Probiotics and Campylobacter spp.

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Campylobacter spp. are widely distributed microorganisms, many of which are commensals of gastrointestinal tract in multiple animal species, including poultry. Most commonly detected are C. jejuni and C. coli. Although infections are usually asymptomatic in poultry, poultry meat and products represent main sources of infection with these bacteria to humans. According to recent EFSA report, campylobacteriosis is the most commonly reported zoonotic disease. In 2018, EFSA Panel on Biological Hazards indicated that use of feed and water additives is the second most likely strategy that can be successful in minimizing Campylobacter spp. colonization rate in broiler chickens. One of those feed and water additives are probiotics. From numerous research papers it can be concluded that probiotics exhibit plenty of mechanisms of anti-Campylobacter activity, which were evaluated under in vitro conditions.

Keywords: poultry ; Campylobacter spp. ; probiotics ; prevention

1. Introduction

Bacteria from the genus *Campylobacter* spp. are Gram-negative, widely distributed microorganisms, many of which are detected as commensals of the gastrointestinal tract (GIT) in multiple animal species, including poultry, domestic, and wild birds. *Campylobacter* spp., of which the two most common species are *C. jejuni* and *C. coli*, are thermophilic and microaerophilic bacteria that find favorable conditions for colonization in the GIT of all warm-blooded animals and birds, especially the latter due to their higher body temperature (approximately 41 °C) than other animal species [1].

Since the isolation of the genus *Campylobacter* from *Vibrio* spp. in 1963, *Campylobacter* spp. [2] infections have become the most important cause of foodborne bacterial gastroenteritis in humans in many developed countries [3]. For years, there have been an ongoing discussion and number of studies were performed in order to establish and evaluate potential strategies to overcome these issues. In this review paper, we have paid special attention to the potential of probiotics, which together with other food and water additives were jointly classified as the second most likely strategy capable of minimizing *Campylobacter* spp. prevalence in poultry by the European Food Safety Agency (EFSA) Panel on Biological Hazards [3].

2. *Campylobacter* spp. in Poultry—Colonization, Carcass Contamination, and Prevalence

Campylobacter spp. colonizations are more common in domestic than in free-living birds [4]. Although these bacteria do not pose a serious threat to birds per se, or to large-scale poultry production in general, colonizations with them are extremely important because poultry products represent the main source of *Campylobacter* bacteria in humans, and also because human campylobacteriosis has for many years been the most frequently diagnosed zoonotic enteropathy, surpassing even salmonellosis [3][5].

As thermotolerant microorganisms, *Campylobacter* spp., find favorable conditions for colonization in the gastrointestinal tract of birds, which have a higher body temperature than other animal species. The predominant species detected in birds include *C. jejuni* and *C. coli*, the latter having mainly been isolated from turkeys, but *C. lari* bacilli have also been sporadically isolated from chickens [6][7][8][9][10].

The colonization of the GIT of poultry with *Campylobacter* spp. usually proceeds without any distinct clinical manifestations and affects the small intestine, the cecum, and the cloaca. The consequences of colonization have been demonstrated to depend on the chicken breed and age. Humphrey et al. [11] have shown that the varying extents of the appearance of clinical symptoms in various chicken breeds can be ascribed to differences from breed to breed in the immune system responsiveness to infection with *Campylobacter* spp. They have also demonstrated that in some chicken breeds, the prolonged inflammatory reaction in the intestinal mucosa is due to a lack of interleukin (IL) 10 production, which, in turn, leads to diarrhea. Some works have also indicated that a clinical course of campylobacteriosis is most often noted in young birds [12][13] and manifests with enteritis and watery diarrhea, sometimes with mucus and blood in the excreta. This condition can lead to poorer body weight gains, reduced feed conversion ratio, and ultimately to differences in bird growth within the flock. Similar symptoms including diarrhea and lower body weight gains were observed in young turkeys [5]. In addition, cases of campylobacteriosis reported in flocks of laying hens weakened laying performance and egg hatchability [14].

The incubation period of campylobacteriosis in the cases with diarrhea was reported to range from 2 to 5 days [14]. The colonization of the intestines by these bacteria primarily takes place in the jejunum, then in the ileum, and finally in the cecum [15], where their population number peaks [16], and they can be detected and excreted in feces for a prolonged period [1]. *Campylobacter* spp. reaches higher concentration in distal parts of avian GIT [1]. For instance, the concentration of bacteria in the crop was significantly higher than in the gizzard [17], which results from growth pH requirements.

Even though *Campylobacter* spp. can be detected in the liver and other internal organs, deep muscles, and blood of infected birds [18], it is believed that the majority of incidences of contamination with these bacteria found in bird carcasses result from the contact of live birds or carcasses during slaughter with a contaminated external environment in a slaughterhouse or on the production farm. Hue et al. [6] demonstrated that the level of carcass contamination with *Campylobacter* spp. correlated directly with the degree of intestine invasion by these bacteria. In addition, Berrang et al. [19] demonstrated that the number of *Campylobacter* spp.-positive breast skin sponge samples increased after bird defeathering during slaughter from 1 (prior to defeathering) to 120 out of the 120 tested. Additionally, after defeathering, the *Campylobacter* spp. population count reached $\log_{10}$ 4.2 colony forming units (CFU) per sample. The same authors recorded an increase of *Campylobacter* spp.-positive breast skin samples from 0 to 13 out of 120 tested samples from an experimental group of slaughtered birds, the cloacae of half of which were plugged with tampons and sutured closed. According to these authors, an increase in the recovery of *Campylobacter* after defeathering can be related to the escape of contaminated feces from the cloaca during the process. Other risk factors increasing the likelihood of poultry carcass contamination include cross-contamination during transport, scalding, plucking, evisceration, and chilling operations [20][21][22]. Moreover, *Campylobacter* can survive on the surface of equipment used for bird slaughter despite cleaning and sanitizing, and the persistence of the bacteria can contribute to cross-contamination of carcasses during the slaughter process [23].

It has been demonstrated, that only 35 *Campylobacter* spp. CFU are sufficient to establish colonization in the bird gastrointestinal tract [24]. The transmission rate of *Campylobacter jejuni* was estimated to be 2.37 ± 0.295 infections per infectious bird per day. It means that in a flock consisting of 20,000 broilers, 95% of birds will become infected within 4.4–7.2 days after colonization of the first broiler [24]. The same study showed that the mean age at which birds become infected with *Campylobacter* spp. was 21 days of life. Based on selected papers published after the year 2000, it can be concluded that *Campylobacter* spp. prevalence in poultry flocks ranged from 3.5% to 71.5% [25][26]. Besides the immediate threat to consumers, such a widespread prevalence of *Campylobacter* bacteria in the poultry population poses an additional risk. Namely, that in a given location where large-scale production takes place, the constant presence of these microorganisms and the widespread use of chemotherapeutic agents facilitates the selection of *Campylobacter* spp. strains resistant to antimicrobials, which, unfortunately, translates to the results returned in monitoring studies [27][28]. For example, Woźniak and Wieliczko [29][30] showed an increase in the percentage of enrofloxacin-resistant *Campylobacter* strains isolated from poultry in Poland from 52.1% in 1994 to 93.6% in 2008. A similar trend was noted by these authors regarding resistance to tetracyclines. An additional disturbing aspect is the emergence at the beginning of the 21st century of multi-resistant *Campylobacter* strains that were not found in the 1990s [29][30]. These bacteria can pose a direct risk to consumers since these strains could be passed to humans via the food chain or by direct contact and they can additionally act as donors of antibiotic resistance genes to other bacteria.

In the light of the above and taking into account the EU policy to reduce the antibiotic usage in animal husbandry, it is worth emphasizing, that the use of antibiotics is not considered as a preventive option for *Campylobacter* spp. infections in poultry [3].

3. Benefits from Using Probiotics in Poultry

Probiotics are living microorganisms which, when supplemented in the right dose, have a positive effect on the microbial ecosystem of the host gut by ensuring a favorable balance between commensal and pathogenic microflora [31].

The bacteria most often used as probiotics include those from the following genera: *Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Pediococcus*, and *Streptococcus*. Probiotics are also produced from selected species of fungi and yeasts (e.g., *Saccharomyces*).

The main beneficial effects of probiotics relate primarily to their raising of feed digestibility and bioavailability, stimulation of the immune system, improvement of health, and provision of superior organoleptic properties and chemical composition of carcasses [32][33][34][35][36][37][38][39][40].

One of the first reports on the beneficial effects of probiotics in poultry comes from 1973, when Tortuero [41] noticed an improvement in weight gain coinciding with the use of *L. acidophilus* in chicks during the first 5 days of life. In addition, in this experiment, the group receiving the probiotic was characterized by *Lactobacillus* dominance among the gastrointestinal microflora and by the simultaneous reduction in enterococci population. Considering the enterococci population in the probiotic-treated group, Tortuero [41] obtained a result similar to that in the group of birds receiving the antibiotic instead of the *Lactobacillus* culture in the same period. In addition, Nurmi and Rantal [42] showed that protection against *Salmonella infantis* could be obtained in broiler chicks by the per os administration of bacterial flora isolated from the intestines of healthy adult chickens. This concept was later referred to as competitive exclusion.

Probiotic bacteria are able to inhibit the growth of pathogenic microflora in the gastrointestinal tract of birds. This is due to the depletion of nutrients in the environment, the blocking of target receptors for pathogens on the surface of epithelial cells, or the production of natural antibacterial agents known as bacteriocins [43]. Probiotic bacteria also exhibit strong immunomodulatory effects, improving the local immune mechanisms in the gastrointestinal tract. For example, their regular and occasional uses in poultry have been shown to have an immunostimulating effect on interferon production; activities of macrophages, heterophiles, lymphocytes, and natural killer (NK) cells; and the production of specific antibodies [32][33][35][36][38]. In addition, it was previously concluded that probiotics exert a non-specific effect on the stimulation of the gut-associated lymphoid tissue (GALT), but as antigens with relatively low immunogenicity, they do not contribute to the excessive development of the inflammatory reaction nor activate the immunological mechanisms aiming at their complete elimination [37]. Through these properties, probiotics enhance the responsiveness of the immune system to an infecting pathogen [37]. These phenomena induced by probiotics minimize the risk of colonization or limit the population size of a wide range of microorganisms potentially pathogenic to poultry [7][10][13][35][42][44][45][46][47][48].

4. Probiotics and Poultry *Campylobacter* spp. Infection and Colonization

Various systems are used to assess probiotic efficacy in minimizing the consequences of infections with *Campylobacter* spp., and they have been previously reviewed [10][31][49]. In this review article, we would like to present the general scope of probiotics' modes of action against *Campylobacter* from molecular, in vitro, and in vivo studies and in conclude the work to present the results obtained in field experiments performed under commercial broiler farm conditions. General modes of probiotic anti-*Campylobacter* activity are summarized in Table 1.

Table 1. Potential mechanisms of anti-*Campylobacter* activity of probiotics, established based on selected in vitro and in vivo studies.

Probiotic Strain(s) (Origin)	Mode of Action (Experiment Conditions)	Result (Summarized Based on Different References)	References
Different *Lactobacilli* (chicken) Different LAB strains (9 strains from environmental samples of chicken farms; others—not specified) Different *Lactobacilli* strains (human)	**Organic acids production (in vitro)**	**Reduced pH inhibits *Campylobacter* growth**	**Dec et al. [50] Dubois Dauphin et al. [51] Fernández et al. [52]**
Different *Lactobacilli* (chicken)	**Hydrogen peroxidase production (in vitro)**	**Suggested to be involved in antimicrobial activity of probiotics**	**Dec et al. [50]**
Different *Lactobacilli* (chicken) Different LAB strains (fermented pickles, health infant feces and fermented dairy products)	**Hydrophobicity (in vitro)**	**Suggested to correlate with probiotic adhesion to intestinal cells ability and therefore competitive exclusion**	**Dec et al. [50] Wang et al. [53]**
Different LAB strains (chicken)	**Bacteriocins production (in vitro)**	**Direct anti-*Campylobacter* activity**	**Messaoudi et al. [54]**

Probiotic Strain(s) (Origin)	Mode of Action (Experiment Conditions)	Result (Summarized Based on Different References)	References
***Lactobacillus acidophilus* ATCC 4356 (human)** **Different *Lactobacilli* (chicken)**	**Attenuation of *Campylobacter* (in vitro; cell lines)** **Adhesion of probiotic strain to epithelial cells (in vitro; cell lines)**	**Decreased expression of *Campylobacter* virulence related genes** **Decreased *Campylobacter* adhesion to human intestinal epithelial cells by over 30%** **Decreased *Campylobacter* invasion into human intestinal epithelial cells by over 80%**	**Campana et al. [55] *** **Taha-Abdelaziz et al. [56]**
***Lactobacillus acidophilus* ATCC 4356 (human)**	**Therapeutic properties (in vitro; cell lines)**	**Displacement of *Campylobacter* by probiotics in human intestinal epithelial cells**	**Campana et al. [55]**
***Bacillus subtilis* C-3102 (Calsporin®)** ***Lactobacillus salivarius* SMXD51 (chicken)** **Different LAB strains and *Saccharomyces cerevisiae* (chicken, turkey, carp, and plant silage)**	**Modulation of gut environment (in vivo; on farm studies; broiler chickens)**	**Decreased population of *Campylobacter* in GIT and/or on the carcass in vivo**	**Fritts et al. [57]** **Saint-Cyr et al. [43]** **Śmiałek et al. [58]**
Different *Lactobacilli* (chicken)	**Immune system stimulation (in vitro; cell lines)**	**Enhanced macrophages phagocytosis ability of *C. jejuni*. Immunomodulation.**	**Taha-Abdelaziz et al. [56]**

* Gene expression was not evaluated in the paper by Campana et al. [53].

After entering the avian GIT, *Campylobacter* spp. use different mechanisms to establish their population in the gut environment, like motility, chemotaxis, adhesion, intracellular infection, and the capability to synthesize entero- and cytotoxins, as reviewed by Mohan [10]. Given the set of mechanisms, the bacteria can exploit, most research works addressing the choice of potential probiotic bacteria have been focused on the evaluation of their potential to overcome these properties of *Campylobacter* spp.

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