The use of probiotic bacteria to stimulate gut health in turkeys

The genetic potential of poults can only be realised if we ensure a proper 'start' during their early development. The quality of the care received by the poults during the brooding period heavily influences their health status throughout the production cycle.

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Additionally, it directly determines whether the optimal production performance is achieved after the cycle is finished.

Early colonisation of the gastrointestinal tract

Within the large variety of probiotic bacteria used in poultry farming, three groups in particular have become widely used: Bacillus, Enterococcus, and Lactobacillus.

Comparative studies have shown that these specific groups of bacteria bring the most physiologically beneficial properties to the avian digestive system and significantly improve the performance of the birds.

Bacterial species of the Bacillus genus belong to the so-called non-colonising intestinal flora, therefore, their activity is limited to their passage through and presence in the intestinal lumen.

A particular characteristic unique to these bacteria is their ability to produce polysaccharide-peptide capsules (spores) that protect bacteria against harmful environmental factors.

This resistance has been exploited, for example in the manufacture of complete feed mixtures (supplemented with probiotic bacteria) due to the high temperature of the technological process (>75°C).

Enterococcus and lactobacillus strains, in contrast, possess the ability to colonise the intestinal mucosa of birds.

This property enables prolonged presence in the intestinal lumen, allows the bacteria to proliferate, and confers other host-beneficial traits. As such, these micro-organisms are particularly suitable for young poults.

There is a current tendency to promote healthy intestinal tract function from the very initial stages of poult rearing. Exposing young poults to a variety of probiotic bacterial strains at an early point in time undoubtedly constitutes a part of this trend, as part of the general practice of competitive exclusion of pathogenic bacteria.

In essence, this competitive exclusion theory calls for colonising the gastrointestinal tract with favourable intestinal flora that adheres to the intestinal mucosa (the colonisation should be performed as early as possible), thus preventing pathogenic bacteria from colonising the mucous membrane.

Table 1. Enzyme activity of selected strains of probiotic bacteria (✓ enzyme produced, ✗ enzyme not produced).

Enzyme	Lactobacillus spp.	Enterococcus spp.	Bacillus amyloliquefaciens
Lipase	✓	✓	✓
Peptidases (leu., cys., val.)	✓	✓	✓
Trypsin	✗	✓	✗
α-chymotrypsin	✗	✓	✓
Alkaline phosphatase	✓	✓	✓
Acid phosphatase	✓	✓	✓
α-galactosidase	✓	✗	✓
B-galactosidase	✓	✓	✓

Group		Body weight (kg)	Villi length (μm)	Crypt depth (μm)
K2 enclosure	Control	0.996	993.402	154.8964
K2 hall	Experimental	1.124	1016.369	148.3265
K1 enclosure	Control	0.871	1012.129	166.4026
K1 hall	Experimental	1.074	1046.055	160.809

Table 2. Bird body weight and morphometric examination of the jejunum at the end of the rearing period (n = 5, measurements were taken in at least 11 places).

In addition, probiotic bacteria have the ability to produce bacteriocins – natural substances that inhibit the growth of competitive bacteria within a homologous or closely related group.

Examples include eenterocins produced by Enterococcus faecium bacteria, acidocin A and B produced by Lactobacillus acidophilus, and barnases from Bacillus amyloliquefaciens.

The primary antibacterial mechanisms of action of these probiotic strain metabolites are: destruction of the bacterial cell wall, disruption of membrane potential, inhibition of DNA and RNA synthesis, ion leakage, blocking cell membrane transport channels, limitation of ATP production, and disruption of cell wall synthesis.

Owing to this large diversity in action mechanisms, most bacteriocins have a bactericidal effect on susceptible organisms and reduce the bacterial loads shortly after direct contact occurs.

Beneficial intestinal microflora also competes with enteropathogenic bacteria for access and utilisation of nutrients necessary for growth.

The microflora synthesises volatile fatty acids (acetic acid, butyric acid) through fermentation. These acids not only directly damage the cell walls of pathogenic bacteria, but also regulate the pH of the intestinal environment, the latter of which indirectly inhibits the exponential growth of pathogenic bacteria during log phase.

The protective effect on the intestinal tract is conferred primarily through the generation of a biofilm – a multi-layer structure formed by probiotic bacteria that closely adhere to each other.

The biofilm, which is formed on the intestinal mucosal surface, protects the enterocytes from the damaging effects of pathogens and the toxins produced by pathogen metabolism.

Stimulation of the immune system in poults

When analysing the properties of probiotic bacteria through the lens of their importance in the early brooding period, it is crucial to include their role in the stimulation of gut-associated lymphoid tissue (GALT).

The GALT is comprised of the caecal tonsils, Meckel's diverticulum, Peyer's patches, the bursa of Fabricius, and the lymphoid nodules of the intestinal wall.

The feed and the microbiome entering the digestive tract lumen are the very first source of antigens that GALT comes into contact with during the first few days after hatching.

It is hypothesised that the released bacterial antigens (lipopolysaccharides, peptidoglycans) activate the animal's original innate immunity and induce heterophile antibody secretion in combination with a maternal antibody (mIgA) response and a subsequent supplementary mucosal reaction. This process is conditioned by the immature state of the mucosal immune system within the first week after hatching, thus antigen recognition is assisted by passively acquired antibodies. Auto-synthesis of immunoglobulins (IgA) begins after six days from hatch.

Support of nutrient digestion

Maintaining the proper dynamics of digestive enzymes secretion is another important process in young turkeys.

Enzymes produced endogenously by the gastrointestinal tract and related organs may not be sufficient to ensure proper digestion of all nutrients entering the tract.

For example, the peak amylase secretion has been recorded as early as 10 days after hatching, indicating early enzymatic activity in the gastrointestinal tract of poults and therefore high digestibility of polysaccharides.

This stands in contrast with the dynamics of trypsin, chymotrypsin and lipase secretion. The secretion of these enzymes was found to be greatly diminished in turkey poults within 14 days from hatch.

Probiotic bacteria have the capacity to produce a wide range of enzymes that accelerate metabolic processes in birds during the first weeks of life (Table 1).

Induction of small intestinal microstructure development

Early colonisation of the small intestinal mucosa with beneficial bacteria also promotes the development and proper functioning of the intestine. Among other benefits, early application of probiotics increases the absorbing surface area of the duodenum, as mature enterocytes are stimulated to migrate towards the tip of the villi.

A number of studies have reported the beneficial effects of probiotics, such as their role in increasing the length and number of intestinal villi. This process may result from the said properties of the bacteria – for example, their capacity to induce the formation of short-chain organic acids.

Acids generated within the intestinal lumen reduce the pH of the intestinal contents, which inhibits the growth of the (predominantly alkalophilic) pathogenic bacteria.

The organic acids produced via fermentation processes also stimulate the proliferation of external epithelial cells. Additionally, probiotic bacteria reduce the amount of pathogenic bacteria by competing for adherence to the intestinal epithelium, thus protecting the intestines from the development of local lesions and inflammation.

Pathogenic bacteria reduction in the intestinal lumen leads to increased availability of nutrients that can be fully utilised to fuel proper growth and regeneration of intestinal morphological structures.

Morphometric tests usually include the following measurements: length and width of intestinal villi, depth of intestinal crypts, villi length: crypt depth ratio, and thickness of intestinal muscular layer.

- Intestinal villi length

Interpreting villi height is difficult, as the length of the villi is usually determined by a multitude of factors, such as: the type and composition of the feed, the presence of antinutritional factors in feed ingredients, and exposure of poults to residual disinfectants and/or infectious agents.

Production technology and genetic factors also play a role.

It should be noted that the villi exhibit an anatomical pattern: the longest villi are located in the duodenum, the shorter villi are located in the jejunum, and the shortest – in the ileum.

In the course of intestinal infections, both bacterial and viral, the height of the villi is significantly reduced and the outer brush border typically suffers damage.

Table 3. Body weight of birds at the end of the rearing period (in kg) and summary of production parameters at the end of the production cycle (Test No. 2).

Parameter	Experimental	Control
Body weight at the end of the rearing period (4 weeks)	1.36	1.21
Body weight at week 15	10.50	10.21
Final body weight	10.79	10.51
FCR	2.28	2.3
Mortality (%)	4.4	4.81

	Villi length (μm)	Crypt depth (μm)
Experimental	1182.438	190.7435
Control	1122.083	182.6812

Table 4. Morphometric examination of the jejunum at day 27 of the production cycle (n = 5, measurements were taken in at least six places).

It is generally assumed that increased villi height correlates with better digestion and nutrient absorption.

- Intestinal villi width

Increased thickness of the intestinal villi, especially in the duodenal area, occurs as a response to irritants. If the irritant is a virus, the histopathological image will also show pancreatic vacuoles.

- Intestinal crypt depth

The size of the crypts of Lieberkühn is also a function of the gastrointestinal health status and the tract's exposure to infectious agents, damaging agents, and/or irritants.

Crypt deepening indicates the occurrence of one of the aforementioned factors as an interrelated phenomenon.

- Thickness of the small intestinal muscular layer

The smooth muscle layer that surrounds the connective tissue of the submucosa is the thickest in the duodenum and becomes gradually thinner towards the lateral sections. All layers should increase in thickness with age, as the intestines develop.

Selected strains of probiotic bacteria were evaluated in the present study in terms of their impact on the histopathological structure of the small intestine.

Probiotics were used in turkey farms where episodes of intestinal viral infections (astrovirus, rotavirus, coronavirus) were recorded during previous production cycles.

Due to recurrent infections, the flocks were placed under serological monitoring and micro-/macroscopic gut health investigation from hatching onward.

Concurrently, material for morphometric analysis was collected in the form of small intestine tissue samples (specifically from the duodenum, jejunum and ileum).

Organs were extracted from a representative group of poults (5-10 control and experimental subjects) within no more than 10 minutes after euthanasia (performed by cervical dislocation).

Intestinal samples were collected at various points in the production cycle, as per the experiment design. The material was stored in a 10% formaldehyde solution and subsequently forwarded for histopathological examination.

A mixture of probiotic bacteria strains (the Enterocid Grower formula – Bacillus amyloliquefaciens $1x10^{10}$ cfu/g, Enterococcus faecium $1x10^{10}$cfu/g) was administered to poults from the experimental groups via drinking water. Poults from the control group were concurrently given regular water.

Test No. 1

Test No. 1 consisted of male turkeys. Line: BIG 6. Experimental group K1 – 11,600 birds, and K2 – 10,480 birds, control group – 30 birds housed in two enclosures ($3m^2$) set up in each barn.

- Dosage: in week 1: 100g/1000 litres of water per day continuously, afterwards, from week two until the end of the rearing period: 100g/1000 litres in half of the daily water ration, four days per each week.

Test No. 2

Test No. 2 consisted of female turkeys. Line: BIG 6.

- Rearing – two enclosures (one control and one experimental) for 30 birds each were set up in rearing house number one.
- Dosage: from seventh day of life onwards – 400g/1000 litres of water, in half of the daily water ration.
- Fattening – experimental barn (10,220 birds), control barn (11,000 birds).
- Dosage: weeks 5-9: 200g/1000 litres of water, in half of the daily water ration, four days per each week, from week 10 onwards – 200g/1000 litres of water, in half of the daily water ration, three days per each week.

The study has shown that administration of probiotic bacteria to poults throughout the early brooding period promotes intestinal villi growth, reduces excessive deepening of intestinal crypts, and stimulates smooth muscle development (Table 4).

Longer villi length has a positive impact on the digestive function and may also be an indicator of decreased gut structure susceptibility to pathogens and irritants.

The beneficial effect of the probiotic strains is further indicated by the increased weight of the experimental poults and their improved health status (Table 3). ■