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EFFECT OF THE PROBIOTIC BAYKAL EM-1 ON THE GROWTH PERFORMANCE, BLOOD PARAMETERS AND BEHAVIOR OF WEANED PIGS

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Abstract

The aim of the study was to investigate the effect of the probiotic preparation Baykal EM-1 on the growth performance, blood parameters and behaviour of weaned pigs. A research experiment with a total of 96 growing pigs, divided into two groups - control (48) and experimental (48), fed with the probiotic Baykal EM-1 in the amount of 10 ml/kg feed, was carried out. The indicators average live weight, feed consumption, daily gain, feed conversion, blood parameters and behaviour of pigs, were studied. The following conclusions were made: The addition of Baykal EM-1 (10 ml/kg feed) in weaned pig diets improved the average daily gain by 11% (P = 0.031). The higher number of leukocytes and lymphocytes in pigs from the experimental group (P<0.001), compared to the control group, may be an indicator of better health and higher immunity. A trend for better comfort in animals with the microbiological supplement in the feed, contributing to the better absorption of nutrients, has been established. The results obtained in this study show that the combination of probiotics Baykal EM-1 has the potential for use as a dietary supplement in weaned pigs.

Key words*: behaviour, blood, pigs, probiotics, weight.*

INTRODUCTION

The ban on the use of antibiotics in pig diets as growth promotors was introduced on 1 January 2006 due to concerns about antibiotic residues in food products of animal origin and the development of bacterial resistance (Pluske et al., 2002). The search for alternative strategies to improve animal production and health has enforced the use of probiotics in animal husbandry (Gu et al., 2006; Wang et al., 2009). The main reason for their application is the achievement of some beneficial effects, such as maintaining the balance of the intestinal microbiota and effectiveness in the fight against pathogens to both animals and consumers (Markowiak & Śliżewska, 2018). Probiotics have the ability to trigger the activity of the immune system and increase the body's resistance to diseases (Chomakov et al., 1990; Vieco-Saiz et al., 2019). They directly affect the intestinal microflora, the secretion of enzymes and their activity. This improves the functions of the digestive tract and improves metabolism.

A well-balanced intestinal microflora is able to affect the integrity of the intestinal barrier against colonization by pathogens through its metabolic function and thus stimulates the immune system to overcome inflammation. The physiological and psychological stress during the weaning period of the pigs - separation from the mother, regrouping, change in diet, etc. (Lalles et al., 2007), compromises the intestinal microbiota and leads to intestinal dysfunction. The use of probiotics to restore intestinal microbial balance is particularly relevant at this time (Ahmed et al., 2014). In recent years, numerous studies advocate probiotics provide improved nutrient utilization, protection against pathogens, and increased productivity in the pig industry, have been published (Chaucheyras-Durand & Durand, 2010; Ezema, 2013; Devi & Kim, 2014). The aim of the current study was to establish the effect of Baykal EM-1 on the productivity, blood parameters and behaviour in growing pigs.

MATERIALS AND METHODS

The experiment was carried out at the State Enterprise Experimental Farm at Agricultural Institute, Shumen, Bulgaria. A total of 96 weaned pigs from the Danube white breed, divided into two groups - control (48) and

experimental (48), were used. Each group consisted of 6 pens with 8 pigs in a pen. The equalization of the animals was done by origin, age, live weight and sex (equal number of males and females in one pen). The initial average live weight of the pigs was 9.7-9.8 kg. The experiment lasted for 39 days after weaning the pigs until reaching 24-25 kg live weight. Both groups of pigs were fed *at libitum* and received standard feed for the category (NRC, 2012). The contents of the diet and its composition are shown in Table 1.

Table 1. Component composition and analysis of compound feed for weaned pigs

Components	**%**	**kg**
Maize	25.25	252,500
Barley	10.00	100,000
Wheat	27.00	270,000
Bioconcentrate – 12[a]	29.60	296,000
Wheat bran	8.00	80,000
Synthetic lysine, 98%	0.15	1,500
Total:	100.00	1000,000
One kg compound feed contains:		
Metabolizable energy, kcal	3017	
Crude protein, g	18.5	
Lysine, g	1.00	
Methionine + cystine, g	0.63	
Threonine, g	0.68	
Tryptophan, g	0.23	
Crude fats, g	2.52	
Crude fibres, g	5.15	
Calcium, g	1.00	
Phosphorus, g	0.53	

Legend:
[a]The bio-concentrate BC12 contents: 39.90% Crude protein, 1.05% Crude fats, 5.70% Crude fibres, 14.70% Crude ash, 2.72% Calcium, 1.22% Phosphorus, 0.90% Digestible phosphorus, 1.00% Threonine, 1.93% Lysine, 0.86% Methionine, 1.54% Methionine + cystine, 0.40 mg/kg Sodium, 465.00 mg/kg Zinc oxide, 190.00 mg/kg Iron sulphate, 165.00 mg/kg Manganese oxide, 85.00 mg/kg copper sulphate, 1.30 mg/kg Sodium selenite, 3.65 mg/kg Calcium iodate, 40000 UI/kg Vitamin A /retinyl acetate/, 6000 UI/kg Vitamin D3 / cholecalciferol /, 320 mg/kg Vitamin E, 40 mg/kg Antioxidants.

Water was provided by nipple drinkers, one for each pen. The microbiological preparation Baykal EM-1 (10 ml/kg of feed) was added to the feed of pigs from the experimental group. The probiotic Baykal EM-1 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, in the form of a clear liquid without sediment with light-to-dark brown colour, pH 2.8-3.5, and a pleasant smell of kefir silage.

During the experiment, the growth performance indicators of pigs were monitored. Live weight was recorded individually, and all pigs were weighed at the beginning and at the end of the experiment.

Feed consumption was recorded daily, on a pen level, by weighing the feed immediately before feeding. Any residues were removed and weighed from the feeders (if feed left) and subtracted from the quantity of the feed for the previous day. The average daily gain and the average feed conversion were calculated. In order to identify possible differences in pig comfort between the two groups, the behaviour of pigs was monitored on two consecutive days for 24 hours at the beginning and at the end of for the previous day. The average daily gain and the average feed conversion were calculated.

In order to identify possible differences in pig comfort between the two groups, the behaviour of pigs was monitored on two consecutive days for 24 hours at the beginning and at the end of the experiment. Monitoring of behaviour was carried out by video cameras, which were located on the ceiling above the pens and covered four pens in each of the two treatments (Fig. 1). The video recordings were analysed every three minutes, taking into account the behavioural reactions such as movement, lying down and eating.

Figure 1. Observation of behaviour in growing pigs through video observations

At the end of the experiment, blood samples were taken from the eye sinuses from 10 pigs from each groups and were tested for differences in blood parameters in a laboratory with specialized pig kits. The full blood count indicators were analysed with the Diatron Abacus 5 Haematology Analyzer and methods as follows:

- Leukocytes - by conductometric and visual optical method;
- Differential blood count - laser MAPSS technology;
- Hemoglobin - by cyan-methaemoglobin method;
- Erythrocytes and MCV - by conductometric method;
- Erythrocyte indices – calculated;
- Hematocrit - by an indirect method based on conductometric methods;
- Platelets, Red Cell Distribution Width (RDW) - by conductometric method after erythrocyte flotation.

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All the data were processed using statistical software Minitab 16.1 and analysed by ANOVA to establish statistically significant differences between groups.

RESULTS AND DISCUSSIONS

Growth performance results of pigs are shown in Table 2. It can be seen that all growth performance indicators were in favour of the experimental group. There were no statistically significant differences in the average final live weight, although it was higher by 6.52% in the group consuming probiotic. The average total gain and the average daily gain per pig between groups differ significantly (P = 0.031) by 11.17% and 11.14%. There was no difference in daily feed intake (n.s.). Average feed to gain ratio was better in the experimental group by 11.79%.

These results could be probably attributed to the use of the preparation Baykal EM-1. The Lactobacillus bacteria included in Baykal EM-1 increase the activity of bile, which promotes the absorption of fat-soluble vitamins A, E, D and fats. The intestinal micro flora is normalized, thus improving the nutrient absorption from feed.

Table 2. Weight development of weaned pigs, reared with and without the addition of Baykal EM-1 (mean ± SEM)

Indicators	Control group (n = 48)	Experimental group (n = 48)	Significance (P)
Average live weight at the beginning of the the experiment, kg	9.813±0.298	9.694±0.232	0.754
Average live weight at the end of the experiment, kg	24.131±0.878	25.813±0.572	0.112
Average total gain for the period, kg	14.319±0.724	16.119±0.391	0.031
Average daily gain, kg/pig/day	0.367±0.019	0.413±0.010	0.031
Average feed intake, kg/pig/day	0.959±0.024	0.952±0.023	0.830
Average feed/gain, kg	2.613	2.305	-

In a study conducted in Russia, it was found up to 70% increased instead of the usual 30% (SMR, 2005). At 4 months of age, the trial pigs, fed with Baykal EM-1, had a higher average live weight than the control group by 11% ($P<0.05$). These results are in one line with ours and with the results of a study of Dlamini et al. (2017). They observed no statistically significant differences in feed consumption between weaned pigs, fed with one or a combination of probiotics from the Lactobacillus group. However, there were statistically significant differences in average daily gain. Highest average daily gain - by 28.07% compared to the control group, by 29.82% compared to the group with *L. reuteri* and by 17.54% compared to the group with *S. salivarius* ($P<0.05$), had been obtained in the group consuming a combination of probiotics. The best feed utilization was registered in the same group. Improved growth performance of pigs was found in other studies (Huang et al., 2014; Zhao et al., 2018) and may be due to the effect of lactic acid bacteria included in the composition of the used probiotics. They are safe microorganisms with the ability to produce various inhibitory compounds, such as bacteriocins; organic acids such as lactic acid, hydrogen peroxide, diacetyl and carbon dioxide (Vieco-Saiz et al., 2019). They can inhibit harmful microorganisms with their arsenal or

through a mechanism based on competition for nutrients. Bacteria from the *Lactobacillus* group, through specific enzymatic functions (amylase, protease, etc.), can improve the absorption of nutrients, as well as stimulate the immune system of animals. It has been proven that the administration of *L. delbrueskii* subsp. *bulgaricus*, *L. acidophilus* and *L. casei* triggers the activity of macrophages in the body, enhances phagocytosis and increases cellular resistance of the organism (Chomakov et al., 1990). Our results from the study of the blood parameters of the animals, shown in Table 3, are in line to this statement. In both groups of pigs all values of blood parameters fit within the reference range (Friendship et al., 1984), with the exception of MCHC - the average concentration of hemoglobin in the erythrocyte, but it is calculable. They indicate that statistically significant differences, with a high degree of significance (P<0.001), were found in leukocyte content. It was higher by 19.83% in the trial group compared to the control group, and the lymphocyte content was higher by 20.91% (P<0.001). Similar results were found in the study of Lien (2012), examining the effect of probiotics and organic humic acids on blood parameters. The highest values of leukocytes in weaning pigs was measured in pigs consuming probiotics. Leukocytes' main function is to protect the organism from foreign invaders, such as bacteria, viruses and others. In this case, the higher content of leukocytes and in particular lymphocytes in the experimental group may be an indicator of better health, better adaptability and higher immunity than those of the control, due to the use of Baykal EM-1.

Table 3. Blood parameters in growing pigs, reared with and without the addition of Baykal EM-1 to the feed

Blood parameters	Control group		Experimental group		Reference values**
	Mean±SEM	C	Mean±SEM	C	
WBC/Leukocytes, G/L	20.25±0.82**a**	12.82	25.26±0.59**a**	7.32	8.7-37.9
LYM/Lymphocytes, G/L	10.48±0.35**a**	10.46	13.25±0.56**a**	13.29	2.2-16
MID/Monocytes, G/L	1.50±0.09	19.88	1.66±0.17	31.78	0.001-5.0
GRAN/ Granulocytes,G/L	8.28±0.29	10.83	8.52±0.13	4.97	
RBC/Red Blood Cells, T/L	6.63±0.32	15.28	6.56±0.15	7.22	5.3-8.0
HGB/Hemoglobin, G/L	119.30±5.89	15.60	114.40±3.67	10.14	90-140
HCT/Hematocrit, L/L	0.40±0.02	15.39	0.38±0.01	11.28	0.26-0.41
MCV/Mean corpuscular volume, fl	59.67±0.65	3.46	57.54±1.14	6.28	42-62
MCH/Mean corpuscular hemoglobin, pg	18.01±0.27	4.67	17.40±0.31	5.65	14-21
MCHC/Mean corpuscular hemoglobin concentration, g/L	301.80±2.17	2.28	302.70±1.37	1.44	320-360
RDW/Red blood cell distribution width, CV	0.16±0.01	9.25	0.17±0.01	20.94	
PLT/Platelet, G/L	564.10±54.48	30.54	618.90±91.99	47.00	
MPV/Mean platelet volume, fl	9.57±0.15	4.93	9.49±0.15	5.01	
PCT/Procalcitonin, L/L	0.23±0.01	16.01	0.23±0.01	18.81	
PDW/ Platelet distribution width, %	12.45±0.28	7.22	12.70±0.30	7.57	

Note: *Statistically significant differences are marked with the same letters, a - P<0.001
**Reference values are according to Friendship et al. (1984)

The results from this study indicate that the addition of Baykal EM-1, which is a mixture of probiotic strains and their products, in the feed for weaned pigs improved the immune system. They are in sync with those obtained by Dlamini et al. (2017), who found a higher content of immunoglobulin G in the blood of pigs consuming a combination of probiotics.

The results from growth performance and blood parameters indicate that a combination of probiotics added to weaned pig's diets has the potential to be used as a dietary supplement.

The results from the pig behavioural observations during the different periods of the experiment are shown in Figures 2 and 3.

The highest activity in both groups was observed in the hours of the feeding of the animals. In most of the cases it was in coincidence in the peak of movement.

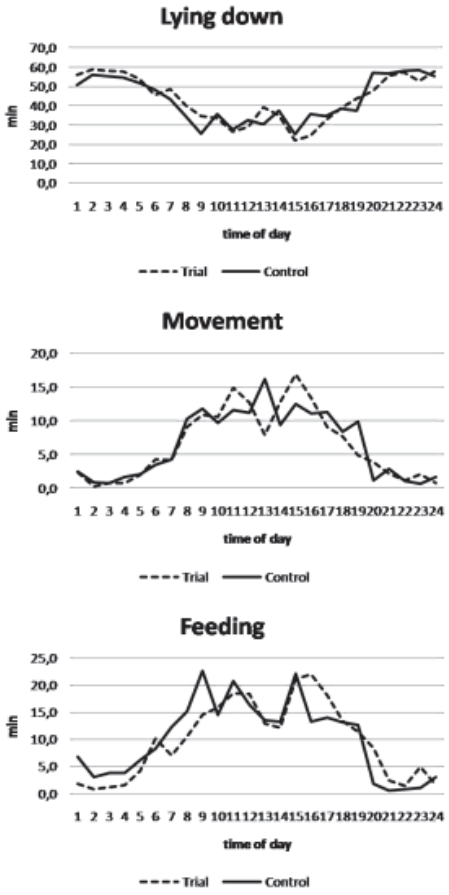

Figure 2. Behavioural reactions of growing pigs at the beginning of the experiment, reported at every hour (first observation)

The lowest values in the time of lying down was found in the time of feeding of the pigs. An interesting feature of the diagrams showing the movement of pigs in the two periods was that in the control group they are characterized by several peaks, and in the trial group the peak values were noted only at two points.

These details indicate that the animals in which the microbiological preparation was administered were calmer and moved only when their feed was being placed until all the animals in the pen were fed at the same time. In the control group, animals were active for longer periods of time - i.e. lower-ranking animals were able to eat "the leftovers", when leaders had already finished.

Although this evidence is circumstantial, it speaks to the better comfort of animals kept with the addition of a microbiological preparation in the feed, as it contributes to better absorption of nutrients.

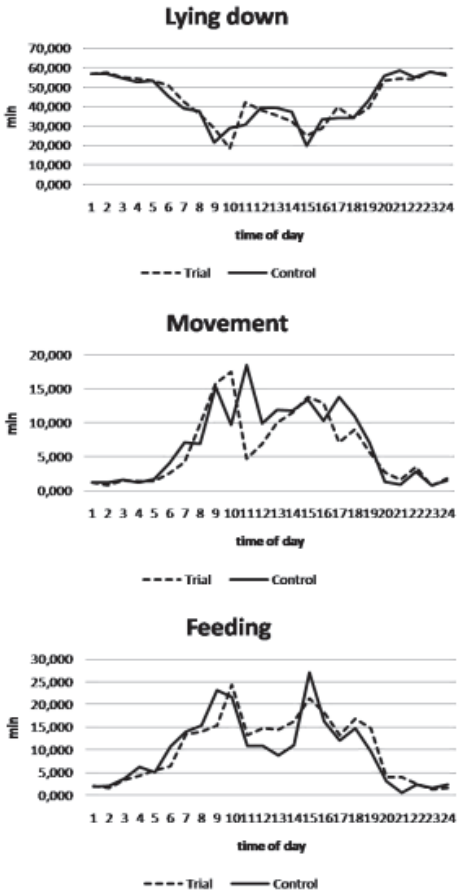

Figure 3. Behavioural reactions of growing pigs at the end of the experiment, reported at every hour (second observation)

CONCLUSIONS

The addition of Baykal EM-1 (10ml/kg feed) in weaned pig diets improved the average daily gain by 11.14% (P = 0.031).

The higher number of leukocytes and lymphocytes in pigs from the experimental group (P<0.001), compared to the control group, may be an indicator of better health and higher immunity.

A trend for better comfort in animals with the microbiological supplement in the feed, contributing to the better absorption of nutrients, has been established.

The results obtained in this study show that the combination of probiotics Baykal EM-1 has the potential to be used as a dietary supplement in weaned pigs.

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REFERENCES

Ahmed, S.T, Hoon, J., Mun, H.S. & Yang, C.J. (2014). Evaluation of *Lactobacillus* and *Bacillus*-based probiotics as alternatives to antibiotics in enteric microbial challenged weaned piglets. *African Journal of Microbiology Research*, *8*(1), 96-104, DOI: 10.5897/AJMR2013.6355

Chaucheyras-Durand, E. & Durand, H. (2010). Probiotics in animal nutrition and health. *J. Benef. Microbes*, 1, 3-9.

Chomakov, H., Boicheva, S., & Grozeva, S. (1990). The probiotic Anticolin and its application in pig breeding. *Biotechnology & Biotechnological Equipment*, *4*(2), 24-27, DOI: 10.1080/13102818.1990.10819339

Devi, S. M. & Kim, I. H. (2014). Effect of medium chain fatty acids (MCFA) and probiotic (*Enterococcus faecium*) supplementation on the growth performances, digestibility and blood profile in weaned pigs. *Vet. Med*., 59, 572-535.

Dlamini, Z.C., Langa, R.L.S., Aiyegoro, O.A. & Okoh, A.I. (2017). Effects of probiotics on growth performance, blood parameters, and antibody stimulation in piglets. *S. Afr. J. Anim. Sci., 47*(6), 766-775.

Ezema, C. (2013). Probiotics in animal production: A review. *J. Vet. Med. Anim. Health*, 5, 308-316.

Friendship, R.M., Lumsden, J.H., McMillan, I. & Wilson, M.R. (1984). Hematology and Biochemistry Reference Values for Ontario Swine. *Can J Comp Med*, 48, 390-393.

Gu, X., Li, D., Lu, W., Piao, X. & Chen, X. (2006). Screening of Bacillus strains as potential probiotics and subsequent confirmation of the in vitro effectiveness of *Bacillus subtilis* MA139 in pigs. Antonie. *Van. Leeuwenhoek*., 90, 139-146.

Huang, C.H., Qiao, S.Y., Li, D.F., Piao, X.S. & Ren, J.P. (2014). Effects of lactobacilli on the performance, diarrhea incidence, VFA concentration and gastrointestinal microbial flora of wean-ing pigs. Asian-Australas. *J. Anim. Sci*., 17, 401-409.

Lallès, J.P., Bosi, P., Smidt, H. & Stokes, C.R. (2007). Nutritional management of gut health in pigs around weaning. *Proceedings of the Nutrition Society*, 66, 260–268, DOI:10.1017/S0029665107005484

Lien, N. (2012). Effects of Fulvic Acid and Probiotic on Growth Performance, Nutrient Digestibility, Blood Parameters and Immunity of Pigs, *Journal of Animal Science Advances*, Corpus ID:8303955.

Markowiak, P. & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*(21), 1-20, https://doi.org/10.1186/s13099-018-0250-0

NRC (2012). Nutrient Requirements of Swine: Eleventh Revised Edition. Animal Nutrition Series, National Research Council of the National Academes, 420 pp. DOI: https://doi.org/10.17226/13298

Pluske, J.R., Pethick, D.W., Hopwood, D.C. & Hampson, D.J. (2002). Nutritional influences on some major enteric bacterial diseases of pigs. *Nutr. Res*., 15, 333-371.

SMR (2005). Scientific and methodological recommend-dations on the use of the microbiological preparation "Baykal" EM-1-Y for the fermentation of fodder in pigs. The recommendations of the Ministry of Agrarian Policy of Ukraine. Kvasnitsky LLC "EM-center Ukraine", KHARKOV, http://shablin.ru/em-biblioteka/em-biblioteka/594-svinovodstvo.html, last accessed on 15.01.2021.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I. & Drider, D. (2019). Benefits and Inputs From Lactic Acid Bacteria and Their Bacteriocins as Alternatives to Antibiotic Growth Promoters During Food-Animal Production. *Front Microbiol., 10*(57), doi:10.3389/fmicb.2019.00057

Wang, Y., Cho, J.H., Chen, Y.J., Yoo, J.S., Huang, Y., Kim, H.J. & Kim, I.H. (2009). The effect of probiotic BioPlus 2B (R) on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. *Livestock Science*, 120, 35-42.

Zhao, P., Zhang, Z., Lan, R., Li, T. & Kim, I. (2018). Comparison of efficacy of lactic acid bacteria complex and Enterococcus faecium DSM 7134 in weanling pigs, *Journal of Applied Animal Research, 46*(1), 888-892, DOI: 10.1080/ 09712119.2017.1420655.