MODULATION OF E-CADHERIN AND INTERFERON-ALPHA EXPRESSION IN THE DUODENUM OF BROILER CHICKENS EXPOSED TO SCFA-M

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Introduction. The application of organic acids has recognized as strategic approach to addressing animal health issues. Despite organic acids are active only in the upper parts of the digestive tract, hence their esterification with glycerin is necessary to manifest activity throughout the entire digestive tract. Being safe to use, monoglycerides do not have harmful effects on vital organs of birds. A significant advantage of the monoglyceride form is the pH-independent antimicrobial activity. The monoglyceride molecule remains lipid-active over a wide pH range, whereas free acids typically require a low pH to exert optimal antibacterial effect.

Markers of intestinal barrier function including E-cadherin and interferon-alpha (IFN-α) perform a range of vital functions in the mammalian intestine, including interferons which belong to the cytokine family produced by cells after viral infection, capable of suppressing virus growth and demonstrating strong antiviral properties. IFN-α, the first obtained using recombinant DNA technology, has great potential in regulating cell growth and differentiation as well as influencing immunological control and other processes. E-cadherin is a key molecule in intercellular adhesion of epithelial tissues, localized on the surface of epithelial cells at sites of intercellular contact known as adherens junctions. Intercellular connections via E-cadherin are primarily responsible for cell recognition as well as initiating and maintaining intercellular adhesion and polarization. The main protein of the junctions is E-cadherin, a transmembrane protein associated with the catenin family of cytoplasmic proteins. E-cadherin plays a crucial role through its interaction with β-catenin and the actin cytoskeleton.

Considering the above, a current direction of scientific research is establishing the influence of SCFA-M on the intestinal barrier function in broiler chickens, which will allow the development of new methods to enhance animal productivity and resistance with minimized antibiotic use.

The aim of this study was to investigate the dynamics of E-cadherin and interferon-alpha content in the duodenum of broiler chickens exposed to short chain fatty acids blended with its monoglycerides (SCFA-M).

Materials and Methods. The study was conducted from 2021 to 2024 at the Department of Animal Physiology, Biochemistry, and Laboratory Diagnostics of DDAEU. Laboratory investigations were carried out at the Scientific Research Center for Biosafety and Environmental Resource Control of the Agricultural Complex "Biosafety-Center" of DDAEU. The research was conducted on Cobb 500 broiler chickens in the conditions of an industrial poultry farm. For the study, two groups of day-old chicks were formed – experimental (55,000 chicks) and control (36,000 chicks), which were housed in separate identical poultry houses. Chicks in the experimental groups were raised according to the standard protocol of the enterprise. Simultaneously, the experimental group birds were administered a preparation of monoglycerides with fatty acids - C3, C4, C8-C10 (SCFA-M) in the drinking water from day 16 to 22, from day 24 to 29, and from day 31 to 36 at a dose of 0.5 liters per ton of water.

For the investigation of molecular markers of intestinal barrier function, 6 broiler chickens were selected from each group at 16, 22, 29, 36, and 45 days of age and euthanized. Tissues of the duodenum (3–5 cm) were collected from the chickens and stored frozen at -18 to -22°C for analysis. Western blot method by Towbin H., 1988, was used to investigate the expression of molecular markers. The results were expressed as percentages relative to the control group. Experimental

research was conducted in compliance with the requirements of the Law of Ukraine No. 3447-IV dated 21.02.06 "On Protection of Animals from Cruelty."

The obtained results were statistically processed with using Microsoft Excel data analysis package. The arithmetic mean (M) and its standard error (m) were calculated. The significance of differences between groups was determined by Student's criterion. Changes in indicators were considered significant at $p \leq 0.05$ (including $p \leq 0.01$ and $p \leq 0.001$). The correlation coefficient (r) was calculated using the Pearson method with the help of the applied software package "Microsoft Office Excel 2016".

Results. It was found that the content of IFN-α in the duodenum of 16-day-old broiler chickens in the control and experimental groups did not significantly differ. From day 16 to 45 of life, the expression of IFN-α in the duodenum of control group chickens remained relatively stable, with the regression equation being $Y = 0.072X + 101.9$; $p=0.628$. In contrast, in the experimental group chickens, the content of IFN-α in the duodenum increased by 78% by day 22 ($p\leq0.001$), gradually decreased thereafter, reaching a decrease of 26% by day 29 ($p\leq0.001$), 16.4% by day 36 ($p\leq0.05$), and further decreasing by 10.4% by day 45. It's noteworthy that the level of IFN-α expression in the duodenum of 22-, 29-, 36-, and 45-day-old chickens in the experimental group was significantly higher, by 71.6% ($p\leq0.001$), 47.8% ($p\leq0.001$), 29.7% ($p\leq0.001$), and 21.0% ($p\leq0.01$) respectively, compared to the control group chickens. The regression equation for the content of this marker in the duodenum of experimental group chickens was $Y = -0.032X + 138.7$ ($p=0.954$).

Throughout the experiment, modulation of E-cadherin expression in the duodenum of control group broiler chickens showed a tendency to decrease, as indicated by the descending regression line, with the regression equation being $Y = -0.1005X + 105.4$ ($p=0.47$). Administration of SCFA-M to the experimental group chickens had a significant impact on the expression of this membrane protein in the duodenum. Specifically, from day 16 to 22, the content of E-cadherin in the duodenum of experimental group chickens increased by 35.3% ($p\leq0.001$), continued to increase by 8.7% until day 29, and then gradually decreased by 21.0% by day 43 ($p\leq0.001$). A higher level of E-cadherin expression was observed in experimental group chickens throughout the entire study period. In the duodenum of 20-, 27-, 34-, and 43-day-old broiler chickens, the content of this protein was respectively higher by 30.4% ($p\leq0.001$), 38.8% ($p\leq0.001$), 32.6% ($p\leq0.001$), and 25.8% ($p\leq0.001$) compared to the control group chickens. The regression equation for the content of E-cadherin in the duodenum of experimental group chickens was $Y = 0.5612X + 112.8$ ($p=0.07$).

Conclusions. A significant protective of SCFA-M exposure on the intestinal health of broiler chickens confirmed in respect with molecular markers of both intercellular adhesion and innate immunity. There was established the modulation of E-cadherin and interferon-alpha contents in the duodenum while an increase in E-cadherin expression and a decrease in interferon-alpha expression were identified from day 22 to day 45 of life. Observed effect of SCFA-M exposure on molecular markers expression confirms the modulation the intestinal cell machinery to support the epithelial barrier integrity in broiler chickens. Detected balance in expression and compartmentalization of molecules which provide intercellular adhesion and regulate innate immunity could be assessed as prospective manner to characterize intestinal health. reactions through alternative anti-inflammatory signaling pathways. Further study of the intestinal barrier integrity in broiler chickens and its modulation with SCFA-M exposure required to minimize the risks in post-antibiotics era in industrial poultry farming.