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Posted Date: 29 November 2024

doi: 10.20944/preprints202411.2332.v1

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Article

Study of the Efficiency of a Carotene-Containing Probiotic Feed Additive in the Diet of Growing Pigs

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Abstract: The effect of carotene-containing probiotic feed additive on the digestibility and assimilation of feed and zootechnical indices of growing piglets was studied. The study involved 40 crossbred male piglets (F1: Duroc–Landrace) with a live weight of 20–25 kg. Two groups were formed, each with 20 piglets, based on the principle of analogues. The experiment was performed during a fattening period and lasted 30 days. The experimental group received a complete feed supplemented with the studied feed additive that resulted in a 6.8% increase in the daily weight gain compared to the control group. Piglets from the experimental group showed increased digestibility coefficients for dry matter (+2.2%), organic matter (+2.5%), and protein (+2.1%) compared to the control group. Additionally, the experimental group demonstrated a trend towards increase in the protein fractions in blood serum as well as the same trend for the phagocytic activity, phagocytic index, and phagocytic count. The study also demonstrated a significant reduction in the number of *Salmonella* and *Enterococcus* cells in the experimental group. The obtained results allow us to recommend this feed additive for use in the diet of fattened piglets.

Keywords: feed additive; piglets; microflora; digestibility; daily weight gain; biochemical blood parameters; nonspecific immunity parameters

1. Introduction

Being a source of protein and other essential nutrients, pork plays a crucial role in human nutrition and represents one of the most widely consumed types of meat [1]. A survey conducted in 2022 revealed that pork made up to 34% of the global meat consumption, with poultry and beef accounting for 40% and 22%, respectively [2]. The global pork consumption has increased by 77% from 63.5 million tons in 1990 to 113 million tons in 2022. According to some forecasts, the demand for pork will continue to rise due to the rapid growth of the global population [2,3].

Gastrointestinal diseases (GIT) take the second place after viral infections and are the primary cause of piglet mortality [4,5]. However, some researchers suggest that disorders in the normal gut formation and functioning in young animals may be associated with factors beyond the development of infectious diseases [6]. For example, it is known that poor nutrition (characterized by insufficient intake of nutrients and vitamins) of piglets immediately after weaning may result in negative morphological and functional changes in the intestine [7–10]. The innate and adaptive immune systems of weaned piglets are underdeveloped [8,11,12]. Piglets also face various stress factors caused by weaning and establishing new social relationships within their group [13]. These unfavorable factors combined with non-compliance with veterinary and sanitary requirements during piglet suckling may cause the development of gastrointestinal diseases followed by the death of an animal [9,14].

One of the main approaches to prevent and treat gastrointestinal diseases in piglets on farms includes the use of antibiotics [5,11,12,15]. However, a long-term use of antibiotics, particularly those with the broad-spectrum activity, has led to the development of antibiotic resistance in pathogenic and opportunistic microflora [5,16]. In 2016, the UN General Assembly recognized the use of antibiotics in animal husbandry and poultry farming as one of the primary causes of antibiotic resistance in humans. Since 2006, the European Union has banned the use of antibiotics in these agricultural sectors [16,17].

Following the ban on antibiotic use, new approaches to treating gastrointestinal diseases have been developed based on the restoration of the natural microflora of the organism using biological active products (probiotics), which activity is comparable to that of antibiotics and chemotherapeutic agents [11,12,18]. According to the existing data, probiotic feed additives provide a complex effect on the organism via normalization of digestive processes and enhancement of non-specific immunity and contribute to the survival of piglets and increased productivity of agricultural animals [11,12].

Since the normal gut flora of animals represents a complex polymicrobial ecosystem, probiotic preparations should be based on a microbial consortium of mutually complementary beneficial bacteria possessing a range of valuable probiotic properties [12,19–21]. Taking this aspect into consideration, multi-component probiotic products are developed in recent years including those based on indigenous microorganisms with different mechanisms of biological activity; such approach provides a wide range of their application [21–23].

The performed review of published data shows that *Bacillus* bacteria are promising strains for developing feed additives, which probiotic activity is associated with the synthesis of antimicrobial compounds, enhancement of non-specific and specific immunity, stimulation of the normal gut flora growth, and secretion of digestive enzymes [24–26]. In recent decades, researchers demonstrate an active interest in application of the bacteria from this genus as probiotic products for the pig farming.

Factors, which contribute to the reduction of the organism resistance, may cause illness and death of young animals. For example, vitamin deficiency in the diet of piglets during fattening as well as their poor absorption (malabsorption) negatively influence on their metabolic processes and immune status [27]. According to some publications, vitamin A functions are not limited by its influence on the visual system development [28]. This vitamin is important for the maintenance of a normal cell growth and differentiation as well as for the development of both innate and adaptive immunity [29–33]. The positive role of the vitamin A in the organism adaptation to various stresses has been also shown [27]. Therefore, the existing data allow us to state that vitamin A-containing feed additives can become the part of an effective strategy for strengthening swine health and provide a complex approach to control infectious agents, parasitic threats, and environmental stress factors in the swine industry [33–36].

In view of the aforementioned, the purpose of this study was the assessment of the effect of a carotene-containing probiotic feed additive on the average daily weight gain, feed digestibility, and biochemical, morphological, and immunological blood parameters in growing piglets during their fattening.

The feed additive (FA) tested in this study was developed at the Russian Biotechnological University (ROSBIOTECH); it contains the mix of two strains of spore-forming bacteria *Bacillus subtilis* VKM B-3826D and *Bacillus licheniformis* VKM B-3825D and an inactivated biomass of *Mycobacterium neoaurum* strain VKM Ac-3067D at a weight ratio of 0.5 : 0.5 : 1, respectively. One gram of the feed additive contains no less than 5×10^9 CFU (colony-forming units) of spore-forming *Bacillus* bacteria and no less than 250 µg of carotenoids; the content of crude protein $21.5 \pm 1.6\%$.

2. Materials and Methods

2.1. Nutrient Media for Isolation of Microorganisms

Isolation of *Lactobacillum*, *Bifidobacterium*, and *Escherichia* species was performed using Lactobacagar, Bifidum medium, and Endo-GRM agar, respectively. Isolation of *Salmonella*, *Enterococcus*, and *Clostridium* species was performed using Bismuth-sulfite-GRM agar, Enterococcus agar, and polymyxin-containing Sulfite agar, respectively. Fungal species were isolated using

Sabouraud agar. All media were manufactured by the State Research Center for Applied Microbiology and Biotechnology (Obolensk, Russia). *Staphylococcus* species were isolated using a special selective salt medium manufactured by Biotekhnovatsiya Ltd. (Electrogorsk, Russia).

2.2. Experimental Design

The study involved 40 crossbred male piglets (F1: Duroc–Landrace) entered the fattening period. The live weight of animals purchased in the Klimovskaya Eco-farm Ltd. (Klimovskaya village, Kaluga region, Russia) was 20–25 kg. Based on the principle of analogues, two groups of animals (experimental and control) were formed, each including 20 piglets. The experiment lasted 30 days. The experimental group was fed by the standard ration supplemented with FA (1.0 kg per ton of feed); the control group was fed by the standard ration only. The scheme of the experiment is shown in Table 1.

Table 1. Scheme of the experiment.

Group	Number of animals per group	Ration ¹
Physiological experiment (25 days)		
1 (control)	20	SMF
2 (experimental)	20	SMF + FA (1.0 kg/ton)
Registration period of the balance experiment (5 days)		
1 (control)	10	SMF
2 (experimental)	10	SMF + FA (1.0 kg/ton)

¹ SMF, standard mixed fodder; FA, feed additive.

2.3. Diet and Conditions

The standard mixed fodder (SMF) used in the study as the basal diet complied with the energy and nutritional requirements for the piglets of the given age and weight class. Animal housing conditions including temperature, humidity, and light regimes as well as the air composition in the pen were similar for all groups and met to the zoohygienic norms. The composition and nutritional value of SMF are presented in Supplementary Materials 1.

After group formation, animals from both control and experimental groups were grown in individual pens under the same conditions (16–25°C, relative humidity 45–70%, natural day/night alteration).

2.4. Determination of Average Daily Weight Gain

The overall and average daily weight gains of piglets were evaluated using a TV-M-300.2-A1 electronic floor scales (Russia). The weighing was performed each morning prior feeding in the course of the whole experiment.

The overall (absolute) weight gain (*A*) was calculated using the following formula:

$$A = W_1 - W_0,$$

where *W*₀ and *W*₁ represent the weight of animals (kg) in the beginning and at the end of the experiment.

The average daily weight gain (*D*) was calculated using the following formula:

$$D = A/d,$$

where *A* is the overall weight gain and *d* is the number of days (duration of the experiment).

2.5. Balance Experiment

At the end of the experimental period, 10 piglets from each group were moved into special balance cages equipped with individual feeders and urine and feces collectors. The duration of the recording period was 5 days.

To determine the FA effect on the feed consumption, the amount of both provided feed and its residues was fixed on a daily basis for each piglet throughout the whole recording period.

After completion of the balance experiment, the averaged samples of feeds, feces, and urine were subjected to the chemical analysis at the Laboratory of Immunobiotechnology and Microbiology of the All-Russian Research Institute of Animal Physiology, Biochemistry, and Nutrition using standardized methods.

The initial moisture content was determined by drying the samples at a temperature of 60–70°C to a constant weight; the hygroscopic moisture content was determined by drying the samples at 100–105°C.

The total nitrogen content in the dried samples was determined by the Kjeldahl method; the crude protein content was determined by multiplying the nitrogen content (%) by a coefficient of 6.25.

2.6. Determination of Hematological Parameters

Upon a completion of the experiment, blood was collected from the milk vein of each piglet into two sterile tubes under aseptic and antiseptic conditions. Blood in one tube was stabilized with EDTA, while another tube was used to obtain serum for the further analysis of biochemical and hematological parameters using an automated ERBAXL-100 biochemical analyzer (ErbaLachema, Czech Republic) and an automated Mindray BC-2800 Vet hematological analyzer (Mindray, China). The analyzed biochemical parameters of the serum included: total protein, g/L; albumin, g/L; globulin, g/L; urea, mmol/L; creatinine, mmol/L; bilirubin, $\mu\text{mol/L}$; alanine transaminase (ALT), IU/L; aspartate transaminase (AST), IU/L; alkaline phosphatase, mmol/L; cholesterol, mmol/L; triglycerides, mmol/L; phospholipids, mmol/L; glucose, mmol/L; calcium, mmol/L; and phosphorus, mmol/L. Hematological parameters included erythrocyte and leukocyte counts, hemoglobin concentration, and hematocrit.

2.7. Determination of the Retinol Content in Blood Serum

The retinol content in a blood serum was determined using an Agilent HPLC 1200 system (Agilent Technologies, United States) equipped with a G1315A DAD diode-array detector (Agilent Technologies, United States), controlled via a personal computer with an installed “ChemStation” software for collecting and processing chromatographic data (Agilent Technologies, United States). The following reverse-phase columns were used: Luna NH2 (250 × 4.6 mm) and Luna Phenyl-Hexyl (150 × 4.6 mm) (both from Phenomenex, United States), Zorbax SB-C18 and Zorbax SB-C8 (150 × 4.6 mm) (both from Agilent, United States). The particle size of the used sorbent was 5 μm . The chromatographic analysis was performed under isocratic elution conditions using the acetonitrile-water mix (90:10) as a mobile phase; the flow rate was 1.0 mL/min. Retinol was detected at 325 nm based on its retention time (min).

The calibration curve was built using a reference retinol solution (Sigma-Aldrich, United States). Calibration solutions were prepared by diluting the stock retinol solution (1 mg/mL) in absolute ethanol.

The carotene assimilation from the studied feed additive was evaluated by the vitamin A concentration in blood serum measured using an AIFR-01 UNIPLAN immunoassay analyzer (Pikon, Moscow, Russia) and a Human Vitamin A, VA ELISA kit (CUSABIO, Houston, TX, United States).

2.8. Determination of Non-Specific Resistance Parameters

The non-specific resistance parameters determined in this study included the phagocytic activity of blood cells (PP, phagocytosis percentage; PI, phagocytic index; and PC, phagocytic count) [37].

The culture of *E. coli* (0.5 mL) was added to 0.5 mL of blood and incubated on a shaker at 37°C for 30 min. The resulting sediment was smeared, fixed with 96% methanol, stained according to the Romanowsky–Giemsa method, and microscopied at a 90× magnification. *E. coli*-engulfed neutrophils were counted as positive cells. The analysis included 100 neutrophils per a slide. The following parameters were determined:

$$\text{PP} = (\text{Number of neutrophils involved in phagocytosis} / \text{total number of neutrophils}) \times 100\%;$$

PI = Number of ingested *E. coli* cells /100 active neutrophils;
PC = Number of phagocytosed bacterial cells/all neutrophils.

2.9. Study of the Microflora of the Gastrointestinal Tract

The microflora of the gastrointestinal tract was studied by the Koch method. The most significant groups of microorganisms were investigated, including *Bifidobacterium*, *Lactobacillus*, *Salmonella*, *Staphylococcus*, *Escherichia*, *Enterococcus*, *Clostridium*, and *Candida* spp.

An aliquot (1 g) of the averaged sample of feces was placed into a sterile tube containing 9 mL of a physiological solution. The resulting sample was then diluted tenfold in a sterile physiological solution. From each dilution, a 100-μL inoculum was taken and spread onto the surface of selective agar medium in 9-cm Petri plates using a Drigalsky spatula. The plates were then incubated at 38°C for 24 h. To cultivate *Bifidobacterium* and *Clostridium* species, the plates were placed into an Anaerogaz anaerobic incubator with gas packs (Biomer LLC, Krasnoobsk, Russia). To ensure accurate results, the inoculation was performed in triplicate. The number of grown colonies was counted visually.

The number of CFU (colony-forming units) per gram of feces was calculated using the following formula:

$$M = \frac{a \cdot 10^n}{V},$$

where *a* is the average number of colonies obtained from the given dilution, 10 is the dilution factor, *n* is the serial number of the dilution, from which the inoculation was performed, and *V* is the volume of a suspension taken for inoculation (mL).

The final number of bacteria was considered as the arithmetic mean of the colony counts on plates with inoculums taken from two sequential dilutions.

2.10. Statistical Data Treatment

The obtained results were statistically treated by variational statistics procedures using the Student’s t-test at a significance level of *p* < 0.05 and a Microsoft Excel 2010 program package.

3. Results

3.1. Effect of Feed Additive on the Average Daily Weight Gain of Growing Fattening Pigs

The performed experiments showed that the FA application promotes more intensive growth of animals of the experimental group (Table 2). At the end of the experiment, there was a tendency to an increase in the live weight of piglets from the experimental group; the average daily gain in these animals calculated for the whole period exceeded that in the control group by 6.8% (*p* < 0.05; Table 2). At the end of the experiment, the gross weight gain in piglets from the experimental group exceeded the same value for the control group by 15.8% (*p* < 0.05). No difference in the mixed fodder consumption by piglets from the experimental and control groups was observed.

Table 2. Dynamics of the growth of experimental piglets (mean ± SD, *n* = 20).

Parameter	Piglet group	
	Control	Experimental
Duration of the experiment, days	30	
Live weight at the beginning of the experiment, kg	25.3 ± 0.8	25.2 ± 0.7
Live weight at the end of the experiment, kg	44.3 ± 1.3	47.3 ± 1.2*
Absolute live weight gain, kg	18.9 ± 2.2	22.1 ± 1.2*
Average daily weight gain, g	630 ± 52	730 ± 22*
Live weight, % of the control	100.0	106.8

**p* < 0.05.

3.2. Balance Experiment Results

The daily registration of the consumed fodder and produced feces in relation to their chemical composition made it possible to calculate digestibility coefficients for fodder nutrients (Table 3). Compared to the piglets from the control group, animals from the experimental group, which received FA together with a fodder, demonstrated an increase in the coefficients of digestibility of dry matter, organic matter, and protein by 2.2, 2.5, and 2.1%, respectively ($p < 0.05$).

Table 3. Nutrient digestibility coefficients (%) in control and experimental groups of fattening piglets ($M \pm m$, $n = 10$).

Nutrient	Piglet group	
	Control	Experimental
Dry matter	75.13 $\pm$ 0.76	76.82 $\pm$ 0.66*
Organic matter	73.34 $\pm$ 0.70	75.23 $\pm$ 0.72*
Protein	75.60 $\pm$ 0.57	77.20 $\pm$ 0.43*

* $p < 0.05$.

During the balance experiment, the balance and utilization of nitrogen, phosphorus, and calcium by piglets were calculated to study the protein metabolism. FA application affected the amount of digested and assimilated nitrogen. Nitrogen accumulation in the bodies of piglets from the experimental group exceeded that in the control group by 1.9% (Table 4).

Table 4. Average daily balance and nitrogen utilization by fattening piglets (mean $\pm$ SD, $n = 10$).

Nutrient parameter	Piglet group	
	Control	Experimental
Received with the fodder, g	58.40 $\pm$ 0.5	58.90 $\pm$ 0.5
Released with feces, g	4.91 $\pm$ 0.55	4.98 $\pm$ 0.40
Digested, g	53.48 $\pm$ 0.65	53.93 $\pm$ 0.27
Released with urine, g	13.92 $\pm$ 0.33	13.71 $\pm$ 0.19
Accumulated in the body, g	39.50 $\pm$ 0.30	40.25 $\pm$ 0.23*
Utilized, % of:		
Received nitrogen	67.63 $\pm$ 0.35	69.36 $\pm$ 0.24
Digested nitrogen	73.85 $\pm$ 1.16	74.63 $\pm$ 1.22

* $p < 0.05$.

A comparison of data on the calcium and phosphorus balance and utilization in piglet groups showed that animals from both groups received almost the same amount of calcium with feed (Table 5). Calcium retention in the experimental group exceeded that in the control by 1.01%. Analyzing data on the use of phosphorus, we observed the similar tendency for the calcium assimilation; however, animals from the control group demonstrated an increased phosphorus assimilation (+40.3%, $p < 0.05$; Table 5). Supplementation of the diet of experimental piglets with FA provided a better utilization of calcium and phosphorus from the fodder.

Table 5. Average daily balance and utilization of calcium and phosphorus in fattening piglets (averaged for the group, mean $\pm$ SD, $n = 10$).

Nutrient parameter	Calcium Piglet group		Phosphorus Piglet group	
	Control	Experimental	Control	Experimental
Received with the fodder, g	14.30 $\pm$ 0.2	14.30 $\pm$ 0.2	9.0 $\pm$ 0.1	9.0 $\pm$ 0.1
Released with feces, g	7.03 $\pm$ 0.38	7.22 $\pm$ 0.38	5.80 $\pm$ 0.38	5.92 $\pm$ 0.15
Released with urine, g	0.50 $\pm$ 0.03	0.20 $\pm$ 0.10*	2.21 $\pm$ 0.11	1.55 $\pm$ 0.07*

Accumulated in the body, g	6.49 ± 0.30	6.61 ± 0.29	1.14 ± 0.22	1.60 ± 0.17*
Utilized, % of received amount	45.38 ± 1.05	46.71 ± 1.03	12.67 ± 1.11	18.50 ± 1.22

*p < 0.05.

3.3. Effect of Feed Additive on Biochemical, Morphological, and Immunological Characteristics of the Blood of Fattened Piglets

In order to study the effect of FA on metabolic processes in the organisms of experimental piglet, the data obtained from the biochemical and morphological blood studies were analyzed (Table 6). The studied blood parameters generally fell within reference values [38,39]. However, there was a trend of increasing content of total protein in the experimental group compared to the control one as well as the similar trend of increasing content of protein fractions in the blood serum of experimental animals.

The content of urea representing the end product of a protein metabolism was higher in the experimental group by 2.5% that correlates with an increase in the creatinine concentration by 3.2%.

Table 6. Biochemical and morphological blood parameters in experimental and control groups of piglets (mean ± SD, n = 20).

Parameter	Piglet group		
	Control	Experimental	Reference values ¹
Total protein, g/L	64.72±1.82	66.8±2.22	58.0–85.0
Albumin, g/L	24.08±3.06	25.01±2.05	20.0–45.0
Globulin, g/L	40.94±2.18	41.74±4.56	23.0–54.0
Urea, mmole/L	6.24±0.86	6.36±0.46	4.9–8.5
Creatinine, mmole/L	130.02±25.12	135.25±14.07	113.0–172.0
Total bilirubin, µmole/L	5.14±0.12	7.29±0.05	0.3–8.2
ALT, ME/L	41.22±26.22	47.06±8.90	30.0–94.0
AST, ME/L	34.25±9.09	35.13±6.27	13.0–97.0
Alkaline phosphatase (ALP), mmole/L	246.30±70.00	256.01±40.10	74.0–454.0
Carotene, mmole/L	0.65±0.12	0.79±0.09*	–
Vitamin A, mmole/L	0.39±0.03	0.45±0.04*	–
Cholesterol, mmole/L	2.20±0.53	2.10±0.41	2.1–3.5
Triglycerides, mmole/L	0.33±0.08	0.29±0.10	0.12–0.60
Phospholipids, mmole/L	3.61±0.10	3.39±0.08	–
Glucose, mmole/L	4.50±0.67	4.71±0.92	3.7–6.4
Calcium, mmole/L	1.98±0.16	2.01±0.09	1.8–3.7
Phosphorus, mmole/L	2.13±0.14	2.39±0.26	2.3–4.8
Leucocytes, 10 ⁹ /L	10.10±1.91	9.10±2.60	8.0–16.2
Erythrocytes, 10 ¹² /L	6.90±0.24	6.20±0.27	6.0–7.5
Haemoglobin, r/L	124.78±32.41	124.85±5.5	90.0–130.0
Hematocrit,%	46.72±5.48	47.12±4.35	40.0–50.0

¹ According to [38,39].

According to the data shown in Table 6, blood samples of the experimental group were characterized by an increased content of calcium and phosphorus that correlates with the data on the assimilation balance of calcium and phosphorus presented in Table 5. The analysis of these data showed the use of the studied feed complex promotes an increase in the carotene content in blood of animals by 15.3%. The analysis of hematological parameters did not show any significant differences between groups in the content of erythrocytes and haemoglobin as well as in hematocrit.

3.4. FA Effect on Nonspecific Resistance Indices

Improvement of criteria determining immunological status of animals plays the key role in the livestock safety and the intensive weight gain. The effect of FA on the nonspecific resistance indices are shown in Figure 1.

According to the obtained data, the PP index in the experimental group of piglets increased that in the control group. The same tendency was revealed for the PI and PC indices. These results indicate a positive effect of FA on the immunity of animals.

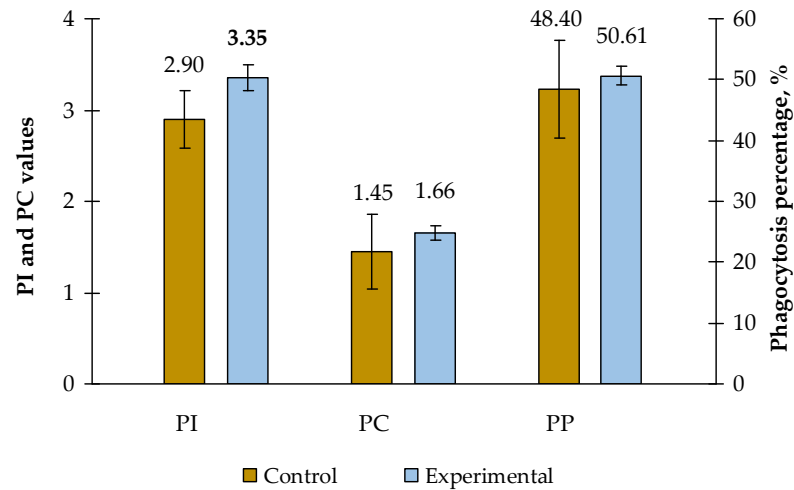


Figure 1. Indices of nonspecific immunity in the control and experimental groups of piglets (mean $\pm$ SD, $n = 20$). PI, phagocytic index; PC, phagocytic count; PP, Phagocytosis percentage. Values significantly ($p < 0.05$) differing from the control are indicated in bold.

3.5. Microbiological Profiling of the Intestine Content

The results of a comparative analysis of the intestinal microflora of piglets from the experimental and control groups at the end of the experiment showed a significant effect of FA on its composition (Figure 2). The number of *Lactobacteria* and *Bifidobacteria* cells in the intestine of piglets fed with FA was an order of magnitude higher than that in the control group. A statistically significant reduction in the number of *Salmonella* and *Enterococcus* bacteria was observed for the animals from the experimental group.

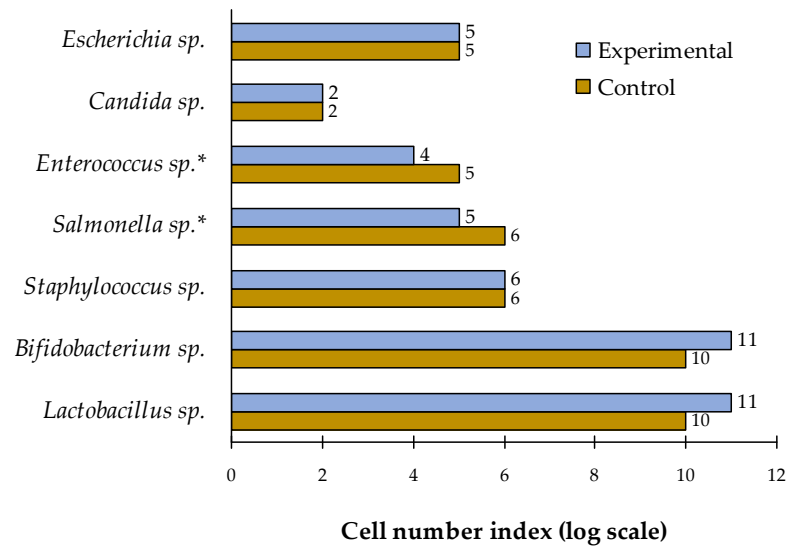


Figure 2. Comparative diagram for the amount of intestine microflora in the experimental and control groups of piglets (mean $\pm$ SD, $n = 20$). Values significantly ($p < 0.05$) differing from the control are indicated with the asterisk.

Thus, the performed analysis revealed differences in the quantitative composition of the intestinal microflora of piglets from different groups. The content of fungal species as well as *Escherichia coli* and *Staphylococcus* did not differ significantly between the groups.

Therefore, FA introduction in the diet of fattening piglets results in some changes in the composition of the intestinal microbiocenosis, such as the tendency to the reduction of quantitative indices of opportunistic microflora.

4. Discussion

Reduced feed consumption by piglets during the weaning period provides a negative impact on the intestine development and functioning. This factor, together with the immaturity of the immune system, predisposes piglets to the development of gastrointestinal disorders. Therefore, the care of piglets during the weaning period is one of the most challenging tasks in the industrial pig breeding [9]. Recently developed new approaches for the prevention and treatment of gastrointestinal diseases are based on the recovery of the natural intestine microflora by probiotic preparations capable of non-specific controlling populations of the opportunistic pathogenic microflora by its displacing from the intestinal microbiocenosis [18,26,40]. The positive effect of *Bacillus*-based probiotic feed additives on the animal productivity is well known [41,42].

The level of satisfaction of piglet needs in energy, nutrients, minerals and biologically active substances, as well as the quantitative and qualitative evaluation of feed rations in experimental piglets are assessed by the dynamics of the live weight and its gain. At the beginning of the experiment, the average live weight of experimental and control piglets was almost the same making 25.2 and 25.3 kg, respectively (Table 2). At the end of the experiment (30 days), the average live weight of piglets fed by the probiotic feed additive was 47.3 ± 1.2 kg that exceeded that in the control group by 3.0 kg (Table 2). The average daily weight gain of the experimental animals was 15.8% higher than in the control (Table 2). The analysis of these data shows that the supplementation of the diets of growing piglets with a probiotic protein-carotene feed additive promotes better utilization and accumulation of nitrogen, and, therefore, a higher live weight gain. An active growth and increase in the average daily weight gain of piglets from the experimental group correlates with higher coefficients of nutrient digestibility (see Table 3), since the digestibility improvement is a key factor in increasing the weight productivity of animals. This result agrees with the available data, according to which *Bacillus* bacteria synthesize a number of enzymes (proteases, lipases, amylases) contributing to the digestion process [43]. Probiotic *Bacillus* strains are also known as producers of amino acids, vitamins and other factors optimizing metabolism and promoting more active growth [42].

Metabolism of animals represents the main factor providing their health and high productivity as well as duration of their economical use. The scale and the speed of metabolic processes can be indirectly determined by changes in the metabolite concentration in blood.

According to the obtained data, the dynamics of a calcium concentration in the blood of animals fed by FA tended to increase within the limits of the physiological norm. According to the data of Table 5, the amount of calcium and phosphorus obtained with feed was the same in both groups. However, one should note an increase in the calcium retention in the experimental group by 1.01% and an increase in the phosphorus assimilation by 40.3% compared to the control.

Being an internal environment of an body and linking all systems and organs into a single whole, blood serves as the main indicator of changes occurring in the organism. In addition, it stabilizes homeostasis, which is necessary for the vital activity of cells and tissues and provides the functional integrity of the organism. Taking into account this fact, some hematological parameters were determined in experimental animals. Our experiments showed that the concentration of the studied metabolites in the blood of animals of all groups fell within the range of permissible physiological norms (Table 6). Use of spore-forming bacterial strains promoted positive changes in the protein and

carbohydrate–lipid metabolism in piglets. Animals from the experimental group demonstrated an increase in the total protein content in blood serum (+3.1%), which correlated with an increased content of albumin (+3.8%) and globulin (+1.9%). The similar results of the probiotic administration were obtained by other researchers [44].

A formation of the vitamin A status during the ontogenesis is associated with its involvement in some vital processes. This vitamin is necessary for the normal functioning of metabolic and growth processes. The content of its metabolically active form (retinol) in the blood is homeostatically maintained at a certain level due to the vitamin A reserves in the liver, which are formed when it is supplied with food. The synthesis of retinol-binding protein in the liver plays an important role in this process, since it forms a complex with retinol to provide its transportation in the blood [32]. The use of a protein-carotene probiotic feed additive provides a constant supply of carotene, a retinol precursor, and our studies confirmed this fact.

An increased albumin content may indirectly indicate an increase in the protein-forming function of the liver, since albumin synthesis occurs in this organ. Positive changes in the protein metabolism are confirmed by the transamination enzyme activity, in particular, the revealed tendency to increase the level of alanine transaminase (ALT) in piglets of the experimental group (+14.2%). Simultaneously, a 2.5% increase in the AST level was observed in the experimental group of animals, while the general tendency to the decrease in the AST/ALT ratio was registered for experimental group of animals.

Activation of the nitrogen metabolism by spore-forming bacteria resulted in a 3.8% increase in the creatinine concentration in the experimental group of piglets. The content of creatinine in blood is known to be dependent on the protein metabolism intensity in an organism [45]. Therefore, an increase of this parameter may indirectly indicate activation of the energy metabolism via phosphocreatine, a reserve energy accumulator for the protein synthesis. Phosphocreatine is a phosphate donor for ADP, which conversion to ATP increases the energy potential of cells. Creatinine is synthesized in an organism at a constant rate, so its concentration in a serum blood is usually stable and correlates with the volume of muscular tissues. Based on the fact that piglets from the experimental group were characterized by a greater body weight, the higher creatinine content observed in this group may have some physiological reasons [45]. The content of urea, a final product of the protein metabolism, was also higher in the experimental group (+2.5% of the control) that correlates with the increased creatinine content. Another indirect indication of a more intensive protein metabolism is an increased phosphorus content in blood of experimental animals (+12.5% of the control, see Table 6).

ALP (alkaline phosphatase), which catalyses the hydrolysis of monoaminophosphoric acid, is a marker enzyme indicating the state of the energy and mineral exchange in an organism. An increase in ALP level by 3.9% in piglets from the experimental group can be explained by a more active bone growth, which is confirmed by a higher live weight gain and an increased (+1.5%) calcium levels in blood compared to the animals from the control group (Table 6) [22].

The level of glucose representing the primary source of energy for all cellular processes, is an important indicator of the carbohydrate metabolism. In both groups of animals, the values of this index remained within the normal physiological range. Nevertheless, an increase in the glucose concentration by 4.6% in piglets from the experimental group may indicate an improved energy provision for their organisms and correlates with the more intense growth of animals fed by the probiotic feed supplement.

The effect of use of the studied FA on the lipid metabolism was manifested via the observed trend towards a decrease in the levels of cholesterol, triglycerides, and phospholipids in the blood of animals from the experimental group by 4.5, 12.1, and 6.0%, respectively, compared to the control. This fact can indirectly indicate a more active utilization of lipids as an energy source. The level of bilirubin in both groups of animals remained within the physiological range; at the same time, one should note an increase of this index in the experimental group by 41.8%. Under normal conditions, bilirubin is produced due to the cleavage of hem-containing proteins, such as hemoglobin, myoglobin, and cytochrome. In the case of intensive protein metabolism, an increase in the bilirubin

level and an increased muscle mass formation are observed; as a result, the level of creatinine as a final product of the protein metabolism also increases. In our study, we observed a correlation between the increase in the bilirubin level, increase in the creatinine level, and increased growth rate in the piglets of the experimental group.

The analysis of hematological indices did not show any significant difference between the control and experimental groups of animals concerning the content of red blood cells and hemoglobin as well as hematocrit.

Non-specific resistance is the ability to maintain optimal functioning of an organ or a system under both standard and changing conditions influenced by various factors. This is the first defensive barrier preventing the penetration of an infectious agent. Natural resistance of animals to various unfavorable factors is ensured by non-specific protective factors, which present in the organism since the birth and are maintained for the whole life. The key role in this process is played by the phagocytosis represented protective cellular mechanisms. This means that phagocytes (macrophages and polymorphonuclear leukocytes) take up a unique position among other protection factors [22]. The level of the protection against microbial agents in animals fed by the developed FA is manifested via the increased PP, PI, and PC levels (+4.6, +15.5, and +14.5%, respectively, compared to the control). These data may indicate a more stable non-specific cellular immunity increasing the resistance of animals in experimental groups to a possible infection [45].

Gastrointestinal microbiota (normal flora) plays a vital role in the food digestion and maintains the homeostasis in an organism. Moreover, numerous studies proved the role of normal flora in maintaining natural resistance mechanisms via competing with pathogens for receptors of the mucous membrane of the intestinal tract at the stage of their primary adhesion and colonization. Normal microflora activates the complement system and phagocytosis, which are critically important in protecting the organism against an intestinal infection [46]. The function of normal flora can be disordered by various factors [47]. Spore-forming *Bacillus* bacteria are known to do not belong to the normal microbial communities of animals. However, their numerous properties allow an organism to maintain normal flora at the natural level. During this study, we showed that the supplementation of the diet of experimental animals with the probiotic feed additive positively influences on the development of a normal intestinal microbiota: the number of lactobacilli and bifidobacteria increased by an order of magnitude (see Table 8). This result may indicate an improvement of the microbial profile of the rectum content, which, in turn, will have a positive effect on the development and functioning of the gastrointestinal tract and stimulate the organism's protective properties. A significant decrease in the number of *Salmonella* and *Enterococcus* bacteria was also observed in the experimental group of animals.

5. Conclusions

The supplementation of a complete mixed fodder with a probiotic carotene-containing feed additive provided an increase in the average daily weight gain of fattened piglets by 6.7% compared to the control animals. Piglets from the experimental group also demonstrated an increased coefficients of digestion of dry matter (+2%), organic matter (+2.2%), and protein (+1.6%) compared to the control. The analysis of biochemical and morphological blood parameters of animals from both control and experimental groups did not reveal any negative influence on their health. Therefore, this feed additive can be recommended for use in different variants and forms of pig fattening at farms and large-scale agricultural complexes.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Composition and feeding value of a mixed fodder used in the study.

Author Contributions: Conceptualization, V.D. and E.G.; methodology, N.K. and M.K.; software, K.O.; validation, E.G., N.K., and V.Y.; formal analysis, V.D.; investigation, N.K, K.O., data curation, V.Y. and K.O.; writing—original draft preparation, N.K. and M.K.; writing—review and editing, V.Y., K.O. and E.G.; visualization, V.Y. and K.O.; supervision, E.G. and M.K.; project administration, V.D. and V.Y.; funding acquisition, V.D. All authors have read and agreed to the published version of the manuscript.

Funding: The research was carried out within the framework of the State Assignment of the Ministry of Science and Higher Education of Russian Federation (theme No. 123012000071-1).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Ernst Federal Research Center for Animal Husbandry (date of approval: September 01, 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

Conflicts of Interest: The authors declare no conflicts of interest.

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