

Alleviation of intestinal permeability and reduction of serum zonulin levels in canine atopic dermatitis using low-volume manual colonic hydrotherapy: A "treat-to-target" approach focused on the gut-brain-skin axis.

Alivio de la permeabilidad intestinal y reducción de los niveles séricos de zonulina en la dermatitis atópica canina por medio de la hidroterapia manual de colon de bajo volumen: Enfoque de «tratamiento por objetivos» centrado en el eje intestino-cerebro-piel

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ABSTRACT

Practical usage of colonic cleansing known as colon hydrotherapy for promoting total health status and beneficial effects still remain unclear among veterinary field population. Both interventions have been frequently claimed as adjuvant therapy for enhancing of longevity/vitality, and as therapeutic resource against chronic degenerative disease. Evidence supporting the use of colon hydrotherapy for detoxification has not been fully investigated in small animal practice. Therefore, we hypothesized that low volume colon hydrotherapy could be capable of alleviating gastrointestinal discomfort. Out of 218 dogs examined within the scope of the study, 79 met the study criteria. Clinical examinations were conducted on days 0 and 7, and the Canine Atopic Dermatitis Extent and Severity Index version 4. In addition, the Visual Analog Scale, Polycheck in vitro allergy test, epidermal corneometric analyses for skin mapping, and dermatoscopy were used. To assess intestinal permeability, serum zonulin levels were measured. Boston Bowel Preparation Scale scores were measured with a median of 2.00 (0.00–7.00) before treatment and a median of 6.00 (0.00–9.00) after treatment. Zonulin levels were decreased from 28.8 ± 1.16 to 3.95 ± 0.27 ng/mL after treatment. In conclusion, colon hydrotherapy in dogs with dermatological problems provides favorable improvements in bowel cleansing, improves leaky gut conditions, and contributes to the recovery of dermatological diseases.

Keywords: Canine atopic dermatitis, colon hydrotherapy, veterinary, zonulin

RESUMEN

El uso práctico de la limpieza de colon, conocida como hidroterapia de colon, para promover la salud integral y generar efectos beneficiosos sigue sin estar claro en el ámbito veterinario. Ambas intervenciones se han señalado frecuentemente como terapias coadyuvantes para aumentar la longevidad y la vitalidad, así como recursos terapéuticos frente a enfermedades crónicas degenerativas. La evidencia que respalda el uso de la hidroterapia de colon para la desintoxicación no se ha investigado exhaustivamente en la clínica de pequeños animales. Por ello, planteamos la hipótesis de que la hidroterapia de colon de bajo volumen podría aliviar las molestias gastrointestinales. De los 218 perros examinados en el estudio, 79 cumplieron con los criterios de inclusión. Se realizaron exámenes clínicos los días 0 y 7, empleando el índice CADESI-4 (*Canine Atopic Dermatitis Extent and Severity Index*, versión 4). Asimismo, se utilizaron la escala visual analógica, la prueba de alergia *in vitro* Polycheck, análisis corneométricos epidérmicos para el mapeo cutáneo y dermatoscopia. Para evaluar la permeabilidad intestinal, se midieron los niveles séricos de zonulina. Las puntuaciones en la escala de preparación intestinal de Boston (*Boston Bowel Preparation Scale*) mostraron una mediana de 2,00 (0,00–7,00) antes del tratamiento y de 6,00 (0,00–9,00) después del mismo. Los niveles de zonulina disminuyeron de $28,8 \pm 1,16$ a $3,95 \pm 0,27$ ng/mL tras el tratamiento. En conclusión, la hidroterapia de colon en perros con problemas dermatológicos favorece la limpieza intestinal, mejora las condiciones asociadas al síndrome de intestino permeable y contribuye a la recuperación de enfermedades dermatológicas.

Palabras Clave: Dermatitis atópica canina, hidroterapia de colon, veterinaria, zonulina

INTRODUCTION

In recent years, there has been increasing evidence that skin diseases may be associated not only with local epidermal factors but also with systemic gastrointestinal, immune, and microbial factors (Janssen et al., 2024). The gut-immune-skin axis, particularly in humans, suggests that increased intestinal permeability, microbiota dysbiosis, and the type 2 immune response play a central role in the development of conditions such as food allergies and atopic dermatitis (Janssen et al., 2024). Similarly, in dogs showed a significant change in both the skin and intestinal microbiota have been identified in cases of atopic dermatitis characterized by chronic pruritus and epidermal barrier dysfunction (Huang et al., 2024). In this context, it has been suggested that gastrointestinal interventions such as probiotic administration or rectal irrigation may not only improve intestinal health but also alleviate dermatological symptoms (Ural et al., 2021). Regulation of inflammation through modulation of the gut microbiota has been supported, particularly by lowering gut permeability biomarkers such as zonulin (Alic Ural et al., 2022a, 2022b). Zonulin is a key regulator of tight-junction function and serves as a biomarker of intestinal permeability, making it relevant for evaluating gut-skin axis disturbances (Alic Ural et al., 2021a, 2021b).

In recent decades, with the widespread use of non-invasive supportive treatments and the maintenance of gastrointestinal health, colonic irrigation (colon hydrotherapy) has increased significantly (Acosta and Cash, 2009). Clearing the colon of residual material not only increases the success rate of diagnostic procedures such as colonoscopy but also offers potential benefits in reducing intestinal inflammation and regulating intestinal permeability (Bacsur et al., 2023; Rezamand et al., 2023). Objective assessment of colonic cleansing is crucial for clinical practice. The Boston Bowel Preparation Score (BBPS), developed for this purpose, allows for the determination of a total cleansing score by separately evaluating three anatomical regions of the colon (right, transverse, and left). Numerous studies have demonstrated that the BBPS is a reliable and valid tool for assessing residual content after irrigation (Rezāmand et al., 2023; Bacsur et al., 2023). Furthermore, the BBPS is not only limited to assessing mucosal visibility but can also be used as an indirect marker in the monitoring of inflammatory conditions. Indeed, this scale has been used not only with classical laxative protocols but also to measure the effectiveness of adjunctive practices such as exercise that increase intestinal motility (Rezāmand et al., 2023). Taken together these findings suggest that colon hydrotherapy is not merely a supportive cleansing procedure but can also be an important component in holistic approaches to gastrointestinal health.

The aim of this study was to evaluate the effects of colon hydrotherapy via rectal irrigation on gastrointestinal cleaning, intestinal inflammation, and permeability in dogs through the Boston Bowel Preparation Score (BBPS) and zonulin levels to investigate the potential implications of this practice on dermatological symptoms related to the gut-skin axis.

MATERIAL AND METHODS

Demographic data

In a total of 218 dogs inspected, evaluated and diagnosed/treated, 79 met inclusion criteria. All animals were client-owned dogs presented to the Aydın Adnan Menderes University, Animal Hospital Small Animal Veterinary- Internal Medicine Clinic Intestinal Permeability Measurement Center (İPÖM). Complete physical examination were performed on days 0 and 7. First inclusion criteria involved The Canine Atopic Dermatitis Extent and Severity Index version 4 (CADESI-04); this severity scale has been in general practice validated for grading cutaneous lesions comprising twenty body sides selectively affected among atopic dogs with proposed benchmarks for mild (10), moderate (35), and severe (60) respectively (Olivry et al., 2014), Visual Analog Scale, designated as a ruler pointed out as 0 (normal dog), >5 (mild itching), >6 (moderate itching), >8 (severe itching) which has been observed by the animal owner or the referring veterinary surgeon (Cosgrove et al 2013, Hill et al 2007), Polycheck in vitro allergy

test; in vitro allergen specific Immunoglobulin E (IgE) among dog sera was preferred comprising twenty common allergens for determining underlining etiology), epidermal corneometric analytes (hydration and pH) for skin mapping (Ural, 2022, Erdoğan et al 2024), dermatoscopy (Ural et al 2023). The case records for canine atopic dermatitis were searched from early 2024 to late June 2025. Inclusion criteria consisted of a confirmed diagnosis of canine atopic dermatitis, availability of CADESI-04 scoring, VAS pruritus assessment, and completion of day-0 and day-7 examinations. Dogs with systemic illness, concurrent infections, recent corticosteroid/immunosuppressive treatment (within 4 weeks), or incomplete medical records were excluded.

Outcome measures

The primary outcome measures were CADESI-04, epidermal corneometric analytes (Ural, 2022, Erdoğan et al 2024) BBPS (Lai et al 2009), intestinal permeability measurement where analyzed at İPÖM facilities by use of serum zonulin levels as detected by Sandwich ELISA through usage of previous methodology (Alic Ural et al 2021a, Alic Ural et al 2021b, Alic Ural, 2022a, Alic Ural, 2022b, Alic Ural, 2022c, Alic Ural, 2023, Alic Ural and Ural, 2023). Regarding relevant analysis, responders will be synonymous with dogs receiving a full response (clinical remission involving erythema, alopecia and pruritus, as the vast majority of clinical signs along with diminished CADESI-04 scores, lowered BBPS and zonulin levels. Secondary outcome measurement was breath nitric oxide determination by usage of Sunvou Breath Analyzer for detecting fractional exhaled nitric oxide, as a related marker of Type 2 inflammation.

Diagnostic algorithm

The present investigation was performed as a prospective assembly of obtained laboratory work and relevant results at unofficial Intestinal Permeability Measurement Center (İPÖM) located at Faculty of Veterinary, Department of Internal Medicine. This study was thoroughly approved by the Aydın Adnan Menderes University Local Ethics Committee (ADÜ-HADYEK) with document no: 64583101/2023/162. Furthermore, written owner consent was collected. All dogs were subjected to i) fractional exhaled nitric oxide (FeNO) through Sunvou Nano Coulomb Breath Analyzer CA2122 (Huang et al 2021, Lei et al 2021) in an attempt to detect low grade systemic inflammation, ii) canine atopic dermatitis inclusion criteria.

Classification of responders and non-responders

Even if a dog was detected as non-responder [covering dogs with partial or no response] an even if relapse episodes were described (both by owners and veterinary surgeon performing physical examination daily/weekly by face to face or by photographic records). Outcome measures were archived at the time of the last follow-up. Even if CADESI-04 scores were diminished and epidermal corenometric analytes (through increased epidermal hydration and pH) were changed, this case was classified as responder. Moreover, during cH, BBPS scoring was used to pre- and post-treatment colonic cleansing efficacy during cH.

Low dose cH methodology

Briefly rectal placement of catheter performed as was previously described (Ural et al 2021, Ural et al 2022, Ural, 2024). A rectal catheter was placed 15 cm apart from rectum (a) (Ural et al 2021, Ural et al 2022, Ural, 2024) and Lactated Ringer' solution (Ural, 2024) was given in low volume, 50 mL per each consecutive session for 10 times in a total reached volume of 500 ml (Figure 1).



Figure 1. Rectal catheter was placed a) Low dose lactated Ringer's Solution was infused, b) cleansing and draining c) residual fecal material located at catheter, d) exactly fulfilled emptying of bowel ingredient.

BBPS scoring

In an attempt to detect BBPS scoring, all 79 out of 218 dogs were subjected to colonoscopy with a relatively short-term local anesthesia given from rectal route; prior to cH and BBPS scoring. Regarding topographical anatomical features of colon (each location), were denoted a "segment score" via 0 to 3. Subjective terminology from excellent to poor, were substituted through a 4-point scoring classification intended to the right colon (involving cecum and ascending colon), the transverse colon (also composed of hepatic and splenic flexures), and the left colon (sum of descending/sigmoid colon parts and rectum).

To obtain the final score, the sum was calculated to obtain a total BBPS score ranging from 0 to 9. The maximum score for a cleansed colon free of any debris was 9, and the minimum BBPS score for an unqualified colon was 0 (Lai et al. 2009).

If an endoscopy was canceled during the examination, the segment in the proximal area that could not be observed was assigned that score. In this context, the 10-point BBPS, developed by the endoscope operator to help assess bowel preparation after all cleansing maneuvers were completed, was used (Lai et al. 2009). Although the BBPS was originally developed for endoscopic assessment, no endoscopy was performed in this study. The reference to canceled endoscopy reflects the original scoring method described by Lai et al. (2009) and does not represent a procedure used in our protocol.

Determination of serum zonulin levels

To those of dogs enrolled (n=79) withdrawn 0.7 ml blood samples from *vena jugularis* into anticoagulated (Lithium heparin) tubes, were then separated and stored at 80° C (Celsius degree), for at least for 8 weeks. All samples were then analyzed with Sandwich ELISA (Alic Ural et al 2021a, Alic Ural et al 2021b, Alic Ural, 2022a, Alic Ural, 2022b, Alic Ural, 2022c, Alic Ural, 2023, Alic Ural and Ural, 2023) reader by use of commercially available dog zonulin kits (MyBiosource ELISA kits, USA). Reference ranges were deemed available based on manufacturer and methodology was similar to what have been reported (Alic Ural et al 2021a, Alic Ural et al 2021b, Alic Ural, 2022a, Alic Ural, 2022b, Alic Ural, 2022c, Alic Ural, 2023, Alic Ural and Ural, 2023). Reference ranges for Sandwich ELISA were deemed available as maximum-minimum 100 ng/ml -1,56 ng/ml, respectively [sensitivity 0.5 ng/ml]. Briefly, serum samples were added into pre-coated wells together with the HRP-conjugate, incubated for 1 h at 37 °C, and the wells were washed five times. Substrate solution (TMB) was then added and allowed to develop for 10 min, followed by the stop solution. Absorbance was read at 450 nm, and zonulin concentrations were calculated from the standard curve.

Statistical analyses

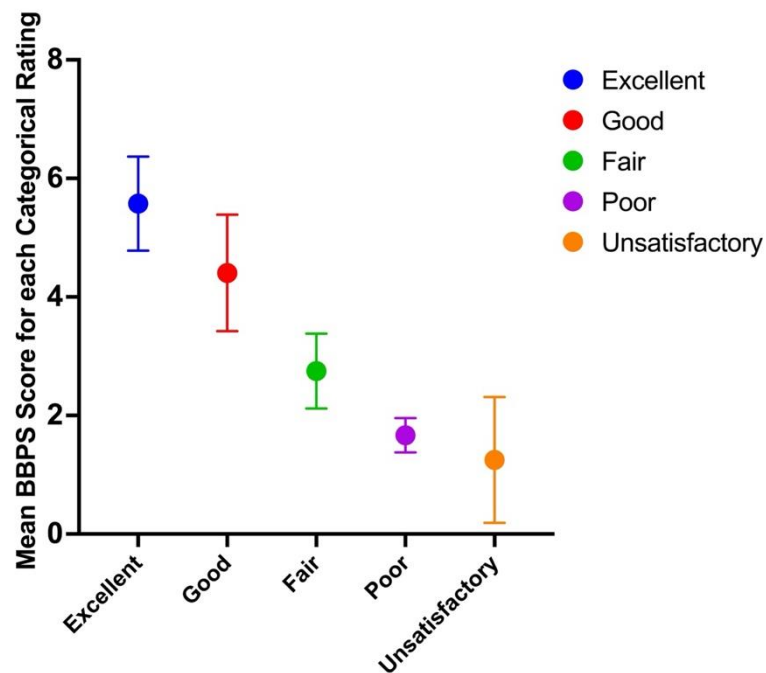
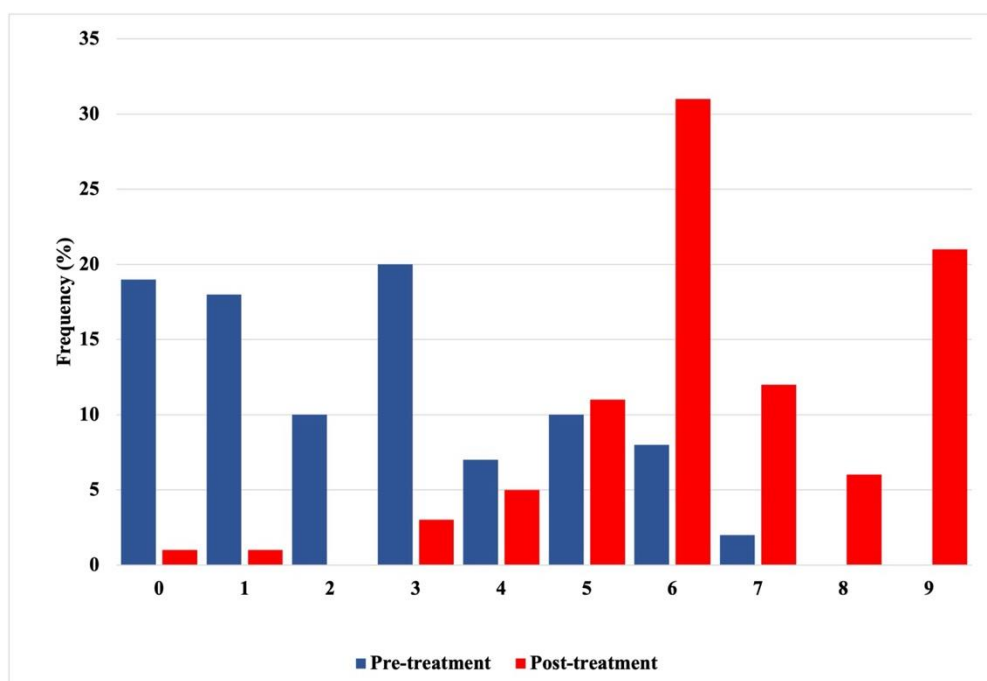
Data were analyzed using GraphPad Prism software (GraphPad Software, USA). Statistical significance was set at $p < 0.05$. The variables evaluated in this study included CADESI-04 scores, VAS pruritus scores, epidermal corneometric parameters including skin hydration and skin surface pH, BBPS scores, serum zonulin concentrations, and fractional exhaled nitric oxide (FeNO) values. Descriptive statistics were calculated for all variables. The normality of continuous variables was assessed using the Shapiro–Wilk test. According to the Shapiro–Wilk test results, BBPS scores and serum zonulin concentrations did not meet the assumptions of normal distribution; therefore, non-parametric tests were used for these variables. Pre- and post-treatment BBPS scores were compared using the Wilcoxon signed-rank test. Similarly, pre- and post-treatment serum zonulin concentrations were compared using the Wilcoxon signed-rank test. Numerical data are expressed as mean $\pm$ standard error of the mean (SEM) and/or median with minimum–maximum values.

RESULTS

Descriptive statistics of BBPS scores, including median and minimum–maximum values, are summarized in Table 1. BBPS scores ranged from a median of 2.00 (min–max: 0.00–7.00) before treatment to a median of 6.00 (min–max: 0.00–9.00) after treatment. Paired data analysis using the Wilcoxon signed-rank test revealed a statistically significant change ($p = 0.001$). The median difference between pre- and post-treatment scores was 5.00 (Table 1, Figure 3, figure 4).

Table 1. Boston Bowel Preparation Scale (BBPS) score data in diseased dogs.

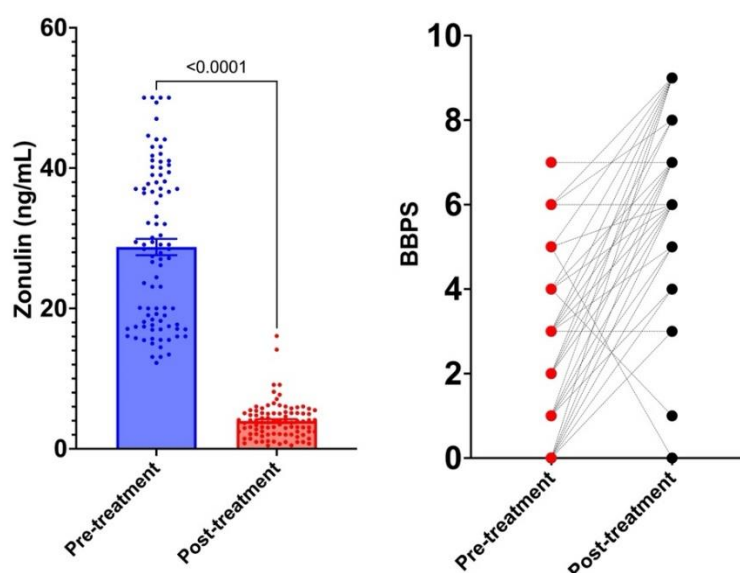
BBPS	Pre-treatment	Post-treatment
Median	2,00	6,00
min-max	0,00-7.0	0,00-9.0
Wicoxon p value	0.001	
Mean of Difference Median	5	

Figure 3. Distribution of BBPS scores in diseased dogs.**Figure 4.** Distribution frequencies of cases according to BBPS scores

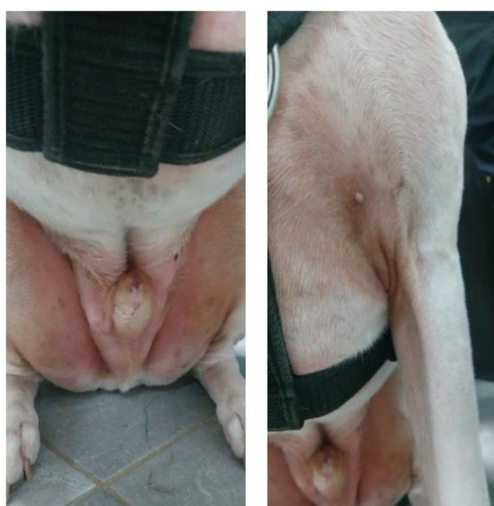
Zonulin levels were measured at a mean of 28.8 ± 1.16 ng/mL before treatment, but this value decreased significantly to 3.95 ± 0.27 ng/mL after treatment ($p=0.001$). The 95% confidence interval for the pre-treatment group was 26.4 to 31.1 ng/mL and for the post-treatment group, it was 3.41 to 4.48 ng/mL (Table 2, Figure 2).

Table 2. Serum zonulin levels before and after treatment in diseased animals.

Serum Zonulin level	Pre-treatment (ng/mL)	Post-treatment (ng/mL)
Mean	28.8	3.95
SEM	1.16	0.27
Lower 95% CI of mean	26,4	3,41
Upper 95% CI of mean	31,1	4,48
Mann-Whitney U test	0.001	

**Figure 2.** Serum zonulin levels and BBPS scores before and after treatment in diseased animals.

While 0% of cases scored at the "Excellent" level before treatment, this rate increased to 34.2% after treatment. Similarly, the 28% of cases in the "Unsatisfactory" category decreased to 1.3% after treatment. The median BBPS score was 2 (IQR: 1–3) before treatment and 7 (IQR: 6–9) after treatment, and the difference was statistically significant ($p < 0.001$) (Figure 3, Figure 4). Dermatological improvement was visually evident after treatment (Figure 5).

**Figure 5.** Dermatological appearance before treatment (left) and after treatment (right)

DISCUSSION

Trans-rectal irrigation has aroused growing interest and has been recognized as minimally invasive intervention against constipation and fecal incontinence (Christensen et al 2006, Emmanuel et al 2013, Christensen et al 2009). Short period of trans-rectal irrigation exhibited beneficial effects to individuals with functional bowel disorders (Falletto et al 2023). In a newly published study trans-rectal irrigation with low volume water against functional faecal incontinence in 47 children. This treatment modality exhibited safe, efficacious active, less invasive and low in cost features (Hougaard et al 2025). These studies, which demonstrate the effectiveness of trans-rectal irrigation as a minimally invasive intervention for constipation and fecal incontinence, have demonstrated that even short-term applications in functional bowel disorders provide clinical benefits. Base on this information, trans-rectal irrigation could be adapted to veterinary clinical practice in situations where similar pathophysiological mechanisms may be involved.

This treatment modality not solely showed efficacy against neurogenic bowel dysfunction, but also to disease activity at gut–brain interaction and postsurgical disorders (Emile et al 2023, Falletto et al 2023, Vollebregt et al 2023). This treatment approach has been reported to be effective not only in neurogenic bowel dysfunctions but also in bowel-brain interaction disorders and post-surgical disease activity reveals the breadth of its application.

In an attempt to detect sufficient intestinal preparation, a well-recognized and established scoring system might be warranted. To this context BBPS, as was also preferred at this study, has been frequently preferred regarding several intestinal preparation scoring utilities since 2009 (Lai et al 2009, Parmar et al 2016). The latter scoring system were composed of a 4-point scoring algorithm (ranged from 0 to 3) denominated to each of the 3 topographical locations of the colon (right, transversal, and left colon) in which each point dedicated to one of 3 colonics segments are summed up for a total BBPS score (ranged from 0–9). Someone might ask the advantage for using BBPS, to those of which this validated scoring has been referred to evaluation following washing and vacuuming colonic ingredient, which is capable of mirroring the proportions of colonic mucosa actually inspected (Lai et al 2009). In this study this scoring system has been preferred as because of visualizing and probably making interpretation for residual volume of colon after cH, to those of calculating its efficacy for diminishing intestinal inflammation. However, it should not be unwise to draw preliminary conclusion that apart from human studies, we herein focused on ascending, transverse and descending colon for interpretation, as because of its nature in topographic anatomy of dogs for colon segments. Based on the need for a reliable and valid scoring system for assessing bowel preparation, the BBPS was chosen for this study. The BBPS allows three segments, each representing a different anatomic region (right, transverse, and left colon), to be scored between 0 and 3, resulting in a total score ranging from 0 to 9. One of the most significant advantages of this system is that it reflects the truly assessable portion of the colonic mucosa after irrigation and aspiration. In our study, after colon irrigation with low-volume lactated Ringer's solution via a rectal catheter, the BBPS allowed objective visual assessment of residual material. The median BBPS score before treatment was 2, and this score increased to 6 after treatment, demonstrating a significant effect of irrigation on colonic cleansing and mucosal visibility. Furthermore, unlike human studies, segmental colon assessment was based on the ascending, transverse, and descending colon, taking into account the anatomical structure specific to dogs. This approach highlights the adaptive and novel nature of the study regarding the use of BBPS in veterinary medicine. Furthermore, the BBPS score was evaluated not only as a measure of cleanliness after irrigation but also as an indirect reflection of intestinal inflammation. When evaluated in conjunction with the zonulin levels used in our study, zonulin levels were found to decrease from 28.8 ng/mL to 3.95. This change was found to be reflected not only in BBPS scores but also in zonulin levels, a key biomarker for the assessment of leaky gut.

The vast majority of researches determined BBPS score ≥ 6 as sufficient (Kluge et al 2018, Clark et al 2016, Calderwood and Jacobson, 2010), dissimilar repercussions were obtained. For instances selected literature suggested ≥ 7 (Clark and Laine, 2016). Kim et al (2024) emphasize that different threshold values may occur in terms of BBPS scores in the intestines and that this is related to the severity of the application performed during bowel preparation depending on the practitioner in different centers. As noted in the studies mentioned above, different threshold values have been suggested for the evaluation of BBPS scores. In our study, BBPS scores obtained after irrigation were detailed using data from three segments of the colon (right, transverse, and left) and analyzed based on the total score. While the median BBPS score was 2.00 before treatment, this value increased to 6.00 after treatment. Furthermore, the proportion of cases achieving excellent cleansing reached 34.2%, demonstrating the cleaning effectiveness of irrigation. These results demonstrate both a significant increase in the score and, with their effects on improving clinical colonic observability, are consistent with findings in previous human studies.

CONCLUSION

In conclusion, this study demonstrates that low-volume colonic irrigation via rectal catheterization in dogs with dermatological complaints provides significant improvements in bowel cleansing, paralleling changes in BBPS scores. The concurrent decrease in serum zonulin levels suggests that this procedure may have positive effects on intestinal permeability. The findings demonstrate that such non-pharmacological and minimally invasive approaches have the potential to contribute not only to colon cleansing but also to the regulation of intestinal inflammation. Larger, controlled studies are warranted to evaluate the long-term clinical effects of this method on gastrointestinal and dermatological veterinary diseases.

Conflict of interest: The authors declare no conflict of interest.

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