Bulgarian Journal of Agricultural Science, 28 (No 2) 2022, 242–249

Effect of the probiotic BAYKAL EM-1 on some hematological and biochemical parameters and faecal score of suckling pigs

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Abstract

Ivanova, S., Nikolova, T. & Dimitrova, A. (2022). Effect of the probiotic Baykal EM-1 on some haematological and biochemical parameters and faecal score of suckling pigs. *Bulg. J. Agric. Sci., 28 (2)*, 242–249

The effect of the probiotic Baykal EM-1 on some hematological and biochemical parameters of suckling pigs was tested in an experiment with 15 equalized by breed, origin, piglet age, farrowing sequence and litter size litters. A total of 154 pigs were included into the experiment, 82 pigs (8 litters) in the experimental and 72 pigs (7 litters) in the control treatment. The feed additive, a multi-purpose microbiological product containing a complex of living, useful microorganisms, Baykal EM-1, was added to the feed of the trial group, both to the feed of sows and piglets. The dosage was 10 ml/kg of feed, from the day 8th after birth to the day 35th at weaning of the pigs. On the day 35th, at weaning, blood samples were taken and hematological and biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein level were tested. Through the whole experimental period, pigs were observed for clinical signs of diarrhea and a faecal scoring system was applied to indicate its presence and severity. A trend for a positive effect of adding the probiotic Baykal EM-1 on white blood picture in suckling pigs has been found, although not clearly demonstrated. Better biochemical status in piglets, consuming multi-strain probiotic ($P < 0.01$), was established. Elevated above reference values biochemical parameters in the control group were indicative of an existing, though not manifested, bacterial infection.

Keywords: pigs; probiotics; hematological; parameters; ALT; AST; total protein; faecal condition scoring

Introduction

In recent years, microbiological preparations are increasingly used in the practice of feeding farm animals, since, unlike antibiotics, biological additives to the diet do not contribute to the creation of resistant strains of pathological bacteria and do not accumulate in the body of animals. The positive effect of such drugs can be defined as follows: neutralization of toxins; oppression of pathogenic and conditionally pathogenic microflora; direct antibacterial effect; decrease in the adhesion of pathogenic microflora and increase in useful activity; stimulation of immunity. The use of potent antibiotics and chemotherapy drugs, unbalanced nutrition, extreme conditions, unfavorable environmental conditions, all this is accompanied by significant changes in the composition of the intestinal microflora, which leads to serious diseases. Microbiocenoses are especially vulnerable during their formation in the process of birth and later in suckling period. Therefore, the task of maintaining and correcting the normal microbiocenosis is of primary importance.

Lactic acid bacteria are the leaders among probiotic bacteria, that is live cultures of microorganisms that can positively affect the animal body by normalizing the intestinal microflora. Therefore, they are widely used not only in bacteriotherapy to correct the functions of the digestive system, but also in nutrition. Recently, there has been a trend to use special food products as an alternative to antibiotics – liquid or dry, fermented or unfermented.

Probiotics have been increasingly attracted the attention of researchers, veterinarians, feed manufacturers and farmers, with the aim of increasing animal growth, improving their health and breeding conditions. They affect the intestinal microflora, secretion and enzymatic activity, activate the functional activity of the digestive tract and improve metabolism. Their use in premixes, compound feeds and rations for pigs in industrial complexes improves the use of feed nutrients and increases productivity. It is known that in industrial pig farms, infection of both premises and the body of animals with pathogenic and conditionally pathogenic microorganisms is observed quite often. Gastrointestinal diseases occur most often in suckling piglets. At the same time, the growth and development of piglets is delayed, mortality can reach 50%. Therefore, purposeful colonization of newborn piglets with useful microflora of the gastrointestinal tract, as well as during their rearing period, is one of the conditions for reducing the mortality of young animals, increasing natural resistance and productive qualities.

Probiotics that have been tested in Bulgaria, are: Bio-Pro-I (Gudev et al., 2003; Ignatova, 2003), Enterosan (Dahterov et al., 2002; Enikova, 2002; Ignatova, 2005), Lactina (Gudev et al., 2008; Gudev et al., 2012; Ignatova, 2004), etc., with a good effect on productivity, feed conversion ratio, gastrointestinal microflora, immune status, blood and biochemical parameters, and in line with the recent trend in pig production for healthy, antibiotic-free production (Dowarah et al., 2017; Liao and Nyachoti, 2017).

Russian scientists have developed the multi-purpose microbiological product Baykal EM-1, which is recommended to be included permanently or periodically in the feed throughout the whole animal's growth period (SMR, 2005). It is a safe probiotic with no acute, subacute and chronic toxicity and has no mutagenic, teratogenic, carcinogenic, allergenic or pyrogenic action (Blinov et al., 2008). It does not visually alter the status of internal organs, but it has a positive effect on hematological and biochemical parameters of the blood and some functions of the liver (Belookov, 2013). It was established that Baykal EM-1 enhanced immunity, stimulated metabolism, improved oxygen supply to tissues, normalized liver function, and did not cause abnormalities in the generative function of animals (SMR, 2005). For example, when using the EM-preparation in feeding pigs during the entire suckling period, the survival of piglets was higher and the yield per sow was also higher (Grachev, 2006). In our previous study with suckling pigs, weight development was not influenced, but microbiological status of treated pigs was better (Ivanova et al., 2018). In another study, the addition of Baykal EM-1 in weaned pig diets improved the average daily gain by 11% and increased the number of leukocytes and lymphocytes in pigs from the experimental group ($P < 0.001$), compared to the control group (Ivanova & Nikolova, 2021). The use of the probiotic Baykal EM-1 as a feed additive for different species of animals prevents the destruction of the membranes of erythrocytes in an osmotically inadequate environment, i.e., it has a membrane-protective effect, and also improves the function of the hematopoietic organs (Blinov et al., 2008).

This paper is aimed at establishing the effect of Baykal EM-1 on some hematological and biochemical parameters of the liver, as well as on faecal condition scoring in suckling pigs.

Material and Methods

This experiment was conducted in accordance with Art. 14 of Part V. Breeding and Livestock Units form European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, regulations of the European Union (Council Directive 2008/120/EDC, Council Directive 2010/63/EU, Commission Recommendation 2007/526/EC) and the national legislation of the Republic of Bulgaria (Recommendation No 20/2012). The experiment was approved by the Bulgarian Scientific Ethics Committee and all procedures performed were in accordance with the ethical standards of the institution. It was carried out at the Experimental Unit of the Agricultural Institute in Shumen.

A total of 154 suckling pigs, originating from 15 litters, were allotted into two groups by the method of analogues regarding lineage, age, parity and litter size. In the Control group 9C) were included 72 pigs from 7 litters, and in the Experimental group (EM-1) – 82 pigs from 8 litters, supplemented by the multi-strain probiotic product "Baykal EM-1". This feed additive is a culture liquid containing bacterial cells and metabolic products of bacteria *Lactobacillus casei* 21, *Lactococcus lactis* 47, *Saccharomyces cerevisiae* 76 and *Photopseudomonas palistris* 108 and it is a transparent liquid without sediment with color from light to dark brown, with a pleasant kefir-silage smell. The product characteristics, established in Saratov State Agrarian University "N. I. Vavilov", Department of Biotechnology, Organic and Biological Chemistry (Blinov & Sazonova, 2008), are given in Table 1.

The probiotic supplement was added to the feed of the trial group, both to the feed of sows and piglets from the day 1 after farrowing of sows and from the day 8 after birth of pigs, together with the start of the feeding with creep feed, to the day 35 at the weaning of pigs. The dosage was 10 ml probiotic to 1 kg of feed. The reason for the inclusion of the probiotic in the feed both of lactating sows and suckling pigs

Table 1. Product characteristics of the microbiological product Baykal EM-1 (Blinov & Sazonova, 2008)

Physico-chemical properties	
pH	3.38 ± 0.13
Titrated acidity	25.7 ± 0.42 T°
Buffered capacity	0.033 ± 0.004 g-equiv.
Isoelectric point	5.3
Protein content	
Protein	2.55 ± 0.19 g/l
Urea	10.043 ± 0.45 mmol/l
Ammonia	0.028 ± 0.002 μmol/l
Glutamine	0.01 ± 0.0 μmol/l
Content of mono and polysaccharides	
Glucose	27.16 ± 0.016%
Sialic acid	55.0 ± 0.8 cond. units

was found in the study of Gudev et al. (2008). Their results showed a better effect of this technology on the non-specific resistance than separate treatment of either sows or piglets. The feed of the control group was fed without any supplements.

Body weights of the pigs were recorded on the day one, 21st, and 35th after birth. Ten rectal swab samples from both groups were taken and microbiologically analyzed for pathogen bacteria on the day 35. The results from this study were published elsewhere (Ivanova et al., 2018). At weaning, blood samples were taken from the ocular sinus of 10 pigs from both groups. The samples were analyzed for complete blood count in an approved standard laboratory by special kits for swine. The study was performed with a Diatron Abacus 5 Optical Hematology Analyzer on a visual-optical method for leukocytes (WBC), conductometric method for red blood cells (RBC) and medium volume of Erythrocytes (MCV); indirect, based on conductometric analysis method, for hematocrit (HCT), cyanmethemoglobin method for hemoglobin (HGB), Laser MAPSS Lymphocyte Indicator (LYM), Monocyte (MID), and Granulocyte (GRAN); conductometric method for platelet counts after erythrocyte flotation (PLT) and mean platelet volume (MPV) and calculated method for mean hemoglobin content in erythrocytes (MCH), mean hemoglobin concentration in erythrocytes (MCHC) and platelets (PCT). Liver enzyme parameters Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were analyzed by the IFCC UV kinetic method, and total protein content – by a photometric colorimetric method, with an Olympus AU640 (Beckman Counter/Olympus) automatic chemical analyzer. The results of the study were processed by the methods of variation statistics.

The pigs were observed for clinical signs of diarrhea and a scoring system (Vila Camps, 2005) was applied to indicate its presence and severity (Figure 1). Every litter received a faecal consistency score from 1 to 4, as follows:

1 – normal firm dry stools;
2 – slightly loose yellow or brown stools;
3 – spilling yellow or brown stools;
4 – white or yellow watery liquid stools.

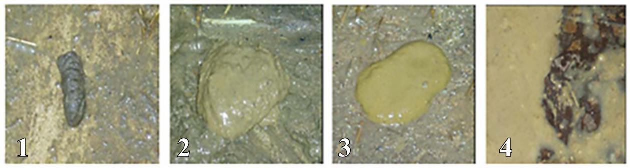

Fig. 1. Presence and severity of diarrhea according to faecal condition

Faecal scoring was done daily for individual pens according to prevailing condition of the faeces and started on day one of application of the experimental diet and continued until day 35th. Scores given for every litter were multiplied by days observed in this condition and presented as percentage of all days, because of unequal number of litters in the two groups.

Results

The results of hematological parameters are shown in Table 2.

Due to the lack of a single source in the literature for weaned pigs, multiple sources were used for the reference values (Friendship, 1984; Schmidl, 1978; Ventrella et al., 2017). The data in the table indicated that the average leukocyte values in the two groups were within the reference values, but higher by 5.39% in the trial group ($P > 0.05$). The lymphocyte values were also within the reference values and were without any differences between treatments, while the monocyte levels in the trial group exceed those in the control by 16.49% ($P > 0.05$). The granulocytes in the control group were close to the reference value. In the trial group they were by 16.31% ($P > 0.05$) lower in comparison to the control. The erythrocyte counts in both groups were also aligned within the reference values and without any significant differences between treatments.

The values of average volume, average hemoglobin content and average hemoglobin concentration in erythrocytes were slightly higher in the control group and the deviation of erythrocytes (RDW) was higher in the trial group. Pertaining to hemoglobin, the reported values of both groups were within the reference values, with a slight superiority (by 7.97%) of the control group. Hematocrit values in both

Table 2. Hematological parameters of suckling pigs, reared with and without Baykal EM-1 supplement

Indicators	Groups						P	Reference values
	Control group (C) (n = 10)			Trial group (EM-1) (n = 10)				
	$\bar{x} \pm S\bar{x}$	C	E	$\bar{x} \pm S\bar{x}$	C	E		
WBC, G/L	17.82 0. 76	13. 47	4. 26	18.78 1. 72	27. 42	9. 14	n.s.	10.50 – 21.30*
LYM, G/L	10.87 0.69	20.12	6.36	10. 93 0.61	16.77	5.59	n.s.	6.3 – 15.5*
MID, G/L	0.810.11	43.34	13.70	0.97 0.13	33.15	13.53	n.s.	0.001-5**
GRAN, G/L	6.13 0.52	26.67	8.44	5.13 0.76	36.40	14.86	n.s.	6.2*
RBC, T/L	6.03 0.14	7.10	2.25	6.06 0.14	6.74	2.25	n.s.	5.81 – 8.13*
HGB, G/L	123.40 2.57	6.60	2.09	113.56 4.58	12.09	4.03	n.s.	108 – 148*
HCT, L/L	0.40 0.01	6.79	2.15	0.37 0.01	8.13	2.71	n.s.	0.33 – 0.45*
MCV, fl	64.26 1.51	7.41	2.34	61.34 1.35	6.60	2.20	n.s.	42-63**
MCH, pg	20.48 0.31	4.76	1.50	18.74 0.62	9.86	3.29	n.s.	14-21**
MCHC, g/L	313.70 1.92	1.93	0.61	304.89 4.24	4.18	1.39	n.s.	265-336***
RDW, CV	0.13 0.01	11.78	3.73	0.17 0.01	26.14	8.71	n.s.	–
PLT, G/L	567.60 50.83	28.32	8.96	657.89 44.69	20.38	6.79	n.s.	192-832***
MPV, fl	10.45 0.29	8.53	2.70	9.34 0.25	7.87	2.62	n.s.	6.5 – 12.7***
PCT, L/L	0.28 0.03	28.92	9.14	0.21 0.02	24.58	8.19	n.s.	0.18 – 0.91***
PDW, %	13.36 0.52	12.40	3.92	12.04 0.45	11.26	3.75	n.s.	–

Notes: *Reference values according to Schmidl (1978); **Reference values according to Friendship (1984);
***Reference values according to Ventrella et al. (2017)

groups were close to each other and within the reference values. Platelet counts data showed a higher level in the trial group by 13.72% (P > 0.05) compared to the control group. Mean platelet volume, platelet counts and platelet deviation (PDW) were higher in the control group. It is remarkable that no statistically significant differences in all blood parameters between the two groups were found, probably because of the small number of examined animals and the high variation within the groups (especially in agranular and granular cells, platelet counts and mean platelet volume).

Blood serum test data of liver enzymes and total protein are reported in Table 3.

It is clear that the mean value of ALT in the trial group was within the reference value and that in the control group was significantly higher (P < 0.01) by 41.14 % than that in the experimental group and above the upper limit of the reference by 26.7%. There was a difference of 12.61% in favor of the experimental group in the levels of AST (P > 0.05). Also, a slight extend (3.48% – P > 0.05) over the limit was discovered in the control group. The data of total protein showed that its level in the control group exceeded the upper limit of the reference value by 38.33%. This value was higher by 46.42% (P < 0.01) than the value of the probiotic treated pigs, which was within the reference limit.

All faecal score conditions of all piglets multiplied by days with given values from 1 to 3, summed and presented as percentages for comparison between groups (because of not equal numbers of pigs in a group), are given in Figure 2. Severe diarrhea (water white or yellow faeces), marked by condition 4, was not observed trough the experimental period neither in the trial, nor in the control group. Faecal score 1 – normal firm dry stools, were registered in 88.41% of the trial group and in 86.58% from the control group. The percentage of the condition 2 – slightly loose yellow-brown stools, was similar in both groups. Only 1.22% from the experimental and 2.44% from the control group showed spilling yellow stools, which disappeared within few days. These

Table 3. Biochemical parameters (liver enzymes and total protein) of suckling pigs reared with and without Baykal EM-1 supplement

Indicators	Groups				Significance (P)	Reference values*
	Control group (C) (n = 10)		Trial group (EM-1) (n = 10)			
	$\bar{x} \pm S\bar{x}$	C	$\bar{x} \pm S\bar{x}$	C		
ALT (GPT), U/l	119.10 5.53	17.79	70.10 13.08	19.01	P < 0.01	21 – 94
AST (GOT), U/l	47.60 2.39	15.88	41.60 4.96	17.42	n.s.	8 – 46
Protein Total (PT), G/l	120.00 25.39	16.90	64.305.51	13.40	P < 0.01	44 – 74

Note: *Reference values according to Schmidl (1978)

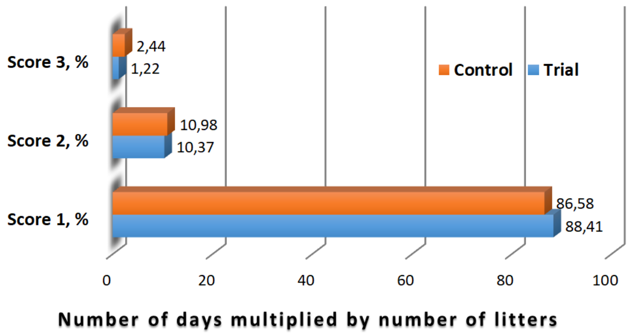

Fig. 2. Faecal condition score of suckling pigs reared with and without Baykal EM-1 supplement

conditions, showing the presence of a diarrhea, were reported in some of the piglets of both groups, with predominance in the control group.

Discussion

Probiotics may influence the immune system function. To protect organism from pathogenic bacteria and viruses, the most important role is attributed to white blood cells (leucocites). Pollmann et al. (1980) observed that the number of leucocytes in blood increased by administration of *Lacobacilus acidophilus* to piglets. Namioka et al. (1991) have reported that administration of *Bifidobacterium* elevated immunity in weanling piglets. In our study, a slight increase in leukocyte values of nearly one unit in trial animals was noticed as well. When looked in detail at the type of white blood cells, more pronounced differences are found between the groups. Increased level of monocytes (mainly consisted of macrophages) in the trial group by 16.49%, although not statistically significant, was probably an indicator of better status of the immune system. The number of granulocytes, consisted of basophils, neutrophils and eosinophils, in the control group was close to the reference value and was higher by 16.31% (n.s.) in comparison to the trial group. Neutrophils are the main amount of leukocytes and in bacterial infections there is an increase in their number. These findings confirmed the results of the microbiological study (Ivanova et al., 2018) for better microbiological status and accordingly better disease resistance of pigs consuming probiotics. Our results were in the same direction with the results of the studies of Czech et al. (2018). Their research has proved that the probiotic effect on the hematological parameters and intestinal microbes was better compared to the effect of yeast. A certain increase in the amount of leukocytes from 10.4 g/l to 12.5g/l at the expense of lymphocyte and monocyte content was found in the study made in Russia (SMR, 2005). Our results coincided with these results and showed higher values, but no statistically significant differences between groups were registered. In this regard, we could state that only a trend of the positive effect on white blood picture of applied multi-strain probiotic existed. According to Budnichenko (2015), Baykal EM-1 had a positive effect on the blood composition of animals. Addition of the fermented food treated with the preparation helped to increase the amount of hemoglobin from 120 g/l to 132 g/l and the erythrocytes – from 6.2 to 7.8 million/mm^3. Red blood cells showed linear increase trend associated with the inclusion of multi-strain probiotic in the study of Lan et al. (2016) as well. Such an increase in red blood picture count in our study has not been found. Lien (2012) also did not find any statistically significant differences between treatments in blood parameters, when studied effect of fulvic acid and probiotic supplementation alone and in combination in weaned pigs.

Aminotransferases are essential for protein metabolism and carbohydrate metabolism. With the help of aminotransferases, a reversible reaction of the transfer of an amine group to a keto acid is catalyzed, as a result of which amino acids and a new keto acid are formed. In diagnostics, aminotransferases are indicator enzymes, the activity of which changes in the case of pathology or damage of tissues. ALT and AST exist inside cells and when the cellular structure is destroyed, enzymes enter the blood serum and could be identified there with increased values. It was reported that the probiotics have the capacity to modulate animal immune system by enhancing the systematic antibody response to soluble antigens in serum (Christensen et al., 2002).

In this study, the lower serum activities of ALT and AST in the probiotic group indicated that antibiotics had a certain protective effect on cell function for piglets. It should be the case in our study as well, indicating the protective role of the combination of probiotics because both liver enzymes had higher values in the control group and exceeded the reference values. Especially for the ALT, the significant difference between trial and control group and an increase of 26.7% above the reference values were established. In the same time, studies in Russia showed an increase in ALT and AST activity in pigs fed supplemented with the same probiotic (Baykal EM-1) feed (Budnichenko, 2015; SMR, 2005), which was an indicator of better health condition. Other researchers also came to this finding. In the recent study of Xu et al. (2020), the effect of dietary supplementation with different levels of compound probiotics and berberine on biochemical indices have been tested.

The results showed increased levels of ALT and AST in probiotic treated groups in comparison to positive control group with antibiotics and decreased levels in comparison to negative control. Our results were in the same direction – higher levels in the control group, raised without antibiotics, and the values were similar to the results of Xu et al. (2020). In the opposite, the results of Gudev et al. (2012), who studied the impact of the Lactina probiotic on ALT and AST parameters in growing pigs, showed different trend. ALT activity in 35 days old probiotic treated pigs was significantly higher in comparison to control group, but associated with increased levels of stress at the time of blood collection.

Total protein level gives information about total immunoglobulin concentrations. Feeding animals with probiotic supplemented food stimulated the functional state of the liver (Kim et al., 2021), which was manifested by an increase in the synthesis of serum albumin. An increase in albumin level in the blood of 120 days old piglets by 1.3 times and the level of total blood serum protein from 62.3 g/l to 70.5 g/l was found as an effect of the application of the probiotic Baykal EM-1 (SMR, 2005). In our study the total protein level in the blood of the pigs from the control group was increased in comparison to the trial group, but also above the reference level by 38.33%, which normally is a sign of inflammation. Together with the increased levels of the liver enzymes, we think this could be a sign of some pathological condition that could not be found without blood examination. As a confirmation of this statement, the data about observed every day health condition of pigs from both groups – faecal condition scoring, presented in Figure 2, did not differ between groups and did not give a clue for an existing health problem.

For this reason, except examination of rectal swabs for microbiological analysis, we performed biochemical blood analyses. It is known that transaminases are involved in a number of biochemical processes and their study was relevant to both liver function and the presence of a disease that may not be clinically manifested. The aforementioned test data were indicative of an existing, though not manifested (seen from the observed faecal condition scoring), bacterial infection in the control group. The obtained results confirmed the view of Angelov (2012) that leukocytosis and elevated levels of the liver enzymes ALT and AST and that of the total protein were detected in animals which have been exposed to the toxigenic bacteria. In this case these were isolated strains of Escherichia coli, typified as enterotoxigenic (ETEC) of serogroup O139: F4 (Ivanova et al., 2018). Decreased number of E.coli counts ($P < 0{,}05$), when applying multi-strain probiotic in the same concentration like in our study, was found also by Lan et al. (2016).

It is also very important to mention that our experiment was performed with suckling piglets. They did not receive any antibiotics before weaning. The only way to protect them in the critical weeks after birth is through passive immunity supplied by the sow's colostrum. Colostrum does not only provide the vital energy and proteins essential for the piglets to start suckling and to grow, it is also enriched with the maternal antibodies (immunoglobulins) (Farmer et al., 2006). Furthermore, colostrum intake is negatively associated with mortality during the nursery period (Declerck et al., 2016). In our study, mortality rate was zero in both groups and no antibiotics were applied, but the effect of the probiotics in this phase of pigs' immunity development, could not be clearly seen. From the literature, it is known that the young piglet is capable of active immune responses to live viruses and dietary components by three weeks of age (Lalles et al., 2007). In this experiment the pigs were weaned two weeks later, at the age of 35 days. Probably for this reason, we could not observe remarkable effect of probiotics on the incidence of diarrhea, like Abe et al. (1995) reported. Even more, no challenge with pathogenic bacteria was applied, like other researches did (Afonso et al., 2013; Untervweger et al., 2018). Although no visible signs of severe diarrhea in both experimental and control groups were monitored and almost no differences in faecal condition scoring were registered, trends for better hematological and biochemical status in piglets, consuming multi-strain probiotic, were established.

Conclusions

A trend for a positive effect of supplementing probiotic Baykal EM-1 on white blood picture in suckling pigs has been found, although not clearly demonstrated.

Biochemical parameters elevated above reference values in the control group were indicative of an existing, though not manifested, bacterial infection. Better biochemical status in piglets, consuming multi-strain probiotic ($P < 0.01$), could be indicative of the protective role of the probiotic Baykal EM-1 on piglet's health and its possible application in suckling piglets.

Acknowledgements

Authors acknowledged the contribution of Prof. Simeon Yordanov from the National Diagnostic Research Veterinary Medical Institute – Sofia, to the interpretation of the results.

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Received: March 10, 2021; *Accepted:* November 24, 2021; *Published:* April, 2022