Effect of diet and nutraceuticals on antioxidative status in dogs with chronic enteropathies

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Effect of diet and nutraceuticals on antioxidative status in dogs with chronic enteropathies

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ABBREVIATIONS

AREs= Antibiotic-responsive enteropathies

CAT= Catalase

CCECAI= Canine chronic enteropathy clinical activity index

CE= Chronic enteropathies

CIBDAI= Canine inflammatory bowel disease index

CUPRAC= Cupric reducing antioxidant capacity

FOX= Ferrous oxidation-xylenol organ

FRAP= Ferric reduing ability of the plasma

FREs= Food responsive enteropathies

GPx= Glutathione peroxidase

GSH= Glutathione

HPLC= High-performance liquid chromatography

IBD= Inflammatory bowel disease

IREs= Immunosuppressant-responsive enteropathies

MDA= Malondialdehyde

MrMRE= Microbiota-related modulation responsive enteropathy

MSCs= Mesenchymal stem cells

NADPH= Nicotinamide adenine dinucleotide phosphate

NREs= Non-responsive enteropathies

Nrf2= Nuclear factor erythroid 2-related factor 2

PON1= Paraoxonase-1

RER= Resting energy requirement

ROS= Reactive oxygen species

SCCAI= Simple clinical colitis activity index

SCFA= Short-chain fatty acid

SOD= Superoxide dismutase

T-AOC= Total antioxidant capacity

TBA= Thiobarbituric acid

TBARS= Thiobarbituric acid reactive substances

TEAC= Trolox equivalent antioxidant capacity

TSOD= Total superoxide dismutase

UC= Ulcerative colitis

CONTENTS

1. INTRODUCTION

Canine chronic enteropathies (CE) are a group of gastrointestinal diseases characterized by persistent or recurrent signs of digestive dysfunction. The chronic nature of the condition is defined by clinical signs lasting for 3 weeks or longer, and includes a range of gastrointestinal signs such as vomiting, diarrhea, abdominal discomfort, borborygmus, belching and/or weight loss. Exclusion of other diseases is required for a diagnosis to be made. Diseases such as parasitosis, infectious diseases, neoplasms and various intestinal or extra-intestinal disorders present with clinical signs similar to chronic enteropathies and must be ruled out early in the diagnostic process (DANDRIEUX, 2016).

The pathogenesis of CE is understood to stem from an intricate relationship involving gut microenvironment, the immune system, genetic predisposition and environmental factors provoking intestinal inflammation (SIMPSON and JERGENS, 2011). Genetic predisposition influences the onset of ulcerative colitis and Crohn's disease in humans, which supports the belief that host genetics of dogs, in a similar way, contribute to the pathogenesis of CE (JERGENS and HEILMANN, 2022). One study demonstrated that 5 dog breeds, specifically Weimaraner, Border collie, German Shepherd, Rottweiler and Boxer are of high risk of developing IBD in south-eastern UK (KATHRANI et al., 2011). However, the most commonly presented dog breeds in a study from Sweden were West Highland White Terrier, Rottweiler, Cavalier King Charles Spaniel, Shetland Sheepdog, French Bulldog, Norwegian Lundehund, Miniature Poodle, Border Terrier and Boxer (HOLMBERG et al., 2022).

CEs are categorized based on their responsiveness to treatment into food-responsive enteropathies (FREs), antibiotic-responsive enteropathies (AREs), immunosuppressant-responsive enteropathies (IREs), and non-responsive enteropathies (NREs). IREs and NREs are classified as forms of inflammatory bowel diseases (IBDs), characterized by a lack of response to dietary change and antibiotics, with evidence of inflammation and a requirement of immunosuppressive therapy (DANDRIEUX, 2016; DUPOUY-MANESCU et al., 2024). Protein-losing enteropathy is an additional group of canine chronic enteropathies that is characterized by loss of protein across the gut wall. The disease can occur due to various causes such as lymphangiectasia, diffuse infiltrative intestinal disease, infectious diseases and neoplasia (SCHMITZ, 2019). PLE can be further classified as food-responsive PLE or non-

food-responsive PLE, with the latter requiring immunosuppressive therapy (JERGENS and HEILMANN, 2022).

An update of the classification of canine chronic enteropathies has been proposed in a recent paper, in which the authors suggest a replacement of AREs by microbiota-related modulation responsive enteropathy (MrMRE). The utilization of antibiotics as a means of treating AREs has led to detrimental and lasting outcomes. As a result, new alternative therapies have developed with the aim of restoring the intestinal microbiota through the use of probiotics, prebiotics, fecal microbiota transplantation and bile acid sequestrants (DUPOUY-MANESCAU et al., 2024)

FREs constitute the largest portion of chronic enteropathies observed in dogs, with a concluded prevalence of over 50% in several studies (ALLENSPACH et al., 2016; KAWANO et al., 2016). Allenspach et al. (2016) demonstrated that dogs with FREs were younger (median age: 3 years, range 0-12 years) than dogs with IREs (median age: 6 years, range 1-13 years). Additonally, dogs belonging to the AREs group were the youngest with a median age of 2 years old and a range of 0-11 years. The clinical improvement was remarkably better in dogs with FREs compared to the ones with AREs and IREs, and could be noticed at 4-8 weeks as well as up to one year after discharge. Moreover, Allenspach et al. (2007) described that FREs are more commonly associated with large intestinal disease than what IREs and PLEs are. Additionally, findings indicated that the clinical signs in dogs with FREs were less severe compared to other groups, suggesting that a clinical presentation of a young dog with mild clinical signs and associated large intestinal disease has a higher chance of improvement with dietary therapy alone. Another study done by Volkmann et al. (2017) supports these findings.

Oxidative stress plays a key role in the development of IBD by contributing to inflammation and tissue damage. It results from an overproduction of reactive oxygen species (ROS) that overwhelm the body's antioxidant defenses (REZAIE et al., 2007). Studies have demonstrated a reduction in antioxidant biomarkers, along with increased markers indicating oxidative damage, in serum of dogs with IBD (RUBIO et al., 2017). Consequently, there has been a rising interest in nutraceuticals with antioxidant properties as potential strategies to combat oxidative stress in managing chronic enteropathies.

The aim of this study is to determine the effects of feeding a hypoallergenic home-made diet with a novel protein source or a hypoallergenic extruded diet with hydrolyzed proteins and chestnut tannin supplements on the antioxidant status of dogs with chronic enteropathies. We

hypothesize that the antioxidant status monitored through the concentration of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) indicates a higher level of antioxidant capacity with the addition of chestnut tannin as a dietary supplement.

2. LITERATURE REVIEW

2.1. Diagnosis

Anamnesis, laboratory results, inflammatory biomarkers and histologic abnormalities have not demonstrated reliable distinction between the different forms of CE. Therefore, treatment trials offer the most effective means of distinguishing the diverse forms of CE. The majority of dogs respond to an elimination dietary trial with a resulting FRE diagnosis. However, the ones that do not respond require further investigation and a more in-depth diagnostic workup plan (JERGENS and HEILMANN, 2022). Antibiotics (primarily metronidazole or tylosin) are often recommended as a next step for those that fail to respond to a dietary change. In case the clinical response is sufficient, the patient will fall into the ARE category. A lack of response to the antibiotic therapy requires further examination, such as a gastrointestinal biopsy to detect mucosal inflammation and to exclude neoplastic growth. The patient is subsequently put on immunosuppressive therapy and categorized into the IBD group, followed by subcategorization into IRE or NRE according to the clinical response (DUPOUY-MANESCAU et al., 2024). However, suspected severe disease warrants an exception to sequential treatment trials. For example, PLE is associated with a worse prognosis, leaving little time at diagnosis. In these cases, dogs are typically biopsied early for histological examination and are immediately started on immunosuppressants alongside dietary changes (DANDRIEUX, 2016). Antibiotic therapy in chronic enteropathies is nowadays heavily criticized due to the marked dysbiosis that occurs after its use.

2.2. Clinical scoring systems

Jergens et al. (2003) conducted a study with the objective of developing a scoring system to assess the IBD severity, its response to therapy and the long-term improvement of patients. They concluded that, at the time of diagnosis and throughout the treatment process, the severity of clinical signs can be evaluated using clinical scoring systems. The canine IBD activity index (CIBDAI) is based on a system of scoring 6 gastrointestinal signs from 0 to 3. Each clinical sign is assessed separately, and the scores are later added together, giving a total CIBDAI score that demonstrates if the disease is clinically insignificant, or if it is of mild, moderate or severe

form. The variables under evaluation are attitude and activity, appetite, vomiting, stool consistency, stool frequency, and weight loss.

The canine chronic enteropathy clinical activity index (CCECAI) is a more extensive scoring system developed by Allenspach et al. (2007) to provide a more accurate estimation of treatment response and prognosis in CE cases. The new system expanded on the six variables of the CIBDAI scoring system by incorporating additional factors such as albumin levels, pruritus, severity of ascites and peripheral edema, all of which strongly correlate with clinical outcomes. This makes CCECAI a valuable tool for both diagnosis and management of chronic enteropathies. Unlike the earlier study by Jergens et al. (2003), which focused solely on IRE, Allenspach et al. (2007) included PLE, FRE, and IRE cases, making it applicable across a broader range of chronic enteropathies. Therefore, CCECAI provides a more comprehensive approach to assessing CE, not only guiding diagnostic and therapeutic decisions but also offering a more reliable tool for evaluating outcomes in clinical practice (ALLENSPACH et al., 2007).

2.3. Gut microbiota

The bacteria, fungi, protozoa and viruses that inhabit the gastrointestinal tract are collectively referred to as the gut microbiota (BLAKE and SUCHODOLSKI, 2016). Bacteria account for the biggest part of the microbiota, where facultative anaerobes are the most numerous (SUCHODOLSKI, 2022). The microbiota is prone to fluctuations between different parts of the gut, as described in a study done by Suchodolski et al. (2008). They found that bacteria belonging to the phylum Firmicutes were the most numerous and exhibited the highest diversity in the gastrointestinal tract of dogs. In duodenum and jejunum, Clostridiales was the predominant order, whereas in ileum and colon, the most prevalent orders were Fusobacteriales and Bacteroidales, which belong to the phyla Fusobacteria and Bacteroidetes, respectively.

The gut microbiota contributes to several health benefits of its host, including formation of intestinal lining, supporting defense mechanisms, promoting immune activity and ensuring nutritional support through various metabolic pathways (SUCHODOLSKI, 2011). The short-chain fatty acids (SCFAs) are produced through the microbial fermentation of indigestible carbohydrates and they play a role in regulating the immune system. They also serve as an energy supply for colonocytes and reinforce tight junctions, improving the functionality of the

intestinal lining (JERGENS and HEILMANN, 2022). Maintaining an acidic environment is another function of the SCFAs, which prevents the proliferation of enteropathogenic bacteria, such as Enterobacteriaceae (SUN and O´RIORDAN, 2013).

Eubiosis refers to the balanced state of the diverse microbes that constitute the microbiota community. This harmony supports the health and functionality of the gastrointestinal tract and suppresses the proliferation of pathogenic bacteria. However, any disturbance of the balance may lead to a state of dysbiosis (MONDO et al., 2019). There are various forms of dysbiosis, and a patient may present with more than one form simultaneously. It can manifest as decreased function of the microbiota, leading to shifts in the metabolic pathways, and alteration of bacterial composition, including changes in abundance and/or species. The potential outcomes differ based on the initial cause. For instance, shifts in metabolic pathways may exacerbate the overgrowth of pathogenic bacteria, increased bacterial adhesion to the intestinal lining can induce a heightened inflammatory immune response, and an enhanced formation of microbial byproducts may lead to diarrhea (ZIESE and SUCHODOLSKI, 2021).

Numerous studies on the disease progression of chronic enteropathies have revealed a modification in the gut microbiota composition. Suchodolski et al. (2012) observed an overgrowth of the phylum Proteobacteria, whereas bacteria belonging to Fusobacteria, Firmicutes and Bacteroidetes were decreased in dogs with idiopathic IBD. Suchodolski et al. (2010) found similar results, concluding that members of Proteobacteria had the strongest correlation with idiopathic IBD. Another key observation is the frequent reduction of Clostridia-class bacteria, including Lachnospiraceae, Ruminococcaceae and Facealibacterium, in gastrointestinal disorders. The loss of these bacteria is associated with a reduced production of SCFAs, which may weaken the suppression of abnormal intestinal immune response (HONNEFFER et al., 2014). However, it remains unclear whether dysbiosis is a cause or a consequence of disease progression in chronic enteropathies (ZIESE and SUCHODOLSKI, 2021).

2.4. Nutritional management

Nutrition impacts the gastrointestinal tract in several ways. It can alter the microbiota, control gene expression, affect the immune system, strengthen the epithelial barrier function and change the motility. (KATHRANI, 2021). The primary goal of dietary changes is to reduce antigenicity, which in turn helps to lower the inflammatory response in the gut (RUDINSKY et al., 2018). However, not only the food-responsive enteropathies could benefit from dietary therapies, nutritional intervention can be advantageous in all forms of chronic enteropathies. Dogs with CE are often undernourished, have a lessened nutrient absorption or have mucosal inflammation due to a decreased tolerance to dietary or microbial antigens. Therefore, evaluation of the nutritional status and performing a specific nutritional intervention play a central role in the management of CE (TOLBERT et al., 2022). The varying response to dietary trials depends on many factors, such as genetic susceptibility and the surrounding environment. Thus, therapeutic dietary trials should always be individualized (KATHRANI, 2021). Furthermore, the full benefits of dietary modification are not always thoroughly explored in some dogs before progressing to other therapies. A study observed that 68% of the initially diagnosed NRE dogs could be reclassified as FRE following a novel dietary trial. This finding suggests the importance of trying several diet types before moving on to other treatment options (DUVERGÉ et al., 2022).

2.4.1. Energy requirement

The energy need of each dog is highly variable and depends on the disease seriousness. Elevated energy needs can be seen in dogs with an impaired digestion and absorption, diarrhea or vomiting, elevated energy consumption from catabolism or corticosteroid treatments. Thus, if feeding a low-calorie and low-fat diet, the requirements can be difficult to meet in dogs with a decreased food consumption. Factors such as tolerance for volume, energy density, how often the dog is fed and the capability of the owner to implement the tailored plan are important to consider. The caloric need is estimated by calculating the resting energy requirement (RER) and for dogs that are under or overweight, an optimal bodyweight is determined (TOLBERT et al., 2022)

2.4.2. Dietary options in the treatment of chronic enteropathies

Common dietary options for dogs with CE typically encompass low-fat, highly digestible, fiber-enriched, novel or hydrolysed protein, limited ingredient protein or home-made diets (RUDINSKY et al., 2018; TOLBERT et al., 2022). Trying out several dietary trials may be needed, since the response to diet change is highly individual. Most dogs will improve within a few days, and in some cases it may even take up to 2 weeks before a response is evident. Thus, it's important that enough time is given to the trials before giving the diagnosis of a non-food responsive disease (TOLBERT et al., 2022).

Fibers are carbohydrates derived from plants, with a characteristic of being indigestible by the mammalian enzymes. Bacteria in the intestinal lumen have the capability of fermenting fibers into short-chain fatty acids (SCFAs) that play a role in regulating the immune system. They also serve as an energy supply for colonocytes and reinforce tight junctions, improving the functionality of the intestinal lining (JERGENS and HEILMANN, 2022). Fibers are either soluble or insoluble. Soluble fibers, such as psyllium, pectins and gums, are easily fermented and dispersed in water, resulting in increased moisture and less firm feces (LENOX, 2021). Additionally, they promote growth of the microbiota and supply energy to the enterocytes (RHIMI et al., 2022). Conversely, insoluble fibers, such as cellulose, do not readily disperse in water but helps to increase fecal volume (LENOX, 2021). Highly digestible or fibre-enriched diets are often recommended as the primary therapy for dogs with subtle illness, no skin changes, and blood work within reference values. Dogs with small intestinal disease are usually given a highly digestible therapeutic diet with a relatively small amount of fiber, whereas dogs with large intestinal disease are given a therapeutic diet with added fiber, so called fibre-enriched diet (TOLBERT et al., 2022). A recent study highlighted the usefulness of psyllium husk in managing chronic large intestinal diarrhea in working dogs. The addition of psyllium husk to their diet led to positive outcomes, including reduced defecation frequency, increased fecal volume and weight gain (ALVES et al., 2021).

Fat, in addition to its important function in the gut health, has the highest energy density among the macronutrients, making it an important component in enhancing the caloric content of food. However, reducing dietary fat is a significant factor to consider for dogs with GI motility disturbances, pancreatitis, lymphangiectasia or idiopathic PLE (TOLBERT et al., 2022). The positive effect of fat restriction was highlighted in a study on dogs with intestinal lymphangiectasia, a type of PLE. A significant proportion (79%) of the dogs showed improved

clinical signs following dietary fat reduction. Additionally, prednisone administration could be reduced or even discontinued 2 months after the dietary modification (OKANISHI et al., 2014). Furthermore, diets rich in fat may delay stomach emptying and exacerbate gastrointestinal signs, such as regurgitation and vomiting (TOLBERT et al., 2022). Additionally, excessive fat levels in patients with impaired fat absorption can result in an overgrowth of microbiota, leading to an enhanced production of compounds that trigger diarrhea (RUDINSKY et al., 2018). However, decreasing the amount of dietary fat means that the volume of the food has to be increased, which can be an issue for dogs with anorexia or sensitivity to volume load. On the contrary, keeping a moderate to high fat content in the diet may be beneficial for dogs that do not exhibit signs of motility disturbances, pancreatitis or fat malabsorption, as it reduces volume and enhances palatability (TOLBERT et al., 2022).

Protein often plays a role in the complex etiology of chronic enteropathies, functioning as an antigen that activates the mucosal immune system. Thus, the immune response to dietary protein can be reduced by feeding a diet composed of a novel or hydrolysed protein. (TOLBERT et al., 2022). The macronutrient structure is modified during enzymatic hydrolysis, resulting in a hydrolyzed diet composed of small polypeptides, which reduces antigenicity (RUDINSKY et al., 2018). Since completely eliminating the antigenic properties of proteins with hydrolyzed diets isn't always achievable, it's recommended to opt for a hydrolyzed diet to which the patient is not believed to be sensitized (TOLBERT et al., 2022). One study demonstrated the potential benefit of a hydrolysed protein diet to a highly digestible diet in dogs with chronic small intestinal enteropathy. While no significant differences were observed between the groups in the early stages, the long-term outcome was superior in the group receiving a hydrolysed protein diet. This suggests that it could serve as a viable alternative for dogs that do not respond to a highly digestible diet as their primary therapy (MANDIGERS et al., 2010). There are a limited number of studies comparing the outcomes of a novel protein diet and a hydrolysed protein diet, and whether to use one diet over the other has been debated. One study demonstrated that hydrolyzed protein diets generated significantly lower cytokine production (IL-10 and TNF-alpha) as opposed to commercial intact protein diets in dogs with CE (KATHRANI and HALL, 2019). Conversely, another study found that there were no significant differences in the clinical response between groups of FRE dogs fed an elimination diet or a hydrolysed diet (ALLENSPACH et al., 2016). Furthermore, a study evaluated the benefit of feeding a novel egg-based protein to 15 dogs. Based on the CIBDAI score, 12 dogs were presented with a significant improvement with a marked reduction of the score, demonstrating the effectiveness

of a novel-based protein diet (TØRNQVIST-JOHNSEN et al., 2020). Additional studies are necessary to ascertain whether hydrolyzed diets offer superior efficacy compared to novel or single-source protein diets. Currently, the selection between novel and hydrolyzed diets is frequently based on palatability and preference for specific nutrients, such as fiber and fat content (TOLBERT et al., 2022).

Some dogs do not tolerate commercial diets, necessitating the use of a home-made diet to manage their clinical signs effectively. Such diets should be prepared with the assistance of a board-certified veterinary nutritionist (KATHRANI, 2021). A home-made diet may be advantageous for patients with PLE that require a low-fat regimen levels or a novel ingredient, particularly when no commercial diets can meet these specific needs (RUDINSKY et al., 2018).

2.5. Nutraceuticals

Numerous studies indicate that imbalances in gut microbial communities are closely linked to the development of chronic enteropathies. While antibiotics have shown promise in treating dogs with chronic enteropathies, research has demonstrated that antibiotics, such as metronidazole, can alter microbiota composition (PILLA et al., 2020). Furthermore, rising antimicrobial resistance complicates treatment efforts. As a result, recent research has explored alternative therapies to address dysbiosis without these side effects, including probiotics, prebiotics, postbiotics, synbiotics and fecal microbiota transplantation (DUPOUY-MANESCAU et al., 2024). Probiotics are living microorganisms that can provide various health benefits to the host, such as stimulating the immune system, producing substances that act against pathogens and neutralizing bacterial toxins (JERGENS and HEILMANN, 2022). Commonly used probiotics include strains of *Bifidobacterium*, *Enterococcus faecium, Lactobacillus* and the yeast *Saccharomyces boulardii* (DANDRIEUX and MANSFIELD, 2019). While a few studies have evaluated the efficacy of probiotics, their results are inconsistent. One study compared the outcomes of a probiotic VSL#3 strain with combined therapy of metronidazole and prednisone therapy in dogs with IBD. VSL#3 consists of multiple strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, which has shown promising results in humans with ulcerative colitis. This study demonstrated significant improvements in CCECAI for both groups. However, the group receiving VSL#3 also exhibited enhanced expression of tight junction proteins, indicating positive effects on intestinal barrier integrity (ROSSI et al., 2014). Conversely, other studies have failed to demonstrate a significant

advantage of probiotic treatment compared to dietary therapy alone in FRE dogs (SAUTER et al., 2006; SCHMITZ et al., 2015). The current body of research is challenging to compare for several reasons. Various probiotics were tested across many studies targeting different forms of CE, the probiotic strains used are not always clearly identified, which poses a problem since their efficacy is strain-dependent, and the majority of studies are significantly underpowered. Therefore, more studies are necessary to validate and expand upon the findings (JERGENS and HEILMANN, 2022).

Prebiotics are non-living nutrient sources that nourish the beneficial microbiota, and may also alter the composition and activity of them in the intestinal environment (DUPOUY-MANESCAU et al., 2024). Several studies investigated the effects of prebiotics on clinical signs, composition of the microbiota, biochemical markers, and histological and endoscopic lesions (SEGARRA et al., 2016; GLANEMANN et al., 2021; SAHOO et al., 2022; BELA et al., 2024). In a study conducted by Segarra et al. (2016), chondroitin sulfate and prebiotics (beta-glucans, mannan-oligosaccharides, and resistant starch) were administered to dogs with IBD, and the results were compared to a placebo group. Both groups received a hydrolyzed diet. While the CIBDAI and histological scores improved in both groups, the supplementation did not show any advantage over the dietary treatment alone. However, the group receiving the supplements exhibited an increase in the antioxidant enzyme paraoxonase-1 (PON1), suggesting a reduction in oxidative stress. Another study evaluated the effect of prebiotic, chondroitin sulphate, and glycosaminoglycan supplementation on the relapse rate of FRE dogs returning to a normal diet 10 weeks following treatment with a hydrolyzed diet, with results compared to a placebo group. None of the groups experienced recurrence of illness. Additionally, there were no statistically significant changes of biochemical markers, fecal SCFAs, or histological and endoscopic scores in either group (GLANEMANN et al., 2021). However, similar to the probiotic studies, the two studies described above were underpowered, making it challenging to interpret the findings and generalize results (SEGARRA et al., 2016; GLANEMANN et al., 2021).

Synbiotic is a product that incorporates both prebiotics and probiotics, that work synergistically to improve gut health. Bela et al. (2024) investigated the effect of a nutraceutical supplement called Microbiotal cane, containing synbiotics (inulin, fructo-oligosaccharides, *Lactobacillus reuteri* DSM 32203) on the microbiota of sporting dogs with dysbiosis. Researchers observed an increased abundance of health-promoting bacteria from the genera *Feacalibacterium, Fusobacterium, Turicibacter, Blautia* and *Clostridium* in the period

following the competition, in contrast to the control group, which showed a decrease in these beneficial bacteria. Harmful bacteria, specifically *E. coli* and *Streptococcus* were more abundant in the control group. Additionally, the control group exhibited poorer fecal consistency compared to the group receiving Microbiotal cane. These findings suggest that stressful events and highly demanding activities significantly contribute to dysbiosis seen in sporting dogs. Additionally, supplementation with Microbiotal cane appears to counteract some of these negative effects.

Increased antimicrobial resistance is a growing global threat linked to excessive antimicrobial use, highlighting the need to avoid unnecessary use of these drugs in dogs with diarrhea and to consider alternative therapies. A study was conducted to analyze the effects of standard antimicrobial treatment (metronidazole and spiramycin) in comparison to nutraceuticals in dogs with acute diarrhea. The nutraceuticals used were tannic acid, zinc oxide, probiotics, mannan-oligosaccharides, vitamin B12 and chestnut extract, among others. Improved scores in appetite, stool consistency and frequency were recorded early on in the group receiving the supplements. Interestingly, no variation in the bacterial taxa was found between the two groups (PIGNATARO et al., 2021). However, other studies have demonstrated an increased dysbiosis index and growth of harmful Proteobacteria, along with decreased abundance of bacteria belonging to the phyla Fusobacteria and Bacteroidetes, in healthy dogs treated with metronidazole (PILLA et al., 2020). These findings suggest that nutraceuticals may be a beneficial alternative to antimicrobials for treating acute diarrhea, potentially avoiding their side effects.

2.6. Oxidative stress and its role in chronic enteropathies

Oxidative stress has been shown to be involved in the pathogenesis of IBD in humans (REZAIE et al., 2007) and in dogs (RUBIO et al., 2017), where it contributes to the inflammatory process and ensuing tissue damage. It occurs when there is an overproduction of reactive oxygen species (ROS) relative to the antioxidants needed to neutralize them. ROS are created as byproducts of the physiological oxygen metabolism and they are extremely reactive due to their unpaired electrons. The body relies on antioxidant defense systems to mitigate their harmful effects (REZAIE et al., 2007). DNA, lipids and proteins are particularly vulnerable to oxidative damage from excessive ROS, which can impair cellular components, reduce

functionality of the intestinal epithelium and initiate a cascade of inflammatory mediators, factors that can further drive the development of IBD (MURO et al., 2024).

Rubio et al. (2017) conducted a study to evaluate the oxidative damage and the antioxidant response in dogs with idiopathic IBD. Ferrous oxidation-xylenol organ (FOX), thiobarbituric acid reactive substances (TBARS) and ROS concentration in serum were measured to determine the oxidative status. To evaluate the antioxidant response, trolox equivalent antioxidant capacity (TEAC), cupric reducing antioxidant capacity (CUPRAC), ferric reducing ability of the plasma (FRAP), paraoxonase-1 (PON1) and total thiol concentration were measured in the serum. The results showed that TEAC and CUPRAC, serum thiol and PON1 that function as antioxidant biomarkers, were significantly decreased in dogs with IBD compared to the control group. Additionally, oxidative damage was indicated by an increased serum concentration of FOX, ROS and TBARS. These results give an explanation to a part of the complex pathogenesis of the CEs, in which oxidative damage serve as a key factor.

Previous studies have shown that allogeneic adipose-derived mesenchymal stem cells (MSCs), known for their anti-inflammatory properties, can positively impact the management of chronic enteropathy in dogs (CRISTÓBAL et al., 2021; CRISTÓBAL et al., 2022). To explore this further, a new study was conducted to evaluate the plasma levels of oxidative biomarkers following MSC administration. The biomarkers assessed included malondialdehyde (MDA), glutathione (GSH) and albumin. MDA serves as a marker of lipid peroxidation, a process implicated in various inflammatory and malignant conditions. GSH, recognized for its antioxidant capacities, plays a crucial role in mitigating oxidative damage and has been linked to various pathological conditions. Albumin is commonly measured to evaluate the overall nutritional health and protein stores. Additionally, it acts as an antioxidant molecule, aiding in the assessment of oxidative stress in the body. Moreover, the present study found no significant differences in MDA and GSH levels between dogs with CE and healthy controls, whereas albumin levels were significantly lower in the CE group. Following treatment with MSCs and prednisone, MDA and GSH levels remained unchanged, while albumin levels increased significantly (CRISTÓBAL et al., 2023). The reduced albumin levels corroborate findings from other studies on dogs with CE. However, those studies did primarily focus on albumin as a nutritional marker and a predictor of poor prognosis, without emphasizing its antioxidant role (ALLENSPACH et al., 2007; CRISTÓBAL et al., 2021).

As previously discussed in the section on nutraceuticals, Segarra et al. (2016) explored the effects of administering chondroitin sulfate and prebiotics to dogs with IBD, all of which

were on a hydrolyzed diet. One of the key parameters measured in the study was paraoxonase-1 (PON1), an enzyme known for its antioxidant properties. Reduced levels of PON1 have been correlated to a heightened inflammatory response in both humans (BOEHM et al., 2009) and dogs with IBD (RUBIO et al., 2017). In this study, PON1 levels were elevated in the group receiving the supplement, suggesting that such supplementation may help mitigate oxidative stress in dogs with IBD and that PON1 levels could serve as a marker for the inflammatory process.

2.7. Therapeutic benefits of tannins

Tannins, belonging to the group of polyphenols, have been identified in nearly every plant species analyzed (SAHAKYAN et al., 2022). They offer numerous health benefits and are recognized for their anti-inflammatory, antiviral, antiparasitic, antidiarrheal, and antioxidant properties (TONG et al., 2022). Additionally, studies have shown that polyphenols exhibit prebiotic effects. They have shown potential in supporting gut health by modulating the microbiota and promoting the growth of beneficial bacteria (YANG et al., 2022). Furthermore, research across various animal species has shown promising antioxidative capacities of tannins, including elevated levels of SOD, T-AOC, and GPx, along with reduced levels of MDA (LIU et al., 2011; LIU et al., 2013; YANG et al., 2022). These therapeutic benefits make tannins a promising area of research for improving animal health, particularly in managing gut health-related conditions.

3. MATERIALS AND METHODS

3.1. Animal study design

The study included dogs, patients of the Clinic of Internal Diseases of the Faculty of Veterinary Medicine at the University of Zagreb. Consent to participate in the research and to the collection and processing of personal data is given by the owners by signing the consent form. After signing the form, the owners complete the questionnaire - nutritional history, which is used to assess whether the animal meets the criteria required for inclusion in the research and to collect the data required for processing. 8 dogs with symptoms of chronic enteropathy (diarrhea, soft and frequent stools, vomiting) over a period of longer than 3 weeks that are older than one year, with no other comorbidities, were included in the study. A prerequisite for inclusion in the study is the anamnestic information that the dog has not been treated with antibiotics and/or immunosuppressants in the last 2 weeks. The control group consisted of 7 healthy dogs, each older than 1 year. The feeding protocol included balanced home-made diet using a hypoallergenic vitamin-mineral supplement (Novomineral sensitive, Napfcheck) or hypoallergenic food with hydrolyzed proteins (Purina Hypoallergenic) and the addition of Farmatan, Tanin Sevnica (sweet chestnut extract). The dogs were initially placed on an elimination diet for 30 days, which continued throughout the entire study period. After the 30-day elimination period, dogs were then supplemented with chestnut tannins at a dose of 16,5 mg/kg/day for the next 15 days. Blood was collected twice as part of a check-up at the Internal Disease Clinic, following good veterinary practice to monitor the clinical condition. The first blood sample was collected before diagnosis to rule out diseases of other organ systems. The second blood sample was collected after the full 45-day elimination diet and 15 days of chestnut tannin supplementation. The study „Effect of diet and nutraceuticals on symptoms, antioxidative status, microbiome and short-chain fatty acids in feces of chronic enteropathies dogs“ has been approved by the Ethical Committee of Veterinary Faculty, University of Zagreb, Croatia.

3.2. Analysis of MDA

3.2.1. Preparation of the mobile phase and standard solutions

The mobile phase for the determination of malondialdehyde consisted of a 50 mM solution of KH2PO4 in distilled water (HPLC grade, Sigma Aldrich, Germany) and methanol (HPLC grade, ≥99.9% Sigma Aldrich, Germany) in a 50:50 ratio. The solvents used as mobile phase should be of high purity and must be freed from dissolved gasses or suspended solids using microporous filters under vacuum.

A standard stock solution of 4 M tetraethoxypropane (TEP) (1,1,3,3-tetraethoxypropane, ≥96%, Sigma Aldrich, Germany, TEP) is prepared by pipetting 50 µl TEP 96% into a volumetric flask with a volume of 50 ml, the rest of the volume is supplemented with ethanol (ethanol, HPLC grade, ≥99.8%, Sigma Aldrich, Germany).

The standard solutions were prepared fresh daily, immediately before the measurement, by pipetting an appropriate amount of the standard stock solution into volumetric flasks with a volume of 5 or 10 ml. Dilution is carried out by adding a certain volume of the mobile phase. The prepared standard solutions are then mixed using a vibromixer. The concentration of TEP in the working standard solutions was 0.96 µM, 4.8 µM, 9.6 µM, 20 µM, 40 µM. The concentrations of the standard solutions used for the determination of MDA in plasma were adjusted to the expected concentrations in the tested sample. The volume is topped up with water (HPLC grade, Sigma Aldrich, Germany) (Figures 13 and 14).

3.2.2. Preparation of the plasma sample

To a test tube containing 150 µl of plasma, 50 µl of distilled water (HPLC grade, Sigma Aldrich, Germany) and 50 µl of NaOH 3N was added. The contents of the test tube are then mixed using a vibromixer for 1 minute. The contents of the test tube are then incubated in a shaking water bath at a temperature of 60 °C for 30 minutes. Then 250 µl H3PO4 6% and 250 µl TBA 0.8% are added to the contents of the test tube and mixed for 1 minute using a vibromixer. The contents of the test tube are then incubated again in a water bath at a temperature of 90 °C for 45 minutes.

After cooling, 250 µl of methanol was added, the contents were mixed again on a vibromixer and 100 µl of sodium dodecyl sulfate 10% was added. The test tubes were centrifuged with a microcentrifuge at a speed of 3600 x 9 for 10 minutes and the contents were transferred to vials so that the measurement could be carried out on the chromatograph. The measurement conditions on a Shimadzu 2010 liquid chromatograph (Shimadzu, Japan) were: Column temperature 40°C, mobile phase flow rate: 1 ml/min, injection volume: 20 µl, UV detector at a wavelength of 532 nm.

The analysis of MDA in plasma was determined by the HPLC method on a Shimadzu 2010 liquid chromatograph (Shimadzu, Japan) using an InertSustain C-18 column (4.6–150 mm -5 µm) (GL Sciences Inc., Japan) and a UV/Vis detector. The method is based on the reaction of MDA with thiobarbituric acid (TBA), which gives a red color and forms a complex of thiobarbituric acid reactive substances (TBARS), the concentration of which is determined.

3.3. Analysis of SOD and GPx

The analysis of SOD and GPx was performed with commercial kits using the ELISA method from whole blood. For this purpose, red blood cells were washed with saline. Plastic test tubes containing whole blood are centrifuged (3000 rpm for 10 mins), and plasma is removed. 1 mL of 0.9% saline is added and centrifuged at 3000 rpm for 10 mins. The supernatant is removed. This procedure is repeated 3 times, and red blood cells are considered washed out after three times. The activity of glutathione peroxidase is determined using commercial Ransel kits from Randox Laboratories (Randox Laboratories, Crumlin, UK) on Cobas c 111 automated biochemical analyzers (Roche, Rokreuz, Switzerland) at a wavelength of 340 nm. The method is based on the fact that GSH-Px catalyzes the oxidation of glutathione (GSH) with hydrogen peroxide and cumene with hydroperoxide. In the presence of glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized form of glutathione (GSSG) is immediately converted to the reduced form by the oxidation of NADPH to NADP+. The entire glutathione peroxidase oxidizes glutathione with hydrogen peroxide. Total superoxide dismutase (Balsom, Söderlund et al.) activity is determined using ready-to-use Ransod kits (Cat. No. SD 125) from Randox Laboratories (Randox Laboratories, Crumlin, UK) from the whole blood on Cobas c 111 automated biochemical analyzer (Roche, Rokreuz, Switzerland) at a length of 505 nm. The method is based on the generation of

superoxide radicals from xanthine using xanthine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazole chloride to form a red coloration of azazan. TSOD activity is measured as the degree of inhibition of this reaction. Enzymatic activities of both enzymes are expressed as UI/ml of full blood.

3.4. Statistical analysis

Data were analyzed using GraphPadPrism10 software between the groups and within the groups CON and CE using the unpaired and paired t-test (GraphPad Software, Inc., San Diego, CA, USA). The normality of distribution was tested with the Shapiro-Wilks test. All data are presented as means ± standard deviation. The significance was set at $p < 0.05$.

4. RESULTS

MDA analysis was conducted on blood plasma and measured in micromoles (μM), whereas the analyses of SOD and GPx were conducted on whole blood and measured in units per milliliter (U/ml). The mean concentrations and standard deviations of each oxidative marker analyzed for both the control group and the chronic enteropathy group at baseline (T0) and post-treatment (T1) are depicted in Table 1.

4.1. Analysis of MDA

There was no statistically significant difference in MDA concentration between the baseline (T0) and post-treatment point (T1) in CE patients, as shown in Figure 1. Similarly, as shown in Figure 2, MDA concentrations in the control group showed no statistically significant difference between these two time points. Additionally, the comparison of MDA concentration at T0 between the CE and control group did not reveal any statistically significant differences, as shown in Figure 3. However, in contrast to the baseline comparison, Figure 4 shows that at T1, there was a statistically significant difference in MDA concentrations between patients with chronic enteropathy and the control group ($p < 0.0167$). Specifically, MDA levels were significantly higher in the CE group compared to the control group after the treatment period, as indicated by the asterisk (*).

Table 1: The mean concentration and standard deviation of MDA, GPx and SOD at baseline (T0) and post-treatment (T1) in patients with chronic enteropathy (CE) and the control group (CON).

Biomarkers	**CON**		**CE**	
	T0	T1	T0	T1
MDA (μM)	10.92±2.324	10.62±0.5774	10.23±1.781	11.40±1.233
SOD (U/ml)	310.5±112.6	288.3±43.45	138.7±140.2	75.06±28.71
GPx (U/ml)	9.613±1.015	9.781±1.708	9.420±1.924	8.916±1.816

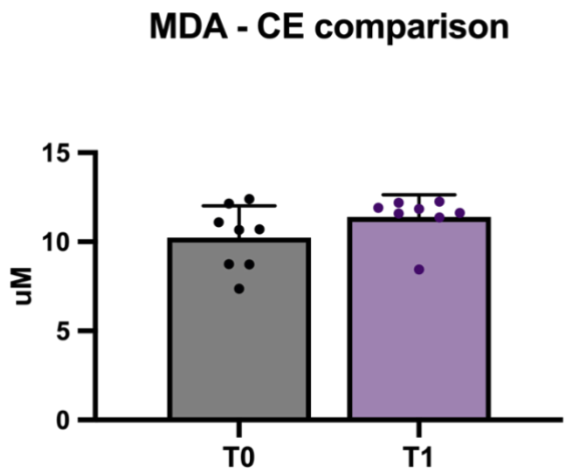

Figure 1: Comparison of malondialdehyde (MDA) concentration at T0 and T1 in patients with chronic enteropathy (CE).

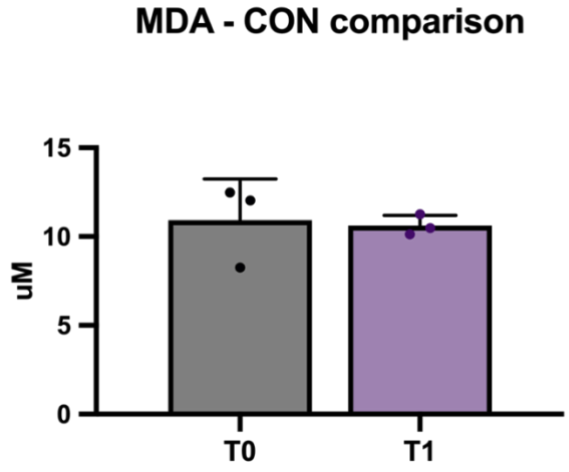

Figure 2: Comparison of malondialdehyde (MDA) concentration at T0 and T1 in the control group (CON).

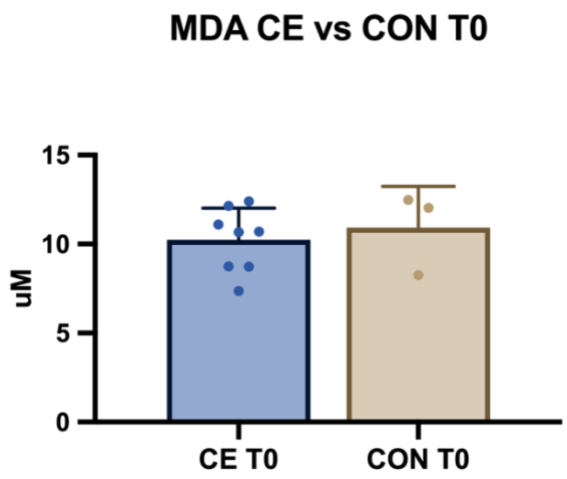

Figure 3: Comparison of malondialdehyde (MDA) concentration at T0 between patients with chronic enteropathy (CE) and control group (CON).

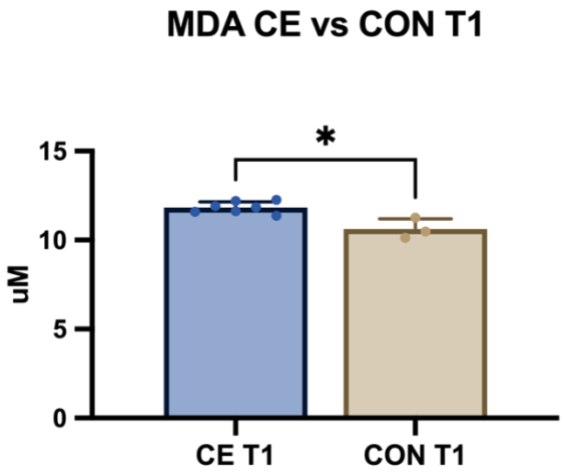

Figure 4: Comparison of malondialdehyde (MDA) concentration at T1 between patients with chronic enteropathy (CE) and control group (CON). The asterisk (*) indicates a statistically significant difference ($p < 0.05$).

4.2. Analysis of SOD

There was no statistically significant difference in SOD concentration between the baseline (T0) and post-treatment point (T1) in CE patients, as shown in Figure 5. Similarly, as shown in Figure 6, SOD concentration in the control group showed no statistically significant difference between these two time points. However, Figure 7 shows that at T0, there was a statistically significant difference ($P < 0.0224$) in SOD concentrations between patients with

CE and the control group. Specifically, SOD levels were significantly higher in the control group compared to the CE group, as indicated by the asterisk (*). Additionally, as shown in Figure 8, the comparison of SOD concentration at T1 between patients with chronic enteropathy and control group demonstrated a statistically significant difference ($P < 0.0001$). Specifically, SOD levels were significantly higher in the control group compared to the CE group, as indicated by the asterisks (****).

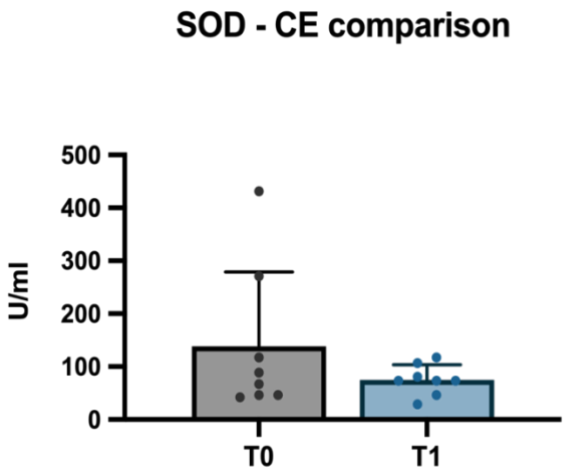

Figure 5: Comparison of superoxide dismutase (SOD) concentration at T0 and T1 in patients with chronic enteropathy (CE).

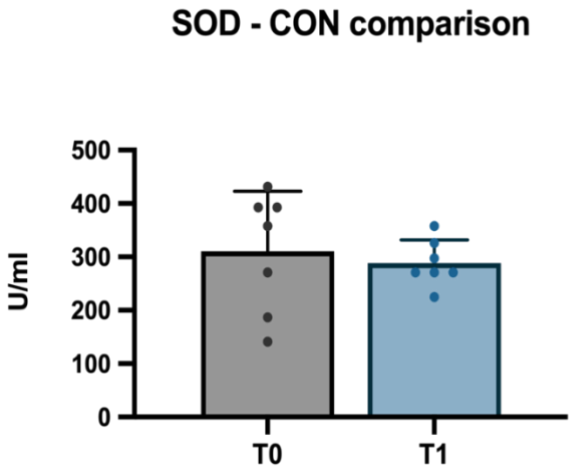

Figure 6: Comparison of superoxide dismutase (SOD) concentration at T0 and T1 in the control group (CON).

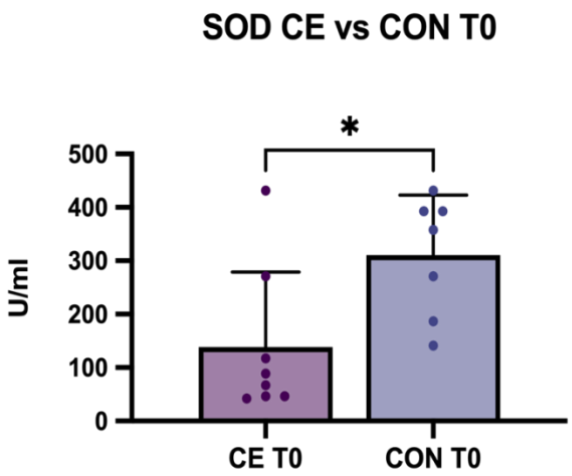

Figure 7: Comparison of superoxide dismutase (SOD) concentration at T0 between patients with chronic enteropathy (CE) and control group (CON). The asterisk (*) indicates a statistically significant difference ($p < 0.05$).

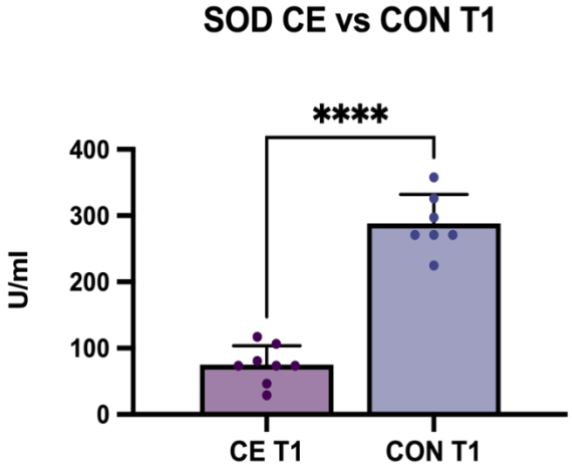

Figure 8: Comparison of superoxide (SOD) concentration at T1 between patients with chronic enteropathy (CE) and control group (CON). The asterisks (****) indicate an extremely statistically significant difference ($p < 0.0001$).

4.3. Analysis of GPx

There were no statistically significant differences in glutathione peroxidase (GPx) concentrations when comparing T0 and T1 within the chronic enteropathy (CE) group (Figure 9) or the control group (Figure 10). Additionally, no statistically significant differences in GPx levels were observed between the CE and control groups at either T0 (Figure 11) or T1 (Figure 12).

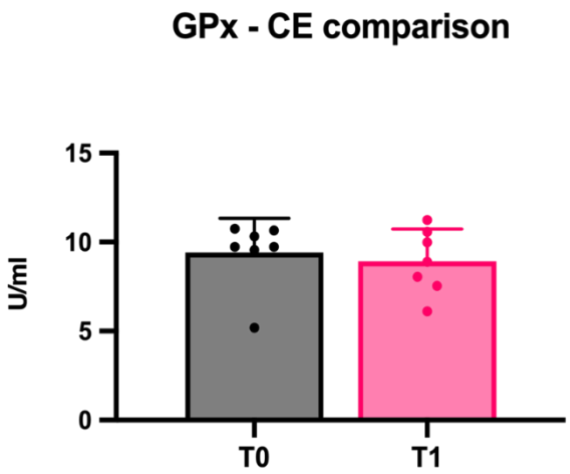

Figure 9: Comparison glutathione peroxidase (GPx) concentration at T0 and T1 in patients with chronic enteropathy (CE).

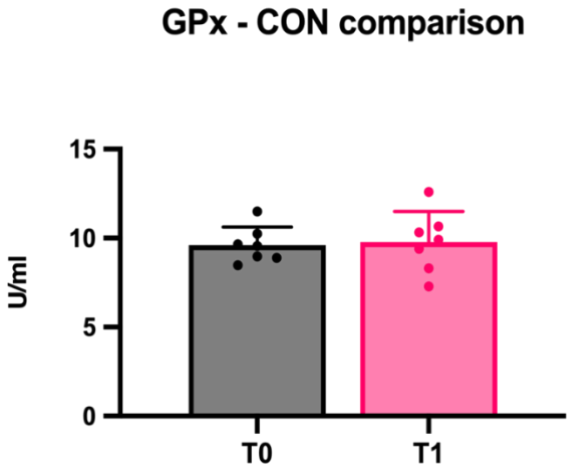

Figure 10: Comparison of glutathione peroxidase (GPx) concentration at T0 and T1 in the control group (CON).

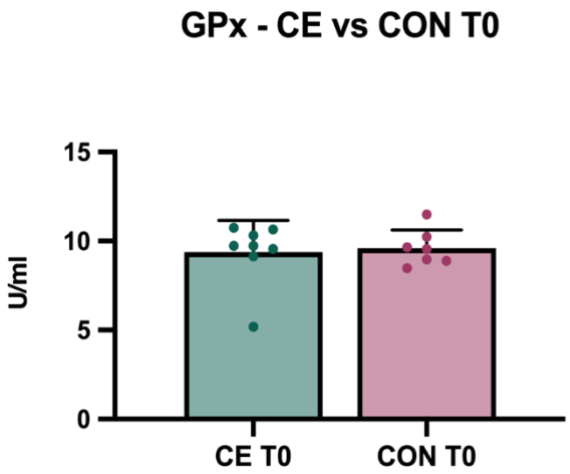

Figure 11: Comparison of superoxide dismutase (SOD) concentration at T0 between patients with chronic enteropathy (CE) and control group (CON).

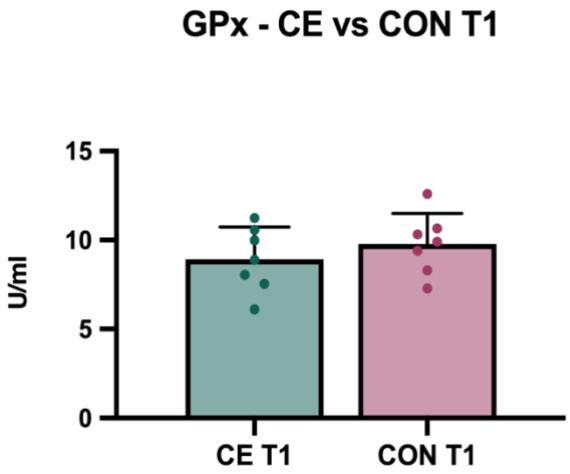

Figure 12: Comparison of glutathione peroxidase (GPx) concentration at T1 between patients with chronic enteropathy (CE) and control group (CON).

5. DISCUSSION

The pathogenesis of CE is understood to stem from an intricate relationship involving gut microenvironment, the immune system, genetic predisposition and environmental factors provoking intestinal inflammation (SIMPSON and JERGENS, 2011). In addition, oxidative stress has been shown to play a key role in this process. In this study, we aimed to determine the effects of chestnut tannin supplementation on the antioxidant status of dogs with chronic enteropathies, fed either a hypoallergenic home-made diet with a novel protein source or a hypoallergenic extruded diet with hydrolyzed proteins. We hypothesized that the antioxidant status monitored through the concentration of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) indicates a higher level of antioxidant capacity with the addition of chestnut tannin as a dietary supplement

Among these markers, MDA serves as an indicator of lipid peroxidation, reflecting the damaging effects of ROS on cell membranes. The generation of lipid peroxides, such as MDA, can further contribute to cellular damage (MURO et al., 2024). In the present study, we measured MDA concentrations before and after tannin supplementation to evaluate changes in oxidative stress. MDA concentrations did not change significantly between baseline (T0) and the post-treatment point (T1) within either the CE or control group, indicating that oxidative stress levels remained relatively stable over time in each group. However, a significant difference in MDA concentration ($p < 0.05$) was observed between the CE and control group at T1, with MDA levels significantly higher in the CE group. Furthermore, SOD and GPx are two key antioxidant enzymes that protect cells from damaging molecules by scavenging them. SOD catalyzes the transformation of the highly reactive superoxide anion into oxygen and hydrogen peroxide, while GPx enables the breakdown of hydrogen peroxide into water (MURO et al., 2024). In addition to MDA, SOD and GPx were analysed at the same time points (T0) and (T1). Similar to the MDA analysis, SOD concentrations did not change significantly between baseline (T0) and the post-treatment point (T1) within either the CE or control group, indicating that oxidative stress levels remained relatively stable over time in each group. However, at T0, SOD levels were significantly elevated in the control group compared to the CE group ($p < 0.0224$). This trend persisted at T1, where SOD levels remained significantly higher in the control group compared to the CE group ($p < 0.0001$). Furthermore, the GPx analysis showed no significant differences in GPx concentrations within CE or control groups between T0 and T1, nor between the CE and control groups at either time point. These findings contrast with previous studies

across various animal species. Liu et al. (2011) demonstrated that oxidative stress parameters can be positively influenced by the administration of chestnut tannins in rabbits. Plasma levels of GPx, total antioxidant capacity (T-AOC) and SOD, along with liver GPx levels, were significantly elevated. Conversely, MDA levels were reduced in both plasma and liver tissues. Similarly, transition dairy cows receiving chestnut tannins showed elevated plasma levels of SOD, GPx and T-AOC, as well as elevated SOD and GPx levels in liver, with reduced MDA levels in both plasma and liver (LIU et al., 2013). In addition to the beneficial effects on microbiota and metabolites, Yang et al. (2022) also demonstrated an elevated level of serum GPx along with reduced MDA levels in gallic acid supplemented dogs exposed to stress. Furthermore, pomegranate peel extract supplementation improved the erythrocyte antioxidant status in dogs, namely by increasing GPx, GSH, catalase (CAT) and glutathione S-transferase (JOSE et al., 2017). The exact mechanism underlying the antioxidant activity of tannins remains unclear. However, Liu et al. (2011) suggested that chestnut tannins may help counteract the reduction in antioxidant enzyme activity by directly scavenging free radicals. Another potential function is to upregulate gene transcription and activate the nuclear factor erythroid 2-related factor 2 (Nrf2), leading to the expression of antioxidant enzyme genes. Nrf2 protein was previously described in a study by Yeh and Yen (2006), which suggested that this protein serves a crucial function in the antioxidant gene activation promoted by phenolic acids.

The absence of significant changes in MDA, SOD, and GPx levels in the present study suggests that these oxidative stress markers may not directly reflect the clinical improvements observed in patients. Despite no significant changes in the oxidative markers, patients exhibited visible improvements in clinical signs, which could indicate that tannins exert their therapeutic effects through mechanisms other than modulating oxidative stress systemically. One possible explanation is that the clinical improvements may be due to changes in the gut microbiome and a reduction in localized inflammatory markers, which were not captured by the systemic markers. This idea is supported by other studies that investigated the effects of tannins on gut microbiome. For instance, Yang et al. (2022) investigated the effects of gallic acid supplementation on stressed beagle puppies. In addition to reducing diarrhea, gallic acid was found to inhibit the growth of harmful bacteria, such as *Escherichia-Shigella* and *Clostridium sensu stricto 1*, while promoting the proliferation of beneficial bacteria like *Lactobacillus* and *Faecalibaculum,* belonging to the phylum Firmicutes. This led to an increase in total SCFAs, attributed to the proliferation of these beneficial bacteria. The positive effects on stress management were further demonstrated by normalizing the metabolism of amino acids,

carbohydrates, lipids, cofactors and vitamins, while also decreasing the serum levels of stress hormones. Similarly, Zhang et al. (2023) found an increased abundance of bacteria belonging to the phylum Firmicutes, specifically *Ruminococcus torques* group, *Faecalibacterium* and *Lachnospiraceae NK4A136* group, in dogs with IBD that were supplemented with grape seed proanthocyanidin. These findings suggest that polyphenols can positively influence gut dysbiosis in dogs presented with gastrointestinal dysfunction.

Furthermore, the absence of significant changes in oxidative stress markers in the present study could be attributed to the short duration of supplementation before the second blood collection at T1. With only 15 days of supplementation, this period may have been too short to produce measurable changes in oxidative stress markers. Additionally, the optimal dosage of chestnut tannins for achieving antioxidant effects in dogs with chronic enteropathy is still unknown and may require further investigation to determine the most effective therapeutic dose. The small sample size in the CE group may have also limited the ability to detect more subtle effects. Future studies with a longer supplementation period, larger sample size, and a focus on dose optimization are needed to further evaluate the impact of tannins on oxidative stress markers in dogs with chronic enteropathy.

Given the potential of antioxidants in managing chronic enteropathies, alternative compounds like curcumin may offer additional therapeutic benefits. Curcumin, a component of *Curcuma longa*, has demonstrated antioxidant, anti-inflammatory and immune-stimulatory properties (LIN et al, 2022). Studies conducted on humans have reported positive outcomes following curcumin administration in patients with ulcerative colitis (UC). Lang et al. (2015) demonstrated that patients receiving curcumin achieved a symptom-free state and showed significant improvements in endoscopic scores compared to the control group, along with a more favorable clinical response as indicated by the simple clinical colitis activity index (SCCAI). Similarly, Masoodi et al. (2018) found a significant reduction in SCCAI in patients with UC receiving curcumin compared to the control group. To date, limited information is available on the effects of curcumin in dogs. However, a study by Campigotto et al. (2020) found that dogs receiving curcumin-supplemented food showed decreased levels of ROS and increased activities CAT, SOD and GPx. Additionally, elevated levels of antioxidant capacity against peroxyl radicals, non-protein sulfhydryls and protein sulfhydryls were observed, indicating curcumin's important role in maintaining redox balance.

Quercetin, belonging to the group of flavonols, has also gained increasing interest due to its positive effects in various studies, making it a subject of further research. Its beneficial impact on health can be explained by its antioxidative properties through radical scavenging and inhibition of lipid peroxidation. In a study on rats with colitis, those fed quercetin showed increased levels of GSH, along with decreased lipid peroxidation products, nitrite/nitrate levels, and myeloperoxidase levels, all of which play a role in oxidative status (DODDA et al., 2014). In addition to improving oxidative stress parameters, quercetin also enhanced microbiota composition in rats with colitis by increasing Firmicutes and decreasing Proteobacteria, suggesting a prebiotic action of quercetin (HONG and PIAO, 2018). Furthermore, dogs fed a combination of bromelain, quercetin and *Lentinula edodes* showed an increased abundance of Bifidobacteriaceae and Lactobacillaceae, bacteria important in the production of SCFAs (ATUAHENE et al., 2024). Additionally, this combination promotes anti-inflammatory effects in kennel dogs prone to stress, by lowering levels of fecal calprotectin, cortisol, N-methylhistamine, and indole/skatole, while increasing SCFAs (ATUAHENE et al., 2023).

The present study contributes to the growing body of evidence suggesting that polyphenolic compounds, such as tannins, curcumin, and quercetin, may offer alternative therapeutic benefits in the treatment of chronic enteropathies, potentially through mechanisms such as gut microbiome modulation and antioxidative effects. Limitations of the study, including the short duration of tannin supplementation, unknown optimal dosage, and a small sample size, emphasize the need for further research. Future studies should explore the ideal duration and dose of tannin supplementation and investigate the potential gut-specific mechanisms to better understand tannins' therapeutic role in chronic enteropathy.

6. CONCLUSIONS

- No statistically significant changes in oxidative stress markers (MDA, SOD and GPx) were observed following chestnut tannin supplementation in dogs with chronic enteropathy (CE) or in the control group.
- Despite the lack of significant systemic changes in oxidative stress markers, clinical improvement was observed in the CE group following elimination diet protocol.
- Tannins may exert therapeutic effects through mechanisms other than modulating oxidative stress systemically, such as changes in gut microbiota or localized inflammation.
- The supplementation period of 15 days was likely too short to produce measurable changes in oxidative stress markers.
- The small sample size in the CE group may have limited the ability to detect subtle effects.
- The optimal dosage of chestnut tannins for therapeutic efficacy in dogs with CE remains undetermined and warrants further investigation
- Further studies with a longer supplementation period, larger sample size and dose optimization are needed to fully understand the impact of tannins on oxidative stress markers and their therapeutic effects in dogs with chronic enteropathy.

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8. SAŽETAK

Utjecaj hrane i nutraceutika na antioksidativni status pasa koji boluju od kroničnih enteropatija

Ida Vikars

Kronične enteropatije kod pasa pojam je koji opisuje prisutnost gastrointestinalnih simptoma u trajanju duljem od 3 tjedna pri čemu su isključene bolesti ostalih organskih sustava, neoplazije te ostali infektivni uzročnici. Klinički, sindrom kroničnih enteropatija se dijeli na temelju odgovora na tretman: hipoalergena hrana, antibiotska terapija i terapija imunosupresivima. U prethodno provedenim istraživanjima utvrđeno je da će na tretman koji uključuje hipoalergeni obrok visoke probavljivosti odgovoriti više od 50% pasa u vidu stabilizacije simptoma. Jedan od ključnih faktora koji utječe na patogenezu kronične enteropatije je i oksidativni stres koji potiče upalne procese čime se narušava integritet probavnog sustava. Stoga će intervencije koje za cilj imaju pozitivan učinak na antioksidativni status, pozitivno djelovati na progresiju bolesti pasa sa kroničnim eneropatijama. Tanini kestena čine opsežnu grupu botaničkih spojeva koji posjeduju antimikrobno, protuupalno, antiviralno te antidiaroično djelovanje. Stoga je cilj istraživanja bio ustanoviti učinak pripravaka kestenovog tanina uz eliminacijski protokol hranjenja na antioksidativni status pasa sa kroničnim enteropatijama. 8 pasa sa dijagnozom kronične enteropatije uključeno je u istraživanje. Analiza MDA u serumu utvrđena je HPLC metodom na tekućinskom kromatografu Shimadzu pomoću InertSustain C-18 kolone i UV/Vis detektora. Analiza SOD i GPx utvrđena je putem komercijalnih kitova postupkom ELISA-e (Ransel i Ransod). Istraživanjem nisu utvrđene značajne razlike u kontrolnoj i pokusnoj skupini na razini markera oksidativnog (MDA) i antioksidativnog statusa (SOD i GPx) ukazujući na pozitivan učinak tanina pored antioksidativnih svojstava. Provedba dugoročnijih istraživanja na većem broju pas te optimizacija doze i trajanja suplementacije pomoći će u boljem razumijevanju učinaka tanina na markere oksidativnog stresa i njihov potencijalni terapeutski učinak kod pasa sa kroničnim enteropatijama.

Ključne riječi: kronične enteropatije, psi, tanin kestena, oksidativni status

9. SUMMARY

Effect of diet and nutraceuticals on antioxidative status in dogs with chronic enteropathies

Ida Vikars

Chronic enteropathies in dogs refer to gastrointestinal symptoms persisting for more than 3 weeks, excluding diseases of other organ systems, neoplasia, and infectious agents. Clinically, chronic enteropathy syndrome is categorized according to response to treatment: hypoallergenic food, antibiotic therapy, and immunosuppressive therapy. Previous studies have shown that more than 50% of dogs respond to treatment that includes a highly digestible hypoallergenic diet with stabilization of symptoms. One critical factor in the pathogenesis of chronic enteropathy is oxidative stress, which contributes to the underlying inflammation and tissue damage in the gastrointestinal tract. Given its role in disease progression, improving oxidative status may be key to alleviating symptoms in affected dogs. Chestnut tannins, a group of phytochemicals with antioxidant, anti-inflammatory, antiviral and other therapeutic properties, have been suggested as a potential supplement to address this imbalance. Therefore, this study aimed to evaluate the effects of a hypoallergenic home-made diet with a novel protein source and a hypoallergenic diet with hydrolyzed proteins, supplemented with chestnut tannins, on the antioxidant status of dogs diagnosed with chronic enteropathy. 8 dogs with chronic enteropathy were included in the study. Blood was collected twice, before and after an elimination diet and tannin supplementation. Plasma MDA levels were measured by the HPLC method on a Shimadzu liquid chromatograph using an InertSustain C-18 column and a UV/Vis detector, while the activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD) was measured using commercial kits (Ransel and Ransod, respectivley) on an automated biochemical analyzer. Despite no significant changes in the oxidative stress markers, clinical improvement was observed, suggesting effects of tannins beyond their antioxidant properties. Further studies with larger sample size, extended supplementation duration and dose optimization are needed to better understand the impact of tannins on oxidative stress markers and their therapeutic effects in dogs with chronic enteropathy.

Key words: chronic enteropathy, dog, chestnut tannin, oxidative status

10. CURRICULUM VITAE

My name is Ida Vikars, and I was born and raised in a small town in Finland. Swedish is my mother tongue, as I grew up in a Swedish-speaking region of the country.

As a child, I was always curious and eager to explore a wide range of subjects, which made it difficult to pinpoint my true passion. Therefore, it wasn't until my last year in upper secondary school that I decided to pursue the path of becoming a veterinarian. I took a gap year after graduation to fully focus on the courses needed to pass the entrance exam in Finland, however, I didn't get admitted to the university. During that same year, I discovered the many other opportunities there is to study veterinary medicine in Europe, and that's when the Faculty of Veterinary Medicine in Zagreb came into the picture.

In September 2018, I enrolled in the faculty as part of the 3rd generation of the English program, motivated to devote myself to the demanding study and practice required to become a veterinarian.

I dedicated much of my free time to playing futsal with fellow students from the faculty. We formed a team and competed against other faculties in various leagues and tournaments. I also took part in the Humanijada event three times, and I am proud that we secured the gold medal on every occasion.

I participated in the Erasmus mobility program twice. In 2021 I joined a veterinary team for two months at Eläinklinikka Saari in Vaasa, Finland. In 2024, I decided to combine the mandatory internship of my final study year with the mobility program, which allowed me to spend two valuable months with Distriktsveterinärerna in Stockholm, Sweden.

In Finland, veterinary students have the opportunity to apply for a temporary license after completing a certain number of internship hours. Therefore, during the summer of my 6th year of study, I had the opportunity to work as a substitute municipal veterinarian in my home region. This was a truly rewarding experience that significantly enhanced my confidence, strengthened my problem-solving skills, and expanded my knowledge of veterinary medicine.