USE OF PREBIOTIC MANNANOLIGOSACCHARIDE FOR FEEDING ADULT GERMAN SHEPHERD BITCHES

Algirdas Januškevičius[1], Gražina Januškevičienė[2], Donatas Čepaitis[1], Paulius Gabinaitis[1], Jūratė Kučinskienė[2]
[1]*Department of Animal Nutrition, Veterinary Academy, Lithuanian University of Health Sciences*
Tilžės g. 18, LT-47181 Kaunas, Lithuania. Tel. +37037363408, E-mail: jalgis@lva.lt
[2]*Department of Food Safety and Animal Hygiene, Veterinary Academy, Lithuanian University of Health Sciences*
Tilžės g. 18, LT-47181 Kaunas, Lithuania

Abstract. Complete dry pet food with 0.1% content of prebiotics mannanoligosaccharides (MOS) was made in Lithuania. This pet food, called "Araton" has been used for the adult German Shepherd dog breed feeding. This food was used to feed bitches at rest, preparing to mate, mating and during gestation and lactation periods. MOS affected females stool consistency in shape and smell what is important from the ecological point of view. Under the effect of prebiotic, faeces of females in the test group contained 36.91 % of dry matter or by 2.15 % ($p<0.05$) more compared with controls. Assimilation of dry material by females in the test group was 76.41 %, or by 2.22 % ($p<0.001$) better than in the control group, organic material 83.41 % or by 1.29 % ($p<0.001$) better. Assimilation of green ash was by 0.80 %, crude protein 1.98 % ($p<0.05$), crude fat 0.15 %, crude fibre 0.53 % ($p<0.05$), non-nitrogenous extractive material 0.14 % better. The blood of the experimental females showed the general trend of inrease of the content of proteins. In comparison with the control group, the content of common proteins in the blood of the test group was by 1.97 g L^{-1} ($p<0.05$) higher; the level of glucose in the blood two months after the beginning of the test was 5.94 mmol L^{-1} or by 4.04% lower in the experimental group compared with glucose levels in the blood from control group. At the end of the test, before weaning, the observed cholesterol levels in the blood of female dogs fed with prebiotics supplement was 4.43 mmol L^{-1} or by 20.54% ($p<0.01$) lower compared with the results of the control group (period of lactation).

Keywords: prebiotic, dog, blood, digestibility, stool, dry food.

PREBIOTIKAS MANANOOLIGOSACHARIDAS VOKIEČIŲ AVIGANIŲ VEISLĖS SUAUGUSIŲ KALIŲ ĖDALE

Algirdas Januškevičius[1], Gražina Januškevičienė[2], Donatas Čepaitis[1], Paulius Gabinaitis[1], Jūratė Kučinskienė[2]
[1]*Gyvūnų mitybos katedra, Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas*
Tilžės g. 18, LT-47181 Kaunas; tel. (8~37) 36 34 08; el. paštas: jalgis@lva.lt
[2]*Maisto saugos ir gyvūnų higienos katedra, Veterinarijos akademija, LSMU*
Tilžės g. 18, LT-47181 Kaunas

Santrauka. Lietuvoje pagamintas sausas visavertis ėdalas su 0,1 proc. mananooligosacharidų (MOS). Visaverčiu sausu ėdalu „Araton" buvo šeriamos suaugusios vokiečių aviganių veislės kalės ramybės būklės, ruošiant kergti, kergimo laikotarpiu bei vaikingumo ir laktacijos metu. MOS turėjo įtakos kalių išmatų konsistencijai ir formai bei kvapui, o tai labai svarbu ekologiniu požiūriu. Dėl prebiotiko poveikio tiriamosios grupės kalių išmatose nustatyta 36,91 proc. sausosios medžiagos, arba 2,15 proc. ($p<0,05$) daugiau palyginti su kontrole. Sausoji medžiaga tiriamosios grupės kalių buvo įsisavinama 76,41 proc., arba 2,22 proc. ($p<0,001$) geriau, organinė medžiaga – 83,41 proc., arba 1,29 proc. ($p<0,001$) geriau, žali pelenai – 0,80 proc., žali baltymai – 1,98 proc. ($p<0,05$), žali riebalai – 0,15 proc., žalia ląsteliena – 0,53 proc. ($p<0,05$), neazotinės ekstraktinės medžiagos – 0,14 proc. geriau. Tiriamosios grupės kalių kraujo sudėtyje buvo stebima bendrųjų baltymų kiekio didėjimo tendencija; palyginti su kontroline grupe bendrųjų baltymų tiriamosios grupės kalių kraujyje buvo 1,97 g L^{-1} ($p<0,05$) daugiau. Ištyrus kraują praėjus 2 mėn. nuo bandymo pradžios, gliukozės kiekis buvo 5,94 mmol L^{-1}, arba 4,04 proc. mažiau palyginti su kontrolinės grupės kalių krauju; ištyrus kraują bandymo pabaigoje, prieš šuniukus atjunkant, nustatytas sumažėjęs cholesterolio kiekis kraujyje kalių, kurios gavo prebiotikų, – 4,43 mmol L^{-1}, arba 20,54 proc. ($p<0,01$) mažiau palyginti su kontroline grupe (laktacijos metu).

Raktažodžiai: prebiotikas, šuo, kraujas, virškinamumas, išmatos, sausas ėdalas.

Introduction. In recent years, we have become more and more aware of the importance of relationship between diet and health for both ourselves and our pets. Recent pet food accessories have made this relationship even more evident for pet caretakers. As a result, consumers are now looking for food sources for their pets that support health above and beyond providing basic nutrition. In this article, we are going to discuss beneficial food ingredients called prebiotics and probiotics that are present in natural foods or can be added to prepared foods. For a food ingredient to be classified as a prebiotic, it has to be demonstrated that it is: not broken in the stomach or absorbed in the gastrointestinal tract, fermented by the gastrointestinal microflora, selectively stimulates the growth and activity of intestinal bacteria associated with health and well-being.

Like most mammals, the large intestine of healthy dogs contains a complex microbial ecosystem in which streptococci, bifidobacteria, lactobacilli, *Bacteroides* and *Chlostridium* constitute the main predominant bacterial genera. The large number of bacteria (10^{10} per g dry faeces) contributes significantly to the colonic fermentation of complex carbohydrates and proteins leading to the production of beneficial short chain fatty acids and several toxic putrefactive components (ammonia, indoles, phenols), respectively. The relative ratio in which these components are produced is highly dependent on the bacterial balance and substrate availability in the colon (Gibson, Roberfroid, 1995).

Carbohydrates that are indigestible by mammalian enzymes can influence the composition and metabolic activity of the intestinal mikroflora and are therefore of interest for the formulation of pet food and specific veterinary diets (Zentek et al., 2002).

The vast majority of the prebiotic feeding studies involving dogs are based on the analysis of physiological effects, nutrient digestibility, stool quality and faecal concentrations of fermentative end products (Flickinger et al., 2003; Hesta et al., 2003; Propst et al., 2003). Several dose-response experiments conducted in dogs with oligofructose and inulin have shown beneficial effects towards an improved small intestine absorption capacity and colonic epithelial cell proliferation, whereas future trends in prebiotic dog feeding aim at the controlled decrease of faecal putrefactive components and at an improved nutrient digestibility (Flickinger et al., 2003).

Prebiotics are nondigestible or partially digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or more a limited number of bacteria in the colon, and thus improve host health (Gibson, Roberfroid, 1995; Gibson et al., 2004; Schrezenmeir, de Vrese, 2001). Individual prebiotics may stimulate the growth and activity of some indigenous probiotics but not others.

Prebiotics are efficiently used to increase stool frequency and to treat constipation (Den Hond et al., 2000; Kleeseen et al., 1997).

Prebiotics, such as the type of fibre known, can be used as a dietary supplement for dogs to maintain gastrointestinal health. Prebiotics used as a dietary supplement can help maintain a healthy gastrointestinal tract and aid in restoring the balance between beneficial bacteria and pathogenic bacteria within the gut (Swanson et al., 2002). The results of the study demonstrated that pathogenic bacteria were inactivated in the animals fed diets supplemented with mannanoligosaccharides. This prebiotic was effective in the control of pathogenic *Escherichia coli*.

Flickinger, Wolf, Garleb (2000) indicated that glucooligosaccharides resisted hydrolytic digestion in dogs and were fermented in the large intestine. Swanson et al. (2002a); Gibson, Roberfroid (1995) introduced the concept of prebiotics, which alter the microbial populations of the gut, and consequently, improve the health of the host.

By definition, prebiotics are considered to selectively stimulate growth and activity of potentially health-enhancing intestinal bacteria (Roberfroid et al., 1998). However, at this stage, there are no indications to claim that a potential stimulation of *S. Lutetiensis* by fructan administration exerts positive or negative effects on the health of the dogs. Clearly, additional research is needed to unravel the ecology of this organism in the canine gut and to investigate its role in the gut of healthy dogs. Mannanoligosaccharides resulted in a lower faecal pH, ammonia excretion and apparent digestibilities of crude protein, nitrogen-free extracts and dry matter, compared to those of the control periods and the other carbohydrates. The percentage of total faecal water increased and the unbound water decreased substantially during this period, which can be interpreted as a change in the physical properties of the intestinal chyme. This higher water binding could have influenced the solubility of nutrients, which might explain the lower digestibility and also the activity of the intestinal microflora.

The **aim of the study is** to analyze the effect of prebiotic mannanoligosaccharide (MOS), enriched with yeast, on nutrient absorption level of the adult German Shepherd dog breed and to monitor their health according to some haematological parameters.

Research methods. In 2010, the research was carried out in a kennel of German shepherd dogs. The dogs were selected based on the principle of analogues with respect to their weight, health condition, development and gender (Januškevičius, 1992). The dogs were fed a complete dry food which contained mannanoligosaccharides supplemented with yeast. Examinations of food and faeces were performed based on accepted methodology (Januškevičius, Januškevičienė, 2010).

The blood for the first time was drawn in two months after the beginning of the research (in calm state) and for the second time when puppies were weaned from the bitches.

Biochemical blood indicators were determined with an automatic biochemical analyser "DIALAB Autolyzer 20010D" (USA); the morphological blood test was performed by MELET SCHLOISING LABORATORIES analyzer (French). The blood for testing was taken from *venus safena* – for morphological testing in test-tube EDTA, for chemical testing in test-tube free of preservatives.

Statistical results – arithmetical average, error of arithmetical average, reliability criteria and degree of reliability – were determined according to Student and a statistical programme was used (Sakalauskas, 1998).

The selected dogs were divided into two analogous groups, each with 3 female dogs. The keeping conditions were identical. Every female dog was kept in clean aviaries, protected from draughts and side impact of the environment.

The food for female dogs contained poultry flour, corn, wheat, poultry lard, sunflower oil, corn gluten, sugar beet pulp, egg powder, minerals, vitamin supplement, antioxidants and preservatives. Part of minerals from dry food for dogs from research group was replaced with 0.1% prebiotics MOS supplemented with yeast.

The female dogs were fed with specially made complete food "Araton" in calm state, when preparing for mating, after mating, during pregnancy and during lactation period.

Levels of analytic ingredients of dry food for both groups were identical: humidity – 10%, crude proteins – 26%, crude fats – 14%, crude fibre – 3%, crude ash – 5.5%. The energetic value of the complete dry food was 19.65 MJ kg^{-1}.

Chemical composition of (*Agrimos*) MOS was: humidity – 3.3%, crude proteins – 18.3%, B-glucanes[1] – 30.2%, manans[2] – 26.3%, pH (2% solution) – 6.0.

After feeding the female dogs with complete dry food with prebiotics for three months a digestion test was performed; for this, a precise account of consumed food and excreted faeces was conducted. The digestibility test was performed without preparatory period, because we used the same complete dry food for feeding and monitored that there were no food residues (from feeding of bitches).

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).

Research results. One of the most important aims of keeping dogs is to maintain normal dog's health condition, allow good mating, and create conditions for a normal litter as well as health and further growth pace of puppies born. We weighted the female dogs before and after digestibility research. There were no big differences between weights because during that period the female dogs were in a calm state. Female dogs ate 980 g of dry food per day and excreted 830 g of faeces.

Table 1. **Chemical content of female dog's faeces**

Indicators, %	Control group n=3	Experimental group n=3
Dry substance	34.76±0.46	36.91±0.54*
Organic substance	25.00±0.28	27.02±0.42***
Crude ash	9.76±0.16	9.89±0.22
Crude protein	7.52±0.17	8.62±0.18***
Crude fats	0.84±0.03	0.92±0.07
Crude fibre	5.59±0.08	4.76±0.09***
Non-nitrogenous extractive substances	12.72±0.19	11.05±0.28***

*p<0.05; ***p<0.001

Even before establishing the chemical content it was noticed that faeces of female dogs from the test group were dryer, lighter and smelled less. There were 36.91% of dry substance in the faeces of female dogs from research group or by 2.15% (p<0.05) more compared with the control group. The tendency of larger quantities of all substances in the faeces of female dogs from the test group compared with the faeces of female dogs from the control group is further noticeable. There were 27.02% of organic substance or by 2.02 % (p<0.001) more, 8.62% of crude protein or 1.10% (p<0.001) more, 0.92% of crude fats or 0.08% more, 4.76% of crude fibre or 0.83% less (p<0.001), 11.05% of non-nitrogenous extractive substances or 1.67% (p<0.001) less compared with the control group.

Table 2. **The level of assimilating nutrients**

Indicators, %	Control group n=3	Experimental group n=3
Dry substance	74.19±0.16	76.41±0.40***
Organic substance	82.12±0.11	83.41±0.19***
Crude ash	14.27±0.30	15.07±0.22
Crude protein	76.47±0.21	78.45±0.52*
Crude fats	99.35±0.35	99.50±0.15
Crude fibre	24.00±0.18	24.53±0.13*
Non-nitrogenous extractive substances	99.31±0.06	99.45±0.11

*p<0.05; ***p<0.001

The assimilation of nutrients by female dogs was approximately on the same level; therefore the error of arithmetical average is not large. In all cases, the digestibility of nutrients was higher in the bodies of female dogs from the experimental group. Female dogs from the experimental group digested dry substance to 76.41% or by 2.22% (p<0.001) better, organic substance 83.41% or by 1.29% (p<0.001) better; crude ashes in the testing group were assimilated to 0.80%, crude proteins 1.98%, crude fats 0.15%, crude fibre 0.53% (p<0.05) and non-nitrogenous extractive substances 0.24% better.

In order to assess the health status of bitches and positive or negative impacts of prebiotics, blood was drawn from the female dogs and examined. The blood for

the first time was drawn two months after the beginning of the research (in calm state) and for the second time when puppies were weaned from the bitches. The blood was drawn from three female dogs from control group and three female dogs from experimental group.

In order to assess the healthiness of female dogs, the main indicators were examined: total protein, albumin, globulin, glucose and cholesterol and blood morphological composition.

Table 3. **Biochemical parameters of female dog's blood**

Parameters	First time		Second time	
	Control group n=3	Experimental group n=3	Control group n=3	Experimental group n=3
Total protein, g L^{-1}	64.18±0.45	65.31±0.56	63.89±0.62	65.86±0.36*
Albumin, g L^{-1}	34.16±0.26	34.98±0.45	34.47±1.01	34.76±0.86
Globulin, g L^{-1}	26.21±0.26	27.38±0.24*	27.62±1.02	29.31±0.47
Glucose, mmol L^{-1}	6.18±0.43	5.94±0.37	5.97±0.16	5.59±0.28
Cholesterol, mmol L^{-1}	5.12±0.08	5.59±0.12*	5.34±0.24	4.43±0.08**

*p<0.05; **p<0.01

Total protein in the blood of female dogs from the control group slightly decreased before the time of mating. A tendency of total protein increase in the blood of female dogs from the experimental group was noticeable. The quantity of albumins and globulins was higher due to the impact of prebiotics, although the quantities of these blood protein components were also larger in the blood from the control group. After examining the blood for the first time, a large difference between glucose levels was noticeable – 5.94 mmol L^{-1} or by 4.04% lower in the experimental group compared with glucose levels in the blood from control group (calm state). After examining the blood for the first time, a slight increase of cholesterol levels in the blood of female dogs from the experimental group was noticed – 5.59 mmol L^{-1} or by 9.18 % higher (p<0.05) compared with the control group. After examining the blood for the second time, a decrease of cholesterol levels in the blood of female dogs from the experimental group was noticed – 4.43 mmol L^{-1} or by 20.54% (p<0.01) lower compared with the results of the control group (period of lactation).

In addition to biochemical parameters morphological analysis of blood (white blood cels (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cels (RBC), hematocrit, hemoglobin) also was performed.

Table 4. **Morphological parameters of female dog's blood**

Parameters	Control group n=3		Experimental group n=3	
	in the start	in the end	in the start	in the end
WBC x 10^9 L^{-1}	17.65±0.13	12.09±0.42	17.62±0.27	16.58±0.81***
Neutrophils, %	60.03±0.03	62.43±0.11	59.17±0.22***	60.04±0.11
Lymphocytes, %	23.93±0.12	22.74±0.11	24.15±0.09	24.06±0.11***
Monocytes, %	3.85±0.11	3.58±0.03	3.62±0.14	3.74±0.12
Eosinophils, %	11.83±0.21	10.87±0.21	12.71±0.12*	11.88±0.11**
Basophils, %	0.36±0.11	0.38±0.11	0.35±0.06	0.28±0.08
RBC x 10^{12} L^{-1}	7.29±0.16	7.47±0.19	7.38±0.27	7.81±0.13
Hematocrit	46.23±0.41	52.14±0.32	49.65±0.41***	51.12±0.36
Hemoglobin, g L^{-1}	164.86±31.06	172.71±22.70	167.63±18.85	169.03±24.03

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

In the beginning of the test, the WBC in control and investigated groups differed very little. At the end of the test, blood leukocyte reduction was observed in both groups. In the control group it was 12.09 x 10^9 L^{-1} or by 27.08% lower compared with the experimental group (p<0.001).

In both cases, lymphocyte counts were higher in the experimental group. In the beginning the test rate was 24.15%, or by 0.22 % higher, at the end – 24.06% or by 1.32 % higher (p<0.001). At the end of the test, an increase of neutrophil 60.04 % (p<0.01) was observed in the experimental group. More eosinophils in both cases were found in the blood of females from the experimental group (p<0.05), (p<0.01).

Discussion of research results. Prebiotics MOS added to the complete dry food for the feeding of female dogs at calm state, during preparation for mating as well as mating, pregnancy and lactation had a positive influence over the healthiness of female dogs and digestibility levels of nutrients. It also influenced the pace of puppies growth and healthiness, and this means that the activity of female dogs mammary glands was more

intense and they produced more milk that contains nutrients valuable for the growth and development of puppies. Other authors also agree that mannanoligosaccharides and transgalactosaccharides produce positive influence on dogs body (Zentek et al., 2002).

MOS were used for the first time in Lithuania to produce a final product – complete dry food for dogs. When fed with dry foods that contain the aforementioned prebiotics, dogs excrete less humid and smelly faeces (Swanson et al., 2002). Our observations are in agreement with the findings of many other authors who also have noticed the influence of prebiotics over the decrease of concentration of hydrogen ions and undesirable bacteria colonies in dogs faeces (Newman, Lyons, Jacques, 1994; Vickers et al., 2001). Authors also point out that the prevalence of pathogenic bacteria *E. coli* in digestive tract decreased in 85.71% of animals.

MOS decreases the formation of pathogenic bacteria colonies in individual sections of digestive tract, decrease the possibility to have cancer or a disease that is prevalent among dogs – gastroenteritis. Many authors share the same opinion (Gouveia et al, 2006).

The dog blood biochemical analysis showed that in dogs fed beef-based diets, blood cholesterol was greater ($p<0.05$) with the inclusion of MOS, but was lower ($p<0.05$) in dogs fed chicken-based diets including MOS (Beloshapka et al., 2010).

Swanson et al. (2002) reported that the dogs, supplemented with MOS, on all blood morphological treatments had RBC, haemoglobin, hematocrit, eosinophil, basophil and monocyte concentrations that fell within normal ranges for dog. The percentage of lymphocytes in the total WBC count in blood collected from dogs fed MOS was significantly elevated. Beloshapka et al. (2010) reported that WBC count was lower ($p<0.05$) in dogs fed diets containing MOS, but was unaffected by protein source.

Conclusions. The following conclusions can be drawn from the results received after feeding dogs with complete dry food "Araton" with MOS supplemented with yeast:

- 36.91% of dry substance or by 2.15% ($p<0.05$) more was detected in the faeces of female dogs from the experimental group compared with the control group;
- female dogs from the experimental group assimilate dry substance to 76.41% or by 2.22% ($p<0.001$) better, organic substance 83.41% or by 1.29% ($p<0.001$) better; crude ashes by 0.80%, crude protein 1.98% ($p<0.05$), crude fats 0.15%, crude fibre 0.53% ($p<0.05$) and non-nitrogenous extractive substances by 0.24% better;
- a tendency of total protein increase in the blood of female dogs from the experimental group was noticeable; compared with the control group, the total protein was by 1.97% ($p<0.05$) higher; 2 months after the beginning of the research, glucose levels in blood were 5,94 mmol L^{-1} or by 4.04% lower compared with blood glucose levels in the blood from the control group; after examining blood at the end of research before weaning the puppies, a notable decrease of cholesterol in the blood of female dogs who received prebiotics was detected – 4.43 g L^{-1} or by 20.54% ($p<0.01$) lower compared with the control group;
- in the beginning of the test, the WBC in the control and test groups differed very little; at the end of the test, the WBC in the control group was 12.09 x 10^9 L^{-1} or by 27.08% lower compared with the experimental group ($p<0.001$);
- in both cases, lymphocyte counts were higher in the experimental group; in the beginning the test rate was 24.15%, or by 0.22 % higher, at the end – 24.06% or by 1.32 % higher ($p<0.001$); at the end of the test, increase of neutrophils 60.04 % ($p<0.01$) was observed in the experimental group; more eosinophils in both cases was found in the blood of females from the experimental group ($p<0.05$), ($p<0.01$).

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