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Use of different concentrations of enzymesporin probiotic in feeding of growing young pigs

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The study, which included four groups of growing young pigs (each consisting of 30 animals), was arranged under conditions of the Bryansk meat-processing plant and laboratories of the Ernst Federal Research Center of Animal Husbandry. The duration of the experiment was 113 days. Animals of the group 1 (control) were fed with the SK-4, SK-5 and SK-6 mixed fodders without any probiotic additives. The Groups 2 and 3 were fed by a mixed fodder supplemented with the Enzymesporin probiotic (0.5 and 1.0 kg t^{1}, respectively). The Group 4 was fed by a mixed fodder supplemented with the Virginiamycin antibiotic (250 g t^{-1}). The feeding of different concentrations of the Enzymesporin probiotic complex and Virginiamycin antibiotic to the growing young pigs of the experimental Groups 2, 3, and 4 provided the average daily liveweight gain equal to 771.4, 775.4, and 818.8 g (5.8, 6.5, and 12.2%), respectively, as compared to the control. Addition of different dosages of the probiotic complex or the antibiotic to the fodder fed to the growing young pigs (Groups 2, 3, and 4) did not result in a significant increase in the lysozyme and bactericidal activity of blood serum and the phagocytic activity, though a tendency to the phagocytic index increase was observed. Addition of the probiotic complex to forage did not provide any negative impact on the intestinal microbiocoenosis; moreover, it positively influenced on the growth of lactobacteria.

Keywords: Growing young pigs; Weight gain; Probiotics; Antibiotics; Resistance; Microflora

Introduction

There are four groups of biologically active substances able to increase the forage assimilation efficiency: feed antibiotics, feed enzymes, probiotics, prebiotics and synbiotics. Antibiotics are produced by a microbiological or chemical synthesis and inhibit the development of both pathogenic and beneficial microflora of the digestive tract. Enzymes destroy cell membranes of grain, reduce a chyme viscosity and, therefore, re-arrange the nutrient flow from microorganisms to the host. Probiotics represent therapeutic and prophylactic preparations based on live microorganisms, which provide a beneficial effect on the physiological and biochemical functions of the animal organism via its microflora optimization. Finally, prebiotics include small organic compounds, yeast cell derivatives, etc. (Tarakanov, 2003; Laptev, 2004; Panin et al., 2009; Nekrasov et al., 2010; Ushakova et al., 2010; Gamko et al., 2015; Tellez and Latorre, 2017). Today the global annual production of antibiotics for animal husbandry is estimated to be equal to ~$4 billion. The annual production of veterinary and feed antibiotics in United States makes 2700 tons. In the value terms, the use of antibiotics in animal husbandry reaches $250 million that makes 45% of the total antibiotic production. In United States, every dollar spent for the production of feed antibiotics provides a profit equal to 2–5 dollars; feed antibiotics are used for production of ~80% of poultry, 75% of pigs and dairy cattle, and 60% of beef cattle. Antibiotics from the virginiamycin group (E711) are especially important for the animal husbandry. These antibiotics are used as feed additives to improve the growth of poultry, pigs and cattle (Stewart, 2010; Li et al., 2017). Virginiamycin is produced by *Streptomyces virginiae* and possesses a bacteriostatic activity towards Gram-positive and Gram-negative bacteria, which have the R-factor. This antibiotic is included into a number of drug preparations, such as eye and ear drops, capsules and ointments. An alternative to the antibiotic use includes the use of feed supplements and preparations of the probiotic and prebiotic action (Fuller, 1998), enzyme preparations, and complex therapeutic and preventive additives intended to stimulate non-specific immunity, as well as to prevent and/or treat mixed gastrointestinal infections and digestive disorders (Roselli et al., 2017). As an alternative to highly efficient foreign preparations, Russian scientists developed a new spore-based probiotic Enzymesporin characterized by an increased concentration of beneficial bacteria, such as *Bacillus subtilis* and *Bacillus licheniformis* (including strains of a targeted action). Thus, the investigation of the efficiency of different dosages of Enzymesporin comparing to Virginiamycin in relation to feeding of growing young pigs is very interesting from the theoretical and practical point of view.

The aim of the study was a comparative assessment of the efficiency of the Enzymesporin probiotic and Virginiamycin antibiotic preparations in feeding of growing young pigs.

Material and Methods

The study was arranged at the Bryansk meat-processing plant ("Tsar-Myaso" agroholding, Bryansk region) and in laboratories of the Ernst Federal Research Center of Animal Husbandry. The study was carried out using four groups of 36-day-old piglets, each group consisting of 30 animals. The total duration of the experiment was 113 days. Piglets of the Group 1 were fed with a mixed fodder without any additives. Piglets of the Groups 2 and 3 were fed by a mixed fodder supplemented with the Enzymesporin probiotic at the dosage of 0.5 and 1.0 kg t^{-1}, respectively. Piglets from the Group 4 were fed with a mixed fodder containing Virginiamycin preparation (250 g t^{-1}). A chemical analysis of a fodder, blood, and faeces was performed according to the standard methods and protocols of the Laboratory of Chemical Analysis of the Ernst Federal Research Center of Animal Husbandry. Samples of a large intestine content were collected from three animals of each group at the end of the growing period and also during the first and second fattening periods. During the first and second fattening periods the blood of growing young pigs (three animals from each group) was analyzed for the nonspecific resistance. Bactericidal activity was determined by photonephelometric method, lysozyme activity was analyzed by the Mutovin method, and phagocytic activity of blood cells was determined by the absorbing and digestive capacity of blood cells. The economic basis for the Enzymesporin application in mixed fodders for pigs was calculated using a common method for calculation of the economic efficiency comparing to the control. The obtained data were biometrically treated using a Student's t-test.

Results and Discussion

Live weight is an important parameters characterizing the growth and development of animals. Depending on the productivity, a conclusion about the growth rate of animals and results of their growing and finishing is made. The effect of feeding of different dosages of Enzymesporin on the growth rate of young pigs is shown in Table 1.

Table 1. Dynamics of the live weight gain and feeding costs.

Parameters	Group			
	1 (control)	2 (experimental)	3 (experimental)	4 (experimental)
Live weight at the beginning of the experiment, kg	11.35 ± 0.14	11.31 ± 0.17	11.66 ± 0.21	10.89 ± 0.19
Live weight at the end of the growing period, kg	25.59 ± 0.54	28.43 ± 0.59**	29.06 ± 0.67***	29.76 ± 0.60***
Average daily live weight gain, g	395.2 ± 1.23	475.6 ± 14.62**	483.1 ± 15.86***	524.1 ± 13.75***
In % versus control	100	120.3	122.2	132.6
Live weight at the end of the 1st fattening period, kg	55.5 ± 1.24	60.8 ± 1.51*	62.7 ± 1.65***	68.8 ± 1.55***
Average daily live weight gain, g	729.1 ± 20.06	789.1 ± 26.29*	819.5 ± 26.20**	953.5 ± 26.88***
In % versus control	100	109.5	112.9	124.0
Live weight at the end of the 2nd fattening period, kg	93.8 ± 2.12	98.5 ± 1.99	99.5 ± 2.42*	103.4 ± 2.58**
Average daily live weight gain, g	1064.09 ± 35.23	1046.9 ± 26.09	1022.6 ± 32.96	960.0 ± 36.32
In % versus control	100.0	98.4	96.1	90.2
Gross gain for the whole experimental period, kg	82.46 ± 2.05	87.20 ± 1.87*	87.81 ± 2.31*	92.52 ± 2.48**
Average daily live weight gain for the whole experimental period, g	729.7 ± 18.15	771.4 ± 16.52*	777.05 ± 20.47*	818.76 ± 21.99**
In % versus control	100.0	105.8	106.5	112.2
Fodder consumption, kg/head/day	2.08	2.09	2.16	2.40
Fodder consumption per 1 kg of a live weight gain	2.857	2.711	2.785	2.927
In % versus control	100	94.9	97.5	102.4

Here and further, the results are significant at * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

At the beginning of the experiment, the live weight of piglets did not significantly differ between the groups and was equal to 10.89–11.66 kg ($P>0.05$). At the end of a 36-day growing period, the live weight of pigs from the Group 4 reached the maximum among the experimental groups: comparing to the control, the live weight of this group significantly increased by 4.17 kg (16.3%, $P<0.001$). The average daily live weight gain in the piglets of the Group 4 was 524.1 g, whereas in the control group it was only 395.2 g; thus, the difference was 128.9 g or 32.6% ($P<0.001$). As for the piglets, which received the Enzymesporin complex at the dosages of 0.5 and 1.0 kg t^{-1} of fodder, the average daily live weight gain in these groups was slightly lower than that in the Ggroup 4, but was still 20.3 and 22.2% higher than in the control group ($P<0.001$). Note that by the end of the first fattening period, the live weight of animals from the Groups 2, 3, and 4 reliably increased by 5.3 ($P<0.05$), 7.2 ($P<0.001$), and 13.3 ($P<0.001$) kg, or 9.5, 12.9, and 24.0%, respectively; the mean daily gain exceeded that in the control group by 60.02 ($P<0.05$), 90.42 ($P<0.001$) and

224.41 (P<0.001) g, or 8.2, 12.4, and 30.8%, respectively. During the second fattening period, the growth rate of animals in the experimental groups was slightly lower than in the control (p>0.05), especially in the group 4 fed by the antibiotic-containing preparation. However, the total effect of the studied feed additives calculated for the whole experimental period still remained positive. The average daily live weight gain in the Groups 2, 3, and 4 for the whole experimental period exceeded the corresponding control value by 5.8%, 6.5%, and 12.2%, respectively, with a simultaneous decrease in a feed consumption. Immunological parameters of blood determined for growing piglets fed by different dosages of Enzymesporin and Virginiamycin are shown in Table 2.

Table 2. Immunological parameters of blood of experimental animals (M ± m, n=3).

Parameters	Groups			
	1 (control)	2(Experimental) Growing period	3 (Experimental)	4 (Experimental)
Lysis, %	52.53 ± 1.01	78.79 ± 1.75***	78.28 ± 0.51***	64.65 ± 11.88
Lysozyme, μg/mL of serum	0.89 ± 0.01	2.80 ± 0.29**	2.67 ± 0.13***	1.80 ± 0.55
E/mg of protein	4.23 ± 0.09	6.44 ± 0.16***	6.26 ± 0.45**	4.66 ± 0.87
Blood bactericidal activity (BBA), %	37.03 ± 3.36	51.35 ± 0.78**	48.11 ± 0.47*	38.20 ± 8.16
Phagocytic activity (PA), %	29.72 ± 3.0	56.61 ± 4.88**	48.29 ± 2.76**	32.57 ± 4.36
Phagocytic index (PI)	2.20 ± 0.21	2.71 ± 0.04	2.24 ± 0.16	2.24 ± 0.11
Phagocytic number (PN)	0.65 ± 0.04	1.54 ± 0.15**	1.08 ± 0.03***	0.74 ± 0.12
First fattening period				
Lysis, %	36.52 ± 11.57	43.17 ± 12.10	27.46 ± 4.08	20.05 ± 1.56
Lysozyme, μg mL^{-1} of serum	0.55 ± 0.23	0.85 ± 0.38	0.41 ± 0.07	0.26 ± 0.04
E/mg of protein	3.73 ± 0.74	3.66 ± 0.54	2.92 ± 0.33	2.35 ± 0.10
Blood bactericidal activity (BBA), %	57.50 ± 1.44	46.67 ± 8.82	49.17 ± 3.00	42.50 ± 15.00
Phagocytic activity (PA), %	55.20 ± 6.74	46.42 ± 2.42	50.49 ± 4.36	43.17 ± 4.00
Phagocytic index (PI)	2.28 ± 0.13	2.60 ± 0.27	2.40 ± 0.12	2.53 ± 0.03
Phagocytic number (PN)	1.27 ± 0.21	1.20 ± 0.12	1.20 ± 0.07	1.09 ± 0.11
Second fattening period				
Lysis, %	40.46 ± 10.28	63.12 ± 3.03	53.75 ± 6.59	46.26 ± 14.09
Lysozyme, μg/mL of serum	0.73 ± 0.19	1.37 ± 0.22	1.07 ± 0.22	0.91 ± 0.34
E/mg of protein	2.93 ± 0.61	4.11 ± 0.34	3.810.61 ±	3.58 ± 0.97
Blood bactericidal activity (BBA), %	66.67 ± 0.68	64.63 ± 1.80	65.31 ± 1.18	63.27 ± 1.18
Phagocytic activity (PA), %	20.97 ± 5.28	17.19 ± 3.46	21.05 ± 3.18	28.00 ± 4.36
Phagocytic index (PI)	1.52 ± 0.20	1.74 ± 0.33	1.83 ± 0.32	1.65 ± 0.41
Phagocytic number (PN)	0.34 ± 0.13	0.29 ± 0.06	0.41 ± 0.13	0.50 ± 0.20

A higher resistance level in animals of the experimental groups was observed during the whole experimental period though being the most manifested at the growing period. Virginiamycin administration during this period contributed to the doubling of a lysozyme content in blood (P>0.05) and increase in the blood bactericidal activity (BBA) by 0.91 μg ml^{-1} (1.17%) comparing to the control animals; in addition, we observed an increase in the phagocytic activity (PA), phagocytic index (PI), and phagocytic number (PN) by 2.85%, 0.04, and 0.09, respectively. Feeding of piglets with Virginiamycin during the growing period had a positive effect on the protective properties of their organisms. However, the effect of the Enzymesporin probiotic complex was even more significant. For example, the lysis percentage in the Groups 2 and 3 increased by 25.8–26.3% (p>0.001); this effect was caused by the following changes: an increase in the concentration and specific activity of lysozyme in 3.0 (P>0.01)–3.1 (P>0.001) and 2.0 (P>0.01) – 2.2 E.a. (P>0.001) times, respectively; BBA increase by 11.1 (P>0.01) and 14.3% (P>0.001), respectively; PA increase by 18.6 (P>0.01) and 26.9% (P>0.001), respectively; PI increase by 0.04 and 0.5, respectively; and PN increase by 0.43 (P>0.01) and 0.89 (P>0.001), respectively. The analysis of immunological parameters of blood during the first and second fattening periods showed that the further feeding of the probiotic complex and feed antibiotic to animals from the Groups 2, 3, and 4 did not result in a significant increase in the lysozyme and bactericidal activity of blood serum, as well as the phagocytic activity (P>0.05). At the same time, we observed a tendency to increase the phagocytic index in animals of experimental groups. The state of the intestinal microbiocoenosis is an important factor influencing on the assimilation of nutrients, as well as immunity and productivity of animals (Table 3). During the growing period, piglets from the experimental groups demonstrated a decrease in the content of lacto- and bifidobacteria by 1.28 and 35.8 times, respectively, comparing to the control group. For these groups, we also registered an increased content of streptococcocci and lactose-positive *E. coli* (9.37 and 68.63 times higher, respectively, than in the control group). During the first fattening period, piglets from the group 4 also showed a decrease in the content of lacto- and bifidobacteria (12.6, P<0.01) and by 1.6 times lower than in the control group). The analysis of the contents of large intestines of piglets showed that, in the case of animals from the experimental groups, the content of streptococci was 47 times lower, while the content of lactose-positive *E. coli* was 48.1 times higher than in the control group. At the end of the experiment, the content of positive microflora in the large intestine of pigs from the group 4 was similar to that in the control group.

Table 3. Microfloral composition of piglet faeces (averaged, n=3).

Parameters	Group			
	1 (control)	1 (control)	1 (control)	1 (control)
	Growing period			
Lactobacteria, CFU g^{-1}	1.82×10^9	5.17×10^8	3.50×10^8	1.42×10^9
Bifidobacteria, CFU mL^{-1}	3.34×10^{10}	4.40×10^8	1.40×10^9	9.33×10^8
Hemolytic microorganisms, CFU g^{-1} including:				
- streptococci	7.17×10^3	n/d	1.0×10^5	6.72×10^4
- *E. coli*	n/d	n/d	n/d	n/d
colibacillus, CFU g^{-1} including:				
- lactose-positive	1.53×10^4	1.68×10^5	6.62×10^4	1.05×10^6
- lactose-negative	n/d	2.0×10^4	7.0×10^4	3.50×10^6
Fungi from the genus *Candida*, CFU g^{-1}	n/d	n/d	n/d	n/d
	First fattening period			
Lactobacteria, CFU/g	3.4×10^8	5.5×10^8*	4.9×10^8	2.7×10^7**
Bifidobacteria, CFU/mL	8.3×10^8	9.0×10^8	9.2×10^8	5.1×10^8
Hemolytic microorganisms, CFU g^{-1} including:				
- streptococci	4.7×10^4	1.3×10^4*	2.0×10^3***	1.0×10^3
- *E. coli*	n/d	n/d	n/d	n/d
colibacillus, CFU/g including:				
- Lactose-positive	2.7×10^3	3.3×10^3	3.4×10^5***	1.3×10^5
- Lactose-negative	n/d	n/d	n/d	n/d
Fungi from the genus *Candida*, CFU g^{-1}	n/d	n/d	n/d	n/d
	Second fattening period			
Lactobacteria, CFU g^{-1}	3.3×10^7	7.4×10^7*	11.6×10^7	3.2×10^7
Bifidobacteria, CFU mL^{-1}	4.5×10^8	2.5×10^8	3.4×10^8	3.5×10^8
Hemolytic microorganisms, CFU g^{-1} including:				
- Streptococci	2.2×10^4	0.2×10^4	1.7×10^4	2.7×10^3
- *E. coli*	n/d	n/d	n/d	n/d
colibacillus, CFU g^{-1} including:				
- Lactose-positive	2.3×10^5	0.1×10^5	3.8×10^5	9.0×10^5
- Lactose-negative	n/d	n/d	5.0×10^2	2.2×10^5
Fungi from the genus *Candida*, CFU g^{-1}	3.3×10^3	5.0×10^3	1.7×10^3	n/d

Conclusion

During the growing period, piglets from the Groups 2 and 3 did not show a significant difference with the control animals in relation to the microbiological content of the large intestine (including lacto- and bifidobacteria). However, during both fattening periods, the content of lactobacteria in the intestines of animals from the group 2 and 3 exceeded that in the control group by 1.2–1.6 (P<0.05) and 1.4–4.8 times, respectively. In general, feeding of piglets with the antibiotic preparation reduced the content of microorganisms in the large intestine in the beginning of the experiment with the further stabilization at the control level to the end of the experiment. At the same time, feeding of animals with the probiotic complex did not negatively influence on the intestine microbiocoenosis; moreover, use of this complex positively influenced on the growth of lactobacteria. The revealed increase in the level of nonspecific immunity of experimental animals, which total biological effect results in more intensive growth of piglets, evidences that, in the case of a well-balanced ration in relation to the energy, nutrients, and biologically active substances, its supplementation with the tested probiotic complex provides the same efficiency as the antibiotic-containing preparation.

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