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Original research

Modulation of molecular markers in the duodenum and jejunum of piglets induced by an isotonic protein mixture

O. A. Sheptukha , D. M. Masiuk
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

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

Tel.: +8050-636-62-37
E-mail: *shepto@icloud.com*

[#]**Corresponding author:**
O. A. Sheptukha
shepto@icloud.com

Abstract. The article presents an analysis of the results that allow to evaluate isotonic protein mixture (IPM) effect on the barrier function of the digestive tract of piglets in the early postnatal period, which is important for development of new preventive and curative strategies in pigs farming. The study was carried out with involving the piglets of the early postnatal period, which were divided in control and experimental groups. The expression of molecular markers in the duodenum and jejunum was determined in the animals of 9, 21 and 35 days of age. It was found that in IPM-exposed piglet group on the 9th day of life E-cadherin level in duodenum intestines exceeded control group content by 50.1% (P<0.001), and the level of fibronectin (FN) exceeded control group content by 55.3% (P<0.001). After weaning another increase of following factors was observed: E-cadherin level exceeded control values by 59.5%, and the level of FN – by 56.3% (P<0.001). The level of tumor necrosis factor-α (TNF-α) in piglets of the experimental groups was much lower than in controls animals: on the 9th day its concentration was decreased by 18.0% (P<0.01), and after weaning remained 62.0% lower (P<0.001). The level of caspase-3 (Casp-3) in the duodenum intestine of experimental piglet group after weaning remained relatively stable and was 60.7% lower (P<0.001) in compare with the control group. The expression of molecular markers in the jejunum of piglets underwent gradual changes, in particular, by the 21st day of life the level of E-cadherin increased by 4.0% (P<0.05), however, after weaning (by the 35th day) its expression increased by another 9.4% (P<0.05). A similar dynamic trend was observed for FN, the level of which reached 113.8 ± 4.0%. At the same time TNF-α levels increased by 72.5% (P<0.01), and Casp-3 by 65.1% (P<0.01). In piglets receiving IPM on the 9th day of life, the level of E-cadherin was 32.1% higher comparing to the control group (P<0.001). Although by the 21st day there was a decrease of E-cadherin content (by 9.0%), contrary, E-cadherin level increased by 22.2% (P<0.01) again after weaning. FN has also demonstrated similar dynamics, with its level being 58.9% higher compared to control (P<0.001). The most drastic changes were observed in the TNF-α content while its level remained 90.6% lower compared to the control group (P<0.001). The level of Casp-3 in the thin intestine of experimental piglet groups remained stable low, being 60.1% lower than the control level (P<0.001). The present results demonstrated a multifactorial beneficial effect of IPM supplementation on the gut barrier functioning, the balance in pro-/anti-inflammatory cytokines, and the inhibition of apoptosis in piglets.

Keywords: piglets; thin intestine; molecular markers; gut immunity; isotonic formulations; weaning.

Модуляція експресії молекулярних маркерів у дванадцятипалій та порожній кишці поросят за дії ізотонічно-протеїнової суміші

Анотація. У статті представлено аналіз результатів досліджень, які дозволяють оцінити вплив ізотонічної протеїнової суміші (ІПС) на бар'єрну функцію травного тракту поросят у ранньому постнатальному періоді, що має важливе значення для розробки нових профілактичних і лікувальних стратегій у свинарстві. Дослідження проводили на поросятах раннього постнатального періоду, яких розділили на контрольну та дослідну групи. У тварин визначали експресію молекулярних маркерів у дванадцятипалій та порожній кишці у 9-, 21- та 35-денному віці. Встановлено, що у поросят, які отримували ІПС на 9-й день життя рівень Е-кадгерину (E-CAD) у дванадцятипалій кишці перевищував контрольні показники на 50,1% (P<0,001), а рівень фібронектину (FN) – на 55,3% (P<0,001). Після відлучення відзначалося повторне їх підвищення: рівень E-cadherin перевищував контрольні значення на 59,5%, а рівень FN – на 56,3% (P<0,001). Рівень фактору некрозу пухлин-альфа (TNF-α) у поросят дослідної групи був значно нижчим, ніж у контрольних тварин: на 9-й день його концентрація була знижена на 18,0% (P<0,01), а після відлучення залишалася на 62,0% нижчою (P<0,001). Рівень каспази-3 (Casp-3) у дванадцятипалій кишці поросят дослідної групи залишався після відлучення відносно стабільним та був на 60,7% нижчим (P<0,001), ніж у контрольній групі. Експресія молекулярних маркерів у порожній кишці поросят зазнавала поступових змін, зокрема, до 21-го дня життя рівень E-cadherin зріс на 4,0% (P<0,05), однак після відлучення (до 35-го дня) його експресія збільшилася ще на 9,4% (P<0,05). Подібна динаміка спостерігалася і для FN, рівень якого досяг 113,8 ± 4,0%. Водночас рівень TNF-α зростав на 72,5% (P<0,01), а Casp-3 на 65,1% (P<0,01). У поросят, які отриму-

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вали ІПС на 9-й день життя рівень E-cadherin був на 32,1% вищим порівняно з контрольною групою (P<0,001). Хоча до 21-го дня спостерігалося зниження цього показника (на 9,0%), після відлучення рівень E-cadherin знову зріс на 22,2% (P<0,01). Аналогічну динаміку продемонстрував і FN, рівень якого був на 58,9% вищим порівняно з контролем (P<0,001). Найбільш виражені зміни спостерігалися у регуляції TNF-α – його рівень залишався на 90,6% нижчим порівняно з контрольною групою (P<0,001). Рівень Casp-3 у порожній кишці поросят дослідної групи залишався стабільно низьким, будучи на 60,1% нижчим за контрольний рівень (P<0,001). Представлені результати свідчать про багато-спрямований корисний ефект ІПС стосовно бар'єрної функції тонкого кишечника, балансу про-запальних та анти-запальних цитокінів та гальмування апоптозу у поросят до та після відлучення.

Ключові слова: поросята; тонкий кишечник; молекулярні маркери; інтестинальний імунітет; ізотонічно-протеїнова суміш відлучення.

Introduction

Research into the mechanisms of modulation of the intestinal barrier function of piglets in the early postnatal period is an extremely relevant area of modern veterinary medicine (Modina et al., 2019). The first weeks of piglets' lives are characterized by high sensitivity to the influence of external and internal stress factors, which can lead to disruption of intestinal homeostasis, microbiome imbalance, metabolic disorders and, as a result, a decrease in the efficiency of nutrient absorption (Campbell et al., 2013). This, in turn, impairs growth rates, overall animal viability and pig productivity. In this context, the search and scientific justification of methods for correcting and maintaining intestinal barrier function are key to improving piglet health and reducing economic losses in the industry (Modina et al., 2019; Xiao et al., 2021).

Recent studies have shown that the state of the intestinal barrier is directly related to the composition of the intestinal microbial community. The microbiota performs a number of critically important functions, including the synthesis of biologically active metabolites, regulation of immune responses, maintenance of the density of adhesive contacts between epithelial cells, and resistance to colonization by pathogenic microorganisms (Gieryńska et al., 2022). Disturbances in the composition of the microbiome of various origins can lead to changes in metabolism, increased permeability of the intestinal wall, inflammation, and systemic metabolic disorders (Tommaso et al., 2021). All this together leads to inhibition of the digestive and barrier functions of the intestine, increases risks of enteric infections, inhibits daily growth of animals, and reduces the profitability of pig farming. That is why maintaining the normal composition of the microbiome, optimizing metabolic processes, and maintaining stable intestinal functioning are critically important tasks in piglet farming.

One of the promising approaches to improve the intestinal barrier is the use of biologically active substances, in particular isotonic protein mixture (IPM). Bioactive additives together with milk proteins provide stimulation of metabolism and, at the same time, contribute to the development of the digestive system of piglets. Such a stimulating effect increases the resistance of piglets to the invasion of enteropathogens (Masiuk et al., 2024). It is assumed that IPM can contribute to the normalization of the structural and functional state of the intestinal epithelium, stimulate the development of local immunity, stabilize the microbiome and improve energy metabolism in enterocytes (Buzoianu et al., 2020; Masiuk et al., 2023). The use of similar technologies in piglet feeding can not only promote increase their stress resistance, but also to optimize metabolic processes that ultimately has a positive effect on intestinal function, growth rates and productivity (Zhang et al., 2022; Xu et al., 2025).

Given the urgency of finding effective and inexpensive feed additives, the development of novel mixtures with multi-factorial effects requires an understanding of the molecular mechanisms of their effects. In addition, the implementation of similar agents into veterinary practice requires detailed experimental verification of their effectiveness. All aforementioned together requires comprehensive studies of the morphofunctional state of the intestine, as well as determination of the impact on the main components of the digestive system.

Current strategies for assessing gut health involve the use of a panel of molecular markers of the most critical components of gut function, including the epithelial barrier, immunity, and cell viability. Among the wide range of specific markers of gut health, tight junction and adherens junction proteins, pro-inflammatory and anti-inflammatory cytokines, pro-apoptotic proteins, and specific microbiome metabolites are being considered (Masiuk et al., 2025). Of particular interest are molecular markers of cell-cell and cell-extracellular matrix interactions. All of these connection types are dynamic structures and are responsible for a wide range of cellular functions, including biological barriers, cell migration, organ development, and the formation of unique tissue-specific structures (Faraj et al., 2022; Nedzvetsky et al., 2020). Thus, the creation of a panel of molecular markers will be useful for improving methods for assessing gut function in productive animals and for testing the effectiveness of feed additives.

The aim of this study was to assess the effect of IPM supplementation on the gut barrier maintenance, the balance of pro-inflammatory and anti-inflammatory cytokines, and caspase-3 content as a marker of apoptosis in the small intestine of piglets.

Materials and methods

The cohort of 2-day-old three-breed hybrid piglets of DanBred genetics (n = 168) were purchaused from industrial swine farm. The piglets were selected and randomly divided into two groups: control and experimental (84 animals per group). The performance in both control and experimental group of piglets was assesses through the measuring live weight, average daily gain and level of survival. The experimental manipulations were carried out taking into account the relevant regulatory framework and current national legal requirements described in the Law of Ukraine No. 3447- IV, 22.02.2006 "On the Protection of Animals from Cruelty" as well as all procedures were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe, 1986) and current principles of bioethics were applied for every manipulation with selected animals (Vaughn, 2023). In addition, every experimental protocol was approved by the Institutional Animal Care and Use Committee of Dnipro State Agrarian and Economic University, Ukraine (Protocol No. 11-052024).

Piglets of the experimental group were supplemented with 500 ml of IPM per litter from 3th to 8th day of life. Animals of each group (n = 6) were randomly selected at 9, 21, and 35 days of age, euthanized, and small intestine samples were collected. Piglets were slaughtered in compliance with all modern veterinary, sanitary, and ethical requirements. Samples were immediately frozen and stored at minus18–22 °C for no more than 2 weeks before the beginning of the study.

To assess barrier function, the adherens junction protein

E-cadherin (E-CAD) was selected, the content of which reflects the adhesion density of the epithelial layer (Masyuk et al., 2024). The content of fibronectin (FN) was determined to assess cell adhesion and extracellular matrix (ECM). The content of tumor necrosis factor-α (TNF-α) was determined to assess the stimulation of the pro-inflammatory process in the intestine (Plantone et al., 2024). The content of interferon-α was determined to assess the balance between the pro-inflammatory and anti-inflammatory response (González-Navajas et al., 2012). The content of caspase-3 was determined for the predictive analysis of apoptosis activation in intestinal cells (Tang et al, 2019).

To form a sample pool, 150 mg of small intestinal tissue, including the duodenum and jejunum, was selected. To determine the content of molecular markers in intestinal tissue, immunoblotting (Western blot – WB) samples were previously homogenized in phosphate buffer solution (PBS, pH 7.4) containing a cocktail of proteinase inhibitors (Kuryata et al., 2020). The concentration of total protein in the homogenates was determined by the Bradford method to normalize the protein content to 50 μg/track during electrophoresis (Bradford, 1976). The next step was the separation of proteins by electrophoresis in a polyacrylamide gel with an acrylamide gradient from 7 to 18% (Nedzvetsky et al., 2020). After completion of electrophoresis, the gels were stained with 0.1% Coomassie G-250 solution to control the quality of protein distribution. Proteins were transferred from the gel to a nitrocellulose membrane at a current of 150 mA for 60 minutes in tris-glycine transfer buffer containing 2 M urea and 20% methanol. After transfer, the membranes were washed three times in PBS and the areas of nonspecific antibody sorption were blocked with a 2% solution of skimmed milk powder in PBS. The blocked membrane was incubated for 60 min at room temperature with a solution of the appropriate primary antibodies against selected molecular markers, namely, anti-caspase-3, anti-fibronectin, anti-E-cadherin, anti-TNF-α, and anti-IFN-α (sc-7272; sc-2710984; sc-8426; sc-52746; sc-373757 respectively). Subsequently, the membrane was washed three times in PBS and incubated for 60 min at room temperature with a solution of secondary antibodies conjugated with horseradish peroxidase (goat anti-mouse IgG-HRP, sc-2005). Visualization of peroxidase staining of the corresponding polypeptide bands was performed by enhanced chemiluminescence (WB Luminol Reagent: sc-2048).

The obtained results of the total protein content were displayed as the number of mg per 1 ml of extract. The relative content of every molecular markers was calculated as the ratio of the intensity of staining of samples of the experimental groups to the corresponding samples of the control group. The content values were expressed as a percentage relative to the values of the control group, which ensured reliable comparison and objective interpretation of the obtained data. Statistical processing of the obtained results was performed using the Microsoft Office Excel 2019 Data Analysis package. The sample parameters presented in the work had the following designations: M – sample mean; SD – standard deviation. Changes in indicators were considered significant at $P<0.05$ (including $P<0.01$ and $P<0.001$).

Results

The content of the intercellular adhesion protein E-CAD was determined to assess the density of the epithelial barrier of the small intestine of piglets in the control group and piglets that consumed additional IPM. The results of the determination of E-CAD in the duodenum of piglets in the control group showed a moderate increase from the 9th to the 21st day of life and a subsequent slight decrease from the 21st to the 35th day (Fig. 1). In particular, the increase in the relative content of E-CAD was approximately 10% ($P<0.05$), which reflects a moderate increase in the density of intercellular connections and the stability of the epithelial barrier. The results of the determination of E-CAD in the duodenum of piglets in the experimental group showed the opposite dynamics, i.e. a decrease in the content of E-CAD from the 9th to the 21st day and a subsequent increase from the 21st to the 35th day of life.

Similar dynamics in changes of E-CAD were observed in the duodenum of piglets of the control group where content of FN increased by 8.3% ($P<0.05$) from the 9th to the 21st day of life and a further decreased from the 21st to the 35th day (Fig. 1). Thus, before weaning, the level of cell-cell and cell-ECM adhesion markers changed slightly over the entire observation period. On the other hand, in the control group, the level of both of these markers decreased after weaning, which may serve as an indicator of metabolic complications and the development of stress due to a change in feeding.

Piglets treated with IPM had higher levels of E-CAD (50.1% higher than control, $P<0.01$) and FN (55.3% higher, $P<0.01$) expression already on the 9th day of life, which may indicate the early formation of a more stable intestinal barrier. Although the above markers decreased by day 21, their levels remained significantly higher than in the control group, and after weaning they increased again (E-CAD 59.5% higher than control, FN 56.3% higher, $P<0.01$).

The results of determining the content of immune markers INF-α and TNF-α showed a progressive increase in both of these indicators in the duodenum of piglets of the control group (Fig. 1). The level of increase in the relative content of TNF-α from the 9th to the 21st day of life was 3 1.3% ($P<0.01$). At the same time, changes in the relative content of TNF-α from the 21st to the 35th day of life were close to The level of such changes that were determined for the previous period from the 9th to the 21st day in this group. In contrast to the control group, the changes in the content of TNF-α in the IPM-exposed group were less significant and amounted to a 21% increase for the period from the 9th to the 21st day. A slight decrease in this indicator was observed for the period from the 21st to the 35th day of life (Fig. 1).

Comparative analysis of the same age groups showed that the level of TNF-α was statistically lower in the experimental group piglets – 18.0% less ($P<0.01$) on the 9th day compared to the control. In the group of IPM- exposed piglets after weaning on the 35th day, the level of TNF-α was found to be 62.0% lower than in the control group piglets ($P<0.001$). This result indicates a progressive decrease in TNF-α as an indicator of inflammatory stress.

The results of determining the content of INF-α showed a statistically significant increase in the IPM-exposed group by 47% during the period from the 21st to the 35th day of life (Fig. 1). Thus, the detected IPM-induced changes in the immune markers INF-α and TNF-α were oppositely directed and together contributed to the inhibition of the inflammatory process.

The content of the molecular marker of apoptosis Casp-3 was determined in order to assess the viability of small intestinal cells. The results showed a statistically significant increase in Casp-3, which was 29% ($P<0.05$) in the control group for the period from the 9th to the 21st day of life. At the same time, a more progressive increase in the content of Casp-3 by 64.2% ($P<0.01$) was detected in samples of piglets from the IPM-exposed group for the period from the 9th to the 35th day of life.

Comparative analysis of Casp-3 content in the control and IPM-exposed groups revealed the most significant difference ($P<0.01$) between the groups on day 35 of life. The obtained results indicate an anti-apoptotic effect of IPM in terms of preserving intestinal epithelial cells and maintaining their viability.

The results of determining the content of molecular markers in the jejunum of piglets of the control group, as well as in the duodenum, were variable during the period from the 9th to the 35th day of life. The changes detected primarily reflected the process of adaptation to changes in nutrition and the effect of weaning (Fig. 2). The increase in the content of E-CAD from the 9th to the 21st day

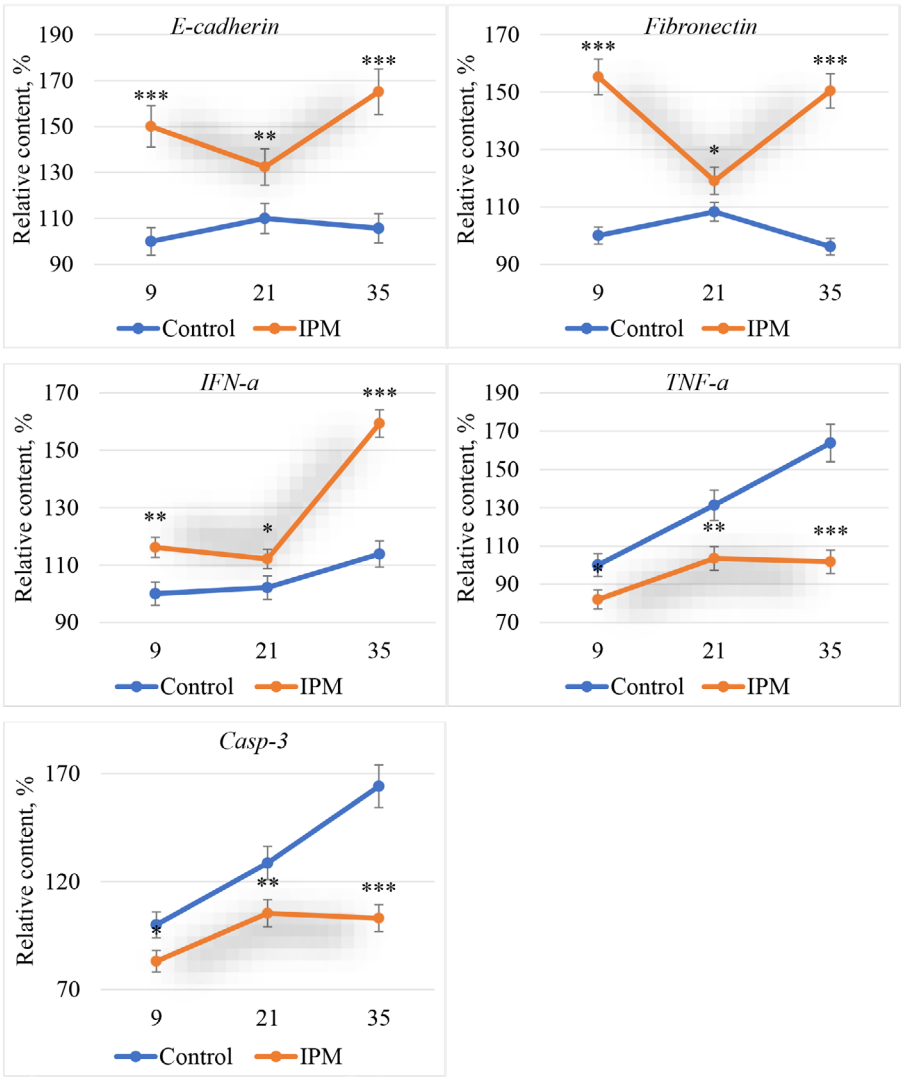

Fig. 1. The content of molecular markers in the duodenum of piglets of the control and IPM-exposed groups. The relative content is presented in percentages, where the value of the corresponding marker in the control group on the 9th day of life is taken as 100% (M ± SD; n = 5). Significance of differences: * – P<0.05, ** – P<0.01, *** – P<0.001.

by 4.0% (P>0.05) was statistically insignificant, but to some extent indicated an increase in the number of intercellular contacts (Fig. 2).

However, this process was progressive after weaning while the growth in the group of animals on the 35th day was equivalent to 9.4% (P<0.05). Observed results obtained indicate the strengthening of metabolic pathways that provide compensatory reactions of the body. Similar trends were observed for FN, the increase in the level of which was 113.8 ± 4.0%. Considering the role of FN in the formation of ECM, the increase in the level of this protein is accompanied by a gradual compaction of cell-matrix interactions and the tissue matrix itself. At the same time, the level of TNF-α increased quite progressively and amounted to an increase of 72.5% (P<0.01). Together, these results indicate a significant activation of inflammatory processes associated with weaning stress.

The results showed that in piglets of the IPM-exposed group there was a significant variability in the content and dynamics of changes in molecular markers. On the 9th day, the level of E-CAD was 32.1% higher (P<0.01), which indicated the early formation of a more effective intestinal barrier. Despite the fact that by the 21st day a slight decrease of 9.0% was observed, protein level increased by 22.2% (P<0.01) after weaning, which contributed to the improvement of the density of intercellular connections. Similarly, FN showed a significant increase - 58.9% more than in the control group (P<0.01), which indicated the active support of the architecture of the intestinal epithelium. The most striking changes were observed in the dynamics of TNF-α - its level remained 90.6% lower than the control (P<0.001), indicating a significant reduction in inflammatory stress. Similar changes to TNF-α were found in the level of Casp-3, which remained consistently low (60.1% lower than the control level, P<0.001), indicating the preservation of the intestinal cellular composition and lower activation of apoptosis.

The use of IPM significantly improved the productive indicators of piglets, in particular live weight, average daily gain and level of survival. Already on the 9th day, the body weight of piglets in the experimental group exceeded the control values by 4.8%, which indicates the early effect of IPM on growth and development processes. By the 21st day, this advantage increased to 18.1% (P<0.01), which indicates an increase in the efficiency of nutrient

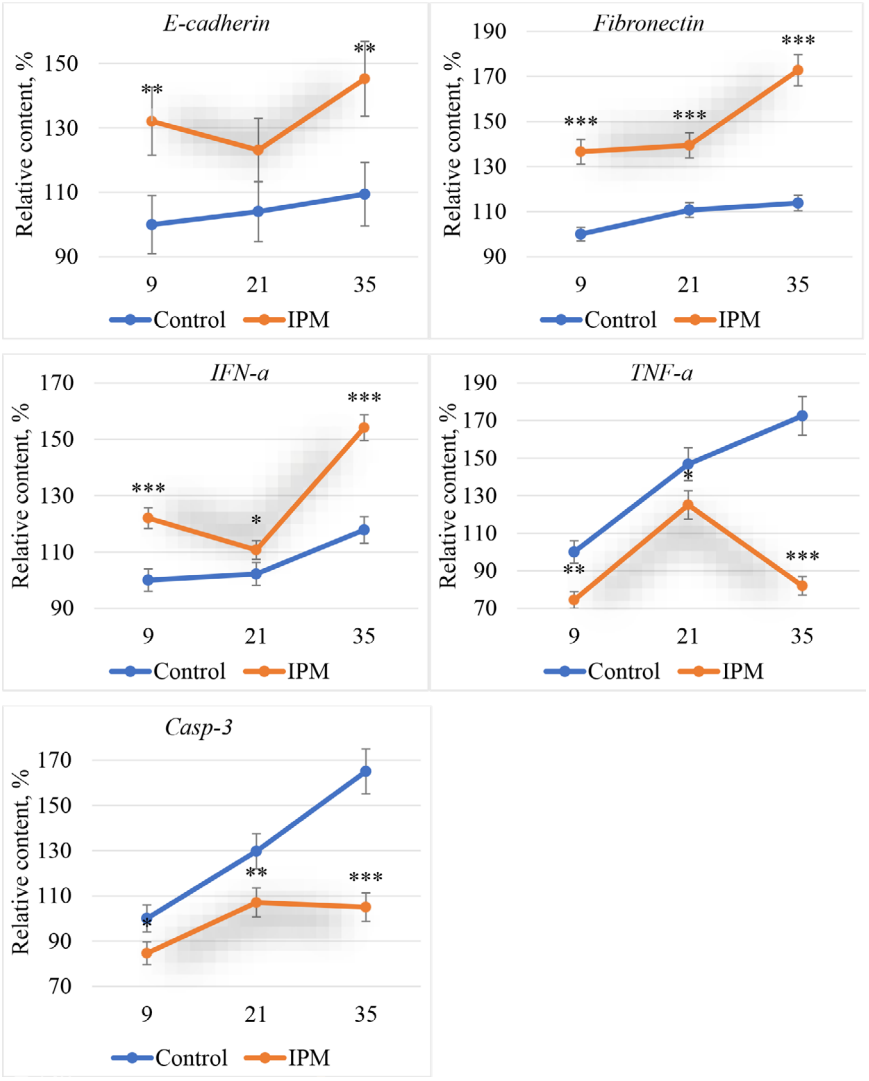

Fig. 2. The content of molecular markers in the jejunum of piglets of the control and IPM-exposed groups. The relative content is presented in percentages, where the value of the corresponding marker in the control group on the 9th day of life is taken as 100% (M ± SD; n = 5). Significance of differences: * – P<0.05, ** – P<0.01, *** – P<0.001.

absorption and intensification of metabolic processes. By the 35th day, the body weight of piglets in the experimental group remained stably higher by 9.5% (P<0.05) compared to the control group, which confirms the long-term effect of IPM on the formation of live weight. In addition to increasing body weight, piglets in the experimental group had a higher average daily gain of 11.0% (P<0.05) compared to the control group, indicating improved feed conversion and more efficient absorption of macro- and micro-nutrients.

Discussion

The early postnatal period of piglets is characterized by intensive morphofunctional changes in the digestive tract aimed at adapting to autonomous nutrition (Tang et al., 2022). Immediately after birth, the intestine is characterized by increased permeability, which is due to the morphological and functional immaturity of the epithelial layer and insufficient development of intercellular contacts. The formation of the intestinal barrier is a multicomponent process that includes the structural differentiation of the epithelium, the maturation of immune mechanisms and the gradual colonization of the intestine by commensal microorganisms (Ding et al., 2024). These adaptive changes play a crucial role in the establishment of the intestinal barrier function, providing protection against pathogens and optimal absorption of nutrients (Zhao et al., 2021).

The results of the presented study indicate a multidirectional beneficial effect of IPM on the integrative properties of the intestinal barrier of piglets in the first weeks of life and, in particular, during the period of stress caused by weaning and change of feeding. The presented results indicate a significant support and development of barrier function through the modulation of the content of E-CAD and FN (Fig. 1). The important role of these molecular markers has been demonstrated recently in the model of stress in piglets induced by enteric viral injection (Masiuk et al 2024) of the immune response (Fig. 1). In addition, the detected IPM-caused modulation of the content of E-CAD and FN indicates the activation of tissue repair processes including epithelial barriers.

Any stress is accompanied by an imbalance of regulatory

pathways of pro-inflammatory and anti-inflammatory processes. The results of determining the content of TNF-α and IFN-α showed that the consumption of IPM by piglets in the first week after birth significantly improves the balance of production of these cytokines. In turn, this inhibits excessive activation of the inflammatory response in response to stress and prevents pro-inflammatory damage and metabolic disorders.

The status of molecular markers of the intestinal barrier, such as adhesion proteins (occluding, E-CAD, FN), pro-inflammatory cytokines (TNF-α), anti-inflammatory cytokines (IFN-α) and pro-apoptotic factors (Casp-3) is an important and sensitive indicator of the functional integrity of the intestine. Assessment of changes in the expression of these markers allows us to detect the initial stages of intestinal barrier disorders, determine the mechanisms of inflammatory and degenerative processes, and also timely diagnose the risks of developing pathologies of the digestive tract (Du et al., 2023). In addition, monitoring of these molecular indicators contributes to the justification of effective strategies for the prevention and treatment of intestinal barrier dysfunctions, and also allows us to assess the effectiveness of the use of modern feed additives and therapeutic agents in maintaining animal health and productivity (Masiuk et al., 2023).

The most realistic mechanism of action of IPM may be the cellular response to the biologically active compounds of this mixture on metabolic pathways that are associated with the complex modulation of cell adhesion, immune response and regenerative processes in the piglet intestine (Hu et al., 2021). The high level of E-CAD and FN in piglets of the experimental group may indicate the stimulation of intercellular interactions and the enhancement of the intestinal barrier function, which prevents the penetration of pathogens . In addition, IPM-induced modulation of the content of E-CAD and FN makes a certain contribution to the maintenance of the architecture of the intestinal epithelium through both types of cell-cell and cell-ECM interactions that was demonstrated in recent report (Purich et al., 2025).

The relative decrease in TNF-α levels observed in the present study indicates an anti-inflammatory effect of IPM, which contributes to a decrease in the level of chronic inflammation in the intestine. Also, the decreased expression of Casp-3 may indicate a protective role of IPM in reducing apoptosis of intestinal epithelial cells, which contributes to the preservation of its integrity after weaning (Pan et al., 2021; Li et al., 2025). Therefore, it can be assumed that IPM acts by improving intercellular adhesive bonds, regulating the immune response and reducing the level of inflammatory processes in the intestine. All this together provides better adaptation of piglets after weaning, which is the most critical factor for preventing stress complications.

The formation of intercellular junctions in young piglets is a critical process that determines the functionality of the intestinal barrier and its protective properties. The immaturity of tight junctions in the first days of life ensures the absorption of immunoglobulins, but also increases the risk of pathogen penetration (Wang et al., 2016). However, the gradual maturation of intercellular junctions under the influence of hormonal, microbiological and nutritional factors contributes to the stabilization of the intestinal barrier function and its increased resistance to adverse factors (Masiuk et al., 2024).

The obtained results suggest that the isotonic-protein mixture has a complex effect on the intestinal barrier, strengthening it through stimulation of the expression of E-CAD and FN, which contributes to the maintenance of the intestinal cellular structure. The anti-inflammatory effect, confirmed by a decrease in the level of TNF-α, is likely to be realized through the modulation of signaling pathways associated with NF-κB, which plays a key role in inflammatory processes (Li et al., 2022). The simultaneous decrease in the level of Casp-3 (Fig. 1) can reflect a decrease in apoptosis which may be the result of the protective effect of IPM on epithelial cells (Pan et al., 2021). Observed in present study significant increase in Casp-3 levels (by 65.1%, $P<0.01$) in control group reflect apoptosis initiation, which is likely a consequence of destabilization of the intestinal barrier function, as demonstrated by the results of recent report (Zhao et al., 2021).

Thus, it can be hypothesized that the main mechanism of action of IPM is associated with the modulation of molecular pathways that maintain cellular integrity and regulate inflammatory processes, which ultimately contributes to better adaptation of piglets to weaning.

It is obvious that the improvement of piglet productivity is associated with a number of effects that have been noted by other researchers, in particular, with the improvement of the functional state of the intestine (Masiuk et al., 2024). Furthermore, recent data in respect with a decrease in the level of apoptosis of the intestinal epithelium and stabilization of the processes of nutrient absorption which was previously demonstrated by us in the analysis of biochemical and molecular indicators (Wang et al., 2022; Zhang et al., 2023). An important factor confirming the beneficial effect of IPM on the health and adaptive capabilities of piglets is an increase in the level of survival by 13.1% in present study. In general, the results obtained in our study indicate the beneficial effect of IPM, which is realized by reducing stress load, supporting the immune response and improving the general condition of the body.

Thus, the results of the study indicate that the use of IPM in piglet feeding contributes to the optimization of protein, energy and mineral metabolism, stabilization of intestinal barrier function, reduction of inflammatory response and apoptosis, as well as normalization of the microbial environment. The use of IPM may be an effective strategy to reduce weaning-related stress, maintain health, intensify growth, increase survival and improve adaptation of piglets in the critical postnatal period.

Conclusions

The results of the study showed multifactorial effect IPM supplementation detected with molecular markers applying. IPM supplementation induced dynamic modulation of intestinal barrier integrity, supporting immunity and the balance between pro-inflammatory and anti-inflammatory regulatory links, inhibits the initiation of programmed cell death. All this together improves intestinal functioning. After weaning, in the duodenum of piglets, there is a decrease in the concentration of structural proteins (E-cadherin by 59.5% and FN by 56.3%; $P<0.001$) and an increase in pro-inflammatory and pro-apoptotic markers (TNF-α, Casp-3), which indicates a disfunction of the intestinal barrier. The use of an isotonic protein mixture contributed to an increase in the levels of E-cadherin (by 59.5%) and FN (by 56.3%; $P<0.001$), as well as a decrease in the concentrations of TNF-α and Casp-3 (by 60.7%; $P<0.001$), which indicates its significant protective effect, stabilization of barrier function and reduction of inflammatory and apoptotic processes in the intestinal epithelium of piglets after weaning.

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Conflict of interests

The authors of this article declare no conflict of any interests or personal relationship regarding this manuscript.

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