EFFECT OF PROBIOTIC PREPARATIONS ON THE GROWTH AND ASSIMILATION OF NUTRITIVE SUBSTANCES IN DIFFERENT BREEDS OF PUPPIES

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Abstract. A newborn puppy faces the environment being absolutely sterile and during relatively short time acquires microflora typical for the species. The digestive system of the dog is constantly affected by harmful environmental factors. Due to these factors, the balance of microorganisms prevailing in the intestine system is disturbed and consequently the probability to get sick with the diseases of digestive system is increasing. Probiotics are bacterial preparations of live cultures of microorganisms intended for the correction and treatment of the digestive system microflora. Probiotics, contrarily to antibiotics, have no negative effect on normal microflora. For this reason probiotics are widely used for prevention and treatment of disbacteriosis.

During the investigation, immediately after the birth puppies of three different breeds were given 5 g of probiotic preparation „Fermactiv“. It was defined that probiotics positively affected the daily weight gain of the puppies: for the puppies of small breeds the daily weight gain was on the average by 10.7%, medium breeds by 2.1%, and large breeds by 0.75% higher compared to the control group of puppies.

Probiotic preparation „Fermactiv“ had an influence on the assimilation of different nutritive substances as well. It was defined that small breeds of puppies given probiotics were able to assimilate crude fat by 1.59% and organic matter by 3.43% better compared to the control group. Puppies of medium breeds assimilated dry matter by 7.66% ($p<0.01$), fat by 0.14%, fiber by 1.72% ($p<0.05$), and organic matter by 1.58% better. Puppies of large breeds assimilated dry matter by 1.34% ($p<0.001$), crude protein by 2.09% ($p<0.05$), crude fat by 0.68% ($p<0.001$), crude fiber by 8.82% ($p<0.001$), crude ash by 10.06% ($p<0.001$), and organic matter by 6.72% ($p<0.001$) more effectively than the control group.

It was defined that the probiotic treatment influenced the blood chemical composition. Compared with the control group, blood glucose and cholesterol levels in all breeds of puppies decreased, wheareas total protein and calcium levels became higher.

Keywords: probiotics, puppies, assimilation, daily weight gain.

PROBIOTIKŲ ĮTAKA SKIRTINGŲ VEISLIŲ ŠUNIUKŲ AUGIMUI IR MAISTO MEDŽIAGŲ ABSORBAVIMO LYGIUI

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Santrauka. Tik atvestas šuniukas į aplinką patenka visiškai sterilus ir palyginti per labai trumpą laiką įgauna mikroflorą, būdingą šiai gyvūno rūšiai. Šuns virškinamąjį traktą nuolat veikia kenksmingi išorės veiksniai, dėl to suardoma žarnyne vyraujančių mikroorganizmų pusiausvyra. Tas padidina tikimybę susirgti virškinamojo trakto ligomis. Probiotikai – bakteriniai preparatai iš gyvų mikroorganizmų kultūrų, skirti virškinamojo trakto mikrobinei terpei koreguoti bei gydyti. Šie, priešingai nei antibiotikai, nesukelia neigiamo poveikio normaliai mikroflorai, todėl plačiai naudojami disbakteriozių profilaktikai ir gydymui.

Tyrimo metu tik ką atvestiems trijų skirtingų veislių šuniukams buvo suduota po 5 g probiotinio preparato „Fermactiv“. Nustatyta, kad probiotikas turėjo teigiamos įtakos šuniukų paros priesvoriui: mažų veislių šuniukai priaugo 10,7 proc., vidutinio dydžio veislių – 2,1 proc., didelių veislių – 0,75 proc. daugiau palyginti su kontrolinių grupių šuniukais.

Probiotikas „Fermactiv“ taip pat darė įtaką pasisavinant skirtingas maisto medžiagas. Nustatyta, jog, gavę probiotikų, mažų veislių šuniukai žalius riebalus pasisavino 1,59 proc., o organinę medžiagą – 3,43 proc. geriau už kontrolinės grupės šuniukus. Vidutinio dydžio veislių šuniukai geriau sausąsias medžiagas pasisavino 7,66 proc. ($p<0,01$), riebalus – 0,14 proc., ląstelieną – 1,72 proc. ($p<0,05$), organines medžiagas – 1,58 proc. Didelių veislių šuniukai sausąsias medžiagas pasisavino 1,34 proc. ($p<0,001$), žalius baltymus – 2,09 proc. ($p<0,05$), žalius riebalus – 0,68 proc. ($p<0,001$), žalią ląstelieną – 8,82 proc. ($p<0,001$), žalius pelenus – 10,06 proc. ($p<0,001$), organinę medžiagą – 6,72 proc. ($p<0,001$) geriau palyginti su kontrolinės grupės šuniukais.

Nustatyta, jog probiotikas turėjo įtakos kraujo cheminei sudėčiai. Palyginti su kontroliniais visų veislių šuniukų kraujyje sumažėjo gliukozės ir cholesterolio koncentracija, tuo tarpu bendrų baltymų ir kalcio nustatyta daugiau.

Raktažodžiai: probiotikai, šuniukai, pasisavinamumas, paros priesvoris.

Introduction. Probiotics are microorganisms – bacteria or yeast – which supplemented to animal ration enable to influence microflora of digestive system. Probiotics often have been used to treat diarrhoea or to decrease the negative effect of antibiotics on the organism (Biourge et al., 1998). Reasonable use of probiotic preparations allows to minimize treatment with antibiotics or to withdraw antibiotics at all. This way of treatment is considered to be maximally safe, but either untraditional or innovative (Weese, 2003). It has been widely stated recently that probiotics are necessary not only for sick, but as a preventive measure for healthy animals as well. The mechanism of these bacteria action has not been fully revealed yet, however, it can be stated that probiotics in the organism of a healthy animal perform the regulatory function of the digestive system being the source of easily degradable protein and stimulating the immune system. Consequently, it seems rational to use these preparations while breeding animals; especially in cases of high concentration, high growth rate and early weaning (Biourge et al., 1998).

However, during treatment of animals it is impossible to abandon disinfection, treatment with antihelminthic preparations, vaccination, coccidiostatics or antibiotics. After application of these measures, it is necessary to restore normal microflora, as the disturbed mucous of the digestive system is unable to absorb normally nutritive substances (Marteu, 2002).

Microorganisms producing lactic acid, such as *Lactobacilli, Bifidobacteria, Streptococcus, Pediococcus* as well as yeast and filamentary fungi (Parvez et al., 2006) are commonly used as probiotic preparations.

The data of probiotics effectiveness investigation sometimes can vary and be contradictory; due to this fact, the importance of further investigations becomes evident. Moreover, it is necessary to find the most effective ways in order to increase the effectiveness of probiotics. The effectiveness of probiotics is determined by the selection of the most effective bacterial strains, combination of several strains, and stability of microorganisms during technological processes in the live organism as well as in synergistically acting components (Bomba et al., 2002).

Recently experiments have been carried out trying to insert probiotic preparations into the composition of commercial food. Keeping in mind their specificity, the use is possible only in dry food, as producing canned food under the effect of thermal processes some microorganisms are destroyed; thermo stability of some microorganisms reaches only 50 ^{0}C (Weese, 2003).

In 2008, the experiment was carried out by A. Laukova et al. (2008), during which she tried to isolate certain species of enterococcus from 28 commercial food containing probiotics. The obtained results revealed that 12 samples contained enterococcus. *Enterococcus faecium* (*E. faecium*) was the most frequent and accounted for 54.6%. *Enterococcus faecalis* (*E. faecalis)* accounted for 36.4% and *Enterococcus hirae* for 9.0%. The possibility of these probiotics to adhere to the intestinal wall was evaluated in the range of 6.2–7.5 grades. It was defined that the index of adhesion of *E. faecium* EF3, EE3 and *E. faecalis* EE2 to the mucous of the intestinal wall tended to be the highest 7.5; 7.3–7.4; 7.0–7.4 (Laukova et al., 2008; Strompfova et al., 2004).

During the experiment, the preparation "Fermactiv", containing $1*10^5$ *Enterococcus faecium* microorganisms, was used. Enterococcus is one of the most frequently found microorganisms in the digestive system of animals; more over, it is especially popular as a constituent of commercial probiotic preparations. *E. faecium* strains effectively protect from the diseases caused by microorganisms of *Escherichia coli*, *Salmonellae* or *Clostridia* strains. It should be stated that enterococci microorganisms are not suitable for all animal species – different strains vary in their ability to attach to the intestinal walls of certain animal species (Strompfova et al., 2006).

It is known that probiotic strain *Lactobacillus rhamnosus* could significantly reduce concentrations of serum total lipids, triacyglycerol, total cholesterol and phospholipids in rats (Umeki et al., 2004).

The aim of the study is to define the effect of probiotic preparation "Fermactiv", containing $1*10^5$ *Enterococcus faecium* microorganisms, on the growth rate and nutritive substances assimilation, healthiness and eco-friendliness in different breeds of puppies, and to monitor their haematological values.

Materials and methods. The experiments were carried out in dogs breeding centres and the at the Laboratory of Food Investigation, Department of Animal Nutrition, Lithuanian University of Health Science in 2010. Clinically healthy puppies of three different types – small, medium and large breeds –were selected for the experiment, taking into consideration their weight, physiological condition, and stage of the development. The investigations were carried out in three stages: after birth, from 14 to 60 days of life, and during weaning. We formed two analogous groups: one group contained three small and medium breed puppies and another group contained six puppies of large breeds.

The puppies were given the bitch's milk and additionally were fed commercial dry food, which was given to the bitches as well. The puppies of the experimental groups were preventively given 5 g of probiotic preparation „Fermactiv" from the 2-nd to the 5-th day of age. The duration of the clinical observation was 60 days.

The puppies were weighted three times: on the 1-st, 30-th and 60-th day. An electronic scale CAS AP1 was used.

Investigations of faeces and food were carried out according to generally accepted methods (Januškevičius, Januškevičienė, 2010).

Blood samples were taken from *safena venus*. Doing the morphological test, blood samples were collected into tubes with EDTA. For chemical test, the blood samples were collected into tube without preservatives.

Blood biochemical parameters were established by Idexx VetTest chemistry analyzer and morphological blood test was performed by Melet Schloising Laboratories analyzer.

Statistical results – arithmetic mean, error of the arithmetic mean, criteria and degree of reliability – were defined according to Stjudent and a statistical package was used (Sakalauskas, 1998).

Scientific research was performed according to Animal care, keeping and usage act No B1-639 of the Republic of Lithuania, dated 18/12/2008 ("Valstybės žinios", 22/01/2009, No 8).

Results. During the experiment, the daily weight gain of three different breeds of whelps was defined. At the beginning of the experiment, the daily weight gain in the experimental group of small newborn puppies was on the average by 8.4%, the medium group by 5.9%, and the large group by 7.5% higher than in the control group of puppies (Table 1).

Puppies were weighed the second time on the 30-th day of age. It was also defined that daily weight gain in the group of small puppies was by 11.6% ($p<0.05$) higher if compared to the control group, whereas in the experimental group of large breeds the daily weight gain in was by 2.0% lower (Table 1).

Table 1. **Daily weight gain by puppies at different periods of growth**

Breeds	Group	
	Control	Experimental
Daily weight gain during first 30d., g		
Small	33.47±1.03	36.30±2.84
Medium	61.27±2.40	64.89±7.42
Large	84.22±5.57	90.50±3.92
Daily weight gain from 30 to 60 d., g		
Small	88.28±1.20	98.50±3.58*
Medium	134.11±8.88	134.56±9.94
Large	203.67±3.57	199.56±2.78
Daily weight gain during the whole time, g		
Small	60.88±1.08	67.40±3.22
Medium	97.69±3.28	99.72±2.68
Large	143.94±1.13	145.03±0.79

*$p<0.05$

When puppies were weighed on the 60-th day of age, the results obtained revealed that daily weight gain of the experimental puppies of the small breeds group was by 10.7% higher, the medium group by 2.1%, and the large group by 0.8% higher than that in the control groups (Table 1).

Assimilation of nutritive substances by three different types of breeds was also studied during the experiment. At the beginning of the investigation, i.e. on the 2-nd–5-th days, the puppies were given 5 g of probiotic preparation „Fermactiv".

Faeces investigation on the 14-th day of age in the group of small breeds puppies demonstrated that the dry matter digestibility coefficient was by 3.95% ($p<0.001$) lower than in the control group, on the 60-th day it was by 0.80% lower. The coefficient of crude protein digestibility on the 14-th day was by 5.77% ($p<0.001$) lower, on the 60-th day by 1.72% lower than in the control groups. Comparison of the crude fat digestibility coefficients revealed that the level of assimilation in the experimental group of small breeds on the 14-th day was by 0.26% higher than in the control group, however, on the 60-th day it tended to be by 1.59% ($p<0.001$) lower. Fibre digestibility coefficient in the experimental group of puppies on the 60-th day was by 3.47% higher than in the control group. Comparing crude ash digestibility coefficients it becomes evident that on the 14-th day this parameter in the experimental group was by 0.21% lower, whereas on the 60-th day by 4.92% ($p<0.05$) higher than in the control group. Organic matter digestibility coefficients in the experimental group of small breeds on the 14-th day was by 3.7% lower, on the 60-th day by 3.43% higher than in the control group (Table 2).

Having investigated the faeces of the group of medium whelps on the 14-th day of age, it became evident that dry matter digestibility coefficient in this experimental group was by 0.50% higher, on the 60-th day by 7.66% ($p<0.01$) higher than in the control group. The digestibility coefficient of crude protein in the experimental group on the 14-th day was by 0.32 % lower and on the 60-th day by 0.10% lower than in the control group. Crude fat digestibility coefficient in the experimental group on the 14-th day was by 0.46% ($p<0.05$) lower and on the 60-th day by 0.14% higher than in the control group. The crude fibre digestibility coefficient in the experimental group on the 60-th day was by 1.72% ($p<0.05$) higher than in the control group. Investigation of crude ash digestibility coefficients demonstrated that on the 14-th day ash assimilation in this experimental group of puppies was by 2.53% better and on the 60-th day by 0.64% worse compared to the control group. The digestibility coefficient of organic matter on the 14-th day of age was by 1.01% lower and on the 60-th day by 1.58% better than in the control group (Table 2).

Table 2. **Level of nutritive substances assimilation in small, medium and large breeds of puppies** (%)

Parameters	14 day-old puppies		60 day-old puppies	
	Control group	Experimental group	Control group	Experimental group
Small breeds n=3				
Dry substance	93.97±0.23	90.02±0.49***	96.31±0.4	95.51±0.11
Crude protein	94.51±0.2	88.74±0.68***	93.77±0.83	92.05±0.27
Crude fat	99.57±0.13	99.83±0.01	98.78±0.15	97.19±0.13***
Crude fiber	-	-	29.74±1.25	33.21±0.61
Crude ash	16.61±1.34	16.40±2.68	14.66±0.48	19.58±1.23*
Organic matter	96.52±2.53	92.82±1.79	93.20±9.2	96.63±1.72
Medium breeds n=3				
Dry substance	96.62±0.29	97.12±0.19	44.79±1.12	52.45±0.84**
Crude protein	96.36±0.43	96.04±0.31	98.61±0.63	98.51±1.45
Crude fat	99.36±0.14	98.90±0.02*	98.76±0.87	98.90±0.41
Crude fiber	-	-	26.16±0.49	27.88±0.14*
Crude ash	14.91±0.81	17.44±1.27	16.22±2.45	15.58±1.34
Organic matter	94.76±0.76	93.75±0.64	94.21±0.24	95.79±1.67
Large breeds n=6				
Dry substance	75.21±0.91	75.49±0.62	90.55±0.36	91.89±0.22***
Crude protein	80.35±0.76	80.25±0.50	76.05±0.34	78.14±0.70*
Crude fat	97.52±0.13	97.61±0.10	96.85±0.08	97.51±0.10***
Crude fiber	-	-	22.48±0.96	31.30±0.92***
Crude ash	79.98±0.52	75.57±0.27***	18.77±1.24	28.83±0.51***
Organic matter	73.91±0.94	71.29±0.74*	86.79±0.49	93.51±1.29***

*p<0.05; **p<0.01; ***p<0.001

Table 3. **Biochemical blood parameters of small, medium and large breeds puppies**

Parameters	Group	
	Control	Experimental
Small breeds, n=3		
Glucose, mmol L^{-1}	6.74±0.51	6.56±0.32
Cholesterol, mmol L^{-1}	2.46±0.05	2.36±0.09
Total bilirubin, µmol L^{-1}	10.33±0.97	13.89±0.2*
Total protein, g L^{-1}	60.33±2.13	63.67±4.45
Calcium, mmol L^{-1}	2.86±0.10	2.96±0.06
Phosphorus, mmol L^{-1}	1.82±0.02	1.90±0.04
Medium breeds, n=3		
Glucose, mmol L^{-1}	7.48±0.19	6.76±0.18*
Cholesterol, mmol L^{-1}	2.52±0.05	2.47±0.17
Total bilirubin, µmol L^{-1}	9.67±0.96	10.67±0.97
Total protein, g L^{-1}	61.00±2.61	63.33±3.10
Calcium, mmol L^{-1}	3.09±0.06	3.43±0.26
Phosphorus, mmol L^{-1}	2.01±0.08	2.03±0.06
Large breeds, n=6		
Glucose, mmol L^{-1}	6.72±0.29	6.57±0.25
Cholesterol, mmol L^{-1}	2.73±0.08	2.70±0.07
Total bilirubin, µmol L^{-1}	11.33±0.62	11.67±1.12
Total protein, g L^{-1}	61.83±3.80	62.84±3.08
Calcium, mmol L^{-1}	3.09±0.09	3.41±0.12*
Phosphorus, mmol L^{-1}	2.12±0.08	2.16±0.10

*p<0.05

The results of large breeds puppies faeces investigation led to the conclusion that dry matter digestibility coefficient in this experimental group on the 14-th day was by 0.28% higher and on the 60-th day by 1.34% ($p<0.001$) higher compare to the control group. Crude protein digestibility coefficient on the 14-th day in the experimental group was by 0.10% lower whereas on the 60-th day by 2.09% ($p<0.05$) higher than in the control group. The digestibility coefficient of crude fat in the experimental group on the 14-th was by 0.09% higher and on the 60-th day by 0.68% ($p<0.001$) higher than in the control group. Crude fibre digestibility coefficient on the 60-th day in the experimental group was by 8.82% ($p<0.001$) higher than in the control group. Crude ash digestibility in the experimental group of puppies on the 14-th day was by 4.41% ($p<0.001$) lower whereas on the 60-th day by 10.06% ($p<0.001$) higher than in the control group. The coefficient of organic matter digestibility on the 14-th day of age in the experimental group was by 2.62% ($p<0.05$) lower and on the 60-th day by 6.72% ($p<0.001$) higher than in the control group (Table 2).

The blood samples for chemical and morphological tests were taken in the end of experimental period on 60-th day. During the experiment, the main chemical indicators wer tested: glucose, cholesterol, total bilirubin, total protein, calcium and phosphorus. In small breeds group there were no significant differences among control and experimental groups in glucose and cholesterol parameters. Total bilirubin in the experimental group was by 3.65% ($p<0.05$) higher than in the control group. Total protein in the experimental group was by 3.34% higher than in the control group. In the experimental group of medium breeds it was established that glucose level by 0.72% ($p<0.05$) lower than in the control group, and the total protein level was by 2.33% higher than in the control group. But there were no significant difference in others parameters of blood. In large breed puppies group, the level of calcium in blood was by 0.32% ($p<0.05$) higher than in the control group of puppies. Glucose in experimental group was by 0.15% lower than in the control group. As to other blood parameters, there were no significant differences (Table 3).

Table 4. **Morphological blood parameters of small, medium and large breeds puppies**

Parameters	Group	
	Control	Experimental
Small breeds, n=3		
WBC x 10^9 L^{-1}	16.23±0.97	16.13±0.93
Neutrophils, %	59.56±0.96	59.11±1.23
Lymphocytes, %	23.35±1.10	22.98±0.97
Monocytes, %	3.25±0.09	3.53±0.22
Eosinophils, %	11.38±0.31	11.79±0.50
Basophils, %	0.38±0.03	0.29±0.03*
RBC x 10^{12} L^{-1}	7.56±0.20	7.82±0.11
Hematocrit	49.12±0.51	48.50±0.75
Hemoglobin, g L^{-1}	168.00±1.87	165.90±1.41
Medium breeds, n=3		
WBC x 10^9 L^{-1}	14.82±0.85	14.30±1.14
Neutrophils, %	61.55±1.11	60.55±1.09
Lymphocytes, %	22.69±0.95	23.31±0.78
Monocytes, %	3.26±0.03	3.66±0.12*
Eosinophils, %	11.84±0.26	11.87±0.34
Basophils, %	0.35±0.04	0.32±0.03
RBC x 10^{12} L^{-1}	7.22±0.11	7.92±0.13**
Hematocrit	53.49±2.55	53.76±1.99
Hemoglobin, g L^{-1}	167.88±2.58	165.55±2.91
Large breeds, n=6		
WBC x 10^9 L^{-1}	15.6±0.94	15.83±1.35
Neutrophils, %	61.18±0.57	60.64±0.59
Lymphocytes, %	23.89±0.58	23.99±0.56
Monocytes, %	3.34±0.08	3.79±0.13*
Eosinophils, %	10.98±0.31	11.78±0.14*
Basophils, %	0.38±0.01	0.36±0.02
RBC x 10^{12} L^{-1}	7.26±0.07	7.59±0.11*
Hematocrit	50.85±0.45	50.89±0.51
Hemoglobin, g L^{-1}	171.18±0.86	169.59±0.46

*$p<0.05$; **$p<0.01$

During the experimental period, morphological blood parameters of different breeds of puppies were established. In the experimental group of small breeds, basophils were by 0.09% ($p<0.05$) lower than in the control group. As to other parameters, there were no significant differences between the groups. In the experimental group of medium breeds of puppies, the RBC was by 0.7% ($p<0.01$) higher than in the control group. Similar result on RBC parameter was obtained in large breed experimental group: it was by 0.33% ($p<0.05$) higher than in the control group. In large breed puppies group monocytes were by 0.45% ($p<0.05$) higher than in the control group. Eosinophils in the blood of experimental puppies were by 0.8% ($p<0.05$) higher than in the control group (Table 4).

Discussion. To ensure good health of the domestic animals, good nutrition seems to be of primary importance. Properly formed ration guarantees normal functioning of the digestive system and suitable microflora. Dogs as all carnivorous species are characterized by considerably short digestive organs, but fast assimilation of nutritive substances. Among common diseases of small animal species pathology of the digestive system prevails. The main reason of these diseases among dogs is considered to be too large population of pathogenic bacteria, producing enterotoxins which provoke certain diseases. The deteriorated composition of microflora is called disbacteriosis or disbiosis (Šengaut, Januškevičius, 2010).

Recently, probiotic and prebiotic preparations have been widely used to treat diseases of the digestive system. Probiotic preparation is a viable microbial combination able to maintain balance of normal microflora in the digestive system of mammals. Probiotics occupy the mucous of the digestive system, here develop and propagate at the same time preventing attachment of pathogenic microorganisms. It should be pointed out, that positive microorganisms have to reach mucous of the digestive system avoiding acidic medium of the stomach. It is known that pH in the stomach of dogs fluctuates around 3.4 and of cats around 4.2. Exactly due to this reason it is not purposeful to give the dogs products consumed by humans. Therefore, new types of probiotic preparations are being created nowadays for dogs and experiments are carried out aimed at insertion of these preparations into the composition of certain food (Zink et al., 2005). During our investigation, the standard veterinary product probiotic preparation „Fermactiv" was used.

Stress and nutritional changes are the most common reasons of the diseases of digestive system. In these cases, application of probiotic preparations helps normalize intestinal microflora (Strompfova, 2004). New born puppies are very vulnerable. Also they can face a lot of stress when being introduced to new pet food after weaning.

The weaning period is especially important for the development of the immune system. During this period it is purposeful to supplement food with probiotic preparations to normalize the intestinal microflora (Lappin, 2007).

V. Biourge et al. (1998) carried out the investigation during which five adult dogs were given probiotic preparation *Bacillus* CIP 5832. The concentration of this product was 1.5×10^8 CFV/g. One particular individual was given 5 g of the preparation. The results of the experiment revealed that dry matter digestibility coefficient in the dogs, which received the probiotic, was 86.9±0.3 %, whereas in the control group it was 86.4±0.4%. The other values were respectively: protein 84.2±0.6% and 83.4±0.8% and fat 95.6±0.3% and 95.9±0.2% (Biourge et al., 1998).

During our investigation, probiotic preparation 1×10^5 CFV/g *Enterococcus faecium* was given to the puppies of small, medium and large breeds. The results of faeces investigation of these dogs when they were 60 day-old showed that in small breed dogs dry matter digestibility coefficient was 0.80% lower compared to control group, protein digestibility coefficient was 1.72% lower, and fat digestibility coefficient was 1.59% lower compared to the control group. Investigation of medium breed dog faeces, revealed that dry matter digestibility coefficient in 60 day-old dogs which received probiotic was 7.66% higher than in the control group, protein was 0.10% lower, and fat digestibility coefficient was 0.14% lower compared with the control group. The investigation of large dogs breed faeces showed dry matter digestibility coefficient to be by 1.34%, protein by 2.09%, and fat by 0.68% higher than in the control group. It should be noted, that better nutrient absorption was recorded among large breed puppies. Absorption of crude protein is especially important for the essential amino acids and crude fats for the vitamins.

When the puppies ration was supplemented with the positive bacteria *Enterococcus faecium* 5×10^8 cfu/d during the eighth week and on the 10-th, 18-th, 32-nd and 44-th weeks, the food consumption and weight gain differences between control and experimental groups were not found. However, while comparing IgA concentration in faeces, on the 44-th week its amount tended to be higher than in the control group. Comparison of the experimental and control groups revealed that IgG of blood plasma was not influenced by probiotic preparation as well. The amount of IgA in blood plasma started to differ considerably between the puppies of the control and experimental groups from the 18-th week of age; this amount in the experimental group was twice as higher as in the control group ($p<0.05$) (Benyacoub et al., 2003).

It was also defined that the immune response to the CDV vaccine defining specific IgA and IgG on the 31-st and 44-th week in the groups of puppies given *Enterococcus faecium* probiotic, was considerably stronger if compared with the control group ($p<0.05$) (Benyacoub et al., 2003).

Application of probiotic preparations is advisable when dogs are kept in big groups, in zoo-shops – places were risk to get sick with infectious diseases is very high (Weese, Anderson, 2002).

There are indications that probiotic preparations affect the blood biochemical composition. The puppies were

treated with a single dose of probiotic preparation "Bilavet" (4 ml/1 kg body weight) from 1 to 5 days of age and repeatedly as a single dose from 14 to 18 days of age (4 ml/1 kg body weight). A positive influence on blood biochemical parameters was observed. The content of blood proteins increased by 4.34% and the content of globulins by 7.68% respectively. The trial continued for 18 days and showed that the probiotic had a positive influence on blood composition. Furthermore, the mineral calcium in the blood of dogs increased by 14.43 %, inorganic phosphorus by 21.60%, and magnesium by 12.73% compared to the controls (Šengaut, Januškevičius, 2010). The results of chemical and morphological parameters in our investigation showed that total proteins in the experimental small breed puppies were higher by 3.34%, in medium breed puppies by 2.33%, and in large breed puppies by1.01% than in the controls groups of puppies. As to the level of calcium in blood during our investigation, in all breeds of puppies it was a little higher than in the control group. Some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts and therefore affecting the metabolism of cholesterol or by directly assimilating cholesterol (Salma et al., 2007).

Conclusions. The results of the investigations allow concluding that application of probiotic preparation in puppies positively affected daily weight gain and nutritive substances assimilation:

- the daily weight gain in small breeds of puppies during 60 days after the birth was 10.7%, medium breeds – 2.1%, large breeds – 0.8%; puppies developed faster, gained the required body weight and were easier weaned compared to the control group;
- under the effect of probiotic preparation digestibility coefficients of certain nutritive substances increased: puppies of small breeds in certain periods of life were able to assimilate fat by 0.26% better, fibre by 3.47% better, and organic matter by 3.43%; puppies of medium breeds were able to assimilate crude fat by 0.14% better, crude fibre by 1.72% better and crude ash by 2.53% better; the values for puppies of large breeds were: increase of digestibility coefficient by 1.34% ($p<0.001$) for dry matter, 2.09% ($p<0.05$) for crude protein, 0.68% ($p<0.001$) for crude fat, 8.82% ($p<0.001$) crude crude fiber and 10.06% ($p<0.001$) for crude ash. Better assimilation of nutritive substances promotes puppies growth and better utilization of certain substances – vitamins soluble in fat and minerals;
- probiotic preparation affects the blood biochemical composition – increases mineral calcium, total protein and decreases glucose and cholesterol levels in all breeds types of puppies.

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