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Usability of Postbiotics in Ruminant Nutrition and Health

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ARTICLE INFO	ABSTRACT
	Since the ban on the use of antibiotics to promote growth in animal production in 2006, there has been a growing interest in alternative feed additives for animal production. Postbiotics, which have been utilized for this purpose in recent years, are highly promising feed additives that substitute banned substances like antibiotics. In recent years, there have been numerous studies on the utilization of postbiotics in ruminant animal nutrition. This article includes the definition of postbiotics, their properties, methods of obtaining them, and their potential applications in ruminant animal nutrition and health.

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Introduction

Since the ban on the use of antibiotics to promote growth in animal production in 2006, there has been a growing interest in alternative feed additives for animal production. According to the European Commission, feed additives are products used in animal nutrition to enhance the quality of feed and food of animal origin, as well as to improve the productivity and health of animals that consume them (Szuba-Trznadel & Rzasa, 2023). Postbiotics used for this purpose in recent years are very promising feed additives, replacing banned substances such as antibiotics (Sharma et al., 2020).

In recent years, new terms related to biotics have gained attention, including probiotics and postbiotics. Postbiotics are defined as non-living biological by-products that are beneficial to the host. Postbiotics are soluble factors that are secreted by living microorganisms or formed after cellular lysis, and they benefit the host organism. These factors are commonly known as microbial products or metabolic byproducts. Bioactive compounds produced by microorganisms during fermentation include microbial cells, cell components, and metabolites found in postbiotics (Gökırmaklı et al., 2021). This term can elicit

biological reactions by signaling to various organs and tissues within the host organism.

Postbiotics are a dependable alternative feed additive due to their inclusion of diverse signaling molecules with immunomodulatory (regulating immune system responses), anti-inflammatory, anti-obesity, anti-hypertensive, hypocholesterolemic, antiproliferative (inhibiting cell proliferation), and antioxidant properties (Nakamura et al., 2012). Additionally, they can maintain stability in the gastrointestinal system. In addition, it is a highly beneficial feed additive because it has no toxic effects, a longer storage period compared to probiotics, and is resistant to enzymes (Gökırmaklı et al., 2021).

In the field of ruminant nutrition, numerous studies have reported immunomodulatory, anti-inflammatory, antioxidant, and antibacterial effects, as well as improved blood metabolites, rumen and intestinal health in general. The impact of postbiotic utilization in monogastric animals (such as broilers, laying hens, and piglets) is comparable to that in ruminants (Izuddin et al., 2020). This review discusses the effects of postbiotics on ruminant nutrition and health.

Postbiotic Sources

Cell wall components and cytoplasmic extracts of various bacteria, particularly *Lactobacillus* species such as *L. acidophilus*, *L. casei*, *L. fermentum*, *L. rhamnosus*, *L. paracasei*, and *L. delbrueckii subsp. Bulgaricus*, *L. reuteri*, and *L. sakei* are utilized as postbiotics for both humans and animals (Sevin et al., 2021). Furthermore, *Bifidobacterium* species, *Faecalibacterium prausnitzii*, and *Bacillus coagulans* have demonstrated postbiotic properties. *Lactobacillus plantarum* strains, either alone or in combination, are the most prevalent producers of postbiotics (Sharma et al., 2020). *L. plantarum* strains have demonstrated activity in rats, pigs, broiler chickens, laying hens, ruminants, and humans (Sharma et al., 2020). *Saccharomyces cerevisiae*, a type of yeast, has been utilized for anaerobic fermentation in a controlled environment and for producing metabolites following the dehydration of the liquid (Coleman et al., 2023; Thomas et al., 2023). In addition, metabolites obtained after fermentation with a fungal strain of *Aspergillus oryzae* have also been utilized as postbiotics (Kaufman et al., 2021; Rius et al., 2022).

Methods of Obtaining Postbiotics

Postbiotics can be obtained from components of microbial cells and metabolic by-products resulting from microbial activities on a nutrient matrix (Barros et al., 2020). Postbiotic production occurs in three stages. The first stage of postbiotic production involves cell disruption. For this purpose, various methods are employed including heat treatment (at temperatures such as 95°C, 100°C, etc.), high-pressure processing, enzymatic treatment (using lysozyme/mutanolysin), co-cultivation (with Saccharomyces cerevisiae), solvent extraction (using ethyl ether; n-butanol extraction for lipoteichoic acid), chemical processes (such as formalin treatment; ethanol-potassium hydroxide solution), and sonication (at power levels such as 50 W, 300 W) (Dinic et al., 2017; Moradi et al., 2021; Thorakkattu et al., 2022; Coleman et al., 2023; Thomas et al., 2023). Among these methods, the most common are heat treatment and sonication (Shin et al., 2010; Sharma et al., 2011; Tiptiri-Kourpeti et al., 2016; Thorakkattu et al., 2022). However, improper solvent extraction and chemical processes during cell disruption can lead to a decrease in postbiotic activity or some volatile components.

The second stage of postbiotic production is the extraction and purification of the cells. The methods used in this sense are centrifugation, dialysis, filtration and column purification (Izuddin et al., 2019b; Thorakkattu et al., 2022). Among these extraction and purification methods, centrifugation is the most common and cost-effective (Thorakkattu et al., 2022).

For ease of use and storage of postbiotics, it is recommended to subject them to lyophilization and spray drying processes after extraction. Lyophilization aids in the removal of volatile metabolites such as hydrogen peroxide, responsible for antimicrobial activity (Prado et al., 2000), while spray drying helps eliminate volatile metabolites such as ethanol (Hartman et al., 2011).

Effects of Postbiotics on Ruminant Nutrition

The Effects of Postbiotics on Rumen Health

Postbiotics added to the ration of ruminants have various effects, including increasing digestive efficiency, improving fermentation, maintaining acid balance, strengthening the immune system, and increasing methane production (Izuddin et al., 2019a; Izuddin et al., 2020).

According to recent studies, the addition of postbiotics to *in vitro* rumen fermentation does not cause any change in rumen fluid pH, unlike probiotics (Vosooghi-Poostindoz et al., 2014; Izuddin et al., 2019b). The lack of changes in rumen pH may indicate that the rumen environment has adapted and is regulated to the presence of lactic acid resulting from the addition of postbiotics to the diet. Furthermore, the breakdown of dietary protein and non-protein nitrogen by rumen microorganisms, along with the addition of postbiotics, leads to an increase in ruminal NH_3-N levels by elevating the concentration of ammonia in the rumen. At the same time, *in vitro* rumen fermentation results in an increase in the concentration of total and individual unsaturated fatty acids (UFAs) such as acetic, propionic, and butyric (Izuddin et al., 2019b). However, in a living organism, the introduction of postbiotics only elevates the level of propionic acid, similar to the introduction of probiotics. This is attributed to the elevated levels of lactic acid found in postbiotics and the conversion of lactic acid into propionic acid by bacteria, such as *Propionibacterium spp.*, that utilize lactic acid (Izuddin et al., 2019a). Increased propionate synthesis reduces the availability of H_2 by decreasing methane formation and increasing energy intake from the diet. Correspondingly, methane production decreases as a result of a reduced population of ruminal methanogens (Jeyanathan et al., 2014; Izuddin et al., 2019a). In addition, when postbiotics are added to *in vitro* rumen fermentation, the total bacteria, *R. albus*, and total protozoa populations are not affected, while the population of cellulolytic bacteria such as *F. succinogenes* and *R. flavefaciens* increases (Izuddin et al., 2019b). An increased population of cellulolytic bacteria leads to improved digestion of dry matter (DM) and neutral detergent fiber (NDF) in the feed (Izuddin et al., 2019a). In addition, post-weaning administration of postbiotics increases the number of binding proteins in the rumen epithelium that regulate ruminal barrier function, such as TJP (Tight junction protein), OCLD (Occludin), CLDN1 (Claudin 1), and CLDN2 (Claudin 2) (Izuddin et al., 2020).

Effects of Postbiotics on Intestinal Health

Postbiotics enhance intestinal health by reducing inflammation, regulating intestinal permeability, regulating intestinal flora, and aiding in digestive problems (Ji et al., 2023).

Recent research suggests that the inclusion of postbiotics into the ration results in an enlargement and improved structure of rumen papillae (Izuddin et al., 2019b). This increase in rumen papillae dimensions, facilitated by postbiotics, stimulates the production of UFAs, especially butyric acid, in the rumen. The increased production of UFAs contributes to enhanced microbial fermentation.

The observed increase in the size and structure of rumen papillae indicates morphological development of the epithelium. The enlargement of papillae dimensions increases the surface area of these structures, thereby enhancing nutrient absorption and facilitating the adhesion of microorganisms to the rumen wall (Shazali et al., 2014). Importantly, these effects are specific to the rumen and do not affect intestinal villi. The lack of a postbiotic effect on the intestinal villi of ruminants may be due to interactions or modifications of postbiotics in the ruminant's stomach, which may not affect intestinal morphology.

Furthermore, the inclusion of postbiotics in the diet does not impact the overall populations of bacteria, lactic acid bacteria (LAB), or *E. coli* in the small intestine; however, it does result in a decrease in the *Enterobacteriaceae* population (Izuddin et al., 2019b). The reduction in the *Enterobacteriaceae* population may be linked to the activation of the mucosal immune system in the intestine, aiding in the removal of pathogenic bacteria as part of mucosal immunity (Izuddin et al., 2019b).

In a study conducted by Rius et al. (2022), it was observed that administering postbiotics to heat-stressed calves helped reduce the increased permeability of the small intestine. This suggests that such an application could improve water absorption and subsequently enhance intestinal functionality.

Effects of Postbiotics on Milk Yield and Milk Composition

In the field of ruminant nutrition, postbiotics are known to have a positive effects on milk yield and composition (Izuddin et al., 2019b). Postbiotics can enhance milk yield by improving digestive system health and nutrient absorption. They can also increase milk production by reducing stress levels in animals. Postbiotics can significantly increase not only milk yield but also milk components such as milk fat, protein, lactose, and calcium(Izuddin et al., 2019b).

In a particular study, the addition of postbiotics derived from *lactobacilli* in the rations of lactating goats led to a notable increase in milk yield, fat-corrected milk yield, and actual protein levels (Fernandez et al., 2023). In addition to these changes, a decrease in milk urea and N-NH$_3$ levels can also be observed. Another study demonstrated that supplementing the diets of lactating dairy cows with *Saccharomyces cerevisiae* postbiotics not only increased milk yield but also elevated milk components (Poppy et al., 2012; Coleman et al., 2023; Thomas et al., 2023).

In ruminant animal nutrition, postbiotics have been found to possess properties such as strengthening the immune system, reducing inflammation, acting as antioxidants, fighting bacteria, and improving rumen and intestinal health. Due to these properties, postbiotics are considered potential alternatives to antibiotics. However, there is a limited amount of research on their use in ruminant nutrition. If the various mechanisms of action of postbiotics are fully elucidated, their effects on the host organism will be completely revealed. The use of postbiotics as a substitute for antibiotics in ruminant nutrition offers potential for research and commercialization.

Effects of Postbiotics on Ruminant Health

Effects of Postbiotics on the Immune System

Just like probiotics, which play a crucial role in regulating the immune system and host health, postbiotics, the by-products of probiotics, are reported to have positive effects on the immune system (Sak & Soykut, 2021). One of the most significant positive effects of postbiotics is their immunomodulatory properties (Szuba-Trznadel & Rzasa, 2023).

The antigen binds to receptors on the surface of effector cells, such as macrophages, neutrophils, T and B lymphocytes, and monocytes, and stimulates the immune response after the completion of the recognition and activation stages. The activated immune cells produce cytokines, which are a type of cell hormone or pro-inflammatory mediator responsible for their reproduction and proliferation. One of the cytokines, interleukin 1 (IL-1), plays a crucial role in the immune response by activating macrophages, converting T lymphocytes, and secreting other interleukins. If antigens, such as pathogenic microorganisms, are present in the environment, stimulated macrophages will recognize them and initiate a cellular response. In addition, T lymphocytes exert a cytotoxic effect by capturing and neutralizing various toxins. As a result, B lymphocytes stimulate the immune system by initiating humoral immunity and producing antibodies (Davis et al., 2004; Broadway et al., 2015; Naqid et al., 2015; Roselli et al., 2017). In light of this information, lipoteichoic acid derived from bacteria can stimulate the immune system and prevent the excessive response of effector cells. It can also enhance digestion by aiding in the regeneration of the mucosa and intestinal walls (Martyniak et al., 2021). Postbiotics exhibit immunomodulatory effects by participating in these physiological processes.

Several studies have reported immunomodulatory effects of postbiotics in ruminants, humans, and other animal species. The inclusion of postbiotics in the diet in response to the stress caused by premature weaning in ruminants promotes papillae development, reduces the requirement for increased mucosal antibody and antimicrobial peptide production, lowers the intestinal population of pathogens, and diminishes the immune response needed to defend against pathogens. It also increases the production of IL-6 and decreases the production of IL-1β, IL-10, and TNF (Tumour necrosis factor) in the jejunum mucosa. Furthermore, regulating the intestinal barrier contributes to the overall health and integrity of the intestinal mucosa (Izuddin et al., 2019a).

Anti-inflammatory effects of Postbiotics

Postbiotics exert anti-inflammatory effects by regulating the production of cytokines secreted by cells in the intestinal mucosa. This leads to a decrease in inflammatory cytokines (molecules associated with inflammation) and an increase in anti-inflammatory cytokines, which helps to control inflammation in the gut.

Elevated levels of lymphocytes, basophils, and neutrophils in the body are closely linked to inflammation as a response to infection.

Neutrophils are a component of the innate immune system and are immune cells that combat pathogens through phagocytosis (Lee et al., 2003; Rosales, 2018). The rise in lymphocytes and basophils stimulates T and B cells, which are essential for the production of immune substances. T cells play a crucial role in recognizing macrophages and enhancing phagocytosis activity, while cytotoxic T cells effectively eliminate infected cells (Coleman et al., 2023). Although basophils do not phagocytose, they play a role in initiating T2-mediated immunity and regulating innate immunity (Sokol & Medzhitov, 2010). Blood platelets play a crucial role in hemostasis, inflammation, and tissue repair (Jenne & Kubes, 2015; Sonmez & Sonmez, 2017). Platelets are involved in antimicrobial host defense and the promotion of inflammation. In cases of infection or inflammation, platelets facilitate the clearance of pathogens by increasing the inflammatory reaction (Klinger & Jelkmann, 2002).

In studies, the anti-inflammatory effect in ruminants varies based on the quantity and duration of postbiotic administration. Long-term administration of postbiotics in lambs reduces leukocyte and platelet levels, supporting the development of an anti-inflammatory response to infection and inflammation in lambs (Izuddin et al., 2019b). Short-term supplementation with postbiotics does not demonstrate any anti-inflammatory effect (Coleman et al., 2023).

Antioxidant effects of Postbiotics

Postbiotics may help reduce oxidative stress and oxidative DNA damage by neutralizing free radicals in the body and increasing the activity of antioxidant enzymes, especially catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx).

The generation of reactive oxygen species (ROS) and oxygen-centered free radicals occurs as by-products of biochemical and physiological processes (Thibessard et al., 2004; Lin et al., 2018). Elevated ROS levels, stemming from increased production of oxidant species or decreased activity of the antioxidant system, can contribute to the oxidative stress observed in various diseases affecting both humans and animals (Lombardi et al., 2017). In progressive cases, excessive formation and accumulation of ROS can lead to tissue destruction initially and then to organ failure (Halliwell & Gutteridge, 1984; Izuddin et al., 2019b).

Recent studies suggest that administering postbiotics to ruminants can positively affect antioxidant enzymes, increasing in glutathione peroxidase (GPx) concentration (Izuddin et al., 2020) and a decrease in Mg and FRAP (Ferric Reducing Antioxidant Power) levels (Aghebati-Maleki et al., 2022; Coleman et al., 2023). These positive effects are thought to be due to the production of exopolysaccharides, lipoteichoic acid, and cell surface proteins, which are by-products of probiotics (Markov et al., 2015; Lin et al., 2018; Pan & Mei, 2010; Yi et al., 2009).

Anti-bacterial Effects of Postbiotics

Postbiotics exhibit antibacterial effects by reducing the pH levels of the environment, creating an acidic environment, generating antimicrobial compounds known as bacteriocins, and depleting the nutrients necessary for the growth and reproduction of pathogenic bacteria (Sevin et al., 2021). In addition, some postbiotics can show direct antibacterial effects by competitively binding to receptors required by pathogens by lining the intestinal barrier (Humam et al., 2021).

Postbiotics exhibit antibacterial effects that vary depending on the probiotic strain used to produce them, the specific target bacteria (Gram-positive bacteria are more resistant to postbiotic compounds than Gram-negative bacteria), and the concentration of the postbiotic (Moradi et al., 2019). Previous studies have shown that postbiotics derived from bacteria of *Lactococcus* and *Lactobacillus* species exhibit antibacterial properties against pathogens that cause mastitis in ruminants (Bouchard et al., 2013; (2015) Malvisi et al., 2016; Pellegrino et al., 2019). Furthermore, there was a variable susceptibility to cell-free supernatant observed among strains of *S. uberis* and *Enterococcus faecalis*. *S. agalactiae*, *S. uberis*, and *E. faecalis* exhibited high MIC (Minimal inhibitory concentration) values. *In vitro* studies have also shown that postbiotics derived from *Lactobacillus plantarum* act as inhibitory agents, effectively reducing the adhesion and invasive abilities of *Listeria monocytogenes* (Moradi et al., 2019).

Effects of Postbiotics on Blood Metabolites

Postbiotics added to ruminant diets can lead to changes in blood parameters, which depend on the type of probiotic-effective microorganism used and the duration of their administration.

While postbiotics may vary based on nutrient digestibility and nitrogen utilization capacity in the rumen, they have been found to increase blood total protein and BUN (Blood Urea Nitrogen) levels with long-term use. They also increase blood glucose levels by promoting the production and absorption of propionic acid, which is a precursor of glucose, leading to increased glucose production. Furthermore, postbiotics do not alter serum triglyceride and cholesterol levels, as they do not affect on the digestibility of crude fat or the absorption of fat digestion products in the small intestine (Izuddin et al., 2019a). When probiotic microorganisms are used in fermentation to produce a product added to the ration, it has been observed that levels of creatinine, AST (Aspartate transaminase), cholesterol, glucose, and GGT (Gamma-glutamyl transferase) in the blood increase (Coleman et al., 2023). It also significantly increases blood NEFA (Non esterified fatty acids) concentration by regulating energy metabolism and stimulating glycogenolysis, endogenous glucose production, and hepatic glucose output through increased secretion of catabolic stress hormones (Rius et al., 2022).

In ruminant nutrition, incorporating postbiotics into rations leads to significant increases in the production and absorption of propionic acid and glucose, thereby regulating negative energy balance during the transition period. Nevertheless, they may also exhibit ketogenic properties due to the increase in NEFA levels (Tekce & Gül, 2014).

Conclusions

Postbiotics refer to the metabolites and cell wall components that are secreted by living bacteria or released after bacterial degradation. Ruminant nutrition benefits from adding postbiotics to rations, boosting propionic acid and glucose production and absorption, aiding energy balance during transition periods. In the field of ruminant health, postbiotics have garnered attention due to their potential to modulate the immune system, reduce inflammation, act as

antioxidants, and exhibit antibacterial effects. However, a more comprehensive understanding of their nutritional and health effects necessitates further studies.

Conflict of Interest

No potential conflict of interest was reported by the authors**.**

References

Aghebati-Maleki, L., Hasannezhad, P., Abbasi, A., & Khani, N. (2021).(2022) Antibacterial, antiviral, antioxidant, and anticancer activities of postbiotics: a review of mechanisms and therapeutic perspectives. *Biointerface Research in Applied Chemistry*, *12*, 2629-2645. https://doi.org/10.33263/BRIAC122.26292645

Barros, C. P., Guimaraes, J. T., Esmerino, E. A., Duarte, M. C. K., Silva, M. C., Silva, R., Ferreira, B. M., Sant'Ana, A. S., Freitas, M. Q., & Cruz, A. G. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. *Current Opinion in Food Science*, *32*, 1-8. https://doi.org/10.1016/j.cofs.2019.12.003

Bouchard, D. S., Rault, L., Berkova, N., Le Loir, Y. & Even, S. (2015) Inhibition of Staphylococcus aureus invasion into bovine mammary epithelial cells by contact with live Lactobacillus casei. *Applied and Environmental Microbiology*, *79*(3), 877-885. https://doi.org/10.1128/AEM.03323-12

Broadway, P. R., Carroll, J. A., & Burdick Sanchez, N. C. (2015). Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: a review. *Microorganisms*, *3*(3), 417-427. https://doi.org/10.3390/microorganisms3030417

Coleman, D. N., Jiang, Q., Lopes, M. G., Ritt, L., Liang, Y., Aboragah, A., Trevisi, E., Yoon, I., & Loor, J. J. (2023). Feeding a Saccharomyces cerevisiae fermentation product before and during a feed restriction challenge on milk production, plasma biomarkers, and immune function in Holstein cows. *Journal of Animal Science*, *101*, skad019. https://doi.org/10.1093/jas/skad019

Davis, M. E. (2004). Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. *Journal of Animal Science*, *82*, 1882–1891. https://doi.org/10.2527/2004.8261882x

Dinic, M., Lukic, J., Djokic, J., Milenkovic, M., Strahinic, I., Golic, N., & Begovic, J. (2017). Lactobacillus fermentum postbiotic-induced autophagy as potential approach for treatment of acetaminophen hepatotoxicity. *Frontiers in Microbiology*, *8*, 594. https://doi.org/10.3389/fmicb.2017.00594

Fernandez, C., Romero, T., Badiola, I., Díaz-Cano, J., Sanzol, G., & Loor, J. J. (2023). Postbiotic yeast fermentation product supplementation to lactating goats increases the efficiency of milk production by enhancing fiber digestibility and ruminal propionate, and reduces energy losses in methane. *Journal of Animal Science*, *101*, skac370. https://doi.org/10.1093/jas/skac370

Gökırmaklı, Ç., Üçgül, B., & Seydim, Z. (2021). Fonksiyonel Gıda Kavramına Yeni Bir Bakış: Postbiyotikler. *Gıda*, *46*(4), 872-882. https://doi.org/10.15237/gida.GD21035

Halliwell, B., & Gutteridge, J. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, *219*(1), 1. https://doi.org/10.1042/bj2190001

Hartmann, H. A., Wilke, T., & Erdmann, R. (2011). Efficacy of bacteriocin-containing cell-free culture supernatants from lactic acid bacteria to control Listeria monocytogenes in food. *International Journal of Food Microbiology*, *146*(2), 192-199. https://doi.org/10.1016/j.ijfoodmicro.2011.02.031

Humam, A. M., Loh, T. C., Foo, H. L., Izuddin, W. I., Zulkifli, I., Samsudin, A. A., & Mustapha, N. M. (2021). Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. *Poultry Science*, *100*(3), 100908. https://doi.org/10.1016/j.psj.2020.12.011

Izuddin, W. I., Humam, A. M., Loh, T. C., Foo, H. L., & Samsudin, A. A. (2020). Dietary postbiotic Lactobacillus plantarum improves serum and ruminal antioxidant activity and upregulates hepatic antioxidant enzymes and ruminal barrier function in post-weaning lambs. *Antioxidants*, *9*(3), 250. https://doi.org/10.1016/j.psj.2020.12.011

Izuddin, W. I., Loh, T. C., Foo, H. L., Samsudin, A. A., & Humam, A. M. (2019b). Postbiotic L. plantarum RG14 improves ruminal epithelium growth, immune status and upregulates the intestinal barrier function in post-weaning lambs. *Scientific Reports*, *9*(1), 9938. https://doi.org/10.1038/s41598-019-46076-0

Izuddin, W. I., Loh, T. C., Samsudin, A. A., Foo, H. L., Humam, A. M., & Shazali, N. (2019a). Effects of postbiotic supplementation on growth performance, ruminal fermentation and microbial profile, blood metabolite and GHR, IGF-1 and MCT-1 gene expression in post-weaning lambs. *BMC Veterinary Research*, *15*(1), 1-10. https://doi.org/10.1186/s12917-019-2064-9

Jenne, C. N., & Kubes, P. (2015). Platelets in inflammation and infection. *Platelets*, *26*(4), 286-292. https://doi.org/10.3109/09537104.2015.1010441

Jeyanathan, J., Martin, C., & Morgavi, D. (2014). The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal, 8*(2), 250–261. https://doi.org/10.1017/S1751731113002085

Ji, J., Jin, W., Liu, S. J., Jiao, Z., & Li, X. (2023). Probiotics, prebiotics, and postbiotics in health and disease. *MedComm*, *4*(6), e420. https://doi.org/10.1002/mco2.420.

Kaufman, J. D., Seidler, Y., Bailey, H. R., Whitacre, L., Bargo, F., Lüersen, K., Rimbach, G., Pighetti, G. M., Ipharraguerre, I. R., & Rius, A. G. (2021). A postbiotic from Aspergillus oryzae attenuates the impact of heat stress in ectothermic and endothermic organisms. *Scientific Reports*, *11*(1), 6407. https://doi.org/10.1038/s41598-021-85707-3

Klinger, M. H., & Jelkmann, W. (2002). Role of blood platelets in infection and inflammation. *Journal of Interferon & Cytokine Research*, *22*(9), 913-922. https://doi.org/10.1089/10799900260286623

Lee, W. L., Harrison, R. E., & Grinstein, S. (2003). Phagocytosis by neutrophils. *Microbes and Infection*, *5*(14), 1299-1306. https://doi.org/10.1016/j.micinf.2003.09.014

Lin, X., Xia, Y., Wang, G., Yang, Y., Xiong, Z., Lv, F., Zhou, W., & Ai, L. (2018). Lactic acid bacteria with antioxidant activities alleviating oxidized oil induced hepatic injury in mice. *Frontiers in Microbiology, 9*, 2684. https://doi.org/10.3389/fmicb.2018.02684

Lombardi, P., Musco, N., Cutrignelli, M. I., Mollica, M. P., Trinchese, G., Calabrò, S., Tudisco, R., Grossi, M., Mastellone, V., Vassalotti, G., Pero, M. E., Morittu, V. M., & Infascelli, F. (2017). The association of aloe and β-carotene supplementation improves oxidative stress and inflammatory state in pregnant buffalo cows. *Buffalo Bulletin, 36*(3), 497-503.

Malvisi, M., Stuknytė, M., Magro, G., Minozzi, G., Giardini, A., De Noni, I., & Piccinini, R. (2016). Antibacterial activity and immunomodulatory effects on a bovine mammary epithelial cell line exerted by nisin A-producing Lactococcus lactis strains. *Journal of Dairy Science*, *99*(3), 2288-2296. https://doi.org/10.3168/jds.2015-10161

Markov, A. G., Aschenbach, J. R., & Amasheh, S. (2015). Claudin clusters as determinants of epithelial barrier function. *IUBMB life, 67*(1), 29-35. https://doi.org/10.1002/iub.1347

Martyniak, A., Medyńska-Przęczek, A., Wędrychowicz, A., Skoczeń, S., & Tomasik, P. J. (2021). Prebiotics, probiotics, synbiotics, paraprobiotics and postbiotic compounds in IBD. *Biomolecules,* *11*(12), 1903. https://doi.org/10.3390/biom11121903

Moradi, M., Mardani, K., & Tajik, H. (2019). Characterization and application of postbiotics of Lactobacillus spp. on Listeria monocytogenes *in vitro* and in food models. *LWT, 111*, 457-464. https://doi.org/10.1016/j.lwt.2019.05.072

Moradi, M., Molaei, R., & Guimaraes, J. T. (2021). A review on preparation and chemical analysis of postbiotics from lactic acid bacteria. *Enzyme and Microbial Technology*, *143*, 109722. https://doi.org/10.1016/j.enzmictec.2020.109722

Nakamura, S., Kuda, T., An, C., Kanno, T., Takahashi, H., & Kimura, B. (2012). Inhibitory effects of Leuconostoc mesenteroides 1RM3 isolated from narezushi, a fermented fish with rice, on Listeria monocytogenes infection to Caco-2 cells and A/J mice. *Anaerobe*, *18*(1), 19-24. https://doi.org/10.1016/j.anaerobe.2011.11.006.

Naqid, I. A., Owen, J. P., Maddison, B. C., Gardner, D. S., Foster, N., Tchórzewska, M. A., La Ragione Roberto, M., & Gough, K. C. (2015). Prebiotic and probiotic agents enhance antibody-based immune responses to Salmonella Typhimurium infection in pigs. *Animal Feed Science and Technology*, *201*, 57-65. https://doi.org/10.1016/j.anifeedsci.2014.12.005

Pan, D., & Mei, X. (2010). Antioxidant activity of an exopolysaccharide purified from Lactococcus lactis subsp. lactis 12. *Carbohydrate Polymers*, *80*(3), 908-914. https://doi.org/10.1016/j.carbpol.2010.01.005

Pellegrino, M. S., Frola, I. D., Natanael, B., Gobelli, D., Nader-Macias, M. E., & Bogni, C. I. (2019). *In vitro* characterization of lactic acid bacteria isolated from bovine milk as potential probiotic strains to prevent bovine mastitis. *Probiotics and Antimicrobial Proteins*, *11*, 74-84. https://doi.org/10.1007/s12602-017-9383-6

Poppy, G. D., Rabiee, A. R., Lean, I. J., Sanchez, W. K., Dorton, K. L., & Morley, P. S. (2012). A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. *Journal of Dairy Science*, *95*(10), 6027-6041. https://doi.org/10.3168/jds.2012-5577

Prado, C. S., Santos, W. L. M., Carvalho, C. R., Moreira, E. C., & Costa, O. (2000). Antimicrobial activity of lactic acid bacteria isolated from Brazilian dry fermented sausages against Listeria monocytogenes. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *52,* 417-423. https://doi.org/10.1590/S0102-09352000000400022

Rius, A. G., Kaufman, J. D., Li, M. M., Hanigan, M. D., & Ipharraguerre, I. R. (2022). Physiological responses of Holstein calves to heat stress and dietary supplementation with a postbiotic from Aspergillus oryzae. *Scientific Reports*, *12*(1), 1587.

Rosales, C. (2018). Neutrophil: a cell with many roles in inflammation or several cell types?. *Frontiers in Physiology*, *9*, 113. https://doi.org/10.3389/fphys.2018.00113

Roselli, M., Pieper, R., Rogel-Gaillard, C., de Vreis, H., Bailey, M., Smidt, H., & Lauridsen, C. (2017). Immunomodulating effects of probiotics for microbiota modulation, gut health and disease resistance in pigs. *Animal Feed Science and Technology*, *233*, 104–119. https://doi.org/10.1016/j.anifeedsci.2017.07.011

Sak, D., & Soykut, G. (2021). Biyotikler ailesinin yeni üyesi: postbiyotikler. *Genel Sağlık Bilimleri Dergisi*, *3*(3), 259-272.

Sevin, S., Karaca, B., Haliscelik, O., Kibar, H., OmerOglou, E., & Kiran, F. (2021) Postbiotics secreted by Lactobacillus sakei EIR/CM-1 isolated from cow milk microbiota, display antibacterial and antibiofilm activity against ruminant mastitis-causing pathogens. *Italian Journal of Animal Science*, *20*(1), 1302-1316. https://doi.org/10.1080/1828051X.2021.1958077

Sharma, R. K., Chauhan, K., Pathak, A. K., & Khan, N. (2020). Postbiotics for animal health and performance. *Intas Polivet, 21*(2), 288-294.

Sharma, S., Singh, R. L., & Kakkar, P. (2011). Modulation of Bax/Bcl-2 and caspases by probiotics during acetaminophen induced apoptosis in primary hepatocytes. *Food and chemical toxicology*, *49*(4), 770-779. https://doi.org/10.1016/j.fct.2010.11.041

Shazali, N., Foo, H. L., Loh, T. C., Choe, D. W., & Abdul Rahim, R. (2014). Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. *Gut Pathogens*, *6*(1), 1-7.

Shin, H. S., Park, S. Y., Lee, D. K., Kim, S. A., An, H. M., Kim, J. R., Kim, M. J., Ca, M. G., Lee, S. W., Kim, K. J., Lee, K. O., & Ha, N. J. (2010). Hypocholesterolemic effect of sonication-killed Bifidobacterium longum isolated from healthy adult Koreans in high cholesterol fed rats. *Archives of pharmacal research*, *33*, 1425-1431.

Sokol, C. L., & Medzhitov, R. (2010). Emerging functions of basophils in protective and allergic immune responses. *Mucosal Immunology*, *3*(2), 129-137. https://doi.org/10.1038/mi.2009.137

Sonmez, O., & Sonmez, M. (2017). Role of platelets in immune system and inflammation. *Porto Biomedical Journal*, *2*(6), 311-314. https://doi.org/10.1016/j.pbj.2017.05.005

Szuba-Trznadel, A., & Rzasa, A. (2023). Feed additives of bacterial origin as an immunoprotective or imunostimulating factor. *Annals of Animal Science*. https://doi.org/10.2478/aoas-2023-0021

Tekce, E., & Gül, M. (2014). Ruminant beslemede NDF ve ADF'nin önemi. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, *9*(1), 63-73.

Thibessard, A., Borges, F., Fernandez, A., Gintz, B., Decaris, B., & Leblond-Bourget, N. (2004). Identification of Streptococcus thermophilus CNRZ368 genes involved in defense against superoxide stress. *Applied and Environmental Microbiology*, *70*(4), 2220-2229. https://doi.org/10.1128/AEM.70.4.2220-2229.2004

Thomas, M., Serrenho, R. C., Puga, S. O., Torres, J. M., Puga, S. O., & Stangaferro, M. (2023). Effect of feeding a Saccharomyces cerevisiae fermentation product to Holstein cows exposed to high temperature and humidity conditions on milk production performance and efficiency—A pen-level trial. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2022-22516

Thorakkattu, P., Khanashyam, A. C., Shah, K., Babu, K. S., Mundanat, A. S., Deliephan, A., Deokar, G. S., Santivarangkna, C., & Nirmal, N. P. (2022). Postbiotics: Current trends in food and Pharmaceutical industry. *Foods*, *11*(19), 3094. https://doi.org/10.3390/foods11193094

Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Aindelis, G., Tompoulidou, E., Lamprianidou, E. E., Saxami, G., Ypsilantis, P., Lampri, E. S., Simopoulos, C., Kotsianidis, I., Galanis, A., Kourkoutas, Y., Dimitrellou, D., & Chlichlia, K. (2016). Lactobacillus casei exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PloS one*, *11*(2), e0147960. https://doi.org/10.1371/journal.pone.0147960

Vosooghi-Poostindoz, V., Foroughi, A., Delkhoroshan, A., Ghaffari, M., Vakili, R., & Soleimani, A. (2014). Effects of different levels of protein with or without probiotics on growth performance and blood metabolite responses during pre-and post-weaning phases in male Kurdi lambs. *Small Ruminant Research*, *117*(1), 1–9. https://doi.org/10.1016/j.smallrumres.2013.11.015

Yi, Z. J., Fu, Y. R., Li, M., Gao, K.S., & Zhang, X. G. (2009) Effect of LTA isolated from bifidobacteria on D-galactose-induced aging. *Experimental Gerontology*, *44*(12), 760-765. https://doi.org/10.1016/j.exger.2009.08.011