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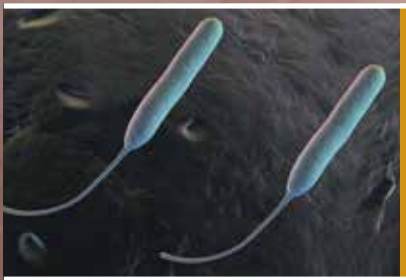
Science & Solutions

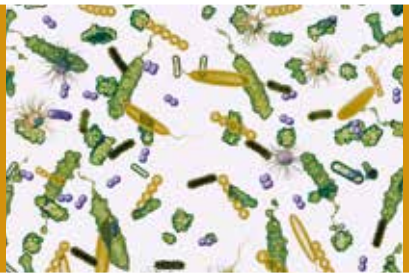
Probiotics & Synbiotics

Protection from day one



Fighting *Campylobacter* in broilers

The proven efficacy of probiotics in controlling enteric pathogens



Synbiotics and microflora

Prevent intestinal dysbiosis and promote gut health in young chicks

Editorial

Containing contaminations

Concerns over food-borne human health problems are not new. During the 70s in the UK, there were periodic scares over *Salmonella* outbreaks coming from incompletely thawed frozen turkey, often during the Christmas period. This changed towards the end of 1988 when a UK health minister informed Parliament that 90% of all layer flocks in the country were infected with *Salmonella enteritidis*. Although the problem began in the UK, the repercussions were felt globally, resulting in massive losses in revenue for the industry.

Following this, *Salmonella* infection in broiler production came under the spotlight. Similar testing regimes used with egg laying stocks were applied to this sector. Despite all the investment, *Salmonella* infection remains the major concern in much of the world as the primary cause of food-borne gastroenteritis. The exception is in Western Europe where *Campylobacter jejuni* infections now exceed those of *Salmonella*. This has led to a European task force looking at ways of reducing the incidence in farms and processing plants, with the ideal of eliminating *Campylobacter* from the food chain.

Since the turn of the century, much effort has gone into developing alternative therapies to antibiotics to reduce the impact of both food-borne and commercially detrimental diseases. Acidifiers have proved successful in reducing microbial contamination in the poultry house, thereby reducing the levels of specific Gram-negative pathogens in the intestinal tract.

Probiotics, first introduced in broilers to control a specific *Salmonella* infection in Finland, have developed with some success in *Salmonella* reduction. Latest research seems to show that they may also be a powerful tool in the reduction of *Campylobacter* incidences.

Looking ahead, I am sure we will see probiotics being used increasingly to reduce levels of contamination of both *Salmonella* and *Campylobacter* in poultry.

Andrew ROBERTSON
Technical Manager, Poultry

Contents

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Fighting *Campylobacter* colonization in broiler chickens

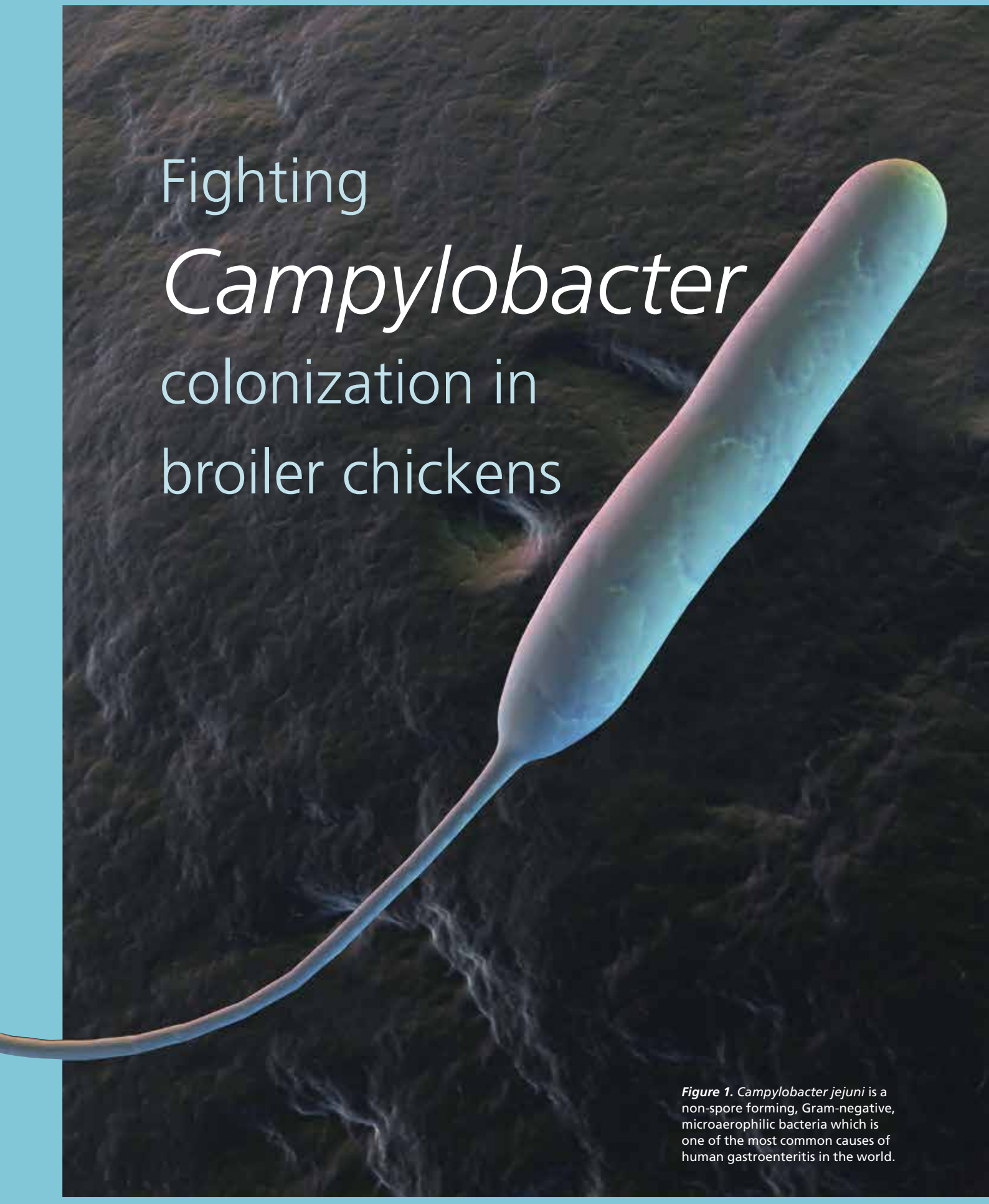


Figure 1. *Campylobacter jejuni* is a non-spore forming, Gram-negative, microaerophilic bacteria which is one of the most common causes of human gastroenteritis in the world.

Wael Abdelrahman
Technical Consultant, Poultry Probiotics

Photo: Shutterstock-Sebastian Kaulitzki

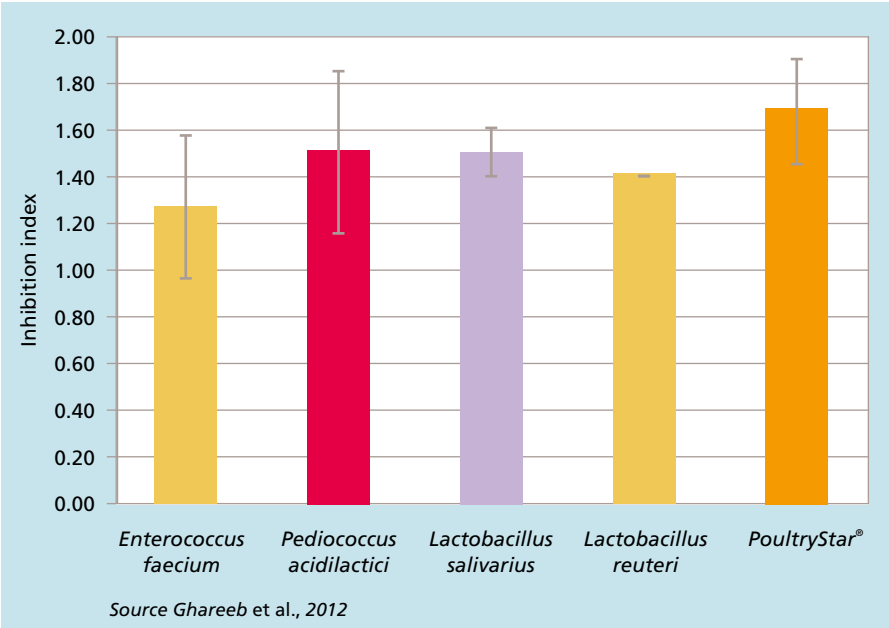
In the last 20 years, *Campylobacter* has emerged as the most commonly reported cause of bacterial gastroenteritis in humans worldwide. Affected humans show clinical signs of acute diarrhea or more severe complications including Guillain-Barré syndrome and arthritis. The cost of campylobacteriosis to 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.

Poultry 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 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].



Source Ghareeb et al., *2012*

Wael Abdelrahman
Technical Consultant, Poultry Probiotics

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.

Table 1. Experiment 1, day 8 & 15. *Campylobacter* content in cecum (log cfu/g) after challenging with 10^5 cfu/ml of a field strain of *Campylobacter jejuni* at day one.

	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	6.67[a]	4.10[b]	>8[a]	3.82[b]

[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 10^5 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 10^5 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 PoultryStar® 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* (PoultryStar®, 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 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.

Table 2a. Experiment 2, day 8. *Campylobacter* content in the cecum, log cfu/g, after challenging with 10^5 cfu/ml of a field strain of *Campylobacter jejuni* at day one.

Bird (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	7.81[a]	<2[b]	<2[b]

Table 2b. Experiment 2, day 15. *Campylobacter* content in the cecum, log cfu/g, after challenging with 10^5 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	7.85[a]	<2[b]	<2[b]

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

Source: Ghareeb et al., *2012*

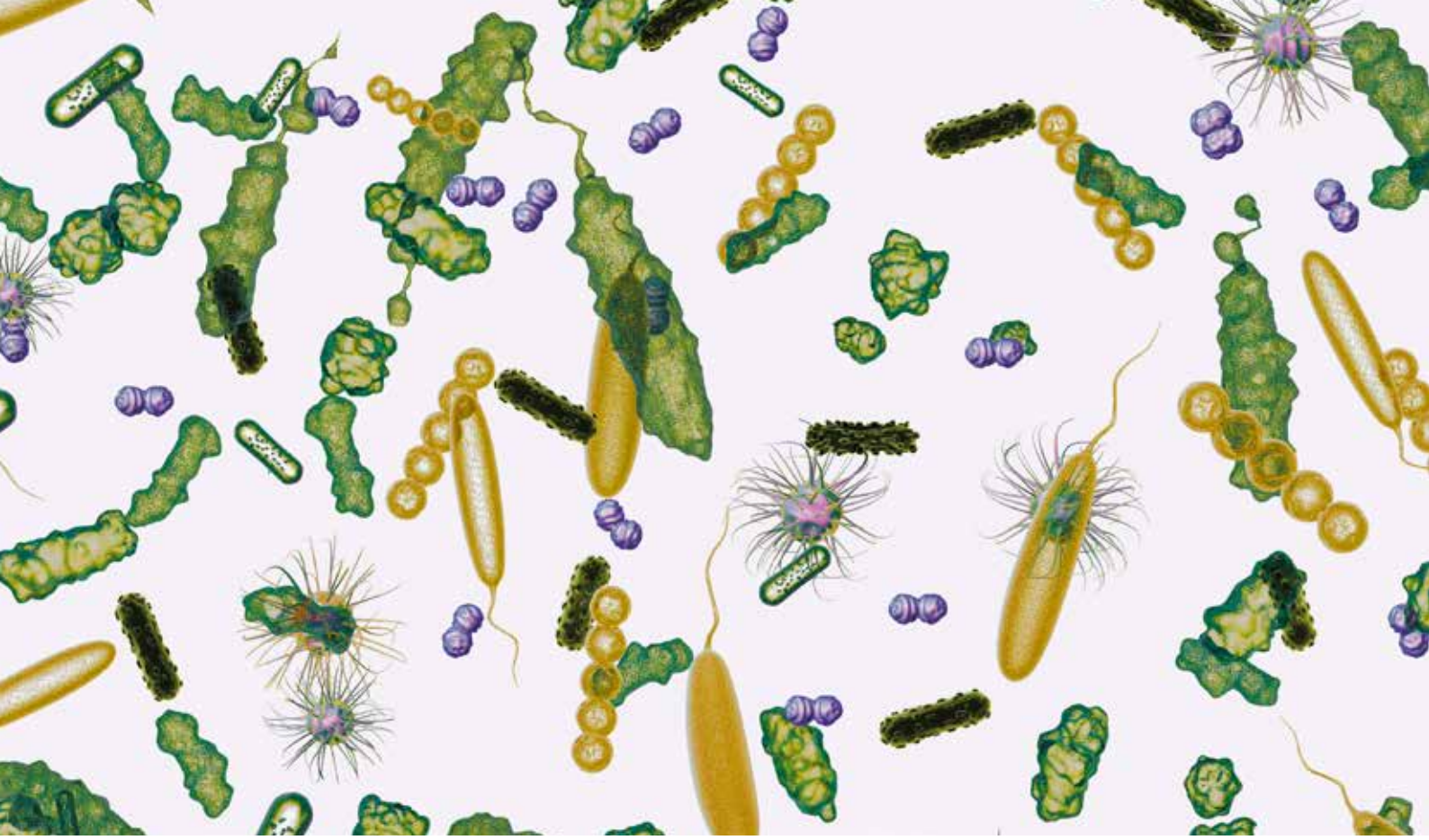
At day 8: Ten of 12 birds in the PoultryStar® group had *Campylobacter* counts that were <3 log cfu/g, which was significantly lower than the mean log count in the positive control group, 6.67 log cfu/g (*P*=0.001).

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*.

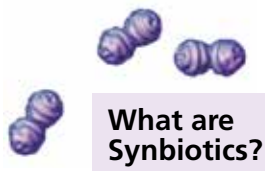
At day 8 & 15: *Campylobacter* counts in the cecal content of the PoultryStar® group were <2 log cfu/g, whereas the mean log counts in the positive control group were 7.81 log cfu/g at day 8 and 7.85 log cfu/g at day 15 (*P*=0.001) respectively (*Tables 2a* and *2b*).

Compared with the controls, the PoultryStar® 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.



Adjusting intestinal microflora with synbiotics

Intestinal microflora play an important role in bird health. In some instances, an imbalance between these beneficial microflora can negatively impact the birds´ health. For this reason, dietary supplementation with probiotics is needed to ensure the propagation of favorable microflora.



What are Synbiotics?

Synergistic combinations of probiotic and prebiotic nutritional supplements.

The formation of microbial microflora occurs in the first days of life. From four days of age, there is a significant increase in bacterial count. Bacterial growth stabilizes from the second week of life. Major challenges arising from the environment may cause unstable microflora.

The sub-therapeutic use of antibiotics as growth promoters is a public health concern because of the transfer of antibiotic-resistant microorganisms, many of which can normally be found in birds' feces. Bacteria expend large amounts of energy to maintain their resistance against antibiotics. Removing or replacing antibiotics with another drug is a common practice in the poultry industry that only exacerbates the problem, leading to the emergence of bacteria that are resistant to several drugs simultaneously.

Intestinal dysbiosis

Some species of *Escherichia coli, Clostridium, Staphylococcus, Blastomyces, Pseudomonas* and *Salmonella* are undesirable flora. Dysbiosis is the imbalance of the intestinal microflora with changes in the population of microorganisms and occurs in many conditions such as prolonged water deprivation or feed fasting, stress and infections (caused by viruses, bacteria, fungi and protozoa), causing an imbalance of flora with a proliferation of undesirable microorganisms.

Under dysbiosis, the undesirable microbial population acts in the gastrointestinal tract (GIT) to reduce the absorption of nutrients, and increase mucosal thickness and the rate of passage of feed. This interferes with the nutritional needs of the host, and increases the turnover of enterocytes, while reducing villous height and crypt depth.

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Technical Manager, Poultry

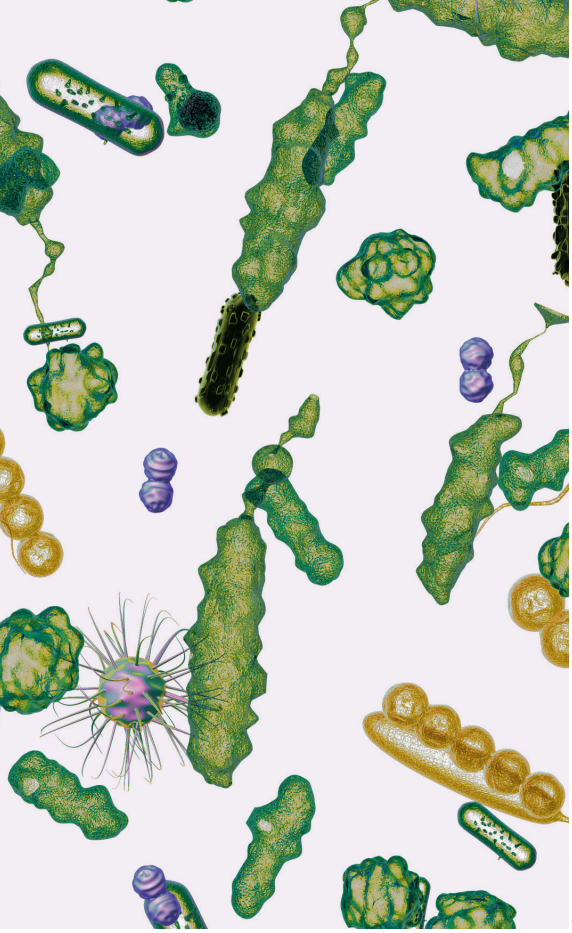


Photo: EKS/Shutterstock

Competitive exclusion

In the intestinal lumen, the microbial population competes with the host for nutrients such as hexoses, amino acids, fatty acids, vitamins and other nutrients that result from the digestion process. This imbalance caused by dysbiosis produces biogenic amines (cadaverine, histamine, putrescine), ammonia and gases, which are highly damaging to mucosal integrity and intestinal health.

The dominance and persistence of desirable flora can be established when microorganisms fix themselves in the intestinal epithelium and multiply faster than their elimination by intestinal peristalsis, as in the case of *Lactobacillus* and *Enterococcus*. Some of these desirable flora may also be found freely in the intestinal lumen even without attaching to the intestinal mucosa.

Integrity of the intestinal tract

The main defense mechanisms against infections caused by enteropathogenic microorganisms are: an intact intestinal mucosa that creates a real barrier; an efficient immune system; and a healthy probiotic population that adhere to the intestinal epithelium, thereby preventing colonization by pathogens.

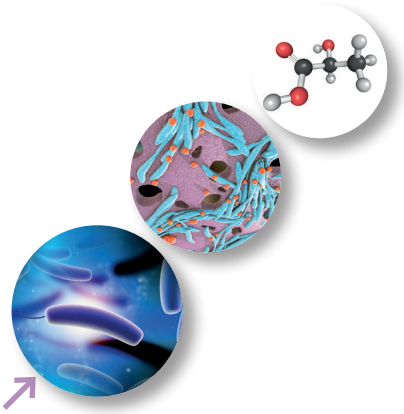
One of the most common mechanisms of digestive tract damage by microorganisms is that which occurs in a specific interaction or fixation between the bacteria and the epithelial cells of the intestinal wall. This mechanism is characteristic of Gram-negative bacterias (eg. *Salmonella*) which have surface structures known as fimbriae (pili). These structures support the connection between the lectins present in their surface and the receptor in the epithelium (*Figure 1*).

The ability of many microorganisms to adhere to the intestinal epithelium is essential for their permanence and development. In this way, they avoid being removed by peristalsis. One method to prevent pathogens from colonizing the intestine is to saturate the epithelial receptors sites, an action most probiotics perform.

Different bacteria have different mechanisms of adhesion; *lactobacilli*, for example, has its adherence controlled by glycocalyx and proteins of its cell wall. Probiotics are microorganisms able to multiply and adapt quickly to the intestines of most animals and capable of preventing unwanted bacteria from attaching themselves in the GIT.

Why protect the GIT?

Sometimes the delicate balance between the microorganisms in the GIT of day-old chicks do not provide the necessary protection against undesirable pathogens. There is a need for a defense strategy that allows a symbiotic relationship be-



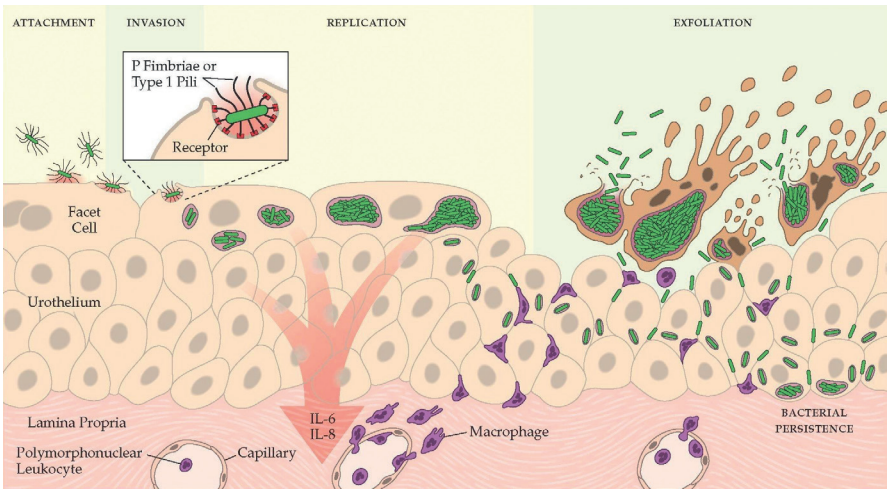
Approximately 90% of intestinal microflora is comprised of facultative anaerobic bacteria that produce lactic acid (*Bacillus, Bifidobacterium, Lactobacillus*) and strict anaerobic bacteria (*Bacteroides, Fusobacterium, Eubacterium*).

The remaining 10% consist of *E. coli, Proteus, Clostridium, Staphylococcus, Blastomyces,* and *Pseudomonas* among others.

Any change in this bacterial composition leads to dysbiosis, enteritis and subsequently poor performance in animals.

Photos: MichaelTaylor3d, dream designs, Iculig

Figure 1. Gram-negative bacteria (e.g. *E. coli*) attach to epithelial cells with P fimbriae or type 1 pili. This facilitates the subsequent invasion, replication and exfoliation of host cells.



Filipe Ribeiro
Technical Manager, Poultry

Wet litter caused by dysbiosis

Diarrhea indicates loss of intestinal integrity. *Clostridium, Salmonella* and *E. coli*, among other pathogens, may be present in the litter.

Probiotics adminstration repopulates the GIT with beneficial bacteria, which curbs the action of pathogens and controls their population.

tween the host and microorganisms with beneficial effects for both.

Newly hatched industrial chicks do not come into contact with mother hens and are placed in a clean, sanitized environment with little opportunity for rapid development of a protective intestinal microflora that can successfully compete with pathogens. The first days of life are a critical period of time with a high risk of infection by pathogens such as *Clostridium, Salmonella* and *E. coli* that may be present in the litter. Thus, probiotic supplementation is a beneficial measure.

Under favorable conditions and in the absence of eutrophic flora, these harmful microorganisms multiply rapidly in the GIT, negatively impacting the birds' health.

Probiotics adminstration repopulates the GIT with beneficial bacteria, which curbs the action of pathogens and controls their population. This is especially useful after stressful events such as drastic changes in the diet, fasting, erratic temperatures, or after exposure to aggressors such as enteritis of bacterial or viral origin in the microbiota, and mycotoxin contamination in feed.

Probiotic and prebiotic interaction

The symbiotic action stabilizes the intestinal environment and increases the number of beneficial bacteria producing lactic acid, favoring eubiosis. As probiotic bacteria and prebiotics are administered, eubiosis and intestinal health are established, preventing the proliferation of pathogens.

Synbiotics have the ability to modulate the immune response, increasing both the number and activity of phagocytic host cells. This action assumes great importance in birds, where the intestinal tract is the organ most responsible for the development of general non-specific immunity.

The birds do not have lymphnodes, and lymphoid organs are scattered along the intestinal tract, represented by Peyer's patches, cecal tonsils and bursa of Fabricius. These lymphoid tissues are sensitive to antigens present in the GIT such as probiotics.

A new and safe alternative

The quality of poultry meat and production of foods of animal origin are evaluated by ever more rigid controls. Food safety standards dictate the need for increasing levels of integration between feed and food production technologies. The combined use of probiotics and prebiotics leads to an innovative product that is natural, stabilizes the gut flora, and enhances animal health and zootechnical performance.

The innovative BIOMIN product PoultryStar® was designed to improve gut health and increase birds' resistance against pathogenic infections. With the development of this synbiotic product line which combines the beneficial effects of probiotics and prebiotics on the GIT, the industry now has alternatives found in natural feed additives that can improve gut health, well-being and the performance of animals.

The mode of action of this product line was investigated by looking at the effect on the histomorphological structure of the GIT and the microflora of chickens in the course of several feeding trials (see page 4). Results from *in vivo* experiments showed that the addition of PoultryStar® to broiler diets had a positive effect on gut morphology, microflora composition, nutrient digestibility and volatile fatty acid pattern, which could be clearly connected to better zootechnical performance.

References are available on request.

Did you know...

...that poultry lameness is linked to pathogens in the gut?

Learn more about the science behind lameness and the solution in this article!

What's wrong with my birds? Part 1: Oral lesions

Cut & Keep Checklist

Science & Solutions presents a handy checklist for diagnosing poultry mycotoxicosis. Cut this out and take it along with you to the farm!

Diagnosing common poultry ailments correctly and precisely can be a challenge even for experienced vets, nutritionists or farm managers. Differential diagnosis is especially difficult in the case of mycotoxin-related problems as symptoms vary greatly and may be further complicated by the synergistic effects caused by the co-occurrence of more than one type of mycotoxin in the feed.

	Potential cause	Description of problem	Check list	Corrective actions
MYCOTOXINS	T-2 toxin (T-2) Diacetoxy-scirpenol (DAS)	T-2 and DAS have a dermatotoxic action, thus causing lesions to the epithelium, increasing the speed of epithelial cell renovation.	☐ Positive for T-2 and/or DAS in raw materials (ELISA) or feed (HPLC) ☐ Origin of raw materials from supplier/ region with history of T-2/DAS contamination ☐ Histopathology: Proliferating epithelial cells and hepatic vacuolization ☐ Overall decrease in flock performance	☐ Check average contamination levels ☐ Use Mycofix® at a correct dosage level ☐ Avoid feed bins or feed/water lines that have become contaminated by stale, wet or moldy feed
NUTRITION	Feed granulometry	Small particles of feed block saliva ducts, which may result in oral lesions.	☐ Pelletized feed: Fine particles >20% ☐ Mashed feed: Check mean particle diameter ☐ Histopathology: Presence of inflammatory cells and bacteria ☐ No overall decline in flock performance	☐ Adjust the pelleting process ☐ Increase the sieve diameter ☐ Use pellet binders to improve pellet quality
MANAGEMENT	Liquid methionine	Methionine dripping in the application system produces points of high methionine concentration in the feed.	☐ Methionine injector dripping inside masher ☐ Histopathology: Infiltration of inflammatory cells and necrotic lesions ☐ No overall decline in flock performance	☐ Clean/replace methionine injectors
	Organic acids	Excessively high concentrations of organic acids in the feed lead to caustic lesions in the oral mucosa.	☐ Acids injector dripping inside masher ☐ Histopathology: Infiltration of inflammatory cells and necrotic lesions ☐ No overall decline in flock performance	☐ Clean/replace acid injectors ☐ Adjust dosage of organic acids
	High temperatures	More frequent drinking during hot periods increases feed residues in beaks.	☐ Histopathology: Infiltration of inflammatory cells and necrotic lesions ☐ Possible decline in flock performance ☐ Increased mortality	☐ Apply vitamins in water ☐ Apply organic acids in water ☐ Increase chlorine level in water
	Copper sulphate	Concentrations between 0.05 to 0.2% in feed and drinking water can promote oral lesions.	☐ Check concentration of $CuSO_4$ in premix ☐ Check concentration of $CuSO_4$ in water ☐ Check if water dosing system is working correctly (if applicable)	☐ Apply group B vitamins and K_3 vitamin in water ☐ Correct set-up of the water dosing system
PATHOGENS	Candida albicans (Candidiasis)	The yeast *C. albicans* can lead to lesions in the crop that can extend to other parts, including the mouth. More common in birds with longer lifespans, such as layers and breeders.	☐ Histopathology: Fungal hyphae present in affected mucosa	☐ Nystatin or diflucan or imidazoles such as ketoconazole, fluconazole, etc. as treatment
	Fowl pox (Avian pox)	Viral disease caused by Poxviridae (Avipoxvirus) often leads to cutaneous lesions on head, neck, legs and feet. • Dry pox: Raised, wart-like lesions on feathered areas (head, legs, vent, etc.) which heal in about 2 weeks. • Wet pox: Canker-like lesions in the mouth, pharynx, larynx, and trachea.	☐ Flock history and presence of typical lesions ☐ Laboratory diagnosis by tissue or transmission studies	☐ Use preventive vaccination depending on prevalence and season (typically fall) ☐ Treat affected birds with antibiotics to reduce secondary infection, although the disease has to run its course
	Protozoans	Protozoans are more prevalent in birds with a longer lifespan, such as layers, breeders and turkeys, game birds and/ or free-range birds.	☐ Histopathology: Microscopic examination of a smear of mucus or fluid from the throat demonstrates the presence of trichomonads	☐ Separate chronically infected birds from breeding birds
	Trichomonas gallinae	First lesions appear as small, yellowish areas on the oral mucosa.	☐ Cankers, also known as "yellow buttons" — yellow, rounded areas with central caseous necrotic foci	☐ Use nitroimidazoles (not approved in US by FDA and prohibited in the EU)
	Histomonas meleagridis	Also known as histomoniasis or blackhead disease. Common in commercial turkeys and chickens.	☐ Cecal inflammation, ulceration, thickening of wall, ceca containing yellowish cheese-like exudate	☐ Use nitroimidazoles (not approved in US by FDA and prohibited in the EU)

For more information, visit **www.mycotoxins.info**

*DISCLAIMER: This table contains general advice on poultry-related matters which, most commonly affect poultry and may be related to the presence of mycotoxins in feed. Poultry diseases and problems include, but are not confined to the ones present in the table. BIOMIN accepts no responsibility or liability whatsoever arising from or in any way connected with the use of this table or its content. Before acting on the basis of the contents of this table, advice should be obtained directly from your veterinarian.

PoultryStar®

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