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Quantitative gastrointestinal ultrasonographic evaluation in brachycephalic dogs with obstructive airway syndrome[1]

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ABSTRACT.- Fabris IA, Facin AC, Rein A, Aires LPN, Gasser B, Lima BB, Slompo MEF, Gujanwski CA, Silva DG, Quitzan JG, Evangelista GCL, Feliciano MAR, Moraes PC. **Quantitative gastrointestinal ultrasonographic evaluation in brachycephalic dogs with obstructive airway syndrome**. *Pesquisa Veterinária Brasileira 45:e07682, 2025.* Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Rua Duque de Caxias 225, Jardim Elite, Pirassununga, SP 13635-900, Brazil. E-mail: gabriela.clopes@yahoo.com.br

Respiratory obstruction in brachycephalic obstructive airway syndrome (BOAS) can lead to secondary gastrointestinal alterations. This study aimed to quantitatively assess the echogenicity of the stomach, duodenum, and jejunum in dogs affected by brachycephalic syndrome. The correlation of these findings with BOAS severity, hematological alterations, and signs of systemic inflammation was investigated. Fifty-two brachycephalic patients and 15 mesocephalic controls, aged between 1 and 8 years, underwent evaluation including hemogram, biochemical analysis, C-reactive protein, and B-mode ultrasonography of the gastrointestinal tract. Brachycephalic animals were categorized based on the severity of BOAS, and owners provided clinical data via questionnaire. In the quantitative analysis, eight regions of interest were defined within the mucosal layer of the stomach, duodenum, and jejunum, and the mean pixel values were quantified for each structure. Leukocyte count ($p \leq 0.001$), eosinophils ($p = 0.002$), monocytes ($p < 0.001$), creatinine ($p \leq 0.001$) and total protein ($p \leq 0.001$) were higher in brachycephalic dogs than mesocephalic dogs. Hematological patterns showed mild leukocytosis, possibly indicating subclinical inflammation. Brachycephalic dogs exhibited elevated echogenicity in the duodenum and jejunum, measured in pixels (duodenum: 18.2 ± 11.3; jejunum: 25.6 ± 15.2), which was significantly higher than mesocephalic dogs ($p < 0.05$; duodenum: 11.04 ± 4.3; jejunum: 9 ± 7), according to variance analysis. Brachycephalic dogs of grades 0, 1, and 2 exhibited higher values than controls and grade 3 dogs. In conclusion, quantitative ultrasonography reduces subjectivity and provides objective insights into gastrointestinal alterations in BOAS-affected dogs.

INDEX TERMS: Ultrasound, brachycephalic syndrome, respiratory obstruction, inflammatory bowel disease.

RESUMO.- [**Avaliação ultrassonográfica gastrointestinal quantitativa em cães braquicefálicos com síndrome obstrutiva das vias aéreas.**] A obstrução respiratória na síndrome obstrutiva das vias aéreas braquicefálicas (BOAS) pode levar a alterações gastrointestinais secundárias. Este estudo teve como objetivo avaliar quantitativamente a ecogenicidade do estômago, duodeno e jejuno em cães afetados pela síndrome braquicefálica. A correlação desses achados com a gravidade da BOAS, alterações hematológicas e sinais de inflamação sistêmica foi investigada. Cinquenta e dois

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pacientes braquicefálicos e 15 controles mesocefálicos, com idades entre um e oito anos, foram submetidos a avaliações incluindo hemograma, análise bioquímica, proteína C-reativa e ultrassonografia modo B do trato gastrointestinal. Os animais braquicefálicos foram categorizados com base na gravidade da BOAS, e os proprietários forneceram dados clínicos por meio de questionário. Na análise quantitativa, oito regiões de interesse foram definidas dentro da camada mucosa do estômago, duodeno e jejuno, e os valores médios de pixel foram quantificados para cada estrutura. Contagem de leucócitos ($p \leq 0,001$), eosinófilos ($p = 0,002$), monócitos ($p < 0,001$), creatinina ($p \leq 0,001$) e proteína total ($p \leq 0,001$) foi maior em cães braquicefálicos do que em cães mesocefálicos. Padrões hematológicos mostraram leucocitose leve, possivelmente indicando inflamação subclínica. Cães braquicefálicos exibiram ecogenicidade elevada no duodeno e jejuno, medida em pixels (duodeno: 18,2 ± 11,3; jejuno: 25,6 ± 15,2), que foi significativamente maior do que cães mesocefálicos ($p < 0,05$; duodeno: 11,04 ± 4,3; jejuno: 9 ± 7), de acordo com análise de variância. Cães braquicefálicos graus 0, 1 e 2 exibiram valores mais altos do que controles e cães grau 3. Concluindo, a ultrassonografia quantitativa reduz a subjetividade e fornece *insights* objetivos sobre alterações gastrointestinais em cães afetados por BOAS.

TERMOS DE INDEXAÇÃO: Ultrassom, síndrome braquicefálica, obstrução respiratória, doença inflamatória intestinal.

INTRODUCTION

Brachycephalic obstructive airway syndrome (BOAS) is characterized by congenital anatomical abnormalities that partially obstruct the upper airways (Lameu et al. 2020). Several authors confirm the association of gastrointestinal signs with respiratory syndrome due to a higher predisposition to hiatal hernia, pyloric stenosis, esophageal deviation, decreased esophageal transit, obesity, incompetent cardia, increased abdominal pressure, and chronic vomiting (Aslanian et al. 2014, Shaver et al. 2017, Broux et al. 2018, Kaye et al. 2018). Such alterations lead to the occurrence of gastroesophageal and/or gastroduodenal reflux (Poncet et al. 2005, Mitze et al. 2022), present in 84% of patients (Appelgrein et al. 2022). A study showed that 97.3% of animals with respiratory changes due to the syndrome had significant endoscopic and histological alterations in the esophagus, stomach, and duodenum (Poncet et al. 2005).

BOAS serves as an experimental model for obstructive sleep apnea (OSA) due to the apneic episodes observed in these dogs, occurring both during sleep and wakefulness (Hendricks et al. 1987). Humans with OSA have a higher predisposition to inflammatory bowel disease (IBD) (Gombert et al. 2019, Orr et al. 2020), attributed to disruptions in the intestinal microbiota caused by hypoxia and alterations in circadian rhythms (Ranjbaran et al. 2007). Circadian rhythms influence various aspects of digestive function, including epithelial renewal, immune function, hepatic metabolism, and intestinal motility (Ghiasi et al. 2017).

Moreover, specific digestive processes, such as intestinal permeability and colonic motility, are linked to genes directly associated with the circadian cycle (Orr et al. 2020). Sleep disorders have been linked to heightened activation of the immune system, primarily facilitated by immune cell migration and the subsequent release of pro-inflammatory cytokines (Ranjbaran et al. 2007). Tang et al (2009) substantiated these findings, showing that acute and chronic sleep deprivation intensify colonic inflammation in experimental rat colitis models. Repeated episodes of apnea in rats induced rapid endothelial cell activation, suggesting a surprisingly rapid response to OSA-induced inflammation (Nácher et al. 2007). There is a robust correlation between OSA and systemic inflammation (Crane et al. 2017, Trzepizur et al. 2018). C-reactive protein (CRP) is a marker for acute and chronic inflammation and has been extensively examined in individuals with OSA (Céron et al. 2005, Hong et al. 2017, Yi et al. 2022). Elevated levels of CRP have also been documented in conditions such as Crohn's disease, ulcerative colitis (Henriksen et al. 2008), and gastric mucosal lesions in dogs (Otabe et al. 2000). It has been proposed that brachycephalic dogs exhibit signs of subacute systemic inflammation (Rancan et al. 2013, Gianella et al. 2019, Facin et al. 2020). Although obesity frequently plays a confounding role in studies on inflammation in BOAS, no correlation was found between the animals' body score and the worsening of the syndrome (Gianella et al. 2019, Facin et al. 2020). Certain investigations have indicated an increase in this biomarker in 14% of such animals, suggesting systemic inflammation in dogs with BOAS may not manifest in the same manner or to the same degree as OSA in humans (Planellas et al. 2012, 2015).

Intestinal wall thickness has limited diagnostic value for idiopathic IBD in dogs, as many inflammatory cases show no thickening. Studies have found no significant differences in wall thickness between affected and healthy dogs, suggesting echogenicity may be a more useful parameter (Rudorf et al. 2005, Gaschen et al. 2008). Nonetheless, ultrasonographic signs, such as altered echogenicity and increased colonic thickness, have been reported in symptomatic dogs (Malancus & Malancus 2017). In human IBD, intestinal thickening may occur even in inactive stages, assisting in disease severity assessment (Haber et al. 2000). The quantitative analysis of ultrasonographic images has been explored to mitigate potential interpretation errors and reduce the subjectivity of examinations, thereby enhancing assessment reliability (Silva et al. 2013, Simões et al. 2018). Numerical pixel value (NPV) estimation relies on the density and composition of macromolecules and water (Griffin et al. 2009) and is represented on a scale ranging from 0 (absolute black) to 255 (absolute white). This method assesses organ echogenicity, with alterations often associated with inflammatory conditions, chronic changes, and neoplasms (Silva et al. 2013, Simões et al. 2018).

The intestinal mucosa typically exhibits low echogenicity in healthy dogs, often described as nearly anechoic (Penninck 2015, Gaschen et al. 2016, Tcygansky et al. 2021). Conversely, an increase in echogenicity, manifesting as spots and hyperechoic streaks, is considered a positive indicator of chronic enteropathies and IBD (Gaschen et al. 2016). Tcygansky et al. (2021) revealed notable alterations in the quantitative assessment of intestinal mucosal echogenicity in dogs affected by parvovirus, showing a 2.54-fold increase compared to values previously reported in healthy dogs by the same authors. Limited information exists regarding ultrasonographic changes in dogs with BOAS, and no study has addressed quantitative ultrasound evaluation in these animals. While measurements of intestinal wall thickness aid in diagnosing and staging inflammatory intestinal diseases in

humans (Haber et al. 2000), intestinal thickness lacks reliable diagnostic utility in dogs, with echogenicity measurements being preferred (Gaschen et al. 2008). This study aimed to evaluate gastrointestinal tract (GIT) echogenicity in brachycephalic dogs with BOAS, compare it to healthy mesocephalic dogs, and investigate potential associations with BOAS severity and systemic inflammation markers.

MATERIALS AND METHODS

Ethical approval. This observational and prospective study was approved by the Institutional Animal Care and Use Committee (protocol number 1471/21). Pet owners signed a consent form for their animal's inclusion in this experiment.

Study design. A minimum sample size of 44 animals was calculated to ensure 95% confidence and 90% statistical power, based on expected differences in echogenicity. Brachycephalic and mesocephalic dogs, male or female, aged between one and seven years and weighing between five and 20 kg, were selected. Dogs with chronic gastrointestinal disease, gastric or intestinal neoplasia, recent anti-acid, anti-emetic, or anti-inflammatory treatment within the last 30 days, aggressive behavior hindering manipulation, or a history of previous surgical procedures in the respiratory tract were excluded. All patients underwent weighing and were subsequently classified according to their body condition score (BCS), ranging from one to nine (Kato et al. 2011). Additionally, all brachycephalic patients were categorized based on the validated BOAS index (Liu et al. 2015, Riggs et al. 2019). The submaximal exercise test was consistently administered in an open environment, maintaining a uniform distance, and utilizing the same evaluator. Animals classified as grade 0 exhibited no symptoms, whereas those categorized as grade III presented severe syndrome (Liu et al. 2015, 2017). The dogs were divided into five distinct experimental groups based on their clinical characteristics: (1) Control group (CG), comprising mesocephalic dogs without any respiratory and gastrointestinal abnormalities; (2) Brachycephalic group without BOAS (BG0); (3) Brachycephalic group with functional classification grade I (BG1); (4) Brachycephalic group with functional classification BOAS grade II (BG2); and (5) Brachycephalic group with functional classification BOAS grade III (BG3).

Clinical assessment. The frequency of gastrointestinal signs (ptyalism, regurgitation, and vomiting) was documented by a specific clinical questionnaire developed for this study (Supplementary data 1), following the criteria outlined by Poncet et al (2005), where the presence of at least one sign in a higher series determined the classification of the animal in that grade. Additionally, tutors were queried about other gastrointestinal signs, including eructation, hiccups, licking of air/noses/lips (a clinical sign indicating reflux), choking, selective appetite, flatulence, and stool appearance. Hematological and biochemical evaluations were conducted on all animals. The biochemical profile included serum concentrations of albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, creatinine, direct bilirubin, indirect bilirubin, total bilirubin, gamma-glutamyl transferase (GGT), total plasma protein, triglycerides, and urea. CRP was measured by the immunoturbidimetry method on an automatic analyzer (LabMax Pleno III, Labtest Diagnóstica, Lagoa Santa, Brazil) using a kit with specific human antibodies (PCR Turbiquest Max, Lagoa Santa, Brazil).

B-mode ultrasonography. Gastrointestinal ultrasonography was conducted using an ACUSON S2000™ ultrasound machine (Siemens®, Munich, Germany), equipped with a high-frequency convex transducer (7.5 MHz), after patients had fasted for 12 hours. All examinations were performed by the same examiner, who possessed over five years of experience in the field. Longitudinal plane images of the stomach regions (fundus, body, and pylorus), descending duodenum, and jejunum were captured and saved as digital files in JPEG format. These images were subsequently analyzed using commercially available image analysis software (Image ProPlus®; Media Cybernetics Inc., San Diego/CA, USA) by a trained operator. An area of interest was delineated, covering at least 65% of the organ parenchyma area. Special attention was given to avoiding areas of shadow artifacts caused by luminal content and gas presence. Subsequently, eight non-overlapping regions of interest (ROIs), represented as circular point meters generated by the computer, were projected to determine the average NPVs of each animal (Fig. 1-3). For the stomach, the ROI was standardized at 12 mm, while for the duodenum and jejunum, it was 30 mm. Reference values for normality in the evaluated dogs were based on a previous study that assessed the echogenicity of the gastrointestinal tract in healthy dogs using pixel data (Tcygansky et al. 2020).

Statistical analysis. Hematological, biochemical, and CRP parameters were examined among skull conformations utilizing the t-test. Normality of continuous variables was assessed using the Shapiro-Wilk test. Homoscedasticity was evaluated using the Bartlett test. Depending on distribution and variance, comparisons were performed using t-tests, ANOVA with Tukey's post-test, or Kruskal-Wallis followed by Dunn's post-test. Correlations were assessed using Spearman's test. All statistical analyses were performed using R Software version 4.2.2 (R Foundation for Statistical Computing, Austria) with a significance level set at $p < 0.05$.

RESULTS

Animals

Fifty-two brachycephalic patients, including French Bulldogs and Pugs, and 15 Beagles met the evaluation criteria. Out of 52 brachycephalic dogs included in this study, 57.7% were females (n = 30), and 42.3% were males (n = 22). Regarding the mesocephalic dogs, 73.3% were females (n = 11), and 26.7% were males (n = 4).

The mean age observed for each breed was 2.74 ± 1.64 years in French Bulldogs, 4.01 ± 1.75 years in Pugs, and 3.64 ± 1.37 years in Beagles. The weight of the selected dogs ranged from 6.9 to 16.9 kg, with a mean weight of 11.51 ± 2.85 kg for brachycephalic dogs and 10.74 ± 1.45 kg for mesocephalic dogs. The average BCS for each breed was 5.91 ± 1.04 for French Bulldogs, 5.78 ± 1.56 for Pugs, and 5.87 ± 0.34 for Beagles. There was no significant difference in BCS between the BOAS grades and the control group by Spearman's rank correlation (correlation coefficient 0.242, $p = 0.0843$).

Assessment of clinical signs of digestive and respiratory symptoms

Twenty-eight owners (53%) responded to the provided questionnaire. Dogs not graded in the gastrointestinal questionnaire were housed in kennels where individual clinical and feeding observations were not feasible due to group housing. The distribution of patients according to the grade of the syndrome and digestive signs is depicted in Table 1, indicating that most brachycephalic dogs (67.8%) received a moderate grade on the questionnaire.

The observed gastrointestinal clinical sign among the evaluated animals in this study was flatulence (25/28 dogs,

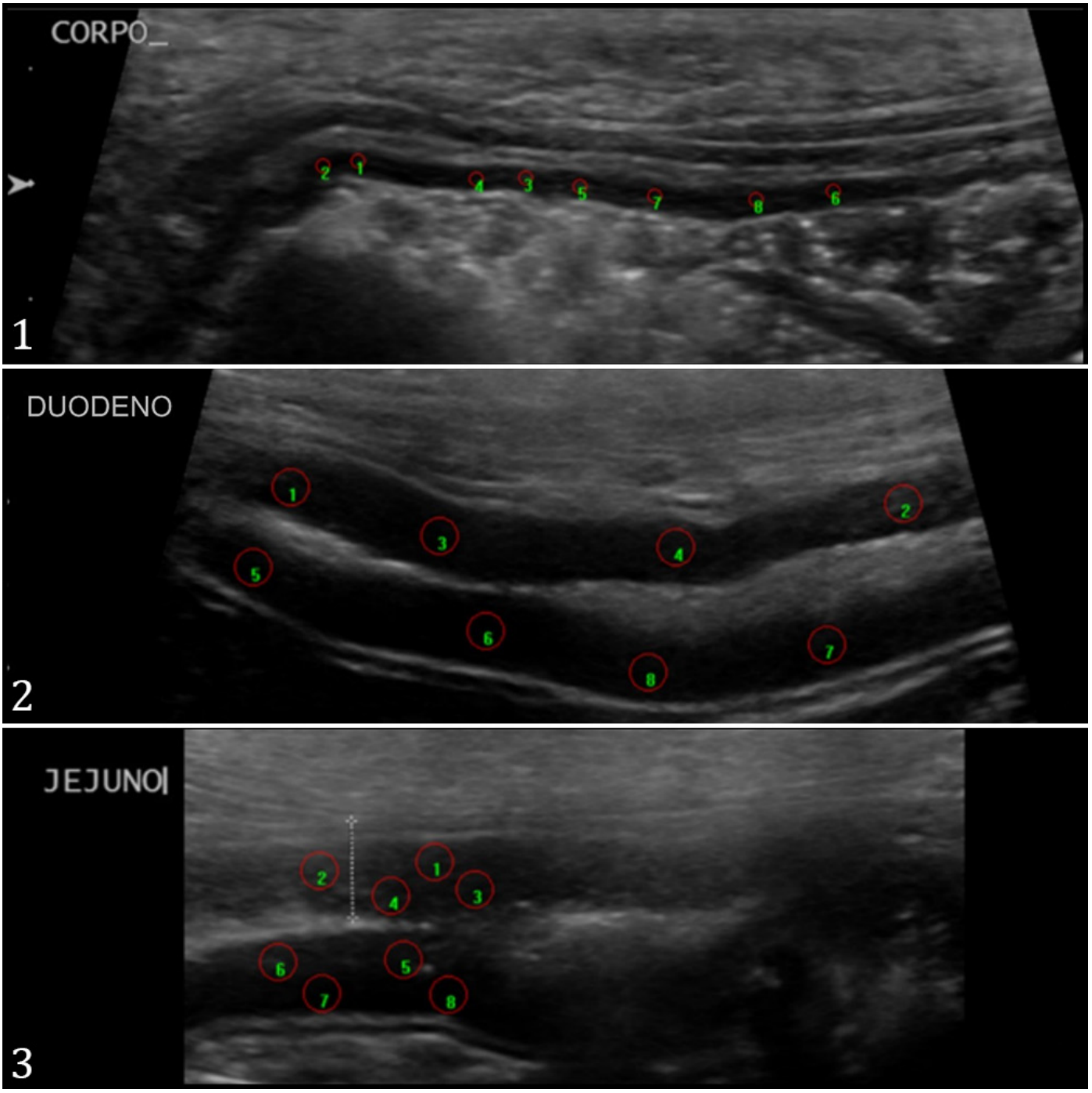

Fig. 1-3. Longitudinal B-mode ultrasound image with ROI (regions of interest) delineations in red using ImageProPlus® software in the following gastrointestinal portions: (**1**) gastric body, (**2**) duodenum, (**3**) jejunum.

Table 1. Modified grading of digestive signs according to the questionnaire filled out by the tutors and proposed by Poncet et al. (2005) within the brachycephalic obstructive airway syndrome (BOAS) groups

	BG0	BG1	BG2	BG3	Total
Grade I	1	3	0	3	7 (25%)
Grade II	3	4	7	5	19 (67.8%)
Grade III	0	1	1	0	2 (7.1%)
TOTAL	4 (14.3%)	8 (28.6%)	8 (28.6%)	8 (28.6%)	

BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade.

89.3%), followed by reflux (21/28 dogs, 75%), eructation (25/28 dogs, 53.6%), regurgitation and vomiting (15/28 dogs, 53.6% and 13/28 dogs, 54.5%, respectively), loose stools, selective appetite, and choking with water (12/28 dogs, 42.9%; 9/28 dogs, 32.1%; 8/28 dogs, 28.6%, respectively). Among dogs with loose stools, occasional episodes accounted for 91.7%, and weekly episodes for 8.3%. Regarding this clinical sign, 25% of dogs belonged to BG0, 33.3% to BG1, 8.3% to BG2, and 33.3% to BG3. Diarrheic stools were occasionally reported in 21.4% (6/28) of dogs, with 33.3% from BG0 and 66.7% from BG1.

Hematological, biochemical, and C-reactive protein evaluation

Table 2 displays the results of the variables concerning skull conformation. Notably, significant differences were observed among the analyzed parameters. Brachycephalic dogs

Table 2. Means and standard deviations of the values of hemogram, serum biochemistry, gastrointestinal tract (GIT) thickness, and pixels obtained by B-mode ultrasound and evaluation of echogenicity between mesocephalic and brachycephalic dogs by the Student's t-test

Complete blood count	Skull conformation		*p*
	Mesocephalic	Brachycephalic	
RBC (106/µL)	7.0 ± 0.7	7.2 ± 0.7	0.335
HGB (g/dL)	16.2 ± 1.9	16.8 ± 1.9	0.294
HTC (%)	48.5 ± 5.2	51.0 ± 4.8	0.077
PLT (10^3/µL)	297.5 ± 95.7	319.9 ± 123.9	0.521
WBC (µL)	7,040 ± 879	10,665 ± 3.685	< 0.001*
Neutrophil (µL)	5,106.4 ± 852.1	7,624.5 ± 3.036	0.002*
Band cells (µL)	82.3 ± 97.7	103.3 ± 103.8	0.486
Lymphocyte (µL)	1,535 ± 469.4	2,138.2 ± 1,172.4	0.088
N/L ratio	3.9 ± 2.4	4.49 ± 2.8	0.484
Eosinophils (µL)	152.7 ± 418.8	515.5 ± 418.8	0.002*
Monocytes (µL)	163 ± 48.4	295 ± 144.8	< 0.001*
	Serum biomarkers		*p*
Albumin (g/dL)	3.3 ± 0.2	3 ± 0.5	0.009*
ALP (U/L)	116.4 ± 69.1	57.6 ± 27.8	< 0.001*
ALT (µL)	47.1 ± 14.3	39.5 ± 35.2	0.418
AST (µL)	36.5 ± 16.5	31.4 ± 8.7	0.505
Cholesterol (mg/dL)	237.2 ± 79.4	216.1 ± 49.6	0.668
CRP	0.5 ± 1	3.4 ± 6.2	0.081
Creatinine	0.85 ± 0.1	1.1 ± 0.19	< 0.001*
DB (mg/dL)	0.07 ± 0.03	0.05 ± 0.04	0.026*
GGT (U/L)	7.6 ± 0	6.6 ± 2.6	0.137
IB (mg/dL)	0.1 ± 0.05	0.13 ± 0.06	0.093
TB (mg/dL)	0.17 ± 0.06	0.18 ± 0.06	0.762
Total protein (g/dL)	6.2 ± 0.3	4.8 ± 0.7	< 0.001*
Triglycerides (mg/dL)	67.4 ± 25.1	88.1 ± 82.7	0.345
Urea	27.3 ± 4.6	32 ± 18.7	0.343
	Thickness of the GIT		*p*
Gastric fundus (mm)	3.4 ± 0.9	4.08 ± 1.5	0.103
Gastric body (mm)	3.6 ± 0.6	3.7 ± 0.8	0.539
Antrum (mm)	3.9 ± 0.9	4.3 ± 1.05	0.159
Pyloric sphincter (mm)	4.2 ± 0.7	4.3 ± 1.1	0.783
Duodenum (mm)	4.3 ± 0.6	4.8 ± 0.7	0.010*
Jejunum (mm)	3.3 ± 0.7	3.6 ± 0.7	0.119
Ascending colon (mm)	1.84 ± 0.4	1.8 ± 0.5	0.759
Transverse colon (mm)	1.6 ± 0.3	1.9 ± 0.7	0.087
Descending colon (mm)	1.5 ± 0.4	1.6 ± 0.4	0.713
	Pixels		*p*
Stomach	27.2 ± 11.2	25.3 ± 10.3	0.553
Duodenum	11.04 ± 4.3	18.2 ± 11.3	0.019*
Jejunum	9 ± 7	25.6 ± 15.2	< 0.001*

GIT = gastrointestinal tract, RBC = red blood cell, HGB = hemoglobin, HTC = hematocrit, PLT = platelet count, WBC = white blood count, N/L ratio = neutrophil-to-lymphocyte ratio, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRP = C reactive protein, DB = direct bilirubin, GGT = gamma-glutamyl transferase, IB = indirect bilirubin, TB = total bilirubin; * Significant variables ($p < 0.05$).

showed elevated values in leukocyte count (10,665 ± 3,685, $p \leq 0.001$), eosinophils (515.5 ± 418.8, $p = 0.002$), monocytes (295 ± 144.8, $p < 0.001$), creatinine (1.1 ± 0.19, $p \leq 0.001$), total protein (4.8 ± 0.7, $p \leq 0.001$), duodenal thickness (4.8 ± 0.7, $p = 0.010$), pixels in the duodenum (18.2 ± 11.3, $p = 0.019$), and jejunum (25.6 ± 15.2, $p \leq 0.001$) when compared to mesocephalic dogs. The observed hematological patterns showed mild leukocytosis, possibly indicating subclinical inflammation. Conversely, albumin (3 ± 0.5, $p = 0.009$), direct bilirubin (0.05 ± 0.04, $p = 0.026$), and ALP(57.6 ± 27.8, $p < 0.001$) exhibited lower values in brachycephalic dogs when compared to Beagles.

All variables were assessed among the BOAS groups and the Control group. Red blood cell variables, hemoglobin, hematocrit, platelets, total bilirubin, and indirect bilirubin did not exhibit a significant difference ($p > 0.05$) (Table 3). However, creatinine demonstrated a p-value of < 0.001, indicating elevated values in brachycephalic dogs compared to the Control group, with higher rates observed in BG2 (1.19 ± 0.17) (Table 3).

Lymphocytes, rod, neutrophil-lymphocyte ratio, urea, ALT, AST, GGT, triglycerides, cholesterol, and direct bilirubin did not exhibit a statistically significant difference ($p > 0.05$) (Table 4). Meanwhile, leukocytes ($p = 0.002$), neutrophils ($p = 0.018$), and monocytes ($p = 0.001$) demonstrated an increase in BG1, while eosinophils ($p \leq 0.001$) displayed higher values in BG2 and BG3 (Table 4). Although lymphocytes did not reach statistical significance ($p = 0.261$), they displayed significant variation in brachycephalic dogs, particularly in BG1 and BG3 (Table 4).

B-mode ultrasonography of the gastrointestinal tract

The thickness of the gastric body, pyloric antrum, duodenum, jejunum, ileum, and ascending colon was analyzed, revealing a significant difference only in the thickness of the ascending colon ($p = 0.02$) between BG1 and BG2 (Table 5). Despite the differences shown in the ascending colon, it is noteworthy that these values remain within the normal range. The variables reflecting the thickness of the gastric fundus, pyloric sphincter, and transverse and descending colon did not exhibit a significant difference ($p < 0.05$), as indicated in Table 6.

Quantitative evaluation

A detailed analysis of echogenicity was presented regarding the grades of BOAS (Table 7) and different segments of the GIT (Table 8). The quantitative assessment of duodenum and jejunum pixels revealed disparities between the BOAS groups and the CG, despite no differences in intestinal wall thickness among groups. Specifically, higher pixel values were noted in BG1 animals within the duodenum. In the jejunum, groups BG0, BG1, and BG2 exhibited a significant increase in quantitative indices.

Upon analyzing the BOAS groups and echogenicity, pixel values were correlated with the BCS variable of brachycephalic dogs and the CG. The stomach and jejunum variables did not exhibit a statistically significant difference ($p = 0.326$ and $p = 0.266$, respectively). Duodenal mucosal echogenicity showed a weak negative correlation with BCS (correlation coefficient = -0.259, $p = 0.0345$), suggesting that adipose tissue may interfere with deeper structure evaluation.

DISCUSSION

This original study conducted measurements of the thickness and echogenicity of different parts of the gastrointestinal tract in dogs with BOAS. This seems to be the first study to quantitatively assess intestinal echogenicity in dogs affected by BOAS. Compared to mesocephalic dogs, brachycephalic dogs exhibited higher echogenicity in the duodenum and jejunum, measured in pixels. Additionally, they exhibited increased thickness of the ascending colon and changes in laboratory tests, including elevated levels of leukocytes, neutrophils, lymphocytes, eosinophils, monocytes, CRP, and creatinine.

The pixel data reference in healthy dogs comes from Tcygansky et al. (2020). In brachycephalic dogs, quantitative measurements of echogenicity in the duodenal and jejunal mucosa were higher, suggesting potential inflammation or alterations in the composition of the intestinal wall, possibly including fibrosis (Penninck et al. 1989). Interestingly, no correlation was observed between intestinal echogenicity changes and the degree of brachycephaly, as no differences were observed in the segments of the GIT of BG3 compared to the CG and other groups. The lack of difference in BG3 may result from a small sample size in this group. The variability of pixels among these animals suggests that a larger sample size of BOAS grade 3 dogs may be necessary to confirm GI

Table 3. Means and standard deviations of hematological and biochemical variables distributed among the different grades of BOAS and control groups, analyzed by ANOVA

Variable	BOAS grade					p
	CG (N=15)	BG0 (N=14)	BG1 (N=15)	BG2 (N=11)	BG3 (N=12)	
RBC (106/µL)	6.99 ± 0.73	7.14 ± 0.79	6.95 ± 0.64	7.6 ± 0.68	7.2 ± 1.95	0.194
HGB (g/dL)	16.21 ± 1.87	16.15 ± 1.53	16.25 ± 1.68	17.97 ± 1.97	17.12 ± 1.95	0.062
HTC (%)	48.47 ± 5.18	49.93 ± 4.48	49.6 ± 3.83	53.82 ± 5.13	51.59 ± 2.3	0.065
PLT (10^3/µL)	297.47 ± 95.67	359.86 ± 152	297.8 ± 131.43	337.64 ± 82.38	319.5 ± 120.83	0.870
Creatinine	0.85 ± 0.10 b	1.12 ± 0.15 a	1.04 ± 0.17 a	1.19 ± 0.17 a	1.08 ± 0.24 a	< 0.001*
IB (mg/dL)	0.1 ± 0.05	0.13 ± 0.06	0.13 ± 0.08	0.11 ± 0.06	0.15 ± 0.05	0.240
TB (mg/dL)	0.17 ± 0.06	0.17 ± 0.06	0.18 ± 131.43	0.16 ± 0.06	0.20 ± 0.07	0.466

BOAS = brachycephalic obstructive airway syndrome, CG = control group, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade 3, RBC = red blood cell, HGB = hemoglobin, HTC = hematocrit, PLT = platelet count, IB = indirect bilirubin, TB = total bilirubin, a,b = means followed by the same lowercase letter in the column did not differ statistically according to the Tukey test ($p > 0.05$); * Significant variables ($p < 0.05$).

Table 4. Medians and 25th and 75th percentiles of hematological and biochemical variables distributed among the different grades of BOAS and the control group, analyzed by the Kruskal-Wallis test

Variable	BOAS grade					*p*
	CG (N=15)	BG0 (N=14)	BG1 (N=15)	BG2 (N=11)	BG3 (N=12)	
Albumin (g/dL)	3.3 [3.2 – 3.5] a	2.8 [2.4 – 3.1] b	2.7 [2.5 -3.4] ab	3.1 [2.7 – 3.4] ab	3.4 [2.9 – 3.5] ab	0.028*
ALP (U/L)	99.5 [76.7 – 124.4] a	66.3 [45.5 – 82.9] ab	49.8 [41.5 – 58.0] b	41.5 [41.5 – 56.0] b	45.6 [37.3 – 58.0] b	< 0.001*
ALT (µL)	41.9 [36.67-57.62]	34.05 [26.19-36.37]	36.67 [22.26-41.9]	31.43 [27.5-41.9]	39.28 [23.57-49.76]	0.073
AST (µL)	31.43 [26.19-45.83]	31.43 [26.19-36.37]	26.19 [20.95-35.36]	31.43 [26.19-29.28]	31.43 [31.43-36.67]	0.322
Band cells (µL)	68 [0-110.75]	97 [0-244]	95 [0-144]	81 [0-96.75]	90.5 [73.5-165]	0.700
Cholesterol (mg/dL)	213.2 [177.32-257.8]	203.5 [184.9-249.4]	193.5 [170.92-221.92]	216 [173.05-223.27]	220.2 [193.75-257.5]	0.848
CRP	0.5 [0.0 – 1.2] b	2.2 [1.3 – 3.8] a	2.4 [0,9 – 3,4] a	1,7 [1,2 – 2,1] ab	1,4 [1,2 – 3,2] a	0.003*
DB (mg/dL)	0.06 [0.06-0.09]	0.03 [0-0.06]	0.06 [0.02-0.09]	0.06 [0-0.06]	0.03 [0.01-0.09]	0.109
Eosinophils (µL)	83 [68 – 189] b	394 [322 – 670] a	306 [154 – 684] a	504 [243 – 753] a	400 [273 – 716] a	< 0.001*
GGT (U/L)	7.65 [7.65-7.65]	7.65 [0-7.65]	7.65 [7.65-7.65]	7.65 [7.65-7.65]	7.65 [7.65-7.65]	0.074
Lymphocyte (µL)	1,518 [1,267.5-1,833]	2,008.5 [1,560-2,684]	1,800 [1,435.5-3,196.75]	1,651 [1,195.25-2,205.75]	2,046.5 [1,228-3,192.5]	0.261
Monocyte (µL)	166 [148 – 196] b	412 [249 – 525] a	264 [159 – 374] ab	216 [150 – 314] ab	242 [192 – 328] ab	0.001*
Neutrophil (µL)	4,899 [4,352 – 5,799] b	8,992 [5,395 – 10,293] a	6,670 [5,046 – 9,114] ab	7,742 [5,644 – 9,087] ab	6,828 [5,694 – 8,181] ab	0.018*
N/L ratio	3.36 [2.98-4.02]	3.53 [2.5-4.87]	3.19 [2.62—4.22]	4.94 [3.87-6.02]	3.63 [2.67-4.81]	0.451
Total protein (g/dL)	6,1 [6,0 – 6,6] b	6,7 [6.6 – 7.1] ab	7.4 [7.0 – 7.7] a	7.1 [6.6 – 7.5] a	7.2 [6.9 – 7.8] a	< 0.001*
Triglycerids (mg/dL)	63.74 [46.2-87.84]	61.35 [54.18-75.69]	60.55 [46.4-89.23]	59.76 [43.42-64.14]	64.53 [4]	0.880
Urea	29.31 [23.63-30.36]	24.52 [10.47-46.66]	27.52 [16.3-46.81]	34.1 [18.1-45.02]	32.9 [20.93-40.68]	0.762
WBC (µL)	6,800 [6,450 – 7,975] b	12,800 [8,300 – 14,700] a	9,500 [7,750 – 13,300] ab	9,800 [8,275 – 12,300] a	9,100 [7,900 – 11,950] ab	0.002*

BOAS = brachycephalic obstructive airway syndrome, CG = control group, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade 3, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRP = C reactive protein, DB = direct bilirubin, GGT = gamma-glutamyl transferase, N/L ratio = neutrophil-to-lymphocyte ratio, WBC = white blood count, a,b = medians followed by the same lowercase letter in the column did not differ statistically according to the Dunn test ($p > 0.05$); *Significant variables ($p < 0.05$).

Table 5. Mean and standard deviation of the thickness of gastrointestinal segments distributed among the different grades of brachycephalic obstructive airway syndrome (BOAS) and the control group (CG), by analysis of variance, with Tukey's post hoc test

Variable (mm)	BOAS grade					*p*
	CG (N=15)	BG0 (N=14)	BG1 (N=15)	BG2 (N=11)	BG3 (N=12)	
Gastric body	3.61 ± 0.6	3.86 ± 0.99	3.77 ± 0.75	3.72 ± 0.94	3.58 ± 0.75	0.889
Antrum	3.89 ± 0.9	4 ± 0.84	4.44 ± 1.02	4.63 ± 1.2	4.17 ± 1.2	0.336
Duodenum	4.27 ± 0.6	4.67 ± 0.5	4.9 ± 0.75	4.95 ± 0.95	4.79 ± 0.86	0.111
Jejunum	3.26 ± 0.7	3.56 ± 0.76	3.58 ± 1.03	3.58 ± 0.61	3.59 ± 0.53	0.748
Ileum	2.8 ± 0.7	2.07 ± 0.39	2.62 ± 0.82	2.81 ± 0.83	2.25 ± 0.35	0.257
Ascending colon	1.84 ± 0.37 ab	1.65 ± 0.38 ab	1.57 ± 0.43 b	2.12 ± 0.49 a	1.92 ± 0.52 ab	0.020*

BOAS = brachycephalic obstructive airway syndrome, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade 3, a,b = means followed by the same lowercase letter in the column did not differ statistically according to the Tukey test ($p > 0.05$); * Significant variables ($p < 0.05$).

alterations. In this context, a larger sample size and further histopathological studies to confirm whether echogenicity changes reflect inflammation or fibrosis are required to elucidate the reasons behind intestinal hyperechogenicity, particularly in dogs affected by grade 3 BOAS, given the absence of validated scores for the quantitative evaluation of the gastrointestinal tract in these patients.

Tcygansky et al. (2020) observed increased echogenicity of the gastric mucosa in comparison to the duodenum and jejunum; nevertheless, the ratio between the pixel values of the mucosa remained consistent. This escalation in echogenicity was associated with the predominance of villi and crypts in the lamina propria of the mucosa in these intestinal segments, rendering them more hypoechoic. This finding supports the quantitative values of the duodenum and jejunum of the CG animals in the present study, which showed no disparity between them but exhibited significant differences in echogenicity compared to the gastric mucosa (Table 2). Notably, an average increase of two to three times in the pixel count was observed in the gastric mucosa relative to the duodenum and jejunum.

Nevertheless, the precise cause behind the heightened gastric echogenicity relative to the intestinal segments in the present study remains uncertain, as there was no discernible difference between the CG and BOAS groups, warranting biopsy for definitive tissue characterization. Several factors could have influenced and limited this outcome, including the type of diet, feeding habits, individual behavior, and other non-standardized criteria in our study. Additionally, in brachycephalic dogs, BOAS itself may predispose them to gastric alterations (Kaye et al. 2018).

The measurement of gastrointestinal thickness is considered a standard tool in ultrasound evaluation and is routinely conducted. However, there is conflicting information in the literature regarding the correlation between intestinal thickness and disease diagnosis. In one study, reference values for intestinal thickness in dogs weighing up to 20 kg were established as ≤ 5.1 mm for the duodenum and ≤ 4.1 mm for the jejunum (Delaney et al. 2003). Another study involving dogs weighing less than 15 kg reported duodenal thickness of 3.8 ± 0.5 mm and jejunal thickness of 3 ± 0.5 mm (Gladwin et al. 2014). The thickness of the colon in the same dogs was

Table 6. Median and 25th and 75th percentiles of the thickness of gastrointestinal segments distributed among the different grades of brachycephalic obstructive airway syndrome (BOAS) and the control groups by the Kruskal-Wallis test

Variable (mm)	BOAS grade					*p*
	CG (N=15)	BG0 (N=14)	BG1 (N=15)	BG2 (N=11)	BG3 (N=12)	
Gastric fundus	3.4 [2.7-3.9]	3.6 [3.2-4.9]	3.4 [3.3-4.37]	3.9 [3.15-4.52]	3.95 [3.7-4.3]	0.351
Pyloric sphincter	4 [4-4.65]	4.5 [3.57-5.37]	4.35 [3.2-5]	4.1 [3,35-5.3]	3.7 [3.3-4.5]	0.541
Transverse colon	1.5 [1.42-1.67]	1.4 [1.17-1.82]	1.6 [1.42-2.65]	1.9 [1.52-2.5]	2.15 [1.55-2.3]	0.450
Descending colon	1.4 [1.3-1.85]	1.45 [1.3-1.8]	1.5 [1.3-1.9]	1.7 [1.35-1.9]	1.4 [1.25-1.6]	0.545

BOAS = brachycephalic obstructive airway syndrome, CG = control group, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade 3.

Table 7. Medians and 25th and 75th percentiles of echogenicity among the different grades of brachycephalic obstructive airway syndrome (BOAS) within groups, analyzed by the Kruskal-Wallis test

Group	Stomach	Duodenum	Jejunum
CG	25.8 [18.3-35.6]	10.2 [7.1-14.9] b	6.9 [4.6-11.7] b
BG0	26.9 [17.6-38.9]	14.9 [11.5-23.8] ab	26.7 [21.7-46.1] a
BG1	28.8 [17.8-31.8]	21.8 [15.6-32.6] a	25.2 [19.7-40.9] a
BG2	19.1 [15.9-25.2]	12.8 [9-26.3] ab	28.6 [15.6-37.4] a
BG3	22.4 [17.5-32.6]	11.4 [7-13.7] b	10.1 [6.4-29.7] ab
p-value	0.659	0.004*	< 0.001*

CG = control group, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, GB3 = brachycephalic dogs classified as functional grade 3, a,b = medians followed by the same lowercase letter in the column did not differ statistically according to the Dunn test ($p > 0.05$); * Significant variables ($p < 0.05$).

Table 8. Medians and 25th and 75th percentiles of echogenicity among the portions of the gastrointestinal tract (GIT) within groups, analyzed by the Kruskal-Wallis test

	CG	BG0	BG1	BG2	BG3
Stomach	25.8 [18.3-35.6] a	26.9 [17.6-38.9] ab	26 ± 9.5	22.2 ± 11.1	22.4 [17.5-32.6] a
Duodenum	10.2 [7.1-14.9] b	14.9 [11.5-23.8] b	24.5 ± 12.6	17.4 ± 9.5	11.4 [7 – 13.7] b
Jejunum	6.9 [4.6-11.7] b	26.7 [21.7-46.1] a	28.3 ± 14.4	28.2 ± 17.9	10.1 [6.4 -29.7] ab
p-value	<0.001*	0.014*	0.714	0.197	0.017*

CG = control group, BG0 = brachycephalic dogs without BOAS, BG1 = brachycephalic dogs classified as functional grade 1, BG2 = brachycephalic dogs classified as functional grade, BG3 = brachycephalic dogs classified as functional grade 3, a,b = medians followed by the same lowercase letter in the column did not differ statistically according to the Dunn test ($p > 0.05$); * Significant variables ($p < 0.05$).

measured at 1.5 ± 0.3 mm (Gladwin et al. 2014). However, this study did not distinguish the measurements between different colonic portions (Gladwin et al. 2014). Despite differences observed in the comparison between cranial conformation and duodenal thickness, as well as among the grades of BOAS, CG, and ascending colon, it is noteworthy that these values remain within the normal range.

In addition to possible changes in laboratory and imaging results, animals with chronic enteropathies may present persistent or intermittent clinical signs, such as increased defecation frequency and decreased fecal consistency and volume (Allenspach & Gaschen 2003). It was not possible to correlate the presence and severity of clinical signs with the severity of BOAS. The limited number of valid questionnaires (n = 28) prevented statistical correlation between clinical signs and BOAS severity. However, it was evident that brachycephalic animals tended to display gastrointestinal alterations, consistent with findings from previous studies (Aslanian et al. 2014, Shaver et al. 2017, Broux et al. 2018, Kaye et al. 2018). According to the questionnaire completed by the owners, most animals experiencing changes in fecal consistency belonged to BG0 and BG1. Other noticeable signs included flatulence, eructation, gastroesophageal reflux, regurgitation, and vomiting, observed in over 50% of the animals. Despite gastrointestinal signs in brachycephalic dogs, increased gastric echogenicity in quantitative ultrasound assessments may not reliably correlate with vomiting and gastritis, as even in clinically asymptomatic dogs, echogenic changes were present.

The increase in eosinophils and monocytes was notable in brachycephalic dogs, although they remained within the normal range. Similar findings were reported previously (Facin et al., 2020), where brachycephalic dogs exhibited elevated counts of leukocytes, eosinophils, and monocytes, as well as lymphocytes and neutrophils, and a neutrophil-to-lymphocyte ratio. The authors of that study suggested a potential subacute inflammatory condition based on hepatic elastography changes observed in these dogs (Facin et al. 2020). However, contrasting results were found in another study, which did not observe disparities in total leukocyte count, neutrophils, and monocytes, and noted lower lymphocyte and eosinophil levels when comparing brachycephalic and non-brachycephalic dogs (Kämpf et al. 2023). The leukocytosis due to eosinophilia and monocytosis observed in this study suggests a subacute inflammation state in brachycephalic dogs.

Although the hematological results were within the normal range, notable variation was observed in the white blood cell subtypes of brachycephalic dogs, consistent with subclinical inflammatory patterns. This variance did not correlate with the severity of BOAS in the studied sample. However, the literature lacks uniform information to substantiate further discussion on such differences, emphasizing the importance of future laboratory standardizations for these breeds, with a larger cohort of animals. Since reference values are frequently determined based on the animal species, breed peculiarities, and the animal profile within the canine species, are not considered.

To investigate a potential correlation between systemic inflammation and the evaluated clinical and ultrasonographic parameters, CRP was assessed. However, these data did not vary according to the severity of BOAS, as there was a distinction between the BOAS and Control groups. Moreover, the CRP values fell within the normal range, typically below 10 to 20 mg/L (Klenner et al. 2010, Hillström et al. 2014, Hindenberg et al. 2018).

Creatinine levels exhibited variances among the examined groups, with brachycephalic dogs demonstrating elevated levels of this enzyme compared to the GC and across the grades of BOAS. In humans, the bidirectional relationship between OSA and chronic kidney disease has been established (Mirrakhimov 2012, Abuyassin et al. 2015). This association is attributed to renal reabsorption of bicarbonate due to hypercapnia episodes (Eskandari et al. 2017). Elevated creatinine levels were observed in obese patients affected by OSA (Agrawal et al. 2009, Pochetti et al. 2020). It is postulated that intermittent hypoxia, coupled with tubular injury and endothelial dysfunction, exacerbates renal function in these patients, particularly in cases involving comorbidities such as diabetes and hypertension (Kato et al. 2011). Similar veterinary studies are lacking, making this finding novel and noteworthy.

The correlation analysis between the pixel values of duodenal mucosa echogenicity revealed that a higher BCS corresponded to lower pixel values for the duodenum, implying that adipose tissue may influence the assessment of deeper structures, as subcutaneous fat may attenuate ultrasound signals, affecting echogenicity. Despite being statistically significant, the correlation between these variables was considered low. A similar observation of a low correlation between BCS and hepatic elastography values was reported in another study (Facin et al. 2020). However, in the cited study, the alteration in elastography values was deemed independent of the influence of the BCS (Facin et al. 2020).

In the present study, the correlation between higher pixel averages in the duodenal and jejunal mucosa of dogs affected by BOAS, the elevation in leukocytes, neutrophils, eosinophils, and monocytes, alongside a high prevalence of gastrointestinal clinical signs in this population, implies a potential involvement of these changes in the context of subacute inflammation commonly observed in these animals. These findings support the hypothesis that intestinal inflammation may represent a secondary manifestation of BOAS.

This study had limitations. Firstly, the age range determined for the study dogs might have resulted in the inclusion of patients in the early stages of gastrointestinal alterations, thereby introducing heterogeneity into the diseased group due to the wide age variation. Secondly, quantitative analysis of the large intestine mucosa was not conducted, precluding a correlation between the thickness of these portions and echogenicity. Thirdly, there was no prior standardization of dog feeding. Standardization is challenging due to the characteristics of each animal, a common challenge in clinical trials as opposed to working with experimental animals. The lack of dietary standardization is a common challenge in clinical veterinary research and should be addressed in future studies. Maximizing efforts to standardize groups and animals to the greatest extent possible is imperative for research involving routine patients.

CONCLUSION

Brachycephalic dogs exhibited higher echogenicity in the duodenum and jejunum compared to mesocephalic dogs. They also showed elevated leukocyte and eosinophil counts, as well as increased C-reactive protein (CRP) levels, although within species reference ranges. These findings reinforce the potential gastrointestinal impact of brachycephalic obstructive airway syndrome (BOAS) and highlight the clinical utility of quantitative ultrasonography as a practical, non-invasive diagnostic tool in veterinary medicine.

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Data availability statement.- The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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