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EFFECT OF BAYKAL EM-1 ON GROWTH DEVELOPMENT AND MICROBIOLOGICAL STATUS OF SUCKLING PIGS

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Abstract

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In an experiment with 15 equalized by piglet age, origin and size litters, of which 8 litters in the trial and 7 litterts in the control group, the effect of Baikal EM-1 feed additive, a multi-purpose microbiological product containing a complex of living, useful microorganisms, on the weight development and the microbiological status of the gastrointestinal tract of the pigs, was studied. The additive was included in the feed of the sows and pigs at a dosage of 10 ml / kg of feed, from the day 8th after birth to the day 35th at weaning of the pigs. On the day 21st and day 35th the body weights of the pigs were recorded, and on the day 35th, 10 rectal tampon samples (RTS) from the two groups were taken and microbiologically examined.

It was found that the data on weight development, total growth and average daily gain did not show any significant differences in the benefit of the trial group, which contradicts the hitherto established for many other feed additives. Microbiological tests showed that 40% of the RTS of both groups were positive for E. coli, of which in the trial group 75% were nonpathogenic and 25% pathogenic (O139:F4) in and in the control group 75% pathogenic (O139:F4) and 25% non-pathogenic, which represents the product as a good prophylactic remedy.

Key words: pigs, feed additives, probiotics, "Baykal EM-1", average daily gain, effectiveness, E. coli.

Introduction

Feed additives are a group of substances with a wide range of action, which are classified as: nutritional additives defined as "separate components" and „additives" and non-nutritional additives, including "nutritional antibiotics" and "alternatives to nutritional antibiotics" (Angelova, 2000; Angelova and Tenchev, 2008). In pig holdings, stationary for colibacteriosis and other gastroenteritis diseases in pigs, besides etiotropic and symptomatic therapy, a number of immunoprophylaxis and metaphylaxis measures are applied (Yordanov, 2008, 2014; Dimitrova, 2009, 2010; Lyutskanov, 2013; Dimitrova et al., 2015).

In the intestinal forms of colibacteriosis with good therapeutic and metaphylactic effect, the antibiotics streptomycin, neomycin, apraplan and spectinomycin have recently been applied. In recent years as a result of the acquired resistance of some coli-strains to them, the aminoglycosides are current – gentamycin, kanamycin and amikacin; amphenicoles-florfenicol and thiamphenicol, some quinolones such as flumectin (Plamb, 2002; Popova, 2009; Vestič, 2012) and fluoroquinolones-ciprofloxacin, pefloxacin, enrofloxacin and

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marbofloxacin (Dimitrova, 2010; Popova,2013; Fraile, 2013; Dimitrova et al., 2014; Yordanov et al., 2016; Petkova, 2017).

The emergence of multiple resistance to antimicrobials focuses on the attention of both human and veterinary doctors on their use. Therefore, each case requires estimation for necessity the need for antibiotics (Close, 2000; Drumev, 2001; FVE, 2003; Dimitrova et al., 2014). In connection with the problem of resistance, the Committee for veterinary medical products of European Union has stopped using some antibiotics and has completely prohibit use of nutritional antibiotics as growth promoters (Yordanov and Dimitrova, 2014).

Various remedies, alternatives to nutritional antibiotics, such as acidifiers, oligosaccharides, enzymes, prebiotics, probiotics and other biologically active substances of plant origin could be successfully applied in the prosperous and relatively prosperous herds for bacterial intestinal infections (Dimitrova et al., 2003; Virtanen, 2004; Yordanov et al., 2006; 2009; Mateva, 2010; Popova, 2013). According to Petkova (2017), the feed additives Tariben and Periben, representing combinations of tannin or pectin with essential oil of oregano and benzoic acid, reduce the carrying of pathogenic and conditionally pathogenic bacteria to 50% and stimulate the improvement of the general and clinical condition of the pigs. Parvulov and Markov (1996) and Murgov and Denkov (2007) pay spetial attention to probiotics and probiotic foods, especially lactic acid products. It is known that there are about 100 bacterial species of in the gastrointestinal tract of humans and animals that can be useful (lactic acids) and harmful (decaying) and are found among themselves in symbiotic or antagonistic relationships. The fact that the gastrointestinal flora is closely related to the health of the host shows that its balance is of great importance for the health (Slavchev, 2004).

Baykal EM-1 is a multi-purpose microbiological product developed by Russian microbiologists, a complex of living, useful microorganisms, used to restore the soil and enhance the immunity of plants, animals and humans. Manufacturer is a company "EM-Corporation", Russia, Ukraine. Used in Canada, Japan, the Netherlands, Austria, Denmark and many other countries. His composition includes nearly 60 strains of microorganisms from the groups of photosynthetic bacteria, lactic acid bacteria, actinomycetes, yeasts and fermenting fungi. The mechanism of action is that the animals received a complex of living, useful microorganisms who form colonies in the animal's body, displace the pathogenic microflora from the active growth zone and enrich the environment with the products of its vital activity. Preliminary studies of Ukrainian farms (INDUSTAR, Kharkov region) show that the product helps balancing the microflora of the digestive tract in animals, and this has a positive effect on the reduction of enterocolitis, dysbacteriosis, diarrhea, etc. In addition, the product has a positive effect on the animal's live weight gain (***, 2005). The aim of this study was to determine the effect of Baykal EM-1 on the weight development and microbiological status of the gastrointestinal tract in suckling pigs.

Materials and Methods

The experiment was carried out in Experimental farm of Agricultural Institute - Shumen. A total of of 154 suckling pigs of 15 litters, of which 7 litters (72 pigs) included in the control group and 8 litters (82 pigs) included in the trial group, equalized by age, origine, sequence of farrowing and number of pigs in the litters, were studied. Combined feed for sows and pigs (Tables 1 and 2) in the trial group was treated with Baikal EM-1, at a dose of 10 ml/kg of fodder, and the feed of the control group was fed without any additives. The trial and control groups received the specified diets from 8th to 35th day of birth.

On the day 1st, 21st and 35th after birth, weights of the pigs of both groups were recorded. Total and daily gains were determined for periods from day 1st to day 21st and from day 22nd to day 35th and generally from day 1 to day 35 days after birth. The data were statistically processed with Microsoft Excel for statistically significant differences between groups using t-test.

At day 35th, 10 numbers rectal tampon samples (RTS) from each group were taken and tested microbiologically for pathogenic bacteria. Generally accepted selective and differentiating feed media for isolation and determination of the fermentation activity of the isolates were used. The occurrences of α- and β-hemolysis or the absence of these were recorded on blood agar. To determine the serologic groups of Escherichia coli (E. coli) isolates and to determine pathogenicity factors, we applied the corresponding serological tests with specific agglutinating sera (O8, O20, O25, O74, O78, O101, O136, O138, O139, O141, O147, O148, O149, O157 and for F4 and F5) and ready diagnostic kits.

Results

During the entire trial period, no sickness in pigs and no mortality in both groups were recorded. No difference in the body status of the pigs in the trial and in the control group during the whole experimental period was mentioned. Data on weight development, total and average daily gain are shown

in Table 3. Pigs from both groups had one and the same live weight at birth (1.579 kg and 1.582 kg) and on the day 21st day a difference of 0.255 kg in advantage of the control group is registered. This differentiation in the weight development of the pigs continued in the next period from the day 22nd to the day 35th day, with control animals exceeding those in the experimental group by 0.367 kg. Expressed in total gain this difference was 0.258 kg in the favor of the control group for the first period and 0.112 kg for the second period, also for the control group. Calculated for the entire trial period, from day 1st to day 35th day after birth, the difference in total gain was 0.369 kg in the favor of the control group or 5.1% less total gain for the trial group.

The estimated average daily gain for the first period in the control group was 0.233 kg, and in the trial group 0.225 kg or 8 g lower, and in the second period, respectively, 0.204 kg and 0.191 kg or 13 g lower than that in the control group. For the entire trial period, the average daily gain was 0.215 kg and 0.205 kg respectively, or 10 g (4.7%) lower in the trial group.

In the microbiological study of RTS taken at the end of the experiment, it was found that from the experimental group 4 RTS (40% of the 10 tested) were positive for E. coli, of which 3 samples were non-hemolytic, non-O-serotype non-having F4 and F5 fimbrial factors and one sample positive for hemolytic E. coli, serogroup O139 positive for F4. From the control group, 4 RTS (40% of the 10 tested) were positive for E. coli, of which 3 isolates were haemolytic, serogroup O139, F4 positive and one is non-hemolytic, O-serotype-non-typing and negative for F4 and F5.

Table 1. Content of energy and nutrients in 1 kg feed for suckling pigs

Components	Unit
Metabolizable energy	10.59 MJ
Crute protein	22.10%
Fiber	3.70%
Crute Fat	2.20%
Lysine	1.40%
Methionine	0.45%
Calcium	1.25%
Phosphorus	0.85%
Sodium	0.03%

Table 2. Component composition, energy and nutrients content in 1 kg of feed of lactating sows

Components	%
Wheat	30.0
Barley	18.5
Maize	14.0
Wheat brain	12.0
Sorghum	10.0
Lysine	0.2
Vitamin-Mineral Premix -15%	0.2
Bioconcentrate X-16	14.0
Limestone	0.5
Dicalcium Phosphate	0.4
NaCl	0.2
Total:	**100.0**
1kg of feed contains:	
Metabolizable energy, MJ	12.13
Crute protein, %	14.48
Crute fat,g	22.22
Fiber, g	46.46
Lysine, %	0.83
Methionine + Cystine, %	0.51
Calcium, %	0.93
Phosphorus, %	0.60

Discussion

According to defined of Working Group by the World Health Organization and the International Scientific Association for Probiotics and Prebiotics (Reid and Friendship, 2003), "oral probiotics are living microorganisms, which have a healthy effect on the recipient when are given in adequate quantities". The results of the clinical examinations of the trial and control pigs during the whole experiment did not show any visible differences. This circumstance hindered extra-laboratory assessment of the prophylactic efficacy in animals receiving Baykal EM-1 additive at a dose of 10 ml / kg of feed. In contrast, publications for other supplements reported by a number of authors (Dimitrova, 2009; Slavchev, 2004; Yordanov et al., 2006, 2009; Mateva, 2010, Yordanov and Dimitrova, 2014; Dimitrova et al., 2015; Petkova, 2017) have shown positive effect of the additives.

The reported data of the weight development of the suck-

Table 3. Growth development of suckling pigs with and without microbiological additive Baykal EM-1

Groups / Indexes	Control n=72			Experimental n=82		
	$\bar{x} \pm S \bar{x}$	C	E	$\bar{x} \pm S \bar{x}$	C	E
Live weight at birth, kg	1.579	18.73	2.19	1.582	15.74	1.73
Live weight at day 21, kg	5.859 ± 0.11	16.18	1.90	5.604 ± 0.10	15.74	1.73
Live weight at day 35- weaning, kg	9.121 ± 0.20	19.07	2.23	8.754 ± 0.18	18.24	2.00
Total gain 1-21 day, kg	4.279 ± 0.10	19.66	2.30	4.022 ± 0.09	19.90	2.18
Total gain 22-35 day, kg	3,262 ± 0,15	38.54	4.51	3.151 ± 0.13	38.62	4.24
Total gain 1-35 day, kg	7.541 ± 0.19	21.89	2.56	7.172 ± 0.17	21.24	2.33
Average daily gain- day 1-21, kg	0.233	38.52	4.51	0.225	38.64	4.24
Average daily gain- day 22-35, kg	0.204 ± 0.01	19.67	2.30	0.191 ± 0.01	19.90	2.18
Average daily gain- day 1-35, kg	0.215 ± 0.01	21.86	2.56	0.205 ± 0.01	21.27	2.33

ling pigs included in the trial and control group, such as the total and the average daily gain, for the period from the day 1st to day 21st day and for the period from the day 22nd to the day 35th day after birth and for the whole experimental period, show statistically significant differences in favor of the control group. This is in contradiction with the opinions of a lot of authors such as Angelova (2000), Virtanen (2004), Angelova and Tenchev (2008), Parvulov and Markov (1996) and Slavchev (2004), investigated the effect of feeding lactic acid products as additives for feed diets. In the same line are findings of Yordanov et al. (2009), who established a good result with the Tanacid and Carbovet supplements, studies of Matteva (2010), investigated the effect of administering the Nadstim immunomodulator to suckling and growing pigs and studies of Virtanen (2004) and Popova (2013), which tested herbal remedies for the decontamination of organic livestock wastes. Our results are also not in agreement with the results from an experiment conducted with fermented feed and application of Baykal EM-1 in Russia, showed 11% increase of the total gain in the trial group of growing pigs.

The established imbalance between the total gain, respectively the average daily gain, in the pigs of the two groups during the first period (up to the day 21st) and the second period (from the day 22nd to the day 35th day) is difficult to be explained only with the physiological condition of the mother sows with manifestations of estrus and reduced milk secretion about 20 days after birth. The question remains, however, why the product Baykal EM-1, in which a large number of useful microorganisms are included, according to the product characteristics – lactic acid bacteria, actinomycetes, yeasts and fermenting mushrooms, has not contributed to the most important effects of the use of probiotics, such as improvement of the body and health condition and improvement of the productive indicators (Angelova, 2000; Slavchev, 2004; Murgov and Denkov, 2007; Angelova and Tenchev, 2008). It is likely that different factors from the external environment have contributed to this. Moreover, it is fairly to be outlined that in the two-month study conducted in Russia

with weaned pigs was used fermented with Baykal EM-1 fodder and especially its variety Baykal EM-1-Y. The probiotic product "Baykal EM-1-Y" is a collection of bacterial cells and metabolic products of the bacteria Lactobacillus casei 21, Lactococcus lactis 47, Saccharomyces cerevisiae 76 and Photopseudomonas palistris 108, a clear, light-to dark solution, having pH 2.8-3.5, with a nice smell of kefir silage. Bacteriological studies of gastric content, wall fragments of the stomach and small intestine of pigs at 2-2.5 months with the addition of a product in animal diets indicate, that Baykal EM-1-Y in combination with the microflora of the gastrointestinal tract in pigs has a certain capacity to inhibit the growth of Proteus, E. coli, coccobacillus and promotes the growth of lactic acid bacteria and yeasts. In addition, an increase in the total aminoacid content in the blood of the pigs from 32.25 mg/100g to 39.95 mg/100g ($P<0.05$) was found. This is due to the fact that during the fermentation process, feeds were enriched with amino acids through the EM-1 additive. Once milled, they are included in the bloodstream and used for protein biosynthesis. Ultimately, this helps to improve the growth of experimental young animals that at 4 months of age had a higher average live weight compared to control ones with almost 11% ($P <0.05$) (***, 2005).

In contrast to the absence of a positive result in the clinical and weight development of the pigs in our experiment, the results of microbiological testing of RTS at the end of the trial period are clear enough and highlight the positive effect of Baykal EM-1 application on sows and pigs of the trial group. Predominantly (75%) non-hemolytic E. coli are isolated from RTS of the experimental pigs, which are not serotyped to the most common in the pigs "O" groups and have no pathogenic factors F4 and F5, therefore are not pathogenic. This fact is an evidence of a change in the intestinal microflora of the pigs, as seen by the presence of only one pathogenic E. coli strain of O139: F4, which corresponds to the findings of Dragoycheva et al. (2011), Lyutskanov (2013), Dimitrova et al. (2014), Yordanov and Dimitrova (2014), Petkova (2017). The positive effect of the product is confirmed by the microbiological data of RTS taken from the pigs in the control group. It is clear that isolated hemolytic E. coli (75%) are in all cases typified as O139 and positive for F4, which determines them as highly pathogenic and specific in pigs before and after weaning. These results are in line with the established by Dragoycheva et al. (2011), Lyutskanov (2013), Dimitrova et al. (2014), Yordanov (2014), Petkova and kol. (2014), Petkova (2017) that the virulence determinants of enterotoxigenic Escherichia coli (ETEC) are dependent on their serotype and pig age. It is also found that probiotics including lactic acid bacteria contribute to the replacement of the pathogenic microflora, a change in the pH of the stomach and gut and have a healthy effect. It is desirable to continue the studies for greater reliability, proof of the efficacy of Baykal EM-1 in pigs and its subsequent application in practice.

Conclusions

The analysis of the weight development of suckling pigs fed with and without addition of Baykal EM-1 from the day 8th to the weaning of the pigs at day 35th did not reveal any significant differences.

The microbiological tests of the rectal tampon samples from suckling pigs receiving Baykal EM-1 feed additive from the day 8th to the weaning of the pigs at day 35th at a dosage of 10 ml/ kg feed presents the product as an effective prophylactic remedy.

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