



Increase of Nonspecific Immunity and Productivity of Piglets when using the Probiotic *Lactobacillus reuteri*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The stress of weaning piglets negatively affects all factors of the body. First of all, this affects the violation of nutrition and a decrease in the activity of the immune system. The use of probiotics allows to reduce the negative consequences caused by weaning from a sow. The aim of the study was to study the effect of probiotics based on *Lactobacillus reuteri* on the indicators of nonspecific resistance and productivity of piglets. The obtained data showed a high efficiency of the use of probiotics. In the experimental group, the phagocytic activity of neutrophils increased by 4.4%, the phagocytic index was 6.6% higher, and the bactericidal activity increased by 29% compared to the control group. In piglets treated with *Lactobacillus reuteri*, body weight increased by 9.3%, and the average daily gains in the control group increased by 15.8%. The use of probiotics in the period of stress after weaning allows you to activate the immune status.

Keywords: Probiotic; *Lactobacillus reuteri*; immune status; productivity; piglets.

1. INTRODUCTION

In natural conditions, the transition from the mother's milk feeding to the basic diet (BD) occurs gradually over several weeks, during the same period, the active colonization of the newborn's body with various microorganisms and the formation of intestinal microflora takes place. Lactobacilli dominate in the microbiota of suckling pigs, while representatives of the genera *Firmicutes* and *Bacteroides* dominate in adult pigs [1]. In the modern practice of intensive pig breeding, piglets are weaned from sows at the age of 15 to 28 days. As a result of stress and a sharp change in the diet, the process of the intestinal microflora formation is disrupted. After weaning, the number of lactobacilli begins to decrease, opening the way for pathogenic microorganisms to colonize the intestines [2]. In addition, the piglets weaning is a serious burden on the developing immune system, which adapts to changing feeding conditions against the background of stress. This leads to a decrease in energy and nutrient consumption, and increases the susceptibility of piglets to various infections [3]. The postweaning period accounts for approximately 70-80% of piglet deaths, 50% of which are losses due to gastrointestinal diseases, and in the animals that have been ill, there is a decrease in growth and development rates by 30 – 50% [4]. For the prevention and treatment of infectious diseases caused by postweaning stress, since the early 1950s, antibiotic drugs have been widely used. However, the irrational use of antibiotics leads to the appearance of drug resistance genes in opportunistic and pathogenic microorganisms, the accumulation of antibiotics in agricultural products, and the transfer of antibiotic resistance genes from animal strains of microorganisms to microbial strains of the human population [5]. In this connection, in 2006, the European Union introduced a ban on the use of feed antibiotics. As an alternative to feed antibiotics and in order to normalize the intestinal microflora, preparations of living microorganisms – probiotics - have become widespread [6].

The action mechanism of probiotics on the macroorganism is diverse and multifaceted, but it is not yet well understood. One of the important functions of probiotic microorganisms is to protect the intestinal mucosa from pathogenic microflora by competing for nutrients, adhesion sites, and the synthesis of various bioactive substances. Intestinal lymphoid tissues, plasmacytes, and macrophages are the first to

respond to intestinal antigens, such as food toxins, bacteria, and viruses. Thus, the intestinal mucosa is the first stage of the immune defense of the digestive system [7]. Probiotics have a positive effect on the condition of the intestinal mucosa. In piglets received probiotics, there is an increase in the height of the villi and the depth of the crypts [8,9]. Macrophages play a key role in intestinal homeostasis maintaining by regulating cytokine secretion and an immune response generation [10]. Some types of probiotic lactobacilli can increase the activity of macrophages, the level of secretory immunoglobulin A, and lead to a decrease in the level of total cholesterol, low-density lipoproteins, and triglycerides [11,12]. A number of authors suggest that probiotics can promote the formation of soluble factors that change the permeability of the villi, which affects the absorption of various substances in the small intestine [13,14]. One of the properties of probiotic strains is an increase in the enzymatic activity of the intestine. Carbohydrates are one of the most important components of the diet. In the digestive tract, carbohydrates are mainly digested by saliva and pancreatic amylases and decomposed into monosaccharides by sucrase, maltase, and lactase, then absorbed in the small intestine. According to a number of authors, the use of probiotic bacilli, lactobacilli and thermophilic streptococcus increases the activity of sucrase, maltase and lactase in the jejunum mucosa, and also leads to an increase in protease activity [15].

The effectiveness of various probiotic preparations is different and depends on the types and strains of microorganisms that make up their composition, the dose of the preparation, the scheme of its use, the age and physiological state of the animals. At the same time, when developing new probiotic preparations, it is necessary to study in detail their effect on the physiological and biochemical status and productivity of the animals of the species for which they are intended, taking into account their physiological state, feeding conditions and maintenance [16].

One of the most promising probiotic strains of *Lactobacillus* is *Lactobacillus reuteri*. It is dominant in the gastrointestinal tract of many mammals, one of the most well - studied lactobacilli, and is widely used as a probiotic in humans and animals. *L. reuteri* is a heterofermentative bacterium that is considered one of the few truly autochthonous species of

Lactobacillus in humans and animals. Various gastrointestinal tract environmental conditions, such as influence of low pH in the stomach, contact with bile in the small intestine, etc., negatively affect on ingested probiotic microorganisms. The adhesion of the probiotic strain to enterocytes of the host intestinal mucosa is important for colonization, competition with pathogens, and interaction with the host intestinal immune cells [17]. A number of authors have shown that *L. reuteri* has high adhesive properties to enterocytes. Possible adhesion mechanism for *L. reuteri* is the binding of bacteria to surface proteins, exopolysaccharide, glucosyltransferase A, and inulosaccharose. Lactobacilli modulate the activity of several genes encoding adhesive proteins, such as E-cadherin and β -catenin [18]. *L. reuteri* also synthesizes various antimicrobial substances, such as lactic acid, hydrogen peroxide, reuterin, and reutericycline [19]. In vitro studies have shown that *L. reuteri* strains inhibit the growth of many intestinal pathogens, including *Escherichia Coli*, *Salmonella Spp*, *Staphylococcus Epidermidis*, *Staphylococcus Aureus*, *Helicobacter Pylori*, and rotavirus [20]. In addition, *L. Reuteri* can produce vitamin B12 and has the ability to synthesize L-lysine and folic acid [21].

2. MATERIALS AND METHODS

The strains *Lactobacillus reuteri* 395 and *Lactobacillus reuteri* 298 were used in the research. The strains were isolated from the intestines of healthy calves, identified by 16S RNA, and deposited in the RNCM.

To obtain the preparation, *L. reuteri* strains 395 and 298 were individually grown on natural skimmed milk in a volume of 2.0 liters for 48 hours at t 38 ° C, after which 5% sucrose and 5% skimmed milk powder were added to the grown culture as cryoprotectors, frozen in a low-temperature freezer and then subjected to lyophilic drying. After drying, the lyophilizate was crushed, homogenized, and the amount of CFU in 1 g was determined by seeding on a nutrient medium (MRS-agar) from a series of ten-fold dilutions, followed by visual counting of the grown colonies according to OFS. 1. 7.2.0008.15.

The research was conducted in the institute vivarium on crossbred piglets (♂ Danish Yorkshire × ♀ Danish Landrace) at 45 days of age immediately after weaning. According to the

principle of analogous pairs, taking into account the live weight, two groups of piglets with 10 heads each were formed. Group maintenance was carried out in cages, drinking was from autodrunkers.

The animals of the control group received a complete SCC-51 (BD). Piglets of the second (experimental) group, in addition to the BD, received 1 g/head of a mixture of *L. reuteri* strains 395 and 298 daily for 30 days. The amount of CFU in 1 g was 10^{10} . To obtain a feed additive, the lyophilizates were mixed in a ratio of 1:1, stored at t 4 ° C.

The duration of the experiment was 1 month, during which the general condition of the animals was monitored daily.

During the experiment, the feed consumption and its consumption per unit of growth were taken into account. The piglets were weighed at the beginning and end of the experiment.

The study of the microflora of the digestive tract was carried out by microbiological investigations of feces, which were obtained by the act of forced defecation.

At the end of the experiment, blood was taken from the mammary vein in animals from each group to study hematological and biochemical parameters. The number of red blood cells and white blood cells, the level of hemoglobin was determined by an automatic hematological analyzer Mindray BC-2800 Vet. The calculation of the leukocyte formula was carried out in smears stained according to Romanovsky-Giemsa [22]. The phagocytic number and phagocytic index were determined using a direct morphological method [23], with the *E. coli* strain 113-3 used as a test culture. The bactericidal activity of blood serum was determined according to the method of Hirsch [25]. Plasma lysozyme activities were measured by the agar plate method of Osserman and lawler [23]. Statistical analysis of the obtained values of the studied indicators was evaluated using the Wilcoxon-Mann-Whitney U-test. The significance of differences was assessed at $P < .05$ by the corresponding age. We used the BioStat 7 program.

3. RESULTS AND DISCUSSION

Throughout the entire experiment, the animals were healthy and ate feed well.

The growth efficiency indicators of piglets when they were kept on diets with the addition of *L. reuteri* were higher than in the animals of the control group (Table 1).

At the beginning of the experiment, the live weight of the experimental piglets was 13.30 ± 0.37 kg. At the end of the experiment, the average live weight of piglets who received *L. reuteri* was 33.80 ± 0.8 kg, and of the control group piglets - 31.0 ± 0.81 kg, which is 9.03% higher. The average daily gains were significantly ($P < 0.05$) higher in piglets who received *L. reuteri* and amounted to 586 ± 28 g, while in the control group it was 506 ± 16 g, which is 15.8% higher compared to the control group. The viability in all groups was one hundred percent.

At the same time, feed costs per 1 kg of gain in piglets who received *L. reuteri* were 13.6% lower compared to the control group, even taking into account the cost of a probiotic supplement, which correlates with the data of other researchers [14]. The use reduced the cost of crude protein by 1 kg of gain in piglets who received *L. reuteri* by 13.4 and exchange energy by 13.6 compared to the animals of the control group.

In the assessment of nonspecific resistance of the organism, there is a certain significance in hematological profile, changes in which are an important indicator of the external environment influence on the body [6]. Hematological parameters in all piglets were within the physiological norm; however, there was a tendency to increase the content of hemoglobin and red blood cells in piglets in the group receiving the *L. reuteri* supplement. Similar results were obtained by other researchers who studied the hematological parameters of weaned piglets when using probiotic preparations [11] (Table 2).

One of the most important properties of probiotic preparations is their ability to increase the immune status of the organism. It is known that the use of probiotic supplements in the diet of animals activates the absorption and digestion capacity of phagocytes, increases the level of lysozyme and secretory immunoglobulins [3]. In our research, when studying the effect of the introduced cultures on the overall immune status of animals, phagocytic and bactericidal activity, as well as the content of lysozyme in the blood serum, were determined. Indicators of nonspecific resistance of piglet blood serum are shown in Table 3.

Table 1. Live weight, average daily gains, consumption of feed, crude protein and metabolic energy in experimental piglets (n=10)

	Control	Experimental group
Live weight at the beginning of the experiment, kg	13,30+0,37	13,30±0.51
Live weight at the end of the experiment, kg	31,00+0,81	33,8±0,80
% to the control		109,03
Live weight gain, kg	17,70± 0,58	20,50±0,96
Average daily gain, g	506±16	586±28*
% to the control		115,8
Feed consumed per period, kg	48±2	48±1,8

Note: * ($P \leq 0.05$) according to the t-test when compared with the control group

Table 2. Hematological parameters of piglets (n=10)

	Control	<i>L. reuteri</i>
Hemoglobin, g / l	130,89±0,15	139,99±1,23
White blood cell count, $10^9/l$	11,05±0,13	10,45±0,17
Red blood cell count, $10^{12}/l$	6,24±0,47	7,01±0,20
Leucogram, %		
Basophils, %	1,6	1,6
Eosinophils, %	4	2,5
Neutrophils:		
Band forms, %	0,6	0,7
Segmented, %	35,1	35,6
Lymphocytes, %	55,6	57,5
Monocytes, %	3,1	2,1

Note: * - $p < 0.05$, compared to the control group

Table 3. Indicators of nonspecific resistance of piglets (n=10)

Indicator	Control	<i>L. reuteri</i>
Phagocytic activity, %	45,2±1,15	47,2±2,2
Phagocytic index	5,40±0,13	5,76±0,14
Blood serum bactericidal activity, %	56,7±3,2	73,4±4,2*
The content of lysozyme in the blood serum, mcg/ml	58,0±2,4	71,2±1,41

Note: * - $p < 0.05$, compared to the control group

As shown in the table, in the blood serum of animals of the experimental groups, the content of lysozyme and the total bactericidal activity significantly increased, while the phagocytosis indicators in all groups were approximately at the same level. As is known, the main phagocytic blood cells are neutrophils. The content of neutrophils in the blood of all the observed animals during the experiment did not significantly differ, the introduced probiotic supplement did not have a significant effect on their activity.

4. CONCLUSION

In the course of the conducted researches, it was found that the introduction of a probiotic strain of *L. reuteri* into the basic diet of weaned piglets has a stimulating effect on the digestive system. It is expressed in the growth rate of piglets, and the weight gains at the end were higher by 9.3% compared to the control group. When recalculating to the average daily weight gain, in the experimental group it was 15.8% higher than in the control group. At the same time, feed costs per 1 kg of growth decreased by 13.6%. Probiotic microorganisms have a stimulating effect on the immune system of the intestinal mucosa and on the nonspecific immune defense of the organism at large. In our research, the addition of *L. reuteri* to the diet of weaned piglets led to positive changes in the microflora of the digestive tract, which affected the increase in the bactericidal activity of blood serum and the content of lysozyme in serum. Thus, the use of probiotics in the postweaning period helps to increase the nonspecific resistance and productivity of piglets.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The procedures used in this experiment were approved by the Institute of Physiology, Biochemistry and Nutrition of Animals (Borovsk, Russian Federation)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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