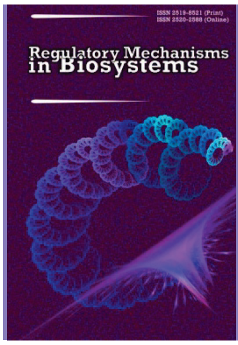

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Morphological characteristics of the duodenum of piglets fed with various feed additives

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Early weaning of piglets from the sow and a sharp transition to granular feeding usually leads to the development of feed stress. As a consequence, changes occur in the small intestine epithelium, which are associated with desquamation of the epithelium of the villi, crypt hyperplasia and atrophy of the villi, colonization of pathogenic microflora, which leads to a decrease in digestion and absorption of nutrients. Hence the aim of this study was to evaluate the effect of various feed additives on the morphofunctional state of the duodenal mucosa of piglets in the pre-weaning period. Forty piglets of the same age were involved in the experiment, which were divided into four groups of 10 head each. The first group of piglets was a control one and received a standard diet and free access to water from the 7th day of life. Additionally to their standard diet, the piglets of group II were given a drug which included a combination of live yeast of the genus *Saccharomyces* and a complex of 6 enzymes (proteases, cellulases, xylanases, α-amylases, β-glucanases and phytases) in the amount of 0.5 kg/t of feed. Piglets of group III received 0.2 kg/t of a feed additive consisting of a mixture of essential oils from plant extracts. Piglets of group IV were given a supplement with dry yeast and egg powder enriched with immunoglobulins. Piglets had constant access to feed and water. On the 14th and 28th day of the experiment, three piglets from each group were euthanized in compliance with ethical standards for animals used in experiments. According to the results of histological studies, it was established that the duodenum mucous membrane in piglets of all experimental groups was characterized by insignificant variability. Mostly, the villi had a leaf-like or finger-like shape. The crypts were mostly closely adjacent to each other. The muscle layer was well developed, represented by smooth muscles that diverged in the places where the excretory ducts of Brunner's glands pass. The lamina of the mucous membrane was represented by a loose connective tissue made of collagen fibers and, to a lesser extent, elastic fibers and small capillaries between which lymphoid cellular elements were located. The enterocytes of the apical surface of the villi had a well-defined brush border and elongated nuclei that occupied a third of the cytoplasm. Morphometric studies showed that, compared to the control group of piglets, the addition of feed additives with a mixture of essential oils increased the taste and smell of the feed, contributing to its better consumption, thus having a positive effect on the condition of the mucous membrane. On the 14th and 28th day, a significant increase in the height and width of the villi was noted. In piglets that received feed supplements with a combination of live yeast, positive dynamics were also observed in terms of increasing the ratio of villi height to crypt depth. Summarizing the experiment results, we can note that various feed additives had a positive effect on the condition of the duodenum mucous membrane, but the feed additive containing a mixture of essential oils from plant extracts was more effective.

Keywords: piglets; feed additives; yeast; plant extracts; digestion; villi; crypts; goblet cells.

Introduction

The intensive pace of pig farming requires early weaning of piglets from sows, which is usually accompanied by various stresses, primarily changes in feeding and adaptation to a new environment. Reduced feed intake and an increased number of piglets in a small area become the main risk factors for disrupting the structure and function of the intestines, which promotes the colonization of pathogenic bacteria in the intestines (Buddle & Bolton, 1992). Therefore, the key goal is a gradual transition from maternal milk feeding to solid feed consumption by piglets.

It is known that glucose is easily absorbed in the gastrointestinal tract of suckling piglets, and lactose is efficiently hydrolyzed to glucose and galactose in the small intestine with the help of lactase, which is intensively secreted in the mucous membrane in the first days after piglets are born. Lactase secretion gradually decreases with age. A newborn piglet cannot digest sucrose and starch from ready-made feed because sucrose is not synthesized in its organism in sufficient quantities. Suckling piglets are unable to absorb xylose and fructose, but from the very birth, they are capable of hydrolyzing and absorbing a large amount of fats (Zakhariv et al., 2020; Canibe et al., 2022). That's why during the first weeks of piglets' lives, there is a sharp transition from sow's milk to dry feed, which, along with the stress from separation, can lead to digestive disorders, causing diarrhea. This, in turn, makes veterinarians use antibacterial drugs, that negatively affect the microflora and morphology of the intestinal mucosa. In addition, a sharp transition to granulated feed may be accompanied by lower consumption with the subsequent development of malabsorption, which leads to piglet growth retardation.

In his research (De Lange et al., 2010), the author summarized data from many scientists who studied metabolic processes in the gastrointestinal tract (GIT) of pigs when using feed additives and noted that an important factor in pig feeding is the composition of diets with optimization and balance of proteins, amino acids, micro- and macro-elements, growth factors, and immunoglobulins, minimizing the content of anti-nutrients. It was noted that a deficiency of total protein in piglets caused their growth retardation, development of anemia, and serous atrophy of fat depots. A shortage of amino acids such as threonine, lysine, or methionine caused fatty degeneration of the liver (Hou et al., 2016; Hasan et al., 2020).

During the suckling period, lactic acid bacteria begin to appear in large quantities throughout the entire gastrointestinal tract of piglets, including the stomach. Large amounts of streptococci and coliforms colonize the lower sections of the intestine. In the first week, the intestine is colonized by *Clostridium welchii* microorganisms, which subsequently decrease (Holland,1990; Buntyn et al., 2016). After 2 to 3 weeks of piglets' life, the number of different types of microorganisms gradually increases in the segment from the duodenum to the caecum, and during the weaning period, their numbers reach maximum levels. Many researchers have proved that the morphology of the small intestine's mucous membrane partly depends on the interaction between the microflora and the diet, and metabolic adaptations associated with specific types of microorganisms and the symbiosis or antagonism relationship on a particular substrate play an important role. However, the composition of the diet has a greater influence on the intestine's flora than the content of microorganisms in it. It should also be noted that as a result of a sharp change in feeding, changes occur in the epithelium of the piglets' small intestine, including villus epithelium desquamation, crypt hyperplasia, and atrophy of villi. This leads to the colonization of pathogenic microflora, which results in decreased digestion and absorption of nutrients. According to Chopra et al. (2010) and Banerjee et al. (2018), there is a dynamic equilibrium between the constant enterocyte production at the crypts' base and desquamation of apical enterocytes of the mucosal layer of the villi in the small intestine of piglets until weaning. Therefore, over two to four days, the epithelium of the mucosal layer returns to its normal conditions. When parietal digestion is disrupted due to dystrophic changes or loss of enterocytes, changes in pH, osmotic pressure, or intestinal microflora changes, and atrophy of the villi can occur, which may be a consequence of reduced cell renewal rate due to decreased cell division in the crypts. These morphological changes can also be caused by changes in the small intestine microflora and dietary factors, which are more noticeable at the early stages of piglet weaning. Hampson (1986) showed that abrupt changes in the feeding and weaning of piglets at two weeks of age led to a decrease in villi length and an increase in crypt depth (Bontempo et al., 2006). According to data from Hampson, within 24 hours after weaning, a 50% reduction in villi height was observed compared to pre-weaning levels, with subsequent slow recovery (Hampson, 1986). In general, researchers (Caspary, 1992; Wiese et al., 2003) claim that the digestion and absorption in the small intestine of piglets become more efficient due to a significant increase in the area of its epithelial surface, which is provided by the mucous membrane. At the same time, villi and microvilli also increase the surface area of absorption. In addition, the small intestine's surface area increases many times due to the numerous folds of the submucosal layer (Caspary, 1992; Biagi et al., 2006). There is evidence that the small intestine of a 10-day-old piglet weighing 3 kg has a total absorption area of 114 m^2, which increases after weaning.

Miller et al. (1983) and Cera et al. (1988) reported in their studies a decrease in the microvilli length three to seven days after weaning, while in piglets that were not weaned, only a slight decrease in villi height was observed. Such morphological changes in the structure of the mucosal membrane of piglets' small intestine are of great significance (Miller et al., 1983; Cera et al., 1988).

In Ukraine, as well as in European Union countries, it is prohibited to use growth stimulants and antibiotics to ensure a stable transition from the pre-suckling period to weaning. Therefore, feed companies are increasingly engaged in developing alternative products, phytobiotics, pre- and probiotics, which can maintain a beneficial microflora of the gastrointestinal tract and affect various aspects of the digestive system development in piglets (Baum et al., 2002; Chen et al., 2019; FresnoRueda et al., 2021; Holanda et al., 2021).

For over 15 years, active research has been conducted worldwide on feeding suckling piglets with probiotics before weaning. The obtained results had several positive effects, such as promoting digestion, reducing pathogenic microflora while increasing positive microflora, contributing to rapid intestinal mucosa recovery, and improving immune response (Prudius & Vishchur, 2022). However, a stable effect of probiotic use was not achieved, as there were variable factors on which the experimental results depended (Di Giancamillo et al., 2008; Desantis et al., 2019). They included, for example, unstable variations in microbial strains, and large divergences in both recommended dosages and feeding periods. In addition to probiotics, natural bioactive compounds derived from plants have been applied in pig farming, which also had a positive impact on piglet growth and health. Extracts from various herbs have antimicrobial, anti-inflammatory, and antioxidant properties, thus promoting nutrient absorption. However, their application in piglet diets mainly relied on their antimicrobial properties.

Our work aims to compare the effectiveness of different feed additives on the duodenum morphology in piglets during the pre-weaning period.

Materials and methods

During the study, ethical requirements for the use of animals in experimental research were fully respected (Strasbourg, 1986; Kyiv, 2002), and the research methodology was approved by the Bioethics Committee at the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine (protocol No. 93-01, dated June 3, 2021).

Research on the effectiveness of feed additives for piglets was conducted at the Barkom farm in Lviv region. For this purpose, four groups of piglets were formed, with ten head of the Large White breed in each group, to which different feed additives were added from 7 to 28 days of age along with the main diet. The piglets of the control group (I) received a standard balanced diet throughout the study; the piglets of group II received a standard diet with the addition of the EnzActivemixdrug – a combination of live yeast of the genus *Saccharomyces* and a complex of 6 enzymes (proteases, cellulases, xylanases, α-amylases, β-glucanases, and phytases) in the amount of 0.5 kg per 1 ton of feed; piglets of group III received Activofeed additive with the main feed, which contains a mixture of essential oils with extracts of plants (*Thymus vulgaris*, *Rosmarinus officinalis*, *Origanum vulgare*) – 17%, and chilli pepper extract – 1.5%. The amount of feed additive was 0.2 kg/ton. Piglets from group IV were given GlobigenJump Start feed additive in addition to their basic feed, which contains dry yeast and egg powder enriched with immunoglobulins (manufacturer EW NutritionGmBH, Germany) in a quantity of 2 kg of additive per 1 ton of feed. The basic feed used in the experiment was given in the form of small pellets, which provided piglets with all the necessary nutrients. Throughout the experiment, the piglets were kept under identical conditions with free access to feed and water. Weaning was conducted at the age of 28 days. On the 14th and 28th day, three piglets from each group were euthanized and their material was selected for histological examination.The duodenum fragments were fixed in a 10% neutral aqueous solution of formalin and Bouin's fluid, dehydrated through an ascending series of alcohols, and were embedded in paraffin blocks after I and II chloroform. From the obtained blocks, 7-μm-thick sections were prepared using the MS-2 microtome, mounted on glass slides, and stained with hematoxylin and eosin after drying. The following indicators were determined on histological preparations using the Aperio ImageScope software: the villi height and crypt depth, the villi width, and the ratio of the villi height to the crypt depth. The statistical analysis of the research results was performed using one-factor ANOVA analysis, taking into account the Bonferroni correction. The StatPlus program (Analyst Soft Inc., USA) was used for statistical analysis. The results are presented as mean ± standard deviation (x ± SD). Differences between animal groups were considered statistically significant at $P < 0.05$. Histological samples were processed and photographed using a Leica DM-2500 microscope (Switzerland) with a Leica DFC450C camera and Leica Application Suite Version 4.4 software.

Results

The duodenum mucous membrane of the control and experimental groups of pigs showed slight variability in the histological picture, specifically, the mucous membrane's thickness ranged from 650 to 895 μm, and this depended mainly on the villi height. The villi were mostly leaf-like in shape, and the crypts were mainly closely adjacent to each other. The mucous membrane's muscular layer was composed of smooth muscles, which diverged at the sites of the Brunner's glands' ducts. The lamina propria of the mucous membrane, which was located between the villi and crypts, was represented by loose connective tissue. Blood vessels and lymphatic vessels, fibroblasts, small lymphocytes, plasma cells of various differentiation degree, and histiocytes were visualized in the lamina propria. The number of different cell forms in the lamina propria of the experimental group was quite variable. The lympho-histiocytic apparatus of the duodenum mucosa was represented by small lymphoid clusters. The fibrous connective tissue of the lamina propria was mainly composed of collagen fibers and, to a lesser extent, elastic fibers. The crypt epithelium was represented by poorly differentiated goblet cells, Paneth cells, and endocrine cells (Fig. 1). Figures of mitosis were visualized in the middle part of the crypt cells. In the lower part of the crypts, as well as on the lateral surfaces of the villi, tall columnar cells with brush borders were predominant. These cells were rich in neutral and acidic mucopolysaccharides, alkaline and acid phosphatases, and other enzymes. Along with the brush border, a small number of goblet cells were located on the villi surface, which contained mucin composed mainly of neutral and acidic mucopolysaccharides. The mucin composition depends on the degree of goblet cells' differentiation. For example, young mucous cells secrete mucin that is rich in neutral mucopolysaccharides, sialo- and sulfomucins. As the goblet cells differentiate, they lose the ability to produce sialo- and sulfomucins, continuing to secrete neutral mucopolysaccharides and, to a lesser extent, carboxymucins. Paneth cells, which are located at the bottom of intestinal crypts, also produce a peptidase-rich secret. Brunner's glands, which are located in the submucosal layer, also produce mucin. The nuclei of these cells are pressed against the base, and the entire apical part is filled with secretory granules containing neutral mucopolysaccharides. The histological picture of the piglets' mucosal membrane on the 14th day of the study was characterized by slight variability. Piglets of group II, which consumed the drug with a combination of live yeast of the genus *Saccharomyces*, had a pronounced polymorphism of villi. The mucosal membrane was significantly thickened due to an increase in the villi height and a change in their shape (Fig. 2b). In piglets of group III, to whom a mixture of essential oils from plant extracts was added, the morphological structure of the villi was also slightly altered, and the apical surface became flat compared to the control group. In addition, there was moderate cellular infiltration of the mucosal membrane plate (Fig. 2c). According to morphometric studies, the average height of the villi in the small intestine of 7-day-old piglets before feeding feed additives was 691.2 μm, and the crypt depth was 394.2 μm. On day 14, after the intake of various feed additives, slight fluctuations were observed, especially between the experimental groups. The average villi height in the small intestine of the control group's piglets was 650.9 μm, while that of the II and III groups increased slightly to 690.0 and 759.8 μm, respectively. In piglets of the IV group, the villi height slightly increased on day 14, compared to the control group, and was 651.6 μm. A significant increase in the villi height in the duodenum of pigs in group III may indicate an increase in the number of enterocytes in the small intestine mucosa, capable of better absorption of available nutrients. Analyzing the parameters of crypt depth, it should be noted that on the 14th day of the experiment, the values varied significantly for all groups and were 352.8, 326.7, 334.8, and 309.2 μm, respectively.

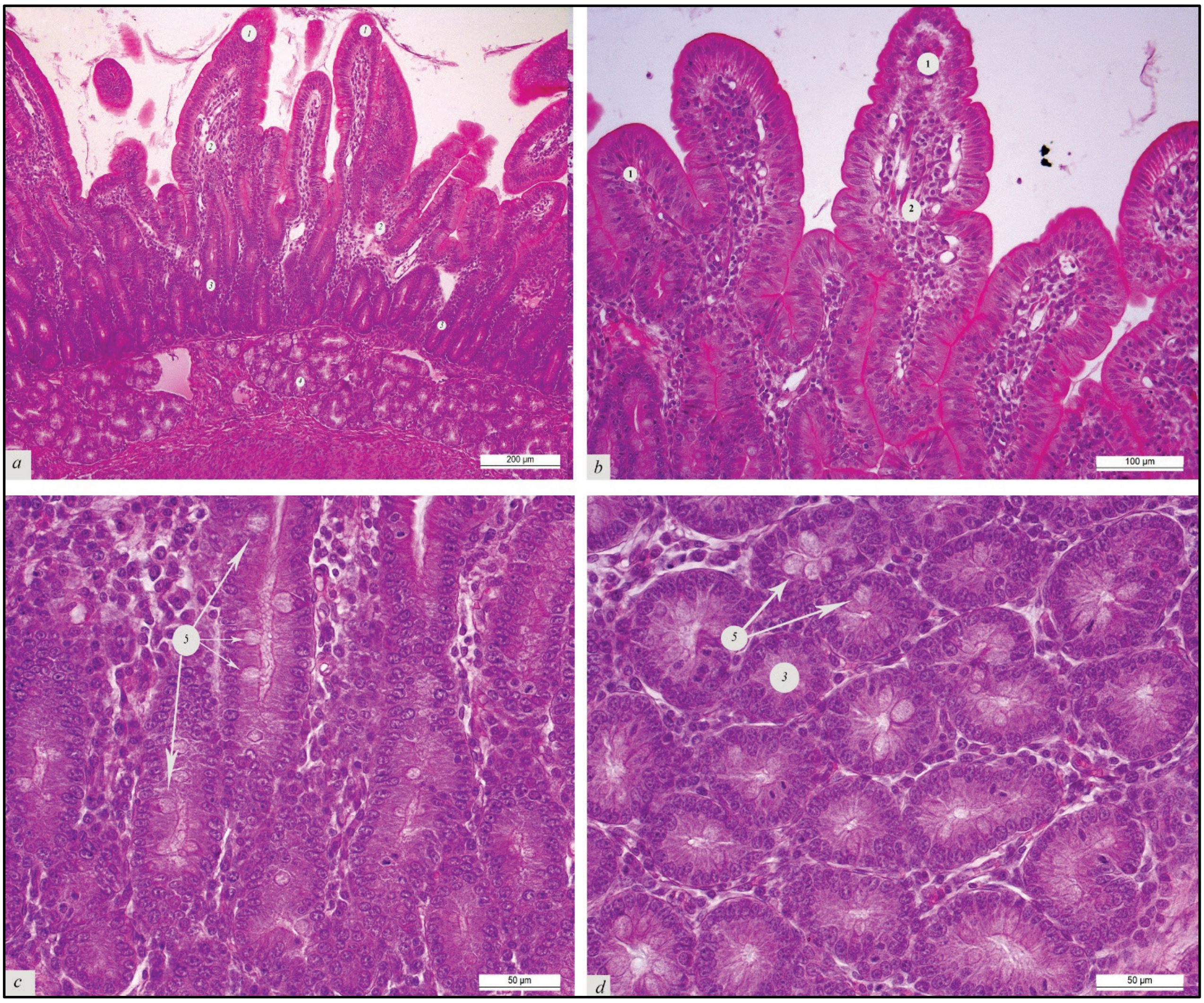

Fig. 1. Mucous membrane of the pig duodenum at 7 days of age: *a*, *b* – mucous membrane, *c* – base of intestinal crypts, *d* – Lieberkuhn glands; *1* – villi, *2* – lamina propria of the mucous membrane, *3* – crypts, *4* – Brunner's glands, *5* – goblet cells; hematoxylin and eosin staining

On day 28, both the experimental and control group showed preserved villi in the duodenum. The lamina propria of the mucosal membrane showed slight changes, and the number of goblet-like cells in the intestinal glands slightly increased. In the piglets of group II, the duodenum villi became thicker, and the lamina propria contained dense clusters of lymphoid cellular elements compared to the villi of the other groups

(Fig. 3b). It should be noted that the intensity of mucin secretion by the goblet cells in the apical part of the duodenum villi in piglets of group III, which received a feed supplement Activo containing a mixture of essential oils and plant extracts, was significantly higher compared to the other groups. It probably happened due to the stimulation of the mucosal membrane cells and increased mucin secretion, polymorphism of the villi, and moderate cellular infiltration of the lamina propria (Fig. 3c). In the duodenum of piglets that received the feed additive with dry yeast and egg powder enriched with immunoglobulins Globigen Jump Start (group IV), no significant changes were found compared to the 14-day-old piglets.

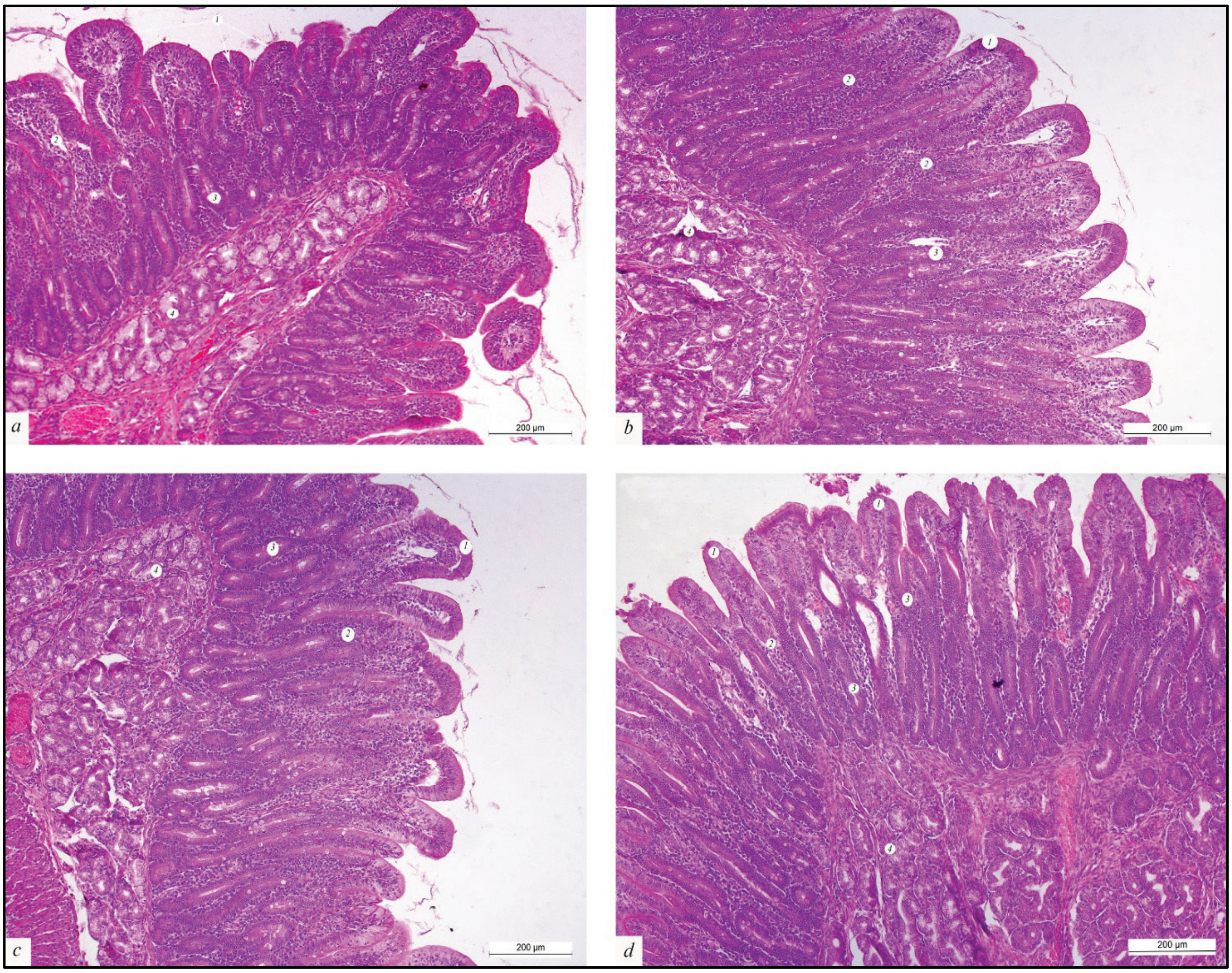

Fig. 2. Histological structure of the mucous membrane of the piglet's duodenum on day 14:
a – control group, *b* – group II, *c* – group III, *d* – group IV; the numbers on all photos represent the following structures:
1 – villi, *2* – the lamina propria of the mucous membrane, *3* – crypts, *4* – Brunner's glands; hematoxylin and eosin staining

Table 1
Morphometric indicators of the piglets' duodenum after feeding various feed additives (n = 5, x ± SD)

Parameters	7th day	14th day				28th day			
	before feeding drugs	control group	II group	III group	IV group	control group	II group	III group	IV group
Villi height, µm	691.2 ± 23.5	650.9 ± 33.9	690.0 ± 34.9	759.8 ± 16.3*	691.6 ± 16.3	717.7 ± 10.1	876.3 ± 11.1	892.1 ± 23.3*	826.2 ± 14.2
Villi width, µm	107.3 ± 7.5	126.1 ± 5.5	139.1 ± 5.5*	138.3 ± 6.3*	117.9 ± 5.8	141.3 ± 6.2	143.4 ± 5.2	139.8 ± 7.2	139.6 ± 5.4
Crypt depth, µm	394.2 ± 14.4	352.8 ± 18.9	326.7 ± 16.0	334.8 ± 15.2*	309.2 ± 12.2	360.0 ± 16.7	347.7 ± 11.6	331.1 ± 17.1	331.6 ± 15.3
Villi height/crypt depth (V/C)	1.76	1.85	2.11	2.27	2.24	1.99	2.52	2.69	2.49

Note: group I piglets received a standard nutritionally balanced diet; group II piglets received a combination of live yeast of the genus *Saccharomyces* and a complex of 6 enzymes (EnzActivemix drug); Group III piglets received a mixture of essential oils from plant extracts (Activo); Group IV piglets received dry yeast and egg powder enriched with immunoglobulins (GlobigenJump Start) as the main feed; * – P < 0.05 differences between the control and experimental groups.

The analysis of morphometric indicators and the condition of the mucous membrane of the piglets' duodenum at 28 days of feeding different feed additives showed consistently high indicators of villi height. The shortening of the villi height in piglets of the control group occurred during the first days after the change in feeding due to the interaction between the introduced feed and intestinal microflora. This usually results from a decrease in the enterocyte number, which subsequently effects a slight shortening of the villi. The changes in the enterocyte number during food stress can be associated with a sharp loss of cells or due to a short-term decrease in the rate of cell production by crypts.

Discussion

The mucous membrane of the gastrointestinal tract is the largest surface where the body comes into contact with viruses, bacteria, food proteins, and other metabolites. It is also the first line of defense against infections caused by pathogenic microorganisms. The barrier functions of the intestinal mucosa include mechanical, biological, chemical, and immunological barriers, among which the mechanical and immunological barriers play an important role (Patience et al., 2015). The integrity of the intestinal mucosa epithelium is closely related to the mechanical barrier function, which protects against the invasion of pathogens and infection. In addition, the thickness of the intestinal wall is also related to the physical barrier function and peristalsis. When the intestinal wall becomes thin, peristalsis slows down, and the accumulation of undigested food causes pathological fermentation, which leads to diarrhea. In addition, a decrease in peristalsis leads to the retention of pathogenic microorganisms in the intestine and increases the likelihood of infection. Therefore, normal intestinal peristalsis is important for food digestion and absorption, as well as for the timely removal of pathogenic microorganisms through defecation (Whitehead, 1996; Gu & She, 2002; Huting et al., 2021; Fresno Rueda et al., 2021).

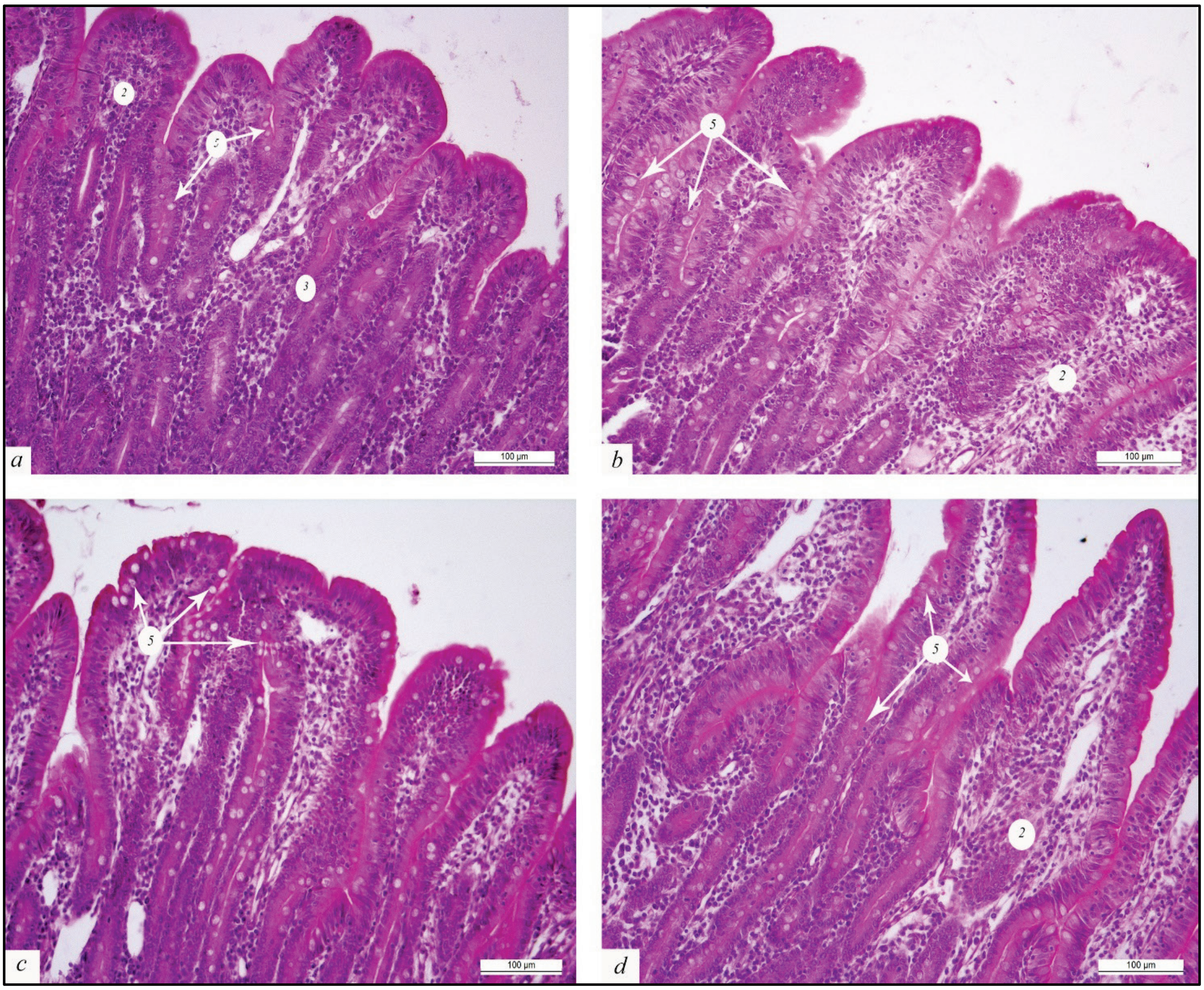

Fig. 3. Histological structure of the mucous membrane of a piglet's duodenum on day 28: *a* – control group, *b* – group II, *c* – group III, *d* – group IV; *2* – lumina propria of the mucous membrane, *3* – crypts, *5* – goblet cells; hematoxylin and eosin staining

One of the first problems that can arise with digestion is the sudden change in feeding type from maternal milk to ready-made feed, especially during the weaning period of piglets. Stress from early weaning causes significant damage to the intestinal tract (Upadhaya & Kim, 2021). The impact on the small intestine's mucous membrane is particularly strong because the morphology, structure, and function of the intestinal epithelium change significantly during this period. Functional changes in the small intestine manifest as a shortening of villi and an increase in crypt depth. For piglets, the small intestine is the main organ for the absorption and transport of nutrients, and a healthy mucous membrane is essential for normal digestive function, as nutrient absorption is largely dependent on the villi of the small intestine. Intestinal villi are histologically represented by finger-like protrusions of the mucous membrane's epithelial covering in the direction of the intestinal lumen and are composed of enterocytes, goblet cells, and enteroendocrine cells, which are responsible for absorption and secretion. To maintain the normal functioning of the pigs' gastrointestinal tract during the transition from suckling to a fully granulated feed, companies are looking for products to improve animal health and growth productivity. In recent years, probiotics have been used in pig feeding (Gu & She, 2002; Buts & DeKeyser, 2006; Biagi et al., 2010; Wang et al., 2019; Zhu et al., 2022). Currently, feed additives with yeast remain relevant. There is a lot of evidence about the direct and indirect effects of yeast on the intestine's microbiota and the immune system, partially mitigating the negative consequences associated with stress and various diseases (Hohenshell et al., 2000; Hedemann et al., 2006; Prudius & Vishchur, 2022). For example, Zhang et al. (2016) reports that yeast culture enhances immunity, and mannan-oligosaccharides obtained from yeast fermentation products have a positive effect on the immune system of weaned pigs (Zhu et al., 2022). Live yeast enhances intestine immunity by regulating the secretion of mucosal IgA and reducing pathogen colonization, thereby improving the intestine health of weaned pigs. Other researchers have reported that adding live yeast to pig diet eases the course of diarrhea caused by *Escherichia coli* enterotoxins, which may be related to improved permeability, innate immunity, and changes in the bacterial profile (Moturi et al., 2021).

Our research showed that a feed additive containing plant extracts and essential oils improved the intestinal mucosa integrity in piglets, with a particularly pronounced effect on the duodenum epithelium, where the villi were wider and longer compared to the control group, and the microvilli were intact and well-formed. The usefulness of using plant extracts in the feeding of weaned piglets is confirmed by the research of other scientists (Placha et al., 2013; Rabelo-Ruiz et al., 2021). We obtained a better effect from feeding piglets a feed additive based on essential oils, as the active ingredients of pepper increased the secretion of saliva with a high content of amylase and mucopolysaccharides, which induces the synthesis of digestive enzymes, and prevents dyspepsia naturally without the use of enzymatic preparations. The essential oil of thyme has a significant bactericidal effect, prevents flatulence, acts as a spasmolytic, bactericidal, and binding agent, promotes the secretion of mucopolysaccharides by goblet endocrine cells, and improves feed intake. Rosemary essential oil stimulites digestion, acts as a good spasmolytic agent, and has a pronounced choleretic effect. It also has antiviral properties. The main effect of oregano is manifested in the stimulation of the digestive glands' secretion, the stimulation of peristalsis and gastrointestinal tract tonus. Moreover, it has an antiviral effect, preventing inflammation of the gastrointestinal tract, promoting the secretion of gastric juice, and stimulating appetite.

Thus, the complex of essential oils used in the feed additive Activo had a positive effect on the taste receptors of pigs, which improved feed consumption. In addition, the prevention of flatulence, diarrhea, and constipation, which often occur when feeding high-protein feeds, was effected. The combination of essential oils also contributes to the prevention of enzyme deficiencies and provides an enhanced synthesis of mucins, which are the main barrier against aggressive microbial associations and an important component in physiological mucosal digestion. The virucidal effects of essential oils prevent many dangerous viral gastrointestinal diseases in pigs. In addition, adding the above-mentioned essential oils reduces the accumulation of peroxide compounds formed as a result of the oxidation of some of its components.

The morphology of the intestine, especially the length and width of the villi and the crypt depth, is one of the main indicators of the pig intestine health. The cells of the apical part of the villi secrete various digestive enzymes, so the longer the villi, the more cells, and the stronger the digestive and absorptive capacity of the intestine. The depth of the crypt reflects the speed of intestinal villi mitosis with the formation of epithelial cells. When the crypt becomes smaller, it indicates the speed of cell maturation and secretory function increases. As a result, the ratio of villi height to crypt depth can reflect the digestive and absorptive function of the small intestine. When the ratio of villi height/crypt depth decreases, the ability to digest and absorb decreases too, which is often accompanied by growth retardation. Conversely, when the number of intestinal epithelial cells increases, the absorption area also increases, as well as the speed of absorption and utilization of nutrients. Impairment of intestinal barrier function is often described as an early manifestation of systemic disorders in the pathogenesis of various intestinal diseases. Such changes lead to increased permeability of various substances in the intestinal tract, such as bacteria and their products (lipopolysaccharides), into the mucosal layer, which can cause local or systemic inflammatory reactions. Based on morphological and structural observations, it can be concluded that the use of feed additives capable of maintaining the intestinal mucosa integrity, improving its immune function and preserving a functionally healthy intestine, should be applied to piglets during the period before weaning and several weeks after weaning.

Conclusion

The histomorphology parameters of the duodenum, such as villi height, crypt depth, and their ratio, are important and informative indicators of intestine health. Higher villi provide a greater number of enterocytes, which increases the surface area and promotes several positive effects such as increased production of enzymes, greater absorption area, and improved transport system for nutrients. Proliferation and differentiation of enterocytes in the crypts provide growth of the villi and play a key role in local immunity.

Based on the conducted research, it was found that in the duodenum of piglets that were fed with the feed additive Activo from day 7 to day 28 of life, there was a significant increase in villi height compared to the control group of piglets. This positively affected the morphofunctional state of the intestinal mucosa, and promoted an increase in the number of goblet cells that induce digestive enzymes.

Our further research will be focused on studying the accumulation of acidic and basic glycosaminoglycans in the small intestine of piglets, determining the immunomodulatory effect on the population of B-lymphocytes in solitary lymph follicles, and the mitotic activity of enterocytes.

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References

Banerjee, A., McKinley, E. T., von Moltke, J., Coffey, R. J., & Lau, K. S. (2018). Interpreting heterogeneity in intestinal tuft cell structure and function. The Journal of Clinical Investigation, 128(5), 1711–1719.

Baum, B., Liebler-Tenorio, E. M., Enss, M. L., Pohlenz, J. F., & Breves, G. (2002). *Saccharomyces boulardii* and *Bacillus cereus* var. *toyoi* influence the morphology and the mucins of the intestine of pigs. Zeitschrift fur Gastroenterologie, 40(5), 277–284.

Biagi, G., Cipollini, I., Paulicks, B. R., & Roth, F. X. (2010). Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. Archives of Animal Nutrition, 64(2), 121–135.

Biagi, G., Piva, A., Moschini, M., Vezzali, E., & Roth, F. X. (2006). Effect of gluconic acid on piglet growth performance, intestinal microflora, and intestinal wall morphology. Journal of Animal Science, 84(2), 370–378.

Bontempo, V., Giancamillo, A. D., Savoini, G., Dell'Orto, V., & Domeneghini, C. (2006). Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. Animal Feed Science and Technology, 129, 224–236.

Buddle, J. R., & Bolton, J. R. (1992). The pathophysiology of diarrhea in pigs. Pig News and Information, 13(1), 41–45.

Buntyn, J. O., Schmidt, T. B., Nisbet, D. J., & Callaway, T. R. (2016). The role of direct-fed microbials in conventional livestock production. Annual Review of Animal Biosciences, 4, 335–355.

Buts, J. P., & De Keyser, N. (2006). Effects of *Saccharomyces boulardii* on intestinal mucosa. Digestive Diseases and Sciences, 51(8), 1485–1492.

Canibe, N., Højberg, O., Kongsted, H., Vodolazska, D., Lauridsen, C., Nielsen, T. S., & Schönherz, A. A. (2022). Review on preventive measures to reduce post-weaning diarrhoea in piglets. Animals, 12(19), 2585.

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption. The American Journal of Clinical Nutrition, 55(Suppl.), 299–308.

Cera, K. R., Mahan, D. C., Cross, R. F., Reinhart, G. A., & Whitmoyer, R. E. (1988). Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. Journal of Animal Science, 66(2), 574–584.

Chen, S., Tan, B., Xia, Y., Liao, S., Wang, M., Yin, J., Wang, J., Xiao, H., Qi, M., Bin, P., Liu, G., Ren, W., & Yin, Y. (2019). Effects of dietary gamma-aminobutyric acid supplementation on the intestinal functions in weaning piglets. Food and Function, 10(1), 366–378.

Chopra, D. P., Dombkowski, A. A., Stemmer, P. M., & Parker, G. C. (2010). Intestinal epithelial cells *in vitro*. Stem Cells and Development, 19(1), 131–142.

De Lange, C. F. M., Pluske, J., Gong, J., Nyachoti, C. M. (2010). Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science, 134(1–3), 124–134.

Desantis, S., Mastrodonato, M., Accogli, G., Rossi, G., & Crovace, A. M. (2019). Effects of a probiotic on the morphology and mucin composition of pig intestine. Histology and Histopathology, 34(9), 1037–1050.

Di Giancamillo, A., Vitari, F., Savoini, G., Bontempo, V., Bersani, C., Dell'Orto, V., & Domeneghini, C. (2008). Effects of orally administered probiotic *Pediococcus acidilactici* on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. Histology and Histopathology, 23(6), 651–664.

Fresno Rueda, A., Samuel, R., & St-Pierre, B. (2021). Investigating the effects of a phytobiotics-based product on the fecal bacterial microbiome of weaned pigs. Animals, 11(7), 1950.

Gu, X., Li, D., & She, R. (2002). Effect of weaning on small intestinal structure and function in the piglet. Archiv fur Tierernahrung, 56(4), 275–286.

Hampson, D. J. (1986). Alterations in piglet small intestinal structure at weaning. Research in Veterinary Science, 40(1), 32–40.

Hasan, M. S., Crenshaw, M. A., & Liao, S. F. (2020). Dietary lysine affects amino acid metabolism and growth performance, which may not involve the GH/IGF-1 axis, in young growing pigs1. Journal of Animal Science, 98(1), skaa004.

Hedemann, M. S., Jensen, B. B., & Poulsen, H. D. (2006). Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. Journal of Animal Science, 84(12), 3310–3320.

Hohenshell, L. M., Cunnick, J. E., Ford, S. P., Kattesh, H. G., Zimmerman, D. R., Wilson, M. E., Matteri, R. L., Carroll, J. A., & Lay Jr., D. C., (2000). Few differences found between early- and late-weaned pigs raised in the same environment. Journal of Animal Science, 78(1), 38–49.

Holanda, D. M., Kim, Y. I., Parnsen, W., & Kim, S. W. (2021). Phytobiotics with adsorbent to mitigate toxicity of multiple mycotoxins on health and growth of pigs. Toxins, 13(7), 442.

Holland, R. E. (1990). Some infectious causes of diarrhea in young farm animals. Clinical Microbiology Reviews, 3(4), 345–375.

Hou, Y., Yao, K., Yin, Y., & Wu, G. (2016). Endogenous synthesis of amino acids limits growth, lactation, and reproduction in animals. Advances in Nutrition, 7(2), 331–342.

Huting, A. M. S., Middelkoop, A., Guan, X., & Molist, F. (2021). Using nutritional strategies to shape the gastro-intestinal tracts of suckling and weaned piglets. Animals, 11(2), 402.

Miller, B., Newby, T. J., Stokes, C. R., Hampson, D., & Bourne, F. J. (1983). The role of dietary antigen in the aetiology of post weaning diarrhoea. Annales de recherches veterinaires. Annals of Veterinary Research, 14(4), 487–492.

Moturi, J., Kim, K. Y., Hosseindoust, A., Lee, J. H., Xuan, B., Park, J., Kim, E. B., Kim, J. S., & Chae, B. J. (2021). Effects of *Lactobacillus salivarius* isolated from feces of fast-growing pigs on intestinal microbiota and morphology of suckling piglets. Scientific Reports, 11(1), 6757.

Patience, J. F., Rossoni-Serão, M. C., & Gutiérrez, N. A. (2015). A review of feed efficiency in swine: Biology and application. Journal of Animal Science and Biotechnology, 6, 33.

Placha, I., Chrastinova, L., Laukova, A., Cobanova, K., Takacova, J., Strompfova, V., Chrenkova, M., Formelova, Z., & Faix, S. (2013). Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. Acta Veterinaria Hungarica, 61(2), 197–208.

Prudius, T., & Vishchur, O. (2022). Efficacy of "EnzActive mix" feed additive in piglet growing. The Animal Biology, 24(4), 27–31.

Rabelo-Ruiz, M., Teso-Pérez, C., Peralta-Sánchez, J. M., Ariza, J. J., Martín-Platero, A. M., Casabuena-Rincón, Ó., Vázquez-Chas, P., Guillamón, E., Aguinaga-Casañas, M. A., Maqueda, M., Valdivia, E., Baños, A., & Martínez-Bueno, M. (2021). *Allium* extract implements weaned piglet's productive parameters by modulating distal gut microbiota. Antibiotics, 10(3), 269.

Upadhaya, S. D., & Kim, I. H. (2021). The impact of weaning stress on gut health and the mechanistic aspects of several feed additives contributing to improved gut health function in weanling piglets – a review. Animals, 11(8), 2418.

Wang, Q., Sun, Q., Qi, R., Wang, J., Qiu, X., Liu, Z., & Huang, J. (2019). Effects of *Lactobacillus plantarum* on the intestinal morphology, intestinal barrier function and microbiota composition of suckling piglets. Journal of Animal Physiology and Animal Nutrition, 103(6), 1908–1918.

Whitehead, W. E. (1996). Psychosocial aspects of functional gastrointestinal disorders. Gastroenterology Clinics of North America, 25(1), 21–34.

Wiese, F., Simon, O., & Weyrauch, K. D. (2003). Morphology of the small intestine of weaned piglets and a novel method for morphometric evaluation. Anatomia, Histologia, Embryologia, 32(2), 102–109.

Zakhariv, O., Vudmaska, I., & Petruk, A. (2020). Features of lipid synthesis from [2-^{14}C] acetate in liver and intestinal mucosa of piglets. The Animal Biology, 22(2), 9–14.

Zhang, D., Ji, H., Liu, H., Wang, S., Wang, J., & Wang, Y. (2016). Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of *Lactobacillus* or an antibiotic. Applied Microbiology and Biotechnology, 100(23), 10081–10093.

Zhu, C., Yao, J., Zhu, M., Zhu, C., Yuan, L., Li, Z., Cai, D., Chen, S., Hu, P., & Liu, H. Y. (2022). A meta-analysis of *Lactobacillus*-based probiotics for growth performance and intestinal morphology in piglets. Frontiers in Veterinary Science, 9, 1045965.