Review Article

Probiotic Products: Appropriate Technologies for Control of Infectious Animal Diseases in the Face of Climate Change and Anti-Microbial Resistance

ABSTRACT

Probiotic products are products which contain probiotics; these are live non-pathogenic microorganisms which provide health benefits to the host when administered in adequate amounts. Currently the world is facing a climate change crisis which among other things, leads to alteration of the abundance, distribution and transmission of animal pathogens leading to increased incidences of animal diseases. Moreover, the world is facing a crisis of development of anti-microbial resistance (AMR) by many strains of pathogenic microorganisms. AMR has made control of infectious animal diseases by administration of prophylactic doses of antimicrobials to be difficult. In attempts to look for alternative technologies for control of infectious animal diseases, many strains of probiotics have been extensively studied. This review provides insights on mechanisms of action and prophylactic efficacy of probiotics against infectious animal diseases.

Keywords: non-pathogenic microorganisms, prophylactic efficacy, health benefits, control of infectious animal diseases.

1. INTRODUCTION

In most developing countries, the livestock sector is a vital component of both individuals' livelihoods and the national economy, contributing significantly to household food security, income generation, draught power, manure production, foreign currency earnings, and employment opportunities. However, despite its pivotal role, the livestock sector faces numerous challenges including diseases outbreaks and low animal performance compounded by the adverse impacts of global climate change. Climate change poses a substantial threat to livestock production systems worldwide; manifesting through changes in mean climate variables and increased climate variability, which directly impact pasture and feed availability, water resources, animal health and productivity. The resulting challenges such as inadequate feed and water resources as well as compromised animal health and performance, have significant socio-economic implications for livestock farmers (Kimaro *et al*., 2017; Magita and Sangenda, 2017; Kimaro *et al*., 2018; Massay, 2020; Ripkey *et al*., 2021; Abazinab *et al*., 2022).

Human activities such as agriculture (crops and livestock production), deforestation and widespread use of fossil fuels have to a large extent contributed to climate change. The burning of fossil fuels like coal, oil, and gas for electricity, heat, and transportation is the main source of human-generated greenhouse gases (GHG) emissions; followed by deforestation, which releases sequestered (stored) carbon dioxide into the air. It is estimated that deforestation releases an average of 8.1 billion metric tons of carbon dioxide per year, accounting for more than 20% of all global carbon dioxide emissions (Turrentine and Denchak, 2021). Similarly, livestock production contributes to climate change by emitting GHG such as carbon dioxide, methane, and nitrous oxide. It is estimated that the livestock sector contributes about 18% of the total GHG emissions caused by human activities (FAO, 2021).

Climate change leads to increased temperatures, changes in the amount of rainfall, shifts in precipitation patterns, increased frequency of extreme weather events, increased heat stress and reduced water availability which have negative effects on livestock production. Increased temperatures and changes in rainfall patterns can alter the abundance, distribution and transmission of animal pathogens leading to emergence of livestock diseases (FAO, 2021; Musa *et al*., 2023), which have negative effects on livestock production as a result increased incidences and prevalence of animal diseases, increased animal mortality rates, and decreased animal productivity.

Studies have shown that sick livestock are less efficient and have higher GHG emission intensities i.e. produce more kilograms of GHG per kilogram of edible output as compared to healthy ones (Özkan *et al*., 2015; Mostert, 2018). Therefore in a livestock farm animal diseases control is important because this will assure a livestock farmer of having healthy livestock with higher production efficiency and lower GHG emission intensities. The conventional approaches which have been used for many decades for prevention of infectious diseases in animals include vaccination of susceptible animals, administration of prophylactic doses of antimicrobials and antihelmintics, and routine dipping or spraying of animals for control of ticks and tick-borne diseases. However, development of anti-microbial resistance (AMR) by some strains of pathogenic microorganisms has made control of some infectious diseases by administration of prophylactic doses of antimicrobials become difficult. Following development of AMR by some strains of pathogens, many studies have been carried out to assess prophylactic efficacy of probiotic products against animal diseases as alternative technologies to anti-microbials. Probiotic products are products

which contain probiotics; these are live non-pathogenic microorganisms which provide health benefits to the host when administered in adequate amounts (Plaza-Diaz *et al*., 2019). The objective of this paper is to review prophylactic efficacy of probiotic products against infectious animal diseases.

2. MATERIAL AND METHODS

Reports of prophylactic efficacy of probiotic products against animal diseases, and mechanisms of action of probiotics were searched using google search engine. After assessment of the reports, appropriate ones were selected, summarized, and globally synthesized.

3. PROPHYLACTIC EFFICACY OF PROBIOTIC PRODUCTS AGAINST ANIMAL DISEASES

Prophylactic efficacies of probiotic products against some animal diseases are as summarized below:

3.1 Prophylactic efficacy of probiotic products against diseases of the digestive system

Reports show that probiotic products have protective effects against some diseases affecting the digestive system. For example, Watkins *et al*. (1982) reported that pre-treatment of gnotobiotic chicks with a culture containing 10□ to 10□ cfu of *Lactobacillus acidophilus*/ml at day 2 of age, followed by a challenge with a culture containing 10□ to 10□ cfu of a pathogenic strain of *E. coli*/ml at day 4 of age; and subsequent treatment with the same dose of *L. acidophilus* at days 6, 8, 10, and 14; significantly decreased the mean chicks mortality due to avian colibacillosis by 89.31%, from 34.6% in the control group to 3.7% in the *L. acidophilus*-pretreated group.

Nisbet *et al*. (1998) reported that oral administration of 0.25 ml of a commercial probiotic product or competitive exclusion (CE) culture of cecal bacteria to day-old chicks, followed by a challenge with 0.25 ml of a culture containing 10□ *Salmolnella gallinarum* /ml (i.e. 2.5 x 10□ *S. gallinarum*/chick) on day 3, had a significant decrease in mortality compared to non-CE treated *S. gallinarum* challenged chicks. The mean mortality for the control chicks was 74% compared with 7.5% for the CE-treated chicks. This implies that pretreatment of the day-old chicks with the CE significantly decreased the chicks mortality by 89.86%. Moreover, the authors found that day-old chicks which were directly infected with 10□ *S. gallinarum* and provided no CE culture had a high *S. gallinarum* horizontal transmission which averaged at 86%, and a high mortality which averaged at 80% during the first 12 days post-hatch. The horizontal transmission of *S. gallinarum* in untreated contact chicks that were commingled with the seeder (directly infected) chicks averaged at 84%, while mortality averaged at 54%; and the horizontal transmission in commingled CE-treated contact chicks averaged at 35% while mortality averaged at 9% during the first 12 days post-hatch. These findings demonstrated that pretreatment of day-old chicks with the CE significantly decreased horizontal transmission of *S. gallinarum* by 58.33%; from 84% of the untreated contact chicks to 35% of the commingled CE-treated chicks.

In a study to determine the efficacy of a new probiotic product containing viable spores of *Bacillus licheniformis* in controlling post-weaning diarrhea syndrome in weaned pigs, mainly caused by enterotoxigenic *Escherichia coli* (ETEC) strains, Kyriakis *et al*. (1999) found that feeding of weaned pigs with a diet supplemented with the probiotic product at 10□ viable spores of *B. licheniformis* per gram of feed for 28 days resulted to a significant decrease in mortality of the pigs from 43.75% in the negative control group to 4.69% in the probiotic product-treated group. This implies that treatment of weaned pigs with the probiotic product significantly decreased mortality of weaned pigs due to post-weaning diarrhea syndrome by 89.28%.

Audisio *et al*. (2000) reported that oral administration of 200 µl of a culture containing about 1 x 10□ cells of *Enterococcus faecium* J96 per chick to 30-hour-old chicks, twice per day at 12 hours interval for 3 consecutive days, followed by a challenge dose of 200 µl of a culture containing about 1 x 10□ cfu of *Salmonella pullorum* M97 per ml on day 4 post-hatch; decreased the chicks mortality due to pullorum disease by 50%; from 50% in the control group, to 25% in the *E. faecium* J96-treated group. In another study, Genovese *et al*. (2000) found that oral administration of 5 ml of a culture of a probiotic at 12 hour and again at 24 hour of age, followed by oral challenge with 1 x 10□ cfu of enterotoxigenic *E. coli* 987 at 48 hour of age significantly decreased the mortality of piglets from 17.5% in the control group to 4.4% in the probiotic-pretreated group. Apart from that, there was a significant decrease in fecal shedding of *E. coli* in the probiotic-pretreated group. These findings demonstrated that pre-treatment of the piglets with the

probiotic significantly decreased mortality of the piglets by 74.86% due to enterotoxigenic *E. coli* 987 infection.

Ogawa *et al*. (2001a) found a significant decrease in the severity of diarrhea in infant rabbits, and 100-fold decreased Shiga toxin-producing *Escherichia coli* (STEC) colonization levels in the gastrointestinal tract (GIT) were noted on day 7 post infection after daily feeding of infant rabbits with sterilized artificial milk supplemented with probiotics *Lactobacillus casei* strain Shirota at a concentration of 10□ cfu/ml twice a day from the day of birth, followed by experimental infection of 3-day-old infant rabbits with STEC O157:H7 strain 89020087 at about 10^3 cfu/animal. Moreover, both histological damage to the intestinal mucosa (Figure 1) and the concentration of Shiga toxins (Stxs), Stx1 and Stx2, in the intestines induced by STEC infection were decreased by the administration of the probiotics *L. casei* strain Shirota. In addition to that, administration of the probiotics increased levels of IgAs against Stx1, Stx2, and formalin-killed STEC cells in the colon about two-, four-, and three-fold, respectively, compared to those of the untreated controls by day 7 post infection.

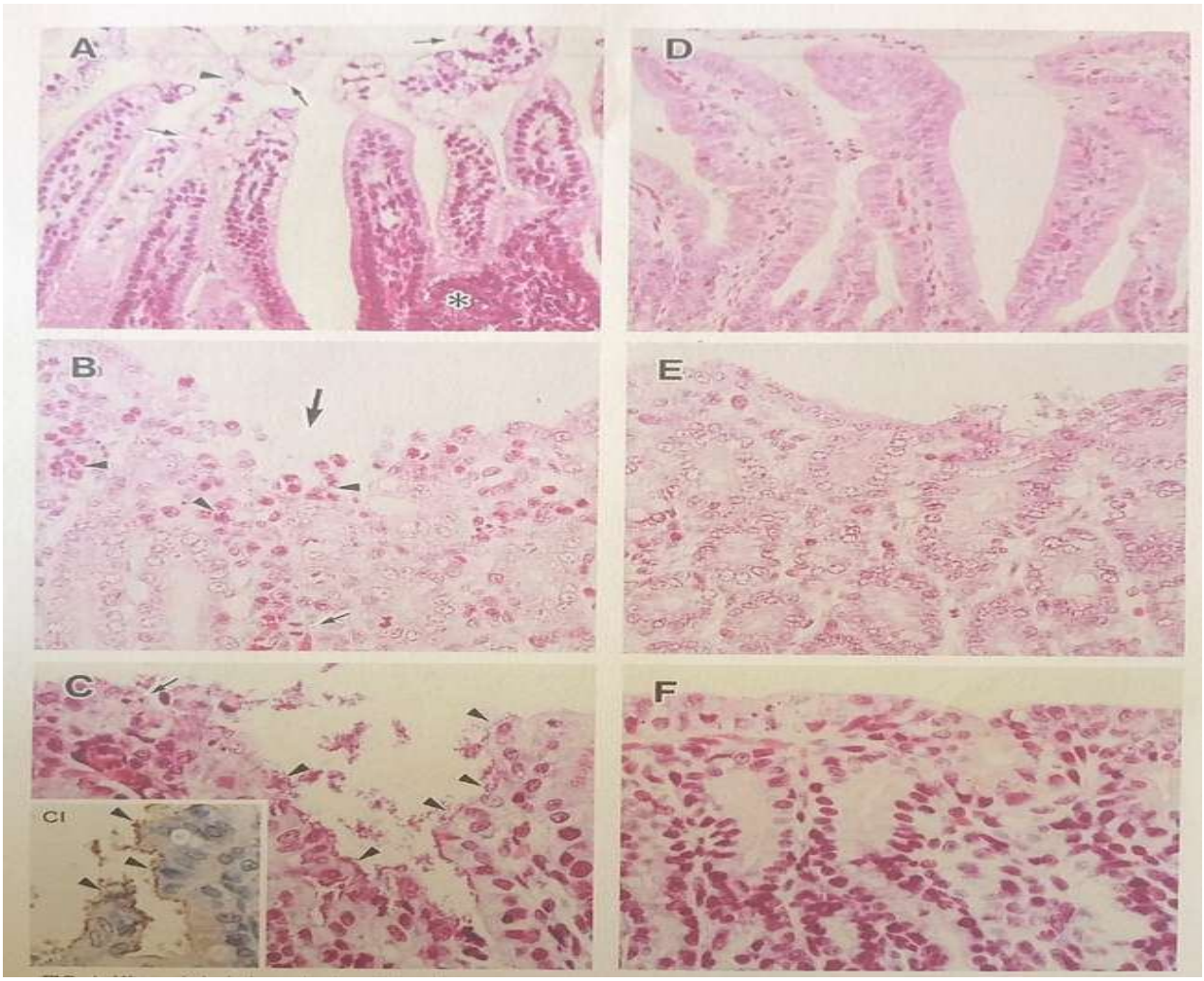

Fig. 1. Images showing histopathological examination of intestinal segments of infant rabbits infected with STEC O157:H7 strain 89020087 on day 7 post-infection. Hematoxylin-and-eosin staining of the small intestine (images A & D), cecum (images B & E), and colon (images C &F) from a control rabbit (images A to C) and an *L. casei*-treated rabbit (images D to F). Image CI shows immunostaining of STEC O157 in a colon section from a control rabbit. In the control group the images show vacuolation of epithelial cells (arrows) with attached STEC cells (arrowhead) on top of the villi and necrosis due to massive growth of STEC cells (asterisk) in the small intestine (image A); exfoliation of epithelial cells (large arrows), pseudo-eosinophil infiltration (arrowheads), and mitotic activity (small arrow) in the cecum (image B); and exfoliation and necrosis (arrow) in the colon due to STEC cells attached to epithelial cells (arrowheads) (image C). In contrast, no

notable pathological changes except for low mitotic activity in the cecum and slight exfoliation of the epithelium in the colon of rabbits in the *L. casei*-treated group (images D to F). Magnifications in both groups: small intestine x260; cecum, x390; colon, x520. This Figure was adapted from Ogawa *et al*. (2001a).

In other studies, von Buenau *et al*. (2005) found that oral administration of 15ml of a suspension containing *E. coli* strain Nissle 1917 at 1 x 10□/ml once per day (on day 1 before the first suckling and from day 2 to day 10 before the first feeding in the morning) significantly decreased the incidence of diarrhea in the *E. coli* strain Nissle 1917-treated group as compared to the control group. Only 22 (26.5%) of the calves in the *E. coli* strain Nissle 1917-treated group developed diarrhea as compared to 58 (65.2%) calves in the control group. These findings indicated that pretreatment of the calves with *E. coli* strain Nissle 1917 significantly decreased the incidence of diarrhea by 59.36%. Schroeder *et al*. (2006) reported that pretreatment of piglets with one capsule of a commercial probiotic product (Mutaflor™) containing 0.5 x 10^{10} to 2.5 x 10^{10} cfu of *E. coli* strain Nissle 1917 serotype O6:K5:H1 per day from day 13 postpartum prevented acute secretory diarrhea following oral experimental infection of the piglets with 5 ml of a suspension containing porcine enterotoxigenic *E. coli* strain Abbotstown serotype O149:K91, at 1.1 x 10^{10} to 2.1 x 10^{10} cfu per 5 ml from day 21 postpartum.

In addition to that, Timmerman *et al*. (2006) found that oral administration of 1-day-old broiler chicks with a chicken-specific probiotic preparation composed of 7 Lactobacillus strains (i.e. *Lactobacillus bifermentans* W204.5, *L. sanfranciscensis* W205.6, *L. sanfranciscensis* W208.6*, L. reuteri* W218.2, *L. reuteri* W223.5, *L. reuteri* W227.3, and *L. fermentum* W227.5) via drinking water from day 0 to 31, at an approximate rate of 4 x 10□ cfu/kg of body weight, decreased the chicks mortality rate by 46.64%; from 7.14% of the control group to 3.81% of the probiotic-pretreated group. Dexian *et al*. (2012) reported that intragastric pretreatment of 7 to 15-day old broiler chicks with *Lactobacillus reuteri* ATCC 55730 once daily for 5 days at a dose of 10□ cfu, followed by intragastric challenge with 300 µl of a culture containing 5 x 10□ cfu of *Salmonella pullorum* ATCC 9120 significantly increased the chicks survival rate in the *L. reuteri* ATCC 55730-pretreated group as compared with the control group. The survival rate in the *L. reuteri* ATCC 55730-pretreated group was 78% while that of the control group was 46%. These findings indicated that pretreatment of the chicks with *L. reuteri* ATCC 55730 increased the chicks' survival rate by 69.57%.

Jayamaran *et al*. (2013) prevented *Clostridium perfringens*-induced necrotic enteritis in broiler chickens by providing the chickens with feed supplemented with spores of *Bacillus subtilis* PB6 at 500g/tone of feed, containing 5 x 10^{11} cfu/kg, throughout the experiment (day 1 to day 35). Chickens in the uninfected control group, and in the infected group provided feed supplemented with spores of *B. subtilis* did not contract necrotic enteritis after being experimentally infected with sporulated oocysts of mixed *Eimeria spp* on day 14 of the experiment at a total of 3 x 10□ oocysts per bird, followed by *C. perfringens* at a dose of 10□ cfu/ml per bird on days 19, 20, and 21 of the experiment. Chickens in the infected control group developed necrotic enteritis characterized by thickened mucosa, hemorrhage, and ballooning of intestine. Villus histomorphometry of the duodenal tissue on day 28 of the experiment (7 days post-infection) revealed distorted and damaged villi in the infected control group due to *C. perfringens* infection. The infected group supplemented with *B. subtilis* PB6 showed intact villi architecture.

In other studies, Wang *et al*. (2019) demonstrated that feeding of chicks with basal feed supplemented with *Lactobacillus casei* DBN023 at 10□ cfu/g of feed from day 1 of age; followed by an oral challenge with 1 ml of a culture of *Salmonella pullorum* CMCC-533 (10□ cfu/ml) per chick when they are aged at 7 days, significantly decreased chicks mortality due to pullorum disease by 66.67%; from 24% in the control group to 8% in the *Lactobacillus casei* DBN023-pretreated group. Yang *et al*. (2022) demonstrated that pretreatment of chicks with *Bifidobacterium lactis* JYBR-190 at a concentration of 1.0 x 10□ cfu/ml in drinking water daily from the age of 1 day prior to oral challenge with 0.5 ml of a culture containing 2.32 x 10□ cfu of *Salmonella pullorum* at 7 days of age, protected the intestinal mucosa of the chicks from the challenge with *S. pullorum*. This was indicated by the intestinal villus height; villus height to crypt depth (V/C) ratio; and muscle layer thickness of the duodenum, jejunum, and cecum being significantly higher in the *B. lactis* JYBR190-treated group than those in the infection group, and antibiotic-treated group. In addition to that, at 14 days of age, the mortality rate of the blank control group was 10%. In the infection group, the mortality rate increased to 70% after challenge with *S. pullorum*. Compared with the infection group, pretreatment of the chicks with *B. lactis* JYBR-190 prior challenge with *S. pullorum* decreased the mortality rate by 52.43%, from 70 to 33.3%.

Furthermore, Wang *et al*. (2023) demonstrated that feeding of the chicks with basal feed supplemented with probiotics *Bacillus subtilis* at 10□cfu/kg of feed from day 1 of age onwards, followed by administration of a 20-fold dose of coccidiosis vaccine per chick by oral gavage on day 15 of age, and oral gavage of 1 ml of a culture containing *Clostridium perfringens* at 2 x 10□ cfu/ml per day on days 18 – 21 of age, significantly decreased the degree of severity of intestinal lesions from a lesion score of 4 in the subclinical necrotic enteritis group where chicks were fed basal feed not supplemented with *B. subtilis* from day 1 of age onwards, followed by administration of a 20-fold dose of coccidiosis vaccine per chick by oral gavage on day 15 of age, and oral gavage of 1 ml of a culture containing *Clostridium perfringens* at 2 x 10□ cfu/ml per day on days 18 – 21 of age; to a lesion score of 2 in the *B. subtilis*-treated group.

3.2 Prophylactic efficacy of probiotic products against diseases affecting systems other than the digestive system

Medina *et al*. (2008) reported that intranasal administration of a probiotic *Lactococcus lactis* NZ9000 in mice at a dose of 25 µl of inoculum containing 10□ cells of *L. lactis* NZ9000/mouse/day for 5 and 7 days prior to intranasal challenge of the mice on days 6 and 8, respectively, in each treatment group with 25 µl of inoculum containing 10□ cells of *Streptococcus pneumoniae* significantly increased the clearance rate of *Str. pneumoniae* from the lung and prevented dissemination of the pathogen into blood. They also found that administration of the probiotics significantly increased the number of neutrophils and lymphocytes in the bronchoalveolar lavages as compared to the control group, and treatment with *L. lactis* NZ9000 significantly increased the number of leucocytes and neutrophils in the blood and specific IgG antibodies in the serum as compared to the control group. Furthermore, they found that treatment with *L. lactis* NZ9000 for 5 days significantly decreased the severity of lung tissue damage as revealed by histopathological examination where signs of moderate inflammation with focal cellular infiltration, without hemorrhage and with conserved airspaces were demonstrated (Figure 2b); as compared to severe lung tissue damage revealed by a gradual and intense inflammatory response with progressive parenchymal involvement, including widespread cellular infiltration, increased fibrosis in bronchial walls and vessels, hemorrhage, and reduction of the alveolar airspaces demonstrated in the control group (Figure 2a). Although *Str. pneumoniae* is maintained in humans, the pathogen is occasionally isolated from asymptomatic or sick animals. The pathogen can be transmitted back to humans from this source (i.e. reverse zoonosis), and can sometimes spread between animals (Spickler, 2020). These findings imply that intranasal administration of a probiotic product containing *L. lactis* could be appropriate for control of respiratory streptococcosis in animals due to *Str. pneumoniae*.

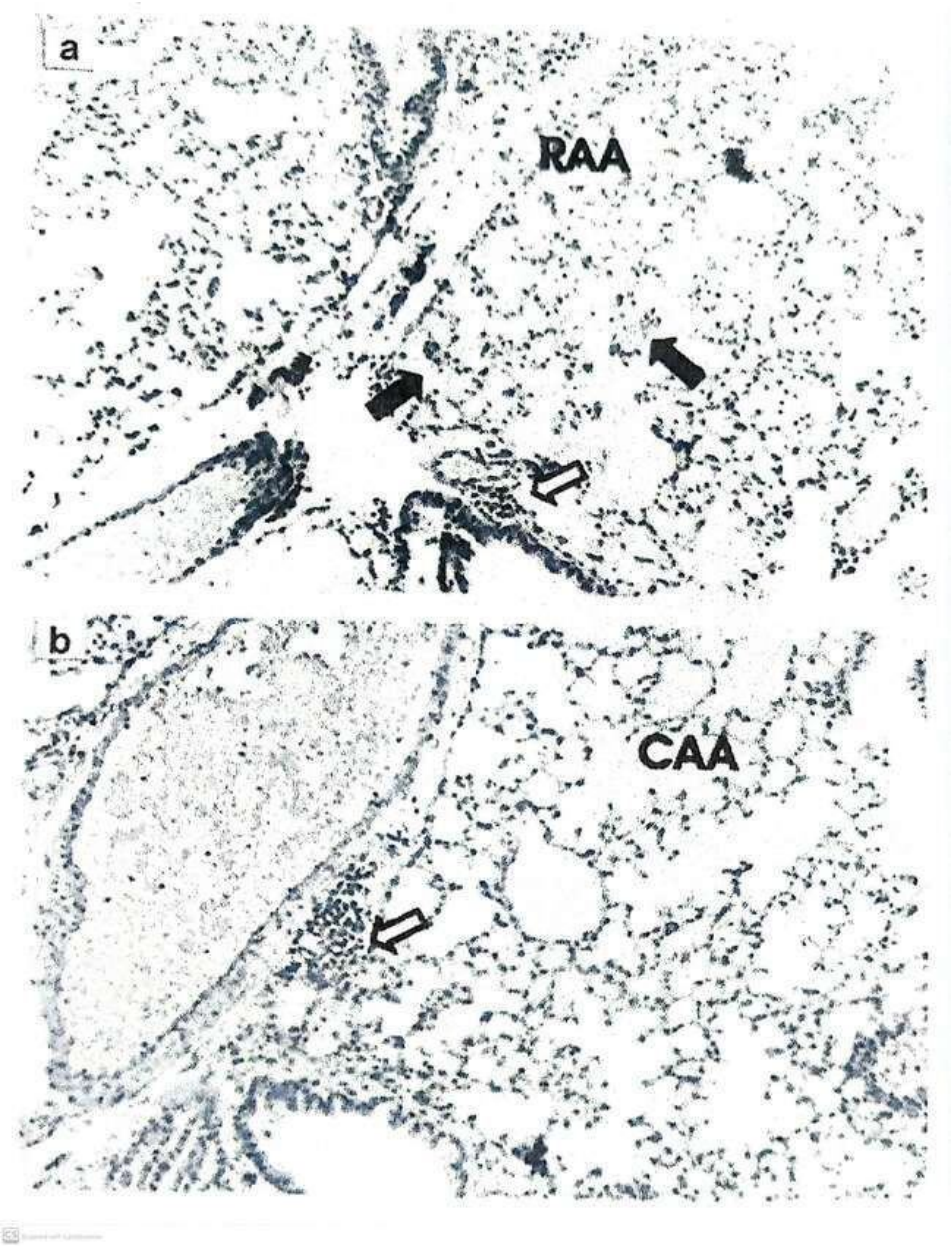

Fig. 2. Images showing histopathological examination of lungs of control mice and probiotic (*L. lactis* NZ9000)-treated mice. Image (a) demonstrates severe lung damage indicated by hemorrhage (black arrow) and reduction of alveolar airspaces (RAA) in control mice on day 10 postchallenge with *S. pneumoniae* at 10□ cells/mouse. Image (b) demonstrates less severe lung damage indicated by signs of a moderate inflammatory response without hemorrhage, conserved alveolar airspaces (CAA), and increased cellularity in the lamina propria of the bronchus-associated lymphoid tissue (white arrows) in mice treated with probiotics (*L. lactis* NZ9000) at 10□ cells/mouse for 5 days prior to challenge with *S. pneumoniae*. This figure was adapted from Medina *et al*. (2008).

Youn *et al*. (2012) demonstrated that intranasal administration of 500 µl of a suspension containing 1.5 x 10□ of *Lactobacillus fermentum* CJL-112 for 7 days pre- and 14 days post-challenge with H9N2 avian influenza virus, significantly decreased the number of chickens with viral shedding from the GIT in the indirect contact chickens, and also significantly decreased viral shedding from the respiratory tract in the challenged and the direct contact chickens as compared to viral shedding in the control group.

Apart from that, in a study to investigate the prophylactic effects of intravaginal administration of a mixture of three strains of lactic acid bacteria (LAB) i.e. *Lactobacillus sakei* FUA 3089, *Peridococcus acidilactici* FUA 3140, and *Peridococcus acidilactici* FUA 3138 on the incidence of metritis in cows clinically presented by purulent vaginal discharges; Ametaj *et al*. (2014) demonstrated that intravaginal administration of 1 ml

of a suspension containing a mixture of the three LAB strains at 10^{10} – 10^{12} cfu/ml once per week during 2 and 1 week pre-partum, and at 1, 2, 3, and 4 week postpartum; significantly decreased the incidence of metritis at 3 week postpartum by 71.12%, from 45.7% in the control group to 13.2% in the LAB-treated group.

In other studies, Genís *et al*. (2018) reported that administration of two intravaginal doses of a mixture of three LAB strains i.e. *Lactobacillus rhamnosus* CECT 278, *Pediococcus acidilactici* CECT 5915, and *Lactobacillus reuteri* DSM 20016 per week during 3 week pre-partum with a final cell count of 4.5 x 10^{10} cfu/dose and a proportion of 25/25/2, respectively; significantly decreased the prevalence of metritis in cows by 57.15%; from 31.11% in the control group to 13.33% in the LAB-treated group. Urakawa *et al*. (2022) reported that oral supplementation of the cows with *Bacillus subtilis* C3102 at 3.0 x 10 cfu/cow from about one month before calving to three months after calving significantly decreased the incidence of mastitis compared to the control group, and mastitis incidence in the previous lactations where the cows were not supplemented with *B. subtilis* C3102.

Moreover, in a study to investigate the clinical, antiviral, and immunological effect of spraying broiler chickens with a cocktail of *Bacillus spp* (i.e. *B. subtilis*, *B. licheniformis*, and *B. indicus*) and *Lactobacillus spp* (i.e. *L. acidiphilus*, *L. plantarum*, and *L. rhamnsus*) as a single dose or a mixture of a cocktail of *Bacillus spp* and cocktail of *Lactobacillus spp*; Rasaei *et al*. (2023) found that spraying of walls of rooms (where cages containing the chickens were kept) with the probiotics at 9 x 10 cfu/m² daily from 1-day-old to 35-day-old; followed by challenging the chickens in the challenge groups on the 22nd day of age by H9N2 avian influenza virus (H9N2 AIV) at a dose of 10 EID_{50} via the eye route (100 µl/bird) and nasal route (100 µl/bird), significantly decreased the severity of clinical signs, gross lesions, and histopathological lesions in some days (Figure 3). It also decreased viral shedding in the probiotics-sprayed chickens as compared to the positive control chickens. However, there were no significant differences in serum antibodies against H9N2 AIV between the positive control group and the probiotics-sprayed group.

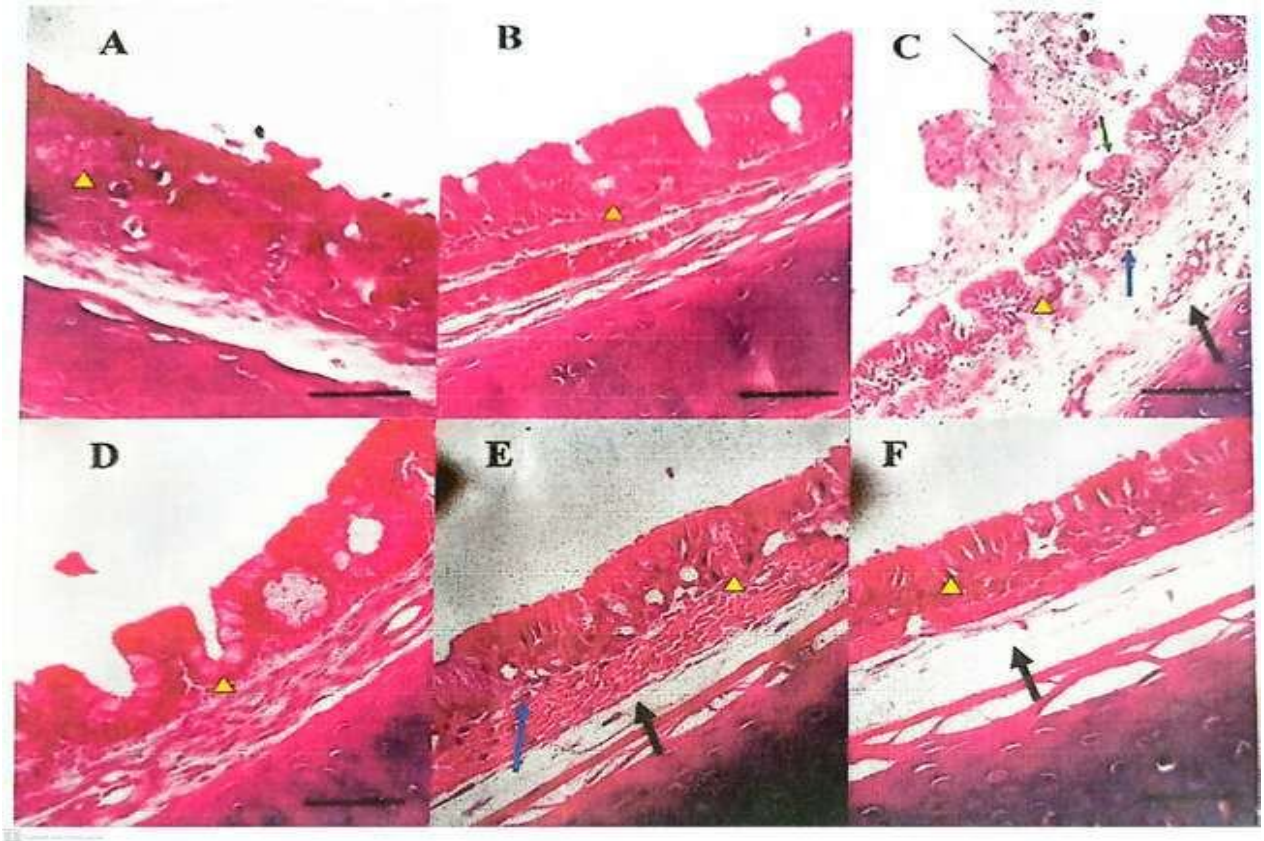

Fig. 3. Images demonstrating microscopic changes of the trachea in chickens which received three different cocktails of probiotics sprays and experimentally infected with H9N2 AIV. Image A - negative control (no H9N2 AIV challenge and no probiotics sprays); image B - received distilled water as spray with no H9N2 AIV challenge and no probiotics sprays; image C - positive control (challenged with H9N2 AIV and no probiotics sprays); image D – sprayed with a cocktail of *Bacillus*

***spp* and challenged with H9N2 AIV; image E – sprayed with a cocktail of *Lactobacillus spp* and challenged with H9N2 AIV; image F – sprayed with a mixture of a cocktail of *Bacillus spp* and cocktail of *Lactobacillus spp*, and challenged with H9N2 AIV. The images demonstrate massive mucous exudate (thin black arrow), edema in submucosal layer (bold black arrow), multifocal deciliation (green arrow), goblet cell hyperplasia (yellow arrowhead), and infiltration of inflammatory cells (blue arrow) (H&E, bar = 40 µm). This figure was adapted from Rasaei *et al*. (2023).**

4. MECHANISMS OF ACTION OF PROBIOTICS

In order to protect their hosts against diseases, probiotics employ various mechanisms of action, some of which are described below:

4.1 Competitive exclusion of pathogens

Studies have demonstrated the protective effect of probiotics to their hosts through competition with pathogens for receptor-binding sites in the mucosal tissues. For instance, Ushe and Nagy (1985) reported that oral administration of culture of *Streptococcus faecium* M74 given in milk suspension significantly decreased colonization of enterotoxigenic *E. coli* O101:K30:K99:NM in the ileum of piglets. Apart from that, Nemcová *et al*. (2007) reported that oral administration of 2 ml of a culture of *Lactobacillus plantarum* at 1 x 10 cfu/ml daily for seven consecutive days; combined with oral administration of prebiotics, maltodextrin Maldex 150® and Raftifeed IPX®, four times a day at a dose of 0.3 g for seven consecutive days significantly inhibited *E. coli* 08:K88 from adhering to the jejunal and colonic mucosa in piglets.

Moreover, Bourchard *et al*. (2013) demonstrated inhibition of adhesion of *Staphylococcus aureus* RF122 on bovine mammary epithelial cells (bMECs), and inhibition of internalization of *S. aureus* RF122 and *S. aureus* Newbould 305 into bMECs by *Lactobacillus casei* CIRM-BIA 667. Assis *et al*. (2015) reported inhibition of adhesion of *S. aureus* Newbould 305 on bMECs; and inhibition of internalization of *S. aureus* RF122, *S. aureus* Newbould 305, *E. coli* P4, and *E. coli* K08 into bMECs by *Lactococcus lactis* V7.

4.2 Production of compounds with antimicrobial effects against pathogens

Probiotics produce different types of compounds which have been proven to have antimicrobial activity against pathogenic microorganisms. These compounds include organic acids particularly short-chain fatty acids (SCFAs) which are also known as volatile fatty acids (VFAs) such as acetate, propionate, butyrate, lactic acid, formic acid, phenyllactic acid, and benzoic acid; hydrogen peroxide; carbon dioxide; acetaldehyde; acetoin; diacetyl; bacteriocins such as lacticin, lactocin, pediocin, pisciolin, enterocin, reuteri, reuterin, plantaricin, enterolysin, colicin, acidocin, macedocin, lactococcin, and nisin; bacteriocin-like inhibitory compounds; siderophores; and biosurfactants. For instance, Nemcová *et al*. (2007) demonstrated the production of lactic acid and acetic acid in the jejunum, ileum, and colon of piglets following oral administration of 2 ml of a culture of *Lactobacillus plantarum* at 1 x 10 cfu/ml daily for seven consecutive days; combined with oral administration of prebiotics, maltodextrin Maldex 150® and Raftifeed IPX®, four times a day at a dose of 0.3 g for seven consecutive days. Neljat *et al*. (2019) demonstrated the production of SCFAs particularly lactate, acetate, propionate, isobutyrate, and n-butyrate in the chicken cecum by the probiotic *Bacillus subtilis* DSM29784 after feeding the birds with a diet containing *Bacillus subtilis* DSM29784 as feed additive. In addition to that, Fantinato *et al*. (2019) reported the production of bacteriocin by *Streptococcus salivarius*; and Ahire *et al*. (2021) reported the production of lactic acid and hydrogen peroxide by *L. plantarum* UBLP40. The produced lactic acid and hydrogen peroxide had antimicrobial effect against *Micrococcus luteus* MTCC 106, methicillin-resistant *Staphylococcus aureus* subsp. *aureus* ATCC BAA-1720, *Pseudomonas aeruginosa* MTCC 1688, and *Escherichia coli* MTCC 1687.

4.3 Decreasing the luminal pH to levels which are unfavourable for pathogens

Probiotics can decrease the luminal pH levels through production of organic acids especially SCFAs/VFAs. In *in vitro* studies, Wolin (1969) demonstrated the inhibitory effect of VFAs particularly acetic, propionic, and butyric acids on *E. coli* which was pH-dependent. Little inhibition was observed at pH 7.0, and inhibition increased with decreasing pH. A combination of acetate, propionate, and butyrate in concentrations usually found in bovine rumen contents gave 96%, 69%, and 2% inhibition at pH 6.0, 6.5,

and 7.0, respectively. Rumen fluid gave 89% and 48% inhibition at pH 6.0 and 6.5, respectively. Ogawa *et al*. (2001b) demonstrated that the inhibition of *in vitro* growth of Shiga toxin-producing *E. coli* (STEC) O157:H7 strain 89020087 by *Lactobacillus casei* strain Shirota and *L. acidophilus* YIT0070 was attributed to the production of lactic acid and low pH.

In an *in vivo* study using mice, Asahara *et al*. (2004) demonstrated the inhibition of growth of STEC O157:H7 by probiotics *Bifidobacterium breve* strain Yakult and *B. pseudocatenulatum* DSM 20439. These probiotic strains produced a high concentration of acetic acid (56 mM) and lowered the pH of the intestinal contents (to pH 6.75) compared to the infected mice in the control group (acetic acid concentration, 28 mM; pH, 7.15) in which STEC O157:H7 growth was not inhibited. These effects were thought to be related to the anti-infectious activity of these strains because the combination of a high concentration of acetic acid and a low pH was found to inhibit Shiga toxin production during STEC growth *in vitro*.

4.4 Immunomodulation of the immune system of the host through regulation of the innate and adaptive immune responses by modulating immune cells and production of cytokines

Studies have shown that administration of probiotics to animals leads to immunomodulation of the immune system of the hosts through regulation of the innate and adaptive immune responses by modulating immune cells. For instance, Çetin *et al*. (2005) reported that feeding of turkeys with a diet supplemented with a commercial probiotic product (Primalac®454) containing *Lactobacillus acidophilus* at 4.52 x 10□ cfu/g, *Lactobacillus casei* at 1.32 x 10□ cfu/g, *Enterococcus faecium* at 2.8 x 10□ cfu/g, and *Bifidobacterium thermophilus* at 1.36 x 10□ cfu/g; at 1g/kg of feed to eighth week, then 0.5g/kg of feed for 15 weeks significantly increased the serum IgG and IgM levels, and significantly decreased the peripheral blood T lymphocytes percentage compared with those of the control group. Crispie *et al*. (2008) demonstrated that intramammary infusion of a live culture of probiotics *Lactococcus lactis* stimulated substantial recruitment of immune cells particularly lymphocytes and polymorphonuclear (PMN) leucocytes including neutrophils, eosinophils, basophils, and mast cells to the mammary gland. For instance, in one assay, quarters infused with the probiotic experienced significant increase (about 20,000-fold) in neutrophils over the first 48 hours period from an average value of 83.6 cells/ml pre-infusion to 1.78 x 10□ cells/ml 48 hours post-infusion.

Cytokines are regulators of host immune responses and inflammation. Pro-inflammatory cytokines act to make diseases worse. They include interleukin (IL)-1β, IL-12, and tumor necrosis factor (TNF) (Dinarello, 2000; Al-Qahtani *et al*., 2024). Anti-inflammatory cytokines serve to reduce inflammation and promote healing. They include IL-1 receptor antagonist (IL-1 ra), IL-4, IL-6, IL-10, IL-11, IL-13, and transforming growth factor-β (TGF-β). Specific cytokine receptors for IL-1, tumor necrosis factor-α (TNF-α), and IL-18 also function as pro-inflammatory cytokine inhibitors (Opal and DePalo, 2000; Zhang and An, 2007; Al-Qahtani *et al*., 2024).

Reports indicate that the immune system of the host can be immunomodulated through regulation of cytokines production. For example, Wang *et al*. (2019) reported that expression levels of IL-4, IL-17, TNF-α, TGF-β, and interferon gamma (IFN-γ) in the infected group of chicks (fed non antibiotic basal feed not supplemented with *L. casei* DBN023 from day 1 of age, and orally challenged with 1 ml of a culture of *S. pullorum* CMCC-533 per chick at 10□ cfu/ml when they were aged at 7 days) increased by 31.09%, 15.77%, 29.15%, 19.50%, and 29.17%, respectively, and these levels were significantly higher than those of the blank control group (fed non antibiotic basal feed not supplemented with *L. casei* DBN023 from day 1 of age, and orally administered 1 ml of phosphate-bufered saline per chick when they were aged at 7 days). The expression level of IL-10 in the infected group significantly decreased by 17.50%, compared with that in the blank control group. In addition to that, the expression levels of IL-17, TNF-α, TGF-β, and IFN-γ in the prevention group (fed non antibiotic basal feed supplemented with *L. casei* DBN023 at 10□ cfu/kg of feed from day 1 of age, and orally challenged with 1 ml of a culture of *S. pullorum* CMCC-533 per chick at 10□ cfu/ml when they were aged at 7 days) decreased by 15.45%, 16.39%, 10.34%, and 16.35%, respectively, compared with those in the infected group.

In another study, Terada *et al*. (2020) administered 500 µl of water supplemented with 2 x 10□ cfu of *Lactobacillus reuteri* per chick in the first group of broiler chicks, and the same amount of water supplemented with 1.3 x 10□ cells of *Clostridium butyricum* per chick in the second group of the broiler chicks from day 1 to day 6 post-hatch. The treatments caused the expression levels of IL-1β in the *L. reuteri*- and *C. butyricum*-treated groups, and TGF-β2 in the *C. butyricum*-treated goup to be significantly

higher in the ileum of the chicks in the probiotics-treated groups than in the control group. Furthermore, the authors demonstrated that as a result of the probiotic treatments, in the caecum, the expression levels of TGF-β3 in the *L. reuteri*-treated group and that of TGF-β4 in the *L. reuteri*- and *C. butyricum*-treated groups were significantly higher than that in the control group.

4.5 Improvement of mucosal barrier functions by stimulating the production of mucin proteins and regulating the expression of tight junction proteins

Tight junctions (TJs) are areas where membranes of two adjacent cells join together to form a barrier. They are composed of transmembrane proteins such as claudins (CLDNs), occludin (OCLN), junctional adhesion molecules (JAMs), tight junction-associated marvel proteins (TAMPs) (Lee *et al*., 2018; Vermette *et al*., 2018; Zeisel *et al*., 2018), and zonula occludens (ZOs) (Yang *et al*., 2015). Generally, CLDNs, OCLN, JAMs, TAMPs and ZOs regulate the permeability of TJs. TJs play a major role in maintaining the integrity and impermeability of the barrier of mucosal tissues. Consequently, they are ideal targets for pathogens to promote their translocation through the mucosal tissues and invade their hosts (Paradis *et al*., 2021).

Mucins are high-molecular-weight glycoproteins which are synthesized, stored, and secreted by epithelial mucosal cells, especially goblet cells (Dayan *et al*., 2004) and are involved in cell signaling and barrier protection (Cox *et al*., 2023). In the past decades mucins were thought to exclusively represent the main constituent of mucus, protecting and lubricating epithelial surfaces within the human body (Mahomed, 2011). The family of MUC-type mucins consists of many members designated as MUC1, -2, -3A, -3B, -4, -5AC, -5B, -6, -7, -8, -9, -11, -12, -13, -15, -16, -17, -19, -20 and -21. Based on their structural and functional properties the MUC-type mucins are divided into two major categories: the secreted or gel-forming mucins which include MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9 and MUC19; and the membrane-bound mucins which include MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20 and MUC2 (Mahomed, 2011; Grondin *et al*., 2020).

Studies have shown that administration of probiotics in animals can lead to improvement of barrier functions of mucosal tissues by stimulating the production of mucins and regulating the expression of tight junction proteins. For instance, Smirnov *et al*. (2005) reported that feeding of chicks with a diet supplemented with a commercial probiotic product containing *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium bifidum*, and *Enterococcus faecium* (minimum 1.0 x 10□ cfu/g) at a concentration of 2g/kg of feed from day of hatch to 14 days of age significantly increased *mucin* mRNA expression and mucin glycoprotein levels in the jejunum of the probiotic-fed chicks as compared to the control chicks.

Aliakbarpour *et al*. (2012) found that feeding of broiler chicks with feed supplemented with a commercial probiotic product (Calsporin®) containing *Bacillus subtilis* at a level of 50 mg of Calsporin® per kg of feed for 42 days, significantly increased intestinal mRNA MUC2 expression levels as compared to the control group. The authors also found that feeding of chicks with feed supplemented with another commercial feed (Primalac®) containing lactic acid bacteria (LAB) particularly *Lactobacillus casei*, *L. acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*; at a level of 1000 mg of Primalac® per kg of feed for 42 days, increased mRNA MUC2 expression levels in the intestine as compared to the control group.

5.0 LITERATURE SYNTHESIS

Reports summarized in sections 4.1 – 4.5 indicate that when probiotic products are strategically prophylactically administered to healthy animals, the bioactive constituents of the products, i.e the probiotics, act against pathogens by employing many mechanisms of action simultaneously. These mechanisms of action include competitive exclusion of pathogens, production of compounds with antimicrobial effects against pathogens, decreasing the luminal pH to levels which are unfavourable for pathogens, immunomodulation of the immune system of the host through regulation of the innate and adaptive immune responses by modulating immune cells and production of cytokines, and improvement of mucosal barrier functions by stimulating the production of mucin proteins and regulating the expression of tight junction proteins.

Administration of adequate amounts of probiotics in animals enhances both local and systemic forms of innate and adaptive immune responses. Innate immune responses (the first line of defense) are not specific to a particular pathogen, and depend on cytokines and phagocytic cells that recognize conserved features of pathogens and become quickly activated to help destroy them. Unlike the innate immune responses, the adaptive responses are highly specific to the particular pathogen that induced them, and they can provide long-lasting protection. Generally, cytokines play a key role in downregulation of inflammation and upregulation of both innate and adaptive immune responses.

The combined effect of probiotics-enhanced innate and adaptive immune responses leads to enhanced resilience of the animals to diseases which ultimately lead to prevention or control of the diseases. This is indicated by a significant decrease in horizontal transmission of pathogens by 58.3%, from 84 to 35% (Nisbet *et al*., 1998); significant decrease in diseases incidences by 59.36%, from 65.2 to 26.5% (von Buenau *et al*., 2005) and by 71.12%, from 45.7 to 13.2% (Ametaj *et al*., 2014); significant decrease in disease prevalence by 57.15%, from 31.11 to 13.33% (Genís *et al*., 2018); and significant decrease in disease severity (Ogawa *et al*., 2001a; Medina *et al*., 2008; Rasaei *et al*., 2023) leading to significant decrease in animals mortalities by 89.31%, from 34.6 to 3.7% (Watkins *et al*., 1982); 89.86%, from 74 to 7.5% (Nisbet *et al*., 1998); 89.28%, from 43.75 to 4.69% (Kyriakis *et al*., 1999); 50%, from 50 to 25% (Audisio *et al*., 2000); 74.86%, from 17.5 to 4.4% (Genovese *et al*., 2000); 66.67%, from 24 to 8% (Wang *et al*., 2019); and 52.43%, from 70 to 33.3% (Yang *et al*., 2022); and significant increase in animals survival rate by 69.57%, from 46 to 78% (Dexian *et al*., 2012) demonstrated in sections 3.1 and 3.2.

However, this review has revealed that most reports reported the prophylactic efficacy of probiotic products against diseases of the digestive system. The studied diseases include colibacillosis in gnotobiotic chicks (Watkins *et al*., 1982) and piglets (Genovese *et al*., 2000; Schroeder *et al*., 2006), fowl typhoid in chicks (Nisbet *et al*., 1998), pullorum disease in chicks (Audisio *et al*., 2000; Dexian *et al*., 2012; Wang *et al*., 2019; Yang *et al*., 2022), necrotic enteritis in chickens (Jayamaran *et al*., 2012), subclinical necrotic enteritis in chicks (Wang *et al*., 2023), post-weaning diarrhea syndrome in weaned pigs (Kyriakis *et al*., 1999), and diarrhea in calves (von Buenau *et al*., 2005) and infant rabbits (Ogawa *et al*., 2001a). Few reports reported the prophylactic efficacy of probiotic products against diseases affecting systems other than the digestive system. They include a report by Youn *et al*. (2012) which demonstrated significant prophylactic efficacy of a probiotic product against avian influenza in chickens which affects the respiratory system; reports by Ametaj *et al*. (2014) and Genís *et al* (2018) which demonstrated significant prophylactic efficacy of probiotic products against metritis in cows which is a diseases of the reproductive system in female animals; and a report by Urakawa *et al*. (2022) which demonstrated significant prophylactic efficacy of probiotic products against bovine mastitis which affects the mammary gland. This could imply that currently there is scarcity of reports on the potential of probiotic products for use in prevention of animal diseases affecting systems other than the digestive system because most studies focused on evaluating the efficacy of probiotic products against infectious diseases of the digestive system. Therefore, researchers could also focus on studies aiming at determining the prophylactic efficacy of different strains of probiotics against animal diseases affecting systems other than the digestive system.

Apart from that, this review shows that most studies focused on assessing the prophylactic efficacy of probiotic products against bacterial diseases. In order to have a full picture of the spectrum of activity of specific species of probiotics, researchers could also focus on studies to establish the prophylactic efficacy of probiotic products against animal diseases caused by other types of etiological agents such as viruses, protozoa, and helminths.

Studies to determine prophylactic efficacy of probiotic products against animal diseases are of paramount importance. This is because following demonstration of significant prophylactic efficacies of probiotic products against various diseases; the products are commercially produced by manufacturing companies and sold to livestock farmers for use in routine animal husbandry practices (Várhidi *et al*., 2022). However, based on the fact that these products (technologies) are new and not well known by livestock farmers and other stakeholders in different agricultural value chains (Várhidi *et al*., 2022); informed stakeholders such as researchers, agricultural extension workers, manufacturers and distributors could focus on dissemination of the products (technologies) to livestock farmers and other stakeholders (especially in developing countries), by using different dissemination pathways including workshops, seminars, agricultural exhibitions, trade fairs, and farmers field schools.

7. CONCLUSION

Based on this review it is concluded that probiotic products have significant prophylactic efficacy against infectious animal diseases. The products (technologies) are appropriate for use in climate adapted livestock farming because apart from contributing to enhancement of animal health and productivity, they contribute to adaptation of some of the impacts of climate change in livestock farming (particularly increased incidences of animal diseases) by enhancing the resilience of livestock to diseases; and climate change mitigation by decreasing GHG emissions intensity through enhanced animal health.

COMPETING INTERESTS

The author has declared that no competing interest exist.

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