# Effects of autoclaving on compressive strength of bovine bones and their use as chewing agents for dogs

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**ABSTRACT:** This study aimed to evaluate the effect of autoclave processing on compressive stress of spongy and cortical bones, and the effect of autoclaved bones as chewing agents to reduce dental calculus in adult dogs. Spongy and cortical bones were autoclaved (1 ATM, 30 min, and 120°C) and compressive strength was evaluated in autoclaved and raw bone specimens. Autoclaved bones were offered to ten Beagle dogs divided into two groups of 5 dogs each: Group 1 – received a portion of the autoclaved bovine cortical bone (ACB) and Group 2 – received a portion of the autoclaved bovine spongy bone (ASB). Prior to the experimental period (1-d) and every two days thereafter, oral photographs were taken on both sides of the dental arch to evaluate dental calculus reduction over time. The vestibular surface of the canines, premolars, and molars teeth was evaluated using integration software to measure the proportion between the area covered by calculus and the total teeth area. The effect of bone type, treatment (raw vs. autoclaved), and their interaction were evaluated using the PROC GLIMMIX procedure of SAS (version 9.4). Linear equations were generated to estimate calculus reduction over time for ACB and ASB. Compressive strength was higher ($P < 0.05$) in cortical bones compared to spongy bones. However, the autoclaving procedure did not affect ($P > 0.05$) compressive strength, regardless of the bone type. The teeth area covered by calculus of dogs that were offered ACB reduced from 41% to 32% in 5 days, and at the end of 15 days a reduction of 62.2% was observed, resulting in a remaining of 15.5% of teeth area covered by calculus. In this group, the dental calculus area reduced by 57.7% after 5 days, and at the end of the trial, only 5.4% of teeth were still covered by calculus, which represents a reduction of 81%. The linear regression analysis revealed no significant difference between the slopes for the ACB and ASB equations ($P > 0.05$). No health complications such as tooth fracture, intestinal obstructions, and oral lesions were observed throughout the study. Our results demonstrated that the autoclave processing did not impair compressive strength of spongy and cortical bones. This corroborates with the results observed in vivo, which suggests that autoclaved bones are chewing agents for adult dogs with additional benefits of lower risk of bacterial contamination.

**Key words:** autoclaved bones, dental calculus, teeth



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## INTRODUCTION

The senior dog population has increased over the years mostly due to improvement in health care. However, periodontal disease is still considered one of the most common diseases in older dogs – with up to 80% of animals affected (Harvey and Emily, 1993; Harvey, 1998). In this condition, bacterial plaque is considered one of the most significant causative factors (Riggio et al., 2011). Gram-positive aerobics are the predominant microorganisms in the supragingival plaque formed above and along the gingival margin (Estrela et al., 2010). Growth and maturation of supragingival plaque precedes the subgingival plaque development, which is formed within the gingival sulcus and is composed mostly of gram-negative anaerobic bacteria (Logan, 2006). Marx et al (2016) recently reported that raw bovine bones could effectively promote dental calculus removal in dogs in a short period of time. Chewing bones are not only a natural method to remove calculus, but are also considered a pleasant toy for dogs as it helps to satisfy their natural instinct to chew. Although raw bones resemble the feeding behavior of the dog's ancestor, raw food is still a major concern in the pet food industry because of its potential for bacterial contamination (e.g., Salmonella and Listeria; Nemser et al., 2014) in dogs, and most importantly, in household members – raising public health concerns. Autoclaving is an effective method to sterilize food products, and this process might be used to effectively sterilize raw bovine bones. However, autoclaving may impact the bovine bone texture, impairing its use as a chewing agent. The aim of this study was to determine the effect of autoclaving on the texture of compact and spongy bovine bones, and on the effectiveness of autoclaved bovine bones in removing dental calculus in adult Beagle dogs. We hypothesized that raw bovine bones had a texture that promotes dental calculus removal, and that autoclaving would affect the bone texture and decrease its effectiveness as a chewing agent.

## MATERIALS AND METHODS

The study was developed in two phases: the first phase aimed to evaluate the compressive strength of autoclaved and raw bovine bones, and the second phase aimed to test autoclaved bovine bones as chewing agents in Beagle dogs.

### *Phase I – Compressive Strength of Bovine Bone*

Raw bovine femurs certified by the federal inspection services of the Brazilian government were acquired from commercial butchery. Specimens of 4 cm long were sectioned from diaphysis and epiphysis of the bovine femur, representing the compact and spongy bones, respectively (Fig. 1). Those pieces were placed inside plastic bags prior to autoclave (Phoenix Luferco - Araraquara, São Paulo, Brazil), where they remained under 1.0 ATM, at 121°C for 30 min. Raw and autoclaved 4-cm long bone specimens were machined into 2.5 × 2.5 cm using an electric tape saw (Implemis IP55 – Santa Rosa, Rio Grande do Sul, Brazil). Those pieces were further cut in a symmetrical and rectangle-shaped size of 2.0 × 1.0 cm (base × height) using a disc sander (Gamma 375W-Quatro Barras, Paraná, Brazil). Seven specimens per treatment were selected for compressive strength evaluation. Bone samples were submitted to a resistance test using a compression procedure (ATS universal test machine, model 1105C, EastBrook Lane, Butler, PA, United States) and a flat-ended cylindrical probe at a test speed of 0.5 mm/min. Compressive strength was defined as the maximum amount of compressive load the sample could withstand before fracturing. The highest peak load in MPa was taken as a measure of compressive strength. Data were

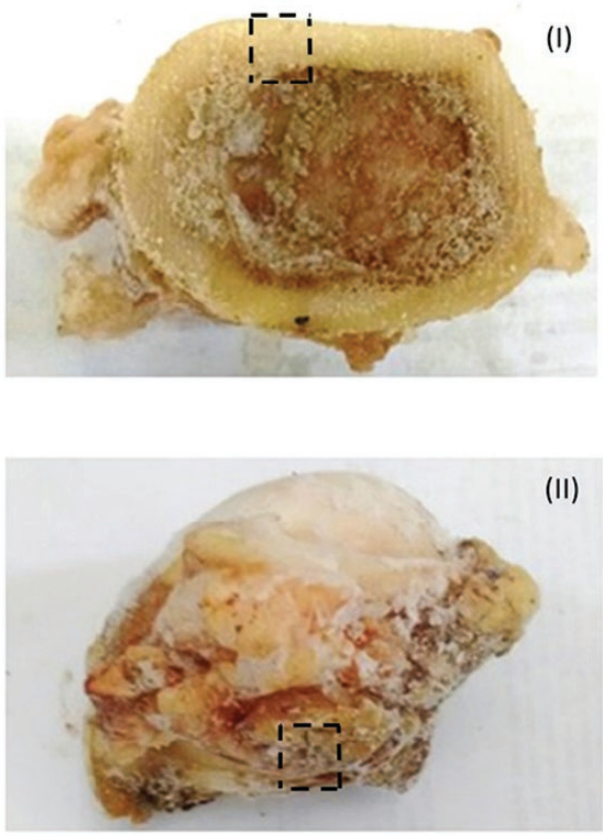


**Figure 1.** (I) Autoclaved compact bone; (II) Autoclaved spongy bone. Dashed squares represent the portion removed for mechanical property analysis.

analyzed using the GLIMMIX procedure of SAS (9.4 v, SAS Institute, Inc., Cary, NC) in a 2 × 2 factorial with two levels of bone type (compact and spongy), and two levels of treatment (raw and autoclaved). Main effects of bone and treatment and their interaction were tested. Results were considered significant at $P < 0.05$.

### *Phase II – Supragingival Calculus Assessment*

The Animal Care Committee from Universidade Federal do Rio Grande do Sul approved the procedure according to the Brazilian national guidelines under the protocol number 25685.

The second part of the study was conducted at the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Ten healthy Beagle dogs (5 males and 5 females) of the same age (3 years old) were used in this study. During the experimental period, dogs were individually housed in metabolic cages located in a controlled temperature environment and were fed a commercial extruded diet (Don Perro Premium Especial Pequeno Porte, NutriBarraSul, BR) twice daily (0800 h and 1700 h) to maintain body weight. Dogs were taken to 20-min walks twice a day. The trial followed a completely randomized design with a period of 15 days. Dogs were randomly assigned into two groups: Group 1 – dogs received autoclaved pieces (4 cm in length) of bovine femoral diaphysis, as an autoclaved compact bone (ACB); and Group 2 – dogs received autoclaved pieces (4 cm in length) of bovine femur epiphyses, as an autoclaved spongy bones (ASB; Fig. 1). The bones were offered to the dogs after the morning feed and remained available for 22 h. After this period, the bones were replaced with a new portion of similar size. The 2 h per day in which the bones were not available to the dogs were used for feeding, cleaning, and enrichment. Prior to the starting of the experimental period (1-d) and at every two days during the experimental period (3-d, 5-d, 7-d, 9-d, 11-d, 13-d, and 15-d), oral photographs were taken on both sides of the dental arch focusing on vestibular surface of the teeth. The area of canines, premolars, and molars were evaluated on both sides of the dental arch. Teeth areas were analyzed using Windows software (Image-Pro), through the integration surface tool. This procedure was adapted from Abdalla et al. (2009). Linear equations were generated to estimated calculus reduction over time according to bone type, and the slopes were compared using Statgraphics 6.0 (Statistical Graphics Co., 1993). The affected area from the vestibular surface was defined as the teeth area covered by calculus, and the reduction rate was calculated using Equation 1:

$$\text{Reduction rate } (\%): \frac{\textit{Total teeth area} * 100}{\textit{Affected area}} \quad (1)$$

Comparison of the reduction rate between the two experimental groups at each time point and between two-time points in the same experimental group was performed using the GLIMMIX procedure of SAS (9.4 v, SAS Institute, Inc., Cary, NC).

## RESULTS/DISCUSSION

In this study, we attempted to use spongy and cortical bones as natural chewable products in order to promote dental calculus removal through mechanical action. According to the study proposed by Marx et al. (2016), raw compact or spongy bones might act on calculus removal. However, the use of raw pet foods and treats is an emerging concern due to the presence of zoonotic bacteria (Bottari et al., 2020). Jones et al. (2019) used whole-genome sequencing to investigate the presence of zoonotic foodborne bacteria in pet illness fed raw pet food, and a close relation between animal clinical isolates and raw pet food bacterial isolates was observed. The autoclave sterilization process can effectively kill bacteria under the adequate steam, pressure, and time conditions (Edley et al., 1993). Thus, it can be used to sterilize raw bones and overcome potential health issues. However, this process could affect the mechanical properties of the product (e.g., compressive strength), decreasing friction and its ability to remove dental calculus. The first objective of the present study was to evaluate the effect of autoclave sterilization on the compressive strength of spongy and cortical bones. Although autoclaved sterilization decreased compressive strength by 16% in both cortical and spongy bones (Table 1; Fig. 2), there was no statistical difference ($P > 0.05$). Borchers et al. (1995) observed a reduction in adjusted strength and adjusted modulus of bovine trabecular bone after autoclaving, and attributed this result to damage of bone structure due to heating. A potential limitation of the present study is the number of samples ($n = 7$ per treatment) used to evaluate compressive strength–mechanical property tests, which includes assessment of fracture force, are very sensitive to the variation in strength (Røhl et al., 1991); thus, a greater sample size might be beneficial to observe significant differences. However, we were able to detect an effect of bone type on compressive strength even with a limited sample size. Cortical

**Table 1.** Compressive strength of autoclaved and raw bones

| | Bone | | | | *P* value | | | |
|---|---|---|---|---|---|---|---|---|
| | Cortical | | Spongy | | | | | |
| Item | Autoclaved | Raw | Autoclaved | Raw | SEM[a] | B[b] | T[c] | B × T[d] |
| Compression, Mpa | 101.7 | 121.3 | 9.34 | 11.14 | 6.59 | <0.0001 | 0.1178 | 0.1897 |

[a]SEM = standard error of the mean based on seven observations.

[b]B = main effect of bone source.

[c]T = main effect of thermal treatment.

[d]B × T = interaction effect between bone source and thermal treatment.

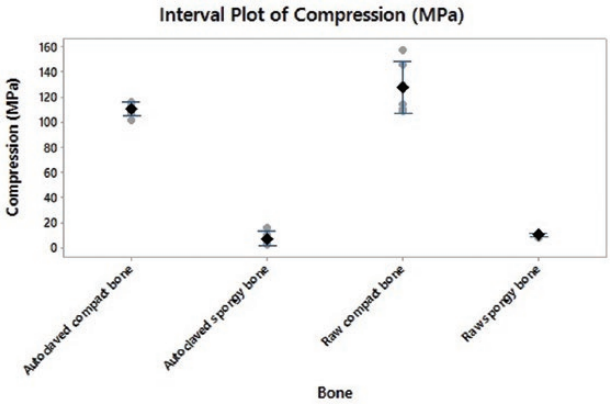


**Figure 2.** Compression stress averages (MPa): Autoclaved compact bone: 110 MPa (DP:5.2); Autoclaved spongy bone: 7.3 MPa (DP 5.2); Raw compact bone: 127 MPa (DP:20.1); Raw spongy bone:10.1 MPa (DP:1.1).

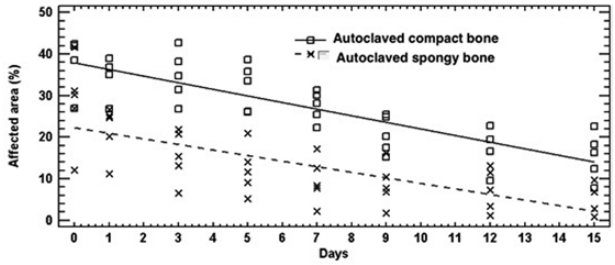


**Figure 3.** Dental area affected by calculus (%) over time of dogs fed autoclaved compact or spongy bone. Linear equations generated are 1) autoclaved compact bone (solid line): y = −1.5895x + 37.87 ($R^2$ = 69%) and 2) autoclaved spongy bone (dashed line): y = −1.3464x + 22.32 ($R^2$ = 51%). The comparison between slopes was not significant ($P$ = 0.3857).

bones presented a greater ($P < 0.05$) compressive strength when compared with spongy bones (111 vs. 10.2 MPa, respectively). Cortical bone is a very dense and strong material while the spongy bone is much more porous, having open spaces connected by trabeculae. This can explain the lower compressive strength observed in our study. The thickness and direction of the trabecula directly impact compressive strength (Gomez and Nahum, 2002); thus, it was our intent to test samples of similar sizes.

The second objective of this study was to evaluate the effectiveness of autoclaved cortical and spongy bones on calculus removal of Beagle dogs. Raw bones were not tested as they were evaluated in a previous study (Marx et al., 2016). The in vivo assay showed that autoclaved bones were well accepted by the dogs. Dogs were allowed to chew the bone sample for 22 h, which resulted in consumption of the inner portion. We did not evaluate the total time that each dog effectively chewed the bones within those 22 h. Future work should consider assessing chewing time as it may impact dental calculus removal. While the bone remained intact in ACB (Group 1), the ASB (Group 2) bone samples were either greatly reduced in size or were almost completely consumed by the dogs. Data regarding dental area affected by calculus revealed that both ACB and ASB treatments were able to reduce dental calculus over the 15-day period (Fig. 3).

At 1-d, dental calculus was covering 41% of the total area of the dental arches of dogs fed with ACB (Group 1). The area affected by dental calculus decreased by 7.3% ($P < 0.05$) and 21% on 3-d and 5-d, respectively. A continuous reduction of dental calculus was observed over time, reaching 62% reduction when compared with 1-d ($P < 0.05$). At the end of the trial, only 15.5% of the analyzed teeth area was covered by calculus in dogs from Group 1.

Dogs fed with ASB (Group 2) exhibited a lower dental calculus area at 1-d when compared with the ones fed with ACB (28.4% vs. 41%, respectively). After exposure to bones for 3 days, a reduction rate of 24% ($P < 0.05$) was observed in dogs from Group 2. Reduction rate continued to increase, and at 5-d it reached 57% compared to 1-d ($P < 0.05$). Similarly, as observed in Group 1, the area affected by calculus continually reduced over time, leading to a reduction of 81% when compared with 1-d at the end of the evaluation. At the end of the trial, dental calculus covered only 5.4% of the analyzed teeth area ($P < 0.01$). Linear regression equations were generated in order to demonstrate the effect of bone type on the reduction of dental calculus over the days (Fig. 3). Both ASB and ACB bones presented significant reductions

after supplementation. It was not observed a significant difference ($P > 0.05$) between the slopes from the linear equations (Fig. 3) which indicates that both ACB and ASB reduced calculus over time at a similar rate. The lack of difference observed in our study between bone types may be due to the fact that the dogs had a small amount of supragingival calculus at the time of the study. In the studies from Marx et al. (2016) and Pinto et al. (2020), raw bones and autoclaved bones, respectively, were tested as chewing agents in Beagle dogs. In the study of Marx et al. (2016), a greater reduction in dental calculus for raw spongy bones compared with raw compact bones (56.5% vs. 35.5%, respectively) was observed after three days of supplementation. However, this difference was not detectable after 12 days of supplementation (81.6% vs. 70.6% reduction, respectively). In our study, the effect of bone type was carried until the end of the experimental period (15-d).

A previous study demonstrated that the mechanical action of chewing by dogs has a significant impact on oral health, such as reduction in calculus and plaque accumulation, gingival inflammation, and periodontal bone loss (Harvey et al., 1996). Logan et al. (2006) also reported the positive effects of the consumption of cartilaginous materials and chewable strips for oral health. Chewing materials can also stimulate salivary flow, thus providing cleaning of the oral cavity as saliva contains antimicrobial agents (Gorrel, 2000). Besides the positive effects on oral health, chewing agents can also affect animal behavior. A recent study reported that the use of calf horn as a chewing agent increased exploratory behavior and decreased inactivity behavior in laboratory dogs (Ketter et al., 2020). Although evaluation of dog behavior was beyond the scope of this study, future work may consider the impact of bovine bones on dog behavior.

Our study indicates that autoclaved bone femurs were able to partially remove supragingival calculus, and may be used as chewing agents. However, an important aspect to consider is the animal population used in our study. While the use of kennel-dogs is more convenient for the research, allowing more control of data acquisition, client-owned dogs do not behave similarly and may present different results regarding food acceptance and preference (Griffin et al., 1984). An in-home test may provide additional data, representing a "real-world" scenario, and may also provide additional insights on the acceptance of bovine bones as chewing agents not only by the dog but also by the owner. Another aspect that must be taken into consideration is the effect of bones as a chewing age on oral health. Concerns remain present regarding feeding this product to dogs as they may favor dental fracture (Lopes et al., 2005) and gingiva laceration due to their hardness. Unfortunately, other parameters regarding oral health (e.g., gingivitis and dental plaque) were not evaluated in the present study. Although teeth root and enamel fractures and digestive tract injury were not observed by Pinto et al (2020) when autoclaved bones were fed to Beagle dogs, lesions were found on the gingiva and pieces of spongy bones were found between teeth – which warrants further attention regarding the used of autoclaved bones as chewing agents. Another major concern regarding the use of bones as chewing agents by dogs relates to the fact that small bone fragments could be swallowed and become lodged in the esophagus. Although none of these problems were faced in our study, pet owners should supervise their dogs while the product is being provided to prevent possible health issues. We reinforce that based on previous studies (Marx et al., 2016; Pinto et al, 2020) is extremely important to remove the leftover pieces of bones at the end of each day to avoid health complications due to ingestion of small bone fractions. A complete professional teeth cleaning may be performed less often if bones are provided as chewing agents, but it is still highly recommended.

## CONCLUSION

Compressive strength of ASB and ACB was not affected by the autoclaving procedure. Although ASB presented lower compressive strength when compared with ACB, both types of bones were able to create sufficient friction and promote partial dental calculus removal. Visible injuries in the teeth surface and digestive tract injuries were not observed throughout the period of this study.

*Conflict of interest statement*. None declared.

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