.05$) of plaque coverage and thickness, calculus coverage, and day 27 volatile sulfur concentrations compared to controls. Additionally, Dr. Lyon's dental treats also reduced volatile sulfur compounds on day 14 when compared to controls. Bones and Chews Dental Treats reduced ($p<0.05$) calculus coverage and day 27 volatile sulfur concentrations compared to controls. Our results suggest that the dental chews tested in this study may aid in reducing the risk of periodontal disease in dogs.

To My Parents

Thank you for your endless love and encouragement.

I would not be anything without your constant support.

I owe all of my accomplishments to you.

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CHAPTER 1

INTRODUCTION

Periodontal disease (PD) is the most common clinical condition in adult dogs, affecting 44% to 63.6% of dogs over the age of 3 years (Davis et al., 2013). Periodontal disease is characterized by both gingivitis and periodontitis, the inflammation of the gingiva and the inflammation of the nongingival periodontal tissues, respectively (Harvey, 2005). These conditions develop as a result of plaque and calculus buildup on the teeth, which harbor bacteria that can cause an immune response and inflict damage to oral tissues. This chronic, progressive disease is preventable and can often be reversed if treated before permanent damage occurs. When allowed to progress, PD can result in tooth loss, bone loss, oral malodor, and chronic pain, which may lead to reduced food intake and behavioral problems.

Several methods for oral care are available to aid in the prevention of PD. Most importantly, it is recommended that dogs have an examination by a veterinary dentist once per year for teeth cleaning and polishing. This is done under anesthesia using ultrasonic tools, which remove plaque and calculus from all tooth surfaces. In addition to this regular veterinary therapy, it is helpful to use at-home techniques to slow the buildup of plaque and tartar. In doing so, owners may be able to extend the time between veterinary cleanings. Daily tooth brushing is considered to be the gold standard of at-home canine oral care (Gorrel and Rawlings, 1996; Gorrel and Bierer, 1999). However, this method is not always feasible as a long-term plan due to lack of dog cooperation and/or owner compliance. Daily dental chew consumption may provide a convenient alternative to slowing the progression of plaque and calculus buildup, which ultimately lead to PD. As novel dental chews enter the market, there is growing interest in evaluating their ability to prevent plaque and calculus buildup through mechanical action. If successful, dental chews may be a convenient tool for the prophylaxis of PD, while providing dogs an enjoyable treat.

The objective of this study was to determine the differences in gingivitis, plaque, and calculus scores and halitosis of adult dogs consuming novel dental chews compared to control dogs consuming only a dry, extruded diet. We hypothesized that gingivitis, plaque, and calculus scores and halitosis would be lower in adult dogs consuming novel dental chews compared to controls eating a kibble diet only, and as low as dogs consuming a leading brand chew.

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CHAPTER 2

LITERATURE REVIEW

INCIDENCE OF PERIODONTAL DISEASE

Periodontal disease (PD), plaque-induced disease of the periodontium, affects 44% to 63.6% of dogs over the age of 3 years (Davis et al., 2013). The incidence of PD is higher in small breed dogs in comparison to large breed dogs and also increases with advancing age across breed sizes (Harvey, 1994; Kylar and Witter, 2005). The increased prevalence of PD in older dogs is not necessarily because it is an age-specific disease, but because older animals have had more time to accumulate plaque and calculus (Logan et al., 2010). If appropriate oral care is provided, older animals can maintain good periodontal health. As the average life expectancy of dogs continues to rise, it is increasingly important to maintain the oral health of dogs to prevent discomfort and ensure a good quality of life throughout their entire lifetimes. In addition to age and breed, many factors can contribute to a dog's PD susceptibility, including nutritional status, immunologic capability, differences in salivary components, and concurrent infections (Harvey, 2005).

Periodontal disease is a chronic disease characterized by inflammation of non-gingival tissues. Its main contributing factor, plaque, is a constant threat and therefore must be frequently removed in order to stave off the effects of advancing disease. Disease progression begins when oral bacteria form plaque that adheres to the teeth. Plaque is a sticky biofilm comprised of oral bacteria, salivary glycoproteins, polysaccharides, and epithelial and inflammatory cells. Plaque is clear to pale yellow in color and can be brushed or scraped away; however, if not removed it will interact with salivary and crevicular calcium and phosphate salts to harden into calculus (Logan, 2006). Calculus is a solid, mineralized substance comprised mostly of calcium carbonate (Logan et al., 2010). Calculus is yellow to brown in color and is best removed by ultrasonic tools used by veterinarians. Once formed, calculus is again covered with plaque and buildup continues. Supragingival calculus formation often can indicate more serious subgingival problems. As plaque is allowed to accumulate below the gingival margin, bacterial enzymes and toxins cause gingival inflammation and elicit an immune response from the host. Host white blood cells (WBC) and inflammatory chemical signals move into the space between the gum and the tooth where WBC work to destroy bacterial invaders, but can become overwhelmed causing a release of chemicals that also cause damage to tissue around the tooth. Continual plaque buildup and the host's subsequent immune response advances tissue damage. Characterized by mild edema and bleeding upon probing, gingivitis is the reversible first stage of oral disease and can serve as a visual indication of declining oral health. Although gingivitis does not always result in PD, it does establish a basis for irreversible PD development.

There are many potential consequences of PD. Pain and discomfort often arise, namely from associated edema, bleeding, loose teeth, and bone loss. This can lead to changes in behavior, such as an unwillingness to eat and consequent weight loss, as well as signs of aggression. Monetary cost to the owner from veterinary visits and tooth removal procedures may also result. These consequences have the potential to strain owner-pet relationships and cause preventable stress to both dog and owner. Although signs of PD may be noticed by the owner, obvious signs of discomfort are not always evident, as much of PD occurs below the gingival margin, making regular veterinary examinations crucial in confirming good oral health.

ORAL ANATOMY AND PERIODONTAL DISEASE

The term periodontium refers to all periodontal tissues involved in holding teeth in the mouth. They include the alveolar bone, periodontal ligament, gingiva, and supporting connective tissue and blood vessels (Figure 2.1). While gingivitis is limited to the gingiva, advanced oral disease affects the non-gingival structures and is subsequently classified as PD.

Teeth

Adult dogs have 42 permanent teeth, including 3 incisors, 1 canine, 4 premolars, and 2 molars on each side of the maxilla and 3 incisors, 1 canine, 4 premolars, and 3 molars on each side of the mandible (Gioso and Carvalho, 2005). Small breed dogs have been found to have proportionally larger mandibular first molars relative to mandibular height when compared to measurements of larger dogs. This contributes to an increased susceptibility for periodontitis (Gioso et al., 2001). Periodontal disease and its soft tissue destruction may cause the teeth to become loose or completely detached. Missing and loose teeth can cause additional pain as well as difficulty eating.

Periodontal Ligament

The periodontal ligament serves to hold the teeth in the jaw, firmly attaching each one to the alveolar process. The ligament provides shock absorption to prevent tooth damage by spreading the mechanical force of mastication across the root of the tooth. This mechanical force, in turn, helps to maintain the integrity of the alveolar bone and the periodontal collagen of the ligament (Harvey, 2005). Inflammation and infection can cause damage to the periodontal ligament, reducing its function of shock absorption and potentiating bone damage and tooth loss.

Alveolar Process

The alveolar process, also known as the alveolar bone, is the portion of the jawbone that contains the teeth and the alveoli from which they are suspended. It is composed of the cribriform plate, trabecular bone, and the cortical plate. The structure of the alveolar bone is tooth-dependent, is formed via the eruption of the teeth, and is reabsorbed following tooth extraction (Gioso and Carvalho, 2005). There are multiple tunnels called Volkmann canals that run through the alveolar process and are connected to the periodontal ligaments. Blood vessels, lymphatics, and nerves pass into these Volkmann canals (Verstraete, 1999). Chronic infection causes bone remodeling, sometimes causing alveolar bone expansion or thickening as a sign of PD. Damage to the alveolar process can be a sign of more advanced PD (D'Astous, 2015).

PATHOLOGY OF PERIODONTAL DISEASE

Without plaque control, oral bacterial colonization increases. Oral microbial populations can shift in relation to periodontal health status. The most prevalent oral bacterial species have been identified in dogs with normal health and gingivitis and periodontitis disease states. Routine bacterial culture has shown that in dogs without evidence of oral disease, uncultured bacterium (12.5%) were most prevalent, while *Bacteroides heparinolyticus/Pasteurella dagmatis* (10.0%) and *Actinomyces canis* (19.4%), were most prevalent in dogs suffering from gingivitis and periodontal disease, respectively. Using 16S rRNA gene sequencing, the most prevalent bacterial species in healthy dogs, dogs with gingivitis, and dogs with periodontitis were reported to be *Pseudomonas spp.* (30.9%), *Porphyromonas cangingivalis* (16.1%), and *Desulfomicrobium orale* (12.0%), respectively (Riggio et al., 2011). In another study of 223 canine plaque samples, *Porphyromonas* was the most prevalent genus in all oral health stages, particularly in dogs without oral disease. *Moraxella* and *Bergeyella* were also common. *Peptostreptococcus*, *Actinomyces*, and *Peptostreptococcaceae* were the most abundant genera measured in the plaque of dogs with mild periodontitis (Davis et al., 2013).

In addition to local damage, studies have reported that oral bacteria can have detrimental effects on other areas of the body, including the heart, liver, and kidneys. This is sometimes referred to as "periodontal disease burden", which increases with the severity of PD (Pavlica et al., 2008). These associations result from bacterial migration via lymphatic and blood vessels. Blood-borne bacteria may colonize sites far from the oral cavity; impaired immune and organ function have been documented. Associations have been identified between PD and cardiac conditions, which may impact atherosclerotic processes, including elevated concentrations of inflammatory mediator serum amyloid (p=0.02) and antichymotrypsin (p=0.04) when compared to dogs with only one or neither condition, and an increase of C-reactive protein in subjects with both diseases (p=0.04; Gulrich et al., 2002). A historical cohort observational study using 59,296 dogs with a history of PD identified significant associations between PD severity and risk of endocarditis and cardiomyopathy (Glickman et al., 2009). Liver and kidney changes, such as histopathological lesions, also have been documented in PD patients (Pavlica et al., 2008). Those researchers reported that for each square centimeter of PD burden, there was a 1.4 times higher likelihood of changes to the left atrioventricular valves [odds ratio (OR) point estimate = 1.43], as well as 1.2 and 1.4 times higher likelihood for elevated liver and kidney pathology (OR = 1.21; OR = 1.42), respectively.

DIAGNOSIS AND TREATMENT OF PERIODONTAL DISEASE

Periodontal disease has a wide range of clinical signs and can vary in severity from tooth to tooth in the same dog. Dogs also may experience periods of remission and recurrence. General classification of the PD stages are illustrated in **Table 2.1.**

Prominent signs of PD include accumulation of dental substrates on tooth surfaces, gingival redness, swelling and bleeding of the gingival margin, gingival recession and periodontal pocket formation, accumulation of purulent material in the periodontal pocket, and tissue destruction with loss of attachment, furcation exposure, and tooth mobility (Logan et al., 2010). **Figure 2.2** illustrates the progression of the visible signs of PD. “Periodontal pockets” refer to the free space around the tooth as the gingiva detach due to inflammation. These pockets deepen as the disease progresses and often are measured (mm) by veterinarians using a periodontal probe during assessment.

Halitosis (oral malodor or bad breath) often is associated with PD and is caused by the colonization of oral microbiota and their metabolism of proteinaceous substrates in the mouth. Volatile sulfur compounds (VSCs) generated by these bacteria cause malodor and are indicative of PD pathogenesis (Culham and Rawlings, 1998). By reducing the buildup of microbiota and food particles remaining in the mouth, halitosis may be reduced, reflecting a decreased risk of PD progression. The reduction of VSCs may not only reflect a reduction in PD but also in the disruption of human-animal relationships due to oral malodor. Halitosis may be documented subjectively, but may also be assessed objectively through the use of a halimeter, an instrument for measuring VSCs in the mouth. A small, flexible tube extends from the side of the halimeter and can be placed in the mouth of the subject being evaluated. Measurements often take less than one minute and values are recorded in parts per billion (ppb). By using the criteria listed above, veterinarians can identify the development of disease and assign disease stages to individual teeth or classify the mouth as a whole.

METHODS OF PERIODONTAL DISEASE PREVENTION

Optimal oral health is achieved through a combination of professional therapy and consistent homecare. In addition to annual veterinary examination, scaling, and polishing, several homecare methods of plaque and calculus reduction exist. Efficacy of these products vary among individuals and rests heavily on overall contact with the teeth.

Tooth Brushing

Tooth brushing has long been considered the gold standard of oral care for dogs as it is in humans. However, it is recognized that many owners do not wish to brush their dog's teeth every day or simply do not have the ability due to the dog's disposition. For this reason, studies have been conducted to determine the effects of varying frequencies of tooth brushing on oral health outcomes. Significant differences have been shown between mean plaque scores for dogs whose teeth were brushed daily or every other day when compared to a control group receiving no brushing ($p<0.01$ for both groups). Dogs whose teeth were brushed daily and dogs whose teeth were brushed every other day had 37% and 25% lower mean plaque scores than the control group, respectively. In comparison, mean plaque scores for dogs brushed weekly was only 10% lower and dogs brushed every other week were 2% higher, but both of these were not significantly different than the control. Additionally, calculus scores were reduced ($p<0.01$) when teeth were brushed daily (80%), every other day (62%), or weekly (23%).

A linear relationship (R^2=0.99, p<0.01) was observed between frequency and effectiveness of tooth brushing for dogs (Harvey et al., 2015). When compared to dogs consuming a daily dental chew and dogs consuming a prescription dental diet, daily tooth brushing with veterinary toothpaste was reported to be more than three times as effective at controlling plaque accumulation (Allan et al., 2018). Tooth brushing may reduce oral bacteria as a result of plaque removal. In 2016, Watanabe et al. compared oral bacterial proliferation in 12 beagles over time. Results showed a reduction in the number of oral bacteria in dogs whose teeth were brushed daily for 8 weeks relative to baseline values and those that did not (control) and baseline values (p<0.05). Gorrel and Rawlings (1996) reported that the daily addition of a dental chew to an every other day tooth brushing regimen reduced plaque, calculus, and gingivitis, observing that daily dental chews were a beneficial addition to oral homecare when daily brushing was not feasible. This study and others like it created a new category of pet products targeting oral care.

Oral Health Kibble Diets

Oral health kibble diets often utilize several tactics to ward off gingivitis and PD. Perhaps the most important aim is to promote increased contact with the teeth and provide a scrubbing effect, reducing plaque accumulation. Often featuring large kibble size and a strong structure, dental diets are designed to withstand more pressure from teeth before crumbling. Teeth pierce the kibble causing large, abrasive pieces to break around the tooth, resulting in a greater scrubbing effect on the teeth and keratinization of the gums as the dog chews.

Dental health diets often contain functional ingredients that may influence PD susceptibility of dogs. While many of these ingredients are added to diets, they also may be used in treats and chews, achieving similar goals. Fibrous ingredients can provide an abrasive quality to the food, thus facilitating the removal of plaque. Antioxidants like vitamins E and C and selenium prevent oxidative stress and may work to reduce inflammation in many areas of the body, including the oral cavity and periodontal tissues. Supplementation of these and other antioxidants may therefore reduce gingivitis and bleeding of the gums. Some dental products include calcium chelators to bind salivary calcium so it is less likely to mineralize into dental calculus. Sodium tripolyphosphate and sodium hexametaphosphate are commonly used for this purpose (Logan, 2006).

The effects of kibble size and calcium chelate types on calculus accumulation in beagles was evaluated by Hennet et al. (2007). In that study, four treatments were tested in dogs 12 to 24 months of age for four weeks using a completely randomized experimental design. The four treatments were: a diet having 10-mm diameter kibbles (F10); the same formula having 15-mm diameter kibbles (F15); the 15-mm diet coated with 0.6 % sodium hexametaphosphate (H15); and the 15-mm diet coated with 0.7 % sodium tripolyphosphate (T15). Results showed that calculus was reduced ($p<0.05$) in group F15 dogs when compared to F10 dogs. Additionally, calculus scores were reduced ($p<0.05$) in H15 dogs (36.0%) and T15 dogs (55.0%) compared to F15 (Hennet et al, 2007).

Chew Toys, Rawhides, Cartilaginous Products, and Other Chewing Devices

Many types of inedible toys and edible chews are available for oral healthcare and often are considered to be convenient methods for the owner, while being enjoyable and low stress for the dog. Examples of these products include plastic, rope, and rubber toys, rawhide in various shapes, bully sticks, hooves, bovine and porcine bones, and antlers.

Harvey et al. (1996) conducted a study in 1,350 client-owned dogs to investigate differences between feeding regimens consisting of dry foods and those including other food types. Differences were identified between dogs fed dry food only and those fed other than dry food only. There was progressively less ($p<0.05$) calculus accumulation, gingival inflammation, and periodontal bone loss observed in dogs given access to more types of chewing devices (rawhides, bones, biscuits, chew toys) compared with dogs given access to fewer or no chewing devices. When the effects of individual chewing devices were analyzed, access to rawhides had the greatest preventative effect on calculus and this response was more pronounced in dogs fed dry food only compared with those fed a mix of food types (Harvey et al., 1996).

A study conducted in 16 adult beagles (age 6-11 years) by Stookey (2009) reported that daily feeding of a soft rawhide chew for 4 weeks resulted in reductions in dental calculus (28.0%; $p=0.0003$), dental plaque (19.0%; $p=0.0048$), and gingivitis (46.0%; $p=0.0001$) when compared to a diet-only control. Another study in 22 beagles (age 13 to 22 months) tested an enzymatically treated rawhide chew fed twice daily vs. diet-only control for 7 days. In that study, rawhides were shown to reduce ($p<0.05$) plaque accumulation by 19% (Hennet, 2001).

Although certain chewing devices may be effective in reducing oral disease parameters, care must be taken. Toys and chews that are too hard may crack teeth, break teeth, and/or cause soft tissue damage upon chewing. There also is potential for ingestion of pieces too large to pass through the gastrointestinal tract. When edible chew toys are used, swallowing large pieces should be avoided and the digestibility of the product should be confirmed to be appropriate for ingestion. Softer products may be safer in this regard; however, the hardness of chews often provide them with oral health function.

Rawhide chews are among the most popular chew types. *In vitro* disappearance characteristics of selected rawhide dog treats has been found to vary widely. de Godoy et al. (2014) measured gastric dry matter disappearance (DMD) values of 14.2% to 73.1%. The use of slowly digestible treats like rawhides should, therefore, be discouraged for dogs prone to consuming large pieces of food without much mastication, as it could pose a risk for choking and gastric blockage. Such consumption tendencies may negate any potential abrasive, plaque-reducing effects.

Hooda et al. (2012) investigated the *in vitro* DM digestibility of expanded pork skin chews and rawhide chews, as well as apparent total tract digestibility (ATTD) and gastrointestinal transit time in healthy adult beagles (age 5-5.5 years) fed a weight-control commercial diet. *In vitro* analysis showed that the gastric digestibility of expanded pork skin chews was higher than that of rawhide chews. *In vitro* pork skin gastric digestibility increased over time, being 54.7%, 58.6%, 76.4%, and 86.4% digestible after 6, 12, 18, and 24 hours, respectively. Rawhide chew gastric digestibility was 7.6% at 6 hours and slowly increased, reaching a maximum of 41.6% after 18 hours but increased to 85% after 24 hour gastric + 18 hour small intestinal digestion. *In vivo* ATTD of dry matter (DM), organic matter (OM), and nitrogen (N) were shown to be greater ($p<0.05$) for dogs fed expanded pork skin chews along with the basal diet compared with the basal diet alone. Chew intake did not change transit time measured with a wireless motility device; however, motility index and contraction pattern of the colon were altered ($p<0.05$) when chews were fed relative to the control. In conclusion, expanded pork skin had a greater DM digestibility than the rawhide chews.

Dental Chew Treats

Dental chew treats present an additional method of plaque buildup prevention for dogs, and often are considered to be a convenient method for oral health support. Dental chews are unique in that they, unlike rawhides and other cartilaginous products, are formulated to have specific properties. Ingredients may be included for their structural or nutritional properties, or both. The ability to adjust hardness and digestibility lend them additional appeal over some of the previously mentioned chewing devices. Often having a compressed semi-moist consistency, dental chew treats should be hard enough to pose chewing resistance and to provide a scrubbing effect while remaining digestible. Dental chew treats are variable in formulation and shape. Some common formats are star shaped sticks, X-shape, bone shape, and those resembling toothbrushes. Research has shown evidence of dental chew treats improving several measures of oral health status in dogs.

One study reviewed the effects of a vegetable dental chew on PD parameters, including gingivitis, halitosis, plaque, and calculus in 16 toy breed dogs (Clarke et al., 2011). A 70-day two-period crossover design was used to test client-owned dogs. Eighteen teeth were evaluated, following two treatments: dry diet only or the same dry diet plus a vegetable dental chew given 4-8 hours after the meal daily. Following an acclimation period of 14 days, teeth were scaled at day 0 and then gingivitis and halitosis were evaluated. On days 28 and 56, gingivitis, plaque, calculus, and halitosis were measured. Results showed an 11.25% reduction in mean gingival scores in dogs receiving the vegetable chew ($p=0.01$). Halitosis scores were numerically reduced by 6.6%, but this change was not statistically significant ($p=0.41$). Plaque and calculus score analysis was only conducted on the first period due to evidence of a sequence effect. Mean plaque scores were 37% lower when dogs received the dental chew (p=0.001) and mean calculus scores were 70.2% lower (p=0.0005) (Clarke et al., 2011).

Another study investigated the efficacy of a novel dental hygiene chew with or without an undisclosed, proprietary natural antimicrobial agent additive (Brown et al., 2005). Gingivitis, plaque, and calculus were evaluated in 12 dogs used in a replicated 3x3 Latin square design. Dogs were fed for 4 weeks and received either diet only (control) or a single daily dental chew with or without the microbial agent. Dogs receiving a daily chew had less gingivitis (p=0.02), plaque (p=0.0004), and calculus (p=0.0001) compared with dogs in the control group. The inclusion of the antimicrobial agent did not improve dental chew efficacy (Brown et al., 2005).

Overall, studies that have evaluated the efficacy of various dental chews have shown varying, but largely positive results. Differences in treat formulation and physical shape largely contribute to these differences. The Veterinary Oral Health Council (VOHC) has established guidelines for dental product testing that, when successful, results in an approved VOHC seal to be displayed on product packaging for plaque and/or tartar (calculus) reduction. The VOHC seal is not required for oral health products, but is an indication to customers that a product has met certain efficacy standards for its intended use in plaque and/or calculus reduction. VOHC protocol requirements include, but are not limited to, good general health of animals and presence of all teeth to be scored (maxillary I3, C, P3, P4, Ml and mandibular C, P3, P4, Ml), use of a clean mouth model, scoring and cleaning procedures performed under sedation, a minimum trial period of 28 days, and a total of two studies using independent sets of dogs. A minimum reduction of 15% is required in the test group for all PD parameters or a minimum 20% average reduction between studies. While the current study did not seek to obtain VOHC approval, it followed similar standards and provided clear support for PD parameter reduction.

Many studies have investigated the effects of various dental chew types on PD parameters including gingivitis, plaque, and calculus scores, and halitosis. The objective of this thesis was to determine the differences in gingivitis, plaque, and calculus scores and halitosis of adult dogs consuming novel dental chews compared to control dogs consuming only a dry, extruded diet. We hypothesized that gingivitis, plaque, and calculus scores and halitosis would be lower in adult dogs consuming novel dental chews compared to controls eating a kibble diet only, and as low as dogs consuming a leading brand chew.

TABLE AND FIGURES

Table 2.1. Stages of Periodontal Disease*	
Stage 0	**Clinically normal** No gingival inflammation or periodontitis is clinically evident.
Stage 1	**Gingivitis only** Gingivitis only without attachment loss; the height and architecture of the alveolar margin are normal
Stage 2	**Early periodontitis** Less than 25% attachment loss or Stage 1 furcation involvement in multirooted teeth.
Stage 3	**Moderate periodontitis** 25 to 50% attachment loss or Stage 2 furcation involvement in multirooted teeth.
Stage 4	**Advanced periodontitis** Greater than 50% attachment loss or Stage 3 furcation involvement in multirooted teeth.
*Adapted from Wolf and Rateitschak (2005).	

Figure 2.1. Healthy periodontal anatomy (Logan et al., 2010)

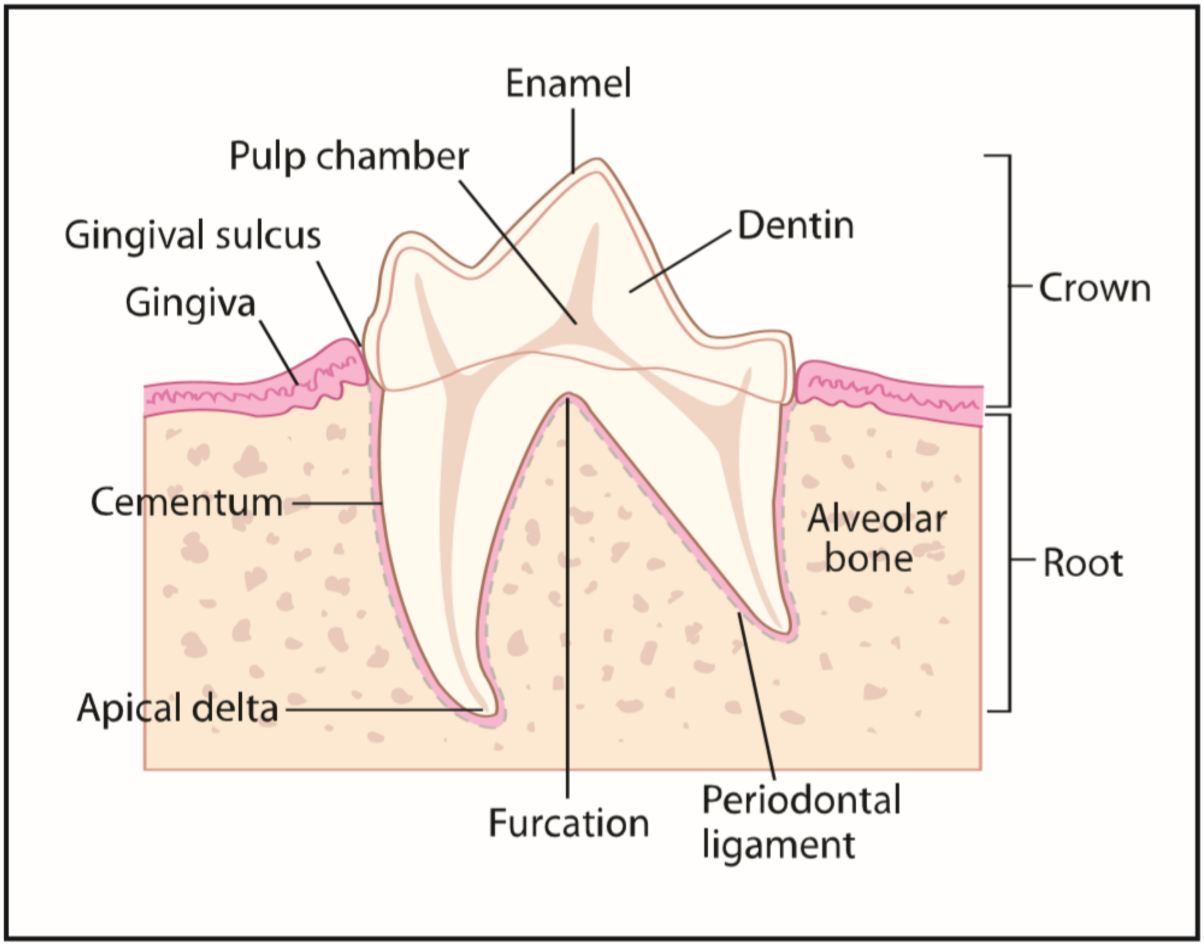
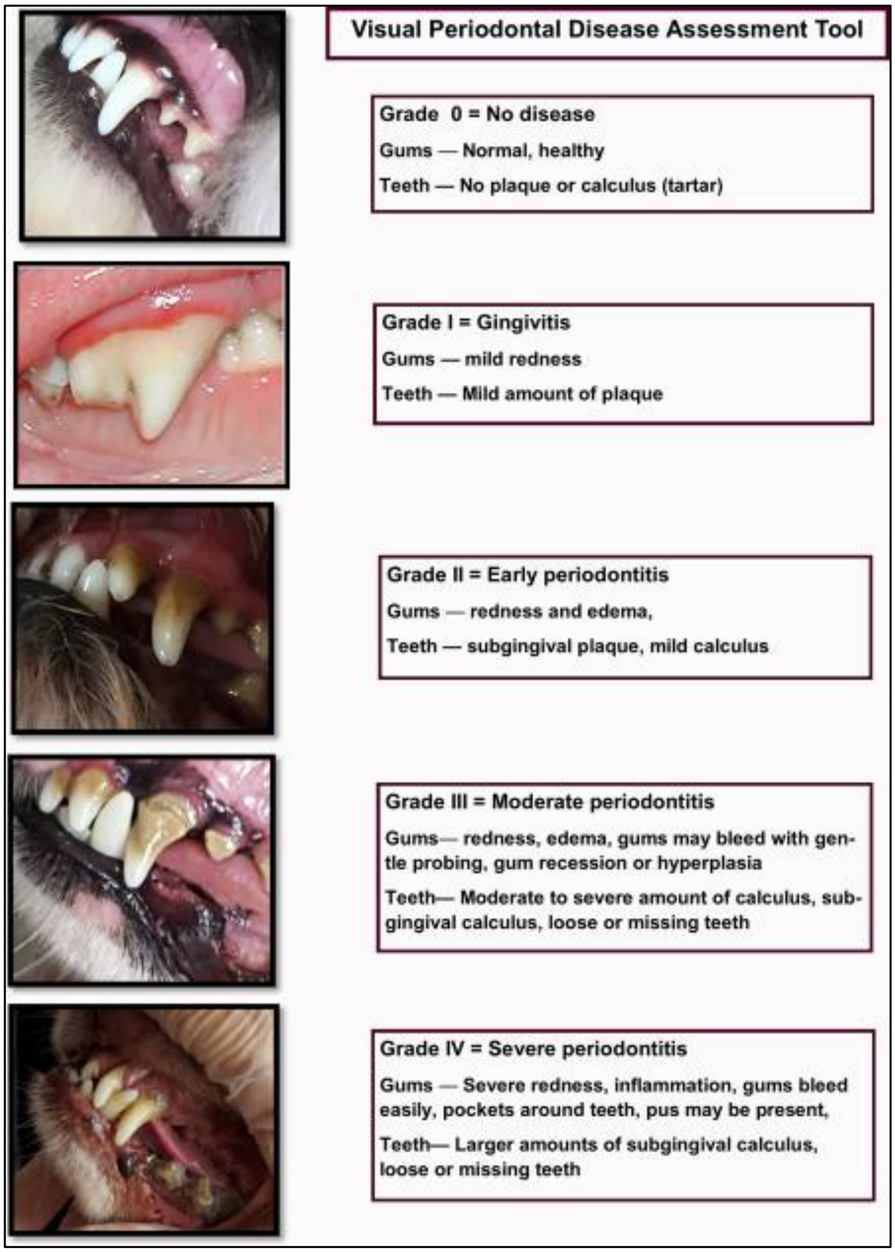


Figure 2.2 Visual signs of PD stages (Stella et al., 2018)



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CHAPTER 3

EFFECTS OF NOVEL DENTAL CHEWS ON ORAL HEALTH OUTCOMES AND HALITOSIS IN ADULT DOGS

ABSTRACT

Periodontal disease (PD) is the most common clinical condition occurring in adult dogs and cats, but is preventable. The objective of this study was to evaluate the benefits of daily dental chew administration on oral health outcomes in adult dogs. Twelve adult (mean age = 5.31 ± 1.08 years; mean BW = 13.12 ± 1.39 kg) female beagles were used in a replicated 4x4 Latin square design consisting of 28-day periods. All animal care and experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. On day 0 of each period, teeth were cleaned by a veterinary dentist blinded to treatments. Teeth then were scored for plaque, calculus, and gingivitis by the same veterinary dentist on day 28 of each period. Breath samples were measured for malodor (volatile sulfur compounds) on days 1, 7, 14, 21, and 27 of each period. All dogs consumed the same commercial dry diet throughout the study. Control dogs were offered the diet only, while treatment groups received the diet plus one of three dental chews. Two novel chews [Bones & Chews Dental Treats (Chewy, Inc., Dania Beach, FL); Dr. Lyon's Grain-Free Dental Treats (Dr. Lyon's, LLC, Dania Beach, FL)] and a leading brand chew [Greenies Dental Treats (Mars Petcare US, Franklin, TN)] were tested. Each day, one chew was provided four hours after mealtime. All tooth scoring data were analyzed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC). Halimeter data were analyzed using repeated measures using the Mixed Models procedure of SAS and testing for differences due to treatment, time, and treatment*time interaction. Data are reported as LS means ± SEM with statistical significance set

at $p<0.05$. Dr. Lyon's Dental Treats performed at the same level as the leading brand, Greenies, as both resulted in a reduction ($p<0.05$) in plaque coverage and thickness, calculus coverage, and day 27 volatile sulfur concentrations compared to controls. Additionally, Dr. Lyon's dental treats reduced volatile sulfur compounds on day 14 when compared to controls. Bones and Chews Dental Treats reduced ($p<0.05$) calculus coverage and day 27 volatile sulfur concentrations compared to controls. Our results suggest that the dental chews tested in this study may aid in reducing the risk of periodontal disease in dogs.

INTRODUCTION

Periodontal disease (PD) is the most common clinical condition in adult dogs, affecting 44% to 63.6% of dogs over the age of 3 years (Davis et al., 2013) and is considered to be the most undertreated animal health condition (Niemiec, 2008). PD is characterized by both gingivitis and periodontitis, the inflammation of the gingiva (gums) and the inflammation of the nongingival periodontal tissues (the periodontal ligament and the alveolar bone), respectively (Harvey, 2005). These conditions develop as a result of plaque and calculus buildup on the teeth, which harbor bacteria that can cause an immune response and inflict damage to oral tissues. This chronic, progressive disease is preventable and can be reversed if treated before permanent damage occurs (Niemiec, 2008). When allowed to progress, PD can result in tooth loss, bone loss, halitosis (oral malodor), and chronic pain, which often can lead to reduced food intake and behavioral problems. Periodontal disease incidence increases with dog's age and as the average life expectancy of dogs continues to rise, it is increasingly important to maintain the oral health of dogs to prevent discomfort and ensure a good quality of life throughout their entire lifetime.

Several methods for oral care are available to aid in the prevention of PD. Most importantly, it is recommended that dogs see a veterinary dentist once per year for teeth cleaning and polishing. This is done under anesthesia using ultrasonic tools, which remove plaque and calculus from all tooth surfaces. In addition to this periodic cleaning, it is helpful to use at-home techniques to slow the buildup of plaque and tartar. In doing so, owners may be able to extend the time between veterinary cleanings. Daily tooth brushing is considered to be the gold standard of at-home canine oral care (Gorrel and Rawlings, 1996; Gorrel and Bierer, 1999). However, this method is not always feasible as a long-term plan due to lack of dog cooperation and/or owner compliance. Daily dental chew consumption may provide a convenient alternative to slowing the progression of plaque and calculus buildup, which ultimately may lead to PD. As novel dental chews enter the market, there is growing interest in evaluating their efficacy in preventing plaque and calculus buildup through mechanical action. If successful, dental chews may be a convenient tool for the prevention of PD while providing dogs an enjoyable treat.

The objective of this study was to determine the differences in gingivitis, plaque, and calculus scores and halitosis of adult dogs consuming novel dental chews compared to control dogs consuming only a dry, extruded diet. We hypothesized that gingivitis, plaque, and calculus scores and halitosis will be lower in adult dogs consuming novel dental chews compared to controls eating a kibble diet only and as low as dogs consuming a leading brand chew.

MATERIALS AND METHODS

Animals, treatments, and experimental design

Twelve adult female beagles (mean age = 5.31 ± 1.08 years; mean BW = 13.12 ± 1.39 kg) were used in a replicated 4x4 Latin square design. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. Prior to the start of the study, all dogs underwent a physical examination and serum chemistry values were evaluated. A dental evaluation was performed by a veterinary dentist to confirm the presence and integrity of all teeth to be scored in order to confirm trial eligibility.

Dogs were housed individually in pens (1.0 m wide by 1.8 m long) in a humidity- and temperature-controlled animal facility. The experiment consisted of four 28-day periods. On day 0 of each period, dogs were transported to the University of Illinois Veterinary Teaching Hospital where their teeth were cleaned and polished by a veterinary dentist. The same blinded veterinary dentist then scored teeth on day 28 of each period. Day 28 of each period then served as day 0 for the subsequent period in order to maintain a clean mouth model. In order to maintain consistency in scoring and cleanings from the same veterinarian, dogs were randomly assigned to four groups of three and start days were staggered over a 4-day period. Three dogs were evaluated each day, and this schedule was maintained for the duration of the study. Breath samples were measured for malodor on days 1, 7, 14, 21, and 27 of each period.

Dogs had access to fresh water at all times and were fed once a day to maintain BW. All dogs were fed a commercial diet (American Journey Salmon & Sweet Potato Recipe, American Journey, LLC, Dania Beach, FL) throughout the study. Proximate analysis was performed on the diet to verify chemical composition (See Appendix C). No additional treats, chew toys, or other dental interventions were permitted for the duration of the study. No active anti-plaque or calculus substances were included in chew formulations. Dogs were allotted to one of four treatments in each experimental period (see Appendix D for dental treat nutritional information and Appendix E for treat images):

- Diet only (control) **(CT)**
- Diet + Bones & Chews Dental Treats **(BC)** (Chewy, Inc., Dania Beach, FL)
- Diet + Dr. Lyon's Grain-Free Dental Treats **(DL)** (Dr. Lyon's, LLC, Dania Beach, FL)
- Diet + Greenies **(GR)** (Mars, Inc., Franklin, TN)

Dogs were fed at 0800 each morning and were given one hour to consume their food. Leftover food was weighed each day to calculate intake. Four hours after eating their diet, dogs receiving a dental chew were given the chews. Due to fasting prior to anesthesia, dogs did not receive a chew on day 28 and were, therefore, fed their diet only upon return to their kennels. When given chews, all dogs were monitored to ensure consumption and to prevent swallowing of large pieces and/or choking. They were given one hour to consume their dental chew. Any remaining treats and treat pieces were collected and weighed. Dogs consuming less than 85% of their assigned chews (by weight) over the course of each experimental period were excluded due to lack of compliance. As a result, data from two Dr. Lyon's, one Bones & Chews, and one Greenies treatment were excluded (n= 10, 11, and 11, respectively). When allotted to dental chew treatments, food intake was adjusted to compensate for the energy provided by the chews.

All dogs were weighed once per week prior to feeding and the BW of all dogs remained constant throughout the duration of the study. On days 1, 7, 14, 21, and 27, breath samples were analyzed for volatile sulfur compounds using a halimeter (Interscan Corp, Simi Valley, CA). Halimeter measurements were conducted three hours after dental chew administration. Each dog was measured three times and a mean score was calculated.

On day 28 of each period, gingivitis, plaque, and calculus scoring were conducted according to Gorrel et al. (1999) (see Appendix B). The same veterinary dentist conducted all gingivitis, plaque, and calculus scoring and was blinded to all treatment regimens. For each measurement, the maxillary I3, C, P3, P4, and M1 and the mandibular C, P3, P4, and M1 were scored. This selection allowed for scoring of various types of teeth, including those used to nip and tear food (incisors and canines) as well as teeth used to shear and crush food (premolars and molars). Prior to tooth scoring, images were taken of each mouth so a visual representation could be used to compare against scores. Plaque scoring was completed using Trace Disclosing Solution (Young Dental, Earth City, MO).

Evaluation methods

To assess gingivitis, a periodontal probe was placed subgingivally on the buccal side of each tooth and values were assigned via visual assessment of inflammation and bleeding upon probing. The sum of scores was divided by total scores for each dog to obtain mean scores for each measure.

Plaque levels were evaluated by using Trace Disclosing Solution to cover the teeth followed by a gentle rinse of water to remove the excess. Plaque was hence revealed and subsequently scored for coverage and thickness on both the gingival and occlusal portions of the tooth (see Appendix B). Calculus scores were based on visual assessment of coverage and thickness on the mesial, buccal, and distal portions of the tooth. When all scoring was complete, supra- and sub-gingival scaling and supra-gingival polishing was done on all teeth with a fine grade paste to maintain a clean mouth model for subsequent treatment periods.

Although it is a subjective scoring system, the same veterinary dentist scored all dogs for all measures and always was blinded to treatments. In order to ensure adequate time for each assessment and, therefore, improve accuracy, dogs were randomly assigned to 4 groups of 3 and treatment start days were staggered so that scoring and cleaning procedures were done over a four-day period.

Halitosis measurement

On days 0, 7, 14, 21, and 27, three halitosis measurements were obtained for each dog using a clean plastic straw as an extension of the halimeter air drawing hose. A clean straw was used for each measurement. The tube was placed over the dog's tongue and approximately even with the maxillary fourth premolar. The mouth was held gently shut while ensuring that the straw was not bent by the teeth or blocked by the tongue of the dog. The highest reading of VSCs over a period of approximately 30 seconds was displayed by the halimeter and recorded. The machine was allowed to return to 0 (about 60-120 seconds) before the next measurement was taken. Score sums were averaged to determine mean halitosis scores for each dog.

Anesthesia for dental scoring

Dogs were premedicated with butorphanol (0.3 mg/kg). Twenty to 30 minutes after pre-medication, the fur over the cephalic vein was clipped, the site was aseptically prepared, and a 20 gauge intravenous (IV) catheter was placed in the cephalic vein for administration of anesthetic agents and IV fluids. Dogs were pre-oxygenated and anesthesia was induced with etomidate following either midazolam (0.3 mg kg^{-1}), lidocaine (2 mg kg^{-1}), or physiologic saline (1 mL) administered intravenously. Heart rate, invasive arterial blood pressure, respiratory rate, and intraocular pressure were recorded following butorphanol sedation, after co-induction administration, after etomidate administration, and following intubation. Dogs were orotracheally intubated and transferred to isoflurane to maintain anesthesia. IV fluids were run at 5 mL/kg/hr. throughout anesthesia and active heating with a forced air warmer was provided to maintain normothermia. Cardiovascular and respiratory function was monitored continuously using an anesthetic multiparameter monitor (electrocardiogram, oscillometric blood pressure, capnograph, pulse oximeter, and temperature). Supplementary anesthetic agents and cardiovascular support were administered as needed based on the decision of the attending anesthesiologist.

Proximate analysis

Diet and treat samples were dried at 55°C in a forced-air oven and then were ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen and then analyzed for dry matter (DM), organic matter (OM), and ash according to AOAC (2006; methods 934.01 and 942.05). Crude protein content of diets and treats was calculated from Leco (TruMac N, Leco Corporation, St. Joseph, MI) total nitrogen values according to AOAC (2006; method 992.15). Total lipid content as acid-hydrolyzed fat (AHF) was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Diet and treats were analyzed for gross energy (GE) as measured by bomb calorimetry (Model 6200, Parr Instruments Co., Moline, IL).

Statistical analysis

All tooth scoring data were analyzed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC). Halimeter data were analyzed using repeated measures using the Mixed Models procedure of SAS, testing for differences due to treatment, time, and treatment*time interaction. Data are reported as LS means ± SEM with statistical significance set at $p<0.05$.

RESULTS

Plaque

Plaque coverage was 12.26% lower for dogs consuming DL and 13.33% lower for dogs consuming GR compared to control dogs ($p=0.003$; $p=0.0002$, respectively). Plaque thickness also was reduced by 17.43% for dogs consuming DL and 15.53% for dogs consuming GR compared to control dogs ($p=0.0001$; $p=0.0002$), and 11.58% lower ($p=0.0471$) for dogs consuming DL compared to dogs consuming BC (**Figure 3.1**).

Calculus and gingivitis

Calculus coverage was 36.87% lower for dogs consuming DL, 31.64% lower in dogs consuming GR, and 20.39% lower ($p\leq0.0001$) for dogs consuming BC compared to control dogs. Calculus coverage was 20.70% lower for dogs consuming DL and 14.12% lower for dogs consuming GR compared to dogs consuming BC ($p=0.0009$; $p=0.02$**; Figure 3.2)**. Calculus thickness was not affected by treatment. Gingivitis scores were not different among treatment groups **(Figure 3.3)**.

Halitosis

A significant (p<0.0001) treatment*time interaction was observed for breath malodor in the form of volatile sulfur compounds as measured in ppb by a halimeter (**Figure 3.4)**. At day 14, breath volatile sulfur compounds were lower (p=0.02) for dogs consuming DL compared to control dogs. At day 27, breath volatile sulfur compounds were lower (p<0.0001) for dogs consuming BC, DL, or GR compared to control dogs.

DISCUSSION

Periodontal disease is a common yet preventable disease. The onset of PD may not only lead to animal discomfort but also to a disturbance in owner-pet relationships via oral malodor, poor appearance of teeth, changes in dog behavior, and veterinary costs. Tooth brushing is considered to be the gold standard of oral care for dogs. However, this often is not a feasible option due to lack of dog cooperation and/or owner compliance. Dental chews are a promising method of PD prevention in dogs due to their convenience for the owner and acceptance by dogs.

The Veterinary Oral Health Council (VOHC) has established guidelines for dental product testing that, when successful, results in an approved VOHC seal to be displayed on product packaging for plaque and/or tartar (calculus) reduction. The VOHC seal is not required for oral health products, however, but is an indication to customers that a product has met certain efficacy standards for its intended use. While the current study did not seek to obtain VOHC approval, it follows similar standards and provides clear support for PD parameter reduction. Many studies have investigated the effects of various dental chew types on PD parameters including gingivitis, plaque, and calculus scores, and halitosis.

One study used similar assessments to determine the effects of dental chews akin to those investigated in the current study. Quest (2013) conducted an independent study to determine the effects of daily administration of a commercial edible dental chew using 60 beagles (age 2-8 years). Thirty dogs received a daily dental chew and diet ration, while 30 control dogs received only the diet for a 28-day period. Plaque was reduced (p=0.0005) in test group dogs while dogs in the control group experienced increases in plaque scores (p=0.0004). Day 28 mean mouth plaque scores for dogs in the test group were 32% less than the mean mouth plaque scores of control dogs. Dogs in the test group also had lower day 28 calculus scores when compared to control dogs (p<0.0001). Mean calculus scores for dogs in the test group were 60% lower than the mean mouth calculus score for dogs in the control group. Gingivitis was reduced (p<0.0001) in the test group while dogs in the control group maintained stable values (p=0.54). Day 28 mean gingivitis scores for the test group were 80% lower than mean scores for control dogs. Halitosis also was reduced in test dogs (p<0.0001), while control dogs maintained stable values (p=0.09). Day 28 mean halitosis scores were 45% lower than mean scores for control dogs. Using a 70 day crossover model with 16 toy breed dogs and daily vegetable dental chew administration, Clarke et al. (2011) showed similar reductions. Mean scores were calculated by multiplying coverage and thickness scores for each tooth and data showed a reduction in gingivitis by 11.2% (p=0.01), plaque by 37% (p=0.01), and calculus by 70% (p=0.0005). However, halitosis was not found to be significantly reduced.

Given the subjective nature of dental scoring and varying treat types, results of dental chew administration have varied. However, numerous studies have shown improvements in multiple PD parameters. In the current study, three commercially available dental chews were compared. Similar to previous studies, parameters often associated with PD were evaluated to assess the efficacy of the dental chews. The scoring system used was adapted from Gorrel et al. (1999), which has been commonly implemented to assess the development of PD in dogs. Calculus thickness was not affected by treatment; this may be due to treatment period length not being long enough to allow for significant buildup. Given that plaque scores varied among treatments, a longer treatment period may result in quantifiable differences in calculus thickness as accumulating plaque mineralizes. A similar conclusion can be drawn in regard to gingivitis results in this study. Gingivitis scores were not different among treatment groups, which may be due to insufficient time to impact inflammatory processes surrounding gum tissue.

Formulation of dental chews affects their hardness and abrasiveness. The dental chews evaluated in this study varied widely in ingredient types (see Appendix D). GR contains wheat gluten, which lends to the semi-moist consistency that provides chewing resistance and, therefore, increased contact with the teeth. In DL, this function is likely provided by pea protein and gelatin, while BC contains pork gelatin. Fiber sources such as oat fiber in GR and powdered cellulose in BC may also support a scrubbing effect during mastication of the treat.

CONCLUSIONS

Novel chews differ in key features like formulation, shape, and hardness and, therefore, effectiveness. However, it has been established that daily consumption of dental chews by dogs can help reduce plaque and calculus accumulation as well as gingivitis and halitosis. The dental treats evaluated in the current study exhibited the ability to reduce several parameters indicated in PD onset and progression. In summary, plaque, calculus, and halitosis measurements were reduced, but varied among treat types. DL performed similarly to GR, as both treats resulted in a reduction in plaque coverage and thickness, calculus coverage, and breath volatile sulfur concentrations compared to controls. BC dental treats reduced calculus coverage when compared to control as well as breath volatile sulfur concentrations at day 27. Longer treatment periods would likely have allowed more insight into long-term differences in calculus thickness and gingivitis development among treatments. Overall, daily administration of DL, GR, and BC dental treats may be helpful in mitigation of PD in dogs.

FIGURES

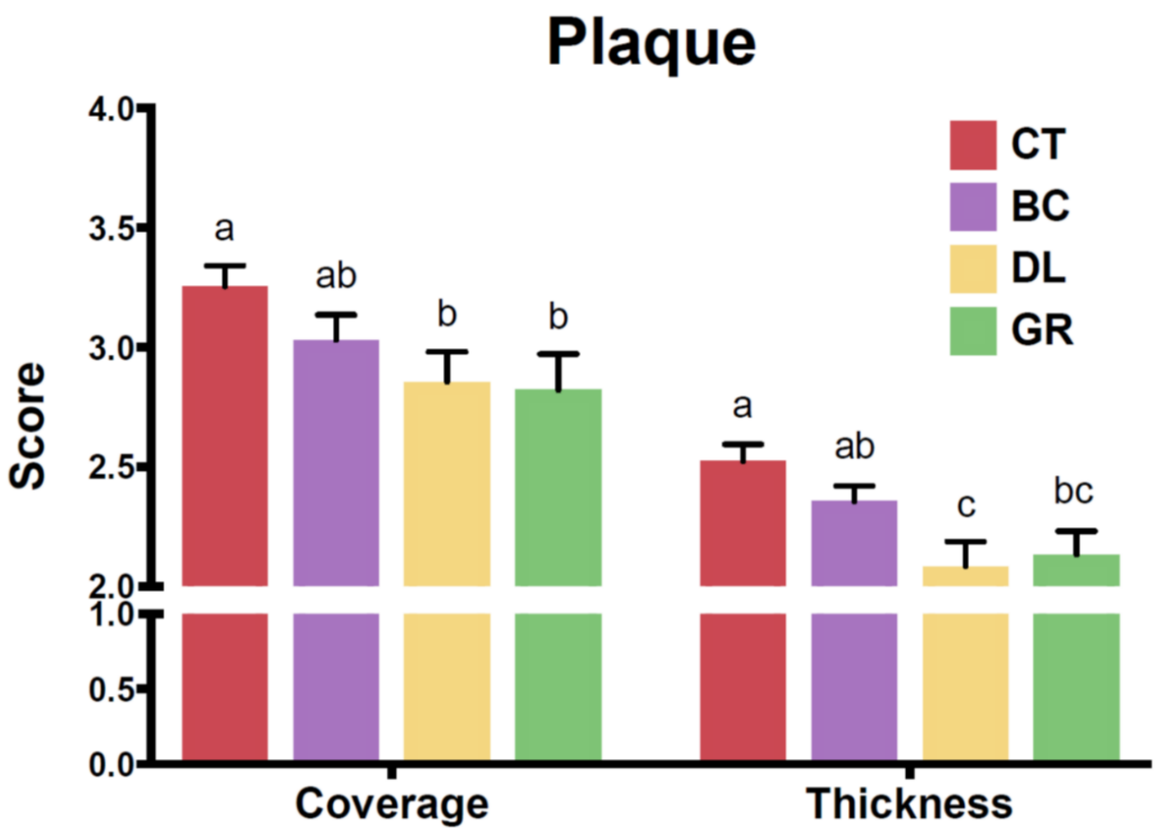

Figure 3.1. Plaque coverage and thickness for dogs consuming dental chews or diet alone. Values represent LS means ± SEM. Control = CT, Bones & Chews = BC, Dr. Lyon's = DL, and Greenies = GR. [a,b,c]Mean values with unlike letters were different ($p<0.05$).

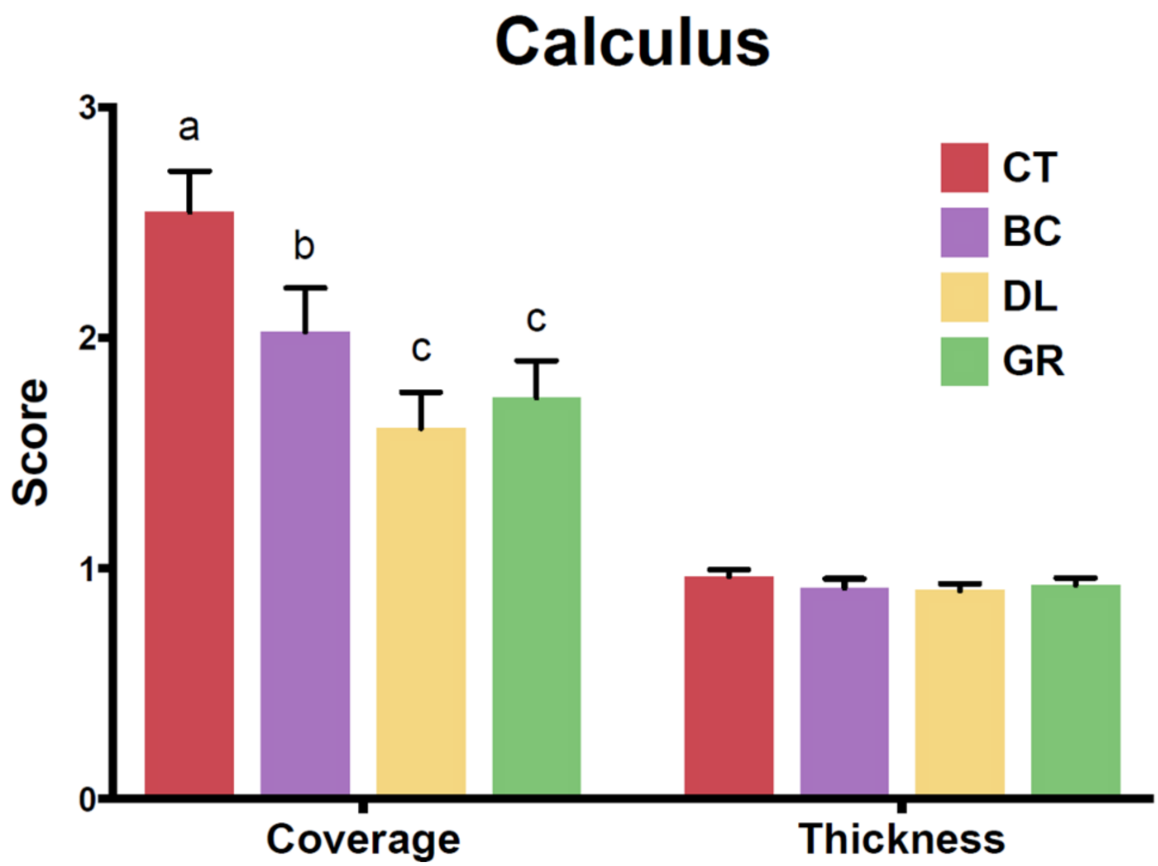

Figure 3.2. Calculus coverage and thickness for dogs consuming dental chews or diet alone. Values represent LS means ± SEM. Control = CT, Bones & Chews = BC, Dr. Lyon's = DL, and Greenies = GR. [a,b,c]Mean values with unlike letters were different ($p<0.05$).

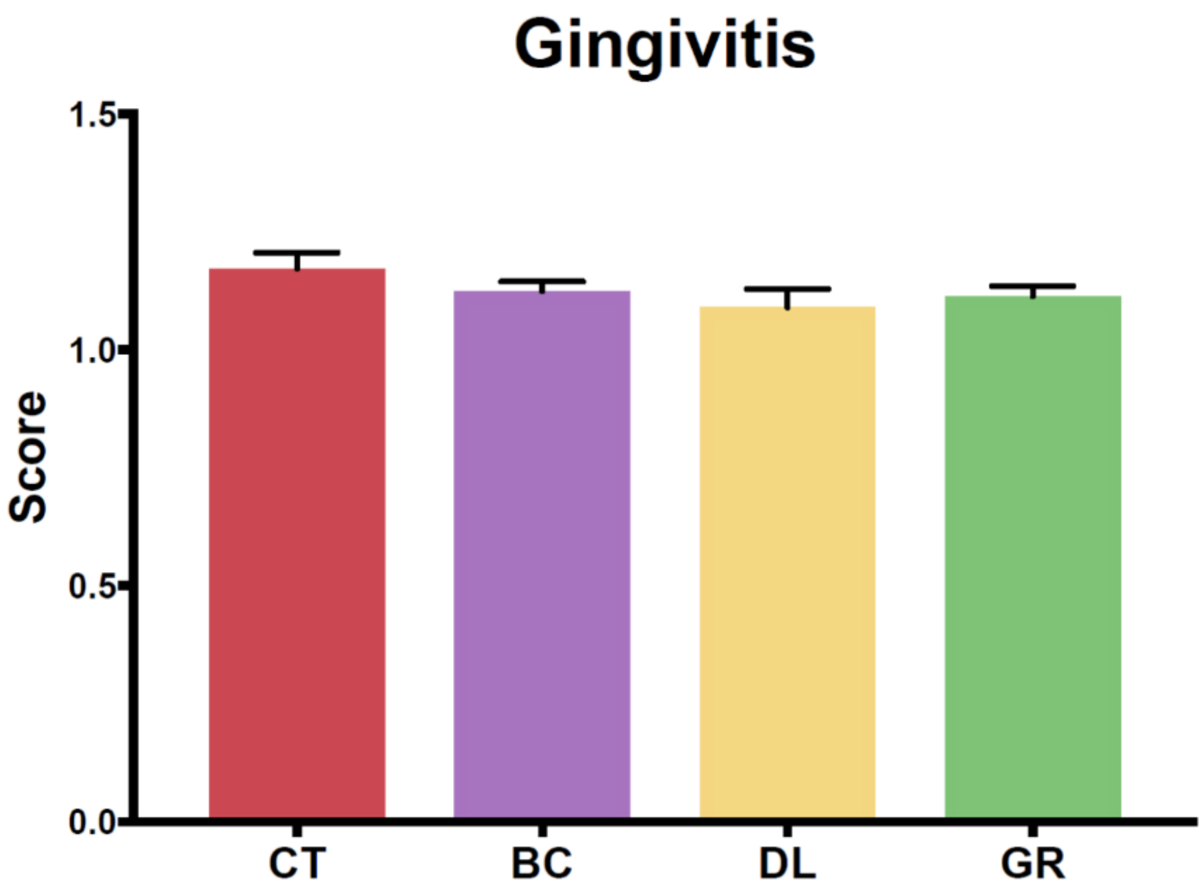

Figure 3.3. Gingivitis scores for dogs consuming dental chews or diet alone. Values represent LS means ± SEM. Control = CT, Bones & Chews = BC, Dr. Lyon's = DL, and Greenies = GR. No differences ($p<0.05$) were observed among groups.

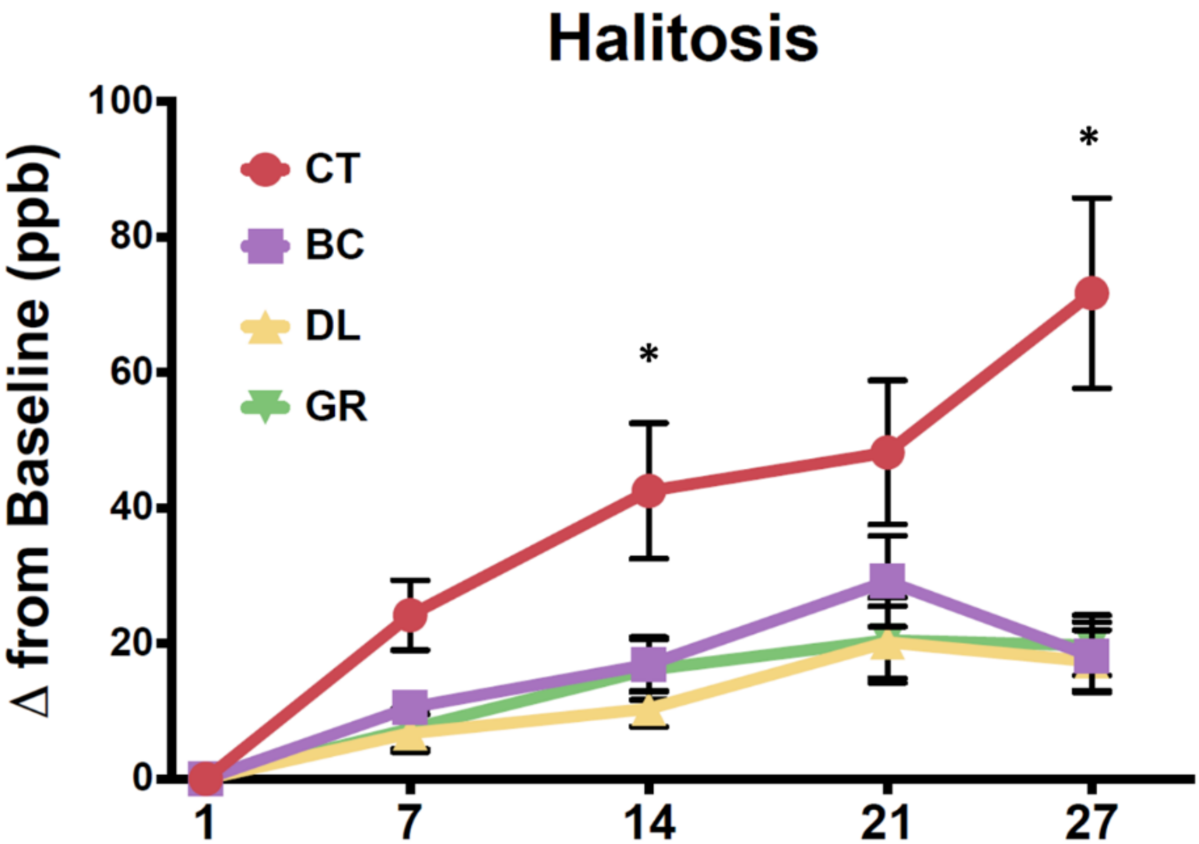

Figure 3.4. Oral malodor for dogs consuming dental chews or diet alone. Values represent LS means ± SEM changes from baseline. Control = CT, Bones & Chews = BC, Dr. Lyon's = DL, and Greenies = GR. *At day 14, DL was lower ($P<0.05$) than CT; at day 27, BC, DL, and GR were lower ($p<0.05$) than CT.

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APPENDIX A. EXAMPLE IMAGES OF TREATMENT RESULTS

Control, day 28

Greenies, day 28

Dr. Lyon's, day 28

Bones and Chews, day 28

Clean mouth

APPENDIX B. GINGIVITIS, PLAQUE, AND CALCULUS SCORING METHODS

Gingivitis Scoring
Teeth scored:
Maxilla: 13, C, P3, P4, Ml
Mandible: C, P3, P4, Ml

Criteria:

0 = no gingivitis
1 = 'incipient' or very mild gingivitis, (red, swollen but no bleeding on probing)
2 = mild gingivitis (red, swollen and delayed bleeding on probing)
3 = moderate gingivitis (red, swollen and immediate bleeding on probing)
4 = severe gingivitis (ulceration, spontaneous hemorrhage and profuse bleeding on probing)

The buccal gingiva for each scored tooth was divided into thirds (mesial, buccal, distal).
Each site was evaluated by the criteria above.

Calculation used
Each tooth was graded by the average of the three scores obtained per tooth. The score for each dog is the mean score for all teeth scored.

Plaque Scoring
Teeth scored:
Maxilla: 13, C, P3, P4, Ml
Mandible: C, P3, P4, Ml

Criteria:
Coverage

0 = no detectable plaque
1 = scattered plaque covering less than 24% of the buccal tooth surface
2 = plaque covering between 25 and 49% of the buccal tooth surface
3 = plaque covering between 50 and 74 % of the buccal tooth surface
4 = plaque covering more than 75% of the buccal tooth surface

Thickness
1 = Light
2 = Moderate
3 = Heavy

Scoring Method
Plaque was disclosed by applying a disclosing solution to the buccal surface of each tooth and immediately rinsing with water. The gingival and occlusal half of each tooth was scored for coverage and thickness.

Calculations
The gingival and occlusal values for each tooth were added together to obtain a tooth total score. The score for each dog is the mean score for all teeth scored.

Calculus Scoring
(Warrick-Gorrel method)
Teeth scored:
Maxilla: 13, C, P3, P4, Ml
Mandible: C, P3, P4, Ml

Criteria:
Coverage

0 = no detectable calculus
1 = scattered calculus covering less than 24% of the buccal tooth surface
2 = calculus covering between 25 and 49% of the buccal tooth surface
3 = calculus covering between 50 and 74% of the buccal tooth surface
4 = calculus covering more than 75% of the buccal tooth surface

Thickness
<0.5 mm = 1
0 .5-1.0 mm = 2
>1.0 mm = 3

Scoring Method
The disclosed plaque was removed by gentle tooth brushing and rinsing with a dental air-water syringe. The tooth was then air-dried. The buccal surface of the tooth was divided vertically into mesial, buccal and distal thirds, and each third assigned a numerical score for both coverage and thickness. A probe was used to verify the visual impression of cover and measure thickness.

Calculations
The tooth score is the sum of the scores for each of the three tooth surfaces. The sum of the teeth scores is averaged to obtain a whole mouth mean calculus score for each animal.

APPENDIX C. DIET NUTRITIONAL INFORMATION

Ingredients

Deboned salmon, chicken meal, turkey meal, peas, sweet potatoes, chickpeas, pea protein, chicken fat (preserved with mixed tocopherols), dried plain beet pulp, natural flavor, flaxseed, menhaden fish meal, blueberries, carrots, salt, salmon oil, dried kelp, fructooligosaccharides, choline chloride, vitamin E supplement, mixed tocopherols (preservative), ferrous sulfate, zinc proteinate, zinc sulfate, iron proteinate, Yucca schidigera extract, niacin supplement, copper sulfate, potassium chloride, sodium selenite, D-calcium pantothenate, copper proteinate, riboflavin supplement, vitamin A supplement, manganese sulfate, thiamine mononitrate, manganese proteinate, pyridoxine hydrochloride, vitamin B12 supplement, calcium iodate, vitamin D3 supplement, folic acid, dried Bacillus coagulans fermentation product, rosemary extract.

Analyzed chemical composition	**%**
Dry matter (DM)	93.7
	% DM Basis
Organic matter	90.6
Crude protein	36.3
Acid-hydrolyzed fat	17.1
Gross energy, kcal/g DM	5.1

APPENDIX D. DENTAL TREAT NUTRITIONAL INFORMATION

Greenies ingredients and analyzed chemical composition

Wheat flour, wheat gluten, glycerin, gelatin, oat fiber, water, lecithin, natural poultry flavor, minerals (dicalcium phosphate, potassium chloride, calcium carbonate, magnesium amino acid chelate, zinc amino acid chelate, iron amino acid chelate, copper amino acid chelate, manganese amino acid chelate, selenium, potassium iodide), dried apple pomace, choline chloride, fruit juice color, vitamins (dl-alpha tocopherol acetate [source of vitamin E], vitamin B12 supplement, D-calcium pantothenate [vitamin B5], niacin supplement, vitamin A supplement, riboflavin supplement [vitamin B2], vitamin D3 supplement, biotin, pyridoxine hydrochloride [vitamin B6], thiamine mononitrate [vitamin B1], folic acid), turmeric color.

Dry matter (DM)	85.5%
	% DM Basis
Organic matter	94.3
Crude protein	33.2
Acid-hydrolyzed fat	6.7
Gross energy, kcal/g DM	4.9

Dr. Lyon's ingredients and analyzed chemical composition

Potato flour, pea protein, vegetable glycerin, pea starch, gelatin, water, natural flavor, sunflower lecithin, ground flaxseed, sunflower seed oil, citric acid, zinc propionate, peppermint oil, mixed tocopherols

Dry matter (DM)	80.8%
	% DM Basis
Organic matter	96.8
Crude protein	25.7
Acid-hydrolyzed fat	3.9
Gross energy, kcal/g DM	4.7

Bones & Chews ingredients and analyzed chemical composition

Rice flour, wheat flour, vegetable glycerin, pork gelatin, natural chicken flavor, calcium sulfate, dried cultured skim milk, powdered cellulose, salt.

Dry matter (DM)	85.2%
	% DM Basis
Organic matter	96.6
Crude protein	16.7
Acid-hydrolyzed fat	1.6
Gross energy, kcal/g DM	4.4

APPENDIX E. IMAGES OF DENTAL TREATS

Greenies regular size dental chew

Dr. Lyon's medium size dental chew

Bones & Chews dental chew

Bones & Chews, Greenies, and Dr. Lyon's