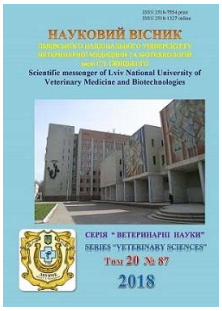

Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Ґжицького

Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies

ISSN 2518–7554 print
ISSN 2518–1327 online

doi: 10.15421/nvlvet8705
http://nvlvet.com.ua/

UDC 611.342:611:018:619:615.3

The influence of probiotic fodder additives on the morphofunctional state of duodenum pigs

G.I. Kotsyumbas, V.M. Lemishevskyi, A.K. Kostyniuk

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Ukraine

Article info

Received 29.01.2018
Received in revised form 06.03.2018
Accepted 12.03.2018

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies,
Pekarska str., 50, Lviv 79010, Ukraine.
Tel.: +38-032-239-26-91
E-mail: info@vetpathology.lviv.ua

Kotsyumbas, G.I., Lemishevskyi, V.M., & Kostyniuk, A.K. (2018). The influence of probiotic fodder additives on the morphofunctional state of duodenum pigs. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. 20(87), 27–31. doi: 10.15421/nvlvet8705

The article is dedicated to the study of the effect of a class of microorganisms and substances of microbial and other origin that are used in feeding pigs to achieve maximum growth in animal mass, as well as therapeutic purposes. Study of the effect of biologically active additives on the state of the pig's digestive system. The article considers the rational nutrition that increase the productivity and reproductive capacity of animals, and also prevent the emergence of profound disturbances of all metabolic processes which leads to a decrease in resistance to productivity, a clinically pronounced disease of adult animals and young animals. The authors pay attention to the morphometric parameters, ultrastructure and content of nucleic acids in the wall of the duodenum of pigs by forage feeding with the addition of probiotic fodder additive «Probion-forte» in dose of 1 g/kg of fodder. Research were conducted on 28 day pigs, breed «Large White». It was formed two groups of piglets per 30 heads; piglets were fed with standard mixed fodders; piglets from the first group were received standard mixed fodder with the addition of probiotic fodder additive «Probion-forte» in dose 1 g/kg of fodder for 42 days. After the slaughter of piglets, pieces of duodenum for histological, histochemical and ultrastructural studies were selected. Statistical significance of differences was determined by Student's t test , assuming 5% estimate error. It was shown that feeding with forage within 42 days from the addition of probiotic fodder additive «Probion-forte» at a dose of 1 g/kg, villus height is increase, crypt depth and a number of plasma cells in the lamina propria of mucosa of the duodenum, which helps the digestive process and increase the area of nutrient absorption in the intestines. The number of plasma cells are increased in the lamina propria of mucosa and testify immunomodulatory effect of fodder additives. Ultra structural alteration of microvilli and changes in the nuclei of duodenal enterocytes of piglets of the first group indicates a more pronounced acfunctional activity of enterocytes and thereby increases the activity of parietal digestion in the intestine. As a conclusion, the task by definition performed of the effective dose of probiotic in feed for pig it can be considered completed and we can state the probiotics can be incorporated, as a alternative to antibiotics and increase the weight gain of animals and also to increase the level of immune resistance of the organism.

Key words: *probiotics, intestine duodenum, villi, enterocytes, plasma cells.*

Introduction

The normal function of the gastrointestinal tract of animals, despite the continuous intake of pathogenic bacteria can be maintained only if the natural balance of gastrointestinal microflora (Lemishevskyi, 2011; Lemishevskyi, 2017). Influence of some external and internal factors (poor quality food, non-sanitary standards, desultory and inappropriate use of antibiotics), leads to disruption of the dynamic equilibrium of microbial associations in a healthy organism, i.e. the development of dysbiosis (Lobsyn et al., 2003).

In the conditions of industrial economy sector it often occurs in animals disruption of normal content of micro flora, which is accompanied by a sharp decrease of symbiotic microorganisms (Sculze and Bathke, 1977). In addition, various diseases of gastro-intestinal tract, mainly characterized by dysbacteriosis of the intestinal micro flora, leading to disturbance of digestion, metabolism, reducing of resistance and productivity of animals.

Probiotic drugs can optimize the environmental conditions in the intestine for the development of their own microflora of the host (Lukashchuk, 2017), and the number of which is growing in Ukrain. However, there is still a need to clarify the effectiveness of probiotics in pigs breeding and basic mechanisms of their action.

Material and methods

All animal experiments were conducted in accordance with the principles outlined in Law of Ukraine № 3447-IV paragraph 26 «On the protection of animals from cruelty». The experiment was carried out in educational and research and production center «Komarnivskyj» Gorodok district, Lviv region, Ukraine on piglets of «Large White», at the age of 28 days. According to the principle of analogues two search groups of piglets were formed per 30 heads in each. Piglets of the first group were fed with mixed fodder according to the rules recommended for «Large White» breed taking into account age categories.

On 42nd day of the experiment per 5 heads from each group were withdrawn from the experiment, pathological and anatomical cutting was conducted with the selection of material. The tissue samples were maintained in 10% neutral-buffered formalin, Karnua liquid, with further pouring in paraffin according to conventional methods. Sections were made at microtome MS-2 («Kharkov factory «Tochmedpribor», Ukraine), a thickness of 7 ± 1 µm. Preparations were stained with hematoxylin and eosin (H&E), methyl green (C.I. 42590) and pironin Y (C.I. 45005) by Brachet (Pyronin / MG Brachet) (Sigma-Aldrich, Germany) (Mulisch and Welsch, 2010). For histological analysis, the tissue sections were photographed using a high-resolution color digital camera mounted on an Olympus CX41 microscope (Olympus, Japan).

To study the ultrastructure of the mucosa of the duodenum of pigs, duodenum were fixed in 1.5% of sodium of glutamic aldehyde in 0.2 molar cacodyl tic buffer (pH 7.2) – 2 hours. Samples were washed in two portions of buffer and fixed in 5% solution of osmium oxide. After washing, dehydration in increasing concentrations of ethanol, contrasted samples of uranil acetate and put in epoxy resin – Epon 812 (Sigma-Aldrich, Germany). Micro taming was performed on ultra microtome, contrasted uranyl with acetate and lead citrate (SPI-Chem, USA). Samples were viewed and photographed in the electron-transmission microscopy Tesla – BS-500 (TESLA, Czech Republic). Carrying out morphometria and being kept a recommendation of G.G. Avtandilova (Avtandilov, 2002), performing at least 50 measurements of each parameter on the same micropreparation.

Statistical analysis the reliability of the difference between statistical characteristics of the two sets of experimental data it was determined by Student's ratio, and considered changes to be credible in the level of significance of $P < 0.05$ (Lapach et al., 2001).

Results and discussion

During microscopic study of histological preparation of duodenum of pigs it was indicated that in the control group of animals mucosal villi tight were placed tightly, not high, had a fingerlike shape (Fig. 1), between prismatic enterocytes it was observed moderate amount goblet cells of round form. A moderate infiltration of the mucosa of lamina propria of lymphocytes was noted (Fig. 2).

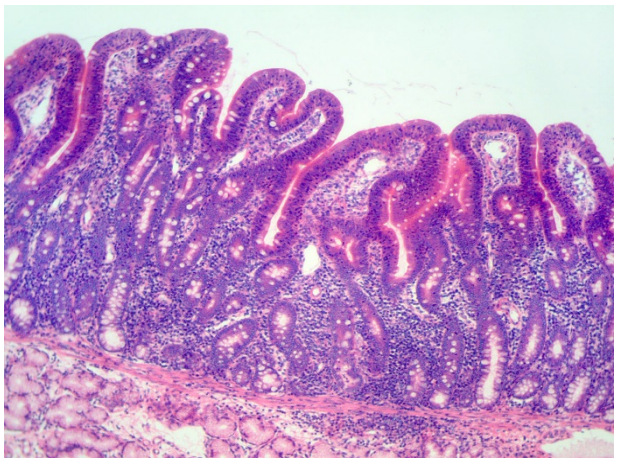

Figure 1. Mucous of duodenum of piglets from the control group. H&E, x100

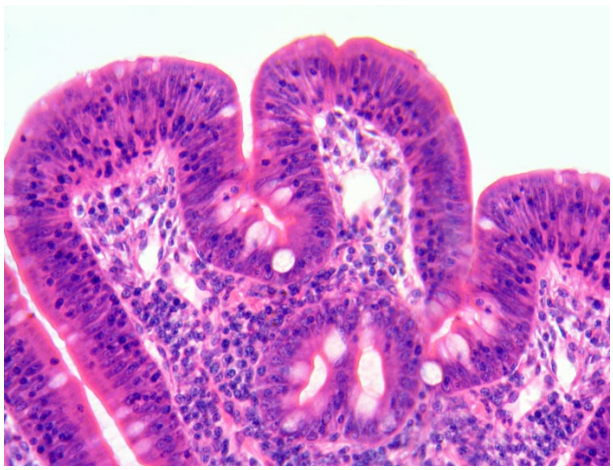

Figure 2. Structure of duodenum villi of piglets from the control group. H&E, x400

In the first group of piglets, which were fed probiotic «Probion-forte» together with fodder in a dose of 1.0 g/kg, the villi in the form of leaves, were well structured (Fig. 3) and slightly higher relatively to the control group of piglets. Prismatic epithelial cells with marked acidophilus border on the apical surface (Fig. 4).

By the morphometric research, villus height of duodenum mucosa of pigs of the control group was 315.13 µm, and in pigs of the first group villus height was significantly increased and reached 374.64 µm, which is more than 59.51 µm in piglets of the control group (Tab. 1). Duodenal villus width of the control group of piglets was 164.96 µm, and in piglets of the first group - 166.32 µm. By increasing the height and width of the villi of the mucosa, are area was increased of contact with the chyme, which contributed to increased activity of digestion and absorption in the intestines of piglets, which were fed probiotic feed additives with the fodder.

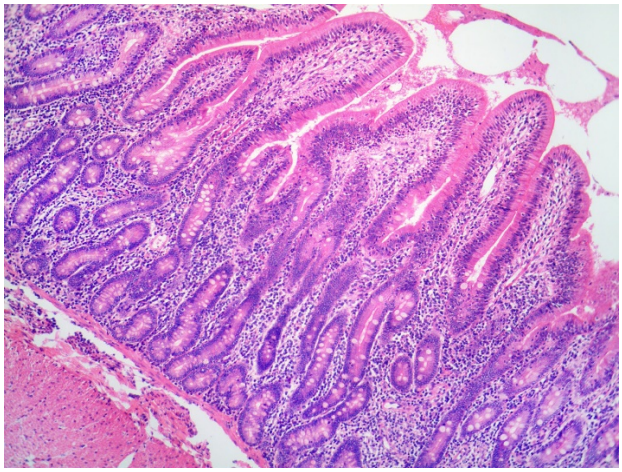

Figure 3. Duodenum mucous of the first group. H&E, x100

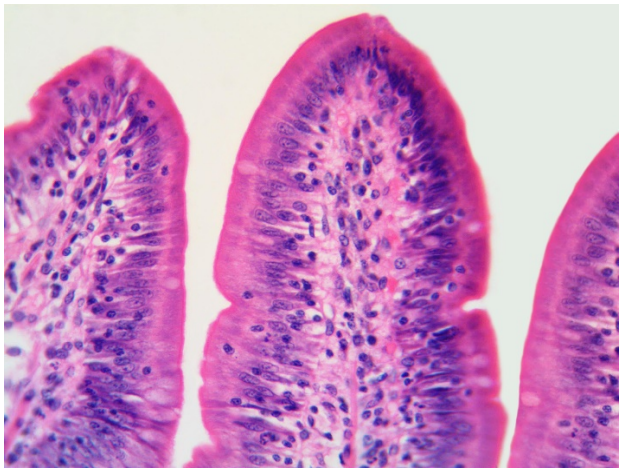

Figure 4. Structure of duodenum villi of piglets from the first group. H&E, x400

Table 1
Morphometric indices of piglets duodenum on the 42nd day of the experiment (M ± m, n = 5)

Indices	The control group pigs	Probion-forte 1 g/kg
Villus height, μm	315.13 ± 1.00	374.64 ± 2.23***
Width villi, μm	164.96 ± 1.31	166.32 ± 1.08
Crypt depth, μm	122.71 ± 1.93	140.97 ± 2.50**
Width crypt, μm	39.87 ± 0.50	46.34 ± 0.53***
Index of villi, con.un.	2.56	2.65***

Note: * – P < 0,05; ** – P < 0,01; *** – P < 0,001.

The depth of the crypts of the mucous membrane of the duodenum of piglets from the controls group of oval shape and their depth was 122.71 μm, and in piglets of the first group crypt depth was significantly increased to 18.26 μm and was 140.97 μm. However, it was noted a modest increase of bowled exocrinocytes in crypts compared with the control group of animals. Width of crypts significantly increased in the mucosa of the duodenum of piglets of the first group and was 39.87 μm, relatively similar parameter of the control group 39,87 μm.

In this connection, with different morphometric indices of duodenum mucous of piglets from the experimental group, villi index was bigger (correlation of villus height to crypt depth). In piglets from the control group, villus index was 2.56 con. un. Whereas, in piglets from the first group, it was 2.65 con. un., and was reliably bigger in comparison with its meaning in piglets from the control group.

Thanks to the histological findings of DNA and RNA in prismatic enterocytes of duodenum mucous of piglets from the control group, which were fed with full-rationed mixed fodders, it was observed the moderate pyroninphilness of erythrocytes cytoplasm and expressed cytoplasm pyroninphilia of plasmatic cells in its lamina propria mucosa (Fig. 5). In mucous of piglets duodenum, which were fed with probiotic additive «Probion-forte» in a dose 1 gr/kg, it was defined more saturated cytoplasm of crimson colour and enterocytes nucleus of blue and green colour, that testified the rise of content RNA and DNA in cells.

As in the control group as in the first experimental group of piglets, the plasmatic cells were settled mainly diffusively, in its own plate of mucous and in the crypt area and in the connective tissues of villi. In crypts of the lamina propria mucosa (Fig. 6)

Piglets from the control group, moderate colouring of cytoplasm of absorb prismatic erythrocytes. And in the first group of pigs, it was observed higher pyroninphilness of cytoplasm of villus absorb enterocytes (Fig. 7) and without rim enterocytes in cryp area of duodenum mucous (Fig. 8) in comparison with the control group of piglets. It was also noted the increase of diffusely situated plasmatic cell with the defined pyroninphilic cytoplasm and eccentrically placed nucleus.

Also we noted differences in the ultrastructure of the mucous of duodenal piglets. In the control group of piglets, the plasmatic layer of enterocytes consisted of two electron dense layers and less dense intermediate on the apical surface of which were microvilli, they have different height and had free disposition side by side (Fig. 9).

In piglets from the first group on the apical surface of enterocytes, the microvilli are noticeably longer and had denser disposition side by side, forming a dense brush border (Fig. 10). Matrix of microvilli was a bit denser in comparison with the main substance of cell cytoplasm.

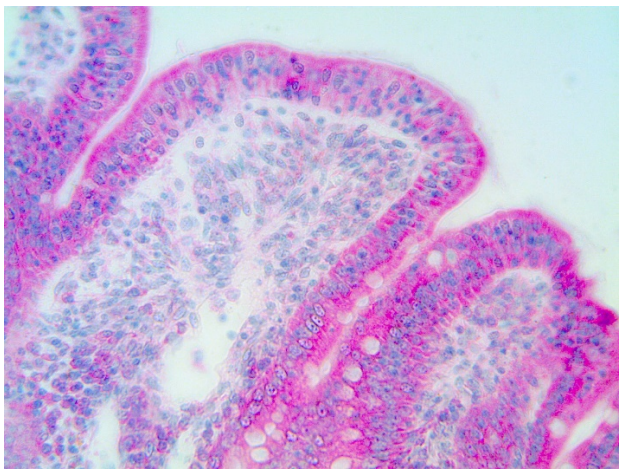

Figure 5. Duodenum villi of piglets from the control group. Pyronine/MG, x400

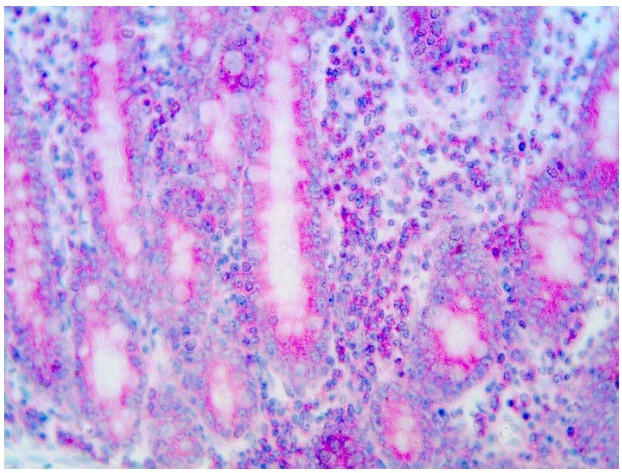

Figure 6. Plasmatic cells in the lamina propria mucosa of the duodenum of piglets from the control group. Pyronine/MG Brachet, x400

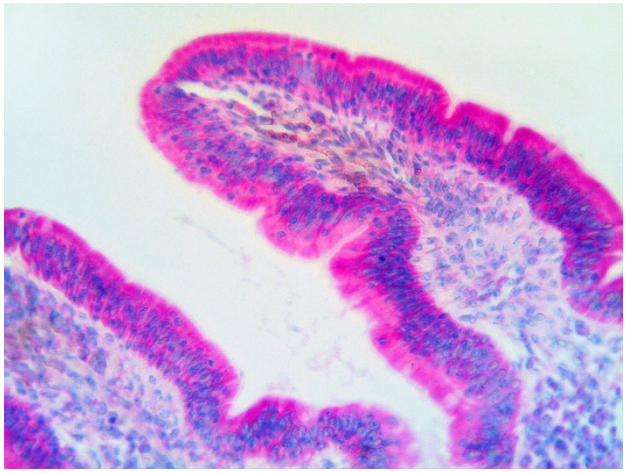

Figure 7. Villus of piglets duodenum from the first group. Pyronine/MG Brachet, x400

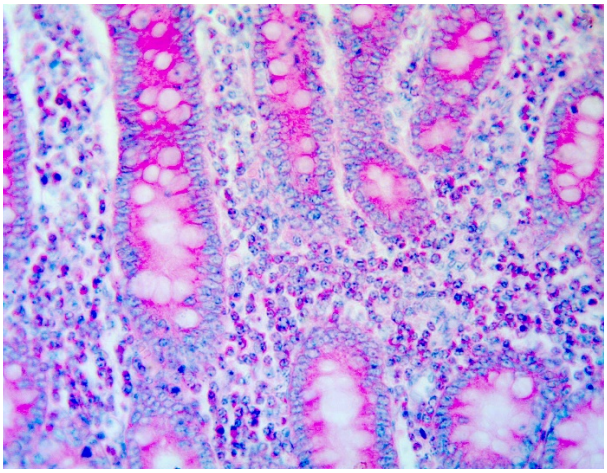

Figure 8. Plasmatic cells in its lamina propria mucosa of piglets duodenum from the first group. Pyronine/MG Brachet, x400

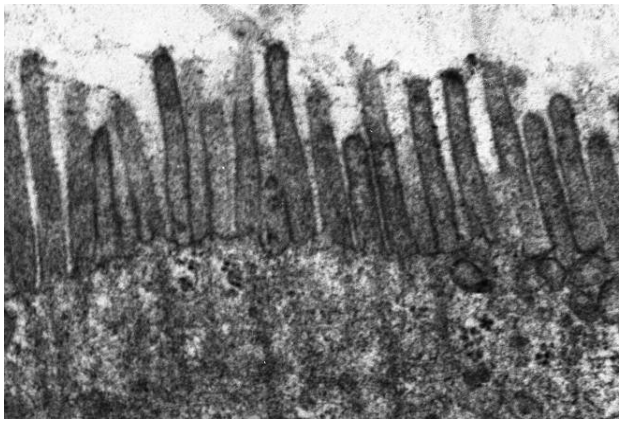

Figure 9. Microvilli of mucous enterocytes of piglets duodenum from the control group. Elecrongram, x24000

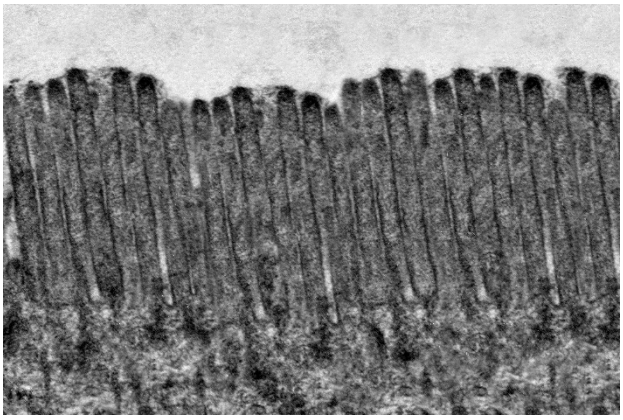

Figure 10. Microvilli of mucous enterocytes of piglets duodenum from the first group. Elecrongram, x24000

Attached to it, you should note the ultra structural differences between nucleus and mitochondrion of enterocytes in animals experimental group. In the control group of piglets the nucleus had chiefly irregular forms with one or several nucleoli, were electron dense and with a moderate content of the chromatin (Fig. 11). Nucleus of piglets erythrocytes of the first experimental group of oval form with the wavy relief of cariolema , insignificantly widened pores and larger content of condensed nuclear chromatine (Fig. 12).

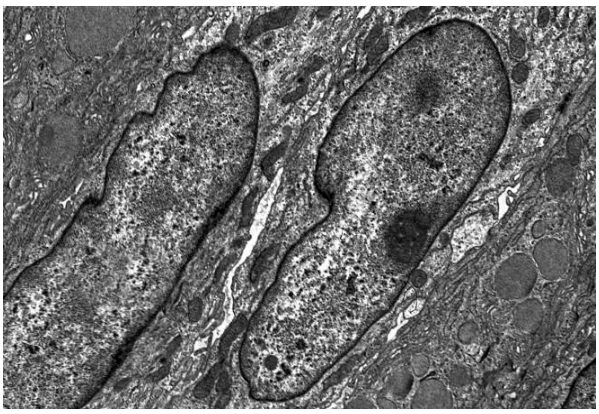

Figure 11. Nucleus of mucous enterocytes of piglets duodenum from the control group. Electrongram, x6000

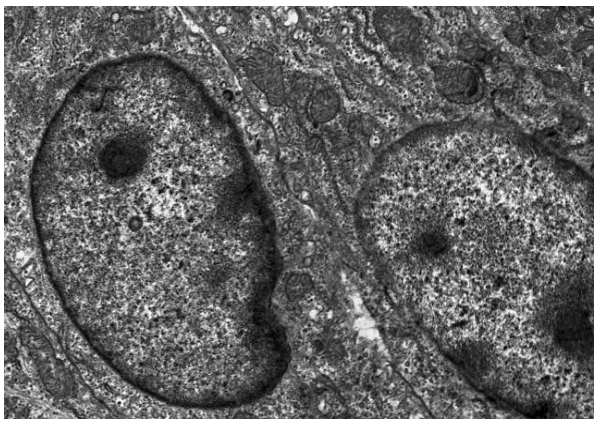

Figure 12. Nucleus of mucous enterocytes of piglets duodenum from the first group. Electrongam, x6000

As in the experimental as in the control piglets groups, mitochondrion in cytoplasm of enterocytes were mostly concentrated in apical cells area, mainly oval, baculiform and round form and different sizes. In the control group of piglets, mitochondrion of enterocytes had dumbbell-visible form and not a big size. It was noted a greater number of mitochondrion in enterocytes of piglets from the first group, which was located a moderate number of cristae.

Conclusions

Pigs foddering with added probiotic fodder additive «Probion-forte» in a dose of 1,0 g/kg for 42-days contributed:

- Increase in villus height and crypt depth, which favoured the process of digestion and absorbtion in the duodenum of piglets;
- Increase in content RNA and DNA in crypt erythrocytes, due to intense proliferative processes in the germinal zone of mucosa of the duodenum, aimed at increasing the index of villous;
- Presence in their lamina propria of mucosa of moderate number of lymphocytes, plasma cells relative to the control group of animals, indicates about immunomodulatory property of probiotic feed additive;
- dense arrangement of microvilli and changes in the nuclei of enterocytes of the duodenum points on the increase of functional activity of enterocytes and a more defined activity of parental digestion relatively similar indices.

References

Lemishevskyi, V.M. (2011). Antagonistic activity of probiotic bacteria and mechanism of the action on organism resistance. Scientific Messenger of Lviv national university of veterinary medicine and biotechnologies named after S.Z. Gzhytskyj. 13(4), 223–227.

Lemishevskyi, V. (2017). Electron-microscopic structure of cells of the mucosa of the large intestine pigs in the physiological norm. Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj. 19(77), 51–54. doi:10.15421/nvlvet7712.

Lobsyn, Y.V., Makarova, V.G., Korvjakova, E.R., Zaharenko, S.M. (2003). Disbacteriosis of intestine (clinic, diagnosis, treatment). For doctors. Spb.: Foliant.

Lukashchuk, B.O. (2017). Influence of probiotic and phytobiotic on parameters of T- and B-cell immunity in weaned piglets with non-contagious gastroenteritis. Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj, 19(73), 173–177. doi:10.15421/nvlvet7336.

Sculze, F., Bathke, W. (1977). Zur quantitativen Zusammensetzung der Magen-Darm-Flora beim Läuferschwein. Arch Exp Veterinarmed. 31(2), 161–185.

Mulisch, M., & Welsch, U. (2010). Romeis Mikroskopische Technik. Spektrum Akademischer Verlag GmbH. http://www.springer.com/de/book/9783642551895.

Avtandilov, G.G. (2002). Basis of quantitative pathological anatomy. M.: Medicine.

Lapach, S.N., Gubenko, A.V., Babych, P.N. (2001). Statistical methods in medical and biological search Excell. K.: Morion.