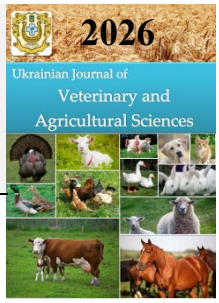
Ukrainian Journal of
Veterinary and Agricultural Sciences
http://ujvas.com.ua

The Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

original article | UDC 636.52/.58:612.015.3:577.161 | doi: 10.32718/ujvas9-2.16

Volume 9
Number 2



Molecular changes in the intestinal barrier function of broiler chickens with bacterial chondro-necrosis and osteomyelitis

Ya. Maksymchuk, D. Masiuk

Dnipro State Agrarian and Economic University, Serhii Efremov Str., 25, Dnipro, 49600, Ukraine

Article info
Received 03.04.2026
Received in revised form 04.05.2026
Accepted 05.05.2026

Correspondence author
Dmytro Masiuk
Tel.: +38-050-636-62-37
E-mail: masiuk.d.m@dsau.dp.ua

Contents

Abstract

The aim of the study was to evaluate the characteristics of gene expression of inflammation, apoptosis, and intestinal barrier function markers in broiler chickens with bacterial chondronecrosis and osteomyelitis, as well as under the influence of a composition of organic (fatty) acids. The study was conducted on ROSS-308 broiler chickens under commercial production conditions. Experimental groups were formed retrospectively according to clinical status. Samples of the small and large intestine, chyme, and feces were collected for molecular analysis. The relative expression levels of the genes TNF-α, IL-1β, Caspase-9, Claudin-1, Occludin, MUC-2, and Fibronectin were determined using the RT-qPCR method. It was established that under pathological conditions, a redistribution of the inflammatory response occurs between the tissue and luminal compartments of the intestine, manifested by decreased TNF-α expression in the small intestine and increased IL-1β expression in chyme and feces. Changes in Caspase-9 expression indicate modification of apoptotic processes, with a tendency toward suppression in tissues and possible enhancement in the luminal environment. Impairment of the intestinal barrier function was revealed, as confirmed by decreased expression of Claudin-1 and MUC-2 in tissues and feces, as well as multidirectional changes in Occludin expression. Increased Fibronectin expression in the luminal compartment indicates activation of extracellular matrix remodeling processes and destructive-reparative changes in the mucous membrane. The obtained results indicate a profound restructuring of the functional state of the intestine in bacterial chondronecrosis and osteomyelitis, accompanied by an imbalance between inflammation, apoptosis, and barrier function. The use of a composition of organic acids may modify the nature of the intestinal response, contributing to the limitation of inflammation in tissues and the maintenance of intestinal barrier integrity.

Keywords: broiler chickens; intestine; barrier function; TNF-α; IL-1β; Caspase-9; Claudin-1; Occludin; MUC-2; Fibronectin; bacterial chondronecrosis; organic acids; gene expression; RT-qPCR.

Citation:
Maksymchuk, Ya., & Masiuk, D. (2026). Molecular changes in the intestinal barrier function of broiler chickens with bacterial chondronecrosis and osteomyelitis. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 9(2), 106–113.

1. Introduction

The intensive development of poultry farming is accompanied by the increasing importance of the intestine as an integrative organ that combines barrier, immune, and metabolic functions and determines the health status and productivity of broiler chickens. The intestinal epithelium, together with the microbiota, forms a complex functional system that ensures the maintenance of homeostasis, regulation of the inflammatory response, and protection of the organism against pathogenic agents (Oakley et al., 2014; Kers et al., 2018). An important role in these processes is played by the intestinal barrier function, which is mediated through the system of tight junctions, the mucus layer, and immune mechanisms of the mucous membrane (Turner, 2009; Peterson & Artis, 2014). Disruption of these structures leads to increased epithelial permeability, development of the so-called "leaky gut," and activation of inflammatory processes (Bischoff et al., 2014).

One of the key mechanisms regulating intestinal homeostasis is the balance between pro-inflammatory cytokines, apoptotic processes, and epithelial regeneration. In particular, TNF-α and IL-1β play a central role in the initiation and maintenance of the inflammatory response, affecting the integrity of the epithelial barrier and the function of intestinal cells (Aggeletopoulou et al., 2024; Yao et al., 2024). Along with this, apoptotic mechanisms mediated by caspases, particularly Caspase-9, ensure the physiological renewal of the epithelium, whereas their imbalance may lead to impaired regeneration and barrier dysfunction (Flynn & Buret, 2008). Important components of the barrier also include tight junction proteins such as Claudin-1 and Occludin, as well as the mucus layer formed by mucin MUC-2, which provides physical separation between the microbiota and the

epithelium (Gallego et al., 2023; Tamchuk & Masiuk, 2024). In addition, the structural integrity of tissues is maintained by the extracellular matrix, a key component of which is Fibronectin (FN), involved in the processes of regeneration and remodeling of the mucous membrane (Cameron et al., 2024; Masiuk & Nedzvetsky, 2025).

Disruption of the functional state of the intestine is considered one of the important factors in the development of systemic pathologies in poultry. In particular, bacterial chondronecrosis and osteomyelitis (BCO) are multifactorial diseases whose pathogenesis is associated not only with infectious agents, but also with impaired intestinal barrier function and bacterial translocation (Wideman, 2016). In this context, the intestine may serve as a key source of microbial penetration into the systemic circulation, followed by damage to bone tissue.

One of the promising approaches to correcting disturbances of intestinal homeostasis is the use of organic acids and their derivatives. It has been established that monoglycerides of medium-chain fatty acids, particularly lauric acid, exhibit pronounced antimicrobial properties by disrupting the integrity of bacterial membranes, and are also capable of modulating the immune response and increasing the expression of tight junction proteins, thereby contributing to the stabilization of intestinal barrier function (Jackman et al., 2020; Masiuk et al., 2024; Dai et al., 2025). In addition, such compounds may influence the interaction between the microbiota and the mucous membrane, reducing the intensity of inflammatory processes and maintaining the structural integrity of the epithelium.

Despite the considerable number of studies devoted to the effects of organic acids on poultry productivity and health, their role in regulating the molecular mechanisms of intestinal barrier function under systemic pathologies, particularly BCO, remains insufficiently studied. Data regarding changes in the expression of genes characterizing inflammation, apoptosis, the state of tight junctions, and the mucosal barrier in different intestinal biotopes are especially limited.

The aim of the present study was to evaluate the expression of genes associated with inflammation, apoptosis, and intestinal barrier function in broiler chickens with bacterial chondronecrosis and osteomyelitis.

2. Materials and methods

The study was conducted on ROSS-308 broiler chickens under the conditions of a commercial poultry farm. Birds were housed in separate poultry houses with a capacity of 35–42 thousand birds each under identical zoohygienic conditions (stocking density of 19.5 birds/m², standard diets, and a unified scheme of preventive and veterinary-sanitary measures). Rearing was carried out using intensive production technology in accordance with the enterprise production protocol and in compliance with biological safety requirements.

From the first day of life, all chicks received the same basal diet supplemented with a composition of short-chain fatty acids and monoglycerides, IP Enterin C3-C12. The composition included monoglycerides (31 %), diglycerides (18 %), triglycerides (1 %), free glycerol (14 %), and fatty acids: propionic (C_3), butyric (C_4), caprylic and capric (C_8–C_{10}), and lauric (C_{12}) acids. During the starter and grower periods, the preparation was added to the feed at doses of 1.5 kg/t and 1.0 kg/t, respectively. All groups underwent a standard vaccination program at the hatchery, including Vectormune, Cevac Vitabron, Cevac Transmune, Cevac I Bird (strain 1/96), and Cefinel. Antibacterial therapy was limited to the administration of enrofloxacin (days 1–5) and the combined antimicrobial preparation Gardizen M (days 6–9).

During the second half of the rearing period, birds additionally received the enteroprotective composition IP Enterin C3-C10 in drinking water at a dose of 0.5 L/t of water during days 19–25 and 28–32, according to the following regimen: 16 hours with the preparation and 8 hours with fresh drinking water. During the intermediate periods (days 26–27 and 33–41), the acid complex IP Cid, based on organic acids, was administered. Vitamin supplementation was provided according to the farm's standard protocol.

No experimental infection of birds was performed. After the completion of the rearing period, chickens were retrospectively divided into two groups according to their clinical status: Healthy – clinically healthy birds without signs of locomotor system lesions, and Diseased – chickens with confirmed signs of bacterial chondronecrosis and osteomyelitis (BCO). The diagnosis was established based on clinical examination, pathological-anatomical assessment, and microbiological investigation. The Diseased group included birds with characteristic lesions of the proximal regions of the tibia and femur.

For molecular analysis, 5 four-week-old chickens from each group were selected by randomized sampling (n = 5). After euthanasia, samples of the mucous membrane of the small and large intestine, as well as intestinal contents for complementary analysis, were collected. The biomaterial was immediately frozen in liquid nitrogen and stored at −80°C until further investigation.

Total RNA was isolated from intestinal tissues using the commercial BioExtract® SuperBall® kit (BioSellal, France). Extraction was performed on a KingFisher Duo automated workstation (Thermo Scientific, USA), which ensured standardization of the procedure and high reproducibility of the results. RNA quality and concentration were assessed spectrophotometrically. Quantitative determination of gene expression levels was performed using one-step RT-qPCR with the NZYSpeedy RT-qPCR Green Kit (Nzytech, Portugal) and the intercalating dye SYBR Green I. Amplification was carried out using the AriaMx Real-Time PCR System (Agilent Technologies, USA). Negative controls without template were included in each reaction series. The expression of TNF-α, IL-1β, Caspase-9, Claudin-1, Occludin, MUC-2, and Fibronectin genes was analyzed. β-actin was used as the reference gene. Relative expression levels were determined using the 2^(-ΔΔCt) method, and the results were expressed in relative units.

Statistical analysis of the results was performed using methods of variation statistics. Data are presented as M ± SD. Normality of distribution was assessed using the Shapiro–Wilk test. Comparisons between groups were performed using Student's t-test or the Mann–Whitney U test depending on the data distribution pattern. Differences were considered statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

All experimental procedures were carried out in accordance with the Law of Ukraine No. 3447-IV "On the Protec-

tion of Animals from Cruel Treatment" and the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study protocol was approved by the Bioethics Commission of Dnipro State Agrarian and Economic University.

3. Results and discussion

3.1. Results

Analysis of TNF-α gene expression in different intestinal biotopes of broiler chickens demonstrated a multidirectional pattern of changes during the development of pathology (Fig. 1). In the small intestine of diseased birds, a 41.8 % decrease in the parameter was observed (0.53 ± 0.12 vs. 0.92 ± 0.09), showing pronounced statistical significance ($P < 0.01$). In the large intestine, the changes were minor, with a 6.4 % decrease (0.82 ± 0.17 vs. 0.88 ± 0.15), and did not reach statistical significance. In the chyme, TNF-α expression increased by 82.6 % (1.62 ± 0.90 vs. 0.89 ± 0.15), which may be characterized as a tendency toward elevation; however, due to the high variability of the parameter, the differences remained statistically non-significant. In feces, the values were practically unchanged between the groups (0.53 ± 0.13 vs. 0.50 ± 0.21). At the same time, a statistically significant difference between the groups was established ($P < 0.01$), indicating an overall activation of the inflammatory response under pathological conditions and its redistribution between the tissue and luminal compartments of the intestine.

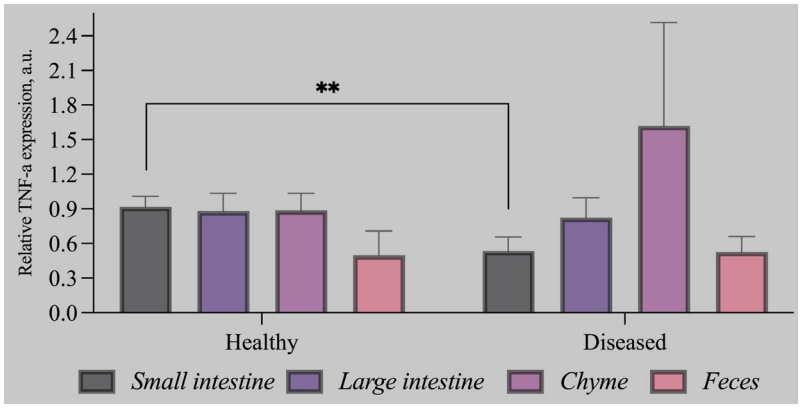


Fig. 1. Relative expression of the TNF-α gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

In the small and large intestine of diseased birds, a decrease in IL-1β gene expression by 18.4–21.8 % was observed, which may be characterized as a tendency (Fig. 2). At the same time, in the chyme, an increase in IL-1β expression by 159.6 % ($P < 0.05$) was established, indicating pronounced activation of the inflammatory process in the luminal environment of the intestine. In feces, an increase in expression level by 129.9 % ($P < 0.05$) was also observed, reflecting enhancement of the inflammatory response. Thus, the obtained data indicate a redistribution of IL-1β expression from the tissue level to the luminal compartment of the intestine under pathological conditions.

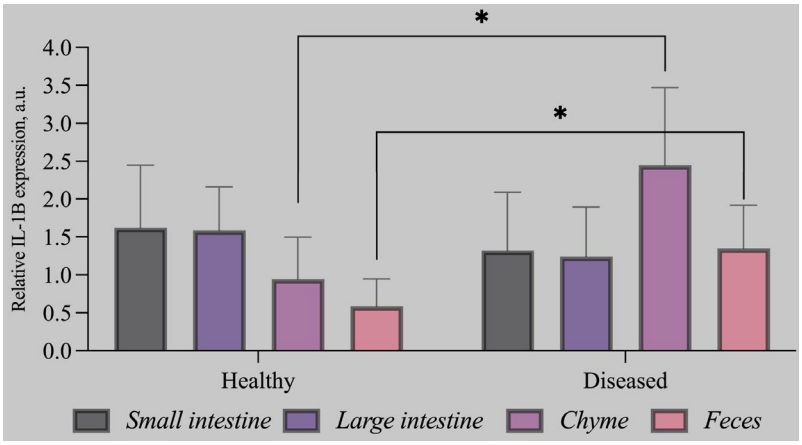


Fig. 2. Relative expression of the IL-1β gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

Analysis of Caspase-9 gene expression in different intestinal biotopes of broiler chickens demonstrated multidirectional changes under pathological conditions (Fig. 3). In the small intestine of diseased birds, a 21.0 % decrease in the parameter was observed, which may be characterized as a tendency. In the large intestine, a 15.2 % decrease in expression was also noted ($P > 0.05$).

In feces, on the contrary, an increase in Caspase-9 expression by 148.2 % (2.56 ± 2.08 vs. 1.03 ± 1.11) was observed, which may indicate activation of apoptotic processes in the luminal environment of the intestine; however, due to the high variability of the parameter, these changes should be regarded as a tendency and are not statistically significant.

Overall, the obtained results indicate suppression of apoptotic activity in intestinal tissues and its possible enhancement in the luminal compartment under pathological conditions.

The expression of the Claudin-1 gene in different intestinal biotopes of broiler chickens showed multidirectional changes under pathological conditions (Fig. 4). In the small intestine of diseased birds, a decrease in expression by 27.7 % was observed ($P < 0.05$). In the large intestine, a more pronounced decrease of 31.3 % (0.75 ± 0.19 vs. 1.09 ± 0.29) was established, although it did not reach statistical significance ($P > 0.05$). In the chyme, the changes were minor, with a 4.5 % decrease, indicating the absence of significant differences between the groups. In feces, on the contrary, an increase in Claudin-1 expression by 18.3 % (1.12 ± 0.49 vs. 0.94 ± 0.32) was observed; however, due to considerable variability, these changes should be regarded as a tendency.

Overall, the obtained results indicate disruption of tight intercellular junctions in intestinal tissues and possible compensatory processes in the luminal environment during the development of pathology.

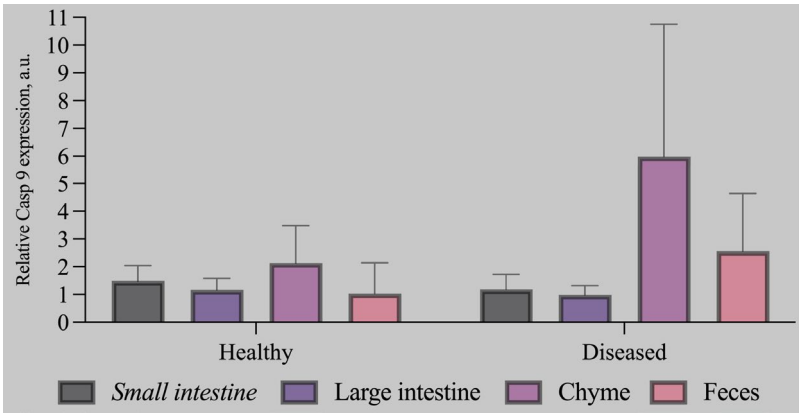


Fig. 3. Relative expression of the Caspase-9 gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

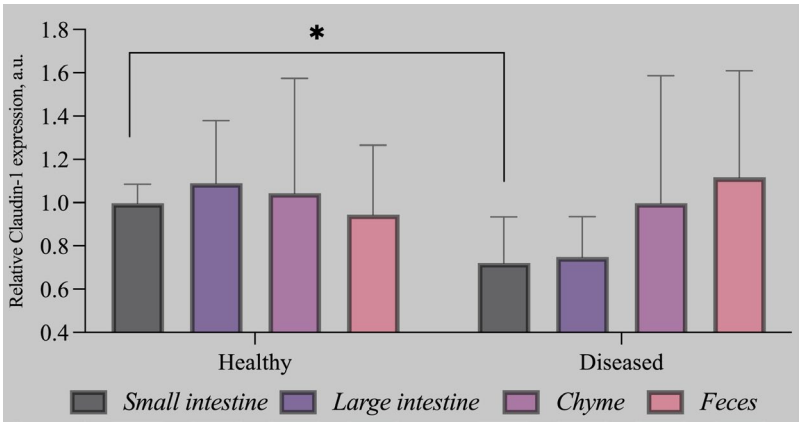


Fig. 4. Relative expression of the Claudin-1 gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

Analysis of Occludin gene expression in different intestinal biotopes of broiler chickens demonstrated multidirectional changes under pathological conditions (Fig. 5). In the small and large intestine of diseased birds, an increase in the expression of this gene by 23.6 % and 7.4 %, respectively, was observed ($P > 0.05$). In the chyme, a more pronounced increase in Occludin expression by 98.0% (1.98 ± 1.22 vs. 1.00 ± 0.42) was established, indicating activation of processes associated with barrier function in the luminal environment of the intestine; however, due to the high variability of the parameter, these changes should be regarded as a tendency. In contrast, in feces, a sharp decrease in expression by 67.7 % ($P > 0.05$) was observed, indicating impairment of barrier function and degradation of tight junction components. Overall, the results indicate an imbalance in the regulation of tight junction proteins, with multidirectional responses in the tissue and luminal compartments of the intestine under pathological conditions.

The expression of the MUC-2 gene in different intestinal biotopes of broiler chickens reflects impairment of the mucosal barrier under pathological conditions (Fig. 6). In the small intestine of diseased birds, a decrease in expression by 28.5 % was observed, representing a tendency without statistical significance ($P > 0.05$). In the large intestine, on the contrary, an increase in the parameter by 13.8 % was noted ($P > 0.05$). In the chyme, a pronounced increase in MUC-2 expression by 87.4 % (1.80 ± 0.82 vs. 0.96 ± 0.28) was established, indicating activation of mucus secretion in the luminal environment of the intestine as a compensatory response; however, due to the high variability of the parameter, these changes should be regarded as a tendency. In feces, a sharp decrease in expression by 60.7 % ($P < 0.05$) was observed, which may indicate depletion of the mucin-producing function or degradation of the mucus layer. Overall, the obtained results indicate an imbalance of the mucosal barrier, characterized by suppression in tissues and com-

pensatory activation in the luminal compartment of the intestine.

The expression of the Fibronectin gene in different intestinal biotopes of broiler chickens indicates extracellular matrix remodeling processes under pathological conditions (Fig. 7). In the small intestine of diseased birds, a decrease in expression by 46.8 % was observed ($P < 0.05$). In the large intestine, a slight decrease of 11.7 % was noted ($P > 0.05$). In the chyme, a significant increase ($P < 0.05$) in FN expression by 129.7 % (1.96 ± 0.40 vs. 0.85 ± 0.75) was established, indicating activation of tissue remodeling and injury-related processes. The most pronounced changes were observed in feces, where expression increased ninefold ($P < 0.05$), which may indicate intensive destructive-reparative processes in the intestine. Overall, the obtained results indicate suppression of FN expression in tissues and its sharp increase in the luminal compartment of the intestine, reflecting profound disturbances in the structural organization of the extracellular matrix under pathological conditions.

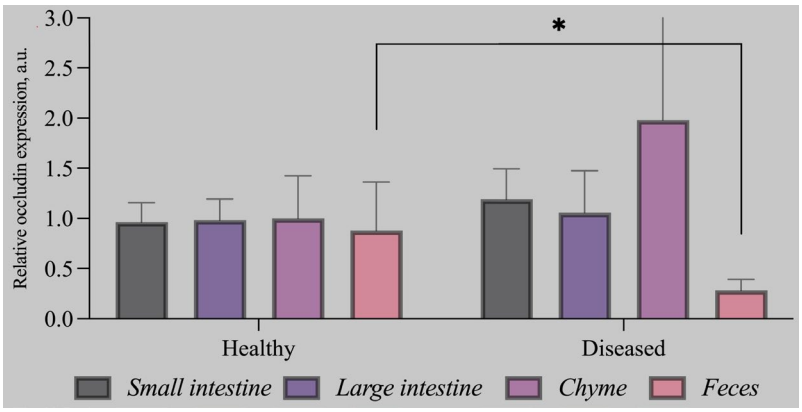


Fig. 5. Relative expression of the Occludin gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

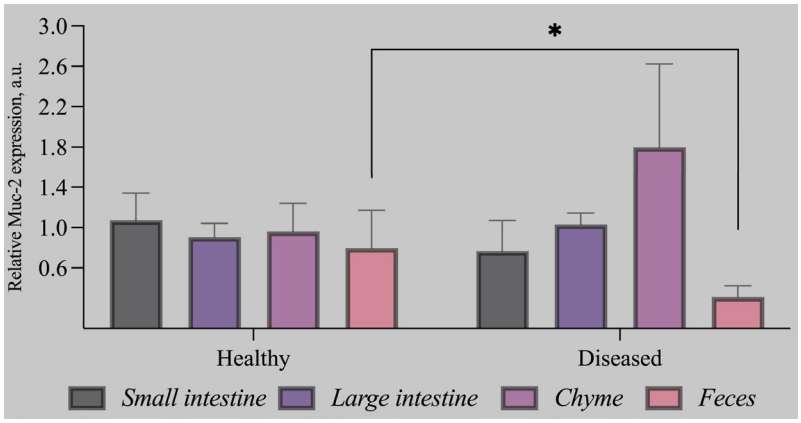


Fig. 6. Relative expression of the MUC-2 gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

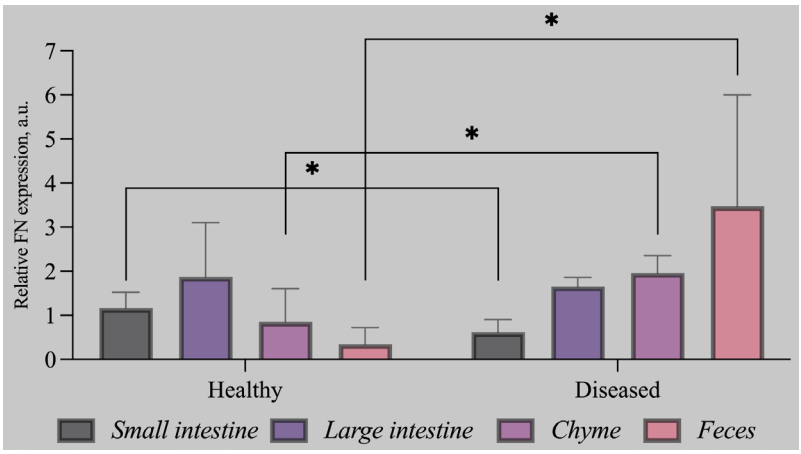


Fig. 7. Relative expression of the Fibronectin gene in the intestine, chyme, and feces of broiler chickens under the influence of a fatty acid composition (relative units; M ± SD; n = 5)

Thus, under pathological conditions in broiler chickens, an imbalance between inflammation, apoptosis, and intestinal barrier function was observed. A tendency toward decreased expression of TNF-α in the small intestine and Caspase-9 in tissues, accompanied by their increased expression in the luminal compartment, was identified, while IL-1β expression increased in chyme and feces. A decrease in Claudin-1 expression in the small intestine and MUC-2 expression in feces, together with opposite trends in Occludin expression in biological materials, indicates impairment of the intestinal barrier, whereas increased Fibronectin expression reflects remodeling processes. Overall, these findings indicate intestinal injury accompanied by compensatory restructuring of its functions.

3.2. Discussion

The identified changes in the expression of pro-inflammatory cytokines (TNF-α, IL-1β) between the tissue and luminal compartments of the intestine may reflect a redistribution of inflammatory activity under conditions of impaired barrier function. It is known that inflammation, particularly TNF-α-dependent mechanisms, leads to destabilization of tight junctions, increased epithelial permeability, and loss of epithelial integrity, accompanied by the release of cellular components into the intestinal lumen (Turner, 2009; Sonnier et al., 2012). In addition, the intestinal epithelium is an active participant in the immune response, capable of secreting pro-inflammatory mediators into the luminal environment, thereby forming a distinct compartment of local inflammation (Peterson & Artis, 2014). Collectively, these mechanisms are consistent with the obtained results and indicate a shift of the inflammatory response from the tissue level to the luminal environment of the intestine under pathological conditions (Bischoff et al., 2014).

A similar tendency was established for IL-1β, confirming the intensification of the pro-inflammatory cascade in the luminal environment. IL-1β is known to be a key effector cytokine of inflammasomes, formed as a result of caspase-1 activation and playing a central role in the initiation of the innate immune response (Yao et al., 2024). Increased expression of this cytokine is closely associated with impairment of intestinal barrier integrity and the development of inflammatory processes in the mucous membrane (Aggeletopoulou et al., 2024). At the same time, inflammasome dysfunction is accompanied by increased intestinal permeability, creating conditions for active interaction between the microbiota and the immune system (Aggeletopoulou et al., 2024). In this context, the elevated levels of IL-1β in chyme and feces may reflect enhancement of the luminal component of inflammation induced by microbial factors, indicating impaired local immune regulation and the potential development of subclinical enteritis in BCO.

Changes in Caspase-9 expression indicate modification of apoptotic processes in the intestine. Activation of caspases, particularly Caspase-9, is recognized as a key mechanism for the induction of apoptosis in intestinal epithelial cells under infectious and inflammatory influences (Flynn & Buret, 2008). Reduced expression of this marker in intestinal tissues may indicate suppression of controlled cell death, leading to the accumulation of functionally defective enterocytes and deterioration of barrier function. At the same time, it has been established that induction of enterocyte apoptosis is accompanied by decreased expression of tight junction proteins, increased permeability, and damage to the epithelial barrier (Lu et al., 2025). Furthermore, disruption of the balance between apoptosis and physiological cell shedding results in enhanced release of cellular material into the intestinal lumen, which is associated with the development of inflammation and barrier dysfunction (Williams et al., 2015). In this context, increased expression of Caspase-9 in the luminal compartment may reflect intensified epithelial cell shedding and activation of destructive processes in the mucous membrane.

Impairment of intestinal barrier function is confirmed by changes in the expression of the Claudin-1 and Occludin genes, which are key structural components of tight junctions and ensure the integrity of the epithelial barrier (Zhao et al., 2025). It is known that proteins of the claudin family form a selective paracellular barrier, and reduced Claudin-1 expression is associated with increased permeability and loss of barrier function (Marsch et al., 2024). In turn, Occludin plays an important regulatory role in maintaining barrier integrity through interaction with proteins such as ZO-1, and its function largely depends on post-translational modifications, particularly phosphorylation (Kuo et al., 2022). Importantly, Claudin-1 is characterized by relative stability within the tight junction structure, whereas Occludin is a dynamic protein capable of rapid redistribution between cellular compartments, which may account for the multidirectional changes in its expression under pathological conditions. Such an imbalance of tight junction proteins leads to increased paracellular permeability, development of the so-called “leaky gut,” and contributes to bacterial translocation and endotoxemia (Moonwiriyakit et al., 2023).

An important component of intestinal mucosal protection is the mucus layer, the basis of which is the mucin MUC-2, forming a physical barrier between the microbiota and the epithelium (Gallego et al., 2023). Maintenance of an adequate level of its synthesis is critical for preserving barrier function and intestinal homeostasis (Liu et al., 2023), while mucins in general serve as a key element of innate immune defense against pathogenic microorganisms (Kang et al., 2022). Reduced MUC-2 expression in intestinal tissues may indicate impairment of the mucosal barrier, facilitating bacterial contact with the epithelium and initiation of inflammation (Qiao et al., 2025). At the same time, increased MUC-2 levels in chyme may reflect compensatory mucus hypersecretion in response to injury, which is consistent with the concept of an adaptive response of the mucous membrane. Subsequent depletion of mucin-producing cells and degradation of mucus, particularly under the influence of the microbiota, may lead to reduced stability of the mucus barrier, as confirmed by decreased MUC-2 expression in feces (Chen et al., 2026).

Changes in Fibronectin expression reflect activation of extracellular matrix remodeling processes, which play a key role in maintaining the structural integrity and regeneration of the intestinal mucosa (Cameron et al., 2024). A decrease in this parameter in intestinal tissues may indicate impairment of regenerative processes and insufficient epithelial repair, which is characteristic of conditions associated with barrier dysfunction (Sylvestre et al., 2023). At the same time, a sharp increase in Fibronectin levels in chyme and feces may be associated with intensive shedding of epithelial cells and the release of extracellular matrix components into the intestinal lumen, which is a characteristic feature of

inflammation and mucosal injury (Lechuga et al., 2023). Such a combination of changes is consistent with the concept of intestinal barrier dysfunction as a systemic factor in pathology that may contribute to bacterial translocation and the development of complications, particularly in bacterial chondronecrosis and osteomyelitis.

Special attention should be paid to the effect of the composition of organic (fatty) acids used in the present study. It is known that monoglycerides of medium-chain fatty acids, particularly derivatives of lauric acid (C12), exhibit pronounced antimicrobial properties by disrupting the integrity of pathogen membranes (Jackman et al., 2020). In addition, they are capable of modulating the intestinal immune response by reducing the expression of pro-inflammatory cytokines while simultaneously increasing the expression of tight junction proteins, thereby contributing to stabilization of barrier function (Dai et al., 2025). Experimental models in broiler chickens have demonstrated that such compounds improve intestinal morphology, reduce inflammation, and promote normalization of the microbiota (Zheng et al., 2023). In this context, the obtained results may indicate that even in the presence of pathology, the use of a fatty acid composition is capable of modifying the nature of the intestinal response by limiting inflammation in tissues and promoting its localization within the luminal compartment, which may be regarded as an adaptive mechanism aimed at preserving the integrity of the epithelial barrier.

Thus, the obtained results confirm that bacterial chondronecrosis and osteomyelitis in broiler chickens are accompanied by profound restructuring of the intestinal barrier, characterized by disruption of tight junctions, imbalance of mucosal protection, altered localization of the inflammatory response, and activation of remodeling processes. This highlights the important role of the intestine as a key component in the pathogenesis of BCO and substantiates the feasibility of using nutraceutical approaches, particularly compositions of organic acids, for correction of these disturbances.

4. Conclusions

In broiler chickens with bacterial chondronecrosis and osteomyelitis, significant changes in the expression of genes associated with inflammation, apoptosis, barrier function, and extracellular matrix remodeling of the intestine were established. The pathological process was accompanied by a redistribution of the inflammatory response between the tissue and luminal compartments, manifested by a 41.8 % decrease in TNF-α expression in the small intestine ($P < 0.01$) and increased IL-1β expression in chyme by 159.6 % and in feces by 129.9 % ($P < 0.05$). Changes in Caspase-9 expression indicate modification of apoptotic processes, with a tendency toward their suppression in intestinal tissues and possible enhancement in the luminal environment. Impairment of barrier function was confirmed by a 27.7 % decrease in Claudin-1 expression in the small intestine ($P < 0.05$), a 60.7 % decrease in MUC-2 expression in feces ($P < 0.05$), and multidirectional changes in Occludin expression, indicating an imbalance of tight junction proteins and mucosal protection. Simultaneously, a 46.8 % decrease in Fibronectin expression in the small intestine ($P < 0.05$), together with its increase in chyme by 129.7 % and a ninefold increase in feces ($P < 0.05$), indicates activation of extracellular matrix remodeling and destructive-reparative processes in the mucous membrane. The obtained data confirm that the intestine is an important component in the pathogenesis of BCO, and the use of organic acid compositions may be considered a promising approach for maintaining barrier function and limiting intestinal epithelial damage.

Conflict of interest

The authors declare the absence of conflict of interests in regard with their scientific writing and research results

References

Aggeletopoulou, I., Kalafateli, M., Tsounis, E. P., & Triantos, C. (2024). Exploring the role of IL-1β in inflammatory bowel disease pathogenesis. *Frontiers in Medicine*, 11. [Crossref] [Google Scholar]

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J. D., Serino, M., Tilg, H., Watson, A., & Wells, J. M. (2014). Intestinal permeability--a new target for disease prevention and therapy. *BMC gastroenterology,* 14, 189. [Crossref] [Google Scholar]

Cameron, O., Neves, J. F., & Gentleman, E. (2024). Listen to Your Gut: Key Concepts for Bioengineering Advanced Models of the Intestine. *Advanced science (Weinheim, Baden-Wurttemberg, Germany),* 11(5), e2302165. [Crossref] [Google Scholar]

Chen, Y., Xu, Y., Li, X., Wu, S., Long, H., Fu, G., & Xiao, S. (2025). The Role of Gut Microbiota and Mucin Barrier in the Pathogenesis of Colorectal Cancer. *Current issues in molecular biology,* 48(1), 16. [Crossref] [Google Scholar]

Dai, S., Li, C., Li, D., & Wang, H. (2025). α-Glycerol monolaurate promotes tight junction proteins expression through PKC/MAPK/ATF-2 signaling pathway. *Frontiers in nutrition,* 12, 1598991. [Crossref] [Google Scholar]

Flynn, A. N., & Buret, A. G. (2008). Caspases-3, -8, and -9 are required for induction of epithelial cell apoptosis by enteropathogenic E. coli but are dispensable for increased paracellular permeability. *Microbial Pathogenesis*, *44*(4), 311–319. [Crossref] [Google Scholar]

Gallego, P., Garcia-Bonete, M. J., Trillo-Muyo, S., Recktenwald, C. V., Johansson, M. E. V., & Hansson, G. C. (2023). The intestinal MUC2 mucin C-terminus is stabilized by an extra disulfide bond in comparison to von Willebrand factor and other gel-forming mucins. *Nature communications,* 14(1), 1969. [Crossref] [Google Scholar]

Jackman, J. A., Boyd, R. D., & Elrod, C. C. (2020). Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. *Journal of animal science and biotechnology,* 11, 44. [Crossref] [Google Scholar]

Kang, Y., Park, H., Choe, B. H., & Kang, B. (2022). The Role and Function of Mucins and Its Relationship to Inflammatory Bowel Disease. *Frontiers in medicine,* 9, 848344. [Crossref] [Google Scholar]

Kers, J. G., Velkers, F. C., Fischer, E. A. J., Hermes, G. D. A., Stegeman, J. A., & Smidt, H. (2018). Host and Environmental Factors Affecting the Intestinal Microbiota in Chickens. *Frontiers in microbiology,* 9, 235. [Crossref] [Google Scholar]

Kuo, W. T., Odenwald, M. A., Turner, J. R., & Zuo, L. (2022). Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival. *Annals of the New York Academy of Sciences,* 1514(1), 21–33. [Crossref] [Google Scholar]

Lechuga, S., Braga-Neto, M. B., Naydenov, N. G., Rieder, F., & Ivanov, A. I. (2023). Understanding disruption of the gut barrier during inflammation: Should we abandon traditional epithelial cell lines and switch to intestinal organoids?. *Frontiers in immunology,* 14, 1108289.
[Crossref] [Google Scholar]

Liu, Y., Yu, Z., Zhu, L., Ma, S., Luo, Y., Liang, H., Liu, Q., Chen, J., Guli, S., & Chen, X. (2023). Orchestration of MUC2 - The key regulatory target of gut barrier and homeostasis: A review. *International journal of biological macromolecules,* 236, 123862.
[Crossref] [Google Scholar]

Lu, Q. Q., Zheng, W. W., Zhang, Z. Y., Cong, P. K., Guo, X., Zhang, Y., Zhang, X. Z., Long, S. R., Liu, R. D., Wang, Z. Q., & Cui, J. (2025). Trichinella spiralis excretory/secretory proteins mediated larval invasion via inducing gut epithelial apoptosis and barrier disruption. *PLoS neglected tropical diseases,* 19(1), e0012842.
[Crossref] [Google Scholar]

Masiuk, D. M., & Nedzvetsky, V. S. (2025). Monoglyceride blend supplementation modulates intestinal molecular markers expression and microbiome status in the duodenum of broiler chickens. *Bulgarian Journal of Veterinary Medicine,* 28(4), 597–612.
[Crossref] [Google Scholar]

Masiuk, D., Nedzvetsky, V., & Maksymchuk, Ya. (2024). The current state and prospects for the use of organic acids and their compositions in poultry feed: A literature review. *Scientific Horizons,* 27(7), 148–157.
[Crossref] [Google Scholar]

Marsch, P., Rajagopal, N., & Nangia, S. (2024). Biophysics of claudin proteins in tight junction architecture: Three decades of progress. *Biophysical journal,* 123(16), 2363–2378.
[Crossref] [Google Scholar]

Moonwiriyakit, A., Pathomthongtaweechai, N., Steinhagen, P. R., Chantawichitwong, P., Satianrapapong, W., & Pongkorpsakol, P. (2023). Tight junctions: from molecules to gastrointestinal diseases. *Tissue barriers,* 11(2), 2077620.
[Crossref] [Google Scholar]

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., … Cox, N. A. (2014). The chicken gastrointestinal microbiome. *FEMS microbiology letters*, 360(2), 100–112.
[Crossref] [Google Scholar]

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nature reviews. *Immunology*, 14(3), 141–153.
[Crossref] [Google Scholar]

Qiao, Y., He, C., Xia, Y., Ocansey, D. K. W., & Mao, F. (2025). Intestinal mucus barrier: A potential therapeutic target for IBD. *Autoimmunity reviews*, 24(2), 103717.
[Crossref] [Google Scholar]

Sonnier, D. I., Bailey, S. R., Schuster, R. M., Gangidine, M. M., Lentsch, A. B., & Pritts, T. A. (2012). Proinflammatory chemokines in the intestinal lumen contribute to intestinal dysfunction during endotoxemia. *Shock (Augusta, Ga.),* 37(1), 63–69.
[Crossref] [Google Scholar]

Sylvestre, M., Di Carlo, S. E., & Peduto, L. (2023). Stromal regulation of the intestinal barrier. *Mucosal immunology,* 16(2), 221–231.
[Crossref] [Google Scholar]

Tamchuk, L.M., Masiuk, D.M. (2024). Modulation of the integrity of the intestinal barrier of broiler chicks associated with changes in the microbiome. *Animal Biology,* 26(3), 26–34.
[Crossref] [Google Scholar]

Turner, J. R. (2009). Intestinal mucosal barrier function in health and disease. *Nature reviews. Immunology,* 9(11), 799–809.
[Crossref] [Google Scholar]

Wideman, R. F., Jr (2016). Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. *Poultry science,* 95(2), 325–344.
[Crossref] [Google Scholar]

Williams, J. M., Duckworth, C. A., Burkitt, M. D., Watson, A. J., Campbell, B. J., & Pritchard, D. M. (2015). Epithelial cell shedding and barrier function: a matter of life and death at the small intestinal villus tip. *Veterinary pathology,* 52(3), 445–455.
[Crossref] [Google Scholar]

Yao, J., Sterling, K., Wang, Z., Zhang, Y., & Song, W. (2024). The role of inflammasomes in human diseases and their potential as therapeutic targets. *Signal transduction and targeted therapy,* 9(1), 10.
[Crossref] [Google Scholar]

Zhao, Z., Liu, X., Zhang, R., Ke, R., Zhang, S., & Chen, Y. (2025). Intestinal Barrier in Inflammatory Bowel Disease: Mechanisms and Treatment. *Journal of Translational Gastroenterology*, *3*(2), 62–73.
[Crossref] [Google Scholar]

Zheng, C., Xiao, G., Yan, X., Qiu, T., Liu, S., Ou, J., Cen, M., Gong, L., Shi, J., & Zhang, H. (2023). Complex of Lauric Acid Monoglyceride and Cinnamaldehyde Ameliorated Subclinical Necrotic Enteritis in Yellow-Feathered Broilers by Regulating Gut Morphology, Barrier, Inflammation and Serum Biochemistry. *Animals*, 13(3), 516.
[Crossref] [Google Scholar]