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The influence of probiotic supplementation in broiler chickens on population and carcass contamination with *Campylobacter* spp. - Field study

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ABSTRACT

Campylobacter spp. is a food-borne pathogen occurring all over the world. According to European Food Safety Authority, in Europe, in 2015 the number of recorded and confirmed cases of *Campylobacter* spp. infections in humans has reached approximately 230,000. Poultry and poultry meat are considered to be the main sources of human infection, which triggers the discussion about the possibility of imposing obligatory control of *Campylobacter* spp. population at the level of primary poultry production.

Recently, the use of probiotics in poultry is considered as a very promising alternative that could reduce infection rate in broiler chickens with *Campylobacter* spp. Although, there were some approaches made *in vivo*, up to date, there were no studies that would evaluate those issues under field conditions.

A study was carried out in order to determine the feasibility of reducing infection rate in broiler chickens with *Campylobacter* spp. raised at a commercial farm, by the addition of multispecies probiotic (Lavipan, JHJ, Poland) that composed of *Lactococcus lactis*, *Carnobacterium divergens*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Saccharomyces cerevisae* to the feed.

Results of our study indicate that probiotic (Lavipan) added to a feed for broiler chickens was capable to reduce the extent of *Campylobacter* spp. invasion in the gastrointestinal tract of birds and, resultantly, to diminish contamination level in bird environment, which eventually contributed to the improved hygienic parameters of analyzed poultry carcasses. Additionally, this probiotic displayed promising immunomodulatory properties that may improve the effectiveness of the specific prophylaxis program applied in a flock of broiler chickens.

1. Introduction

In recent years, campylobacteriosis has emerged as the most frequently reported food-borne disease in humans in Europe (EFSA, 2016; Ghareeb et al., 2012; Hue et al., 2011; Miller et al., 2005; Mohan, 2015; Nachamkin et al., 2002; Nielsen et al., 2000; Zhang, 2008). According to the European Food Safety Authority, in 2015 the number of recorded and confirmed cases of *Campylobacter* spp. infections in humans has reached approximately 230,000 (EFSA, 2016). What is even more disturbing is the fact that the number of recorded cases of campylobacteriosis has been successively increasing - for instance the number of cases from 2015 was equal to the half of the total number of cases recorded between 2008 and 2014 (EFSA, 2016).

The high number of campylobacteriosis cases is associated with the fact that these bacteria exist widely in nature. *Campylobacter* spp. are considered to be a part of saprophytic microflora in a digestive tract of many wild and domestic animal species, including poultry, especially grown commercially (EFSA, 2016; Ghareeb et al., 2012; Mohan, 2015). Poultry and poultry meat are considered to be the main sources of infection to humans, but the role of beef and pork meat is also emphasized in campylobacteriosis epidemiology (EFSA, 2016). For instance in 2015, 46.7% of the 6707 samples of fresh broiler meat were found to be positive for *Campylobacter* spp., which was higher than in 2014 (EFSA, 2016). The very important aspect is that infections of poultry with these bacteria are generally subclinical (Lee and Newell, 2006; Mohan, 2015; Zhang, 2008).

The above situation gives rise to an ongoing discussion about the possibility of imposing obligatory control and reducing *Campylobacter* spp. population at the level of primary poultry production. In the light of the above, one of the alternatives that can easily be implemented at the level of primary production is the use of probiotics.

Probiotics, which are one of the oldest feed additives, are live

Abbreviations: CH, Chicken House; EFSA, European Food Safety Association; ELISA, Enzyme-linked Immunosorbent Assay; IB, Infectious Bronchitis; IBD, Infectious Bursal Disease; MD, Mareks Disease; ND, Newcastle Disease; OD, Optical Density; REO, Reoviruses; S., Salmonella; SD, Standard Deviation; S/P, Sample to Positive Ratio

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microorganisms. If supplemented in the right amount, they can beneficially affect the host's health by: (1) ensuring a favorable balance between commensal and pathogenic microbiota in the gastrointestinal tract, (2) increasing digestibility and assimilability of nutrients, as well as (3) exerting immunomodulatory and immunostimulatory effects (Alkhalf et al., 2010; Balevi et al., 2001; Chichlowski et al., 2007; Dhama et al., 2011; Farnell et al., 2006; Fellah et al., 2014; Galdeano and Perdigón, 2006; Koenen et al., 2004; Samanya and Yamauchi, 2002). It has been demonstrated that the use of probiotics and eventually their domination in birds digestive tract may reduce the growth of pathogenic bacteria such as *Staphylococcus aureus*, *E. coli*, *Salmonella (S.) enteritidis* and *S. typhimurium*, *Clostridium perfrigens*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolica*, *Candida albicans*, as well as coccidia (Bengmark, 1998; Dalloul et al., 2005; Dhama et al., 2011; Fuller, 2001; Ghareeb et al., 2012; Lee et al., 2007; Mohan, 2015; Nurmi and Rantala, 1973; Patterson and Burkholder, 2003; Willis and Reid, 2008). In the light of the above, regular supplementation of poultry with probiotics may both minimize the risk of infection and disease in birds as well as decrease the risk of poultry meat contamination with such pathogens as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and/or *E.coli*.

Up to date, only a few studies have evidenced a possible role of probiotics in preventing the shedding of *Campylobacter* spp. at the level of primary production. Although, there were some approaches made in order to evaluate the potential influence of probiotic supplementation on the rate of broiler chickens infection with *Campylobacter* spp. and shedding of the bacteria *in vivo* (Ghareeb et al., 2012; Willis and Reid, 2008), there were no studies that would evaluate those issues under field conditions.

Considering the above, a study was carried out in order to determine the feasibility of reducing *Campylobacter* spp. infection rate in broiler chickens raised at a commercial farm, by feed supplementation of Lavipan – a multispecies probiotic product (JHJ, Poland), in the final concentration of 0,5 kg of probiotic per every 999.5 kg of the feed.

2. Materials and methods

2.1. Farm

The study was carried out under field conditions on a chicken farm with four poultry houses (CH - chicken house) in four consecutive production cycles. It was conducted in CH1 and CH2 and the number of birds in those houses in each production cycle was (approx.): CH1 – 22,000 and CH2 – 18,000 chickens. Study layout is summarized in Table 1. In each production cycle (1 - retrospective, 2 - experiment, 3 and 4 - control), both CH1 and CH2 were settled with Ross 308 broiler chicks purchased from one hatchery and from one hatch. Feed and water were given to the birds *ad libitum*. Feed was provided by its producer - Wipasz sp. z.o.o. (Poland).

The retrospective microbiological study was conducted on a farm (production cycle 1) in order to establish the presence of *Campylobacter* spp. and the diversity of its population between CH1 and CH2. This study was conducted with environmental samples (freshly excreted droppings). Similarly, after the main experiment (production cycle 2) had been completed, the control study was performed twice (production cycles 3 and 4). Environmental samples (n = 4 / CH) comprised of 10 pooled dropping samples collected from different parts of a chicken house. Environmental samples were collected at 37 days of birds life in each production cycle.

Table 1
Study layout summarized. Only in production cycle 2 birds from chicken house 2 (CH2) received probiotic (Lavipan, JHJ, Poland) in feed.

Sample type	Production cycle no.			
	1/retrospective	2/experiment	3/control	4/control
Environmental sample	+[a]	+	+	+
Feces from intestines	−	+	−	−
Pectoral muscles	−	+	−	−
Blood	−	+	−	−

[a] "+" indicates samples collected during each production cycle.

2.2. Experimental layout

During production cycle 2 (Experiment), chickens from CH2 (group L) received a Lavipan probiotic (JHJ, Poland) in the feed in the final dose of 0.5 kg of probiotic per every 999.5 kg of the feed during the entire production cycle. At the same time, birds from CH1 (group K) received the same feed but without the probiotic. Throughout the experiment, veterinary prophylaxis and therapy schedule and program were the same for CH1 and CH2 (as well as for CH1 and CH2 during three other production cycles). Before birds were placed in CH1 or CH2, 23 birds were selected at random from a transport truck in order to obtain blood samples for the serological evaluation of the time of vaccination against Infectious Bursal Disease (IBD), based on the Deventer formula, as well as for the evaluation of the level of maternally-derived antibodies against Infectious Bronchitis (IB) virus and reoviruses (REO). Vaccination program against Marek Disease (MD), IB and IBD that was executed at the farm in each production cycle is summarized in Table 2. At day 37 of birds life, 20% of the chickens from CH1 and CH2 were transferred for slaughter. At the slaughterhouse, 23 blood samples were collected from birds from K and L group independently for the serological evaluation of the level of antibodies against IB, IBD and REO. At the same time, 5 samples of freshly-squeezed feces from ileum and ceca of the chickens from group K (n = 5) and L (n = 5) were collected for microbiological examination and *Campylobacter* spp. enumeration. Prior to feces samples collection, abdominal skin was disinfected (40% ethanol) and intestines were dissected with the use of sterile surgical equipment (individual equipment was prepared for each bird). Additionally, samples of pectoral muscles (10 g), cut superficially (max. cut depth < 10 mm), with overlaying skin were collected from birds of group K (n = 4) and L (n = 4) for microbiological examination. At the same time, environmental samples were collected from CH1 and CH2 (n = 4 / CH).

In each production cycle, the samples collected for microbiological examination were transferred on ice to a laboratory in < 3 h after they had been collected.

2.3. Probiotic

During production cycle 2 (Experiment) chickens from CH2 received in feed Lavipan probiotic product (JHJ, Poland) which comprises of selected stains of lactic acid bacteria: *Lactococcus lactis* IBB 500 (origin - chicken feces), *Carnobacterium divergens* S-1 (origin - carp gut), *Lactobacillus casei* ŁOCK 0915 (origin - chicken feces) and *Lactobacillus plantarum* ŁOCK 0862 (origin - turkey feces) in the amount of 1×10^9 colony forming units (CFU/g) each and *Saccharomyces cerevisae* ŁOCK 0141 (origin - plant silage) in the amount of 1×10^7 CFU/g.

Table 2
Immunoprophylaxis programme executed at the experimental farm during 4 production cycles.

Disease	Day of life	Vaccine strain
MD	In. ovo	Rispens + HVT
IB	1	H-120
	14	1/96
IBD	16 (Based on Deventer formula)	Winterfield 2512