

Fighting Campylobacter colonization in broiler chickens

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Figure 1. Campylobacter jejuni is a non-spore forming, Gram-negative, microaerophilic bacteria which is one of the most common causes of human gastroenteritis in the world. In the last 20 years, *Campylobacter* has emerged as the most commonly reported cause of bacterial gastroenteritis in humans worldwide. Affected humans show clinical signs of acute diarrhea or more severe complications including Guillain-Barré syndrome and arthritis. The cost of campylobacteriosis to public health and to lost productivity in the EU is estimated by the European Food Safety Authority (EFSA) to be around EUR2.4 billion a year.

oultry are generally recognised to play a significant role in human campylobacteriosis where consumption or mishandling of raw or undercooked poultry meat, or contamination of ready-to-eat foods that have been in contact with raw poultry meat

have been in contact with raw poultry meat are considered the most common sources of infection. As consumption and mishandling of raw or undercooked poultry meat is the main cause of *Campylobacter* transmission to humans, reducing chicken colonization by this bacterium might reduce in the incidence of human infections. One of the challenges associated with campylobacteriosis control is that *Campylobacter* behaves as a commensal microorganism in healthy poultry without causing any clinical diseases. It inhabits the mucus layer of the cecum but does not penetrate the intestinal cells.

Several tools are used to to control enteric pathogens in poultry. Competitive exclusion strategies and the use of specific probiotics and synbiotics have shown to be effective means of manipulating or managing the composition of the microbial population in the gastrointestinal tract of poultry, and thus protecting poultry flocks from pathogenic bacteria.

Figure 2. Antimicrobial activity of probiotic bacterial strains (*Enterococcus faecium, Pediococcus acidilactici, Lactobacillus salivarius* and *Lactobacillus reuteri* and their combination with *Bifidobacterium animalis*) derived from the GIT of chickens against *Campylobacter jejuni* in the co-cultivation agar expressed by inhibition index (diameter inhibition zone [cm]/diameter test strain [cm].



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To control enteric pathogens, the commercial poultry industry uses several management tools such as antibiotics, vaccines, acidifiers, phytogenics, prebiotics and probiotics.

But as more countries ban the use of antibiotic growth promoters (AGPs) in animal feed, and with rising consumer concerns over the indiscriminate use of antibiotics, evaluating alternatives to antibiotics has become more appealing to commercial poultry farming.

	Positive Control	PoultryStar® 2 mg/bird/day	Positive Control	PoultryStar® 2 mg/bird/day
Bird/Age	Day 8		Day 15	
1	7.92	<3	>8	3.59
2	7.74	3.00	>8	3.72
3	3.90	<3	>8	4.10
4	6.45	4.38	>8	3.30
5	4.85	<3	>8	<2
6	7.53	<3	>8	2.78
7	6.79	<3	>8	<2
8	7.86	<3	>8	<2
9	7.51	<3	>8	4.18
10	5.18	<3	>8	<2
11	>8	<3	>8	<2
12	<3	<3	>8	<2
Average	6.67ª	4.10 ^b	>8ª	3.82 ^b

Table 1. Experiment 1, day 8 & 15. Campylobacter content in cecum (log cfu/g) after challenging with 10⁵ cfu/ml of a field strain of Campylobacter jejuni at day one.

^a,^b Means within a row with different superscripts differ significantly (*P*=0.001) Source Ghareeb et al., 2012

Probiotics for poultry

A multinational project funded by the EU brought together five industrial and three research partners for the purpose of developing a well-defined and safe multi-species probiotic product for poultry.

Numerous intestinal bacteria were isolated out of the gut of several healthy

PoultryStar® and Campylobacter control in broiler chickens

Research findings from the CESAC (Centre de Sanitat Avícola de Catalunya i Aragó) revealed that the prophylactic feeding of the poultry-specific, multi-species probiotic PoultryStar[®] to broilers caused a significant decrease in the cecal colonization of *Campylobacter jejuni* in two independent challenge trials with experimentally infected broilers.

Commercial day-old broiler chicks (Ross 308, mixed sex) were procured from a commercial hatchery with certificate of origin and health. The ceca of 10 randomly selected birds were harvested and tested for the presence of *Campylobacter* species to ensure that the experimental birds were *Campylobacter* negative.

The remaining birds were wing-tagged and placed in individual pens with fresh wood shavings litter. Feed and water were provided *ad libitum*. Birds received a standard non-medicated corn-soy based starter diet. Temperature, heating and ventilation followed the commercial recommendation.

Method

Two experiments were conducted to evaluate the efficacy of PoultryStar[®] on *Campylobacter jejuni* colonization in broiler chickens.

Experiment 1: All birds were oral gavaged with 0.1 ml of a solution containing 10⁵ cfu/ml of a field strain of *Campylobacter jejuni* at day 1.

Forty-four day-old broiler chicks were randomly assigned to two groups, a *Campylobacter* challenged positive control group and a *Campylobacter* challenged group which received an additional 2 mg/bird/day of PoultryStar[®] sol via drinking water.

Experiment 2: All birds were challenged with *Campylobacter jejuni* on day 1 by introducing in each group four seeder birds orally gavaged with 0.1 ml of a solution containing 10⁵ cfu/ml of a field strain of *Campylobacter jejuni*.

Seventy-eight day-old broiler chicks were randomly assigned to three groups: a *Campylobacter* challenged positive control group; a *Campylobacter* challenged group which received an additional 2 mg/bird/day of PoultryStar[®] sol via drinking water; and a *Campylobacter* challenged group which received an additional 20 mg/bird/day of PoultryStar[®] sol via drinking water.

At days 8 and 15 of both experiments, 10 birds from each group were euthanized and their ceca harvested for individual quantitative culture of *Campylobacter*.

Results

Experiment 1: The application of 2 mg/bird/day of Poultry-Star[®] sol via drinking water significantly reduced (*P*=0.001) the cecal colonization of *Campylobacter jejuni*. chickens and thoroughly characterized by combining morphological, physiological and genotypic methods. The most promising strains were evaluated for important probiotic criteria like the inhibition of pathogenic bacteria.

Based on these results, a product consisting of strains belonging to the genera *Enterococcus, Pediococcus, Lactobacillus* and *Bifidobacterium* (Poultry-Star[®], BIOMIN GmbH) was designed. As the probiotic strains were able to inhibit *Campylobacter jejuni* (the main cause of human campylobacteriosis) *in vitro*, the efficacy of PoultryStar[®] on *Campylobacter jejuni* was evaluated in experimental challenge trials using experimentally infected broilers.

Improved immunity

The results of these studies showed that the use of probiotics can help to improve the natural defence of birds against enteric bacteria and can be used as an alternative and effective strategy to antibiotics in livestock, thus reducing bacterial contamination of raw poultry meat. The

At day 8: Ten of 12 birds in the PoultryStar® group had *Campylobacter* counts that were <3 log cfu/g, which was significantly lower than the mean log count in the positive control group, 6.67 log cfu/g (*P*=0.001).

At day 15: All the birds from the positive control group had counts higher than 8 log cfu/g. However, in the PoultryStar[®] group, the maximum count was significantly reduced (*P*=0.001) to 4.10 log cfu/g and half the birds had counts <2 log cfu/g (*Table 1*).

Experiment 2: The application of 2 mg/bird/ day and also 20 mg/bird/day of PoultryStar® sol via drinking water significantly reduced (*P*=0.001) the cecal colonization of *Campylobacter jejuni*.

At day 8 & 15: Campylobacter counts in the cecal content of the PoultryStar[®] group were <2 log cfu/g, whereas the mean log counts in the positive control group were 7.81 log cfu/g at day 8 and 7.85 log cfu/g at day 15 (P=0.001) respectively (*Tables 2a* and 2*b*).

Compared with the controls, the Poultry-Star® groups showed a 6 log reduction in the cecal colonization of *Campylobacter jejuni*. The lower dose of PoultryStar® was also effective in reducing *Campylobacter* counts. Table 2a. Experiment 2, day 8. Campylobacter content in the cecum, log cfu/g, after challenging with 10⁵ cfu/ml of a field strain of Campylobacter jejuni at day one.

Bi	rd (day 8)	Positive Control	PoultryStar® 2 mg/bird/day	PoultryStar® 20 mg/bird/day
	1	8.52	<2	<2
	2	7.78	<2	<2
	3	8.15	<2	<2
	4	6.48	<2	<2
	5	6.30	<2	<2
	6	9.02	<2	<2
	7	7.60	<2	<2
	8	9.60	<2	<2
	9	8.38	<2	<2
	10	6.30	<2	<2
	Average	7.81ª	<2 ^b	<2 ^b

Table 2b. Experiment 2, day 15. *Campylobacter* content in the cecum, log cfu/g, after challenging with 10⁵ cfu/ml of a field strain of *Campylobacter jejuni* at day one.

Bird (day 15)	Positive Control	PoultryStar® 2 mg/bird/day	PoultryStar® 20 mg/bird/day
1	8.00	<2	<2
2	7.78	<2	<2
3	7.85	<2	<2
4	7.00	<2	<2
5	7.48	<2	<2
6	8.77	<2	<2
7	7.30	<2	<2
8	8.11	<2	<2
9	8.20	<2	<2
10	8.00	<2	<2
Average	7.85ª	<2 ^b	<2 ^b

^a,^b Means within a row with different superscripts differ significantly (*P*=0.001)

Source: Ghareeb et al., 2012

inclusion of the multi-species synbiotic PoultryStar[®] significantly reduced cecal colonization of *Campylobacter jejuni* in broilers by its marked antimicrobial activities.

This shows the beneficial effects of PoultryStar[®] towards reducing *Campylobacter* prevalence in poultry and subsequently, the incidence of campylobacteriosis in humans.

References are available on request.





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