

Systematic review on microbiome-related nutritional interventions interfering with the colonization of foodborne pathogens in broiler gut to prevent contamination of poultry meat

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ABSTRACT This systematic review aimed to compile the available body of knowledge about microbiome-related nutritional interventions contributing to improve the chicken health and having an impact on the reduction of colonization by foodborne pathogens in the gut. Original research articles published between 2012 and 2022 were systematically searched in Scopus and PubMed. A total of 1,948 articles were retrieved and 140 fulfilled the inclusion criteria. Overall, 73 papers described 99 interventions against colonization by *Escherichia coli* and related organisms; 10 papers described 15 interventions against *Campylobacter spp.*; 36 papers described 54 interventions against *Salmonella*; 40 papers described 54 interventions against *Clostridium perfringens*. A total of 197 microbiome-related interventions were identified as effective against one or more of the listed pathogens and included probiotics (n = 80), prebiotics (n = 23), phytobiotics (n = 25), synbiotics (n = 12), organic acids (n = 12), enzymes (n = 4), essential oils (n = 14) and combination of these (n = 27). The identified interventions were

mostly administered in the feed (173/197) or through oral gavage (11/197), in the drinking water (7/197), in ovo (2/197), intra amniotic (2/197), in fresh or reused litter (1/197) or both in the feed and water (1/197). The interventions enhanced the beneficial microbial communities in the broiler gut as Lactic acid bacteria, mostly *Lactobacillus spp.*, or modulated multiple microbial populations. The mechanisms promoting the fighting against colonization by foodborne pathogens included competitive exclusion, production of short chain fatty acids, decrease of gut pH, restoration of the microbiome after dysbiosis events, promotion of a more stable microbial ecology, expression of genes improving the integrity of intestinal mucosa, enhancing of mucin production and improvement of host immune response. All the studies extracted from the literature described in vivo trials but performed on a limited number of animals under experimental settings. Moreover, they detailed the effect of the intervention on the chicken gut without details on further impact on poultry meat safety.

Key words: nutritional intervention, gut health, microbiome, foodborne pathogen, intervention administration route

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INTRODUCTION

Globally, poultry meat is one of the most commonly consumed meat. Its production is estimated to increase by 16% (about 21 million metric tons) by 2031 (within 10 yr interval) accounting for 45% of growth in meat production worldwide (OECD and Food and Agriculture Organization of the United Nations, 2022). Consumers appreciate poultry meat because it is cheaper

than other meats, the consumption of poultry meat is allowed by all religions and poultry meat is often perceived as healthier than red meat, being characterized by high-quality proteins, vitamins, and minerals important for the human diet (Marangoni et al., 2015). While the demand for poultry meat is growing exponentially, poultry production is facing huge challenges, primary represented by increase of raw material costs and effects of global warming. The latter is quickly changing the microbiomes circulating within and around the poultry food system, raising concerns on occurrence of known and emerging pathogens in both the chickens and derived meat (Talukder et al., 2022; Dietrich et al., 2023; Ibáñez et al., 2023).

Foodborne diseases have been known globally in their dual burden to public health and economy. In 2021, a

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total of 202 strong-evidenced foodborne outbreaks (FBO) associated with the consumption of foods of animal origin were reported in the EU (EFSA and ECDC [European Food Safety Authority and European Centre for Disease Prevention and Control], 2022). They resulted in 2,221 human cases, 316 hospitalizations and 11 deaths (EFSA and ECDC [European Food Safety Authority and European Centre for Disease Prevention and Control], 2022). Broiler meat and derived products accounted for 21 FBOs, resulting in 202 cases, 42 hospitalization and no death (EFSA and ECDC [European Food Safety Authority and European Centre for Disease Prevention and Control], 2022). The gut content is one of the main sources of carcass contamination by foodborne pathogens during slaughtering and there is scientific evidence showing that microbiome-related nutritional interventions may antagonize the growth of foodborne pathogens in the gut and potentially increase the safety of poultry meat. This systematic review aimed to compile the available body of knowledge about microbiome-related nutritional interventions contributing to improve the chicken health and having an impact on the reduction of colonization by foodborne pathogens in the gut. Such reduction was assessed as a statistically significant decrease in Log_{10} CFU/g.

MATERIALS AND METHODS

In the context of this review, the microbiome-related nutritional interventions were considered those interventions based on nutritional strategies able to improve the chicken health modulating the gut microbiome. Examples of those interventions are feed and water additives designed to modulate the gastrointestinal environment to enhance animal performance by improving gut health and functions in broiler chickens (Bean-Hodgins and Kiarie, 2021). Those strategies are included among preharvest meat safety interventions under feed additives (Pessoa et al., 2021). In particular, interventions improving the gut health were considered those 1) resulting in an improvement of the mucosal integrity and a better histological and morphometrical architecture of the gut (Khan et al., 2017; Rivera-Pérez et al., 2021); 2) improving the microbiome composition of the host gut enhancing the host defense mechanisms against foodborne pathogens (Chen et al., 2020; Dame-Korevaar et al., 2020; Shanmugasundaram et al., 2020; Wu et al., 2020; Singh et al., 2021); 3) reducing intestinal lesions caused by colonization of the pathogens (Bozkurt et al., 2014; Deng et al., 2021); 4) facilitating the production of short chain fatty acids (SCFA) (Vermeulen et al., 2018; Bilal et al., 2021; Dauksiene et al., 2021); 5) enhancing the competitive exclusion between beneficial microbes and pathogens (Dame-Korevaar et al., 2020); 6) reducing enteritis (Sokale et al., 2019; Ramlucken et al., 2020; Shanmugasundaram et al., 2020).

The microbiome-related interventions addressed in this review were categorized as prebiotics, probiotics, phytobiotics, synbiotics, enzymes, organic acid and

essential oils. Briefly, prebiotics are nondigestible food ingredients that enhance the growth and/or activity of beneficial microflora (Al-Khalaifah 2018; Al-Khalaifa et al., 2019). Probiotics are viable microorganisms when administered in adequate amounts provide beneficial health effect on the host or live microbial feed supplements, which beneficially affect the host by improving intestinal microbial balance (OECD and Food and Agriculture Organization of the United Nations, 2022; Hill et al., 2014; Al-Khalaifah 2018; Al-Khalaifa et al., 2019). Phytobiotics, also known as phytogetic feed additives, comprise a wide range of plant-derived natural bioactive compounds and include essential oils, botanicals and herbal extracts (Yang et al., 2015). Synbiotics are a combination of prebiotic and probiotic that facilitate the implantation and survival of probiotics into the gastrointestinal tract due to their synergistic relationship.

Search Strategy and Databases

Original research papers which were written in English on selected microbiome-related intervention, were searched online in SCOPUS and PubMed on April 30, 2022. The subject areas of the studies included agriculture and biological sciences; veterinary, medicine, immunology and microbiology; biochemistry, genetics and molecular biology; environmental science; pharmacology, toxicology and pharmaceuticals. The search was conducted by using the key words and search strings described in Annex 1 and refereed to papers published between 2012 and 2022.

Inclusion and Exclusion Criteria

The study selection was done based on the title and abstract. The inclusion and exclusion criteria are detailed in Table 1.

Data Extraction and Management

The information extrapolated from the selected articles was manually entered into an excel sheet categorizing: type of microbiome-related intervention (i.e., prebiotic, probiotic, synbiotic, phytobiotic, essential oils, organic acid, enzyme and their combination); description and details of the intervention (e.g., name and composition of the intervention, route of administration); impact on foodborne pathogens (i.e., statistically significant reduction in the enumeration with $p < 0.05$); effects of the intervention(s) on microbiome modulation categorized as intervention enhancing the concentration of *Lactobacillus* spp. (labelled as A in Supplementary Tables 1–4); interventions enhancing the concentration of lactic acid bacteria (LAB) (labelled as B in Supplementary Table 1–4); interventions modulating multiple microbial groups (labelled as C in Supplementary Table 1–4).

The mechanisms promoting the fighting against colonization by foodborne pathogens included competitive

Table 1. Inclusion and exclusion criteria applied to the papers retrieved in PubMed and SCOPUS.

Inclusion/Exclusion criteria	Descriptions of the criteria
Target species	The target species for this review was broiler chickens. Papers on cattle, goat, swine, salmon, goose, quill, ducks, rabbit, pheasant and other species were excluded
Experimental design	Only papers describing in vivo trials on broilers were included. Studies on mice, cell lines, nematode model, human cell lines etc. were excluded. Studies aimed at epidemiological analysis, pathological or pathogenesis of the interventions, pharmacological or toxicological analysis were excluded.
Scope (area of research interest)	The basic interest of this review is on microbiome-related nutritional interventions contributing to improve the chicken health and having an impact on the reduction of colonization by foodborne pathogens in the gut. Papers on zootechnical parameters, economic analysis, cost-benefit analysis, diagnostic applications, stress biomarkers, alternatives to antibiotics, host diseases were excluded.
Practical application/target commodity	Papers on shelf life of products and poultry meat quality, environmental microbiome and resistance, impact on ammonia emission were excluded.

exclusion, production of short chain fatty acids, decrease of gut pH, restoration of the microbiome after dysbiosis events, promotion of a more stable microbial ecology, expression of genes improving the integrity of intestinal mucosa, enhancing of mucin production and improvement of host immune response.

RESULTS AND DISCUSSION

A total of 1,948 original articles were retrieved using Scopus (n = 869) and Pubmed (n = 1079). A total of 248 papers were excluded because were duplicates. For the others a preliminary screening was performed based on title and abstract and then the full papers were reviewed. At the end of the full text reading, 140 articles were selected for data extraction, while 1,560 were excluded for the following reasons: 356 were related to in vitro or in vivo studies in mice or cell lines; 258 targeted species other than broilers; 127 targeted different commodities; 819 were out of scope.

In the 140 articles selected a total of 197 different microbiome-related interventions were reported to have an impact on chicken health and colonization by foodborne pathogens in the gut. They included probiotics (n = 80), prebiotics (n = 23), phytobiotics (n = 25), synbiotics (n = 12), organic acids (n = 12), enzymes (n = 4), essential oils (n = 14) and combination of these (n = 27). Probiotics were the most frequently reported

microbiome-related intervention (40.6%) while only few papers explored the application of enzymes (2.9%). The identified interventions were mostly administered in the feed (173/197). Additional administration routes identified in the review were oral administration (11/197), administration in the drinking water (7/197), *in ovo* injection (2/197), intra amniotic administration (2/197). Finally, in 2 papers the intervention was applied to the litter or in both feed and water, respectively. The identified interventions enhanced the beneficial microbial communities in the broiler gut, mostly *Lactobacillus* spp. and LAB, or modulated multiple microbial populations.

In the selected papers the microbial population, including foodborne pathogens, was tested using sequencing technology (i.e., 16S rRNA sequencing in 25 papers and shotgun metagenomics in 2 papers); microbiological culture methods (in 76 papers) or polymerase chain reaction (**PCR**), both conventional and real time (in 20 papers). The remaining papers applied a combination of those methods. Both 16S rRNA sequencing, named metataxonomic, and shotgun metagenomics are untargeted methods providing information on the whole population of beneficial and pathogenic microorganisms. On the contrary, both culture methods and PCR are targeted methods. The targeted methods are more accurate for quantitative assessments, while the untargeted methods provide information on the population dynamics able to interfere with the pathogen colonization.

A total of 193 interventions described in 136 selected papers were effective for broilers naturally infected/challenged with the most common foodborne pathogens, namely *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp. and *Clostridium perfringens*, Details on each of these interventions are described in the sections below and in [Supplementary Tables 1 to 5](#).

Microbiome-Related Interventions Against *Escherichia coli*

Seventy of the reviewed papers (50%) described 99 different microbiome-related interventions that were confirmed to be effective against the colonization of *E. coli* and related organisms (e.g., Extended Spectrum β -lactamases (**ESBL**)-producing *E. coli* and Shiga toxin-producing *E. coli* (**STEC**) in broiler gut. Moreover, 3 papers (2%) did not evidence statistically significant ($P < 0.05$) effect to interfere the colonization by *E. coli* ([Supplementary Table 1](#)).

Interventions Enhancing the Concentration of *Lactobacillus* spp

The majority of the papers retrieved in the literature search reported microbiome-related interventions effective against the colonization of *E. coli*, while enriching *Lactobacillus* spp. and lactic acid bacteria (**LAB**) as main mechanism ([Peng et al., 2016](#); [Wang et al., 2017](#); [Tayeri et al., 2018](#); [Al-Khalifa et al., 2019](#); [Ashfaq](#)

et al., 2020; Ikele et al., 2020; Sunu et al., 2021). The interventions promoting the growth of LAB reduce the pH of the gut and create an unsuitable environment for *E. coli* and other potential pathogens resulting in competitive exclusion. Ikele et al. (2020) reported that *Ocimum gratissimum* extract supplementation in the basal diet of broilers elicits the innate immune response that was observed histopathologically in *E. coli* infected broilers by having a mucosal vacuolation which occurred due to mucosal lipidosis in the caeca as result of fermentation by beneficial microbes. This mucosal vacuolation helped to maintain the normal intestinal architecture to avoid infections.

Peng et al. (2016) disclosed that the dietary supplementation with *Lactobacillus plantarum* B1 increased mucosal IgA in the ileum and production of ileal and cecal short chain fatty acids (SCFA). Shokryazdan et al. (2017) showed that supplementation with *Lactobacillus salivarius* strains (strain CI1, CI2, and CI3) in the feed of broilers increased *Bifidobacteria* in the caeca, whereas decreased *E. coli*, total aerobes and harmful cecal bacterial enzymes, such as β -glucosidase and β -glucuronidase. Dietary supplementation of medicinal plants (e.g., *Rhus coriaria*, *Mentha piperita*, *Heracleum persicum*) increased proliferation of cecal *Lactobacilli* ($P < 0.05$) enhancing interferon production helping in phagocytosis of *E. coli* (Vase-Khavari et al., 2019). Elbaz et al. (2022) reported the effects of garlic and lemon essential oils added in feed in reducing *E. coli* ($P < 0.05$) colonization and increasing *Lactobacillus* counts. This study showed that in addition to modifying the intestinal microbial diversity, these essential oils play a role as antimicrobial and anti-inflammatory agents (Elbaz et al., 2022). On the other hand, Gilani et al. (2021) reported that feeding broilers with a blend of organic acids or phytobiotics or the combination of organic acid blend and phytobiotic significantly decreased ($P < 0.05$) total coliforms and *E. coli* in cecal and ileal digesta, while increasing the *Lactobacillus* spp. dynamics. The study indicated a positive correlation between the level of *Lactobacillus* spp. count and the villus height/crypt depth ratio in duodenum of treated broilers (Gilani et al., 2021).

Chang et al. (2022) showed that supplementing 3 strains of *Lactobacillus plantarum* (RG11 or RI11 or RS5) in the feed increased *Bifidobacterium* count ($P < 0.05$) but lowered *Lactobacillus* in the caeca. The significant reduction ($P < 0.05$) of *E. coli* in this study was associated with the increase in *Bifidobacterium* level affecting mucin production, tight barrier junction and epithelial cells turnover. Moreover, the production of IgA contributed to *E. coli* reduction ($P < 0.05$). Dietary supplementation with *Bacillus subtilis* QST 713 (Rivera-Pérez et al., 2021), *Bacillus pumilus* and *Bacillus subtilis* (Bilal et al., 2021) is described in different studies and significantly increased caeca *Lactobacillus* count, while reducing *E. coli* ($P < 0.05$). This effect was explained as the result of 1) deprivation of oxygen consumed by *Lactobacillus* spp. (Rivera-Pérez et al., 2021); 2) increase of the production of mucin by the goblet

cells, resulting in the maintenance of the architecture of the intestine preventing the penetration of *E. coli* or other microorganisms into the intestinal epithelium; 3) expression of IL-17F, a proinflammatory cytokine that plays a role in maintaining the immune homeostasis and the integrity of the intestinal cells (Bilal et al., 2021).

Interventions Modulating Multiple Microbial Groups

The papers retrieved in the literature search described microbiome-related interventions causing a modulation in the dynamics of multiple microbial groups in different intestinal segments and causing a significant reduction in the colonization of *E. coli* and other *Enterobacteriaceae* in the gut. Vermeulen et al. (2018) reported that the supplementation with prebiotic dietary fibers from wheat bran resulted in a significant increase in the richness of the cecal microbiota with an increase of the abundance of butyrate producing bacteria (*Lachnospiraceae* and *Ruminococcaceae*) and significant reduction in *Proteobacteria* ($P < 0.05$), creating unfavorable conditions which reduced the abundances of *Enterobacteriaceae* in the caeca of broilers. In broiler fed with a combination of *Enterococcus faecium* DSM 7134 and Fructo-oligosaccharide (FOS) there is an increase in *Firmicutes* and *Bacteroidetes* and a decrease in *Proteobacteria*, *Actinobacteria* and *Tenericutes* in cecal digesta ($P < 0.05$) (Fuhrmann et al., 2022). The authors described that such microbial modulation facilitated the fermentation processes in the gut and production of microbial metabolites, like L-lactate, n-butyrate and acetate, as well as total SCFA in the caeca resulting in a pH reduction significantly decreasing the concentration of *E. coli* ($P < 0.05$) and enhancing the immune responses (Fuhrmann et al., 2022).

Guo et al. (2021) disclosed that in broiler fed with a diet containing probiotic *Lactocaseibacillus rhamnosus* GG (LGG) strain, there was a significant increase in *Firmicutes*, *Cyanobacteria*, *Proteobacteria*, *Ruminococcaceae* and *Lachnospiraceae* in comparison to the control ($P < 0.05$). This study clarified that broilers fed with this probiotic exhibited an increased level of immunoglobulins (sIgA, IgG, and IgM) and upregulated pro-inflammatory factors such as Myd88, NF- κ B, Il6, and Il8 ($P < 0.05$) as a result of the modulation of the gut members listed above (Guo et al., 2021). Furthermore, Arreguin-Nava et al. (2019) reported that *in ovo* administration of *Bacillus* spp. base probiotic (BBP) (containing a combination of 2 strains of *Bacillus amyloliquefaciens* (AM0938 and JD17) and *Bacillus subtilis* strain AM1002) significantly enriched *Firmicutes*, *Lachnospiraceae* and *Oscillospira*, while significantly reduced *Proteobacteria* and *Enterobacteriaceae*. The increase in the microbiota beta diversity in the chicken gut as result of the treatment was considered the reason of the protection against the pathogenic *E. coli* (Arreguin-Nava et al., 2019). Manafi et al. (2017) reported significant reduction of *E. coli* after feeding broilers with 0.1% *Bacillus subtilis* (Calsporin) ($P < 0.05$). The supplement maintained the balance of the gut microflora

reducing pathogens as *E. coli*, coliforms and *Salmonella* spp. in the caeca. In summary, the findings from these studies (Ahmed et al., 2014; Manafi et al., 2017; Arreguin-Nava et al., 2019; Bilal et al., 2021; Rivera-Pérez et al., 2021) disclosed that *Bacillus* species and different strains of *Bacillus subtilis* have different mechanisms of actions to control the colonization of *E. coli*.

The literature review indicated that there are interventions reducing the colonization of *E. coli* without any significant effect on *Lactobacillaceae* in the broiler gut. Scocco et al. (2017) revealed that oregano aqueous extract added in feed enhanced the quantity and quality of glycoconjugates involved in indirect defense mechanisms against *E. coli*. Coriander (*Coriander sativum*) essential oils had antimicrobial activity reducing *E. coli* by decreasing the pH of the crop and intestine with no effect on LAB counts (Ghazanfari et al., 2015). Rabee and Abdulameer (2018) reported that feeding of herbal *Thymus vulgaris* or probiotic *Lactobacillus plantarum* K KKP 529/p significantly lowered *E. coli* due to an increase in lymphocyte (L) count and to significant decrease ($P < 0.05$) of Heterophile (H) resulting in a reduction of H/L ratio ($P < 0.01$). Similarly, feeding the synbiotic PoultryStar[®] increased gut resorption surface and significantly reduced heterophil/lymphocyte ratio that reduced *E. coli* in the gut (Mohammed et al., 2022).

Phytobiotic *Yucca schidigera* supplemented via drinking water increased the levels of IgG ($P < 0.05$), induced the production of IgM and contributed to increase the immunity against *E. coli* ($P < 0.05$) without any significant change in LAB ($P > 0.05$) (Ayoub et al., 2019). Ahmed et al. (2014) indicated that supplementation of *Bacillus amyloliquefaciens* probiotic (BAP) did not affect the cecal count of *Lactobacillus* and *Bacillus* but had negative linear effect on *E. coli* by significantly increasing serum IgG and IgA ($P < 0.05$).

Interventions With no Effect on *E. coli* Reduction

The papers retrieved in the literature reported interventions that did not originate any significant reduction on the load of *E. coli* (Forte et al., 2018; Galli et al., 2020; Lee et al., 2021). The inefficacy of the treatments observed in these studies can be due to the diversity of probiotic formulation (mono-species/mono-strain, or mono-species/multispecies, or even multigenera); route of administration (specific dosages in feed and/or in water); genotypes of the chicken and husbandry systems that may affect the impact of the treatment making difficult to compare the efficacy of different probiotic products.

Microbiome-Related Interventions Against *Campylobacter* spp

Ten studies (7%) described 15 microbiome-related interventions effective for the control of *Campylobacter* spp. colonization (Supplementary Table 2).

Interventions Enhancing the Concentration of Lactic Acid Bacteria

Froebel et al. (2019) tested the effects of *Saccharomyces*-derived prebiotic refined functional carbohydrates (RFC) (including MOS, β -glucan, and D-mannose) along with yeast culture administered to broilers through feed and drinking water. The results show a reduction of *Campylobacter* spp. count in the caeca and up to 50% decrease in the litter, although the reduction was not significant. This finding was in agreement with a similar study from the same authors testing 1) RFC as prebiotic alone; 2) a probiotic containing multistrain *Bacillus* spp. (i.e., *Bacillus licheniformis* 21, *Bacillus licheniformis* 842 and *Bacillus subtilis* 2084) as direct-fed microorganisms (DFM); 3) the synbiotic RFC plus DFM. These nutritional strategies reduced recovery of *Campylobacter* spp. by up to 1 Log₁₀ CFU/g whereas an increase of 1 Log₁₀ CFU/g in LAB was recovered when broilers were administered prebiotic RFC compared to the untreated (Froebel et al., 2020). The findings from both the studies indicated that the use of probiotic alone or the combination of the prebiotic with the probiotic (as synbiotic) can improved the impact of the prebiotic administered alone against *Campylobacter*. These results showed that the impact of prebiotic, probiotic and their combination is increased in association with RFC, inhibiting *Campylobacter* spp. attachment by binding to the bacterial surface adhesins and prevents colonization. In addition, increasing the LAB count in the caeca and ileal increased the oxygen consumption and development of a fully anaerobic environment unsuitable for *Campylobacter* spp. (Froebel et al., 2020). McMurray et al. (2022) reported that inclusion of the medicinal plant *Smilax glabra Roxb* in broiler diets, from d 14 to 21, significantly increased the abundance of LAB and this leads to a significant reduction of *Campylobacter* spp. cecal colonization at d 14, from 3.3 to 2.2 Log₁₀ CFU/g ($P < 0.01$) and *E. coli* from 8.2 to 7.4 Log₁₀ CFU/g ($P < 0.05$) (McMurray et al., 2022).

Interventions Enhancing Multiple Microbial Groups

Baffoni et al. (2017) highlighted that supplementation of the basal diet of broilers by synbiotic either *Bifidobacterium longum* subsp. *Longum* PCB133 with Xylo-oligosaccharide (XOS) or *Bifidobacterium longum* subsp. *longum* PCB133 with Galacto-oligosaccharide (GOS) increased the abundance of beneficial bacteria (i.e., *Bifidobacteria* spp., and *Lactobacilli* spp.) that competitively reduced *C. jejuni*. *Bifidobacteria* counts were significantly higher in the control group and in group of broilers fed synbiotic from 1st d of life compared to broilers fed the synbiotic from 14th d of life ($P < 0.01$). Baffoni et al. (2017) recognized the benefit of early life synbiotic administration rather than supplementation starting from d 14 of life. Baffoni et al. (2012) demonstrated that the dietary supplementation of a synbiotic

containing *Bifidobacterium* strain (*B. longum* subsp. *longum* PCB133) combined with GOS significantly increased total *Bifidobacteria* spp. while *C. jejuni* count showed significant decrease after 2 wk of treatment compared with the control group.

Nothaft et al. (2017) showed that oral gavage of probiotic prepared for coadministration of the N-glycan-based *E. coli* live vaccine with probiotics *Anaerosporebacter mobilis* DSM 15930 or *L. reuteri* CSF8 reduced *C. jejuni* while increased the abundance of *Clostridium glycolicum*, *C. bartlettii*, *C. metallolevans*, *Clostridioides difficile* and Lachnospiraceae. This study disclosed that administration of these probiotics facilitated the enrichment of the resident microbes that reduced *C. jejuni* through competition and depletion of nutrients available for *C. jejuni* (Nothaft et al., 2017). In other studies, there were data indicating a significant reduction in *Campylobacter* spp. linked to a linear reduction in the load of other microbial populations in the gut. Wealleans et al. (2017) revealed that dietary supplementation of Xylanase, Amylase and Protease with 3 strains of *Bacillus amyloliquefaciens* or Xylanase and Amylase with 3 strains of *Bacillus amyloliquefaciens* determined a significant reduction of *Campylobacter* spp. by $> 2.5 \text{ Log}_{10}$ CFU/g ($P < 0.001$) and *Bacteroides* ($P < 0.02$) in the caeca. The overall conclusion of this study was that the supplementation of multi-strain *Bacillus* probiotic strains alone provided a lower improvement of the gut health than the combination of probiotics and enzymes.

Tabashsum et al. (2020) reported that feeding broilers with *Lactobacillus casei* over-expressing myosin-cross-reactive antigen leads to a significant decrease in colonization of broilers by *C. jejuni* and *S. enterica* that was associated with a significant decrease in abundance of *Proteobacteria*, along with enrichment of bacterial diversity in gut responsible for competitive exclusion of foodborne pathogens.

Microbiome-Related Interventions Against Salmonella

Thirty-six papers (26%) described 54 different microbiome-related nutritional strategies effective to control colonization of *Salmonella* or to reduce its consequences in broilers (Supplementary Table 3).

Interventions Enhancing the Concentration of Lactic Acid Bacteria

Oral administration of *Bacillus subtilis* KKU213 or mixture of *Bacillus subtilis* KKU213 and *Pediococcus pentosaceus* NP reported to be effective against 18 serovars of *S. enterica* by significantly increasing *Bacillus* spp. and LAB counts ($P < 0.05$) (Khochamit et al., 2020). When *Bacillus subtilis* KKU213 and LAB acted synergistically in the gut they adhered to the epithelial cells of the host excluding the possibility for *Salmonella* to colonize the same substrate (Khochamit et al., 2020). Feeding fermented soyabean meal (FSBM) resulted in

a significant increase of LAB in crop, ileum and caeca but reduced *S. Typhimurium* ($P < 0.05$) (Jazi et al., 2019). The increase in the abundance of LAB also corresponded to an increase of bacteriocin production and elicited the immune response by reducing heterophil to lymphocyte ratio ($P < 0.05$) in broilers experimentally infected with *Salmonella* (Jazi et al., 2019). Furthermore, provision of synbiotic mix (containing *Lactobacillus rhamnosus* HN001 and *Pediococcus acidilactici* MA18/5M and 4.5% (0.045 g) of *Agave tequilana* fructans through drinking water enriched 110 strains of LAB in the animal gut. The authors described that the LAB increase was responsible to influence structural changes in the duodenal mucosa, promoting the ability to overcome intestinal infections caused by *S. Typhimurium* and *Cl. perfringens* by inhibiting their growth and decreasing the level of histopathological injuries (Villagrán-de la Mora et al., 2019). Nopparatmaitree et al. (2022) indicated that dietary inclusion of Trimmed *Asparagus* by-products (TABP), which can be a source of prebiotics inulin and FOS significantly enriched LAB and *Enterococcus* spp. as well as the level of acetic, propionic, butyric, and total VFA level decreasing *Salmonella* spp. and *E. coli* in the broiler caeca.

Interventions Enhancing the Concentration of Lactobacillus spp

Wu et al. (2020) indicated that inclusion of 3 or 5% trehalose in the diets of broilers challenged with *Salmonella* Typhimurium decreased the adverse effect of infection in the serum biochemistry (counteract the effect of infection on the serum level of aspartate aminotransferase, triglyceride, and albumin and globulin ratio) and infection associated lesions in liver and caeca. The protective role of trehalose was explained by its action in increasing ($P < 0.05$) the abundance of *Lactobacillus* spp. in the duodenum, jejunum, caeca and feces, contributing to the upregulation of the expression of anti-inflammatory cytokines (IL-10 and IFN α) and downregulation ($P < 0.05$) of proinflammatory cytokine genes in the challenged broilers (Wu et al., 2020).

Dietary supplementation of a phytobiotic called Intebio increased *Lactobacillus* spp. and *Bacillus* spp. and reduced the colonization by *S. Enteritidis* in the caeca contents of challenged chickens (Laptev et al., 2019). In this study it was indicated that Intebio feeding facilitated normalization of the gut microflora and the activation of immunity in the infected birds (Laptev et al., 2019). Peng et al. (2022) showed that supplementation of *Lacticaseibacillus rhamnosus* significantly increased *Lactobacillus* proliferation while *S. Typhimurium*, *Enterococcus* and *Erysipelatoclostridium* decreased in the caeca ($P < 0.05$). *L. rhamnosus* supplementation decreased the adhesion of *Salmonella* spp. and enhanced species richness in the gut microbiota, improving the immune system and maintaining the functions of the intestinal barriers.

Feeding broilers with a combination of probiotic and phytobiotic increased *Lactobacillus* spp. ($P < 0.05$) in caeca, whereas *E. coli* and *Salmonella* spp. decreased ($P < 0.05$) (Gurram et al., 2022). It was described that the increase in *Lactobacillus* spp. facilitated further proliferation of LAB producing short chain fatty acids and reducing intestinal pH (Gurram et al., 2022). Similarly, a significant increase in *Lactobacillus* spp. was reported after supplementation of phytobiotic (Chicory root powder) (Gurram et al., 2021), prebiotic (*Acacia Senegal* (Gum Arabic) (Al-Baadani et al., 2022), microencapsulated phyto-essential oils, organic acid blend or their combination (Islam et al., 2022). The increase in *Lactobacillus* spp. was responsible for lowering the pH in duodenum, jejunum, ileum and caeca and decreased *E. coli* and *Salmonella* spp. in the gut (Gurram et al., 2021; Al-Baadani et al., 2022; Islam et al., 2022). These studies showed strong evidence of the synergistic impact of different groups of microbiome-related interventions to improve gut health (Gurram et al., 2021; Al-Baadani et al., 2022; Islam et al., 2022; Peng et al., 2022).

Interventions Modulating Multiple Microbial Groups

Dietary supplementation of the blend of 3 live spores of *Bacillus subtilis* strains (NP122, B2 and AM0904) significantly increased *Ruminococcaceae*, *Lachnospiraceae* and *Clostridiaceae* families while effectively reducing *S. Heidelberg* colonization in liver and caeca ($P < 0.05$) (Hayashi et al., 2018). The study emphasized that the mechanisms associated with the diversified microbiome dynamics for reduction of *S. Heidelberg* include competitive exclusion between microorganisms and increase of lactic-acid producing organisms ($P < 0.05$) and also acidification of the environment making it unsuitable for *S. Heidelberg* (Hayashi et al., 2018).

Supplementation of Fermented defatted "alperujo" (Olive oil by-product) (phytobiotic) enriched the gut of *S. Typhimurium* challenged broilers with *Bacteroidaceae*, *Lachnospiraceae* and *Ruminococcaceae* or with *Ruminococcaceae* and *Lachnospiraceae* for chickens challenged at 7 and 21 d of life, respectively, ($P < 0.05$) (Rebollada-Merino et al., 2020). The authors demonstrated that the high level of *Lachnospiraceae* contributed to the production of short chain fatty acids reducing *S. Typhimurium* and mitigated the gut dysbiosis associated with the infection (Rebollada-Merino et al., 2020). *Enterococcus faecium* NCIMB 11,181 supplementation ameliorated *S. Typhimurium* infection-induced gut microbial dysbiosis by enriching *Lachnospiraceae* and *Alistipes* and suppressing *Barnesiella* abundance (Shao et al., 2022). The authors revealed that the predicted function analysis showed that there are functional genes of caeca microbiome involved in dibasic acid metabolism; valine, leucine and isoleucine biosynthesis; glycolipid metabolism and lysine biosynthesis that significantly enriched after the *E. faecium* NCIMB 11,181 supplementation in the diet of infected

chickens (Shao et al., 2022). The papers retrieved in this review show that different *Enterococcus faecium* strains are applied alone or in combination with other nutritional strategies as main component of synbiotics.

In another study, LaKast rice bran (as a source of prebiotic) supplementation to the basal diet of broilers challenged with *S. Typhimurium* significantly reduced the abundance of *Proteobacteria* and *Enterobacteriaceae* including *S. Typhimurium* ($P < 0.05$) (Kim et al., 2018). It was explained that LaKast rice treated animals exhibited more diverse gut microbiota playing a role in preventing colonization of *S. Typhimurium* by competitive exclusion and enhancing defense mechanisms (Kim et al., 2018). Xue et al. (2020) reported that dietary supplementation of a combination of plant oils (POC) significantly increased ($P < 0.05$) the relative abundances of *Ruminococcaceae*, *Eisenbergiella*, and *Clostridiales*, whereas decreased ($P < 0.05$) *Faecalibacterium* and *Sellimonas* ($p < 0.05$). Meanwhile, relative abundance of *Escherichia-Shigella*, *Erysipelatoclostridium*, and *Enterococcus* was decreased ($P < 0.05$) after POC supplementation (Xue et al., 2020). The decreased in *Escherichia-Shigella* and *Erysipelatoclostridium* was linked to the antimicrobial property of phenolic acid structure presented in the carvacrol contained in the POC (Xue et al., 2020).

Jazi et al. (2018) described the effects of the mix of *Pediococcus acidilactici*, Mannan-oligosaccharides (MOS) and 0.05% butyric acid as it significantly increased *Lactobacillus* and *Bifidobacterium* spp., while decreased *S. Typhimurium* in the challenged and treated chickens ($P < 0.05$). Moreover, the lowest heterophil to lymphocyte ratio was observed in the treated birds (Jazi et al., 2018). Zhen et al. (2018) revealed that chickens challenged with *S. Enteritidis* and fed diets supplemented with *Bacillus coagulans* showed significantly higher levels of *Lactobacilli* and *Bifidobacterium*, while lower levels of coliforms and *Salmonella* ($P < 0.05$) in the caeca and liver ($0.05 < P < 0.10$).

In broilers fed with diet supplemented either with *Bacillus subtilis* KT260179 or Chromium-enriched *Bacillus subtilis* KT260179, there is a significant increase in *Lactobacillus* and *Bifidobacterium* in the caeca while, *E. coli* and *Salmonella* spp. decreased ($P < 0.05$) (Yang et al., 2016). On the contrary, one study showed that dietary supplementation of oligosaccharides extracts from palm kernel expeller (OligoPKE) significantly reduced *S. Typhimurium* colonization ($P < 0.05$), without significant change on the *Lactobacillus* or *Bifidobacterium* dynamics (Rezaei et al., 2019). The authors described the protective effects of OligoPKE due to an increased in immunoglobulin A (IgA) ($P < 0.05$) in the serum and jejunum and also due to down-regulation of the expressions of Interleukin 8, and 10; interferon- α ; and tumor necrosis factor genes (Rezaei et al., 2019).

Oral administration of essential oils (carvacrol or thyme or oregano) significantly reduced *Salmonella* spp. ($P < 0.05$) with no effect on *Lactobacillus* enumeration (Liu et al., 2019). The authors identified that essential oils have antimicrobial effect, reducing the colonization

of *Salmonella* spp. and *E. coli*, and support the immunity markers of broilers by controlling the stress reactions following infections (Liu et al., 2019). In addition, a significant increase in blood IgG was observed in broilers fed with Oregano ($P < 0.05$) (Liu et al., 2019).

Islam and Yang (2017) reported that oral administration of dry mealworm (*Tenebrio molitor*) or super mealworm (*Zophobas morio*) larvae did not cause any effect on the cecal *Lactobacillus* spp., *Bacillus* spp. and yeast contents ($P > 0.05$) but reduced *Salmonella* Enteritidis and *E. coli* ($P < 0.05$). Moreover, such intervention did not cause any effect on the pH of caeca and fecal content but resulted in a high level of serum IgG and IgA leading to the reduction of these pathogens (Islam and Yang 2017).

Supplementation of the basal diet of broilers with *Punica granatum* L. by-products (PGB) significantly increased the counts of *Saccharomyces cerevisiae* in the ileum and *Bacillus* in the caeca but did not report an effect on *Lactobacillus* ($P > 0.05$). Ahmed and Yang (2017) associated the reduction in *Salmonella* and *E. coli* with the reduction of the intestinal pH, antimicrobial activity of phenolic tannins in PGB and the presence of linear increase in serum IgA and IgG in response to dietary PGB (Ahmed and Yang 2017).

Microbiome-Related Interventions Against *Clostridium perfringens*

Forty papers (29%) described 54 microbiome-related interventions effective against the colonization of *Cl. perfringens* (Supplementary Table 4).

Interventions Enhancing the Concentration of Lactic Acid Bacteria

Moharreri et al. (2021) reported that the supplementation of oil-loaded microcapsules (that has phytobiotic role) increased ($P < 0.05$) LAB in ileum and caeca, thus promoting organic acid production that lowered the pH in the caeca and subsequently decreased ($P < 0.05$) *Cl. perfringens* (Moharreri et al., 2021). Emili Vinolya et al. (2021) reported the effect of supplementation of combinations of organic, inorganic acid and essential oil blends in maintaining of the intestinal health of chickens by increasing LAB, whereas lowering *Cl. perfringens* and its impacts. Moreover, it was also described that increase in LAB responsible to reduce *Cl. perfringens* by providing dietary acidifier had buffering capacity and intestinal antimicrobial activity (Emili Vinolya et al., 2021).

Broderick et al. (2021) reported that supplementation of broilers basal diet with *Bacillus licheniformis* as a direct-fed microorganisms (DFM) with yeast cell wall extract or co-administration with a functional feed additive blend increased the LAB count in ileum and caeca and decreased *Cl. perfringens* count in ileum. The beneficial effect of LAB and the presence of mannan-oligosaccharide (MOS) obtained from the yeast extract was expected to facilitate the production of antimicrobial

non ribosomal peptide (NRP) in the GI tract (Broderick et al., 2021). The lactic acid produced reduced the pH of the ileum and caeca ($P < 0.05$), creating unfavorable environment for *Cl. perfringens* (Broderick et al., 2021) (Supplementary Table 4).

Interventions Enhancing the Concentration of *Lactobacillus* spp

Jeong and Kim (2014) showed that supplementation of *Bacillus subtilis* C-3102 spores increased *Lactobacillus* in the caeca, ileum, large intestine, and excreta and reduced *Cl. perfringens* counts in the large intestine and excreta. The authors explained the mechanisms of action against colonization of the pathogen as a result of the proliferation of *Lactobacillus* resulting in the production of enzymes and competitive exclusion and also producing lactic acid representing a natural antimicrobial that disrupt the outer membrane of Gram-negative bacteria (Jeong and Kim, 2014).

Interventions Modulating Multiple Microbial Groups

Dietary supplementation of microencapsulated organic acids with essential oils blends enhanced ($P < 0.05$) the relative abundance of *Lachnospiraceae* (i.e., Coprococcus, Roseburia, Anaerostipes) and *Ruminococcaceae* (i.e., Fecalibacterium, Anaerotruncus) while reduced *Enterobacteriaceae*, *Helicobacteraceae* and *Cl. perfringens* count in the ileum (Abdelli et al., 2020). The impact of these interventions against the pathogen is associated with the role of these beneficial microbes in the expression of enzymes enhancing the production of butyrate over propionate (Abdelli et al., 2020).

In broilers supplemented with *Lactobacillus plantarum* 16 or *Paenibacillus polymyxa* 10, there was a significant increase in *Firmicutes* and *Proteobacteria* but a decrease in *Bacteroidetes*, which contributed to improve the gut microecology affected as a result of the *Cl. perfringens* infection (Gong et al., 2021). At the genus level these probiotics increased the relative abundance of *Oscillibacter* but decreased *Allistipes* and *Bacteroides* as well as *Bacteroides fragilis* and *Gallibacterium anatis* (Gong et al., 2021). In addition, to keep maintaining the gut microbiome of broilers affected by the infection, the given probiotics reversed metabolic pathways affected by *Cl. perfringens* infection, downregulating peptidoglycan biosynthetic pathway, vitamin B synthesis, pyruvate fermentation to acetate and lactate and improving the intestinal structure, inflammation and anti-apoptosis (Gong et al., 2021).

Li et al. (2017) reported that *Lactobacillus acidophilus* supplementation enriched *Firmicutes* ($P < 0.05$) but reduced *Proteobacteria* and *Escherichia-Shigella* genera ($P < 0.05$) both in the ileum and caeca, playing a key role in restoring the microbial community disturbed during *Cl. perfringens* infection. Furthermore, after supplementation of *L. acidophilus* the relative abundance of

Lactobacillus and concentrations of lactate and short chain fatty acid increased in the intestine of broilers alleviating the damage by *Cl. perfringens* (Li et al., 2017).

Supplementation of broilers with glucose oxidase had an effect on necrotic enteritis by improving intestinal barrier integrity and balancing the microbiota (Zhao et al., 2022a). In the supplemented and *Cl. perfringens* challenged chicken, there was an increase of *Helicobacter* and a decrease of *Streptococcus* and *Cl. perfringens* (Zhao et al., 2022a). The study observed a significant decrease in the ileum pH inhibiting the pathogen proliferation. Moreover, it was observed a modulation of secretion, composition and gene expression of intestinal mucosa (Zhao et al., 2022a).

Feeding chickens with a basal diet supplemented with Sanguinarine-based phytobiotic significantly compensated the reduction of *Firmicutes* and increase of *Bacteroidetes* affected by the infection (Aljumaah et al., 2020b). The authors explained that the microbial change occurred after the treatment facilitated the production of cecal acetic acid reducing the pathogen and associated lesion scores (Aljumaah et al., 2020b). Emami et al. (2021) reported significant reduction on the severity of intestinal lesions and reduction of *Cl. perfringens* counts linked to the modulation of the gut microbiome after supplementation of a synbiotic composed of *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, *Lactobacillus reuteri*, and FOS. The lower lesion score in the synbiotic supplemented group was associated with lower abundance of microbial communities, mainly *Alistipes*, *ASF356*, *Faecalibaculum*, *Lachnospiraceae UCG-001*, *Muribaculum*, *Oscillibacter*, *Parabacteroides*, *Rikenellaceae RC9* gut group, *Ruminococcaceae UCG014*, and *Ruminiclostridium 9* compared with the negative control in the first week. In the 4th wk of life, relative abundance of *Lactobacillus* was lower, whereas abundance of *Bacteroides*, *Barnesiella*, *Butyricicoccus*, *CHKCI001*, *Eisenbergiella*, *Eubacterium hallii*, *Helicobacter*, *Ruminococcaceae UCG-005*, *Ruminococcus torques*, and *Sellimonas* were significantly higher in the control group in comparison to the synbiotic supplemented group (Emami et al., 2021).

Shang et al., (2018) reported that supplementing prebiotic FOS modulated the gut microbiota and improved the gut health by controlling the colonization of *Clostridium* spp., *Salmonella* spp. and *E. coli*. The study identified a significant increase in *Lachnospiraceae* in the ileal mucosa ($P < 0.05$) than the ileal digesta and a decrease in pathogenic organisms as *Helicobacter* and *Desulfovibrio* in the ileal mucosa (Shang et al., 2018). The authors disclosed that high levels of *Lachnospiraceae* facilitated the production of butyric acid supporting the growth of other beneficial microbes to compete with the pathogens (Shang et al., 2018).

Aljumaah et al. (2020a) and Liu et al. (2021) reported that dietary supplementation of *Bacillus subtilis* PB6 restored the gut microbial composition after *Cl. perfringens* infection. Aljumaah et al. (2020a) showed that the supplementation of *Bacillus* strains increased proliferation of *Faecalibacterium* and *Ruminococcus* followed by

Coprococcus, *Oscillospira*, *Lactobacillus* and *Bacteroides* in the caeca. It also exhibited significant increase in acetic and butyric acid in the caeca (Aljumaah et al., 2020a).

Liu et al. (2021) showed the restoring of the microbiota composition by enhancing proliferation of *Clostridium XI*, *Streptococcus*, and *Staphylococcus*. Keerqin et al. (2021) evidenced a significant increase in *Faecalibacterium*, *Oscillospira*, and *Butyricicoccus* and a decrease of *Ruminococcus*, *Lactobacillus* and *Bacteroides* in the caeca of broilers infected with *Cl. perfringens* and supplemented with a probiotic *Bacillus subtilis* 29,784. However, Wang et al. (2021c), applying the same nutritional strategy reported a significant increase in the relative abundance of *Ruminococcaceae* and *Bifidobacterium*. The authors explained the observed effect as an improvement of the composition and metabolism of the intestinal microbiota as well as intestinal structure, and a reduction of the inflammation and apoptosis contributing to control *Cl. perfringens* colonization.

Emami et al. (2021) reported that at d 28 after *Bacillus subtilis* DSM17299 supplementation in chickens, the relative abundance of *Lactobacillus* was lower, whereas the abundance of *Bacteroides*, *Barnesiella*, *Butyricicoccus*, *Helicobacter*, *Ruminococcaceae UCG-005* and *Sellimonas* were significantly higher. Nevertheless, another study that tested the dietary supplementation of direct fed microbial (DFM) *Bacillus subtilis* DSM32315, showed an increase in the abundance of *Bacillus* spp., *Lactobacillaceae* and *Lactobacillus salivarius* and a decrease in *Lachnospiraceae* ($P = 0.04$), *Ruminococcaceae* ($P < 0.01$) and *Cl. perfringens* ($P < 0.01$) in the broiler ileum and caeca (Whelan et al., 2019).

Combining the findings in the 3 studies above, indicated that different strains of *Bacillus subtilis* have different effect against *Ruminococcaceae* and *Lactobacillus* spp. (Whelan et al., 2019; Emami et al., 2021; Wang et al 2021c). Nusairat et al. (2022) reported the impacts of dietary supplementation of Xylanase with multi-strain *Bacillus* spp. as a strategy to significantly reduce *E. coli*, aerobic bacteria count and *Cl. perfringens* in the caeca ($P < 0.05$) measuring also the excretion of the pathogen in the litter. The study highlighted the implication of the intervention on environmental health because it reduced the microbial load excreted in the environment (Nusairat et al., 2022).

A study that applied *B. subtilis* (the specific strain tested not indicated) reported on increase in *Lactobacillus* and *Bifidobacterium* ($P < 0.05$) in ileum and caeca and a decrease in coliforms and *Cl. perfringens* ($P < 0.05$) due to an increase in intestinal short-chain fatty acids production (i.e., increase of lactic acid, succinic acid, and butyric acid in the ileum and caeca; increase of formic acid, isobutyric acid, and isovaleric acid in the caeca) enhancing the gut health (Qiu et al., 2021).

Kim et al. (2022) showed that broilers fed with Xylanase Family 11 in their wheat-based diet displayed a higher abundance of *Bifidobacterium*, *Lactobacillus* and

Enterobacteriaceae and butyric acid while lowered *Cl. perfringens* load ($P < 0.05$) in the caeca at 16 d. The authors explained that the changes in microbiome composition promoted competitive exclusion of the pathogen and also facilitated the production of butyric acid creating unsuitable conditions for the pathogen to growth (Kim et al., 2022).

Pacifici et al. (2017) revealed that intramniotic administration of prebiotics (Raffinose or Stachyose) increased ($P < 0.05$) both *Bifidobacterium* and *Lactobacillus* but decreased ($P < 0.05$) *Clostridium* spp. in the caeca. It was indicated that *Bifidobacterium* and *Lactobacillus* facilitated the production of short chain fatty acid supporting iron bioavailability resulting in the reduction of the abundance of pathogenic bacteria that utilize iron in the colon (Pacifici et al., 2017).

Ślizewska et al. (2019) showed that feeding broilers with synbiotic containing *Lactobacillus* spp., *Saccharomyces cerevisiae* and 2% inulin resulted in a significant increase of *Lactobacillus* and *Bifidobacterium* count in the caeca ($P < 0.05$), while reducing *Clostridium* spp. and *E. coli* in jejunum, caeca and excreta ($P < 0.05$). The mechanisms promoting the reduction of *Clostridium* spp. and *E. coli* were the production of volatile fatty acid and proteolytic enzymes that can attack the membranes of the pathogens (Ślizewska et al., 2019).

Dietary supplementation of Tri-strain probiotics (TSP) containing *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* increased ($P < 0.05$) *Lactobacillus* count in the ileal and caeca and *Bifidobacteria* in the caeca, while reducing ($P < 0.05$) the ileal and cecal *E. coli* and cecal *Cl. perfringens* counts (Hossain et al., 2015). *Lactobacillus* and *Bifidobacteria* reduced the pathogens by competitive exclusion (Hossain et al., 2015). Trials on *Bacillus licheniformis* disclosed that, depending on its effect on microflora, it reduced the load of *Cl. perfringens* in the gut and ameliorated the pathological damage due to the infection (Lin et al., 2017; Kan et al., 2021; Zhao et al., 2022b).

Zhao et al. (2022b) showed that supplementation of *B. licheniformis* H2 increased the count of Bacteroides, Lactobacillus, Ruminococcaceae, Erysipelatoclostridium, Hydrogenoanaerobacterium, Clostridiales and Subdoligranulum positively correlated with metabolic gene expression and negatively correlated with immune gene expression. A decrease of Alistipes, Megamonas, Negativibacillus, Candidatus soleaferrea, Romboutsia, and Phascolarctobacterium in the caeca of treated group was also observed and positively correlated with immune gene expression, and negatively correlated with metabolic gene expression. In the ileum, Romboutsia, Weissella, Faecalibacterium, Rothia, Insolitipirillum, Megamonas, Eisenbergiella, Fusobacterium and Stenotrophomonas decreased in the treated group and were positively correlated with immune gene expression, and negatively correlated with metabolic gene expression.

Lin et al. (2017) fed the same probiotic (*B. licheniformis* H2) and reported higher relative abundance of Bacteroides, Helicobacter, Megamonas and Akkermansia in

the supplemented chicken. Kan et al. (2021) reported that supplementation of the basal diet with *B. licheniformis* enriched *Lachnospiraceae_UCG_010* that improved the metabolism of cofactors and vitamins, amino acid and carbohydrate metabolism pathways to reduce intestinal damage due to *Cl. perfringens*.

In conclusion, this systematic review described different microbiome-related nutritional interventions applied to enhance chicken health while reducing the colonization of broiler gut by foodborne pathogens. Despite lower foodborne pathogen counts in the animal gut do not necessarily result in less contaminated carcasses at the slaughterhouse (De Cesare et al., 2022), the reduction of the colonization in the animal gut certainly decreases the circulation of the pathogens in the farm and later during transport and slaughter (Alali and Hofacre, 2016; Chowdhury et al., 2023; Obe et al., 2023). Moreover, the quantitative risk assessment models for specific foodborne pathogens, as *Campylobacter*, indicated that reducing the *Campylobacter* gut load by 2 Log₁₀ results in a 30 times reduction of human infections associated with consumption of chicken meals (Rosenquist et al., 2003).

The reviewed papers described 99 interventions against colonization by *Escherichia coli* and related organisms; 15 interventions against *Campylobacter* spp.; 54 interventions against *Salmonella* and *Clostridium perfringens*, each. The microbiome related interventions retrieved in this literature search included probiotics (n = 80), prebiotics (n = 23), phytobiotics (n = 25), synbiotics (n = 12), organic acids (n = 12), enzymes (n = 4), essential oils (n = 14) and combination of these (n = 27). The identified interventions were mostly administered in the feed (173/197) or through oral gavage (11/197), in the drinking water (7/197), *in ovo* (2/197), intra amniotic (2/197), in fresh or reused litter (1/197) or both in the feed and water (1/197). The interventions enhanced the beneficial microbial communities in the broiler gut as Lactic Acid Bacteria, mostly *Lactobacillus* spp., or modulated multiple microbial populations. The modulation of beneficial microbes by the microbiome-related nutritional interventions was associated to different mechanisms of actions interfering with the colonization by foodborne pathogens. Those mechanisms were specifically represented by competitive exclusion, production of short chain fatty acids, decrease of gut pH, restoration of the microbiome after dysbiosis events, promotion of a more stable microbial ecology, expression of genes improving the integrity of intestinal mucosa, enhancing of mucin production and improvement of host immune response. In most of the papers described in this review the impacts of the interventions are detailed in terms of reduction in foodborne pathogen concentration in the gut. However, few studies clearly demonstrated the detailed biochemical, physiological, metabolic mechanisms promoting such effects, which can be elucidated using multi-omic methods and advanced data integration strategies (Mengucci et al., 2023). Most of the studies reviewed described *in vivo* experimental trials performed on a limited number of animals under

experimental settings. Therefore, more studies in commercial conditions should be carried out to assess the effect on nutritional interventions on foodborne pathogen reduction. Moreover, in such studies additional information on the interventions should be added as their economic, environmental and social sustainability. Finally, the reviewed papers described the impact of different microbiome related intervention on the chicken gut. However, such impact should be investigated further in poultry carcasses during transport and then slaughtering. Therefore, more longitudinal studies, from farm to the fork, should be planned.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

SUPPLEMENTARY MATERIALS

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