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THE INFLUENCE OF A PROBIOTIC PRODUCT WITH *BACILLUS SPP.* AND *PEDIOCOCCUS ACIDILACTICI* ON HEALTHY DOGS' DIGESTIBILITY

INFLUENȚA UNUI PRODUS PROBIOTIC CU *BACILLUS SPP.* ȘI *PEDIOCOCCUS ACIDILACTICI* ASUPRA DIGESTIBILITĂȚII LA CÂINI SĂNĂTOȘI

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ABSTRACT

The aim of the present study was to assess the influence of a spore probiotic product based on *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* on healthy dogs' digestibility. The probiotic was administered orally once a day to five healthy adult dogs from 1 year to 7 years old for a period of 30 days. Before and after the treatment, all the feces were collected for a period of five days in order to perform faecal digestibility. During the probiotic treatment, on day 1, day 7, day 15 and day 30 the dog owners filled the Optidigest sheet (by Purina) to assess the faecal score weekly. The parameters studied for the digestibility were: dry matter, crude protein, crude fat, crude fiber and nitrogen free extract. Comparing the results regarding the digestibility and faecal score, before and after the treatment, an increase in the digestion capacity was observed after the 30 days probiotic treatment, an observation supported by the obvious improvement of the faecal score. Those findings were observed for all the 5 dogs included in the study.

Keywords: *Bacillus subtilis; Pediococcus acidilactici*; probiotic; digestibility; canine

REZUMAT

Probioticele sunt formule alcătuite din diferite microorganisme, care, administrate în cantități potrivite produc un efect benefic organismului gazdă. Aceste efecte sunt resimțite cu precădere la nivelul tractului gastrointestinal. Scopul prezentului studiu a fost determinarea influenței unui produs probiotic bazat pe spori de *Bacillus subtilis, Bacillus licheniformis* și *Pediococcus acidilactici* asupra digestibilității la câini sănătoși. Probioticul a fost administrat oral o dată pe zi, timp de 30 de zile, la 5 câini adulți, sănătoși, cu vârste cuprinse între 1 an și 7 ani. Înainte și după tratament au fost colectate toate fecalele pentru o perioada de 5 zile pentru efectuarea digestibilității. Pe parcursul celor 30 de zile de tratament, în zilele 1, 7, 15 și 30 proprietarii au completat fișa pentru scorul fecalelor (după modelul Optidigest, conceput de Purina), cu scopul de a determina scorul fecalelor în fiecare săptămână. Parametrii studiați pentru digestibilitate au fost reprezentați de: substanța uscată, proteina, grăsimea, celuloza și substanțele extractive neazotate (SEN). Comparând rezultatele digestibilității precum și a scorului fecalelor obținute înainte de tratament cu cele post-tratament, o creștere a capacității de digestie a fost observată după 30 de zile, lucru completat de o îmbunătățire semnificativă a scorului fecalelor. Aceste observații au fost prezente la toți câinii incluși în studiu.

Cuvinte cheie: *Bacillus subtilis*; *Pediococcus acidilactici*; probiotic; digestiblitate; câini

Probiotics are described as live microorganisms (bacteria or yeast usually) that confer a health benefit on the host when they are consumed in adequate quantity. Probiotics can be integrated in various types of products such as feed/food, therapeutic drugs or feed supplements (1).

Probiotics exercise most of their action at the level of the gastro-intestinal tract. In 1999, in his book, the scientist Michael Gershon described intestine as a "second brain". In fact, the gastrointestinal tract is one of the most complex and important organs of the body, being, in fact, a considerable ecosystem while a trillion (10^{12}- 10^{14}) microorganisms compose the GI microbiota. This number represents about ten times more than the number of all host cells (9, 10). Moreover, probiotics can influence the health status of the host by the enhancement of intestinal barrier function, that is one of the properties of probiotics. That capacity helps in fighting pathogenic microorganisms. More than that, studies have shown the importance of probiotics in production of antimicrobial metabolites and

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their aggregation with pathogens to protect against infection or their beneficial role in cardiovascular diseases. Additional health benefits are carrying by probiotics such as production of vitamins (K vitamin, B2 & B12 vitamins) and oxalate-degradation preventing or solving kidney stones (15). Dogs' dietary behaviour had been drastically modified due to anthropomorphizing (18). Changes in food type and composition, along with feed additives comes with a lot of effects including an increased rate of periodontal disease (16) of even modifications at the level of genetic material of cells (3). Moreover, digestion problems and food allergies are more frequent and perhaps alterations in the normal structure of the gastrointestinal microbiota are present. That phenomena can lead to decreased digestibility of food components. This change may produce a disturbance at the level of gastrointestinal microbiome, and this fact can affect their digestion process. Also, nowadays, antibiotics are used in veterinary medicine at a large scale (13). In this context, probiotics can be used in order to re-establish the gastrointestinal tract and, in the end, to improve the digestion process. A recent in vivo study shows the beneficial effect of a multi-strain probiotic product with Lactobacillus spp. and Bifidobacterium spp. on improving canine feeding intake, gaining weight but also improved the gut microbiota and reinforced the canine immune system (19).

The variety of probiotics at the present moment is huge. Presentation form, composition (single microorganism or combined formulas) or type (what type of microorganisms) are just some classification criteria. When referred to the microorganisms' type, the probiotic market is divided in five categories: *Lactobacillus*, *Bifidobacterium*, spore formers, yeast and others (7). The World Gastroenterology Organization described the most used probiotics containing *Lactobacillus*, *Bifidobacterium* but also *Saccharomyces cerevisiae* yeast and few species of *Escherichia coli* and *Bacillus* (http://www.worldgastroenterology.org/guidelines/global-guidelines/probiotics-and-prebiotics).

Spore formers type of probiotics include *Bacillus subtilis* probiotics. This type of formulas, related to *Bacillus* species, emerged 15 years ago. *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus indicus* are the predominant species (4). Bacillus species are Gram-positive, rod shaped and spore-forming microorganisms. It is defined as an aerobic or facultative anaerobic bacterium (7). *Bacillus* is considered soil organisms but can be also found in air, water, vegetables, and food. Additional studies have shown the presence of *Bacillus* in insects and animals GIT (11). Some *Bacillus* strains present two forms: vegetative and endospore. Both forms have interests used in probiotics. They show different qualities and properties that could be used (7). Bacillus spore forming bacteria have the advantage to be more stable and can be store at ambient temperature without suffering degradation. Moreover, the entire dose of probiotic based ingested will reach the GIT and not be deteriorate before. This confers much more power to spore forming probiotics (4).

Due to the capacity to re-establish the microorganism order at the level of the GI tract, those probiotics may enhance the digestion capacity of the host. The aim of this study was to evaluate the influence of a probiotic product with *Bacillus subtilis* and *Pediococcus acidilactici* on digestibility on 5 healthy adult dogs.

MATERIALS AND METHODS

Study design

A total of 5 adults healthy dogs aged between 1 year and 7 years old entered in the study. The probiotic product composed of *Bacillus subtilis, B. licheniformis* and *Pediococcus acidilactici* was given to the dogs once a day with the meal during 30 days (Table 1). Starting with day - 5 until day 0 was realized the collection of faeces for digestibility. Between day 1 and day 30 the probiotic product was administrated to the animals, respecting the manufacturer recommendations. Between 31 and 36 days were collected faeces for digestibility.

Study population

A total number of 5 dogs (01 – K- Czechoslovakian wolfdog, 4 years, castrated male; 02 – H- Mixed dog breed, 1.8-year, female; 03 – L- Border collie, 3 years, female; 04 – N- Shetland shepherd, 1 year, female; 05 – D- Shetland shepherd,7 years, female) with all vac-

Table 1

Calendar of the activities

DAY/EXPERIMENTAL TIME		ACTIVITIES
day -5 ➔ day 0	Faecal samples collection	Tests digestibility - healthy dogs
DAY 1- DAY 30 ➔ PROBIOTIC ADMINISTRATION FAECAL SCORE ➔ Optidigest sheet ➔ day 1, day 7, day 15 and day 30		
day 31 ➔ day 36	Faecal samples collection	Tests digestibility - healthy dogs

Table 2

Faecal score evolution depending of the day (D) of the study (1, 7, 15, 30)

DOG	Stool consistency				Stool volume				Stool smell				Gut rumbling				Flatulence			
	D1	D7	D15	D30	D1	D7	D15	D30	D1	D7	D15	D30	D1	D7	D15	D30	D1	D7	D15	D30
01-K	4	3	2	2	2	2	2	1	2	2	2	1	-	-	-	-	-	-	-	-
02-H	4	3	3	3	3	2	2	2	3	3	3	3	-	-	-	-	-	-	-	-
03-L	6	5	5	5	2	2	2	2	3	3	3	3	-	-	-	-	+	+	-	-
04-N	5	5	5	5	2	2	2	2	3	3	3	3	-	-	-	-	-	-	-	-
05-D	5	5	4	4	2	2	2	2	2	2	3	3	-	-	-	-	-	-	-	-

cination and no specific external or internal treatment entered in the study. Inclusion criteria for the dogs were: the absence of gastro-intestinal manifestations (diarrhoea, vomiting), without antibiotic treatment in the last 6 months, clinically healthy, one meal/day. Exclusion criteria were: gastro-intestinal manifestations (diarrhoea, vomiting), antibiotic treatment in the last 6 months.

Treatment

The probiotic product tested is composed of two strains of bacteria: *Bacillus subtilis* HU58, *B. licheniformis SL307* and *Pediococcus acidilactici* (FidoSpore® supplied by Microbiome Labs, LLC). The probiotic product is presented as capsules. During 30 days one capsule per day was administered to each dog with the meal. Moreover, an addition of liver powder is used to improve the taste of the probiotic product and facilitates its ingestion.

Faecal score

During the probiotic's treatment, the dog's owners are asked to fulfil the Optidigest clinical sheet made by Purina (https://www.purina.ro/produse/proplan-optidigest). Stool consistency, stool volume and smell, gut rambling and flatulence were assessed by the owners once a week, on day 1, day 7, day 15 and day 30 of the study. The stool consistency part is assessing the stool of the dog during one full day using the faecal score scale. The scale is described as follow 1: very hard and dry, 2: firm but not hard, 3: Log-like, 4: very moist, 5: very moist but has distinct shape, 6: has textured but no defined shape, 7: watery no texture, flat. The stool volume and smell were also recorded during a full day. The volume is graded as 1: small, 2: normal, 3: large, 4: very large. The smell is recorded as 1: very little odour, 2: low odour, 3: moderately unpleasant smell, 4: unpleasant smell, 5: very unpleasant smell. The gut rambling and the flatulence were evaluated during the day and their presence or absence were recorded. At the same time, the dynamics of faecal quantity was observed, in correlation with the quantity of administered food.

Digestibility

The digestibility of fodders is measured using the digestibility coefficient (D.C.) which represents the portion of a feed or nutrient of feed which is not recovered in faeces, i.e., the portion which has been absorbed by the animal. The Apparent Digestibility Coefficient (ADC %) was used (Fig. 1). The levels for dry matter (DM), crude protein - Kjeldahl method (CP), crude fat – Soxhlet method (CF), crude fibre - Weende method (CC), nitrogen free extract (NFE) were assessed. Biological samples were represented by faeces and food samples. Faeces were collected 5 days before the start of treatment and 5 days after the end of the probiotic administration.

$$ADC\ (\%) = \frac{INTAKE - EXCRETION}{INTAKE} \times 100$$

Fig. 1. Apparent Digestibility Coefficient formula

Statistical analysis

Data were analysed using a condition (healthy dogs) × experiment time (pre administration and 31-day post administration) analysis of variance (ANOVA). Significance was set at $P < 0.05$. Location of significant effects was determined using Student-Newman-Keuls Multiple Comparisons Test.

Animal welfare statement

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The study was approved by the decision no 130/20th December 2018 by the Institutional Bioethics Committee. All the procedures were conducted after every owner was fully informed about all the procedures involved and after they voluntarily signed an informed consent. All the procedures were made without producing any harm to animals.

RESULTS AND DISCUSSIONS

Faecal score

In most of the dogs the stool consistency and stool

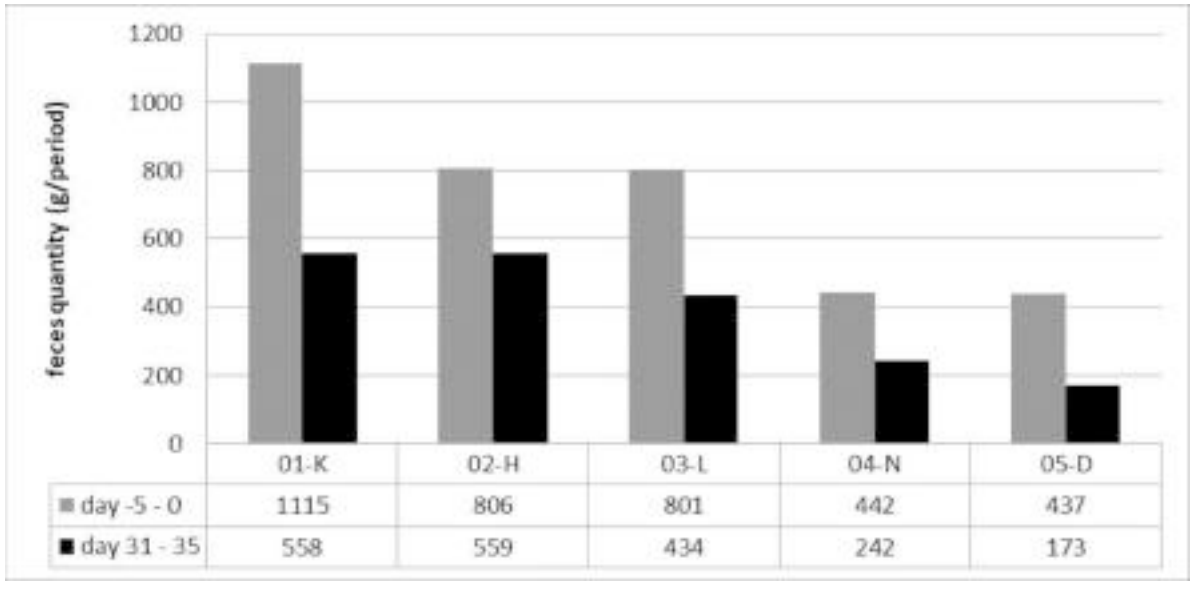

Fig. 2. Evolution of the faeces quantity (average) before and after the probiotic's treatment

volume slightly improved along the probiotic treatment. According to the faecal score analyses, the stool's smell was not improved with the probiotic's treatment. The gut rumbling was not present in any of the dogs before, during and after treatment. However, the flatulence was diminished for the dog that presented flatulence before the treatment (Table 2).

A large variation of faeces quantity before and after the probiotic's treatment is noted (Fig. 2), under the conditions in which the quantity and type of food remained the same during the study. For the five dogs there is an important diminution of faeces production after the treatment.

Digestibility

The study of the digestibility of feedstuffs by measuring the apparent digestibility coefficients shows an important improvement of digestibility of all the studied components (dry matter, crude fat, crude fibre (cellulose) and N.F.E.) with the exception of crude protein where the values decreased in one case (02-H) Table 3; Fig. 3). The increasing of digestibility is statistically important ($p<0.001$) for dry matter, crude protein and NFE.

Dumas et al. (2014) studied the digestibility of different commercial diets in dogs and obtained results for the apparent digestibility, the range of values of crude protein and crude fat were about 70–82 % crude protein and 76–95 % crude fat (5). The results obtained in the present study show a great improvement in the digestion of the crude protein and also for the crude fat after the probiotic administration, for all dogs involved in the research. Moreover, a statistically relevant improvement was observed also for the digestibility of the dry matter and N.F.E. after the treatment, compared with the values obtained before the probiotic administration. Those results demonstrate that the spore-based probiotic with *Bacillus subtilis* and *Pediococcus acidilactici* may have a role in the improvement of food digestion. Also, the digestion process on dogs can be improved by adding dietary fibres that will enter into fermentation and will be transformed in smaller molecules by the intestinal microbiome (6).

A 25 days study conducted in 2010 analysed the digestibility of dogs fed with *Bacillus subtilis* in their diet. The apparent digestibility except for the dry matter, shown an improvement of digestibility with the probiotic treatment. For the control dogs (without probiotics) they obtain an average value of dry matter of 82.1% and for the dogs with a probiotic's treatment an average value of 82.4%. The improvement is not really significant. However, the crude protein digestibility didn't improve (8). An earlier study found a slight improvement in digestibility but no really significative differences in apparent digestibility coefficient (ADC) of dry matter (DM) and crude protein (CP) (2, 8).

In their study, Paap et al. (2016), noticed no changes in dog faecal characteristics (consistency, volume, colour, appearance of blood and mucus) after *Bacillus subtilis* treatment (14). Nevertheless, in their study the faecal odour, flatulence and coat condition was significantly improved. On the contrary, Felix et al. (2010), noticed an improvement in dog faecal consistency with *Bacillus subtilis* treatment (8). The difference between these two studies is that Paap et al. (2016), tested the probiotic on dogs with chronic diarrhoea while Felix et al. (2010) tested the probiotic on healthy dogs like in the study we conducted. Felix et al. (2010) noticed a higher faecal score after probiotic treatment. For precision, the faecal score was evaluated always by the same researcher in a 1 to 5 scale as: 1 = very soft faeces; 2 = soft, unshaped faeces that

Table 3

Evolution of digestibility (%)

Patient code	Dry matter***		Crude protein***		Crude fat		Crude fiber		NFE***	
	Pre-A	Post-A	Pre-A	Post-A	Pre-A	Post-A	Pre-A	Post-A	Pre-A	Post-A
01 - K	80.959	89.588	88.305	92.623	97.638	98.637	22.945	42.273	80.666	90.051
02 - H	77.621	85.146	84.233	75.070	90.524	91.628	23.252	34.004	81.658	89.990
03 – L	77.560	88.763	78.541	87.676	92.957	96.630	24.431	48.633	85.315	94.059
04 – N	76.345	88.190	77.346	87.461	94.310	97.851	24.367	49.539	83.915	93.142
05 – D	69.541	88.605	70.443	86.840	91.175	97.165	25.834	52.727	78.680	94.089
Average	***76.405***	***88.058***	***79.774***	***85.934***	***93.321***	***96.382***	***24.166***	***45.435***	***82.047***	***92.266***
St. dev.	***4.203***	***1.705***	***6.840***	***6.499***	***2.836***	***2.762***	***1.142***	***7.432***	***2.625***	***2.085***

*** p<0.001 - Highly significant

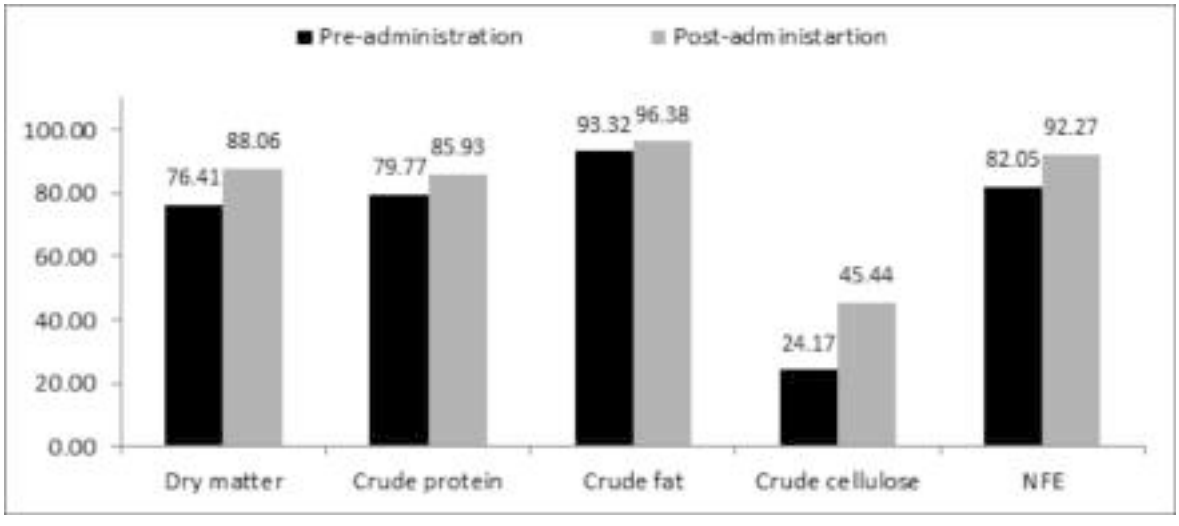

Fig. 3. Dynamic of the average of the digestibility parameters (%) – healthy dogs

took the shape of the collection vessel; 3=soft, shaped and moist faeces that left a mark on the floor; 4 = shaped and solid faeces that did not adhere to the floor; 5 = shaped, dry, and hard faeces (8, 14). Another study carried in 2019 that used *Bacillus subtilis* in the dogs' diet found an improvement in the faecal consistency, a decreased faecal ammonia content and that the synthesis of SCFA was promoted (17). In the present study, in most of the dogs the stool consistency and stool volume slightly improved along the probiotic treatment. An important change was observed also in the stool quantity after the treatment, compared with the quantity before the treatment. The diminution of faeces for the five dogs has an average of 327 g, it is representing a significative change and this diminution can be correlated to the increase of absorption of nutrient. Moreover, an increase in apparent digestibility after probiotic treatment was observed.

Regarding the effect of *Pediococcus acidilactici* on the digestion, the only available data at the present moment came from a study conducted on pigs. In their study Joysowal et al. (2018), have tested the effect of *Pediococcus acidilactici* FT28 probiotic product on pigs. Their results shown the improvement in growth, feed intake and digestibility of crude protein after probiotic treatment. However, the digestibility of dry matter, crude fiber and nitrogen free extract did not show any significant effect (12). According to Xu et al. (2019) the use of probiotics in dog can improve feed intake, weight gain, immunity and intestinal microbiota. Moreover, the elderly dogs showed the strongest response to the probiotics (19).

CONCLUSIONS

Available data about the effect of probiotics on digestibility are variable. The combination between *Bacillus subtilis, B. licheniformis* and *Pediococcus acidilactici* could be a possible explanation for a such improvement in digestibility for the present study. Even if the interaction between these two bacterial strains is not very well understood, it could result in an efficient

combination for a probiotic product. In the present study, a significant improvement in faecal weight and digestibility parameters for all the dogs after administration of probiotics was observed.

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Conflict of interest statement

The funding source had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

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