

The Effect of Various Dosages of Cellobacterin-T on Intestinal Microflora in Brown Nick Laying Hens

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Abstract

The results of using Cellobacterin®-T feed additive in the diets of Brown Nick laying hens have been considered. The dosage of Cellobacterin®-T feed additive equal to 1 kg/t of feed has been proven to be the most effective; it helped increase the useful intestinal microflora and reduce the circulation of bacterial pathogens, thus ensuring the favorable epizootological state of a farm.

Keywords: Laying hens, microflora, intestines, feed additive, diet, microorganisms, cross.

Introduction

Classical microbiological methods combined with modern molecular genetic methods have allowed finding important regularities. Decreased number of bacterial pathogens in internal organs has been detected, as well as changes in the ratio of microorganisms in the caeca: the number of lactobacilli increased, and the number of pathogenic bacteria decreased. The egg

productivity of the Brown Nick hens is increased. The use of antibiotics is not always appropriate, and may not have a positive effect, while the introduction of the feed additive in the diet has a positive effect on poultry health. Feeding was always an important factor in animals' life (Privalo, et al., 2018; Ilona, et al., 2018; Osman, et al., 2019). Modern feed additives can increase productivity and help obtain environmentally friendly products of poultry farming (Yildyrym et al., 2019; Chadfield et al., 2004; Chervyakov et al., 2015; Chervyakov et al., 2015; Abd El-Tawaab, et al., 2019). This study aimed to investigate the effect of Cellobacterin®-T feed additive in the diet of Brown Nick laying hens on the content of microorganisms in the intestinal caeca.

Methods

During the study, the effect of Cellobacterin®-T feed additive in the diets of Brown Nick laying hens on the circulation of bacterial pathogens was studied. For this purpose, an experiment was performed using the Cellobacterin®-T feed additive in the diets of Brown Nick laying hens at the production facilities of the Avangard LLC poultry farm in the Ruzayevka district of the Republic of Mordovia. These studies were performed from October 2018 to February 2019. Two groups were formed: the reference group — without the use of feed additive, and the experimental group that received the feed additive for three months at the dosage recommended by the manufacturer (1 kg/t of the feed). Cellobacterin®-T feed additive manufactured by BIOTROF LLC. At least 50 clinically healthy hens were selected from each group for bacteriological studies.

The organs of the killed hens (liver, lungs, heart, spleen, contents of the head cavities, brain, joints, trachea, ovary, and oviduct) were analyzed for the presence of bacteria from the *Enterobacteriaceae* family, including *Salmonella* spp., *Enterococcus*, and *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Pasteurella*, *Avibacterium*, *Ornitobacterium*, *Mycoplasma*, and other genera. Pathogenic microorganisms were isolated by the method of "direct" inoculation of the test material on nutrient media, as well as by preliminary enrichment of the samples in nonselective media. To detect isolated bacteria, a Lachema micro-test system (Czech Republic), and various diagnostic sera were used. Microorganisms were identified using a mass spectrometry with the use of Bruker MALDI-TOF mass spectrometer and Bruker Taxonomy automated program. T-RFLP analysis is a molecular genetic method based on DNA identification, amplification, and sequencing, followed by comparing the T-RFLP profiles obtained

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with databases. This method allows to rapid and efficient identification of various species of microorganisms, including species that are not grown in special nutrient media and require specific cultivation conditions. Data were statistically processed in RStudio application (Prytkov and Kistina, 2018; Kistina, 2015).

Results

Classical microbiology methods and modern molecular genetic methods allow finding important regularities.

As a result of the studies, 11 isolates of opportunistic microorganisms were identified. Detected pathogens most frequently included microorganisms of the following taxa: staphylococci, enterococci, and, to some extent, streptococci.

After more accurate species differentiation, the most frequently isolated microorganisms were bacteria of the following species: *Staphylococcus equorum* (34 %), while the following species were found only in the reference group: *Staphylococcus gallinarum* (4 %), *Enterococcus cecorum* (13 %), *Avibacterium endocarditidis* (8 %), and *Streptococcus pluranimalium* (2 %).

Poultry staphylococcosis is an infection caused by several types of staphylococci, mainly *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus equorum*, and *Staphylococcus pasteuri*. In most cases, *Streptococcus spp.* and *Staphylococcus spp.* do not cause disease in the upper respiratory tract. However, some isolates may cause it. Staphylococci infection is characterized by local outbreaks in laying hens, in poultry of parent herd, and in broilers older than 30 days. Clinical manifestations are associated with symptoms of general disease, such as weight loss and food refusal, local skin lesions, arthritis, osteomyelitis, and, in severe cases, endocarditis (as a "mixed" infection with other pathogens), pneumonia, and septicemia. Staphylococci often cause infection in chickens in the first days of life, which is characterized by septicemia, and is followed by complications of joints disorders. The effectiveness of antibiotic therapy depends largely on the immunity of the poultry. In the background of stress and immunosuppression, it is usually ineffective (Ritchie et al., 1994; Hermans et al., 2000). Enterococci are considered normal flora, but some species may cause infectious processes in poultry organisms. This mainly refers to *Enterococcus hirae*, *Enterococcus cecorum*, *Enterococcus gallinarum*, and *Enterococcus faecalis*. The infection is pantropic, with a characteristic localization at the points that are hardly accessible for the immunocompetent cells (heart valves, joints, brain). Clinical manifestations include brain injury in chickens in the first days of life, joints pathology in broilers starting at 3–4 weeks, and poultry in parent stock, endocarditis, and lesions of respiratory system.

Intestinal infections and other factors that damage the intestinal villi epithelium may facilitate the ingress of pathogenic strains of streptococci and enterococci that result in potential sepsis and endocarditis (Prytkov et al., 2015). By classification, streptococci and enterococci are closely related, and the diseases caused by them are often considered together. Clinically significant species

of streptococci include *Streptococcus pluranimalium*, *Streptococcus gallinaceus*, and *Streptococcus gallolyticus*. These pathogens are characterized by the predominance of disseminated infection, that involves the respiratory tract and cardiovascular system. Clinical manifestations of infection include pneumonia, endocarditis, rhinitis, and tracheitis; in severe cases, they include septicemia. *Streptococcus pluranimalium* is a new avian pathogen associated with sepsis or with endocarditis and sepsis in adult broiler parents. Preventive measures should be taken to ensure the hygienic status of hatching eggs, the hatching, and litter quality (Hedegaard et al., 2009). *Avibacterium endocarditidis* is a pathogen of "valvular endocarditis" in poultry; it was isolated from chicken with valvular endocarditis in Denmark in 2004. Experimental models proved the pathogenicity of this culture. Its clinical manifestations include sinusitis (often asymptomatic), tracheitis, endocarditis, and liver and spleen damage; in severe cases, they include sepsis and often arthritis. The pathogens of valvular endocarditis may also be *Enterococcus faecalis*, *Staphylococcus aureus*, or *Streptococcus pluranimalium*. With this pathology, some increased mortality is observed in broiler chickens during the last weeks of production (Bisgaard et al., 2010).

In the experimental group, a decrease in the number of pathogenic microorganisms was veraciously observed in the liver, lungs, heart, spleen, cavities in the head, trachea, and brain ($p < 0.05$, Fisher's exact test). The reduction in the number of bacterial pathogens in the internal organs (liver, lungs, heart, and spleen) in the experimental group was 19 %, while in the cavities in the head, trachea, and brain this value was 35 %.

Since there is no specific medicines for the treatment of staphylococcosis, enterococcosis, and streptococcosis, and there is no specific preventive vaccination, antibiotic therapy is recommended after careful determination of antibiotics resistance; however, in severe cases, it may not yield the desired effect.

The walls of a healthy intestine have barrier properties for pathogens. The mucus layer that covers the intestine epithelium contributes to the promotion of chyme and prevents attachment of pathogens. Coarse feed particles, due to their abrasive properties, cause the loss of the mucus layer and damage to the intestinal epithelium, thereby contributing to the penetration of pathogens into the organism.

Bacteria of the genus *Bacillus* in the feed additive feature the multienzyme activity and synthesize the enzymes that contribute to the digestion of hardly digestible components of the feed and strengthen the intestinal walls. To evaluate the effect of Cellobacterin®-T feed additive on the gastrointestinal tract of adult poultry, at the end of the experiment, the caeca contents were studied to determine the content of microorganisms using the T-RFLP analysis method (Terminal restriction fragment length polymorphism), which was performed by BIOTROF LLC. The following groups of microorganisms were found: beneficial microorganisms — cellulolytic bacteria, lactobacilli, bifidobacteria, bacteroids, bacilli, selenomonads; opportunistic microorganisms — enterobacteria, actinomycetes; pathogens of

various infectious diseases — staphylococci, peptococci, fusobacteria, campylobacteria, pasteurellas; and transient representatives of the microflora in the feed — pseudomonads. The main components method makes it possible to simplify and reduce the span of the initial space of indicators and visualize the initial multidimensional data. Bacilli and lactobacilli have the greatest share in the first main component, while bacteroids and cellulolytic bacteria have the greatest share in the second main component. Lactobacilli and a group of actinomycetes, pasteurellas, campylobacteria, peptococci, and pseudomonads are probably negatively related, as they change in different directions as the first major components grow. With bacteroids growth, the second main component grows, while the first main component remains almost unchanged (Prytkov and Kistina, 2016; Prytkov and Kistina, 2017; Kistina, 2017; Prytkov et al., 2016).

The hens that received the feed additive had higher content of the lactic acid bacteria than the reference hens, and lower content of the opportunistic bacteria.

Under the influence of feed additive, the number of lactobacilli increased, and the number of pathogenic representatives (peptococci, campylobacteria, pasteurellas, and actinomycetes) decreased.

Statistically veracious increase in the number of lactobacilli, and a decrease in the number of *Pasteurella* bacteria were observed in the experimental group. According to the available data, there was evidence of antagonistic activity of some strains of *Lactobacillus* sp. to bacterial pathogens; these strains could potentially restore the balance of intestinal microflora in poultry (Dec et al., 2014).

Cellobacterin®-T feed additive contributes to displacement the gastrointestinal microflora in poultry toward increasing the level of beneficial microorganisms and suppressing undesirable pathogenic microflora.

The analysis of the recommended parameters for crossing and comparison the group that received the feed additive for one month to the reference group revealed statistically significant differences between the groups in terms of egg productivity. In the group of hens that received the Cellobacterin®-T feed additive, in 73 % of cases, the egg production increased by 0.5 %, vs 18 % in the reference group (p-value < 0.05, Fisher's exact test).

Conclusion

Cellobacterin®-T feed additive contributes to normalize the microflora in the poultry gastrointestinal tract and reduces the circulation of bacterial pathogens, thus ensuring the epizootologically favorable state of a farm. The cumulative effect of its use is manifested in the increased productivity and the good health of the poultry. The experience reveals the feasibility of using the feed additive regularly. This study have proven the high effectiveness of the feed additive in egg farming.

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