
**CURRENT APPROACHES IN MAINTENANCE GUT
HEALTH IN FARMING ANIMALS.
PART 5. MONOGLYCERIDE BLEND SUPPLEMENTATION
MODULATES INTESTINAL MOLECULAR MARKERS
EXPRESSION AND MICROBIOME STATUS
IN THE DUODENUM OF BROILER CHICKENS**

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INTRODUCTION

The antibiotics were applied in poultry as a growth promoter (GP) and microbial agent to support sustainable animal farming for many decades. However, due to the global increase in the generation of multidrug resistant strains, the antibiotics supplementation in husbandry has been banned in several developed countries¹. Various alternatives of anti-microbial agents and growth promoters were proposed last decade including probiotics, prebiotics, enzymes and organic acids^{2, 3, 4}. However, all aforementioned tools confirmed as alternatives with restricted efficiency in compare to antibiotics features. In spite of current progress in the study of antimicrobial compounds to treat productive animal diseases, the search for alternative strategies to remove antibiotics remains an actual challenge⁵. Particularly, the engineering of antibiotic-free growth promoters for poultry farming is a most important task in a view the fact that modern broilers are fast-growing birds⁶. Poultry performance has

¹ Global Restriction of Using Antibiotic Growth Promoters and Alternative Strategies in Poultry Production / H. M. Salim et al. *Science Progress*. 2018. Vol. 101, No. 1. P. 52–75.

² Nutraceuticals Induced Changes in the Broiler Gastrointestinal Tract Microbiota / E. Tolnai et al. *mSystems*. 2021. Vol. 6, No. 1. Art. e01124-20.

³ Prospects of Organic Acids as Safe Alternative to Antibiotics in Broiler Chickens Diet / R. U. Khan et al. *Environmental Science and Pollution Research International*. 2022. Vol. 29, No. 22. P. 32594–32604.

⁴ Effect of prebiotics administered during embryo development on mitochondria in intestinal and immune tissues of adult broiler chickens / A. Dunislawska et al. *Poultry Science*. 2023. Vol. 102, No. 6. Art. 102663.

⁵ The Immune Enhancing Effect of Antimicrobial Peptide LLv on Broilers Chickens / X. Liu et al. *Poultry Science*. 2024. Vol. 103, No. 2. Art. 103235.

⁶ Dietary tributyrin improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders / J. Wang et al. *Poultry Science*. 2021. Vol. 100, No. 11. Art. 101429.

been regulated with low doses of antibiotics in feed for many years, leading to disease prevention and increased body weight gain⁷. However, even with the use of low doses of antibiotics, poultry health problems still cannot be completely eliminated⁸. Besides, feed additives considered to be prospective manner to maintain profitability in poultry farming⁹.

The application of organic acids in feed is the most promising technic for replacing antibacterial drugs in industrial poultry farming¹⁰. In particular, organic acids with a short (SCFA), medium (MCFA) and long (LCFA) chains, which are able to selectively adjust the growth or activity of particular bacteria are already successfully used today¹¹. Monoglycerides synthesized with use SCFA have several advantages, including a pH-independent antimicrobial effect and improvement of the intestinal barrier function. Monolaurin supplementation exhibited pronounced antibacterial and anti-inflammatory effects¹².

Recent data demonstrated that intercellular adhesion proteins play a key role in ensuring the barrier function of the intestinal epithelium¹³. Unique adhesion proteins form tight intercellular junctions that prevent harmful substances, microorganisms and toxins penetration into the internal environment of the body¹⁴. Recent report has shown that supplementation with different levels of organic acids can stimulate expression of a significant number of genes related to tight junctions (TJ) and immune function in broilers¹⁵. Butyrate can improve intestinal barrier function via increasing Claudin-1 transcription, by improving the association between Sp1 transcription factor and the Claudin-1 promoter¹⁶.

⁷ Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass Yields, Muscle Amino Acid and Fatty Acid Profiles, and Antioxidant Indices of Broilers Supplemented with Synbiotic and/or Organic Acids. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52.

⁸ Abdelli N., Solà-Oriol D., Pérez J. F. Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges. *Animals*. 2021. Vol. 11, No. 12. Art. 3471.

⁹ Mahfuz S., Shang Q., Piao X. Phenolic compounds as natural feed additives in poultry and swine diets: a review. *Journal of Animal Science and Biotechnology*. 2021. Vol. 12, No. 1. Art. 48.

¹⁰ In Vitro Investigation of the Antibacterial Activity of Nine Commercial Water Disinfectants, Acidifiers, and Glyceride Blends against the Most Important Poultry Zoonotic Bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381.

¹¹ Desbois A. P., Smith V. J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Applied Microbiology and Biotechnology*. 2010. Vol. 85, No. 6. P. 1629–1642.

¹² Monobutyryl Reduces Live Cholesterol and Improves Intestinal Barrier Function in Rats Fed High-Fat Diets / T. D. Nguyen et al. *Nutrients*. 2019. Vol. 11, No. 2. Art. 308.

¹³ Use of Short-Chain Fatty Acids for the Recovery of the Intestinal Epithelial Barrier Affected by Bacterial Toxins / D. Pérez-Reytor et al. *Frontiers in Physiology*. 2021. Vol. 12. Art. 650313.

¹⁴ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

¹⁵ Surface Components and Metabolites of Probiotics for Regulation of Intestinal Epithelial Barrier / Q. Liu et al. *Microbial Cell Factories*. 2020. Vol. 19. Art. 23.

¹⁶ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

Organic acids such as butyric, amino acids, and pectin can affect the expression of E-cadherin. E-cadherin is a protein that plays an important role in maintaining the integrity of cell junctions in the intestinal epithelium¹⁷. The changes in E-cadherin expression can affect cell adhesion, migration, and invasion, which are important for the processes of tissue remodelling, inflammatory balance, and wound healing¹⁸. However, to date, the issue of modulation of the molecular markers expression, including E-cadherin, fibronectin (FN), interferon- α (IFN- α) and interferon- γ (IFN- γ) in the duodenum of broiler chickens remains outside the attention of the researchers. Aforementioned markers confirmed to be convenient tools to assess both intestinal barrier integrity and barrier leaking¹⁹.

Microbiome produces many various metabolites which can affect intestinal cells and modulate they functioning thorough both beneficial and detrimental effect. Among of similar metabolites, SCFA exhibit antimicrobial, metabolic, and signalling features. Furthermore, several SCFA blends possess different targeted bioactivity²⁰. However, the effect of SCFA and monoglyceride blend (SCFA-M) on the intestine health also is undiscovered. Therefore, the important field of the animal science is to clarify the effect of SCFA-M on the intestinal barrier integrity, the microbiome community and their interaction in broiler chickens. The understanding of the interaction between microbiome and intestinal cells can advance the development of new approach to increasing the productivity and resistance of animals with the minimization of the use of antibiotics.

The aim of the current work was to study the effect of SCFA-M on the modulation of molecular markers expression, the microbiome composition and to evaluate the correlations between them in broiler chickens.

1. Methodology of the study of intestinal health assessment

The study was carried out in the Research Center of biosafety and environmental control of agriculture resources (Biosafety-center) of Dnipro State Agrarian and Economic University (DSAEU) and poultry farm located in Dnipro region. The broiler chickens of the Cobb 500 cross were used to form the control and SCFA-M-exposed groups.

¹⁷ Modulation of E-cadherin Monomer Folding by Cooperative Binding of Calcium Ions / O. Courjean et al. *Biochemistry*. 2008. Vol. 47, No. 8. P. 2339–2349.

¹⁸ Modulation of E-cadherin Function and Dysfunction by N-glycosylation / S. S. Pinho et al. *Cellular and Molecular Life Sciences*. 2011. Vol. 68, No. 6. P. 1011–1020.

¹⁹ An Isotonic Protein Solution Favorably Modulated the Porcine Intestinal Immune Response and Cellular Adhesion Markers and Reduced PEDV Shedding in Vivo / D. M. Masiuk et al. *Veterinary Immunology and Immunopathology*. 2024. Vol. 271. Art. 110753.

²⁰ Desbois A. P., Smith V. J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Applied Microbiology and Biotechnology*. 2010. Vol. 85, No. 6. P. 1629–1642.

All procedures with the animals were carried out in compliance with the requirements stated in the Law of Ukraine No. 3447-IV dated 21.02.06 “On the Protection of Animals from Cruelty”, agreed with the main principles of the “Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 22 September 2010, Official Journal of the European Union L276, 20 October 2010, pp. 33–79”, adhere to ethical standards concerning animal welfare in accord “Consensus Guidelines on Animal Ethics and Welfare for Veterinary Journals – International Association of Veterinary Editors, Geneva, Switzerland, 2010”. All experimental protocols were approved by the Commission on Bioethics of Experiments on Animals of the DSAEU, Ukraine (Protocol No. 19–072023).

The treatment with SCFA-M carried out to assess SCFA-M effect on the intestinal health of productive animals in the standard conditions of the industrial poultry farming. Two groups of 7 days old chickens were kept in the separate production houses with stocking density in both groups averaged 20.6 chickens/m². The number of broiler chickens in control and SCFA-M-exposed groups was 30.000 and 36.000 chickens approximal.

Broilers were grown according to the company’s standard protocol. Poultry breeding took place according to modern technologies implemented at the enterprise as well as all procedures were performed in accord with the basic requirements for biological safety. All biological safety requirements were implemented in production in the form of relevant work instructions regarding compliance with veterinary and sanitary rules. The birds of the exposed group consumed the SCFA-M blend with drinking water trice on 16–18, 25–29, 32–36 days of life. The final concentration of SCFA-M blend was equal to 1% in a water. The used SCFA-M formulation contained fatty acids (from C-3 to C-10) and monoglycerides as the major compounds of the formulation. Diglycerides, triglycerides, and free glycerol were presented in the blend as the minor components.

Seven chickens were separated from both control and SCFA-M-exposed group. Duodenal tissue samples with contents were collected from chickens on 16th, 22nd, 29th, 36th, and 45th days. The quantitative composition of the microflora was determined by preparing tenfold dilutions according to the standard of Ukraine (ISO 6887-1:2003) and sowing them on appropriate nutrient media. To determine the number of bacteria of the *Escherichia coli* group (BECG), seeding was carried out using Endo’s medium, staphylococci – Beid-Parker’s medium, enterococci – yolk-esculin agar, clostridia – Wilson-Blair’s iron-containing sulphate agar. All studied taxa (*Staphylococcus*, *Enterococcus*, and *Escherichia coli*) were cultivated with aerobic conditions. The crops were cultivated for 20–24 hours in a thermostat at a temperature of

37±1°C. Determination and interpretation of results performed in accordance with ISO 7218. The washed tissue samples were frozen and store at -22°C before western blot (WB) analysis.

The study of the relative content of molecular markers carried out with use the WB method. Briefly, the thin intestinal samples were homogenized in the RIPA buffer contained protease and phosphatase inhibitor cocktail. Total protein concentration in each sample was determined by the Bradford method (Bradford, 1976). After that, the proteins of the intestinal tissue homogenate were separated by electrophoresis in a polyacrylamide gel (PAAG) with an acrylamide gradient 5–20%²¹. Protein bands separated in PAAG were stained in a 0.1% Coomassie G-250 solution to control the quality of the distribution. The transfer of proteins from PAAG to nitrocellulose membrane was carried out using an electric current at 150 mA for 60 min. The blocking of nitrocellulose membrane was performed with bovine serum albumin 1% solution in PBS. After blocking, nitrocellulose membrane was incubated with primary antibodies against E-cadherin (Santa Cruz, sc-271842), fibronectin (Santa Cruz, sc-8422), interferon- α (Abcam, ab191903), and interferon- γ (Santa Cruz, sc-74104) diluted accordingly producer recommendation. After incubation with primary mouse antibodies (anti-E-cadherin, anti-fibronectin, and anti-interferon- γ) 14 hours at 4°C, the membranes were washed 3 times with PBS and probed with secondary anti-mouse (Abcam, ab6789) horseradish peroxidase-conjugated antibody diluted accordingly producer recommendations. Membranes were then washed in PBS three times. Using the same protocol, samples were incubated with anti-rabbit antibody (Abcam, ab205718) for the detection of primary anti-IFN- α . To visualize the polypeptide zones, the nitrocellulose membrane was incubated with a Tris-HCl solution containing diaminobenzidine (0.05%) and hydrogen peroxide (0.02%). The results reflecting the total protein content and immunoblotting were displayed as percentages relative to the similar results in a control group.

Statistical analysis of the obtained data was performed using the specialized software GraphPad Prism 9. The sample parameters presented in the work had the following designations: M – sample mean; SD – standard deviation. Changes in all obtained data were considered reliable at $P < 0.05$ (including $P < 0.01$ and $P < 0.001$).

2. The results on intestinal health assessment

The content of E-cadherin and FN was determined to evaluate the integrity of the intestinal barrier of the small intestine of chickens. The results of WB

²¹ Nedzvetsky V. S., Agca C. A., Baydas G. The Peptidoglycan Fraction Enriched with Muramyl Pentapeptide from *Lactobacillus bulgaricus* Inhibits Glioblastoma U373MG Cell Migration Capability and Upregulates PARP1 and NF- κ B Levels. *Biotechnology Acta*. 2020. Vol. 13, No. 2. P. 65–79.

showed that the content of E-cadherin in the duodenum of broiler chickens of the control and experimental groups on the 16th day of life was not significantly different (Fig. 1). During the period from 16th to 45th day of chickens' life, the modulation of E-cadherin expression in the duodenum of broiler chickens of the control group demonstrated a downward trend, as indicated by a downward regression line, and the regression equation had the form $-Y = -0.1005 \cdot X + 105.4$ ($P = 0.47$).

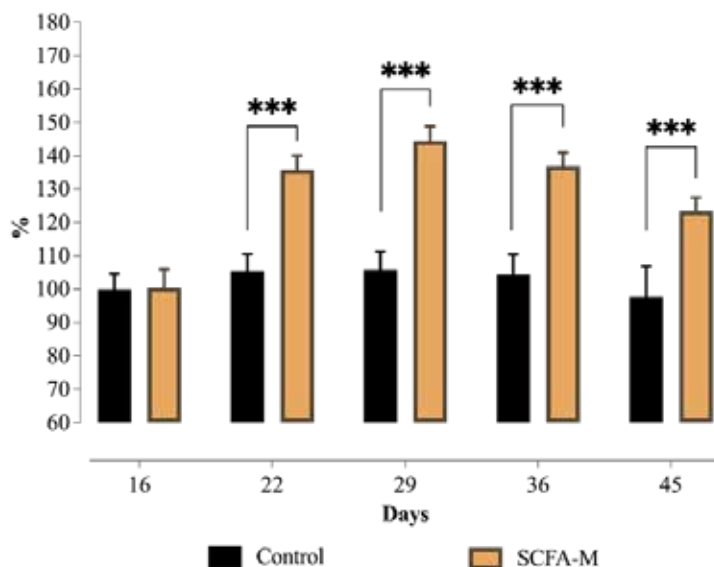


Fig. 1. Modulation of E-cadherin content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$).
Significant differences: * $P < 0.001$**

Consumption of SCFA-M by chickens of the exposed group had a significant effect on this membrane protein expression in the duodenum. In particular, from the 16th to the 22nd day of life the E-cadherin content in the duodenum of broiler chickens of the research group was increased by 35.3% ($P \leq 0.001$). After the 22nd day, the content of E-cadherin increased but somewhat slower until the 29th day of age (by 8.7%), then it gradually decreased by 21.0% ($P \leq 0.001$) until the 45th day of life. We registered higher level of E-cadherin expression in chickens of the research group throughout the entire period of research, so in the duodenum of broiler chickens 22-, 29-, 36-, and 45-day-old,

the expression of this protein was higher respectively by 30.4%, ($P \leq 0.001$), 38.8% ($P \leq 0.001$), 32.6% ($P \leq 0.001$) and 25.8% ($P \leq 0.001$) compared to the indicators of chickens of the control group. The regression equation for the content of E-cadherin in the duodenum of broiler chickens of the research group was $Y = 0.5612 \cdot X + 112.8$ ($P = 0.07$).

The content of FN in the duodenum of 16-day-old broiler chickens of the control and experimental groups did not differ (Fig. 2). During the experiment, the expression of FN in the duodenum of broiler chickens of the control group was increased by 4.8% from 16th to 45th day of life, which determined the upward regression line ($Y = 0.152 \cdot X + 99.1$; $P = 0.123$).

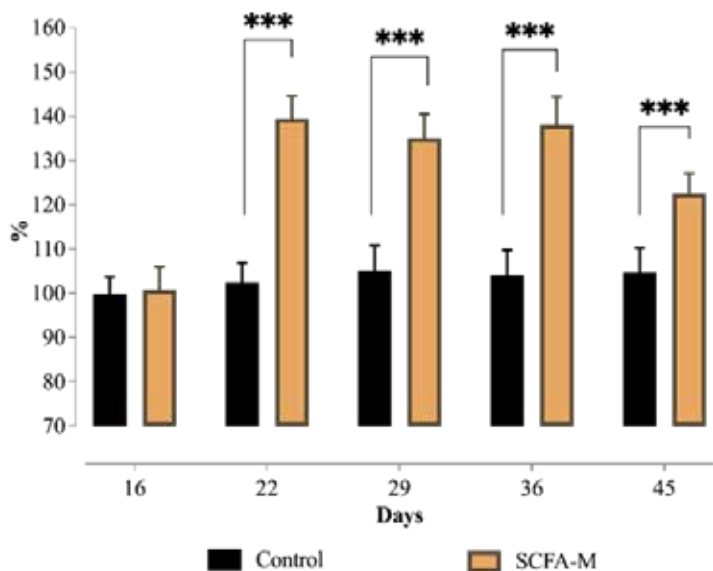


Fig. 2. Modulation of FN content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$). Significant differences: * $P < 0.001$**

The content of FN in chickens of the experimental group duodenum increased by 38.6% ($P \leq 0.001$) from the 16th to the 22nd day of life. The FN content did not change significantly from the 22nd day to the 36th day of life, however, from the 36th to the 45th day of life, the FN content decreased by 15.6% ($P \leq 0.001$). The level of FN expression in the duodenum of the 22-, 29-, 36-, and 45-day-old chickens of the experimental group was higher by 37.0% ($P \leq 0.001$), 30.0% ($P \leq 0.001$),

33.9%, respectively ($P \leq 0.001$) and 17.8% ($P \leq 0.001$) compared to the indicators of chickens of the control group. The regression equation for the content of FN in the duodenum of broiler chickens of the experimental group was $-Y = 0.500 \cdot X + 113.5$ ($P = 0.104$).

The content of IFN- α in the duodenum of 16-day-old broiler chickens of the control and experimental groups was not significantly different (Fig. 3). The expression of IFN- α in the duodenum did not change significantly from the 16th to the 45th day of life of chickens of the control group. Accordingly, the regression equation had the form $-Y = 0.072 \cdot X + 101.9$; $P = 0.628$. Along with this, the content of IFN- α in chickens of the research group duodenum increases by 78% ($P \leq 0.001$) by the 22nd day of life. After that it gradually decreases, in particular, by 29 days of age by 26% ($P \leq 0.001$), by the 36th day by 16.4% ($P \leq 0.05$) and by the 45th day by another 10.4%.

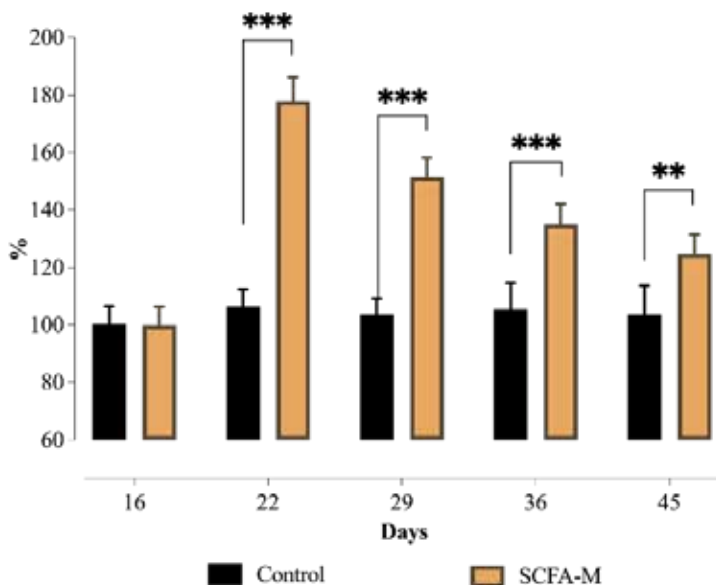


Fig. 3. IFN- α content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$). Significant differences: ** $P < 0.01$, * $P < 0.001$**

Obtained data evidence that the level of IFN- α expression in the duodenum of chickens of the experimental group of 22-, 29-, 36- and 45-days of age was significantly higher, respectively by 71.6% ($P \leq 0.001$), 47.8% ($P \leq 0.001$), 29.7% ($P \leq 0.001$) and 21.0% ($P \leq 0.01$) compared to the indicators of broiler

chickens of the control group. The regression equation for the content of this marker in the duodenum of broiler chickens of the experimental group was $Y = -0.032 \cdot X + 138.7$ ($P = 0.954$). Observed results demonstrated that the content of IFN- γ in the duodenum of broiler chickens of the control and experimental groups on the 16th day of life did not differ significantly and showed a downward trend in both groups (Fig. 4). Thus, the modulation of IFN- γ expression in the duodenum of broiler chickens of the control group was lower by 13.9% than in a control group by the 22nd day of life and, accordingly, the regression equation had the form $-Y = -0.476 \cdot X + 100.9$ ($P = 0.002$).

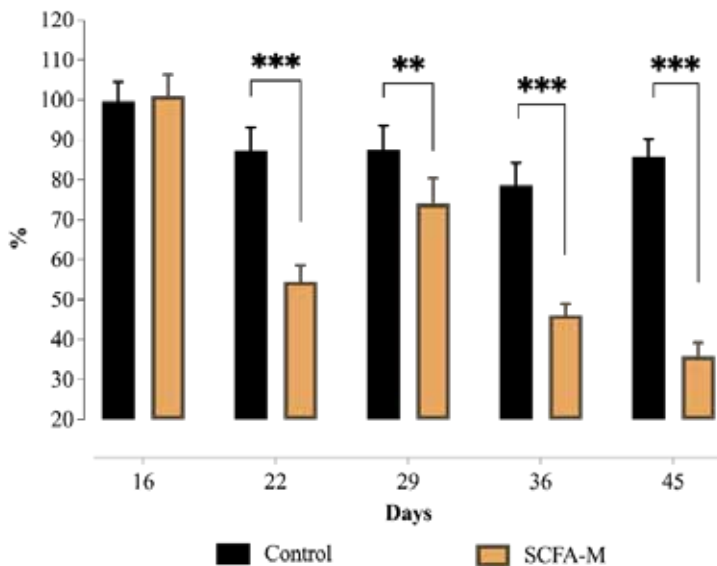


Fig. 4. IFN- γ content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$). Significant differences: ** $P < 0.01$, * $P < 0.001$**

The content of IFN- γ in the duodenum of SCFA-M-exposed group decreased by 46.4% ($P \leq 0.001$) from the 16th to the 22nd day of life, after which it increases slightly (by 19.5%; $P \leq 0.01$) until the age of 29 days. The followed decrease was observed until the age of 36 days (by 28.0%; $P \leq 0.001$). Later, by the 45th day of life, the content of IFN- γ in chickens of the SCFA-M-exposed group drops by 10.2%. The level of IFN- γ expression in the duodenum of the chickens of the SCFA-M-exposed group throughout the experiment was significantly lower than that of the control group chickens, in particular at 22nd,

29th, 36th, and 45th days by 32.7% ($P \leq 0.001$), 13.5% ($P \leq 0.01$), 32.4% ($P \leq 0.001$) and 49.9% ($P \leq 0.001$) respectively. The regression equation for IFN- γ content in the duodenum of broiler chickens of the research group was $-Y = -1.89 \cdot X + 114.5$ ($P = 0.001$).

Correlational analysis of present data on the modulation of molecular markers expression in the duodenum of control group chickens didn't detected significant and/or statistically interrelations between these parameters. However, the direct correlation links between the expression of E-cadherin, FN, and IFN- α were observed in chicken duodenum equal $-r = 0.21-0.58$. On the other side, inverse correlations were found between the expression of IFN- γ and E-cadherin $-r = -0.36$, of IFN- γ and FN $-r = -0.79$, of IFN- γ and IFN- α $-r = -0.82$ (Fig. 5).

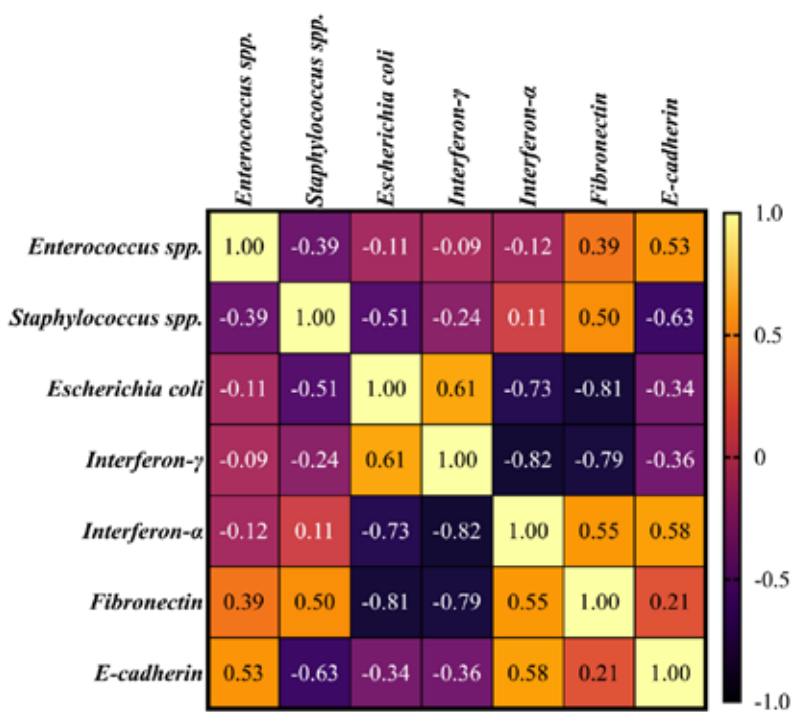


Fig. 5. Correlation matrix of interrelation between molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) and duodenal microbiome broiler chickens of the control group

Furthermore, the supplementation of SCFA-M induced increase in aforementioned correlation coefficients. Besides, statistically significant correlations in SCFA-M-exposed group were found between E-cadherin and FN – $r = 0.96$, between E-cadherin and IFN- α – $r = 0.80$, as well as between E-cadherin and IFN- γ – $r = -0.54$. Similar to the correlation between E-cadherin and other markers, the interrelations between FN and IFN- α exhibited direct dependence (Fig. 6). On the other side, the interrelation between IFN- γ and modulation of FN was decreased – $r = -0.54$ and as well as modulation IFN- α – $r = -0.41$ (Fig. 6).

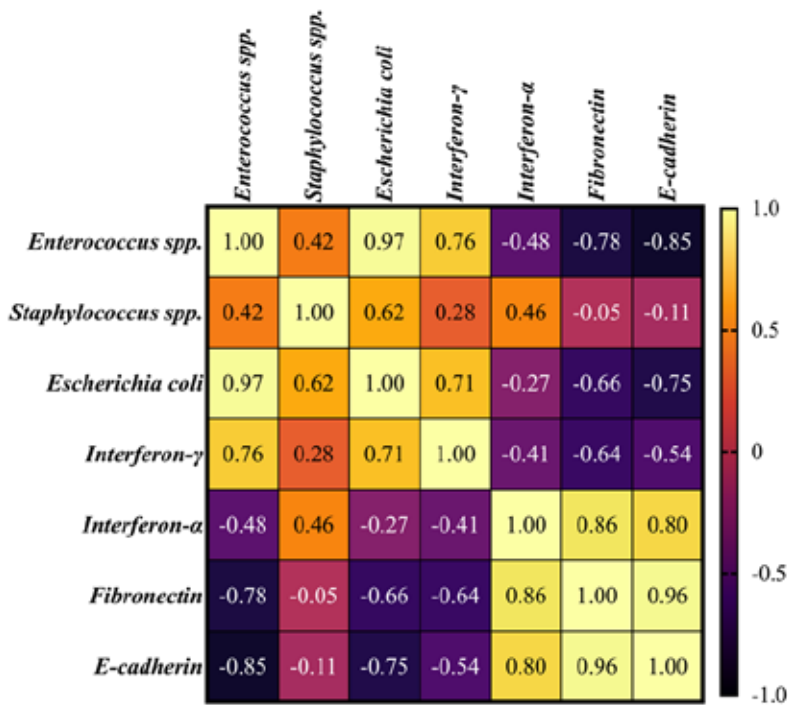


Fig. 6. Correlation matrix of interrelation between molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) and duodenal microbiome broiler chickens of the SCFA-exposed groups (r ; $n=5$)

Observed data on the changes in relative content of the main taxa including *Escherichia coli*, *Enterococcus spp.*, and *Staphylococcus spp.* evidence that all of them permanently involved in concurrent interrelations and participate in the competition to colonize the environment of gut. This fact is supported

by the results of the correlative analysis in respect with the calculated inverse correlations between *Escherichia coli* and *Enterococcus* spp. ($r = -0.11$), *Escherichia coli* and *Staphylococcus* spp. ($r = -0.51$), *Staphylococcus* spp. and *Enterococcus* spp. ($r = -0.39$). However, the SCFA-M supplementation affected multiple interactions into microbiome community and shifted the relative content several taxa. Obtained results have shown that SCFA-M significantly changed correlation between *Escherichia coli* and *Enterococcus* spp. – $r = 0.96$. Furthermore, the direct correlation was observed between *Staphylococcus* spp. and *Escherichia coli* ($r = 0.42$) relative contents as well as between *Staphylococcus* spp. and *Enterococcus* spp. ($r = 0.62$).

The results on main taxa relative content demonstrated inverse correlation in control group of chickens between *Escherichia coli* and the modulation of molecular markers including E-cadherin, FN, and IFN- α ($r = -0.81$ – 0.34). Besides, the direct correlation was observed between *Escherichia coli* and IFN- γ modulation ($r = 0.81$). Similar interrelations were detected in SCFA-M-exposed chicken group.

Direct correlations were detected between *Staphylococcus* spp. content in duodenum of chicken control group and the changes in IFN- α ($r = 0.11$) and FN ($r = 0.50$) contents. However, inverse correlation was observed between *Staphylococcus* spp. content and E-cadherin ($r = -0.63$) and IFN- γ ($r = -0.24$). The treatment with SCFA-M induced the shift of similar to control group parameters, particularly correlation between *Staphylococcus* spp. content in thin intestine SCFA-M-exposed chicken group and E-cadherin modulation was equal $r = -0.11$, while this parameter of FN was equal $r = -0.05$. On the other hand, direct correlations were observed between *Staphylococcus* spp. content and IFN- α changes ($r = 0.28$) and IFN- γ changes ($r = 0.46$).

The relative content of *Enterococcus* spp. in chicken duodenum of control group exhibited the direct correlation in respect with FN and E-cadherin modulation ($r = 0.11$ and $r = 0.50$ respectively). Invers correlation between *Enterococcus* spp. content and IFN- α and IFN- γ changes were observed comparatively low ($r = -0.12$ and $r = -0.09$ accordingly). Moreover, these interrelations were statistically insignificant. Similar to previous described correlations, the SCFA-M exposure induced an increase in inverse correlation coefficients between *Enterococcus* spp. content in thin intestine and modulation of E-cadherin, FN, and IFN- α ($r = -0.85$, $r = -0.78$, and -0.48 respectively). The direct correlation was detected between *Enterococcus* spp. content and changes in IFN- γ ($r = 0.76$) similarly to other studied taxa. Therefore, contrary to aforementioned interrelations in control group, SCFA-M application initiated the decrease in the correlation coefficients almost in all groups from 16th to 45th day of life.

3. The intestinal health maintenance is key task to provide poultry profitability

The correlation analysis demonstrated a complex nonlinear interaction between molecular markers and the duodenal microbiome community of broiler chickens. In the control group, the relationships between molecular markers are weak and statistically insignificant, which indicates the preservation of the balance of the microbiome community within the physiological norm. At the same time, the addition of SCFA-M induced in the study a significant increase in the correlation between markers and the microbiome. This effect may be due to the signaling and regulatory properties of SCFA-M in relation to individual microbiome taxa. In addition, the inclusion of SCFA-M in the energy metabolism pathways of intestinal cells stimulates the expression of individual specific proteins, including intercellular adhesion proteins. All this together can stimulate the complex interaction of the microbiome with intestinal epithelial cells and contribute to the improvement of barrier function.

The observed modulation of the *Escherichia coli* content demonstrated significant inverse relationships with E-cadherin, FN and IFN- α , which reflects the possible negative impact of this bacterium on structural and immune markers. The direct relationship of *Escherichia coli* with IFN- γ indicates the activation of a specific immune response. Similar trends persist in the SCFA-M-exposed group. The changes in *Staphylococcus* spp. content in control group had a direct relationship with the modulation of FN and IFN- α . On the other hand, this taxon had an inverse relationship with the modulation of the content of E-cadherin and IFN- γ . Thus, the results have shown multifaceted regulation of innate immune and structural markers. The effects of SCFA-M significantly modulate the correlation between the microbiome state and the content of the above-mentioned molecular markers. In general, the observed effects of SCFA-M can be associated with differential regulation of individual microbiome taxa by modifying the environment and changing the competition conditions between the main taxa. Changes in the content of *Enterococcus* spp. in the control group showed weak and unreliable relationships with the modulation of molecular markers. However, these relationships in the SCFA-M-exposed group become significant and strengthened. For example, the relative content of *Enterococcus* spp. was inversely correlated with the content of E-cadherin, FN and IFN- α . On the other hand, a direct correlation was found between the content of *Enterococcus* spp. and changes in IFN- γ . Obtained data demonstrated beneficial effect of SCFA-M supplementation, including the commensal microflora modulation via involving *Enterococcus* spp. in the regulation of both the immune system and the intestinal barrier integrity. Therefore, SCFA-M is potent to regulate

interrelation of various microbiome taxa and to switch the communication from synergic type to antagonistic suppression.

Epithelial cells of the intestine, bound together by various intercellular proteins, are the first line of defense against bacteria and their toxins. Claudins, occludins and other TJ proteins are located on the apical surface of the basolateral membrane, form intercellular tight contacts, regulate permeability through the epithelial monolayer and are responsible for cell polarity²². Structural integrity largely depends on the expression levels of intercellular junction proteins²³. We established the fundamental relationships of the modulation of the content of molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) in the duodenum of broiler chickens ($r = 0.98-1.00$; $P \leq 0.001$).

The results of present study have established the dynamics of modulation of the molecular markers expression in the duodenum of broiler chickens for the first time. In particular, the modulation of E-cadherin expression in the cecum of broiler chickens of the control group increases by 5.7% from the 16th to the 22nd day, after which it gradually decreases (by 7.2%) until the 45th day of the chickens' life. At the same time, the expression of E-cadherin in the duodenum of broiler chickens was less dynamics and shown a tendency to decrease from 16th to the 45th day. Recently, it has been shown that the content of E-cadherin in the small intestine can be used as an indicator of the deterioration of the barrier function of the gut²⁴.

FN plays an important role in maintaining the small intestine health of broilers by providing cell adhesion to the extracellular matrix, protection against pathogens, and promoting tissue healing and remodeling processes^{25, 26}. Optimum housing conditions and balanced nutrition are key factors contributing to the maintenance of an adequate level of FN and ensure the health and high productivity of birds²⁷.

IFNs are a family of cytokines that exert biological activity by binding to specific cell membrane receptors and triggering a signaling pathway that

²² Use of Short-Chain Fatty Acids for the Recovery of the Intestinal Epithelial Barrier Affected by Bacterial Toxins / D. Pérez-Reytor et al. *Frontiers in Physiology*. 2021. Vol. 12. Art. 650313.

²³ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

²⁴ An Isotonic Protein Solution Favorably Modulated the Porcine Intestinal Immune Response and Cellular Adhesion Markers and Reduced PEDV Shedding in Vivo / D. M. Masiuk et al. *Veterinary Immunology and Immunopathology*. 2024. Vol. 271. Art. 110753.

²⁵ The Role of the Fibronectin Synergy Site for Skin Wound Healing / I. Gimeno-Lluch et al. *Cells*. 2022. Vol. 11, No. 13. Art. 2100.

²⁶ Kanta J., Zavadakova A. Role of Fibronectin in Chronic Venous Diseases: A Review. *Vascular Medicine*. 2020. Vol. 25, No. 6. P. 588–597.

²⁷ Novel Models for Chronic Intestinal Inflammation in Chickens: Intestinal Inflammation Pattern and Biomarkers / G. C. Dal Pont et al. *Frontiers in Immunology*. 2021. Vol. 12. Art. 676628.

culminates in the induction of interferon-stimulated gene (ISG) transcription and immune enhancing effect^{28, 29}. Mediated by ISGs, IFNs generate a variety of cellular and physiological responses, including antiviral, apoptotic, antiproliferative, antitumor, and immunomodulatory effects³⁰. The expression of IFN- α in the duodenum of chickens didn't changed significantly during the observed experimental period.

Several results have shown that some organic acids can affect the expression level of intercellular adhesion³¹. For example, butyric acid, which is one of the main fatty acids, can reduce the expression of E-cadherin in some cells. This may have important consequences for cell adhesion and other processes occurring in intestinal epithelial layer^{32, 33}.

The relationship between the state of the microbiota and the modulation of intestinal functions has attracted the special attention of researchers. The changes in the content of the main microbiota taxa accompanied by the modulation of adhesion proteins expression. In particular, the microbiota effects on the expression of E-cadherin, occludin, and claudin have been reported³⁴. The microbiota involved into the maintenance of the balance between these proteins, which ensures optimal functioning of the intestinal barrier^{35, 36}. The analysis of present results established interdependencies between the modulation of molecular markers and the duodenal microbiome of the broiler chickens where the content of E-cadherin, FN, IFN- α , and IFN- γ are interconnected with the main taxa ratio. Additionally, obtained data have demonstrated correlation links between microbiome community state and the modulation of intercellular adhesion proteins and innate immune molecular markers first time.

²⁸ Molecular Analysis of Chicken Interferon-alpha Inducible Protein 6 Gene and Transcriptional Regulation / J. W. Park et al. *Journal of Animal Science and Technology*. 2023. Vol. 65, No. 1. P. 183–197.

²⁹ The Immune Enhancing Effect of Antimicrobial Peptide LLv on Broilers Chickens / X. Liu et al. *Poultry Science*. 2024. Vol. 103, No. 2. Art. 103235.

³⁰ Parker N., Porter A. C. G. Identification of a Novel Gene Family that Includes the Interferon-Inducible Human Genes 6–16 and ISG12. *BMC Genomics*. 2004. Vol. 5. Art. 8.

³¹ Surface Components and Metabolites of Probiotics for Regulation of Intestinal Epithelial Barrier / Q. Liu et al. *Microbial Cell Factories*. 2020. Vol. 19. Art. 23.

³² Modulation of Apoptosis and Epithelial-Mesenchymal Transition E-cadherin/TGF-beta/Snai1/TWIST Pathways by a New Ciprofloxacin Chalcone in Breast Cancer Cells / R. Alaaeldin et al. *Anticancer Research*. 2021. Vol. 41, No. 5. P. 2383–2395.

³³ Fomicheva M., Tross E. M., Macara I. G. Polarity Proteins in Oncogenesis. *Current Opinion in Cell Biology*. 2020. Vol. 62. P. 26–30.

³⁴ Regulation of Intestinal Barrier Function by Microbial Metabolites / S. Ghosh et al. *Cellular and Molecular Gastroenterology and Hepatology*. 2021. Vol. 11, No. 5. P. 1463–1482.

³⁵ The Gut Microbiota in Inflammatory Bowel Disease / P. Qiu et al. *Frontiers in Cellular and Infection Microbiology*. 2022. Vol. 12. Art. 733992.

³⁶ Compound Probiotics Can Improve Intestinal Health by Affecting the Gut Microbiota of Broilers / X. Liu et al. *Journal of Animal Science*. 2023. Vol. 101. Art. skad388.

CONCLUSIONS

The application of SCFA-M feed additive induced an increase in the content of E-cadherin, FN, IFN- α and a decrease in the production of IFN- γ in the duodenum. In particular, the effect of SCFA-M in chickens is characterized by statistically significant increase in the content of E-cadherin, FN, and IFN- α through the all periods of the exposure. Inversely, SCFA-M treatment induced statistically significant decrease in the IFN- γ production in compare with the control group. Present results have demonstrated the effect of SCFA-M supplementation on both microbiome community and intestinal barrier integrity of broiler chickens. Furthermore, SCFA-M blend exhibited the modulatory effect on the bilateral interaction between microflora and intestinal epithelial cells.

SUMMARY

The continuous antibiotics application in the animal farming caused the generation of microbial resistant strains. Multi-drug resistant strains are extremely weighted risk factor for both animals and human. The aim of study was to assess the effect of short chain fatty acids plus monoglycerides (SCFA-M) blend on microbiome community and intestinal barrier integrity of broiler chickens. Control group fed with standard diet and antibiotics supplementation. SCFA-exposed group fed with the same diet without antibiotics and additionally supplemented with SCFA-M in a dose 1% four times for 5 days on 16th, 22th, 29th, and 32nd days of life. The microbiome state was estimated via detection of relative content of principal taxa. Intestinal barrier integrity was assessed through the measuring of E-cadherin and fibronectin contents in gut tissue. Innate immunity was assessed via detection of interferon- α and interferon- γ . The administration of SCFA-M induced upregulation in E-cadherin, fibronectin, and IFN- α content ($P \leq 0.01$) in duodenum of SCFA-M-exposed broilers in compare to control group. Inversely, the level of IFN- γ was significantly decreased ($P \leq 0.01$) in the duodenal intestine of the exposed broilers. The correlative analysis demonstrated the link between molecular markers and the changes of main taxa including *Escherichia coli*, *Staphylococcus* spp., and *Enterococcus* spp. Molecular markers content was correlated with microbiome changes where increase in E-cadherin and fibronectin have a positive correlation with *Escherichia coli* content in 22-day-old broiler chickens ($r = 0.79-0.87$) and negative correlation with *Staphylococcus* spp. content in 45-day-old chickens ($r = -0.83 - -0.51$). The complex correlations of all studied molecular markers expression in the duodenum of broiler chickens significantly varied from 16th to 45th day of life in respect with both magnitude and direction of changes. Thus, the application of SCFA-M to broiler chickens has showed a potential to modulate intestinal barrier integrity and microbiome community via non-linear dependence.

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