

CURRENT APPROACHES IN MAINTENANCE GUT HEALTH IN FARMING ANIMALS. PART 1. THE ISOTONIC PROTEIN BLEND INHIBITS PORCINE EPIDEMIC DIARRHEA VIRUS EXCRETION AND AMELIORATES INTESTINAL BARRIER

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INTRODUCTION

Modern requirements for limiting the antibiotic use in animal husbandry are becoming widespread in most countries^{1, 2, 3}. In this regard, research into feed additives as antibiotic substitutes or targeted aids is gaining considerable interest^{4, 5, 6}. The main objective of evaluating antibiotic alternatives for sustainable production is to assess their effectiveness to support intestinal health, immune response, and productivity⁷. A special place among the alternatives is occupied by a feed mixture^{8, 9, 10}. In addition to protein, such

¹ Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health / B. Catry et al. *International Journal of Antimicrobial Agents*. 2015. Vol. 46, No. 3. P. 297–306.

² Tasho R. P., Cho J. Y. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment*. 2016. Vol. 563–564. P. 366–376.

³ McEwen S. A., Collignon P. J. Antimicrobial resistance: one health perspective. *Microbiology Spectrum*. 2018. Vol. 6, No. 2.

⁴ Multidrug-resistant bacteria and alternative methods to control them: an overview / R. Vivas et al. *Microbial Drug Resistance*. 2019. Vol. 25, No. 6. P. 890–908.

⁵ Potential of aromatic plant-derived essential oils for the control of foodborne bacteria and antibiotic resistance in animal production: a review / L. Zhang et al. *Antibiotics (Basel)*. 2022. Vol. 11, No. 11. Art. 1673.

⁶ Apple Polyphenols Improve Intestinal Antioxidant Capacity and Barrier Function by Activating the Nrf2/Keap1 Signaling Pathway in a Pig Model / T. Huang et al. *Journal of Agricultural and Food Chemistry*. 2022. Vol. 70, No. 24. P. 7576–7585.

⁷ Protective effect of berberine against LPS-induced injury in the intestine: a review / F. Izadparast et al. *Cell Cycle*. 2022. Vol. 21, No. 22. P. 2365–2378.

⁸ Yefimov V., Kostushkevych K., Rakytiarskyi V. Effect of feeding treated peat as a supplement on the parameters of cellular immunity, antioxidant status and performance of piglets in early post-weaning period. *Human & Veterinary Medicine*. 2016. Vol. 8, No. 3. P. 133–136.

⁹ Multidrug-resistant bacteria and alternative methods to control them: an overview / R. Vivas et al. *Microbial Drug Resistance*. 2019. Vol. 25, No. 6. P. 890–908.

¹⁰ Effects of supplemental myo-inositol on growth performance and apparent total tract digestibility of weanling piglets fed reduced protein high-phytate diets and intestinal epithelial cell proliferation and function / T. Z. Ogunribido et al. *Journal of Animal Science*. 2022. Vol. 100, No. 7. Art. skac187.

a mixture contains a large number of nucleotides, B vitamins, amino acids and polysaccharides^{11, 12}. Being an important component, nucleotides are particularly important in improving growth performance, regulating immune function and healing the animals' gastrointestinal tract. However, protein mixtures from different manufacturers have an extremely high divergence in respect with biological activity^{13, 14, 15}. That is why different protein mixtures' molecular and cellular effects require detailed study in order to determine their effectiveness and prospects for use as an alternative to antibiotics in animal husbandry.

The benefits for using protein mixtures in poultry production have already been described in recent studies. However, the potential of using protein mixtures in pig production, in particular to maintain piglet health, remains unclear. One of the protein mixtures' protective mechanisms may be the intestinal microbiota modulation and improvement of intestinal resistance to external damage¹⁶. The intestinal barrier function is primarily provided by intercellular adhesion proteins of the intestinal epithelial layers^{17, 18}. The content of such proteins causes the barrier to strengthen, or conversely, their loss leads to growth of intestinal permeability for the number of enteropathogens^{19, 20, 21}. The weakening of the intestinal barrier function leads to microorganisms and toxins entering the integumental system cells²². Similar invasion initiates cellular damage and increases the infectious diseases risk.

¹¹ Effects of dietary addition of heat-killed *Mycobacterium phlei* on growth performance, immune status and anti-oxidative capacity in early weaned piglets / J. F. Zhong et al. *Archives of Animal Nutrition*. 2016. Vol. 70, No. 4. P. 249–262.

¹² Chlorogenic acid improves intestinal morphology by enhancing intestinal stem-cell activity / Y. Qin et al. *Journal of the Science of Food and Agriculture*. 2023. Vol. 103, No. 7. P. 3287–3294.

¹³ Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks / J. Zhao et al. *Poultry Science*. 2010. Vol. 89, No. 10. P. 2147–2156.

¹⁴ Lee M. A. A global comparison of the nutritive values of forage plants grown in contrasting environments. *Journal of Plant Research*. 2018. Vol. 131, No. 4. P. 641–654.

¹⁵ Petry A. L., Patience J. F. Xylanase supplementation in corn-based swine diets: a review with emphasis on potential mechanisms of action. *Journal of Animal Science*. 2020. Vol. 98, No. 11. Art. skaa318.

¹⁶ Weaning stress and intestinal health of piglets : A review / X. Tang et al. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1042778.

¹⁷ Changes in progenitors and differentiated epithelial cells of neonatal piglets / L. Yin et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 265–276.

¹⁸ Intestinal single-cell atlas reveals novel lymphocytes in pigs with similarities to human cells / J. E. Wiarda et al. *Life Science Alliance*. 2022. Vol. 5, No. 10. Art. e202201442.

¹⁹ MicroRNA-29a mediates the impairment of intestinal epithelial integrity induced by intrauterine growth restriction in pig / Y. Zhu et al. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. 2017. Vol. 312, No. 5. P. 434–442.

²⁰ Protecting intestinal epithelial cells against deoxynivalenol and *E. coli* damage by recombinant porcine IL-22 / Y. Li et al. *Veterinary Microbiology*. 2019. Vol. 231. P. 154–159.

²¹ Combined effects of cadmium and ochratoxin A on intestinal barrier dysfunction in human Caco-2 cells and pig small intestinal epithelial cells / S. H. Kim et al. *Mycotoxin Research*. 2022. Vol. 38, No. 4. P. 253–264.

²² Combined Soluble Fiber-Mediated Intestinal Microbiota Improve Insulin Sensitivity of Obese Mice / C. Xu et al. *Nutrients*. 2020. Vol. 12, No. 2. Art. 351.

Small piglets are particularly susceptible to infectious diseases. The intestinal barrier is a critical target for many enteroviruses. One of the most contagious and widespread in the world and in Ukraine is Porcine Epidemic Diarrhea Virus (PEDV)^{23, 24}. There are a lot of results which evidence that PEDV replicates in the absorptive epithelial cells of the small intestine and induces apoptosis and necrosis, epithelial cell detachment and involvement of villi stroma cores^{25, 26, 27}. This leads to a decrease in the ratio of villi height to crypt depth and thereby damages the intestinal barrier^{28, 29}. However, at the present time the molecular mechanisms of intestinal barrier disruption induced by coronaviruses remain unclear. In this regard, the search for products that would contribute to the strengthening and rapid intestinal barrier recovery under the pathogenic effect of enteroviruses is currently an important research area^{30, 31}.

Therefore, our aim was to find out the role of adhesive proteins in maintaining intestinal barrier in piglets affected with coronavirus enteric infection.

1. Materials and methods to assess the intestinal disturbances in PEDV infected animals

A model experiment was conducted with using the PEDV infected suckling piglets to demonstrate the state of the intestinal barrier via measuring molecular markers level. Two groups of piglets (control and experimental) were formed with two weeks aged 30 animals, accordingly 15 animals in each group.

The highly contagious entero-tropic epidemic diarrhea virus (PEDV) was used to initiate the damaging of the intestinal barrier integrity. All piglets 14 days aged were infected with PEDV at the dose of 104 viral particles per

²³ Molecular characteristics of the Porcine Epidemic Diarrhea Virus strains isolated in different regions of Ukraine / D. Masiuk et al. *Biopolymers and Cell*. 2019. Vol. 35, No. 6. P. 486–494.

²⁴ Porcine Epidemic Diarrhea Virus: an updated overview of virus epidemiology, virulence variation patterns and virus-host interactions / Y. Zhang et al. *Viruses*. 2022. Vol. 14, No. 11. Art. 2434.

²⁵ Jung K., Saif L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Veterinary Journal*. 2015. Vol. 204, No. 2. P. 134–143.

²⁶ Cellular entry of the porcine epidemic diarrhea virus / W. Li et al. *Virus Research*. 2016. Vol. 226, No. 2. P. 117–127.

²⁷ BST2 suppresses porcine epidemic diarrhea virus replication by targeting and degrading virus nucleocapsid protein with selective autophagy / N. Kong et al. *Autophagy*. 2020. Vol. 16, No. 10. P. 1737–1752.

²⁸ The influence of porcine epidemic diarrhea virus on pig small intestine mucosal epithelial cell function / X. Y. Wang et al. *Archives of Virology*. 2019. Vol. 164, No. 1. P. 83–90.

²⁹ LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of alix after Porcine Epidemic Diarrhea Virus infection / W. Qin et al. *Journal of Virology*. 2023. Vol. 97, No. 3. Art. e0188422.

³⁰ Alterations of suckling piglet jejunal microbiota due to infection with Porcine Epidemic Diarrhea Virus and protection against infection by *Lactobacillus salivarius* / W. Dong et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 771411.

³¹ Oral administration of Lactic acid bacteria inhibits PEDV infection in young piglets / S. Yang et al. *Virology*. 2023. Vol. 579. P. 1–8.

animal. The piglets of both groups were fed with a whole milk replacer, and the experimental group was additionally fed with 3% isotonic protein mixture (IPM) by free-access manner.

To assess the infection process in control and experimental group of piglets, rectal swabs and blood serum were collected from piglets on day 14 (before virus infection) and on the 21st, 28th, 35th and 42nd day of life. According to this scheme 3 piglets of each subgroup were euthanized to study dynamic changes of small intestine cells. Samples of duodenum tissue were taken from the piglets and stored frozen at -18 to -22°C for examination.

All experimental procedures with piglets were carried out in compliance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.2006 “On the Protection of Animals from Brutal Treatment”, in accordance with the basic principles of the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), and approved by the Local Commission for Bioethics and Animal Experiments of Dnipro State Agrarian and Economic University.

qPCR. The content of PEDV genetic material was determined in rectal swabs by qPCR method.

Nucleic acids were extracted from rectal swabs in several stages. Firstly, rectal swabs were placed in a test tube to which lysis buffer solution ATL Buffer (Biosellal, France) and quartz beads were added according the recommendations of producer. The test tube was placed in the rotor of the FastPrep-24 homogenizer, where the swabs were crushed. A volume of 200 µL was collected from each homogenate sample. Nucleic acids (NA) were extracted using a commercial reagent kit “BioExtract Premium Mag” on a KingFisher Duo automatic nucleic acid extraction device.

The cDNA content was determined in the NA extracts using the “oneMIX & PEDV” test kit (Exolpol, Spain). Amplification and detection of the results were performed using a CFX 96 instrument (BioRad, USA). Thermal cycling was performed according to the test kit instructions.

All samples were analysed in technical triplicate, including the corresponding negative controls (distilled water without DNase/RNase). The PCR efficiency and correlation coefficients of the standard curves were in the 89.20–111.00% range, and R² was from 0.988 to 0.999, indicating a sufficiently high level of linear dependence. The sensitivity limit for qPCR was assessed as 10–100 copies of cDNA from three independent tests.

To verify the qPCR linearity and dynamic range, standard curves were constructed by making 10 times serial dilutions of a positive PEDV cDNA control sample with a certain copy number. The qPCR results were calculated

and analysed using the CFX Manager software, and the amplification efficiency (E) was calculated using the formula $E = (10^{-1/\text{slope}}) - 1$.

ELISA. Serum immunoglobulins of class G (IgG) specific for PEDV antigens were determined by an indirect ELISA method using a commercial diagnostic system "ID Screen® PEDV Indirect" (ID, France) on an ELx800 photometer analyzer (BioTek, USA).

In accordance with the recommendations for the diagnostic kit, the serum was diluted 1/10 before testing. A sample was considered positive if the sum of products of deviations (SP) value was more than or equal to 60%. In accordance with the instructions of the test system used, the level of antibodies in the pig blood serum is proportional to the SP value.

Western blot. The amount of E-cadherin, fibronectin and interferon- α proteins was determined in duodenum tissues by Western blot (WB) method.

To prepare protein extracts of the aforementioned molecular markers small intestine tissue every individual was homogenized with phosphate buffer pH 7.6 containing 1% Triton X100, 0.2% sodium dodecyl sulphate and inhibitors cocktail. The total protein concentration in each sample was determined by the Bradford method (Bradford, 1976). Protein extract samples were separated by electrophoresis in a polyacrylamide gel (PAAG) with an acrylamide gradient of 7–18% (Laemmli, 1970). To control the efficiency of individual polypeptide separation, several tracks were additionally stained with a 0.1% Coomassie G-250 solution. The transfer of proteins from PAAG to nitrocellulose was carried out using an electric field for 120 min at a current of 250 mA. The blocking of unspecific absorbance onto nitrocellulose was performed in a 3% solution of skimmed milk powder at 60 min. After blocking, the nitrocellulose membranes were treated with a 1:25 00 dilution of specific rabbit antiserum (E-cadherin, fibronectin and interferon- α Santa-Cruz), washed in phosphate buffer and incubated for 60 min in a solution of peroxidase-labelled secondary antibodies (anti-mouse Santa-Cruz). To visualize the polypeptide zones, the nitrocellulose membrane was treated with a 50 mM tris-HCl pH 7.4 solution containing 0.05% diaminobenzidine and 0.01% hydrogen peroxide. The results of total protein content and immunoblotting were expressed as a percentage of the control group.

The results were statistically processed using the specialized software Statistica 6 (StatSoft Inc, USA). The differences significance was assessed by the Student's t-test. The sample parameters presented in the paper had the following designations: M – sample mean; m – standard error of the mean. Changes in indicators were considered significant at $P < 0.05$ (including $P < 0.01$ and $P < 0.001$).

2. Results on PEDV excretion dynamics

The results of the PEDV PCR study indicate that before infection both control and experimental group had no virus RNA in rectal swabs (Fig. 1).

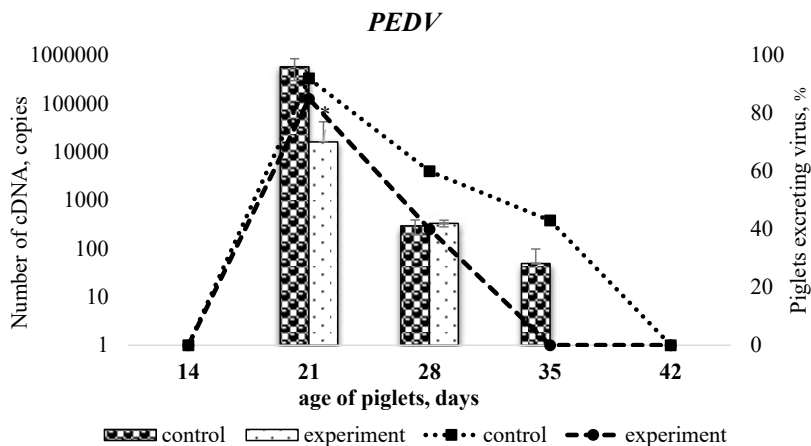


Fig. 1. Duration of PEDV excretion into infected piglets' feces of control group and group consumed IPM ($M \pm SEM$, $n = 16$)

The highest amount of virus was detected in rectal swabs of piglets on the 21st day of life. Over time, the virus excretion level decreases, as indicated by a small amount of cDNA in rectal swabs of control piglets on the 28th and 35th day of life. Complete virus excretion stops only on day 42 after infection. In the experimental group, the virus was detected only at the 21st and 28th day of life. In 21-day-old piglets of the experimental group, the amount of virus in rectal swabs was significantly lower by 10 times, and the number of virus-positive animals was 7% less than in the control group.

There was no difference found between the experimental and control group piglets in the amount of excreted virus on day 28 of life. Besides, there was a significant difference between the percentage of animals with the virus replicating in their bodies. Thus, in the experimental group of piglets, 20% fewer animals containing PEDV cDNA in rectal swabs were detected compared to the values of the control group.

The lowest level of animals excreting PEDV was found in the control group on day 35 of the life. At the same time, the amount of PEDV cDNA in rectal swabs from these animals was the lowest for the entire study period. Besides, there was no PEDV cDNA detected in the rectal swabs of the experimental

group which exposed to IPM. Moreover, all examined rectal swabs from piglets on the 42nd day of life did not contain PEDV cDNA.

The results of the intestinal barrier molecular markers study in the duodenum tissues characterize the changes in the content of E-cadherin, fibronectin and INF- α in piglets of control and experimental groups affected with PED infection (Table 1).

Table 1

Relative content of the molecular markers in the duodenum tissues of the piglets infected with PEDV ($M \pm m$, $n=3$)

Age of piglets, days	E-cadherin		Fibronectin		Interferon- α	
	control	experiment	control	experiment	control	experiment
21	101,2 $\pm$ 3,2	114,3 $\pm$ 5,8	99,7 $\pm$ 3,1	103,1 $\pm$ 5,2	107 $\pm$ 4,7	105 $\pm$ 5,5
28	99,5 $\pm$ 4,7	116,4 $\pm$ 6,2*	98,9 $\pm$ 4,5	106,4 $\pm$ 6,1	195 $\pm$ 7,6	291 $\pm$ 8,2**
35	99,7 $\pm$ 4,5	122,8 $\pm$ 6,4**	101,2 $\pm$ 4,2	112,7 $\pm$ 3,9*	316 $\pm$ 9,2	217 $\pm$ 7,1***
42	102,3 $\pm$ 3,9	111,4 $\pm$ 4,5*	99,4 $\pm$ 3,8	109,2 $\pm$ 3,7*	221 $\pm$ 6,4	151 $\pm$ 5,3**

*Note: changes are considered to be statistically significant at * – $P < 0,05$, ** – $P < 0,05$, *** – $P < 0,001$ relative to the parameters of the animals of the control group.*

There was found that the content of E-cadherin in the piglets' intestinal tissues of the experimental group on day 28, 35 and 42 of life was significantly higher by 17%, 23% and 9% compared to the values of the control group.

Similar results were obtained for fibronectin content alteration into intestine tissue. The level of fibronectin in the duodenum tissues was significantly higher in piglets of the experimental group at 35 and 42 days of age about 11.5% and 9.8% ($P < 0.05$) accordingly compared to the values of the control group.

The results of the IFN- α study showed that its increase in piglets of the experimental group was almost 2 times ($P < 0.05$) on day 28 of life compared to the values of the control group. At 35 and 42 days of life, the IFN- α level in the duodenum tissues decreased sharply and was significantly lower by 2.0 and 1.7 times compared to the values of the control group of animals.

The study results of the PEDV antigen-specific IgG levels in the blood showed a nonlinear rising dynamics of seroconversion processes in piglets from day 21 to day 42 of the modelled infection (Fig. 2).

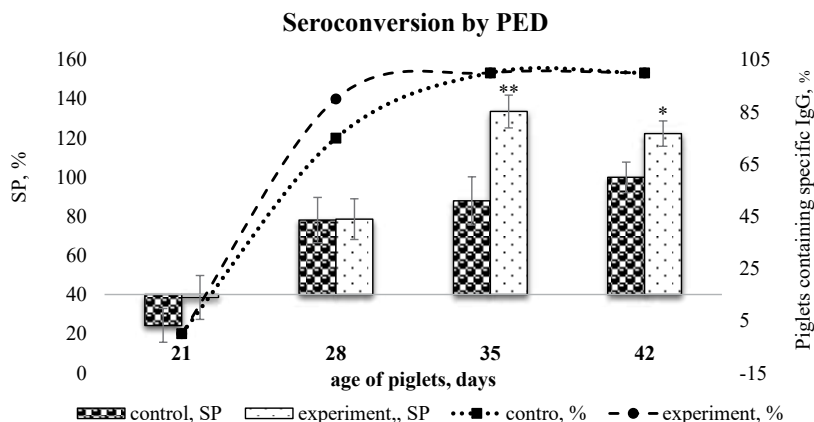


Fig. 2. Dynamics of IgG seroconversion specific against PEDV antigens in the piglet blood of control group and group consumed IPM (M±SEM, n = 16)

The feeding of the experimental piglet group with an IPM increases the number of immune animals by 15% at day 28 compared to the control group. The SP score in piglets of this age did not differ significantly between the experimental and control groups. All piglets of the experimental and control groups developed specific IgG to PEDV antigens in the blood at the 35th and 42nd day of life. The SP value in animals of the experimental group at the 35th and 42nd day of life was significantly higher by 52% and 22% ($P < 0.05$; $P < 0.05$), accordingly, compared to the piglets of the control group.

3. The protective effect of Isotonic Protein Formulation on PEDV proliferation

The obtained results characterize the changes in molecular markers content that the intestinal barrier in piglets at different stages of the infectious process remodeled with experimental PEDV infection. The course of the infectious process was assessed by the timing of virus elimination into environment from the piglets as well as with modulation of immune response in both the blood serum and duodenum tissue.

The PCR results showed that PEDV was excreted in feces up to the 35th day of life. Similar results have been demonstrated in several studies³². It was

³² Immune responses to porcine epidemic diarrhea virus (PEDV) in swine and protection against subsequent infection / V. D. Krishna et al. *PLoS ONE*. 2020. Vol. 15, No. 4. Art. e0231723.

found that the excretion of PEDV in feces lasted for 28 days after inoculation, and the virus persisted in intestinal tissues for a longer time, which, according to the authors, poses additional risks of virus spread into a pig farm.

We have found that the use of IPM in piglets reduces the PEDV excretion time by 7 days and inhibits the excreted virus content by 10 times. Similar results were demonstrated in the study on the treatment piglets with glycyrrhiza extract presented by Gao et al. (2020)³³. The authors note that glycyrrhizin suppresses PEDV infection and reduces the period of virus excretion by attenuating the secretion of proinflammatory cytokines.

Given the presented results and literature data, it can be assumed that the increased rate of PEDV elimination from the infected piglets may be due to intensified immunobiological response and faster restoration of the intestinal barrier integrity³⁴.

This hypothesis is confirmed by the results of present study. Obtained in our study data have shown that the shortening of virus excretion was accompanied by the immune response activation in piglets affected with PEDV infection. Furthermore, immune response could be associated with amelioration in epithelial cell adhesion via modulation tight junction molecular markers in the duodenum.

The results of our study demonstrated dynamic time-dependent changes in expression all of studied molecular markers. The revealed trend towards a more progressive increase in the content of E-cadherin and fibronectin in the experimental piglets' duodenum tissues is due to more significant proliferative processes in the intestine during PEDV infection against the background of feeding piglets with an IPM³⁵. Recently, there was reported that PEDV replication in enterocytes leads to the loss of tight junction proteins, including ZO-1, ZO-2, occludin and some claudins, indicating a disruption of intestinal integrity³⁶. Given that E-cadherin and fibronectin proteins are responsible for intercellular and cell-matrix contacts and ensure the integumentary barrier density³⁷, our results indicate a protective effect of the IPM and enhancement of repair processes in intestinal tissues³⁸.

³³ Glycyrrhizin inhibits PEDV infection and proinflammatory cytokine secretion via the HMGB1/TLR4-MAPK p38 pathway / R. Gao et al. *International Journal of Molecular Sciences*. 2020. Vol. 21, No. 8. Art. 2961.

³⁴ Wiarda J. E., Loving C. L. Intraepithelial lymphocytes in the pig intestine: T cell and innate lymphoid cell contributions to intestinal barrier immunity. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1048708.

³⁵ LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of alix after Porcine Epidemic Diarrhea Virus infection / W. Qin et al. *Journal of Virology*. 2023. Vol. 97, No. 3. Art. e0188422.

³⁶ Jung K., Saif L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Veterinary Journal*. 2015. Vol. 204, No. 2. P. 134–143.

³⁷ Schoutz I., Keita A. V. The intestinal barrier and current techniques for the assessment of gut permeability. *Cells*. 2020. Vol. 9, No. 8. Art. 1909.

³⁸ Developmental changes in intercellular junctions and Kv channels in the intestine of piglets during the suckling and post-weaning periods / J. Wang et al. *Journal of Animal Science and Biotechnology*. 2016. Vol. 7, No. 1. Art. 4.

Taking into the account that a wide range of entero-pathogens destroy the integumentary barrier, protective effect of IPM targeted to the support of enterocytes viability and their ability to form strong adhesive contacts as well as to enhance the proliferation into the intestine affected areas³⁹. All of aforementioned cellular activity alone or together require metabolic energy expenditure. The isotonic protein formulation biologically active components simultaneously stimulate feed intake, cellular energy metabolism, and local immune responses which is confirmed by observed results in present study⁴⁰.

We have found that in an experimental PEDV infection feeding piglets with IPM during all studied period of the suckling from day 14 of life contributes to a rapid increase in IFN- α levels by day 28 compared to the values of control group. Since interferons type I regulate a wide range of nonspecific protective responses in the intestine, it can be assumed that stimulation of IFN- α production could be a useful marker of increased innate immune activity⁴¹.

Feeding piglets with an IPM to enhance immune response against a progress of PEDV infection can restrict the period of seroconversion and reduces the period of immunological tolerance. Obtained results have shown that enhanced IFN- α synthesis in the experimental group piglets' intestinal tissues and increase in the level of seroconversion can reflect the protective effect of IPM exposure. This hypothesis is confirmed with observed data in respect to higher SP value in experimental group of piglets on days 35 and 42 of life. Similar results were obtained in a recent study of experimental infection of pigs with European strains of PEDV⁴². The authors observed the appearance of blood serum IgG specific to PEDV antigens on the 18th and 10th day after virus inoculation. Such changes are usually associated with antigen-stimulated seroconversion⁴³.

Therefore, the intestinal barrier is a critical component in ensuring the pig's health as it has a significant impact on permeability selectivity and the intestinal immune system⁴⁴. Effective recovery of the intestinal barrier integrity leads to a reduction in the period of recovery and viral shedding, while

³⁹ Assessing intestinal health. In vitro and ex vivo gut barrier models of farm animals: benefits and limitations / F. Ghiselli et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 723387.

⁴⁰ Glycyrrhizin inhibits PEDV infection and proinflammatory cytokine secretion via the HMGB1/TLR4-MAPK p38 pathway / R. Gao et al. *International Journal of Molecular Sciences*. 2020. Vol. 21, No. 8. Art. 2961.

⁴¹ Single-blinded study highlighting the differences between the small intestines of neonatal and weaned piglets / C. Yuan et al. *Animals*. 2021. Vol. 11, No. 2. Art. 271.

⁴² Experimental infection of pigs with recent European porcine epidemic diarrhea viruses / C. M. Lazov et al. *Viruses*. 2022. Vol. 14, No. 12. Art. 2751.

⁴³ Tran T. H. T., Everaert N., Bindelle J. Review on the effects of potential prebiotics on controlling intestinal enteropathogens *Salmonella* and *Escherichia coli* in pig production. *Journal of Animal Physiology and Animal Nutrition*. 2018. Vol. 102, No. 1. P. 17–32.

⁴⁴ Wiarda J. E., Loving C. L. Intraepithelial lymphocytes in the pig intestine: T cell and innate lymphoid cell contributions to intestinal barrier immunity. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1048708.

enhancing the body's immune responses and shortening the seroconversion period⁴⁵. Taking into the account aforementioned, the estimation of protein markers including E-cadherin and fibronectin constructs usable way to monitor the enterotropic drugs effect on the intestinal condition⁴⁶. Moreover, this strategy is prospective tool to determine the level of damage and the effectiveness of the intestinal barrier recovery in pigs under the influence of various alternative factors, including PEDV.

CONCLUSIONS

The peculiarities of PEDV excretion by piglets infected with a dose of 104 viral particles per animal showed that starting from 14 days of life, excretion progressively increased for the 21st day and then decreased until the 35th day of age. Starting from the 42nd day of life, the PEDV excretion in feces was finished completely.

Feeding piglets with an IPM stimulate the content increase in the adhesive proteins E-cadherin and Fibronectin in the duodenum tissues by an average of 17% and 11%, accordingly, and enhanced the synthesis of IFN- α and IgG specific to the PEDV antigen by 2 and 1.5 times, accordingly. Thus, the decrease in PEDV excretion may be due to the improvement of barrier function and innate immunity in the animals' intestines induced by the consumption of IPM.

SUMMARY

The intestinal barrier is an extremely important structure to maintain piglet health. However, various enteropathogens, including coronaviruses, are especially aggressive factors for the intestinal barrier destruction. That is why the aim of present study was to clarify the role of adhesive proteins in the piglet intestinal barrier maintaining affected with coronavirus enteric infection. For this purpose, we formed two groups of 14-day-old piglets to construct experimental model enteric disorder. Each animal was infected with porcine epidemic diarrhea virus (PEDV) at a dose of 104 virions. The piglets of the experimental group were additionally fed of isotonic protein mixture (IPM). The intensity of the infectious process was assessed by the duration of virus elimination from the piglets' body, the amount of excreted virus, the immune response efficacy. The level immune response was detected by specific IgG in the blood serum and α -interferon (IFN- α) in the intestinal tissues. The intestinal barrier integrity was assessed by the level of proteins

⁴⁵ Regulation of the intestinal barrier function by host defense peptides / K. Robinson et al. *Frontiers in Veterinary Science*. 2015. Vol. 2. Art. 57.

⁴⁶ Effect of riboflavin on intestinal development and intestinal epithelial cell function of weaned piglets / Y. Qin et al. *Journal of Animal Physiology and Animal Nutrition*. 2023. Vol. 107, No. 2. P. 518–528.

in the tissues that form tight intercellular contacts especially E-cadherin and fibronectin in the duodenum. The results have shown a variability in the duration of the PEDV elimination period in piglets' feces. The exposure to IPM provides a protective effect that enhances the recovery processes in the intestinal tissues. That was also reflected in an increase in the amount of E-cadherin and fibronectin in the duodenum. Furthermore, the isotonic protein mixture's protective effect was reflected in an increase in the body's immune responses into the intestinal level. This effect was evidenced by an increase in the amount of IFN- α in the duodenum and an increase in the synthesis of specific IgG in the blood serum. Obtained results showed a protective effect of IPM treatment while the intestinal barrier and immune responses were enhanced. IPM treatment induced about 10 times decrease in the amount of PEDV excreted in the feces in experimental animals and contributed to a 7-day reduction in the virus excretion period.

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