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ORIGINAL ARTICLE OPEN ACCESS

Dogs

Study of the Effect of Synbiotic Diet on Haematological and Oxidative Indexes Changes in Male Dogs

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Received: 14 April 2024 | **Revised:** 19 November 2024 | **Accepted:** 17 February 2025

Funding: The authors received no specific funding for this work.

Keywords: functional food | haematological parameters | oxidative stress | prebiotic | probiotic | synbiotic

ABSTRACT

Background: Synbiotic products are functional foods/feeds that contain both probiotic and prebiotic strains and have health-promoting effects beyond those of probiotics or prebiotics alone.

Objectives: This study aimed to investigate the effect of synbiotic feed containing the probiotic strain *Lactobacillus acidophilus* La5 (10^{12} cfu/g) and inulin (5%) on changes in haematological and oxidative serum indices in male dogs.

Methods: Twelve male dogs in two groups of six were included in the study. In addition to the basic feed, the dogs in the treatment group also received the synbiotic supplement at 5% in each of their three meals during 29 days. Then, on Day 0 and Day 29, the amount of malondialdehyde was measured based on thiobarbituric acid, and glutathione peroxidase activity and the amount of total serum antioxidants was also measured with the Randox kit; the obtained data were statistically analysed.

Results: The results showed that white blood cells increased (from 10.28 ± 2.89 to 16.48 ± 7.59 [$p = 0.138$]) and red blood cells decreased (from 6.492 ± 0.64 to 5.92 ± 1.14 [$p = 0.461$]) in the treatment group, but these changes were not significant. In the treatment group, it was found that synbiotic food has a significant effect on the increase of MCH (from 24.32 ± 0.93 to 26.30 ± 1.04 pg [$p = 0.003$]) and MCHC (from 33.24 ± 0.32 to 36.58 ± 0.72 g/dL [$p < 0.001$]), but it does not have a significant effect on the decrease or increase of other haematological factors. Also, significant reductions in the amount of malondialdehyde (from 3.2 ± 1.05 to 1.35 ± 1.05 µmol/L [$p = 0.015$]) and increases in the number of total serum antioxidants (from 0.27 ± 0.06 to 0.42 ± 0.09 mmol/L [$p = 0.035$]) were obtained in the treatment group.

Conclusion: It concluded that the consumption of synbiotic feed (*L. acidophilus* + inulin, at the rate of 5%) in male dogs may reduce and prevent oxidative stress, adjust side effects and prevent some disorders. In any case, many studies are needed to definitively prove these results.

1 | Introduction

The term 'synbiotic' is used when a product contains both probiotics and prebiotics. Since this term implies collaboration between these two, it should be used for products in which prebiotics specifically provide a favourable environment for the use of probiotic microorganisms. Some examples of synbiotic combinations include *Bifidobacterium* with FOS and/or GOS, *Lactobacillus* with lactulose and *Saccharomyces* with GOS (Dixit et al. 2016; Norouzi et al. 2019; Schrezenmeir and de Vrese 2001). According to the definitions by the Food and Drug Administration (FDA) and the World Health Organization (WHO), probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits on the host. Positive effects of probiotics on health are observed only when the food contains the minimum required amount of live microorganisms during consumption (Hill et al. 2014; Homayouni-Rad et al. 2020). *Lactobacillus* and *Bifidobacterium* are considered the primary groups of probiotic bacteria. Prebiotics are a type of food that is neither hydrolysed nor absorbed in the upper gastrointestinal tract and is selectively employed as a substrate for beneficial bacteria in the large intestine. Therefore, the use of various types of prebiotics, such as fructooligosaccharides and galactooligosaccharides, alongside probiotic strains, enhances their synergistic health effects on each other. Some of the most important prebiotic compounds include inulin, resistant starch, polydextrose and various types of plant fibres (Collins and Gibson 1999) (Alonge et al. 2020). Intestinal bacteria are essential for the growth of gut-associated lymphoid tissue (GALT), which is the primary site of B lymphocyte proliferation. Thus, intestinal bacteria are crucial for humoural immunity (Adogony et al. 2007). These beneficial bacteria produce short-chain fatty acids, which can promote B cell differentiation into plasma cells, enhance B cell metabolism, increase glycolytic activity in B cells and boost antibody production (Kim et al. 2016; Rad et al. 2022). Regarding dogs, it's worth noting that they were domesticated during the Stone Age and have undergone significant changes alongside humans, transitioning from a hunting and gathering lifestyle to a post-industrial western lifestyle (Frantz et al. 2016; Larson et al. 2012; Wang et al. 2013). The shared dietary resources with humans have enabled dogs to adapt to starch-rich diets and exhibit behavioural changes compared to their ancestors (Axelsson et al. 2013; Ollivier et al. 2016). The digestive system of dogs is host to a highly diverse microbial complex and ecosystem, with most microbial species resembling those commonly found in the intestines of other omnivorous mammals. However, in comparison to mice and pigs, the gut microbiome of dogs has the closest similarity to the human gut microbiome (Coelho et al. 2018; Ley et al. 2008). Several recent studies have shown that the gut microbiota of mammals is connected to the central nervous system (CNS) of the host through various parallel channels, including the vagus nerve, neural signalling mechanisms, neural glands and the production of neurochemical substances. These substances include gamma-aminobutyric acid (GABA), serotonin (5-HT), norepinephrine and dopamine (Forsythe et al. 2014; Fung et al. 2017; Lyte 2013; Neuman et al. 2015). Haematocrit is the ratio of the volume of red blood cells to the total blood volume, measured in units of L/L, expressed as a percentage (conventional method) or a decimal fraction (SI unit) (Mcpherson and Pincus 2011; Pagana and Pagana 2012). Malondialdehyde (MDA) is the end product of lipid peroxidation (Mohammadi et al. 2009). Free radicals initiate lipid peroxidation, producing harmful substances, the most significant of which are aldehydes. Aldehydes exacerbate atherosclerosis and ageing processes and play a role in the pathogenesis of many diseases. Antioxidants, whether endogenous or exogenous, inhibit lipid peroxidation. A reduction in antioxidants increases the levels of free radicals and, consequently, increases lipid peroxidation. As a result, the impact of stress on cardiovascular health intensifies (Satari et al. 2003). Glutathione peroxidase (GPX) is a general name for a family of enzymes with peroxidase activity that primarily protects organisms against oxidative damage (Ettinger and Feldman 2009). The use of probiotics for promoting animal health has been successful (Xu et al. 2019). Production of antimicrobial substances to control colonization of pathogenic microbes, regulation of host immunity and induction of unique systemic responses in T lymphocytes are desirable effects of probiotics (Barletta et al. 2013; Jones and Versalovic 2009; Thomas and Versalovic 2010; Wang et al. 2013). Generally, the consumption of synbiotics can strongly influence gut immune regulation (Roller et al. 2004). The consumption of these products can also enhance immunity against various infections during critical periods of breastfeeding cessation and throughout the lifespan (Rossi et al. 2020). The administration of these nutrients before initiating a vaccination program for dogs accelerates the development and persistence of immunity resulting from vaccination. This phenomenon is due to the maturation of B lymphocytes. In addition to preventive applications, they also have therapeutic uses, as they may enhance the health of dogs exposed to stress and infections (Benyacoub et al. 2003). Some studies showed that probiotics, especially lactic acid bacteria, can enhance adaptive and innate immune responses (Perdigon et al. 1988; Simpson et al. 2009). These bacteria can also increase phagocytosis in mice and humans (Perdigon et al. 1988; Schiffrin et al. 1995). Reports have indicated that the consumption of *Enterococcus faecium* leads to increased colonization of mature B lymphocytes in the intestinal mucosa, followed by increased IgA production (Ansari et al. 2020; Benyacoub et al. 2003; Strober 1990). A study has shown that the use of *Lactobacillus casei* Zhang can reduce abnormalities in red blood cells (Hor et al. 2018). It has been reported that the administration of heat-killed *Enterococcus faecalis* (FK-23) to healthy dogs stimulates non-specific immune responses, including lymphocyte proliferation in response to mitogens and phagocytosis by neutrophils (Kanasugi et al. 1998). A study has demonstrated that the employing of probiotics in the diet does not significantly affect haemoglobin, haematocrit, or mean corpuscular haemoglobin concentration (MCHC) (Hosseini et al. 2014). It has been suggested that antioxidants may help reduce the harmful effects of stress on the body, particularly the cardiovascular system (Satari et al. 2003). Ultimately, the main focus of this research is to demonstrate the impact of synbiotic food on the health of the studied dogs. In addition to enhancing immunity, this product should not lead to excessive stimulation or disruption that could increase the risk of allergies, autoimmune diseases or inflammation. It should also be capable of maintaining physiological levels of red blood cells, white blood cells (neutrophils, lymphocytes, eosinophils, basophils and monocytes), platelets, haemoglobin, MCV, MCH, MCHC, PCV, MDA, GPX activity and total serum antioxidants so that its administration to dogs does not pose harm.

In another study, Kumar et al. (2016) conducted an investigation on the blood biochemical profile and erythrocytic antioxidant indices in response to a canine-origin probiotic (species-specific probiotic *Lactobacillus johnsonii* CPN23 supplementation). The consequences showed that the plasma glucose had a decrease in both dPRO and cPRO groups vis-a-vis the CON group; nevertheless, total protein retained higher in both dPRO and cPRO in comparison to CON. The total cholesterol was lower in the dPRO and cPRO groups as compared to CON. The HDL/LDL ratio became broader in the dPRO and cPRO groups as compared to the CON. The erythrocytic lipid peroxidation and the levels of antioxidants counting reduced glutathione, glutathione S-transferase and catalase continued intact via the probiotic usage; nevertheless, the bustle of GPX and superoxide dismutase was higher in cPRO. They concluded that species-specific probiotic *Lactobacillus johnsonii* CPN23 supplementation was effective as well as having the potential to enhance the antioxidant status in dogs (Kumar et al. 2016).

Several studies have shown that the probiotic microorganisms alone or in combination with prebiotics exhibited antioxidant bustle in all main ways and also released and encouraged the creation of chief free-radical scavenger glutathione and no enzymatic antioxidants. Furthermore, they endorse the construction of specific antioxidant biomolecules. Hereafter, synbiotics may have a possible therapeutic character in intestinal illnesses involving reactive oxygen species (ROS) (Balouei et al. 2024; Kumar et al. 2016; Saghir et al. 2024; Spyropoulos et al. 2011; Srinivas et al. 2024). However, evidence is scarce, mainly of synbiotics and antioxidant modulation in dogs. Therefore, the present study was undertaken to ascertain the effects of synbiotic food on haematological and oxidative changes in male dogs.

2 | Method

2.1 | Animals, Experimental Design and Diet

After the study was approved by the Ethics Committee of the Faculty of Veterinary Medicine (Tabriz, Iran), a total of twelve Sarabi male dogs, or Persian mastiffs (scientific name: *Canis lupus familiaris*) entered the study, of which six entered the treatment group and six entered the control group. The allocation was random. Each dog was assigned a number and six numbers were selected from a bowl containing 12 numbers by lottery. The dogs with selected numbers were allocated to treatment group and the others to the control group. All the selected dogs were native to the area, aged between 2 and 3 years and weighed 50–70 kg. All dogs were kept for 29 days in similar environmental conditions regarding the resting area's temperature, moisture and measurements. The dogs had access to suitable toys at all times to ensure their relaxation. Dogs were allowed to exit the cage three times a week for 1 h to interact with each other and humans. The general health condition of the dogs was evaluated before and after the completion of the study. The researcher and a trained veterinarian monitored the well-being of the dogs during the study.

All dogs received three base rations and routines recommended by the Association of American Feed Control Officials (base feed: extruded dry dog food (22% protein, 10% fat, 8% ash, 13.8 kJ metabolizable energy/g). 400 grams of meat powder, 150 grams of wheat flour, 100 grams or 2 eggs, 50 grams of vegetable oil, 150 grams of chicken broth and 150 grams of vegetables (carrots, potatoes and spinach) were used to prepare 1 kilogram of base food (AAFCO; 5). The participant dogs were randomly distributed in two equal groups (using the random number generated by Excel software). Dogs of the treatment group received synbiotic supplements (5%) besides their base feed in each of their three meals. The synbiotic diet contained the *Lactobacillus acidophilus* La5 probiotic strain and inulin (Figure 1). A base feed of dogs and a 10^{12} cfu/mL load of live bacteria of *L. acidophilus* La5 and inulin (5%) were mixed and dried to prepare the synbiotic supplement (100 g functional feed per 1900 g base feed; animals received 10 g every 10 kg of body weight) (See Figure 2).

FIGURE 1 | Shape and approximate weight of synbiotic pelleted functional feed containing *Lactobacillus acidophilus* (10^{12} cfu/mL) and inulin (5%). Due to the large size of the dogs, the food pellets were also produced in large sizes (5–6 × 2 cm).

2.2 | Experiments

At the beginning of the study and 29 days, blood samples were obtained from each dog in empty tubes for serum and whole blood separation (Alonge et al. 2020; Sun and Kim 2020). Haematological indices, including white blood cell count, red blood cell count, platelets, haemoglobin, MCV, MCH, MCHC and PCV, were analysed using the Alpha Classic automated blood analyser. The serum was separated by centrifugation at 2000 *g* for 30 min at 4°C. MDA levels were measured based on thiobarbituric acid, GPX activity and total serum antioxidant levels were assessed using the Randox kit. Finally, all factors were compared between the two study groups. All tests were conducted at the Laboratory of the Islamic Azad University Tabriz Medical Sciences. Ethical principles and considerations were followed during and after the study.

2.3 | Analytical Methods

Mean results for each parameter were calculated. Since all the studied factors were quantitative continuous variables, the Kolmogorov–Smirnov test was conducted to determine the normality of the data. According to the results of this test, the distribution of variables monocyte at Day 0, band cell at Day 0, WBC at Day 29, monocyte at Day 29, eosinophil at Day 29 and band cell at Day 29 were not normal. Paired samples *t*-test was used to compare the data of Day 0 and Day 29 with normal distribution. Wilcoxon signed-rank test was used for data not normally distributed. Independent samples *t*-test and Mann–

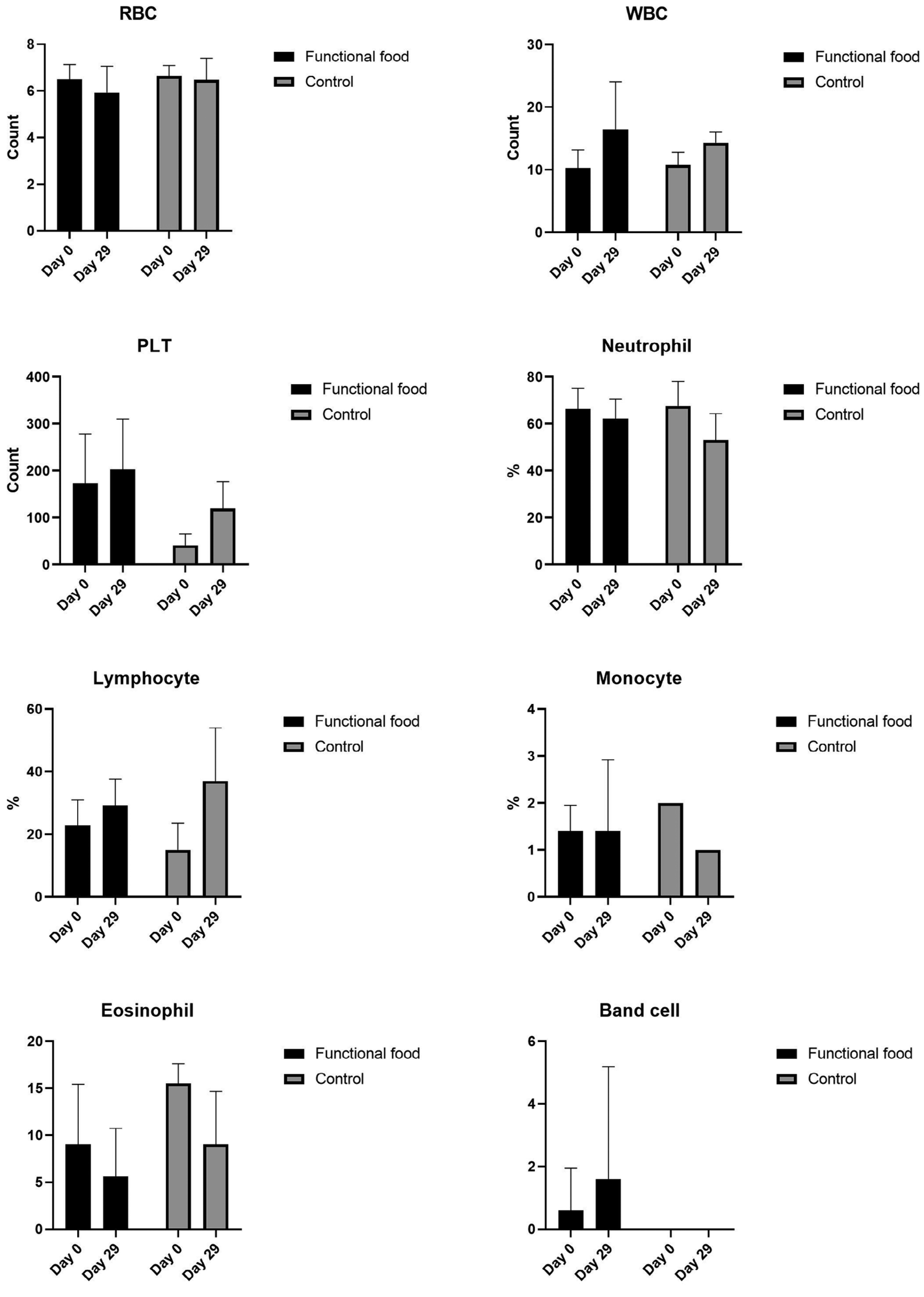

FIGURE 2 | Changes in the functional food user group compared to the control group after 29 days of functional food consumption for RBC, WBC, PLT, neutrophil, lymphocyte, monocyte, eosinophil and band cell. The statistical difference between Day 0 and Day 29 was not significant in any of the treatment and studied groups for any of the factors ($p > 0.05$).

Whitney U test were also used to compare data of each time with normal and without normal distribution, respectively. p values lower than 0.05 were considered statistically significant ($p < 0.05$).

3 | Results and discussion

According to the World Health Organization's (WHO) definition in 2001, probiotics are live microorganisms that, when consumed in sufficient quantities, confer health benefits to the host (Abdou et al. 2018). Since Metchnikoff introduced the concept of probiotics over a century ago (Mazziotta et al. 2023), many different microorganisms have been considered probiotics. These microorganisms are generally classified as *Lactobacilli*, *Bifidobacteria*, other lactic acid bacteria and non-lactic acid bacteria (Amin et al. 2009). In the interaction between the host and probiotics, mechanisms generally involve competition between probiotics and pathogenic organisms for the use of nutrients, particularly in the intestines (Coman et al. 2019; Schmitz and Suchodolski 2016). It has been reported that the beneficial effects of probiotics during their interaction with the regulation of gut microbiota, stimulation and development of the active immune system, increased metabolism of nutrients and prevention and reduction of various diseases such as gastrointestinal disorders and infectious diseases contribute to gut health in the host (Jang et al. 2021; Sivamaruthi et al. 2021). As demonstrated in the present study, the use of synbiotics had a notable impact on the oxidative stress parameters of the dogs.

The results showed that WBC increased (from 10.28 ± 2.89 to 16.48 ± 7.56 [$p = 138$, 95% CI: −1.16 to 13.56]) and RBC decreased (from 6.492 ± 0.64 to 5.92 ± 1.14 [$p = 461$, 95% CI: −1.76 to 0.62]) in the treatment group, but these changes were not significant ($p > 0.05$). In treatment group, it was found that synbiotic food has a significant effect on the increase of MCH (from 24.32 ± 0.93 to 26.30 ± 1.04 pg [$p = 0.003$, 95% CI: 0.71–3.25]) and MCHC (from 33.24 ± 0.32 to 36.58 ± 0.72 g/dL [$p < 0.001$, 95% CI: (2.62–4.06)]), but it does not have a significant effect on the decrease or increase of other haematological factors. Also, significant reductions in the amount of MDA (from 3.2 ± 1.05 µmol/L to 1.35 ± 1.05 µmol/L [$p = 0.015$, 95% CI: (−3.20 to −0.50)]) and increase in the amount of total serum antioxidants (from 0.27 ± 0.06 mmol/L to 0.42 ± 0.09 mmol/L [$p = 0.035$, 95% CI: (0.05–0.25)]) were obtained in the treatment group (See Table 1).

In a similar study, Kim et al. (2016) reported through their investigation into the effects of probiotic bacteria on immune indices in dogs that probiotic therapy can enhance certain immune factors, reduce inflammation by increasing beneficial gut microbiota and simultaneously suppress potential pathogens. Beneficial microorganisms may improve gut function through the production of specific metabolites. As an example, short-chain fatty acids derived from gut microbiota can enhance the differentiation of B cells into plasma cells, increase the metabolism of B cells, enhance glycolytic activity in B cells and increase host antibody production (Kim et al. 2016). In another study, Stene et al. (2022) reported the effects of probiotic bacteria on white blood cells and immunity. The use of probiotic bacteria *Lactobacillus* and *Bifidobacterium* led to a significant reduction in leucocytes and also a decrease in pro-inflammatory

TABLE 1 | Changes in studied factors during 29 days in treatment and control groups.

	Treatment group				Control group			
	Day 0	**Day 29**	**Mean difference and 95% CI**	***p* value***	**Day 0**	**Day 29**	**Mean difference and 95% CI**	***p* value***
HGB (g/dL)	15.78 ± 1.04	15.58 ± 3.03	−0.2 (−3.11 to 2.71)	0.913	16.3 ± 1.98	17.60 ± 3.25	1.30 (−2.16 to 4.76)	0.785
PCV (%)	47.46 ± 3.43	42.64 ± 8.77	−4.82 (−13.39 to 3.74)	0.395	49.4 ± 3.96	48.05 ± 7.99	−1.35 (−9.46 to 6.76)	0.899
MCV (fL)	73.32 ± 2.37	71.90 ± 2.65	−1.42 (−4.65 to 1.81)	0.094	74.65 ± 0.78	74.20 ± 1.70	−45 (−2.15 to 1.25)	0.840
MCH (pg)	24.32 ± 0.93	26.30 ± 1.04	1.98 (0.71 to 3.25)	0.003	24.5 ± 1.27	27.10 ± 1.13	2.60 (1.050 to 4.140)	0.369
MCHC (g/dL)	33.24 ± 0.32	36.58 ± 0.72	3.34 (2.62 to 4.06)	< 0.001	32.9 ± 1.41	36.60 ± 0.71	3.70 (2.260 to 5.13)	0.245
MDA (µmol/L)	3.2 ± 1.05	1.35 ± 1.05	−1.85 (−3.21 to −0.50)	0.015	4.27 ± 0.21	2.74 ± 1.41	−1.53 (−2.83 to −0.23)	0.410
TAC (mmol/L)	0.27 ± 0.06	0.42 ± 0.09	0.15 (0.05 to 0.25)	0.035	0.29 ± 0.08	0.32 ± 0.01	0.03 (−0.04 to 0.10)	0.766
GPX (U/g Hb)	37.64 ± 3.92	41.23 ± 2.68	3.59 (−0.73 to 7.9	0.244	38.33 ± 6.64	39.07 ± 4.26	0.74 (−6.44 to 7.92)	0.736

*Paired samples *t*-test result comparing Day 0 and Day 29 within each group.

cytokine IL-6 levels in the rectal mucosa after TNF-α stimulation following Lp299 consumption. Therefore, probiotic usage results in the reduction of inflammatory factors (Stene et al. 2022). In another study Batatinha et al. (2020) surveyed the effects of probiotic bacteria on serum immunity markers, it was found that the production of pro-inflammatory cytokines by stimulated lymphocytes decreased significantly after probiotic supplementation. A 30-day probiotic supplement maintained CD8 T cell and effective memory cell populations and had an immunomodulatory role in stimulated lymphocytes (Batatinha et al. 2020). Various studies' findings indicate that in cases of disease or stress, probiotics can modulate conditions and bring them closer to normal through various mechanisms of action. Probiotics modulate these factors and increase immunity by affecting inflammatory factors. Mazziotta et al. (2023) reported that the probiotic bacteria can interact and stimulate specific immune functions and immune homeostasis with gut immune cells and the common microbiota. Growing evidence suggests that probiotic bacteria have important properties for promoting health and immune modulation. Therefore, the use of probiotics may be a promising approach to improving immune system activity (Mazziotta et al. 2023). In a similar study to the present research, Jang et al. (2021) reported the effects of probiotics on immune and haematological markers in dogs, demonstrating a significant increase in lymphocytes after probiotic consumption. It is noteworthy that the number of white blood cells (WBC) is an indicator of immunity; therefore, the increased levels of WBC in the present study and the above study can be considered a positive clinical sign that occurred due to the use of synbiotics (Jang et al. 2021). The results obtained in the present study indicated that after 4 weeks of synbiotic intake in dogs, red blood cells decreased, but this decrease was not significant. Cetiṅ et al. (2005) conducted a study on the effects of probiotic bacteria on blood factors and reported that probiotic supplementation led to a statistically significant increase in the number of red blood cells, haemoglobin concentration and haematocrit values. Also, Xu et al. (2019) reported the effects of a probiotic supplement containing three strains of bacteria named *Lactobacillus casei*, *Lactobacillus plantarum* P-8 and *Bifidobacterium* subspecies *lactis* that the use of these probiotic bacteria resulted in a reduction of abnormalities in red blood cells in dogs. In another study, Ooi et al. (2010) reported the effects of synbiotics on red blood cell markers, stating that the synbiotic supplement containing *Lactobacillus acidophilus* CHO-220 and inulin improved the irregularities of red blood cells. In a similar study, Mansouri-Tehrani et al. (2015) reported the effects of probiotics on red blood cells, stating that the consumption of probiotic capsules (including *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve* and *Bifidobacterium longum*) did not significantly alter red blood cell levels or platelet counts. As observed in our study as well, probiotics do not have a significant effect on the haematological markers of WBC and red blood cells. However, an important point is that the most significant impact of probiotics is on oxidative stress and gut microbiota markers. Probiotic bacteria contribute to increasing immunity in dogs by preventing and inhibiting the growth of harmful bacteria in the intestines. Azizpour Maghvan et al. (2022) conducted a study on the effects of probiotics on anaemia and white blood cell count in cancer patients undergoing chemotherapy and radiation therapy. The statistical results did not show a significant difference in the increase of these factors before and after probiotic consumption. However, clinical analysis revealed that probiotics can increase blood factors. Probiotics may have a positive effect on increasing blood factor levels (MCH, MCV, HCT, HGB and WBC) with prolonged intake (Azizpour Maghvan et al. 2022). In the present study, it was also found that probiotics have a significant effect on increasing MCH and MCHC but no significant effect on the decrease or increase of other haematological factors. Proper dosage, timing, dietary regimen and investigating the duration of probiotic consumption may positively influence blood factor levels (MCH, MCV, HCT, HGB and WBC) and improve systemic conditions. The results obtained in this study indicate that after 4 weeks of synbiotic intake, the level of MDA showed a significant reduction, and another factor, TAC, also showed a significant increase during this period in the probiotics group. However, the use of probiotics did not have a significant effect on GPX levels. Therefore, our study findings suggest that the use of probiotics has a very favourable effect on preventing oxidative stress in a way that using probiotics reduces oxidative stress by lowering MDA levels, which is recognized as one of the most important oxidative factors (Cordiano et al. 2023; Del Rio et al. 2005; Pirinccioglu et al. 2010). On the other hand, probiotics, by significantly increasing the total antioxidant capacity (TAC), contribute to preventing oxidative stress. Researchers have reported that probiotics can modulate local inflammation by regulating cytokines such as TNF-α and IL-6 and then enhance host immunity (Maslowski et al. 2009). In addition, the gut microbiota is limited by a multi-layered system consisting of physical and chemical barriers along with an intrinsic and adaptive immune system (Levy et al. 2017). In a similar study to the current one, Zhao et al. (2020) reported that the use of probiotics significantly increased SOD levels by 114% and reduced MDA concentration by 160%, based on their examination of the effects of probiotic bacteria on oxidative stress indicators, including superoxide dismutase, GPX and MDA. The results of this study align with the impact of probiotics on controlling and mitigating oxidative stress, as evidenced by our findings. It is noteworthy that oxidative stress, characterized by a decrease in enzymatic and non-enzymatic antioxidants and an increase in ROS, is closely linked to the health of organisms (Mishra et al. 2015). Many studies have shown that while a certain amount of ROS is essential for vital homeostasis, excessive ROS can induce oxidative stress, thus leading to various diseases. Therefore, probiotics, possessing antioxidant capabilities and properties, can modulate levels of ROS and consequently reduce oxidative stress and inflammation. Zamani et al. (2020) studied the effects of probiotic supplementation on oxidative stress biomarkers; it was reported that probiotic supplements significantly increase TAC. Furthermore, the use of these probiotics resulted in a significant reduction in MDA (Zamani et al. 2020). However, the use of probiotics did not have an effect on glutathione concentration in this study. The results of this study regarding the effect of probiotic bacteria on the concentration of oxidative stress indices, including MDA, TAC and GPX, are fully consistent with the findings obtained in the present study. This indicates that probiotics have a highly significant impact on mitigating oxidative stress. MDA increases during disease, tissue damage, inflammation and oxidative stress, leading to disruptions in bodily functions. The results of our study, similar to the aforementioned study, suggest the effect of probiotics on preventing inflammation and oxidative stress. It is noteworthy that MDA is a product of lipid peroxidation and a key indicator of lipid peroxidation (Mohammadi et al.

2009). Unsaturated fatty acids are easily oxidized by ROS and free radicals. For example, they readily react with hydroxyl radicals to form lipid peroxyl radicals. These radicals, in turn, react with other unsaturated fatty acids and generate more lipid hydroperoxides and lipid peroxyl radicals. Lipid peroxyl radicals also undergo intramolecular double-bond rearrangements to form cyclic endoperoxides, which mostly decompose and produce MDA (Esterbauer et al. 1991).

Correspondingly, according to the study of Pourrajab et al. (2022), a systematic review and meta-analysis of randomized controlled trials, existing evidence proposes that probiotic/synbiotic supplementation can considerably enhance serum TAC, GSH and NO, along with decreasing MDA levels. Consequently, these supplementations possibly will play a role in improving antioxidant indices and decreasing oxidative stress in the body (Pourrajab et al. 2022). In another study by Roshan et al. (2022), a meta-analysis of randomized clinical trials, 16 eligible RCTs with 915 participants were comprised. Results of their study disclosed that probiotics could considerably enhance GSH levels in comparison with the control groups. Moreover, probiotics and synbiotics displayed no significant impact on TAC levels and SOD activity (Roshan et al. 2019).

4 | Conclusions

The results of this study suggest that synbiotics containing a 10^{12} cfu/mL load of live bacteria of *L. acidophilus* La5 and 5% inulin (5%) may have a beneficial role in preventing oxidative stress and consequently preventing various disorders linked to oxidative stress in male dogs. Further studies are needed to assess the effect in a larger population and in several health conditions to conclude.

Author Contributions

Amir Muhammad Armian: investigation, validation, writing – review and editing. **Hadi Pourjafar:** conceptualization, data curation, investigation, methodology, supervision, validation, visualization, writing – original draft preparation. **Mehrdad Neshat Gharamaleki:** supervision, investigation, validation, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We would like to thank Dr. Fereshteh Ansari (Razi Vaccine and Serum Research Institute), Ms. Tayebeh Sadeghian Sharefi, Mr. Ghadir Armian, Ms. Rafiqeh Yazdanian Asr, Ms. Elaheh Armian, Ms. Nava Ashtari Majolan and Dr. Ali Khatami.

Ethics Statement

The authors confirm compliance to the ethical policies of the journal, as noted on the journal's author guidelines page. The study was approved and conducted in accordance with guidelines for the Ethics Committee of Animal Experiment of the Faculty of Veterinary Medicine (Tabriz, Iran; Approval No. IR.IAU.TABRIZ.REC.1401.162).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.70290.

References

Abdou, A. M., R. H. Hedia, S. T. Omara, M. A. E.-F. Mahmoud, M. M. Kandil, and M. Bakry. 2018. "Interspecies Comparison of Probiotics Isolated From Different Animals." *Veterinary World* 11, no. 2: 227.

Adogony, V., F. Respondek, V. Biourge, et al. 2007. "Effects of Dietary scFOS on Immunoglobulins in Colostrums and Milk of Bitches." *Journal of Animal Physiology and Animal Nutrition* 91, no. 5-6: 169–174.

Alonge, S., G. G. Aiudi, G. M. Lacalandra, R. Leoci, and M. Melandri. 2020. "Pre- and Probiotics to Increase the Immune Power of Colostrum in Dogs." *Frontiers in Veterinary Science* 7: 570414.

Amin, M., M. Jorfi, A. Khosravi, A. Samarbafzadeh, and A. F. Sheikh. 2009. "Isolation and Identification of *Lactobacillus casei* and *Lactobacillus plantarum* From Plants by PCR and Detection of Their Antibacterial Activity." *Journal of Biological Sciences* 9, no. 8: 810–814.

Ansari, F., F. Pashazadeh, E. Nourollahi, S. Hajebrahimi, Z. Munn, and H. Pourjafar. 2020. "A Systematic Review and Meta-Analysis: The Effectiveness of Probiotics for Viral Gastroenteritis." *Current Pharmaceutical Biotechnology* 21, no. 11: 1042–1051.

Axelsson, E., A. Ratnakumar, M.-L. Arendt, et al. 2013. "The Genomic Signature of Dog Domestication Reveals Adaptation to a Starch-Rich Diet." *Nature* 495, no. 7441: 360–364.

Azizpour Maghvan, M., P. J. Jafari, and A. A. Anushiravani. 2022. "Investigating the Influence of Probiotic Bacteria on Anemia and White Blood Cells Count in Cancer Patients Undergoing Chemotherapy and Radiation Therapy." *Multidisciplinary Cancer Investigation* 6, no. 3: 1–6.

Balouei, F., B. Stefanon, E. Martello, D. Atuahene, M. Sandri, and G. Meineri. 2024. "Supplementation With *Silybum marianum* Extract, Synbiotics, Omega-3 Fatty Acids, Vitamins, and Minerals: Impact on Biochemical Markers and Fecal Microbiome in Overweight Dogs." *Animals* 14, no. 4: 579.

Barletta, B., G. Rossi, E. Schiavi, et al. 2013. "Probiotic VSL# 3-Induced TGF-β Ameliorates Food Allergy Inflammation in a Mouse Model of Peanut Sensitization Through the Induction of Regulatory T Cells in the Gut Mucosa." *Molecular Nutrition & Food Research* 57, no. 12: 2233–2244.

Batatinha, H., E. Tavares-Silva, G. S. Leite, et al. 2020. "Probiotic Supplementation in Marathonists and Its Impact on Lymphocyte Population and Function After a Marathon: A Randomized Placebo-Controlled Double-Blind Study." *Scientific Reports* 10, no. 1: 18777.

Benyacoub, J., C. Cavadini, T. Sauthier, et al. 2003. "Supplementation of Food With *Enterococcus faecium* (SF68) Stimulates Immune Functions in Young Dogs." *Journal of Nutrition* 133, no. 4: 1158–1162.

Cetiṅ, N., B. Güçlü, and E. Cetin. 2005. "The Effects of Probiotic and Mannanoligosaccharide on some Haematological and Immunological Parameters in Turkeys." *Journal of Veterinary Medicine Series A* 52, no. 6: 263–267.

Coelho, L. P., J. R. Kultima, P. I. Costea, et al. 2018. "Similarity of the Dog and Human Gut Microbiomes in Gene Content and Response to Diet." *Microbiome* 6: 1–11.

Collins, M. D., and G. R. Gibson. 1999. "Probiotics, Prebiotics, and Synbiotics: Approaches for Modulating the Microbial Ecology of the Gut." *American Journal of Clinical Nutrition* 69, no. 5: 1052S–1057S.

Coman, M., M. C. Verdenelli, C. Cecchini, et al. 2019. "Probiotic Characterization of *Lactobacillus* Isolates From Canine Faeces." *Journal of Applied Microbiology* 126, no. 4: 1245–1256.

Cordiano, R., M. Di Gioacchino, R. Mangifesta, C. Panzera, S. Gangemi, and P. L. Minciullo. 2023. "Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update." *Molecules* 28, no. 16: 5979.

Del Rio, D., A. J. Stewart, and N. Pellegrini. 2005. "A Review of Recent Studies on Malondialdehyde as Toxic Molecule and Biological Marker of Oxidative Stress." *Nutrition, Metabolism and Cardiovascular Diseases* 15, no. 4: 316–328.

Dixit, Y., A. Wagle, and B. Vakil. 2016. "Patents in the Field of Probiotics, Prebiotics, Synbiotics: A Review." *Journal of Food: Microbiology, Safety & Hygiene* 1, no. 02: 1–13.

Esterbauer, H., R. J. Schaur, and H. Zollner. 1991. "Chemistry and Biochemistry of 4-Hydroxynonenal, Malonaldehyde and Related Aldehydes." *Free Radical Biology and Medicine* 11, no. 1: 81–128.

Ettinger, S., and E. Feldman. 2009. *Textbook of Veterinary Internal Medicine*. Elsevier Health Sciences.

Forsythe, P., J. Bienenstock, and W. A. Kunze. 2014. "Vagal Pathways for Microbiome-Brain-Gut Axis communication." In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*, 115–133. Springer.

Frantz, L. A., V. E. Mullin, M. Pionnier-Capitan, et al. 2016. "Genomic and Archaeological Evidence Suggest a Dual Origin of Domestic Dogs." *Science* 352, no. 6290: 1228–1231.

Fung, T. C., C. A. Olson, and E. Y. Hsiao. 2017. "Interactions Between the Microbiota, Immune and Nervous Systems in Health and Disease." *Nature Neuroscience* 20, no. 2: 145–155.

Hill, C., F. Guarner, G. Reid, et al. 2014. "Expert Consensus Document: The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic." *Nature Reviews Gastroenterology & Hepatology* 11, no. 8: 506–514.

Homayouni-Rad, A., A. Azizi, P. Oroojzadeh, and H. Pourjafar. 2020. "Kluyveromyces marxianus as a Probiotic Yeast: A Mini-Review." *Current Nutrition & Food Science* 16, no. 8: 1163–1169.

Hor, Y.-Y., L.-C. Lew, A. S.-Y. Lau, et al. 2018. "Probiotic *Lactobacillus casei* Zhang (LCZ) Alleviates Respiratory, Gastrointestinal & RBC Abnormality via Immuno-Modulatory, Anti-Inflammatory & Anti-Oxidative Actions." *Journal of Functional Foods* 44: 235–245.

Hosseini, A., H. Oraji, S. Yegane, and H. Shahabi. 2014. "The Effect of Probiotic *Pediococcus acidilactici* on Growth Performance, Blood and Some Serum Parameters in Caspian Salmon (*Salmo trutta caspius*)." *Iranian Scientific Fisheries Journal* 22, no. 2: 35–45.

Jang, H.-J., S. Son, J.-A. Kim, et al. 2021. "Characterization and Functional Test of Canine Probiotics." *Frontiers in Microbiology* 12: 625562.

Jones, S. E., and J. Versalovic. 2009. "Probiotic *Lactobacillus reuteri* Biofilms Produce Antimicrobial and Anti-Inflammatory Factors." *BMC Microbiology* 9: 1–9.

Kanasugi, H., T. Hasegawa, Y. Goto, H. Ohtsuka, S. Makimura, and T. Yamamoto. 1998. "Single Administration of Enterococcal Preparation (FK-23) Augments Non-Specific Immune Responses in Healthy Dogs." *International Journal of Immunopharmacology* 19, no. 11-12: 655–659.

Kim, M., Y. Qie, J. Park, and C. H. Kim. 2016. "Gut Microbial Metabolites Fuel Host Antibody Responses." *Cell Host & Microbe* 20, no. 2: 202–214.

Kumar, S., A. Pattanaik, S. Sharma, and S. Jadhav. 2016. "Species-Specific Probiotic *Lactobacillus johnsonii* CPN23 Supplementation Modulates Blood Biochemical Profile and Erythrocytic Antioxidant Indices in Labrador Dogs." *Indian Journal of Animal Sciences* 86, no. 8: 918–924.

Larson, G., E. K. Karlsson, A. Perri, et al. 2012. "Rethinking Dog Domestication by Integrating Genetics, Archeology, and Biogeography." *Proceedings of the National Academy of Sciences of the United States of America* 109, no. 23: 8878–8883.

Levy, M., A. A. Kolodziejczyk, C. A. Thaiss, and E. Elinav. 2017. "Dysbiosis and the Immune System." *Nature Reviews Immunology* 17, no. 4: 219–232.

Ley, R. E., M. Hamady, C. Lozupone, et al. 2008. "Evolution of Mammals and Their Gut Microbes." *Science* 320, no. 5883: 1647–1651.

Lyte, M. 2013. "Microbial Endocrinology in the Microbiome-Gut-Brain Axis: How Bacterial Production and Utilization of Neurochemicals Influence Behavior." *PLoS Pathogens* 9, no. 11: e1003726.

Mansouri-Tehrani, H.-A., M. Rabbani-Khorasgani, S. M. Hosseini, F. Mokarian, H. Mahdavi, and M. Roayaei. 2015. "Effect of Supplements: Probiotics and Probiotic Plus Honey on Blood Cell Counts and Serum IgA in Patients Receiving Pelvic Radiotherapy." *Journal of Research in Medical Sciences* 20, no. 7: 679–683.

Maslowski, K. M., A. T. Vieira, A. Ng, et al. 2009. "Regulation of Inflammatory Responses by Gut Microbiota and Chemoattractant Receptor GPR43." *Nature* 461, no. 7268: 1282–1286.

Mazziotta, C., M. Tognon, F. Martini, E. Torreggiani, and J. C. Rotondo. 2023. "Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health." *Cells* 12, no. 1: 184.

Mcpherson, R., and M. Pincus. 2011. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. WB Saunders.

Mishra, V., C. Shah, N. Mokashe, R. Chavan, H. Yadav, and J. Prajapati. 2015. "Probiotics as Potential Antioxidants: A Systematic Review." *Journal of Agricultural and Food Chemistry* 63, no. 14: 3615–3626.

Mohammadi, A. Z., A. M. Khadem, K. B. Jalali, M. J. M. Mosadegh, and S. M. Mahdavi. 2009. "Evaluation of Serum Malondialdehyde Spectrophotometrically and High Performance Liquid Chromatography and its Relationship With Coronary Artery Disease." *Studies in Medical Sciences* 19, no. 4: 289–294.

Neuman, H., J. W. Debelius, R. Knight, and O. Koren. 2015. "Microbial Endocrinology: The Interplay Between the Microbiota and the Endocrine System." *FEMS Microbiology Reviews* 39, no. 4: 509–521.

Norouzi, S., H. Pourjafar, F. Ansari, and A. Homayouni. 2019. "A Survey on the Survival of *Lactobacillus paracasei* in Fermented and Non-Fermented Frozen Soy Dessert." *Biocatalysis and Agricultural Biotechnology* 21: 101297.

Ollivier, M., A. Tresset, F. Bastian, et al. 2016. "Amy2B Copy Number Variation Reveals Starch Diet Adaptations in Ancient European Dogs." *Royal Society Open Science* 3, no. 11: 160449.

Ooi, L.-G., R. Bhat, A. Rosma, K.-H. Yuen, and M.-T. Liong. 2010. "A Synbiotic Containing *Lactobacillus acidophilus* CHO-220 and Inulin Improves Irregularity of Red Blood Cells." *Journal of Dairy Science* 93, no. 10: 4535–4544.

Pagana, K. D., and T. J. Pagana. 2012. *Mosby's Diagnostic and Laboratory Test Reference-E-book*. Elsevier Health Sciences.

Perdigon, G., M. De Macias, S. Alvarez, G. Oliver, and A. P. de Ruiz Holgado. 1988. "Systemic Augmentation of the Immune Response in Mice by Feeding Fermented Milks With *Lactobacillus casei* and *Lactobacillus acidophilus*." *Immunology* 63, no. 1: 17.

Pirinccioglu, A. G., D. Gökalp, M. Pirinccioglu, G. Kizil, and M. Kizil. 2010. "Malondialdehyde (MDA) and Protein Carbonyl (PCO) Levels as Biomarkers of Oxidative Stress in Subjects With Familial Hypercholesterolemia." *Clinical Biochemistry* 43, no. 15: 1220–1224.

Pourrajab, B., S. Fatahi, M. H. Sohouli, M.-A. Găman, and F. Shidfar. 2022. "The Effects of Probiotic/Synbiotic Supplementation Compared to Placebo on Biomarkers of Oxidative Stress in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials." *Critical Reviews in Food Science and Nutrition* 62, no. 2: 490–507.

Rad, A. H., S. Hosseini, and H. Pourjafar. 2022. "Postbiotics as Dynamic Biological Molecules for Antimicrobial Activity: A Mini-Review." *Biointerface Research in Applied Chemistry* 12, no. 5: 6543–6556.

Roller, M., G. Rechkemmer, and B. Watzl. 2004. "Prebiotic Inulin Enriched With Oligofructose in Combination With the Probiotics *Lac-*

tobacillus rhamnosus and *Bifidobacterium lactis* Modulates Intestinal Immune Functions in Rats." *Journal of Nutrition* 134, no. 1: 153–156.

Roshan, H., E. Ghaedi, J. Rahmani, et al. 2019. "Effects of Probiotics and Synbiotic Supplementation on Antioxidant Status: A Meta-Analysis of Randomized Clinical Trials." *Clinical Nutrition ESPEN* 30: 81–88.

Rossi, G., G. Pengo, L. Galosi, et al. 2020. "Effects of the Probiotic Mixture Slab51®(SivoMixx®) as Food Supplement in Healthy Dogs: Evaluation of Fecal Microbiota, Clinical Parameters and Immune Function." *Frontiers in Veterinary Science* 7: 613.

Saghir, S. A., A. M. Al Hroob, A. H. Al-Tarawni, et al. 2024. "Effect of *Lactiplantibacillus plantarum* on the Growth, Hemato-Biochemical, Inflammation, Apoptosis, Oxidative Stress Markers, Involved Gens and Histopathological Alterations in Growing Rabbits Challenged With Aflatoxin B1." *Poultry Science* 103, no. 9: 104002.

Satari, A., S. Askari, G. Naderi, and A. Rezayat. 2003. "Lipid Peroxidation and Antioxidation Capacity in Posttaumatic Stress Disorder." *Journal of Research in Medical Sciences* 8, no. 1: 1735–1736.

Schiffrin, E., F. Rochat, H. Link-Amster, J. Aeschlimann, and A. Donnet-Hughes. 1995. "Immunomodulation of Human Blood Cells Following the Ingestion of Lactic Acid Bacteria." *Journal of Dairy Science* 78, no. 3: 491–497.

Schmitz, S., and J. Suchodolski. 2016. "Understanding the Canine Intestinal Microbiota and Its Modification by Pro-, Pre-and Synbiotics—What Is the Evidence?" *Veterinary Medicine and Science* 2, no. 2: 71–94.

Schrezenmeir, J., and M. de Vrese. 2001. "Probiotics, Prebiotics, and Synbiotics—Approaching a Definition." *American Journal of Clinical Nutrition* 73, no. 2: 361S–364S.

Simpson, K., M. Rishniw, M. Bellosa, et al. 2009. "Influence of *Enterococcus faecium* SF68 Probiotic on Giardiasis in Dogs." *Journal of Veterinary Internal Medicine* 23, no. 3: 476–481.

Sivamaruthi, B. S., P. Kesika, and C. Chaiyasut. 2021. "Influence of Probiotic Supplementation on Health Status of the Dogs: A Review." *Applied Sciences* 11, no. 23: 11384.

Spyropoulos, B. G., E. P. Misiakos, C. Fotiadis, and C. N. Stoidis. 2011. "Antioxidant Properties of Probiotics and Their Protective Effects in the Pathogenesis of Radiation-Induced Enteritis and Colitis." *Digestive Diseases and Sciences* 56: 285–294.

Srinivas, K., A. Das, P. B. Reddy, and A. Verma. 2024. "Supplementation of Tropical Red Seaweeds Improved Gut Health Indices, Antioxidant Status and Immunity in Adult Dogs." *Journal of Applied Phycology* 36: 2183–2198.

Stene, C., A. Röme, I. Palmquist, et al. 2022. "Administration of Probiotics to Healthy Volunteers: Effects on Reactivity of Intestinal Mucosa and Systemic Leukocytes." *BMC Gastroenterology* 22, no. 1: 100.

Strober, W. 1990. "Regulation of IgA B-Cell Development in the Mucosal Immune System." *Journal of Clinical Immunology* 10: 56S–63S.

Sun, H. Y., and I. H. Kim. 2020. "Effect of *Lactobacillus acidophilus* Based Probiotic Product Supplementation on the Blood Profile, Fecal Noxious Gas Emission, and Fecal Shedding of Lactic Acid Bacteria and Coliform Bacteria in Healthy Adult Beagle Dogs." *Korean Journal of Agricultural Science* 47, no. 3: 437–443.

Thomas, C. M., and J. Versalovic. 2010. "Probiotics-Host Communication: Modulation of Signaling Pathways in the Intestine." *Gut Microbes* 1, no. 3: 148–163.

Wang, G.-d., W. Zhai, H.-c. Yang, et al. 2013. "The Genomics of Selection in Dogs and the Parallel Evolution Between Dogs and Humans." *Nature Communications* 4, no. 1: 1860.

Xu, H., W. Huang, Q. Hou, L.-Y. Kwok, and Z. Sun. 2019. "Oral Administration of Compound Probiotics Improved Canine Feed Intake, Weight Gain, Immunity and Intestinal Microbiota." *Frontiers in Immunology* 10: 394673.

Zamani, B., A. Sheikhi, N. Namazi, B. Larijani, and L. Azadbakht. 2020. "The Effects of Supplementation With Probiotic on Biomarkers of Oxidative Stress in Adult Subjects: A Systematic Review and Meta-Analysis of Randomized Trials." *Probiotics and Antimicrobial Proteins* 12: 102–111.

Zhao, J., L. Yu, Q. Zhai, F. Tian, H. Zhang, and W. Chen. 2020. "Effects of Probiotic Administration on Hepatic Antioxidative Parameters Depending on Oxidative Stress Models: A Meta-Analysis of Animal Experiments." *Journal of Functional Foods* 71: 103936.