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Semiquantitative strain elastography of intestinal mucosa of healthy dogs

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ABSTRACT: Semiquantitative strain elastography is a complementary tool for intestinal ultrasonography. This study established reference values for intestinal mucosal strain ratio (SR) in healthy dogs and assessed associations with weight and age. Forty-one clinically healthy dogs were examined using semiquantitative strain elastography, focusing on the proximal duodenum and jejunum. The mean SR in the duodenum and jejunum was 0.87 (95% CI: 0.74-0.99) and 0.80 (95% CI: 0.71-0.90), respectively. The mean region of interest (ROI) was 0.43% $\pm$ 0.24% and 0.71% $\pm$ 0.80% in the mesentery adjacent to the duodenum and in the duodenal mucosa, respectively. In the jejunum, the average mesenteric and mucosal ROIs were 0.43% $\pm$ 0.22% and 0.64% $\pm$ 0.44%, respectively. Pearson's test demonstrated no significant correlation between SR and the dogs' weight or age, except for a weak but significant negative correlation between jejunal SR and age ($r = -0.31$, $P = 0.04$). These findings established reference SR values for the duodenal and jejunal mucosa in healthy dogs. Semiquantitative strain elastography is a feasible and safe tool for assessing intestinal elasticity in dogs. Further studies should include a larger sample and dogs with varying ages, weights, and intestinal diseases to expand diagnostic applicability.

Key words: canine, elasticity, intestine, ultrasonography.

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RESUMO: Este estudo teve como objetivo estabelecer valores de referência para a razão de deformação (SR) da mucosa intestinal em cães hígidos e avaliar associações com peso e idade. Para alcançar o objetivo, quarenta e um cães clinicamente saudáveis foram examinados por meio de elastografia de deformação semiquantitativa, uma ferramenta complementar à ultrassonografia intestinal, com foco no duodeno proximal e jejuno. Os resultados revelam que a média do SR no duodeno e no jejuno foi de 0,87 (IC 95%: 0,74-0,99) e 0,80 (IC 95%: 0,71-0,90), respectivamente. A média da região de interesse (ROI) foi de 0,43% $\pm$ 0,24% e 0,71% $\pm$ 0,80% no mesentério adjacente e na mucosa duodenal. Além disso, no jejuno, as médias das ROIs mesentérica e mucosa foram de 0,43% $\pm$ 0,22% e 0,64% $\pm$ 0,44%. O teste de Pearson não demonstrou correlação significativa entre o SR e o peso ou a idade dos cães, exceto por uma correlação negativa fraca, porém significativa, entre o SR jejunal e a idade ($r = -0,31$; $P = 0,04$). Esses achados estabelecem valores de referência de SR para a mucosa duodenal e jejunal em cães hígidos. Portanto, a elastografia de deformação semiquantitativa mostrou-se uma ferramenta viável e segura para a avaliação da elasticidade intestinal em cães. Estudos futuros devem incluir uma amostra maior e cães com diferentes idades, pesos e doenças intestinais, a fim de ampliar a aplicabilidade diagnóstica.

Palavras-chave: canino, elasticidade, intestino, ultrassonografia.

INTRODUCTION

Elastography is an advanced ultrasound technique that started in the 1990s (FELICIANO et al., 2014). It is a noninvasive method used to examine tissue elasticity, allowing an indirect assessment of the mechanical behavior of the examined structures (SIGRIST et al., 2017). It provides information

on tissue composition and elasticity of organs using elastic models to generate images that reflect mechanical properties (MARONEZI et al., 2019). Two forms of elastography can be applied as diagnostic methods: static and dynamic (APPLEBY et al., 2023). In gastrointestinal imaging, changes in tissue mechanical properties may indicate inflammatory processes, edema, or fibrotic remodeling, which

are commonly observed in canine enteropathies. Therefore, elastography may be considered a complementary, noninvasive method to help assess and monitor intestinal diseases in dogs (JERGENS & HEILMANN, 2022; OLIVEIRA et al., 2024).

The static technique, also known as compression or deformation elastography (BALLEYGUIER et al., 2013), is based on tissue displacement induced by external compression or physiological abdominal motion (MARONEZI et al., 2019; SPUŽAK et al., 2019). The resulting deformation is processed using specific software to generate a color-coded elastogram that represents relative mechanical behavior (HOSKINS, 2012; MARONEZI et al., 2019; ERCOLIN et al., 2024).

The strain ratio (SR) is defined as the relative deformation between a reference region of interest (ROI) and the target tissue ROI. Higher SR values denote lesser target tissue deformation than the reference tissue, indicating reduced elasticity (BALLEYGUIER et al., 2013). Although compression elastography is a qualitative technique, the deformation rate that can be applied provides a semiquantitative assessment (APPLEBY et al., 2023).

In human medicine, intestinal elastography has been considered a complementary technique to evaluate bowel wall mechanical properties and differentiate inflammatory from fibrotic changes, particularly in Crohn's disease (STIDHAM et al., 2011; QUAIA et al., 2018; GIANNETTI et al., 2019). Conversely, veterinary studies examining intestinal elastography in dogs remain limited. Most existing studies have focused on shear wave elastography (SPUŽAK et al., 2019; CORDELLA et al., 2021), and strain elastography has only recently been reported in canine chronic enteropathy (OLIVEIRA et al., 2025a). However, reference values for semiquantitative strain elastography of the canine intestinal mucosa have not yet been established.

Considering the high prevalence of canine enteropathies and different intestinal wall changes, noninvasive assessment of tissue mechanical properties may facilitate diagnosis and monitoring. In using elastography, reference measurements should be established so that these parameters can be reproduced in subsequent studies with sick dogs. Although strain elastography has been previously described in canine intestinal diseases, reference values for semiquantitative strain elastography of the duodenal and jejunal mucosa in clinically healthy dogs have not yet been established. In this perspective, this study examined the intestines of dogs using semiquantitative strain elastography to

establish reference values for the technique and its relationship with weight and age.

MATERIALS AND METHODS

Animals

The following inclusion criteria were determined for the experimental group: domestic dogs, age between 6 months and 15 years, weight between 1 and 35 kg, no history of clinically relevant systemic or gastrointestinal disease in the last 3 months, and normal parameters on physical examination, blood count, serum biochemistry, and B-mode abdominal ultrasound. Exclusion criteria were animals with altered clinical, laboratory, and ultrasound parameters, in accordance with the references for the species. These assessments were conducted to confirm good health.

Clinical evaluation and sample collection

Forty-one healthy male and female dogs were included, aged between 11 months and 13 years, weighing between 2 and 32 kg, without a history of illnesses in the last 3 months. The noncastrated bitches were in anestrus. The animals were treated at the Veterinary Hospital of the Universidade Federal de Goiás. They came from private veterinary clinics in the city of Goiânia, after the owners had given their permission by signing an informed consent form.

Initially, an anamnesis was obtained, followed by a physical examination. Consciousness, hydration, mucous membrane color, capillary refill time, rectal temperature, pulse, palpable lymph nodes, and cardiopulmonary auscultation were examined. To measure systolic blood pressure, a Doppler (DV 610® Veterinary Portable Vascular Doppler), sphygmomanometer, and cuffs with widths of 40% of the circumference of the thoracic limb, below the humerus-radius-ulnar joint, were used.

Subsequently, 5 ml of blood was collected by puncturing the jugular vein. Part of the volume was placed in a tube containing 10% ethylenediaminetetraacetic acid, and the rest was placed in tubes without anticoagulant with clot activator gel to obtain blood serum. The blood was collected after a 12-hour fast.

Hematological and biochemical evaluation

All analyses were conducted within 2 hours of collection. The blood count was done using an automatic analyzer (Celltac α MEC 6550, Nihon Kohden®, Japan), and the parameters of total leukocyte count, red blood cell count, hemoglobin,

mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total platelet count (PLT) were measured. Microhematocrit and refractometry techniques were used to analyze hematocrit and measure total plasma proteins (TPP), respectively.

Blood smears were taken, stained with Romanowsky's stain (Panótico rápido, Laborclin® Ltda, Pinhais, Paraná, Brazil), and analyzed under light microscopy. Only two evaluators analyzed the differential leukocyte counts and cell morphology throughout the experiment. For hematozoa tests, the whole blood in the blood smear was evaluated using the Woo technique.

Tubes containing clot-activating gel were centrifuged for 5 min at 3600 revolutions per minute to obtain the serum. The activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, total cholesterol, and albumin were measured. All analyses were performed on fresh samples using an automated biochemistry device (CM 250, Wiener®, Argentina). The values obtained in this analysis were measured using commercial reagent kits (Biotécnica® and Doles®).

Ultrasound assessment

The animals were fasted for 12 hours on food and 4 hours on water to standardize gastrointestinal conditions and reduce luminal content that could interfere with image quality. For the abdominal ultrasound assessment, we used FT422 (SAEVO®, Ribeirão Preto, Brazil) equipment coupled to microconvex and linear transducers with a frequency of 7.5-10 MHz based on the body condition score of the dog. The animals were placed in dorsal decubitus, and before the assessment, the abdomen was extensively trichotomized, and acoustic gel was applied to ensure better contact with the skin. A general systematic assessment of the abdomen was conducted by checking the size, shape, contour, echogenicity, and echotexture of the structures evaluated.

Ultrasound elastography

The animals were prepared in the same way as for the ultrasound assessment, with a maximum interval between the ultrasound and elastography examinations of 10 days. For strain elastography, an FT422 (SAEVO®, Ribeirão Preto, Brazil) device was used with a linear transducer and a frequency of 5-8 MHz. All evaluations were performed by the same examiner with proficiency in the area (Silva, WPR). During the tests, the transducer was positioned perpendicular to the animals' abdomen, without

lateralization, followed by two cycles of compression and decompression during expiration. Images were acquired during expiration and only when the evaluated intestinal segment was momentarily free of visible peristaltic movement to minimize motion-related artifacts. The elastographic map was generated on a colorimetric scale, superimposed on the B-mode image, in shades ranging from blue to red, with blue indicating lower tissue elasticity, green denoting intermediate elasticity, and red demonstrating higher elasticity. The images were taken with homogeneous color filling in the structures observed, especially in the intestinal mucosa; thus, visual scanning and calculations of the semiquantitative data were performed.

Due to the thickness, size, abdominal distribution of the small intestine, the proximal duodenal and jejunal mucosa were evaluated in cross-section. Double projection of the intestinal images was used as a display for B-mode ultrasound and elastography to maintain the scanning plane during imaging. In addition, the scan generated a waveform indicator known as the elastographic tension curve, which represented transducer compression during the scan.

In addition to visual observation, the proximal duodenal and jejunal mucosa were evaluated by measuring relative elasticity using a cursor in the ROI. This allowed a measurement based on deformation calculations obtained using a ROI selected in the adjacent mesentery (ROI 1) and another in the intestinal mucosa (ROI 2). ROI values were expressed as percentages (%), and the SR was calculated as a dimensionless ratio between the reference and target ROIs.

The designs of ROIs were similar, circular, at the same depth, and in the horizontal direction of the cross-section of the intestinal segments evaluated. The size of the ROIs was adjusted based on the thickness of the intestinal mucosa in each animal, ensuring that measurements were confined to the mucosal layer and did not include adjacent structures. The SR ratio was calculated based on the ROI ratios. An illustrative example of the SR calculation generated by the software is presented in figure 1.

Statistical analysis

The data obtained were presented using descriptive statistics in the form of mean, SD, and coefficient of variation. The Shapiro-Wilk test was used to establish normality. The SR values of the duodenum and jejunum were used to calculate the 95% confidence interval. Additionally, animals

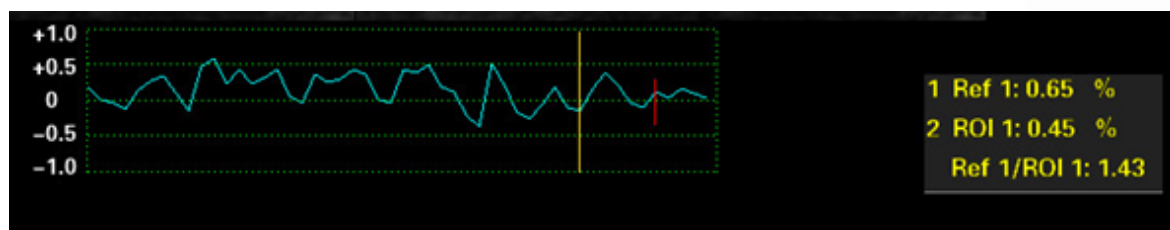


Figure 1 - Example of semiquantitative strain elastography output during jejunal evaluation in a healthy dog. The graphic display shows the compression-decompression deformation curve generated during image acquisition. The software automatically calculates the deformation values for the reference region (Ref, %) and target region (ROI, %) and generates the strain ratio (SR = Ref/ROI), expressed as a dimensionless value. The SR represents the relative deformation between the two regions and is used for the semiquantitative assessment of intestinal elasticity.

Legend: ROI, region of interest; Ref, reference tissue corresponding to ROI 1; SR, strain ratio.

were stratified by age (≤ 2 , $>2-8$, and >8 years) and body weight (≤ 10 , $>10-20$, and >20 kg). Intergroup comparisons were performed using one-way analysis of variance, and homogeneity of variance was assessed using Levene's test. In all analyses, differences were considered significant when $P < 0.05$.

As a secondary exploratory analysis, Pearson's test was used to examine the relationship between SR values and age and body weight to verify whether these demographic variables influenced SR measurements in clinically healthy dogs. To interpret the correlation tests, the magnitude of Pearson's correlation coefficient (r) was classified as follows: 0.00-0.19, very weak; 0.20-0.39, weak; 0.40-0.69, moderate; 0.70-0.89, strong; and ≥ 0.90 , very strong (SCHÖBER et al., 2018). The data were evaluated, and graphs were generated using GraphPad Prism 10.

RESULTS

The animals in this study were confirmed to be healthy through clinical assessment, laboratory tests, and abdominal ultrasound. A total of 50 animals were assessed, of which 41 were included in the study based on the established and presented criteria. All animals were of no defined breed. The average age was 6.45 ± 3.15 years, ranging from 11 months to 13 years. The average weight was 15.07 ± 6.01 kg, ranging from 2 to 32 kg. The descriptive statistics for these parameters are shown in table 1.

The elastography results revealed ROI 1 in the mesentery adjacent to the duodenum with an average value of $0.43\% \pm 0.24\%$ and ROI 2 in the duodenal mucosa, $0.71\% \pm 0.80\%$; ROI 1 of the mesentery adjacent to the jejunum was $0.43\% \pm 0.22\%$ and ROI 2 in the jejunal mucosa, $0.64\% \pm 0.44\%$. The elastographic map generated during the

Table 1 - Descriptive statistics of strain elastography measurements, age, and body weight in 41 healthy dogs.

Variable	Average/Unit of measure	Standard Deviation	Average CI (95%) low	Average CI (95%) high	Coefficient of variation (%)
ROI 1 duodenum	0.43 %	0.24	0.36	0.51	55.95
ROI 2 duodenum	0.71 %	0.80	0.46	0.97	112.9
SR duodenum*	0.87	0.39	0.74	0.99	45.07
ROI 1 jejunum	0.43 %	0.22	0.36	0.50	51.17
ROI 2 jejunum	0.64 %	0.44	0.50	0.78	69.33
SR jejunum*	0.80	0.29	0.71	0.90	36.85
Weight	15.07 kg	6.01	13.17	16.97	39.91
Age	6.45 years	3.15	5.46	7.45	48.86

CI = Confidence interval; ROI = Region of interest and SR = strain ratio.

*Values obtained by strain elastography.

evaluation of study animals, as well as markings of the ROIs between the intestinal mucosa and mesentery, are shown in figure 2.

The mean SR for the duodenum and jejunum was 0.87 ± 0.39 and 0.80 ± 0.29 , respectively. The 95% confidence interval (CI) obtained for the SR of the duodenum and jejunum was 0.74-0.99 and 0.71-0.90, respectively. Therefore, the duodenum showed higher values in the elastography parameters.

There were weak, positive, and negative correlations between the SR of the intestines and weight and age (Figure 3). However, the correlation between jejunal SR and animal age was weak, negative, and statistically significant ($r = -0.31$, $P = 0.04$).

When animals were stratified by age groups (young, adult, and senior), no significant differences were observed in SR values for the duodenum ($P = 0.12$) or jejunum ($P = 0.13$). Homogeneity of variances was confirmed for duodenal SR (Levene $P = 0.17$), whereas jejunal SR exhibited variance heterogeneity (Levene P

$= 0.01$); although no statistically significant differences were observed between groups. Similarly, stratification by body weight (small, medium, and large) showed no significant differences in SR values for the duodenum ($P = 0.68$) or jejunum ($P = 0.33$), with homogeneous variances across all groups (Levene $P > 0.05$).

DISCUSSION

This study established reference SR values for the duodenal and jejunal mucosa in clinically healthy dogs using semi quantitative strain elastography. Establishing baseline values in normal animals is a fundamental prerequisite before evaluating tissue mechanical behavior under pathological conditions.

In human medicine, strain elastography has been widely examined in inflammatory bowel diseases, particularly Crohn's disease, where semi quantitative parameters and color distribution

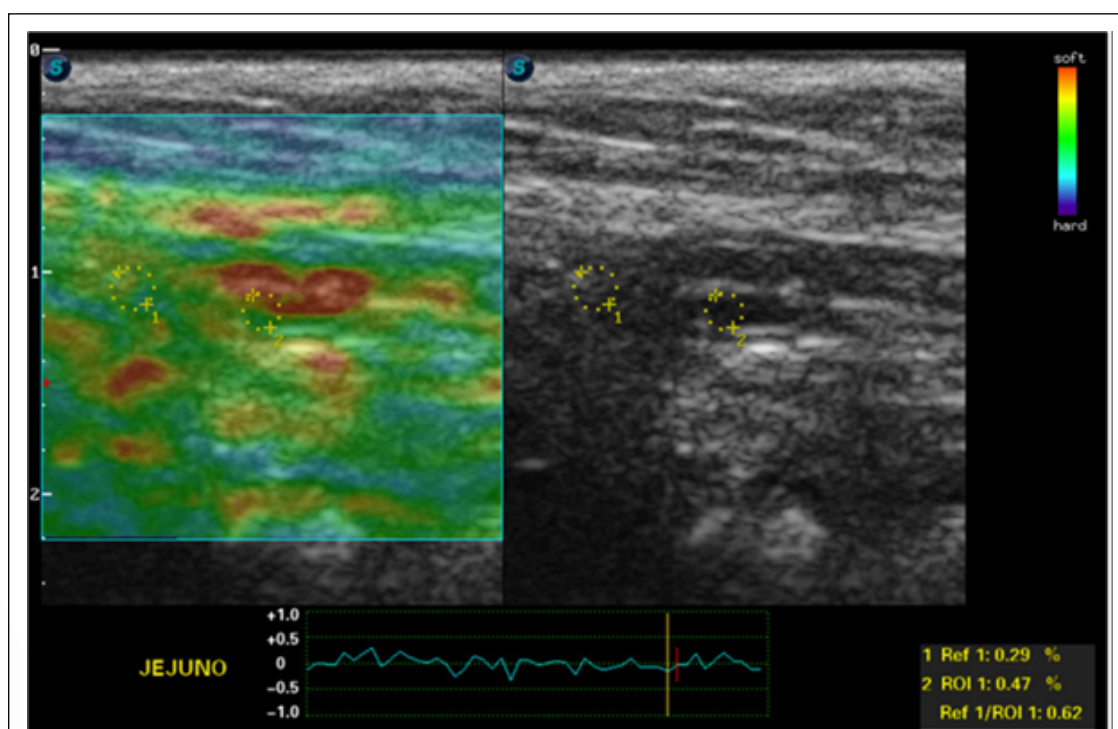
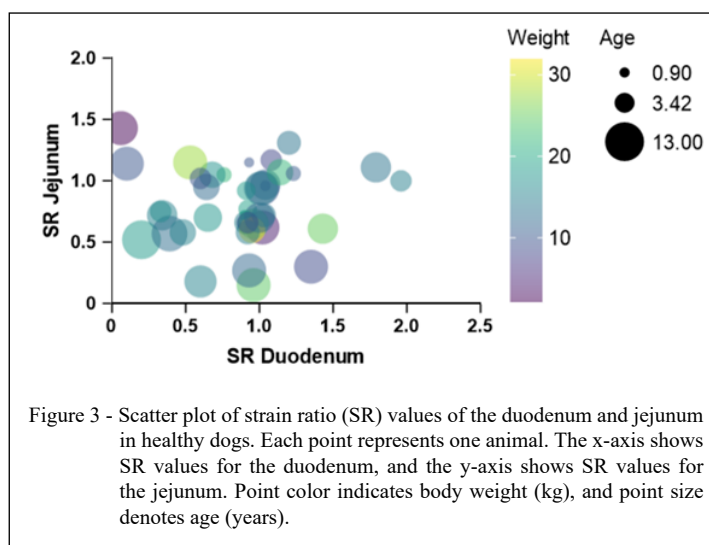


Figure 2 - Representative strain elastography image of the jejunal mucosa in a healthy dog. Color elastogram (left) and the corresponding B-mode ultrasound image (right) obtained in the cross-section of the jejunum. ROI 1 (Ref) was positioned in the adjacent mesentery (reference tissue), and ROI 2 in the dorsal jejunal mucosa (target tissue). The color scale ranges from blue (lower deformation) to red (higher deformation). The calculated strain ratio (SR = 0.62) is shown in the lower right corner.

Legend: ROI, region of interest; Ref, reference tissue corresponding to ROI 1; SR, strain ratio. The image was selected based on adequate signal stability and homogeneous color filling within the region of interest.



patterns have been correlated with inflammatory activity and fibrotic remodeling (GIANNETTI et al., 2019; QUAIA et al., 2018). However, the veterinary literature remains limited. A recent review emphasized the limited number of controlled studies evaluating intestinal elastography in dogs and underscored the need for standardization of acquisition and interpretation protocols (OLIVEIRA et al., 2024). More recently, strain elastography findings were observed in individual canine cases of chronic inflammatory enteropathy and intestinal lymphangiectasia (OLIVEIRA et al., 2025b), revealing that SR values may vary in diseased tissue. Nevertheless, controlled reference data in healthy dogs were not previously available, reinforcing the relevance of this study.

The mean SR values in this study (0.87 ± 0.39 for the duodenum and 0.80 ± 0.29 for the jejunum) indicate preserved mucosal deformability in clinically healthy animals. Based on the general physical principles of SR, higher SR values denote reduced relative deformation of the target tissue compared to the reference region (CHOI et al., 2015). Because all animals were clinically normal and showed no ultrasonographic abnormalities, no increased SR values were expected. Although human studies have reported associations between altered elastographic parameters and histological fibrosis (QUAIA et al., 2018), histopathological correlation was not performed in this study, as biopsy was not ethically justified in healthy animals. Therefore, extrapolation of fibrosis-related interpretations to

canine SR values remains speculative and requires future investigation in diseased populations.

The selection of proximal duodenal and jejunal segments was based on anatomical accessibility and imaging reproducibility, aiming to reduce variability associated with intestinal content and gas interference. Standardizing the evaluation to these segments allowed consistent ROI positioning in all animals. Previous canine studies have also evaluated the proximal jejunal segments (SPUŽAK et al., 2019), whereas human studies frequently examine the terminal ileum (ZHAO et al., 2021). In dogs, distal segments may present greater technical variability, supporting the use of proximal regions for standardization. Thus, proximal segments were considered more appropriate for the initial standardization of the technique.

Methodological standardization is particularly relevant in strain elastography because of its operator-dependent nature. In this study, image acquisition and ROI placement were standardized to ensure consistent SR measurements in all animals. Differences in acquisition parameters compared with other studies are mainly associated with tissue depth and elastography technique (TAMURA et al., 2019; DEL SIGNORE et al., 2021; PUCCINELLI et al., 2023). The mesentery was used as a reference region based on previously described approaches (GIANNETTI et al., 2019).

The weak negative correlation observed between jejunal SR and age ($r = -0.31$) should be interpreted cautiously, as the magnitude of the

correlation denotes limited biological relevance. In addition, when animals were stratified by age groups, no significant differences in SR values were observed, indicating that age did not significantly influence elastographic measurements in this population of clinically healthy dogs. The study population predominantly consisted of adult dogs, and the descriptive design does not allow inference of age-related biomechanical changes. Canine renal elastography studies reported a similar absence of consistent correlations between elastographic parameters and demographic variables (PUCCINELLI et al., 2023), revealing that baseline tissue mechanical behaviors in healthy animals may be relatively stable within typical adult ranges.

Despite its contributions, this study has limitations inherent to its exploratory design. A priori power analysis was not performed, and the relatively small sample size with wide ranges of age and body weight likely increased biological variability, as shown in the coefficients of variation for mucosal ROI measurements. In addition, intraobserver repeatability was not formally assessed, and measurement variability cannot be excluded. Future studies should include larger, stratified populations and reproducibility analyses to refine reference intervals.

Although preliminary case reports indicated that SR alterations may occur in canine enteropathies (OLIVEIRA et al., 2025a), this study did not assess diagnostic performance, disease differentiation, or prognostic capacity. Establishing reference SR values in healthy dogs is a necessary first step; however, histopathological correlation in dogs with chronic enteropathies will be essential to determine whether SR changes truly reflect specific pathological processes, such as inflammation or fibrosis.

CONCLUSION

This study used semiquantitative strain elastography to establish reference SR values for the duodenal and jejunal mucosa of clinically healthy dogs. These baseline measurements provide foundational data for future studies on canine intestinal diseases. Further studies, including diseased populations and histopathological validation, are required before diagnostic or prognostic implications can be determined.

DATA AVAILABILITY STATEMENT

Research data is only available upon request.

ACKNOWLEDGMENTS

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The study was approved by the Ethics Committee for the Use of Animals (CEUA) of the Universidade Federal de Goiás (UFG), under protocol number 41/24.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that no artificial intelligence resources were used in the writing or development of this manuscript.

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