EFFECT OF PREBIOTIC PREPARATIONS ON THE ASSIMILATION OF NUTRIENTS IN DIFFERENT BREEDS OF PUPPIES

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Abstract. The aim of this study is to define the effect of prebiotic preparation containing fructooligosaccharides and mannanoligosaccharides on the assimilation of nutrients, healthiness and eco-friendliness in different breeds of puppies. Three different types of breeds – small, medium and large – were selected for the experiment. Puppies in the experimental group were preventively given a supplement of fructooligosaccharides and mannanoligosaccharides. Duration of the clinical observation was 60 days, divided into two periods – up to 30 and from 30 days old of puppies. Prebiotic preparation influenced the assimilation of different nutrients. The coefficient of crude protein digestibility in the experimental group of small breed puppies was by 1.30%, in puppies of medium breed by 2.29%, and in puppies of large breed by 0.15% higher in the first period. In the second period, the coefficient of crude protein digestibility in the experimental group of small breed puppies was by 0.89%, in medium breed puppies by 3.13% ($p<0.01$), and in large breed puppies by 1.84% higher. The coefficient of crude fibre digestibility was determined to puppies from 30 days old. In the experimental group of small breed puppies the coefficient of digestibility was by 0.79%, in medium breed puppies by 0.36% and in large breed puppies by 0.33% higher. The prebiotic preparations had a positive effect on the crude ash digestibility only in the experimental group of small breed puppies up to 30 days old – by 18.26% higher ($p<0.001$) – and in the experimental group of medium breed puppies from 30 days old – by 14.96% higher ($p<0.001$). The puppies, which received the prebiotic preparation, defecated easier, their faeces consistency was harder and the amount of faeces was smaller.

Keywords: puppies, fructooligosaccharides, mannanoligosaccharides, nutrients, digestibility.

Introduction. Prebiotics are substances that are neither absorbed non digested in the body – they are used to stimulate the growth of beneficial intestinal microorganisms. Feeding prebiotics to pets improves bowel habits and fermentation, resulting in improved faeces quality, measured in terms of consistency and odour (Van Loo, 2007). Non-digestible inulin-type fructans are widely found in many vegetable feed and food ingredients and are perhaps the best studied and documented prebiotics in domesticated animals (Verdonk et al., 2005; Sharma et al., 2011). Companion animals suffer from obesity, cardiovascular diseases, and cancer. Feeding inulin or oligofructose to hyperlipidemic dogs decreased the levels of circulating cholesterol and triglyceride, which is a risk factor of cardiovascular diseases (Van Loo, 2007). Prebiotics beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon (Hesta et al., 2003). Newly born puppies are particularly susceptible to infectious diseases, as well as sensitive to the bitch's milk or changes of pet food. Therefore, the early use of prebiotic products is one of the ways to strengthen puppies organism..

Prebiotic preparations can modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota. In 1990, one study reported that feeding lactosucrose, a prebiotic, to dogs and cats respectively increased bifidobacteria while decreasing pathogenic organisms (*Clostridium perfringens*) (Hussein et al., 1999).

The European Union has banned all in-feed use of antibiotics from 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. This perspective has stimulated nutritionists and feed manufacturers to search for new, safer alternatives. The primary alternatives studied include acidification of the feed by organic acids, feeding probiotic organisms and feeding prebiotic compounds (Verdonk et al., 2005).

It is important to establish accurate relationships between dietary ingredients (especially protein) and the microbial ecology of the colon of dog and cat. Such relationships should provide guidelines for the formulation of diets that contain optimal levels and sources of proteins to meet amino acid requirements and contain prebiotics that support the health of dogs and cats (Hussein et al., 1999).

During the experiment, a commercial preparation containing fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) was used. The term "FOS" may include all indigestible oligosaccharides composed of fructose and glucose units. Specifically, FOS refers to short chains of fructose units bound by – (2–1) linkages attached to a terminal glucose unit. Supplementation of FOS has been shown to enhance gut health in many ways. MOS are moderately fermentable and serve as a substrate for lactic acid bacteria. But most often their role in pathogen resistance and modulation of the immune system is the object of studies (Bolduan, 1999, Sharma et al., 2011).

The aim of this study is to define the effect of prebiotic preparation containing FOS and MOS on the assimilation of nutrients, healthiness and eco-friendliness in different breeds of puppies.

Materials and methods. Animals, diets, treatments: the experiments were carried out in dog breeding centres

and in the laboratory of Food Investigation in the Department of Animal Nutrition of Lithuanian University of Health Sciences in 2011. Puppies of three different breeds – small (Yorkshire terrier), medium (Beagle) and large (German Shepherd) – were selected for the experiment, taking into consideration their weight, clinical status, physiological condition, stage of development. The study included six small-breed puppies (two females and four males), six medium-sized puppies (three males and three females) and ten large-breed puppies (four females and six males). Bitches and puppies were kept in specially adapted and isolated from other animals areas with washable and easily disinfected walls and floors. Small and medium breed puppies up to 60 days of life had no direct contact with the external environment, while from the 30th day of life large breed puppies and the bitch spent several hours per day in the field of tailored aviaries.

The research was carried out in two stages – from 14 to 30 days and from 30 to 60 days of age. The uptake of nutrients was examined during the period when the dietary source mostly consisted of the bitch's milk, and the period when puppies were introduced to the commercial pet food. Puppies were divided into two analogous groups, containing three small and medium breed puppies and five large breed puppies.

The puppies were given bitch's milk and from one month of age in addition were fed commercial dry food. Main ingredients of pet food were corn, chicken meat, animal fat, hydrolysed proteins, beet pulp, fish oils (Table 1). This pet food was given to the bitches as well.

Table 1. **Analytical composition of pet food**

Analytical composition, %	Mini/medium breeds	Large breeds
Crude ash	6.30	4.20
Crude protein	27.80	18.40
Crude fat	19.60	14.50
Crude fibre	2.20	3.50
Dry substance	92.5	94.8

The puppies in the experimental group were preventively given a FOS/MOS supplement with the ratio of 10 g for 10 kg of body weight from the 2nd to the 5th day of age. Duration of the clinical observation was 60 days.

Sample analysis: after each feeding, the faeces of puppies were collected into sterile disposable cups – in each stage at least three times. The faeces were daily delivered to the laboratory. Analysis of faeces and food was carried out according to generally accepted methods. Crude protein was determined by Kjeldahl method in operation of concentrated sulphur acid, crude fat according to its solubility in organic solvents, crude fibre by Kiurshner method, exposing the test substance to nitric and acetic acids, crude ash by burning dry process, and dry matter by deducting the general faecal moisture and organic matter in the faeces. By knowing what food the puppies received and chemical composition of bitch's milk and feed quantities, the coefficients of nutrient digestibility were calculated (Januškevičius, Januškevičienė, 2010).

Statistical results – arithmetic mean, error of the arithmetic mean, criteria and degree of reliability were defined according to Stjudent; we used statistical package (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. For the first 30 days of life dogs mainly received mother's milk. Meanwhile, from the 30th day of age, they were increasingly introduced to the commercial pet food, until the 60th day, when they were fully weaned. During the experimen,t the assimilation of nutrients in three different types of breeds was studied. At the beginning of the research, i.e. from the 2nd to the 5th day, the puppies were given prebiotic preparation containing FOS and MOS with the ratio of 10 g for 10 kg of body weight.

Analysis of faeces on the up to 30th day of age in the experimental group of small-breed puppies demonstrated that the coefficient of dry matter digestibility was by 2.48% higher than in the control group, and from the 30th day it was by 1.68% higher. Up to the 30th day, the coefficient of crude protein digestibility in the experimental group was by 1.30% higher, and from the 30th day by 0.89% higher than in the control group. The coefficient of crude fat digestibility up to the 30th day in the experimental group was by 0.62% lower, and from the 30th day by 0.21% lower than in the control group. From the 30th day, the digestibility of crude fibre in the experimental group was by 0.79% higher than in the control group. Up to the 30th day, the digestibility of crude ash in the experimental group was by 18.26% higher than in the control group, but from the 30th day the experimental group digested by 14.09% less crude ash than the control group. The coefficient of digestibility of organic matter in the experimental group of puppies was by 1.45% higher up to the 30th day, and from the 30th day, it was by 2.82% higher than in the control group (Table 2).

Analysis of faeces up to the 30th day of age in the experimental group of medium-breed puppies demonstrated that the coefficient of dry matter digestibility was by 2.22% higher than in the control group, and from the 30th day, it was by 6.27% lower. Up to the 30th day, the coefficient of crude protein digestibility in the experimental group was by 2.29% higher, and from the 30th day, it was by 3.13% higher than in the control group. The coefficient of crude fat digestibility in the experimental group was by 0.08% higher than in the control group, but from the 30th day, it was by 0.20% lower. From the 30th day, the digestibility of crude fibre in the experimental group was by 0.36% higher than in the control group. Up to the 30th day, the digestibility of crude ash in the experimental group was by 16.20% lower, and from the 30th day, it was by 14.96% higher than in the control group. Up to the 30th day, the

coefficient of organic matter digestibility in the experimental group of puppies was by 3.39% higher than in the control group, but from the 30th day, the experimental group digested by 4.17% less organic matter than the control group (Table 3).

Analysis of faeces up the 30th day of age in the experimental group of large-breed puppies demonstrated that the coefficient of dry matter digestibility was by 0.27% lower than in the control group, and from the 60th day, it was by 2.57% lower. Up to the 30th day, the coefficient of crude protein digestibility in the experimental group was by 0.15% higher, and from the 30th day, it was by 1.84% higher than in the control group. The coefficient of crude fat digestibility in the experimental group was by 0.26% higher than in the control group, but from the 30th day, it was by 0.16% lower. From the 30th day, the crude fibre digestibility in the experimental group was by 0.33% higher than in the control group. Up to the 30th day, the crude ash digestibility in the experimental group was by 6.23% lower, and from the 30th day, it was by 12.58% lower than in the control group. Up to the 30th day, the coefficient of organic matter digestibility in the experimental group of puppies was by 0.24% lower, and from the 30th day, it was by 1.78% lower than in the control group (Table 4).

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

Parameters	up to 30 day-old puppies		from 30 day-old puppies	
	Control group, n=3	Experimental group, n=3	Control group, n=3	Experimental group, n=3
Dry substance	93.36±3.42	95.84±3.12	90.06±16.50	91.74±9.19
Crude protein	97.51±0.39	98.81±0.99	96.76±1.68	97.65±1.05
Crude fat	99.43±0.49	98.81±0.99	98.79±0.16	98.58±0.13
Crude fibre	-	-	8.84±0.91	9.63±1.91
Crude ash	76.23±0.94	94.49±0.61***	56.85±1.79	42.76±5.29
Organic matter	94.57±2.70	96.02±4.85	92.58±17.76	95.40±3.90

***p<0.001

Table 3. **Level of nutritive substances assimilation in medium breeds of puppies** (%)

Parameters	up to 30 day-old puppies		from 30 day-old puppies	
	Control group, n=3	Experimental group, n=3	Control group, n=3	Experimental group, n=3
Dry substance	89.28±9.77	91.50±7.87	87.53±4.49	81.26±7.56
Crude protein	94.98±1.50	97.27±1.57	95.88±0.44	99.01±0.62**
Crude fat	98.78±0.07	98.86±0.26	98.91±0.10	98.71±0.14
Crude fibre	-	-	7.43±0.31	7.79±1.06
Crude ash	78.58±0.45	62.38±2.30***	18.63±2.82	33.59±0.88***
Organic matter	90.37±10.09	93.76±5.57	92.61±3.36	88.44±2.03

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

Table 4. **Level of nutritive substances assimilation in large breeds of puppies** (%)

Parameters	up to 30 day-old puppies		from 30 day-old puppies	
	Control group, n=5	Experimental group, n=5	Control group, n=5	Experimental group, n=5
Dry substance	87.11±2.53	86.84±4.64	90.87±3.14	88.30±2.19
Crude protein	92.62±0.27	92.77±0.55	92.05±0.65	93.89±0.52
Crude fat	98.78±0.06	99.04±0.07	98.99±0.08	98.83±0.90
Crude fibre	-	-	17.28±1.03	17.61±0.82
Crude ash	63.85±0.24	57.62±7.31	77.99±0.58	65.41±0.54***
Organic matter	89.51±2.42	89.27±4.02	91.96±2.75	90.18±1.95

***p<0.001

Discussion. There are a lot of studies used to determine the influence of prebiotics on adult dogs – nutrient digestibility and levels of faecal excretion. However, there is no information about the potential use of prebiotics for puppies. Weaning is a stressful time for puppies due to changes in diet and environment. At that

time they are more susceptible to infection – for example, *Salmonella* spp. C. J. Apanavicius et al. (2007) established, that fructan supplementation, including FOS and inulin, may have beneficial effects on the immune response to bacterial challenge in weanling puppies.

Dietary fibre sources for pet food are often grains, fruits and vegetables, celluloses, gums, and other sources. Beet pulp is commonly used as a fibre source in high-quality dog diets (Guevara et al., 2007, Propst, 2003). Low-quality pet food for dogs often is used in breeding centres. It would be useful to enrich such food with prebiotic products. It is especially important to maintain required cleanliness and hygiene in breeding centres. During the investigation, we noticed that dogs which received the prebiotic preparation defecated easier, their faeces consistency was harder and the amount of faeces was smaller.

J. Zentek et al. (2001) used four dogs to determine the effects of MOS, transgalactosylated oligosaccharides, lactose, and lactulose on faecal characteristics, total tract digestibility, and concentrations of microbial end products in faeces and urine. Conclusions indicated that mannanoligosaccharide supplementation decreased faecal dry matter, faecal pH, faecal ammonia excretion, and apparent dry matter, organic matter, crude protein, and N-free extract digestibilities. By decreasing faecal pH and ammonia, MOS supplementation appeared to improve indices of colonic health (Zentek et al., 2001).

There are proofs that fructans also effectively improve mineral assimilation in rats (Binder, Mehta, 1989). We can assume that it would be useful to give prebiotic products to growing puppies for better calcium assimilation. Our study showed that small breed puppies only up to 30 days and medium breed puppies only from 30 days in the experimental group absorbed crude ash better. Meanwhile, large breed puppies of experimental group in both study periods absorbed crud ash worse.

There are data about connection between dog's age, body size and digestibility. Four breeds of dogs were used. Digestive trials were performed at 11, 21, 35 and 60 weeks of age. Faecal moisture and scoring were recorded at the same periods. Digestibility coefficients of all macronutrients increased significantly with age in all four breeds. Organic matter digestibility increased from 80.2±1.5 to 85.5±1.5% in small breeds; from 80.2±0.9 to 86.3±0.5% in medium breeds; from 79.6±4.2 to 88.9±0.5% in large breeds. Crude protein digestibility increased from 74.5±4.0 to 81.5±1.9% in small breeds; from 74.3±3.9 to 82.6±0.9% in medium breeds; from 71.8±6.0 to 85.9±0.9% in large breeds (Weber et al., 2003). These results show an effect of age and body size on nutrients digestibility, so we can explain why our results are so different depending on age and type of breed.

K. S. Swanson et al. (2002) conducted a study of adult dogs of 40 different breeds. In their study prebiotic preparation was given to 40 dogs and the nutrient uptake was analysed. It was found that dogs which received food enriched with prebiotics, digested dry matter better (1.50–2.30%) than the control group, while the organic matter absorption was by 1.10% lower in comparison with the control group, and crude protein was absorbed 1.80–2.00% less compared with the control group. In our study, small breed puppies in different periods absorbed by 1.30–0.89%, medium breed puppies by 2.29–3.13%, and large breed puppies by 0.15–1.84% more crude protein compared with the control group. Better assimilation of dry matter was determined in all periods of small breed puppies but in medium and large breed puppies only in the first period – up to 30 days of age. Our study shows, that small breed puppies absorbed organic matter better in all periods and medium breed puppies only in the period up to 30 days of age. Meanwhile, the prebiotic preparation had worse effect of organic matter digestibility in large breed puppies.

So it is appropriate to add prebiotic products to the diet of dogs, because there are many approved tests, which demonstrate positive effects of prebiotics on the immune system of puppies, as well as a preventative measure against specific pathogens. But age and breed of puppies should be taken into consideration. Hygiene and cleanliness is especially important in breeding centres. Pprebiotics reduce the amount of faeces and normalize their consistency.

Conclussions. After performing studies with puppies we can confirm that prebiotic preparations containing FOS and MOS stimulate better absorption of different nutrients:

- the coefficient of crude protein digestibility in the experimental group of all breeds of puppies was significantly higher;
- the coefficient of crude fibre digestibility was higher in the experimental group of all breeds of puppies;
- the coefficient of crude ash digestibility was higher in the experimental group of small breed puppies up to 30 days old and in medium breed puppies from 30 days old;
- the coefficient of organic matter digestibility was higher in the experimental group of small breed puppies up to 30 and from 30 days old periods, and in medium breed puppies only up to 30 days old.

It is very important that the experimental group of puppies better assimilated crude protein, which is one of the most important nutrients to ensure the growth and health of puppies. During the investigation we noticed that dogs, which received the prebiotic preparation, defecated easier, their faeces consistency was harder and the amount of faeces was smaller.

References

1. Apanavicius C. J., Powell K. L., Vester B. M., Karr – Lilienthal L. K., Pope L. L., Fastinger N. D., Wallig M. A., Tappenden K. A., Swanson K. S. Fructan supplementation and infection affect food intake, fever, and epithelial sloughing from *Salmonella challeng*e in weanling puppies. Journal of Nutrition. 2007. 137. P. 1923–1930.

2. Binder H. J., Mehta P. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterology. 1989. 96. P. 989–996.

3. Bolduan G. Feeding weaner pigs without infeed antibiotics. Biotechnology in the Feed Industry. Nottingham University Press. 1999. P. 223–230.

4. Guevara M. A., Bauer L. L., Abbas C. A., Beery K. E., Franklin M. A., Cecavaand M. J., Fahey G. C. Chemical composition, *in vitro* fermentation characteristics, and *in vivo* digestibility responses by dogs to select corn fibers. Journal of Agricultural and Food Chemistry. 2008. 56. P. 1619–1626.

5. Hesta M., Roosen W., Janssens G. P. J., Millet S., Wilde R. Prebiotics affect nutrint digestibility but not faecal ammonia in dogs fed increased dietary protein levels. British Journal of Nutrition. 2003. 90. P. 1007–1014.

6. Hussein S. H., Flickinger E. A., Fahey G. C. Petfood applications of inulin and oligofructose. Journal of Nutrition. 1999. 129. P. 1454–1456.

7. Januškevičius A, Januškevičienė G. Augalinių ir gyvūninių pašarų bei produktų tyrimo metodai. Kaunas, Terra Publica. 2010. 170 p.

8. Propst E. L., Flickinger E. A., Bauer L. L., Merchen N. R., Fahey G. C. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrint digestibility, stool quality, and faecal protein catabolites in healthy adult dogs. Journal of Animal Science. 2003. 81. P. 3057–3066.

9. Van Loo J. How chicory fructans contribute to zootechnical performance and well-being in livestock and companion animals. Journal of Nutrition. 2007. 137. P. 2594–2597.

10. Verdonk J. M., Shim S. B., Van Leeuwen P., Verstegen M. W. A. Application of inulin-type fructans in animal feed and pet food. British Journal of Nutrition. 2005. 93. P. 125–138.

11. Sakalauskas V. Statistika su statistika. Statistinė programa statistika for Windows. Vilnius. Margi raštai. 1998. P. 44–59.

12. Sharma A. K., Agarwall V., Kumar R., Chaurasia H., Chaurasia D., Bhardwaj P. Prebiotics: a review of therapeutic potential. International Journal of Pharmaceutical Invention. 2011. 1. P. 28–40.

13. Swanson K. S., Grieshop Ch. M., Flickinger E. A., Bauer L. L, Chow J., Wolf B. W., Garleb K. A., George C. F. G. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. Journal of Nutrition. 2002. 132. P. 3721–3731.

14. Weber M., Martin L., Biourge V., Nguyen P., Dumon H. Influence of age and body size on the digestibility of a dry expanded diet in dogs. Journal of Animal Physiology and Animal Nutrition. 2003. 87. P. 21–31.

15. Zentek J., Marquart B., Pietrzak T. Investigations on intestinal effects of mannoseoligosaccharides, transgalactosylated oligosaccharides, lactose, and lactulose in dogs. The Waltham International Symposium. 2001. P. 43.

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