Technology rejection from antibiotics in pig breeding through the use of probiotics

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Abstract. The article presents innovative technology development results of introduction of lactulose-containing probiotics for pig feeding. The purpose of the work is to study combined effect of biologically active additives «Lactumin» and «Lactusil» in comparison with drug for veterinary use (DP) in feeding young pigs on physiological state and productivity of animals. The scientific research was carried out on basis of farm-breeding plant named after Lenin of Surovikinsky District of Volgograd Region from January 2019 to June 2019. For this, 4 groups of large white piglets were formed. Each group consisted of 30 animals of two months old animals. Animals of the control group received a general economic diet (GED); analogues of experimental group I - GED + DP at a dosage of 6 mg per 1 kg of animal weight within 14 days; II experimental group - GED + a mixture of dietary supplements «Lactumin» and «Lactusil» at a dosage of 0.2 mg / kg of live weight each. In general, the use of biologically active additives «Lactumin» in common with «Lactusil» in diet of farm animals in comparison with DP did not have a negative effect on physiological state and level of humoral immunity. At the same time, the highest indicators of humoral immunity were found in animals of II experimental group, that received feed additive «Lactumin».

1. Introduction

The development of technologies that are safe in every sense in animal husbandry is a paramount task today. Nowadays, pork production is a dynamically developing branch of economics and the use of progressive solutions for raising pigs without the use of antibiotics in early stages of piglets life is one of the ways to address quality and safety issues especially due to the scale of use antibiotics in agriculture and associated selective development of pathogenic and opportunistic microorganisms, there is an acute problem of increasing antibiotic-resistant forms of microorganisms. In this regard, the most important issue in modern zootechnics is development of cultivation technology of young animals without using feed antibiotics. However, full realization of genetic potential of productivity of farm animals, including pigs, in industrial technologies conditions is impossible without the introduction of various biologically active additives into the diet that increase productivity and normalization of homeostasis indicators [1-9].

Many Russian and foreign scientists are developing modern technological solutions that provide for the use of various feed and biologically active additives, taking into account limiting feeding factor. These feed components contribute not only to an increase in live weight, but also to a large extent allow
improving physiological state and increasing the level of natural resistance of animals due to the high balanced and digestibility of animal diet. In addition, there are some studies about positive effect on the immune status of animals of biologically active substances in the diets [10-13].

In our research, we studied drug for veterinary use (DP) widely used in pig breeding, that contains active ingredient colistimethate sodium, which is formed by Bacillus polymyxa var. colistinus. Colistimethate sodium is colistin methanesulfonate. The active components of the drug have a bactericidal effect on gram-negative microorganisms by affecting to structural bonds of cytoplasmic and outer cell membranes.

DP is used against Pseudomonas aeruginosa, Haemophilus influenzae, Acinetobacter spp., Citrobacter spp., Enterobacter spp., Klebsiella spp., Salmonella spp., Shigella spp., but it does not function against Burkholderia cepacia and Staphylococcus aureus. Among methods used to improve the diets of pigs Russian and foreign researchers are often used premixes, feed additives, biologically active additives, in particular lactulose-containing ones [6, 14-20].

In earlier studies of Nikolaev S I, Volkolupov G V, Vodyannikov V I and Shkalenko V V, 2016 studied such lactulose-containing drugs as «Lactumin», «Lactofit» and «Lactoflex as a feed additive for gilts. The study of growth, development and meat productivity of pigs and physiological state of the experimental animals showed a high efficiency of these drugs.

However, studies of the complex action of the drugs in combination with the dietary supplement «Lactusil» on the immune status of animals have not been conducted.

To increase meat production, scientists from the Volga Region Research Institute of Manufacture and Processing of Meat-And-Milk Production have developed biologically active additives (BAA), among which some of the most effective are: BAA «Lactusil» and BAA «Lactumin» (RU Specification TU 9229-156-10514645-08) (State registration certificate within the customs union № RU 77.99.11003. E 001910.01.12 or 13.01.2012 r.). The composition of these preparations includes lactulose, malic and succinic acids, as well as substances obtained by extraction of milk thistle and Jerusalem artichoke, which greatly contribute to the improvement of absorption of feed nutrients. The composition of studied BAA in presented work is shown in table 1.

| Component                              | Mass fraction, % |
|----------------------------------------|------------------|
| Honey extract from sprouted milk thistle seeds | 81.75          |
| Lactulose                              | 18.00           |
| Malic acid                             | 0.25            |
| BAA «Lactumin»                         |                 |
| Lactulose                              | 50.00           |
| Succinic acid                          | 49.70           |
| Honey extract from fresh Jerusalem artichoke tubers | 0.30            |

The mechanism of prebiotics complex is based on positive impact of lactulose to bifidoflora gastrointestinal tract, as well as flavoring and nutritional effect caused by fresh Jerusalem artichoke (contains high-value component inulin) and milk thistle (contains polyphenols - 1.8%, flavonoids - 1.1 % and vitamin E 1.3%).

There are studies proving the high absorption of lactulose in lower intestine of animals due to action of specific enzymes available only there, as well as the growth of lactic acid bacteria as a result [6].

The uniqueness of the experimental work lies in study of the effect DP in comparison with effect of the probiotic complex on productivity and physiological health of pigs.

The purpose of the work is to study the effect of DP in comparison with combined use of biologically active additives «Lactumin» and «Lactusil» in feeding young pigs on physiological state and productivity of animals.
2. Materials and methods
The scientific research were carried out on basis of farm-breeding plant named after Lenin of Surovikinsky District of Volgograd Region from January 2019 to June 2019. For this, 4 groups of large white piglets were formed. Each group consisted of 30 animals of two months old animals.

Animals of the control group received a general economic diet (GED); analogues of experimental group I - GED + DP at a dosage of 6 mg per 1 kg of animal weight within 14 days; II experimental group - GED + a mixture of dietary supplements «Lactumin» and «Lactusil» at a dosage of 0.2 mg / kg of live weight each (table 2).

Table 2. Dosage of tested drugs.

| Index                  | Group                        | I experimental | II experimental |
|------------------------|------------------------------|----------------|-----------------|
| Animal weight, kg      | control                      | 15.15±0.67     | 15.42±0.71      | 15.20±0.52      |
| Dosage of test drugs   | DP                           | 6 mg / kg live weight | «Lactumin» 0.2 mg / kg | «Lactusil» 0.2 mg / kg |

The experimental animals were fed with a complete and balanced compound feed, that contained: exchange energy 12.34 MJ; dry matter 88.24%; crude protein 16.22%; crude fiber 5.86%; lysine 0.62%; methionine with cystine 0.47%; threonine 0.54%; calcium 0.66%; phosphorus 0.52%, as well as a group of vitamins (A, D3, E, K3, B1, B2, B3, B4, B5, B6, B12) and trace elements (iron, copper, zinc, manganese, cobalt, iodine and selenium). Compound feed was given in the morning and evening. Animals have free access to water. The experimental animals were cultivated up to 180 days of age.

During the experiment, blood was taken from the tail vein of animals at the age of 6 months for analysis. The natural humoral immunity of animals was studied by the bactericidal activity of blood serum by standard zootechnical methods.

Control slaughter of 5 animals from each study group was carried out according to methodology generally of All-Russian Institute of Animal Husbandry accepted in Russia (VASKhNIL, 1983). The results, obtained in our research were processed by the methods of variation statistics on a PC using STATISTICA-6 software package and determining the reliability criterion of the difference according to Student-Fisher at three levels of probability.

3. Results and discussion
It is known from literary sources that genotype of animals significantly affects the formation of natural immunity. At the same time, hematological parameters may indirectly indicate an intensification in metabolic processes occurring inside the body and also be an indicator of natural immunity.

The level of protein in the blood is influenced by the feeding factor, especially the amount of fat, which serves as an energy reserve for the body. The importance of lipids in the blood is determined by their ability to retain nitrogen with a lack of energy entering the body, which limits the catabolism of amino acids [21]. Results of laboratory studies of blood samples taken from the experimental animals in the age of 6 months are given below (table 3).

Table 3. Biochemical parameters of blood.

| Index                  | Group                        | I experimental | II experimental |
|------------------------|------------------------------|----------------|-----------------|
| Total protein, g / L   | control                      | 80.46±0.35     | 82.34±0.41      | 82.41±0.37      |
| Albumin, g / L         | 40.56±0.27                   | 42.16±0.33     | 41.26±0.29      |
| Globulins, g / L       | 39.86±0.31                   | 42.36±0.24     | 41.76±0.36      |
| α- globulins, g / L    | 12.36±0.17                   | 12.86±0.14     | 13.21±0.21      |
| β- globulins, g / L    | 8.70±0.09                    | 9.64±0.13      | 8.56±0.11       |
| γ- globulins, g / L    | 18.80±0.26                   | 19.86±0.29     | 19.99±0.34      |
| Calcium, mg%           | 10.80±0.14                   | 10.82±0.11     | 10.89±0.12      |
| Phosphorus, mg%        | 4.69±0.08                    | 4.76±0.07      | 4.74±0.09      |
Data presented in table 3 show the total protein content in the blood from animals of I and II experimental groups surpass the analogs of the control group by 1.88 g / L (P≤0.05), or 2.34% and 1.95 g / l, or 2.42% (P≤0.05); albumin - by 1.60 g / l, or 3.94% (P≤0.05) and 0.70 g / l, or 1.73%; globulins - by 2.5 g / l, or 6.27% (P≤0.01) and 1.9 g / l, or 4.77% (P≤0.05); α-globulins - by 0.50 g / l, or 4.04% and 0.85 g / l, or 6.88% (P≤0.05); γ-globulins - by 1.05 g / L, or 5.64% and 1.19 g / L, or 6.33%, respectively. The content of calcium and phosphorus in blood obtained from the animals of the experimental groups is higher in comparison with the control group, although the difference was insignificant and unreliable. However, higher content of β-globulins in blood of animals of I experimental group in comparison with analogs of the control and II experimental groups by 0.94 g / l, or 10.80% (P≤0.01) and 0.13 g / l, or 0.65%.

The experimental data indicate that higher concentrations of total protein and its fractions, noted in blood of animals of II experimental group, may suggest a more intensive metabolism of lipids, hormones, pigments and minerals that occur in their organs and tissues.

The level of activity of immune system depends on content of globulins in the blood serum of animals, since they are the most active components of immune defense of the body.

Study results of morphological parameters of the blood of experimental pigs is presented in table 4.

**Table 4.** Morphological parameters of blood serum, obtained from experimental animals (n = 4).

| Index                                | Average Group | Group control | I experimental | II experimental |
|--------------------------------------|---------------|---------------|---------------|----------------|
| Erythrocytes, 10^12/L               | 6.00-7.50     | 6.29±0.03     | 6.34±0.01     | 6.41±0.03      |
| Leukocytes, 10^9/L                  | 8.00-16.00    | 8.15±0.01     | 8.24±0.02     | 8.28±0.04      |
| Hemoglobin, g / L                   | 99.0-119.0    | 104.76±0.66   | 106.28±0.86   | 107.32±0.94    |
| Erythrocyte sedimentation rate (ESR), mm / h | 2.0-9.0       | 2.57±0.36     | 3.46±0.32     | 3.67±0.43      |

According to data of table 4, was established an advantage in content of erythrocytes in blood serum of animals of II experimental group in comparison with the analogs of the control and I experimental groups by 0.05 10^12 / L, or 0.79% and 0.12 10^12 / L, or 0.91% (P ≤0.05); leukocytes - by 0.09 10^8 / L, or 1.10% (P≤0.05) and 0.13 10^9 / L, or 1.59% (P≤0.05); hemoglobin - by 1.52 g / L, or 1.45% and 2.56 g / L, or 2.44%; ESR - by 0.89 mm / h, or 34.63% and 1.1 mm / h, or 42.80%, respectively.

However, superiority of erythrocytes content, hemoglobin and ESR in blood serum taken from animals of II experimental group in comparison with the control and I experimental groups does not exceed physiological norm, that characterizes them as conditionally healthy.

The result of calculating indicators of natural resistance of the animal - one of the main evaluation of state of immune system is given in table 5.

**Table 5.** Assessment of the natural immunity of experimental gilts.

| Index                                | Group control | I experimental | II experimental |
|--------------------------------------|---------------|---------------|----------------|
| Lysozyme, µg%                        | 18.67±0.15    | 19.15±0.16    | 19.26±0.17     |
| Attraction for 50 neutrophils, %     | 21.40±0.24    | 21.55±0.25    | 21.56±0.26     |
| The number of phagocytic neutrophils, % | 24.76±0.18    | 24.96±0.15    | 25.32±0.16     |
| Phagocytic index, %                  | 5.30±0.09     | 5.38±0.13     | 5.46±0.11      |

The data of table 5 show that content of lysozyme in the blood of animals I and II experimental groups surpass analogs of the control group by 0.48 and 0.59 mg%; attraction for 50 neutrophils - by 0.15 and 0.16%; the number of phagocytic neutrophils is 0.20 and 0.56%, respectively. It should be noted that the results obtained were significant, but not reliable.

It is generally known that the action of immune mechanisms is based on interactions of cellular and humoral immunities. This can be explained by the presence of two unrelated populations of
lymphocytes: B-cells, which form antibodies, and T-cells, involved in cell-type reactions. To solve this objective, four main participants in the immune system are used: phagocytosis, the complement system, humoral and cellular immunity.

In our case, a stronger response of the immune system was obtained from animals of experimental group I, which received DP. This is consistent with the research data of V S Popov et al., 2016.

The above research data indicate that animals participating in the experiment are healthy, and existing deviations in the parameters of humoral immunity were within physiological norm.

During the experiment, starting from the period from 120 to 150 days of age, the animals receiving the complex of biologically active additives «Lactumin» and «Lactusil» surpassed their counterparts in the control and I experimental group in live weight by 1.54 kg or 2.67% (P≤0.05) and 0.86 kg, or 1.87% (P≤0.05); from 150 to 180 days of age - by 2.14 kg, or 3.25% (P≤0.01) and 1.13 kg, or 2.76% (P≤0.01), respectively. Over entire period of the experiment, animals had a large weight in the control group, 116.98 kg; experimental group I - 118.36 kg; II experimental group - 119.34 kg.

Growth of lactic acid bacteria in gastrointestinal tract contributed to increase live weight gain in experimental animals of II experimental group, who received GED + the complex of dietary supplements «Lactumin» and «Lactusil» in comparison with analogs of the control, who received GED and I of the experimental group, who received GED + DP. The use of DP at the early stage of ontogenesis caused inhibition of the gastrointestinal microflora as a whole, both pathogenic, opportunistic, and beneficial. This circumstance had a negative impact on the gain in live weight during the first months of feeding animals. However, by the end of the experiment, these gilts outperformed analogues from the control group.

It is known that colistimethate sodium is excreted from the body of piglets within 20 days. Therefore, on day 160 of the experimental experiment, we stopped giving the drug.

At the age of 180 days, 5 analogs were selected from the animals participating in the experiment for control slaughter. The results of the control slaughter showed that carcasses obtained from animals of II experimental group in comparison with analogs of the control and I experimental groups were heavier by 3.40 and 2.86%; slaughter weight - by 5.76 and 4.65%; mass of hot carcass - by 5.56 and 4.46%, respectively.

The highest slaughter yield was noted from animals of the II experimental group, that exceeded the analogs of the control and I experimental groups by 1.20 and 0.60%.

In our opinion, improvement in the slaughter indicators could be caused by an increase in the level of enzymes that improve absorption of food in gastrointestinal tract.

Laboratory results of meat samples from experimental animals showed complete lack of traces of colistin. Chemical analysis of the average meat sample showed that pigs from II experimental group, that received the complex of biologically active additives «Lactumin» together with «Lactusil», had a higher protein content in comparison with analogs of the control and I experimental groups by 0.54 (P≤0.05) and 0.26 % (P≤0.05); dry matter - by 0.14 and 0.08%, and in terms of fat content they were lower by 0.22 and 0.18% with an insignificant difference.

4. Conclusion

The use of biologically active additives «Lactumin» in diet of feeding animals together with «Lactusil» in comparison with DP did not have a negative effect on physiological state and level of humoral immunity.

The high level of humoral immunity, established in the blood serum of experimental animals receiving a complex of biologically active additives, can be explained by their ability to stimulate erythropoiesis, lymphocytopeniosis, which contributes to the activation of humoral and cellular factors of nonspecific resistance of the organism: bactericidal, lysozyme, phagocytic activity.

We suppose that heightened content of protein and dry substances in the meat of experimental animals that received a complex of biologically active additives, was due to intensified metabolic processes and increased content of nitrogenous substances in body.
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References
[1] Zadera M I and Gruzdeva A K 2018 Using of antibiotics in cultivation of farm animals. Antibiotics in agricultural products Young scientist 19(205) 20-3
[2] Hedegaard C J and Heegaard P M 2016 Passive immunisation, an old idea revisited: Basic principles and application to modern animal production systems Veterinary Immunology and Immunopathology 174 50-63
[3] Shevtsov A A, Rusaleev V S, Shiryaev F A and Potekhin A V 2008 Economically significant diseases of pigs of bacterial etiology, methods of their diagnosis and means of prevention Industrial and pedigree pig breeding 4 31-5
[4] Tian Z, Cui Y, Lu H, Wang G and Ma X 2021 Effect of long-term dietary probiotic Lactobacillus reuteri 1 or antibiotics on meat quality, muscular amino acids and fatty acids in pigs Meat Science 171(108234)
[5] Baumann S, Schoof S, Bolten M, Haering C, Takagi M, Shin-Ya K and Arndt H 2010 Molecular determinants of microbial resistance to thiopепptide antibiotics Journal of the American Chemical Society 132(20) 6973-81
[6] Gorlov I, Sitnikov V, Shkalenko A, Sivko A and Bushueva I 2007 Increasing productivity of pigs and consumer qualities of their meat Pigbreeding 2 16-7
[7] Makarov D Yu, Ruzheinikov F V, Shkalenko V V and Nikolaev D V 2012 Lactulose-containing feed additives and their effect on the productivity of experimental pigs Pigbreeding 5 61-2
[8] Sinclair K D, Rutherford K M, Wallace J M, Brameld J M, Stoger R, Alberio R, Sweetman D, Gardner D S, Perry V E, Adam C L, Ashworth C J, Robinson J E and Dwyer C M 2016 Epigenetics and developmental programming of welfare and production traits in farm animals Reproduction Fertility and Development 28(10) 1443-78
[9] Neminsuchaya L A, Provotorova O V, Eremets N K, Nezhuta A A and Krashchko P A 2014 Promising of using a new class of probiotics and synbiotics based on them in animal breeding Veterinaria 1 kormlenie 6 21-2
[10] Mach N, Berri M, Estellé J, Levenez F, Lemonnier G, Denis C, Leplat, J, Chevaleyre C, Billon Y, Dore J, Rogel-Gaillard C and Lepage P 2015 Early-life establishment of the swine gut microbiome and impact on host phenotypes Environmental Microbiology Reports 7(3) 554-69
[11] Xin J, Zeng D, Wang H, Sun N, Zhao Y, Dan Y, Pan K, Jing B and Ni X 2020 Probiotic Lactobacillus johnsonii BS15 Promotes Growth Performance, Intestinal Immunity, and Gut Microbiota in Piglets Probiotics and Antimicrobial Proteins 12(1) 184-93
[12] Kryukov V, Glebova I, Zinoviev S and Shevyakov Aleniye 2018 The problem of selenium in feed: biology or technology Compound feeds 3 90-2
[13] Usenko D V 2016 The effect of antibiotics and probiotics on microbiome of gastrointestinal tract Medical Council 16 98-107
[14] Khramtsov A G, Ryantseva S A, Budkevich R O, Akhmedova V R, Rodnaya A B and Marugina E V 2018 Prebiotics as functional food ingredients: terminology, choice and comparative evaluation criteria, classification Problems of nutrition 87(1) 5-17
[15] Bindels L B, Delzenne N M, Cani P D and Wolter J 2015 Towards a more comprehensive concept for prebiotics Nature reviews 12 303-10
[16] Sonnenburg E D, Zheng H, Joglekar P, Higginbottom S K, Firbank S J, Bolam D N and Sonnenburg J L 2010 Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations Cell 141 1241-52
[17] Sukonina V, Lookene A, Olivecrona T and Olivecrona G 2006 Angiopoietin-like protein
4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue Proc. Natl. Acad. Sci. USA 103 17450-5

[18] Nikolaev S I, Volokolupov S I, Vodiyannikov V I and Shkalenko V V 2016 Biologically active fodder additives «laktumine», «lactofen» and «laktofleks» influence on piglets’ hematological parameters Izvestia of the Lower Volga Agro-University Complex 2(42) 147-52

[19] Landwehr B 2019 Feeding of weaning pigs for reduce antibiotic application Efficient animal husbandry 8 11-3

[20] Bagno O A, Prokhorov O N, Shevchenko S A, Shevchenko A I and Dyadichkina T V 2018 Use of phytobiotics in farm animal feeding Agricultural Biology 4 687-97

[21] Slashchilina T V, Semenov S N and Parfyonov G V 2016 Metabolic status of pregnant sows with the use of stevia as a component of their diet Vestnik of Voronezh state agrarian university 2(49) 93-101

[22] Popov V S, Samburov N V, Vorobieva N V and Zorikova A A 2016 Secondary immunodeficiency swine: clinical and immunological characteristics and principles of immunocorrection Bulletin of Kursk state agricultural academy 3 58-62