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Review

Roles of Probiotics in Animal Health

Yiğit SEFEROĞLU^{1*}[©], Şükrü KIRKAN¹[©]

¹Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, AYDIN

ABSTRACT

Probiotics are defined as live microorganisms consumed by humans and animals that affect the intestinal microflora qualitatively or quantitatively or trigger the beneficial effects of the immune system. The discovery of probiotics and the beginning of studies date back to the end of the 19th century. Afterwards, these studies continue on the microorganisms used as probiotics, selection criteria and probiotic microorganisms in the animal microbiota. Today's probiotics are used as immune system modulation and protection against pathogenic microorganisms in veterinary medicine. In recent studies against gastrointestinal system disorders in cats, dogs and poultry and on the immune system before or after treatment, probiotic applications have been found to be successful in ruminants, especially in mastitis cases. Due to important problems caused by the use of antibiotics in animal breeding, such as the increase in populations of antibiotic resistant bacteria, it seems possible to use the latest probiotic applications as an alternative to antibiotics, especially for prophylaxis. In this review, the effectiveness of probiotic microorganisms on the basis of diseases and their effects on the immune system are discussed together with current studies. *Keywords: Animal health, imuune system, probiotic*

Probiyotiklerin Hayvan Sağlığındaki Rolleri

ÖZET

Probiyotikler kalitatif veya kantitatif olarak bağırsak mikroflorasına etki eden ya da immun sistemin faydalı etkilerini tetikleyen, insanlar ile hayvanların tükettiği canlı mikroorganizmalar olarak tanımlanmaktadır. Probiyotiklerin keşfi ve çalışmaların başlaması 19. yüzyılın sonlarına dayanmaktadır. Devamında probiyotik olarak kullanılan mikroorganizmalar, seçim kriterleri ve hayvan mikrobiyotasında bulunan probiyotik mikroorganizmalar ile ilgili araştırmalar devam etmektedir. Günümüz veteriner hekimliğinde probiyotikler, immun sistem modülasyonu ve patojen mikroorganizmalara karşı koruyucu olarak kullanılmaktadır. Kedi, köpek, kanatlılarda gastrointestinal sistem rahatsızlıklarına karşı ve tedavi öncesi veya sonrasında immun sistem üzerinde son yıllarda yapılan güncel çalışmalarda ise ruminantlarda özellikle mastitis vakalarında probiyotik uygulamalarının başarılı olduğu görülmüştür. Hayvan yetiştiriciliğinde antibiyotik kullanımının oluşturduğu, başta antibiyotik dirençli bakteri popülasyonlarının artması gibi, önemli sorunlar nedeniyle son probiyotik uygulamalarının özellikle profilaksi amacıyla antibiyotiklere alternatif olarak kullanımı mümkün görülmektedir. Bu derlemede probiyotik mikroorganizmaların hastalıklar bazında etkinliği, immun sistem üzerindeki etkileri güncel çalışmalar ile birlikte ele alınmıştır. *Anahtar kelimeler: Hayvan sağlığı, immun sistem, probiyotik*

Corresponding author: Yiğit SEFEROĞLU; yigit.seferoglu@adu.edu.tr, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, ORCID: 0000-0002-3033-2634 Received Date: 20.01.2022 – Accepted Date: 01.03.2022 DOI: 10.53913/aduveterinary.1060132

Introduction

The term probiotic was first used by Lilly and Stillwell in 1965 as "materials secreted by a microorganism that stimulate the proliferation of another microorganism" and the opposite meaning of the term antibiotic (Kaur et al., 2002). Probiotics were defined by Parker in 1974 as additional complementary foods that have beneficial effects on the intestinal microflora. In 1989, Fuller defined probiotics as "live microbial nutritional supplements that improve intestinal microbial balance for the benefit of the host animal" (Sullivan and Nord, 2002).

The first study on probiotics was made in the late 19th century by the Nobel Prize-winning Russian biologist Elie Metchnikoff, known as the father of probiotics. Metchnikoff established a relationship between fermented milk consumption and longevity and detected the presence of probiotics (lactic acid bacteria) in milk. Metchnikoff observed that Bulgarian villagers who ate yoghurt containing *Lactobacillus* as a regular part of their daily meal consumption had significant longevity and theorized that lactic acid bacteria extended their lifespan (Schrezenmeir and Vrese, 2001).

Microorganisms Used in Probiotics and Selection Criteria

The compound of the gastrointestinal tract flora differs between individuals and within the same individual throughout life. The flora of this system includes both "friendly" and pathogenic bacteria that exist in a complex symbiosis. Many factors such as aging, stress, diet, medication (especially the use of antibacterial), climate, sickness and lifestyle can corrupt this balance, leading to diarrhoea, mucosal inflammation or other serious diseases (Teshale et al., 2017). The pivotal event in the development of a probiotic approach to animal health was the dosing of newly hatched hens with a suspension of gut ingredients designed from healthy adult chickens to detect Salmonella spp. in the gut was found to be protected against colonization. Microorganisms used in probiotics include those derived from Bacillus, Enterococcus, Streptococcus, Clostridium, Lactobacillus, Bifidobacterium species and Escherichia *coli* (Kruis et al., 2004). Most probiotic bacteria are lactic acid producing bacteria. Lactic acid has been shown to inhibit coliform growth in the gastrointestinal tract. Acidic flora is harmful to various pathogens. The most widely used probiotic strains include lactic acid bacteria and other Gram-positive bacteria that have been used in food production processes (yogurt, cheese, pickles) for centuries (Henker et al., 2007).

An ideal probiotic should have various potential features that being non-pathogenic and non-toxic by nature, beneficial to the host animal, high viability, stable in storage, capable of surviving or colonizing intestinal tissue, and susceptible for cultivation in an industrial area (Teshale et al., 2017). In addition to these, a probiotic should also have properties stomach acidity, resistance to pancreatic enzymes and bile, ability to adhere to intestinal mucosal cells, high survival rate during transportation during storage, and production of antimicrobial clauses against pathogenic bacteria (Boaventura et al., 2012). In the selection of probiotics, the digestive system of healthy animals or sources of microorganisms such as flowers, rotting fruits and other niches should be selected first. Subsequently, the microorganisms intended to be studied are isolated and identified through selective culture media. A new culture is designed with only target colonies for in vivo evaluation by comparing characteristics such as target species pathogenicity, pathogen inhibition, resistance to host situations. If there are no restrictions on the use of the target species, large and small scale in vivo supplementation experiments are performed to check whether there are real benefits to the host. Finally, probiotics can be produced and used commercially, offering substantial satisfactory results. The main bacteria used in probiotic products were showed in Table 1 (Boaventura et al., 2012). Yeasts are rich in protein, B vitamins, exogenous enzymes and trace elements, and they also have a high degree of digestibility. However, very few yeast species are used commercially. Saccharomyces cerevisiae, also known as baker's yeast, is one of the most common commercialized yeast strains (Vanbelle et al., 1990).

Lactobacillus	Bifidobacterium	Other Lactic acid producing bacteria	Non-lactics
L. acidophilus	B. adolescentis	E. faecalis	B. cereus
L. casei	B. animalis	E. faecium	E. coli
L. crispatus	B. bifidum	Sporolactobacillus	P. freudenreichi
L. gallinarum	B. breve	Leuconostoc	
L. gasser	B. infantis	Mesenteroides	
L. johnsonii	B. lactis	S. thermophilus	
L. paracasei	B. longum	P. acidilactici	
L. plantarum			
L. reuteri			
L. rhamnosus			

 Table 1. Bacteria used in probiotic products (Teshale et al., 2017).

Modes of Action of Probiotics

The effect of probiotics was noted only concerning the incidence of the gastrointestinal tract, diarrhea and other intestinal infections (Sullivan and Nord, 2002). However, probiotics general mechanisms of action can be broadly classified as competitive exclusion, bacterial antagonism, and immune modulation (Yirga, 2015).

The competitive exclusion principle is defined as the protective power of normal microflora from the harmful effects of pathogens. This concept is based on the supplementation of the diet of selected cultures of beneficial microorganisms with potentially harmful bacteria for sites of adhesion and organic substrates (main carbon and energy sources). It includes adhesion to the cell wall of the digestive tract, preventing colonization of pathogenic microorganisms or competing for nutrients (Yirga, 2015).

Probiotics show their bactericidal activity effect by fermenting lactose to lactic acid, lowering the pH to a level that harmful bacteria cannot tolerate. In addition to these effects, for example, Lactobacillus species produce hydrogen peroxide and some Enterococcus species prevent the development of pathogenic microorganisms by producing antimicrobial substances such as nisin (McDonald et al., 2010).

Use of Probiotics in Animal Health

Nowadays, probiotic additives are among the chemicals that support growth, especially in animals, and studies that increase resistance against diseases in animals are also supported. Probiotics prevent the deterioration of body balance in animals and increase the development of natural healthy microflora (Boaventura et al., 2012).

Probiotic preparations are in different forms such as powder, granule, pellet, liquid suspension and capsule and can be used by mixing with drinking water or food. Live bacteria, fungi and yeasts used as probiotics have to maintain their viability during storage, application and in the intestinal environment to show their effects (Krehbiel et al., 2003). Probiotic preparations consisting of Lactobacillus, Bifidobacterium and Streptococcus species should be stored at 22-25 °C and in a dry place. They lose their vitality when the storage temperature rises above 30 °C. In addition, yeast Saccharomyces cerevisiae and Bacillus spp. can withstand the pelleting temperature, while Lactobacillus, Bifidobacterium and Streptococcus spp. depend on the pelleting temperature, significant losses occur. With the microencapsulation method applied to bacteria used as probiotics recently, these bacteria can be made to withstand the pelleting temperature of 90-95° C (Vanbelle et al., 1990). When studies for dogs and cats were examined, it was determined that there were more microorganisms in their gastrointestinal tract than humans. While Lactobacillus species have been detected in all intestinal sections in cats and dogs, it has been reported that there are also species found in humans within these Lactobacillus species (Grześkowiak et al., 2015).

Pascher et al. (2008) investigated the impacts of Lactobacillus acidophilus DSM 13241 in dogs with nonspecific dietary sensitivity. As a result of the study, feeding with probiotics improved stool consistency, stool dry matter, and stool frequency. Numerically less C. perfringens and Escherichia spp. were detected in the stools of dogs given probiotic than those not given (control group). In addition, it was determined that Lactobacillus spp. and Bifidobacterium spp. were increased numerically in dogs given probiotics. Sauter et al. (2006) examined by adding two lyophilized Lactobacillus acidophilus (NCC2628 and NCC2766) and one lyophilized Lactobacillus johnsonii (NCC2667) each strain at a density of 10¹⁰ to their feed for four weeks, in 21 dogs with foodborne diarrhoea. As a result of their study, they found useful impacts on intestinal microbiota and cytokine patterns; the number of enterobacteria in the stool was decreased and the number of Lactobacillus spp. increased. Clinical improvement was observed in all dogs treated with probiotics.

Strompfova and Marcinakova (2006) reported that the potential probiotic strain *Lactobacillus fermentum* AD1 isolated from dog feces had high survival (86.54%) at pH 3 in in vitro study and a high adhesion ability to the intestinal layer. *Lactobacillus fermentum* AD1 strain at 10⁹/ml was given to the diet of 15 healthy dogs for seven days. As a result of the research, the number of *Lactobacillus* spp. and *Enterococcus* spp. in the stool was significantly increased.

In a study on probiotic efficacy in dogs with inflammatory bowel disease, twenty dogs were treated with probiotic (a mixture of strains belonging to species Lactobacillus casei, Lactobacillus plantarum, Lactobacillus delbrueckii bulgaricus, Lactobacillus subspecies acidophilus, Bifidobacterium longum, B. breve, B. infantis, and Streptococcus salivarius subspecies thermophilus) for 60 days. As a result of these applications, the protective effect of the probiotic significantly reduced CD3 + T cell infiltration as well as positive clinical and histological findings. They also found a normalization of intestinal dysbiosis in dogs treated with probiotics. The result of this study shows that the probiotics used in the treatment of inflammatory bowel disease in dogs can be successful and more research is needed in the field of probiotics and infectious bowel disease (Jergens and Simpsons, 2012). Strompofova et al. (2014) determined that it increased the amount of organic acid in the blood serum of dogs and decreased the amount of triglyceride and albumin in the study they conducted with Bifidobacterium animalis B/12 strain (10⁹ CFU) of canine origin. In addition, they found an increase in the phagocytic activity of leukocytes. Grześkowiak et al. (2014) found that Lactobacillus. plantarum VET14A, Lactobacillus rhamnosus VET16A, Lactobacillus fermentum VET9A strains isolated from dogs showed successful adhesion to the enteric mucosa. Besides, they stated that it prevents the colonization of widespread enteropathogens such as Clostridium perfringens, Salmonella enterica serovar Typhimurium, Enterococcus canis, in their in vitro studies.

The probiotic product containing E. faecium SF68 increased immune responses to vaccination in puppies and kittens eight to 52 weeks old and seven to 27 weeks old, respectively. Vaccine applications were applied in the first and fourth weeks of the study. An increase in IgA content can be interpreted as a sign of enhanced protection against pathogens. However, increased IgA concentrations may represent a response to antigenic stimulation without increased immunity or to the body's protective mechanism against a noxious stimulus (Veir et al., 2007). Lactobacillus rhamnosus GG probiotic strain has many immunomodulatory effects, such as its use as an adjuvant in allergic diseases and vaccines, as well as its effects on gastrointestinal health, especially in newborns (Segers and Lebeer, 2014). In a study conducted to evaluate puppies sensitive to Dermatophagoides farinae, those not given probiotics were formed as a control group, and those given Lactobacillus rhamnosus GG, which is used to reduce allergic symptoms, were formed as an experimental group. The experimental group was given probiotics containing Lactobacillus rhamnosus GG from three weeks to six months. All puppies used in the study were found to be susceptible to D. farinae. In the intradermal skin tests of the experimental group using probiotics, lower reaction rates and lower IgE titers were detected in the control group. Since all dogs were sensitive, they were followed and the favourable effect was observed three years after the truncation of the

probiotic treatment (Marsella, 2009). Durand et al. (2006) stated that the number of Escherichia coli 0157:H7 was considerably reduced by the application of a probiotic preparation containing Lactobacillus fermentum, Streptococcus faecium, L. plantarum, L. acidophilus and L. casei in sheep feces. Lema et al. (2001) researched the influence of Lactobacillus acidophilus, Streptococcus faecium, a blend of Streptococcus faecium, Lactobacillus acidophilus and a mixture of Lactobacillus casei, L. acidophilus, S. faecium, L. plantarum and L. fermentum in reducing fecal shedding of sheep experimentally infected with Escherichia coli O157:H7. As a result of the research, they found that dietary S. faecium decreased the fecal shedding of E. coli O157:H7. Ohya et al. (2000) investigated the effect of two probiotic bacteria (Streptococcus bovis LCB6, Lactobacillus gallinarum LCB12) isolated from healthy calves on faecal shedding in calves experimentally infected with Escherichia coli O157:H7. As a result of the study, they reported that the treatment of cattle with the probiotics was shown to eliminate fecal shedding of Escherichia coli O157:H7 in experimentally infected calves compared to the control group. Mazmanian et al. (2005) reported that polysaccharides produced by Bacteroides fragilis in ruminants and some mammals play various immunomodulatory roles in directing maturement of the developing immune system, including correcting systemic T cell deficits, regulating helper T cell 1 and T cell 2 derangements and directing lymphoid tissue biogenesis. A recent study by Donaldson et al. (2018) found that the immunoglobulin A antibody

produced by the host in response to the *Bacteroides fragilis* capsule provides a colonization advantage by helping the bacteria bind to the epithelial surface.

The use of probiotics in the dairy industry to treat mastitis of ruminants, especially cattle, is also widely studied and researched. Lactococcus lactis DPC 3147 with broad-spectrum antimicrobial properties has been reported to be successful in its activity against pathogens causing mastitis in in vitro studies. When combined with a bismuth-based product, Lactococcus lactis DPC 3147 has been found to have a protective effect in cases of mastitis caused by Staphylococcus aureus and Streptococcus dysgalactiae, which are frequently seen in dried cows (Hu et al., 2019). Klostermann et al. (2008), compared intramammary Lactococcus lactis DPC 3147 and antibiotic administration in naturally infected cows with subclinical and clinical mastitis. In antibiotic treatment, prednisolone was used together with amoxicillin-clavulanic acid, which was found to be sensitive to the bacterial agent. Nine out of 25 animals treated with intramammary antibiotics and seven out of 25 animals treated with intramammary live Lactococcus lactis DPC 3147 had a similar bacteriological profile at the end of the 12th day. There was no change in the number of somatic cells in the two experimental groups compared to the previous ones. At the end of the research, 15 animals out of 25 treated with Lactococcus lactis DPC 3147 and 18 animals out of 25 treated with antibiotics did not show clinical signs of the disease after treatment. According to the research results, they reported that Lactococcus lactis DPC 3147 showed that mastitis treatment was possible and that it could be as effective as common antibiotic treatments in some cases. Armas et al. (2017) investigated in vitro the antagonist activity adhesion and invasion ability of Lactococcus subsp. lactis LMG 7930 nisin-producing strain. As a result of the study, they found successful in terms of invasion and adhesion to the cow mammary epithelial cell line. They reported that Lactococcus subsp. lactis LMG 7930 as an antagonistic effect inhibited two strains of cow mastitis, S. aureus LMG 16805 and Streptococcus agalactiae LMG 14838. It failed to inhibit Escherichia coli 285-05, Staphylococcus intermedius 146-08 and Streptococcus dysgalactiae 115-06, Streptococcus agalactiae 115-06, S. aureus 357-08, S. epidermidis 175-07, S. epidermidis 200-SA, S. chromogenes 100-SA, Lactococcus cremoris LMG 7951 strains in sheep.

Pellegrino et al. (2019) 12 probiotic bacteria isolated from milk samples (*Pediococcus pentasacaeous* CRL 1831, *P. pentasacaeous* CRL 1832, *Weissella cibaria* CRL 1833, *W. cibaria* CRL 1840, *Enterococcus hirae* 7-3, *E. hirae* CRL 1834, *E. hirae* CRL 1835, *E. hirae* CRL 1837, *E. mundii* CRL 1856, *Lactococcus lactis* CRL 1655, *L. perolens* CRL 724, *L. plantarum* CRL 1716) and evaluated their antimicrobial effects against selected mastitis agents. *S. aureus* ATCC25923, *S. aureus* RC108, *S. epidermidis* ATCC14990, *S. agalactiae* ATCC27956, *S. dysgalactiae* ATCC27957, *S. uberis* 102, *S. uberis* ATCC27958, *S. hyicus* 112249, *S. bovis* ATCC27960, *Enterococcus faecalis* 1943, *E. faecium*

35667, Pseudomonas spp., Escherichia coli 345, E. coli ATTC35218 and K. pneumoniae ATCC10031 were selected as mastitis agents. They rated the antimicrobial effects of bacteria isolated from milk against selected mastitis agents at low, medium and high levels. As a result of the research, it was determined that L. plantarum CRL 1716 did not show any antimicrobial activity against mastitis agents except S. dysgalactiae ATCC27957, E. coli 345, E. coli ATTC35218, Pseudomonas spp. and K. pneumoniae ATCC10031. Lactococcus lactis CRL 1655 E. hirae CRL 1835, E. hirae CRL 1837, E. mundii CRL 1656 showed high, other probiotic bacteria showed moderate antimicrobial activity against S. dysgalactiae ATCC27957. Antimicrobial activity of all probiotic bacteria isolated from milk against S. dysgalactiae ATCC 27957 was the most striking result in this study.

Microorganisms in balance in the gastrointestinal tract of a healthy poultry aid digestion and absorption and increase body resistance against infectious diseases. This balance is disrupted due to stress or illness. In such cases, changes occur in the intestinal flora and thus the balance of the flora is disturbed. The number of lactic acid bacteria in the flora decreases also number of pathogenic bacteria may increase (Kocak et al., 2016). Wang et al. (2017) reported that feeds containing Bacillus spp. were more effective in feed conversion rate and body weight gain rate in poultry. They also stated that the intense presence of Firmicutes species increased the accumulation of acetate in the cecum and the application of Lactobacillus casei in broiler chickens was beneficial for the health and development of chickens by reducing the urease activity in the ileum.

Torshizi et al. (2010) stated that the incidence of Salmonellosis in broilers decreased significantly when a commercial product containing Lactobacillus casei, L. acidophilus, Enterococcus faecium and Bifidobacterium bifidium was given together with feed or drinking water. In the same study, they found that there was a significant improvement in the experimental groups compared to the control groups in terms of body weight and feed conversion rates on the 31st day. Kergourlay et al. (2012) reported the draft genome sequence of Lactobacillus salivarius SMXD51 isolated from the cecum of healthy chickens, showing activity against Campylobacter jejuni, the most common cause of Campylobacteriosis infection. As a result of the study, they determined that Lactobacillus salivarius SMXD51 has interesting properties as a potential probiotic strain. Santini et al. (2010) investigated the antimicrobial activity of 55 isolates (lactic acid producing bacteria and Bifidobacterium species) against Campylobacter jejuni LMG 8842, C. jejuni CIP70.2, and C. jejuni 221/05 strains. As a result of the study, they determined that Bifidobacterium longum subspecies longum PCB 148, B. longum subsp. longum PCB 133, B. breve PCB 110, B. pseudocatenulatum PCB 107, B. longum subsp. infantis PCD 889B, B. thermophilum PCD 359B, B. longum subsp. longum PCD 232B, L. plantarum PCS 20, Lactobacillus spp. PCK 161, L. pseudomesenteroides Roles of Probiotics in Animal Health

PCK 18, L. plantarum PCA 306, L. plantarum PCA 293, L. plantarum PCA 275, L. plantarum PCA 259, L. plantarum PCA 236, L. pentosus PCA 227 showed antimicrobial activity against three of the C. jejuni LMG 8842, C. jejuni CIP70.2 and C. jejuni 221/05 strains. Elraheam Elsayed et al. (2021) investigated the beneficial effects of a potential synbiotic (Lactobacillus delbrueckii subspecies bulgaricus, L. plantarum, L. acidophilus, L. rhamnosus, B. bifidum, E. faecium, S. thermophilus, Aspergillus oryzae, Candida pintolepesii) with a concentration of 2 \times 10⁹ cfu/g, commercial product (%90 lactic acid, %10 formic acid) and multi-strain bacterin formulated from avian pathogenic multidrug-resistant Escherichia coli O26, O78, S. Enteritidis and S. Typhimurium serotypes. They performed a challenge test against E. coli O26, O78, S. Enteritidis and S. Typhimurium after eight days of using that three products together or alone. As a result of the study it was determined the combined use of that three products, especially when applied on the first day, mortality, developed erythrogram parameters, produced the immunomodulatory effect, decreased proinflammatory cytokine levels and enhanced growth performance parameters. Talebi et al. (2014) investigated the antibody response of the probiotic commercial product (L. acidophilus, L. casei, E. faecium, B. bifidium) to the Newcastle and Gumboro vaccine. In both vaccine administrations, maternal antibodies decreased to normal levels by 21 days, but the decrease in titer was slower in the probiotic-treated groups than in the vaccinated or control groups. Stefaniak et al. (2020) investigated the early in ovo administration (on day 12 of embryo incubation) of selected synbiotics in broilers that affects the humoral immune response to experimental antigens. They found that the in ovo application of synbiotic (inulin and Lactococcus lactis susbsp. Lactis) did not significantly influence the humoral immune response against T cell-dependent antigen and IgG value. However at the end of the 35 days, they reported that the while mortality rate was 8.5% in the control group, it was 2.1% in the synbiotic-administered experimental group. Wu et al. (2019) investigated the impacts of Enterococcus faecium NCIMB 11181 on the growth performance and immune reaction of broilers. They found an increase in growth and antibody response at the rates of 1×10^8 and 2×10^8 CFU/kg added to the daily feed of the broilers, but they determined the highest amount of IgG in the serum on the 35th day in the other experimental group at the rate of 5 x 10⁷ CFU/kg. Koenen et al. (2004) reported that chickens fed diets containing liquid Lactobacillus induced higher IgG and IgM responses compared to the others. In a different study, Huang et al. (2004) stated that when they applied Lactobacillus acidophilus and Lactobacillus casei in the feed they consumed daily, they detected a higher IgA response compared to the control group, while the IgG value was not affected.

Conclusion

Probiotic applications are at the forefront of the methods applied in recent years due to important problems

caused by the use of antibiotics in animal husbandry, such as the increase in populations of antibiotic resistant bacteria. Although probiotics are not expected to replace antibiotics in the treatment of an acute disease, it seems possible to use them as an alternative to antibiotics for prophylaxis and growth performance in animals. In recent years, studies on probiotics in ruminants have been conducted in mastitis cases and have shown that intramammary applications can be as effective as antibiotics.

The impacts of probiotics, prebiotics and synbiotics on the immune system and its components have been seen positive in some studies on cats and dogs and poultry, and it has been concluded that longer-term evaluation is required in scientific studies to see their effects in other species.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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