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Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic administration

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ABSTRACT

C. jejuni is considered a food safety concern to both public health authorities and consumers since it is the leading bacterial cause of food-borne gastroenteritis in humans. A high incidence of *C. jejuni* in broiler flocks is often correlated to pathogen recovery in retail poultry meat, which is the main source of human infection. In this work broiler chickens were fed with a synbiotic product mixed with conventional feed using two different administration strategies. The synbiotic was formulated with the microencapsulated probiotic *Bifidobacterium longum* PCB133 and a xylo-oligosaccharide (XOS). 1-day old chicks were infected with *C. jejuni* strain M1 (10^5 cells) and the synbiotic mixture was then administered starting from the first and the 14th day of chicken life (for animal groups GrpC and GrpB respectively). The goal of this study was to monitor *C. jejuni* load at caecum level at different sampling time by real-time PCR, identifying the best administration strategy. The microbiological analysis of the caecal content also considered the quantification of *Campylobacter* spp., *Bifidobacterium* spp. and *B. longum*.

The supplemented synbiotic was more successful in reducing *C. jejuni* and *Campylobacter* spp. when administered lifelong, compared to the shorter supplementation (GrpB). *Bifidobacterium* spp. quantification did not show significant differences among treatments and *B. longum* PCB133 was detected in both supplemented groups evidencing the successful colonization of the strain. Moreover, the samples of the control group (GrpA) and GrpC were analysed with PCR-denaturing gradient gel electrophoresis (PCR-DGGE) to compare the caecal microbial community profiles at the beginning and at the end of the trial. Pattern analysis evidenced the strong influence of the early synbiotic supplementation, although a physiological change in the microbial community, occurring during growth, could be observed. Experimental results demonstrate that the synbiotic approach at farm level can be an effective strategy, combined with biosecurity measures, to improve the safety of poultry meat.

1. Introduction

Monitoring of *Campylobacter jejuni* in broilers from hatching to slaughter is of fundamental importance to preserve consumer health, since transmission to humans could lead to severe consequences. Human campylobacteriosis is the most frequent zoonosis in the European Union with 236,851 confirmed cases in the year 2014 and broiler meat is the most common food vehicle associated with this disease (EFSA, 2015). In addition to gastrointestinal disorders, 1% of cases may develop peripheral neuropathies, including Guillain-Barré syndrome, reactive arthritis and functional bowel diseases such as irritable bowel syndrome (Epps et al., 2013; Spiller and Lam, 2012). Despite biosecurity measures, broiler houses show a high presence of *C. jejuni* in the chicken gut; in 2014, *Campylobacter* was found in 30.7% of the 13,603 units tested within the EU member states with percentages ranging from 70% to 92% in Greece, Portugal and United Kingdom (EFSA, 2015). *C. jejuni* is considered a gut commensal in chickens; however, Humphrey et al. (2014) have recently shown that some chicken breeds used in intensive production systems have a strong inflammatory response to *C. jejuni* infection leading to disease. The high incidence and the need to prevent zoonosis require a common effort to remove and reduce the pathogen load at farm level in order to lower the risk of transmission along the poultry meat chain. In addition to good hygienic practices during slaughtering and decontamination treatments

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Table 1
Diet composition of reared chickens.

Days	Type of feed	Appearance	Composition
1–10	Starter feed	Chopped	Corn, genetically modified soy flour of extraction decorticated and toasted, wheat, seed toasted soybeans genetically modified, maize gluten genetically modified, animal fats, sunflower meal of extraction, peas, hydrolysed pork protein, dicalcium phosphate, calcium carbonate, sodium chloride, sodium bicarbonate, nutritional additives, vitamins, provitamins, trace elements, digestibility enhancers, coccidiostats
11–20	Grower feed (type 1)	Pelleted	Corn, genetically modified soy flour of extraction decorticated and toasted, wheat, seed toasted soybeans genetically modified, maize gluten genetically modified, decorticated sunflower flour extraction, animal fats, peas, hydrolysed pork protein, dicalcium phosphate, calcium carbonate, sodium chloride, sodium bicarbonate, nutritional additives, vitamins, provitamins, trace elements, nutritional additives, amino acids and their salts, digestibility enhancers, coccidiostats
21–30	Grower feed (type 2)	Pelleted	Wheat, corn, genetically modified soy flour of extraction decorticated and toasted, seed toasted soybeans genetically modified, wheat in grains, animal fats, decorticated sunflower flour extraction, peas, dicalcium phosphate, calcium carbonate, sodium chloride, sodium bicarbonate, nutritional additives, vitamins, provitamins, trace elements, nutritional additives, amino acids and their salts, digestibility enhancers, coccidiostats
31–39	Finisher feed	Pelleted	Wheat, seed toasted soybeans genetically modified, genetically modified soy flour of extraction decorticated and toasted, maize, wheat in grains, animal fats, decorticated sunflower flour extraction, peas, dicalcium phosphate, calcium carbonate, sodium chloride, sodium bicarbonate, nutritional additives, vitamins, provitamins, trace elements, nutritional additives, amino acids and their salts, digestibility enhancers

of carcasses or meat products, feeding probiotic/synbiotic supplements to broilers could contribute to food safety from the initial step of the "farm to fork" food chain (Gaggìa et al., 2010). Probiotic microorganisms (*e.g. Lactobacillus* and *Bifidobacterium* strains), both alone or combined with prebiotic ingredients in a synbiotic mixture, can beneficially affect the host, maintaining a healthy gut microbiota in animals and reducing the risk of pathogen infection (Allen et al., 2013; Gaggìa et al., 2010). The combination with prebiotics is considered an effective strategy, taking into account that such ingredients are selectively fermented in the colonic environment by probiotics, thus stimulating their growth (Gibson et al., 2004). This is an important feature in high intensive flocks where dietary changes during chicken lifespan and therapeutic antibiotics may strongly alter the composition of these microbial groups, increasing the susceptibility to gastrointestinal infection and the shedding of food-borne pathogens (Bomba et al., 2002; Oliver et al., 2009). In the last decades, the use of synbiotic supplements in poultry flocks has been largely investigated; however, their efficacy is not fully established because of the variability of the experimental settings. Lactobacilli and bifidobacteria are the most used probiotic strains in animal feeding, combined with galacto-oligosaccharides (GOS), fructo-oligosaccharadies (FOS) or xilo-oligosaccharides (XOS). Studies usually reported the improving of growth parameters (weight, feed intake *etc.*), the modulation of the gut microbiota with the increase of beneficial microbial groups and the reduction of the load of pathogens such as *C. jejuni* and *Salmonella enterica* (Baffoni et al., 2012; Gaggìa et al., 2010; Santini et al., 2010).

This work aimed at evaluating the impact of a synbiotic formula in broilers challenged with *C. jejuni* strain M1, a virulent strain capable of direct transmission from poultry source to humans (Friis et al., 2010). The formula, composed by the microencapsulated *B. longum* subsp. *longum* PCB133 and a xylo-oligosaccharide (XOS), was administered to chicks from the first and the 14th day of chicken life, and its efficacy was evaluated by monitoring *Campylobacter* spp., *Bifidobacterium* spp. and *C. jejuni* in the caecal content by real-time PCR (qPCR). The analysis of *Campylobacter* spp. was also supported by conventional microbiology. Moreover, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) was performed to evaluate the caecal microbial community.

2. Materials and methods

2.1. Synbiotic composition

The synbiotic supplement consisted of the probiotic strain *B. longum* subsp. *longum* PCB133 (Santini et al., 2010) and the prebiotic xylooligosaccharide. The bacterial strain, microencapsulated in a lipid matrix according to Baffoni et al. (2012), was purchased from Probiotical S.p.A. (Milan, Italy) at a concentration of 10^9 cfu/g and was added to feed at 1% (w/w). The prebiotic oligosaccharide was a 35% xylooligosaccharide (XOS35P) purchased from Italfeed s.r.l. (Milan, Italy) and added to feed at 0.2% (w/w).

2.2. Infection, animal management and sampling

C. jejuni strain M1 was used to infect animals and was provided by the University of Liverpool (Chaloner et al., 2014). One hundred and twenty1-day old chicks, obtained directly from hatchery and tested culture negative for *Campylobacter* spp. in Karmali Agar plates (see paragraph 2.3), were divided into three groups. All groups were immediately infected by oral gavages with a 0.1 ml solution containing a challenge dose of *C. jejuni* M1 (10^6 cfu/ml). The three groups of forty animals were named and managed as follows: 1) GrpA - chickens fed *ad libitum* with conventional feed; 2) GrpB - chickens fed *ad libitum* with conventional feed supplemented with the synbiotic product from the 14th day of life; 3) GrpC - chickens fed *ad libitum* with conventional feed supplemented with the synbiotic product starting from the first day of life. The conventional feed is described in Table 1. Birds were reared under hygienic management practices throughout the entire period of the study. During the experiment, breeding conditions in terms of equipment, temperature and hours of daylight were conventionally set.

Five days after challenge, 4 animals per each group were slaughtered and tested culture positive for *Campylobacter* spp. confirming the colonization. Nine/ten broilers belonging to each group were slaughtered at 10, 20, 30, 39 days of life (sampling times ST1, ST2, ST3 and ST4, respectively) and caecal content collected for the microbiological analysis and DNA extraction. Experiments were conducted according to animal welfare and protection (directive no. 86/609/EEC and Italian Law Act, Decreto Legislativo no. 116, issued on 27 January 1992).

2.3. Campylobacter spp. enumeration from caeca with plate count analysis

Campylobacter spp. enumeration was carried out from 1 g of caecal content diluted with 9 ml of buffered peptone water, then ten-fold serial dilutions were set up and 0.1 ml of each dilution was plated on Karmali Agar (Oxoid, Milan, Italy) and incubated in microaerophilic conditions at 42 °C for 48 h. Following incubation, the number of colony forming units per gram (cfu/g) of.

caecal content was recorded and means and standard deviations were calculated. Five suspected colonies for each plate were confirmed to be *Campylobacter* spp. by means of morphology, motility, oxidase and PCR tests according to Wang et al. (2002).