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THE INFLUENCE OF NEW MICROBIAL ASSOCIATIONS ON THE SOME FUNCTIONAL PARAMETERS OF CALVES AND PIGLETS

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Abstract

In gnotobiological experiments, the influence of a new microbial associations developed by combining strains of beneficial microorganisms on the body mass, numerical value of enterococci and some biochemical blood indices of calves and piglets, and their effectiveness was investigated. The remedy under test was administered orally, immediately after the birth of the animals, 30 minutes before receiving colostrum and then once a day, over a day, until the age of 9 days. The animals were monitored for 30 days. It was observed that in animals with intestinal dysfunction the investigated remedy contributed to the disappearance of diarrheal symptoms, thus contributing to the increase of the general resistance of the organism, preventing dehydration of the organism and decreasing mortality.

***Key words**: beneficial microorganisms, farm animals, intestinal microbiota.*

INTRODUCTION

Food security provides not only the provision of sufficient food to the population, but also the provision of high-quality food.

Milk, meat, and eggs, the "animal-source foods" though expensive sources of energy, are one of the best sources of high-quality protein and micronutrients that are essential for normal development and good health (Smith et al., 2013).

Thus, improvement of food quality is an important aspect of animal breeding and has become more important over time having as objectives the increase of the nutritional value (nutritional value), the assurance of the consumers' health, and optimizing processing characteristics.

Healthy animal or poultry is essential requirement for getting safe and good-quality foods of animal origin. Thus, a major objective in ensuring a quality animal production is the elimination of diseases (Collins & Wall, 2004).

Effective functionality of the gastrointestinal tract (GIT) and its health, are important factors in determining animal performance (Kogut & Arsenault, 2016).

By supporting gastrointestinal functionality of farm animals, the animal breeders can improve the weight gain of animals, leading to increased processing yields.

The concept of "intestinal health" has begun to attract interest in the field of the animal science and refers, in principle, to such aspects that promote the performance and welfare of animals as: diet, effective structure and function of the gastrointestinal barrier, host interaction with the gastrointestinal microbiota, effective digestion and absorption of feed and effective immune status (Celi et al., 2017).

It is believed that the appearance of many diseases in farm animals is due to disorders of the gut microbiota (Lee et al., 2016).

It is known that all cavities of the animal body at different ages have a certain obligatory microflora which fulfills a protective, enzymatic and synthetic functions. However, immediately after birth, the adult animals' specific microflora enters the sterile digestive tract of a newborn animals. In most cases this microflora is represented by putrefaction microorganisms, conditioned-pathogenic and pathogenic microorganisms, often leading to the death of young farm animals (Isaev et al., 2010).

According to statistics, the main gastrointestinal diseases, caused by pathogenic microorganisms, which are spread among piglets aged 5-15 and 26-40 days, cause great economic damage to pig farms, including those in the Republic of Moldova (Timoșco, 1990).

In infant piglets, these diseases were manifested by a microbial shock, and in weaned pigs - as dysbiosis (Carol-Dumitriu et al., 1981).
In this regard, further research aims to find ways to increase the natural resistance and productivity of young farm animals.
The positive role of the obligatory gut microbiota in increasing the bactericidal force of blood serum, of the function of macrophages and the general immunological reactivity of the animal organism it was elucidated (Park et al., 2018; Rovira & Melero, 2018).
Thus, the aim of this research was to study the effect of monocultures and associations of non-pathogenic microorganisms of the genera *Bifidobacterium*, *Enterococcus* and *Lactobacillus* on some parameters of body function, as well as their effect on the process of multiplication and development of intestinal enterococci in calves and piglets. Intestinal enterococci are part of the obligatory species of microorganisms of the intestinal flora which through their probiotic action have an influence on the health and resistance of farm animals, and respectively contribute to increasing the productivity of farm animals in industrial conditions.

MATERIALS AND METHODS

The microorganism strains selected and tested in gnotobiological experiments were subjected to verification of their effectiveness in experiments on calves and piglets, in farm conditions.
For this purpose, 30 calves were divided into three lots of 10 each. The first group included clinically healthy animals; group II - calves with intestinal dysfunctions (contamination with associations of bacteria from the conditioned-pathogenic genera: *Clostridium*, *Proteus*, *Staphylococcus* and *Escherichia*); group III - calves identical to group two (according to clinical status), but which received a remedy based on the association of microorganisms of five species required for calves with increased antagonistic capacity (selected in gnotobiological experiments: *Bifidobacterium longum* var. longum, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Streptococcus bovis* and *Streptococcus lactis*). The remedy under test was administered orally, immediately after the birth of the calves, with 30 min before receiving colostrum and then once a day, over a day, until the age of 9 days, i.e., 5 times. The animals were monitored during 30 days.
Strains of microorganisms were also tested on piglets, in the conditions of the technology-intensive enterprise. The experimental animals (400) were divided into five groups, 80 piglets each. Immediately after birth, before the first colostrum intake, they were orally injected with 10 ml of biomass of non-pathogenic microorganisms (obligatory types of intestinal microflora), containing 10^9 microbial cells in 1 ml, and then the same dose was administered six more times over a day, i.e., up to the age of 14 days. Animals from group I received a monoculture of *Bifidobacterium thermophilum*; animals from group II - *Streptococcus faecium*; animals from group III - association of *Bifidobacterium thermophilum* and *Enterococcus faecium*; animals from IV - *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti*; animals from group V did not receive cultures of microorganisms and served as the control group. All experimental piglets were raised according to the technology adopted in an industrial pig farm, with a closed breeding cycle, excluding chemotherapeutic drugs (sulfamides and antibiotics).
Biochemical analysis of blood in farm animals was performed according to the methods described by Vasilieva E. (1982) and Kondrahina I. (2004).
The content of enterococci was determined using classical microbiological methods (Garmasheva & Kovalenko, 2010).
Their inoculation was performed on agarized elective nutrient medium, recommended for enterococci (produced and marketed by the company "Himedia"). Over 72 hours after incubation of the inoculated samples on Petri dishes at 37 ± 1ºC, quantitative indices of enterococci were calculated at 1 g of intestinal contents (by multiplying the number of colonies by diluting the sample). The final results are expressed in decimal logarithms (log) (GOST 30518-97, 2000).

RESULTS AND DISCUSSIONS

As mentioned above, microbiota of digestive tract greatly affects the development of the host animal, mainly at an early age, and plays a very important role in the animal's resistance to infectious diseases. The formation of an obligatory microbiota as soon as possible, beneficial to host organism, consists in its barrier function and in the ability to prevent the implantation of allochthonous microbes in the gastrointestinal tract.

Thus, knowledge of the mechanism of bacterial interactions in an inevitable presupposition if optimization of the consumption of the gastrointestinal microbiota and stimulation of the beneficial effects of the latter on the host animal are desired (Stavric & Kornegay, 1995).

The greatest differences in the composition of the gut microbiota have been shown to occur between ruminants such calves and monogastric animals such piglets (Bomba et al., 2006).

In this aspect in experiments on calves, the action of the association of microorganisms of five obligatory species characteristic of these animals, with increased antagonistic capacity (*Bifidobacterium longum* var. Longum, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Streptococcus bovis* and *Streptococcus lactis*) was investigated.

First, the body mass of the calves at birth and after 30 days of administration of the tested associations of microorganisms was determined (Table 1).

Table 1. The body mass of the calves at birth and after 30 days of administration of the tested associations of microorganisms

Experimental groups of calves	Body mass of calves, kg	
	Newborn calves	30-day-old calves
I	28.5± 0.02	42.12 ± 0.2*
II	29.9 ± 0.01	42.1± 0.12
III	31.3 ± 0.04	46.33± 0.02*

* P≥0.05, ** P≥0.01

It was observed that the growth energy of calves from group III is higher compared to calves from the control group. The weight gain in group III of animals was 15.03 kg, in control group - 13.63 and in group II - 12.22 kg.

So, the daily weight gain is 454.33 g in the control group, in group II (the animals with intestinal dysfunction) - 407.33 g and in group III - 501 g.

Another parameter investigated was the numerical value of intestinal enterococci (Table 2).

Table 2. Quantitative level of intestinal enterococci in calves with and without dysfunctions caused by microbial factor, per 1 g of intestinal contents, decimal logarithms (log)

Groups of animals	Age (days)				
	1	4	7	15	30
I	1.25±0.1	3.34±0.12	5.78±0.1	6.19±0.11	5.47±0.12
II	4.12±0.09	9.76±0.12	9.9±0.11	9.49±0.14	9.21±0.11
III	6.5±0.1**	8.4±0.07*	8.7±0.09	6.9±0.13*	6.4±0.09*

*$P<0.02$; **$P<0.05$*

According to the data presented in the Table 2, there is a clear difference in the quantitative indices of enterococci of the experimental animals. Thus, in calves from group III, which received a remedy based on the association of obligatory microorganisms, the maximum content of enterococci is attested in the first 7 days of life (8.7 g (log)), after which their quantitative level reaches values characteristic of healthy animals (group I) - 6.4 g (log). At experimental animals from group II during the investigation period (30 days) an increase in the numerical value of enterococci was observed from 4.12 to 9.21 g/(log) (Table 2).

At the same time, it was established that in the first 7 days of life, in calves from group III, which received the association of obligatory microorganisms, the diarrheal symptoms disappeared. At animals from group II, diarrheal symptoms characterized by 100% dysmicrobism, manifested until the age of 20 days, causing weakening of general resistance, dehydration of the macroorganism, resulting in mortality of 30% of calves. This indicates the possibility of developing intestinal dysfunction, if the beneficial microbiota is not formed in time in young animals.

It was also found that in animals from groups I and II, the hemolytic streptococci predominated. In experimental group III prevailed bacteria from group D streptococci (enterococci), i.e., useful forms of the macroorganism, such as *Enterococcus faecium* which has the ability to produce strong bacteriocins (enterocin A and enterocin B), which is important for suppressing the excessive growth of the

opportunistic flora that forms in dysbiosis (Salvucci et al., 2012; Cotter et al., 2013).
The determination of the blood indices of general resistance of calves in the experimental groups reveals the prevalence of bactericidal activity of blood serum in calves from group III compared to that from group I and II at the age of 15 days (respectively 15.84% and 18.4%) and at 30 days (respectively with 5.84% and 11.36%) (Table 3).

Table 3. The blood indices of calves without and with intestinal dysfunctions caused by the microbial factor and under the influence of the association of microorganisms of the obligatory genera of the gastrointestinal tract

Studied indexes	Experimental groups of calves	Age of animals, days	
		15	30
Bactericidal activity of blood serum, %	I	83.3±1.28	94.2±0.82
	II	81.5±0.9	89±0.87
	III	96.5±0.93*	96±1.37
Bicarbonate, blood, mg%	I	370±7.06	376.7±12.4
	II	320±9.98	360±11.16
	III	355±12.33*	388±7.2*
Blood glucose level, mg %	I	96.7±0.95	103.8±1.4
	II	79.2±0.89	98.4±1.47
	III	104.9±1.06*	109.3±1.3*

*P<0.05

According to the data on the indices of the alkaline blood reserve, the differences between the experimental groups and according to the age of the animals were not non-essential. At the age of 30 days, this index was higher for calves of group III respectively by 3.03% and 7.78%, compared to those from groups I and II.
The quantitative level of glucose in animals receiving remedies based on the multi-component microorganism association obligatory for the gastrointestinal tract (group III) at calves of 15 days age was with 8.48% and 32.45% higher compared to this index at calves from groups I and II. After 30 days of administration of the tested remedy, this difference was 5.3% and 11.08%.
So, the influence of the remedy based on the association of microorganisms on the young calves (in the first 30 days of life) and on the intestinal microbiota is beneficial.
Piglets as monogastric animals differ from calves (ruminants) by the composition of the intestinal microbiota (Bomba et al., 2006).
It has been established that gradual changes in the composition of the gastrointestinal microbiota that take place within an animal species are related to age (Smith, 1965). At an early age the microbiota of the digestive tract of young animals is very similar. This largely depends on the fact that after birth the intestinal microbiota is determined by breastfeeding. Especially, milk constituents largely determined which microbiome can be implanted in the intestines (Bomba et al., 2006).
It should be noted that newborn piglets possess very efficient selection systems enabling it to favor certain bacterial species among the bacteria of different ecosystems. This selection is influenced by many factors: diet, environmental conditions such as hygienic stage, temperature, the microbial interactions in the digestive tract and the barrier effect of the dominant microbiota against the environmental bacteria (Jiang et al., 2019).
The monocultures and associations of microorganisms studied were tested on piglets for 30 days, during which the animals were monitored, recording weight gain, numerical indices of enterococci in their digestive tract, as well as non-specific resistance (bactericidal activity of blood serum, %, the hemoglobin, g/dL and erythrocytes content × 10^{12}/L) according to classical methods (Vasilieva, 1982; Kondrahina, 2004).
The data regarding the action of investigated microorganism strains on body mass of piglets are given in table 4.

Table 4. Body mass of piglets in testing experiments of monocultures and new microorganism associations

Experimental groups of piglets	Body mass, kg	
	3-day-old piglets	25-day-old piglets
I	0.89± 0.06	6.25 ± 0.31*
II	0.93 ± 0.04	5.25 ± 0.24
III	1.18 ± 0.03	6.2 ± 0.22**
IV	1.01 ± 0.05	6.75 ± 0.27
V	1.22 ± 0.05	4.45 ± 0.25**

* P≥0.95, ** P≥0.99

The tested microorganisms had a positive effect on the productivity of experimental animals. In particular, the average weight of a 25-day-old piglet in group I was 6.25 kg; in group II - 5.25 kg; in group III - 6.20 kg; in group IV - 6.75 kg and in group V - 4.45 kg. Therefore, the daily weight gain of a piglet was 210 g in group I; 170 g - in group II; 204 g - in group III, 230 g - in group V and 134 g in group V.
Also, the same changes were noted regarding the data on the vitality per head of experimental

animals. Thus, in group I it was 95%, in group II - 85%, in III - 90%, in IV - 97.5% and in V - 65%.

The obtained data revealed that the administration of the association of microorganisms – *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti* had the most beneficial effect on the weight gain and vitality of experimental animals.

The effect of monocultures and associations of microorganisms on the multiplication and development of enterococci in the digestive tract of piglets was also investigated. It is known that the optimal content of enterococci in the intestine ensures the proper functioning of the intestinal microbiota.

It is known, that the enterococci are ubiquitous in GI tracts even though they constitute a small proportion of the gut consortium, typically comprising less than 1% of the adult microflora (Finegold et al., 1983; Sghir et al., 2000). Enterococci are primarily localized to the small and large intestine, where enterococci are prominent members of jejunal, ileal, cecal, and recto-sigmoidal consortia (Hayashi et al., 2005).

Therefore, the numerical value of enterococci was documented in 6 segments of the digestive tract: stomach, duodenum, small intestine, ileum, check and rectum.

The results on the numerical indices of intestinal enterococci in piglets are presented in Table 5.

Among the piglets of control group, bacterial gastroenteritis from dysbacteriosis was recorded, resulting in 35% piglet mortality. In the experimental groups, intestinal disorders at piglets were not observed.

Analysis of the numerical values of enterococci in all gut segments of piglet, in the first 30 years of life at administration of remedies based on monocultures and associations of microorganisms, revealed a lower content of enterococci in animals from group I (administration of *Bifidobacterium thermophilum* monoculture) and higher in group II of animals that received the monoculture of *Enterococcus faecium*.

It should be noted that in the stomach the content of enterococci was the lowest compared to the other segments of the digestive tract (gut), and in the check and rectum the highest values of these bacteria were detected.

When monitoring the numerical indices of enterococci during 30 days of administration of the remedies used in the study, it was established that a higher content is attested on the 7th day in animals of group II, III and IV in the stomach, duodenum, small intestine, ileum and check. In the rectum, the highest level of enterococci is revealed on the 3rd day of the experiment in animals from experimental groups II, III and IV.

Table 5. Quantitative indices of intestinal streptococci in suckling piglets that received a remedy, based on monocultures and associations of microorganisms of the obligatory genera of the gastrointestinal tract

Experimental groups of piglets	Age (days)		
	3	7	30
	Stomach		
I	0	0	0
II	5.98±0.13	6.77±0.15*	4.55±0.11**
III	5.17±0.09*	0	5.17±0.05*
IV	5.17±0.07*	5.75±0.09**	2.32±0.11**
V	4.29±0.17	3.88±0.2	3.00±0.21
	Duodenum		
I	0	2.26±0.07*	2.44±0.09*
II	6.71±0.12*	7.6±0.14**	2.24±0.08*
III	5.65±0.1*	6.72±0.11*	6.69±0.09**
IV	5.69±0.1*	6.26±0.08*	2.25±0.1*
V	6.35±0.19	4.67±0.15*	4.49±0.2*
	Small intestine		
I	2.48±0.07*	3.48±0.05*	3.26±0.08*
II	7.7±0.15*	7.82±0.13*	3.52±0.09*
III	6.74±0.12*	7.57±0.1*	2.89±0.05*
IV	2.88±0.13*	6.88±0.11*	3.32±0.06*
V	7.64±0.22	5.8±0.19	5.28±0.17
	Ileum		
I	3.59±0.09*	4.5±0.11*	4.74±0.1*
II	7.93±0.18*	8.29±0.14*	5.46±0.1*
III	7.69±0.13*	8.39±0.15*	4.39±0.1*
IV	7.76±0.09*	7.84±0.08*	4.79±0.1*
V	6.68±0.19	5.83±0.15	5.83±0.15
	Check		
I	4.6±0.11*	5.52±0.12*	5.87±0.1*
II	8.65±0.17*	8.44±0.15*	8.44±0.15*
III	7.69±0.13*	8.67±0.12*	6.07±0.1*
IV	8.59±0.12*	8.4±0.13*	6.83±0.11*
V	6.27±0.2	8.04±0.17	6.86±0.19
	Rectum		
I	4.15±0.1*	5.55±0.12*	5.67±0.11*
II	8.69±0.17*	8.38±0.15*	5.91±0.12*
III	8.62±0.12*	8.23±0.11*	5.45±0.13*
IV	8.55±0.08*	7.54±0.06*	5.82±0.07*
V	5.87±0.17	7.22±0.17	6.35±0.15

After 30 days of study, the numerical value of enterococci decreased in all animals of the experimental groups in all segments of the digestive tract, except for group I, where an increase in enterococci was observed after 30 days of monoculture *Bifidobacterium thermophilum* administration.

It was established that the associations of microorganisms tested in group III and group IV contributed to maintaining of the enterococci content as the same level as in control group in gut segments as: stomach, duodenum, small intestine and ileum

In check and rectum high numerical values of enterococci are characteristic for all experimental groups (except group I), including the control group.

Thus, based on the obtained data on the influence of monocultures and associations of microorganisms on the numerical indices of enterococci in gut segments of the digestive tract we can mention that:

- administration of the monoculture of *Bifidobacterium thermophilum* (group I) resulted in a lower content of enterococci during 30 days of testing in all segments of the digestive tract;
- the administration of the monoculture based on *Enterococcus faecium* (group II) determined the increase of the numerical indices of the enterococci, reaching the highest level in check and rectum (compared to the other experimental groups) in the first 7 days of testing;
- administration of the associations of *Bifidobacterium thermophilum* and *E. faecium* microorganisms (group III) also contributed to the increase in enterococcal contents in the digestive tract segments, in particular in the check and rectum during the first 7 days of testing.
- the administration of associations of microorganisms *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti* (group IV) more or less determined the maintaining of the enterococci content at the same level in gut segments, which reveals the beneficial action of this remedy on the homeostasis of the gut microbiota and respectively on the health of the digestive tract.

It should be noted that the microbes of the genus *Enterococcus* are mainly ancient and highly evolved members of GI tract consortia of various hosts (Lebreton et al., 2014). It is known that the content of enterococci in the digestive tract can vary, and their numerical value can determine either the positive effect or the negative effect.

Usually, many *Enterococcus* spp are regarded as commensals of the intestine tract in mammals, but some members (*E. durans* and *E. hirae*) have also been associated with diarrhoea in suckling animals. The indigenous commensal enterococci, can act as opportunistic pathogens and translocate across the mucosal barrier to cause systemic infection in immune-compromised hosts (Berg, 1996; Donskey, 2004).

Thus, we can assume that the associations of microorganisms studied, as well as the monoculture of *Bifidobacterium thermophilum* have the most beneficial effect, in terms of maintaining a constant or low level of enterococci in the digestive tract of piglets.

In order to highlight the action of remedies based on monocultures and associations of microorganisms on the general condition of piglets, some biochemical indices of blood that reflect nonspecific resistance (bactericidal activity of blood serum, the hemoglobin and erythrocytes content) were determined. The obtained data are shown in figure 1. The determination of bactericidal activity is important because this is a one of parameters of innate immunity.

As a result of the investigations, the positive action of the monoculture of bifidobacteria (group I) and the association of microorganisms (group IV) on the bactericidal activity of blood serum in piglets was found, the level of which increased by 9.6% and 6.7%, respectively at the age of 7 days, and by 3.9% and 6.7%, respectively, at the age of 25 days, compared to the indices of control group (figure 1A).

Bactericidal activity in group III increased compared to the control group by 5.6% in 7-day-old piglets and by 1.9% at the end of the experiment. The animals that received only monocultures of enterococci (group II), a decrease of bactericidal activity was observed, being at the level of the indices from the control group (Figure 1A).

The best results of the studied indices were found in the groups of animals that received monocultures of bifidobacteria and the association of microorganisms, both at the age of 7 days and at the age of 25 days (Figure 1A).

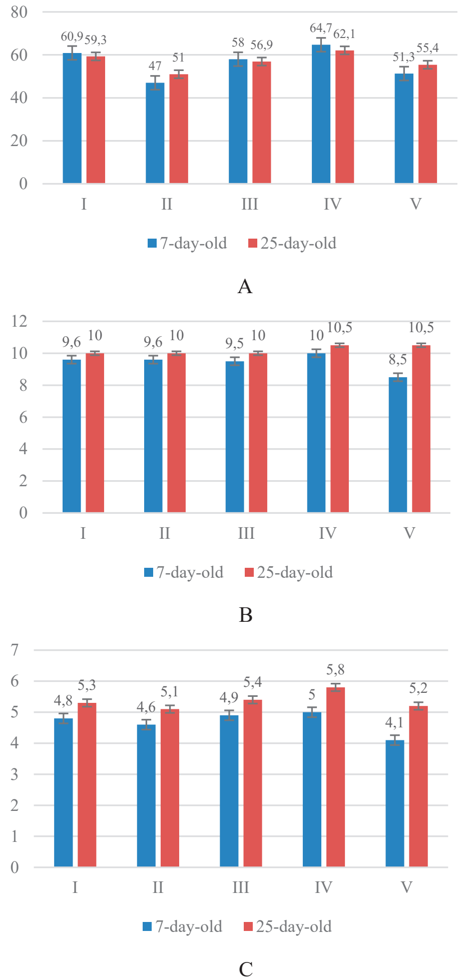

Figure 1. Bactericidal activity of blood serum, % (A), the hemoglobin, g/dL (B) and erythrocytes, $\times 10^{12}$/L (C) content of piglets under the influence of monocultures and association of microorganisms of the obligatory genera of the gastrointestinal tract

The hemoglobin content in the blood of experimental piglets in the first 7 days of investigation was higher compared to the control, but after 25 days, the hemoglobin content became identical in all experimental groups (including control) (Figure 1B). The content of erythrocytes has the same trend of modifications as of hemoglobin parameters: in the first 7 days it is higher in the experimental groups; after 25 days, it is established at the level of the indices identical to the control group (Figure 1C).

This indicates that remedies developed based on monocultures and associations of microorganisms have a more pronounced effect on the indices of nonspecific resistance in the first days of life of piglets. This is important in forming the body's general resistance to pathogens, which in turn causes a decrease in mortality in young animals and an increase their productivity, respectively.

Thus, intestinal integrity is fundamentally important for the growth and performance of food animals.

CONCLUSIONS

All types of tested microorganisms had a beneficial effect on the growth and development of farm animals in industrial farm conditions. In calves, the associations of microorganisms during the experiments ensured the increase of the body mass by 9.8-10%. A monoculture and new developed microorganism association ensured the average daily weight gain of piglets 1.26-1.7 times compared to the control and maintaining its vitality by 20-32.5%.

The investigated associations of microorganisms, as well as the monoculture of *Bifidobacterium thermophilum* have a homeostatic effect on the enterococci content in digestive tract of calves and piglets, which argues their beneficial action on the gastrointestinal microbial cenoses and respectively on the health of the digestive tract.

A direct relationship has been established between the indicators of natural resistance of farm animals in experimental groups and their productivity, which indicates the appropriateness of using bifidobacteria, enterococci and lactic acid bacteria to raise young piglets and calves in industrial farms to increase natural resistance and animal productivity, as well as for the prevention of bacterial gastroenteritis.

The beneficial effect of remedies based on monocultures and association of obligatory microorganisms argue the utilization of microbial strains as alternatives to antibiotics for sustainable food animal production.

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