Comparative ionogram assessment before and after probiotic treatment for healthy dogs and dogs with apparent dysbiosis

Maria-Cătălina MATEI, Victoria BUZA, Daniela NEAGU, Cristian Paul POPOVICI, Andrei Radu SZAKACS, Laura Cristina ȘTEFĂNUȚ
University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine,
3-5 Calea Mănăștur Street, 400372 Cluj-Napoca, Romania
Coresponding author: catalina.matei@usamvcluj.ro

Abstract
Probiotics are considered live formulas composed by microorganisms that, when administered in appropriate amounts, produce a beneficial effect to the host. The effect of probiotics is present both in the gastrointestinal tract and systemically. For this reason, a noteworthy aspect is the impact that these formulas have on commonly systemic investigated parameters. Of these, the main ions are dosed in order to clarify various aspects, being used as a marker in various pathologies. The aim of the present study was to make a comparison between the values of the main ions (calcium, phosphorus, potassium and sodium) obtained before and after a 30-day probiotic treatment. The study population was represented by two groups of dogs: group 1- healthy dogs (n = 5) and group 2- dogs with apparent dysbiosis (n = 6). The treatment was performed with a probiotic product consisting of Bacillus subtilis, Bacillus licheniformis and Pediococcus acidilactici, for 30 days. The analyzed samples were blood serum samples obtained by centrifugation and separation from blood samples collected on anticoagulant on day 0 and day 31 of the study, respectively. Analyzes were performed by dry biochemistry methods using the VetScan biochemistry analyzer. The results obtained by ionograms suggest that probiotic treatment does not have a direct influence on the values of the main ions, neither in the group of healthy dogs nor in the group of dogs with apparent dysbiosis. Variations in ion values were considered physiological, and could not be directly attributed to the treatment performed. In conclusion, the probiotic composed of B. subtilis, B. licheniformis and P. acidilactici does not directly influence the values of the main ions, and can be considered safe for administration in both healthy dogs and dogs with gastrointestinal manifestations.
***Key words**: ions, probiotics, dogs*

Introduction

Probiotics are complex formulas composed from different microorganisms (bacteria, arcanobacteria, fungi, viruses) that are able to confer a benefic effect to the host when it is administered in adequate amounts (Sauter et al., 2006; Barko et al., 2018).

For dog use, most probiotics available at the present moment are composed by lactic acid bacteria like *Lactobacillus* and *Bifidobacterium* (Barko et al., 2018; Lucena et al., 2019). One of the most important characteristics for probiotics in order to provide an effect to the host is their ability to remain viable in unfriendly conditions like the ones meet in gastro-intestinal (GI) tract. This is why it is important for the probiotic bacterial strains to survive on low pH created by the gastric juice and bile acids (Barko et al., 2018).

Sporulated bacteria have a plus compared with lactic acid bacteria because they are more resistant in hard environment conditions (Biourge și colab., 1998; Schmitz and Suchodolski, 2016). Bacteria from genus Bacillus are sporulated bacteria and this is why they are considered to be more resistant in GI tract. At the origins, bacteria from Bacillus genus was considered to have their origin in soil. After few years this concept was unvalidated based on some studies that demonstrated that those bacteria are GI tract commensals (Cutting, 2011).

From the mechanism of action point of view, probiotics are considered to have the capacity to improve the intestinal mucosa health status using different ways of action, synergic or alone. From those mechanisms of actions, the following are considered the principal ones: pathogenic bacteria replacement (Lee et al., 2003), antimicrobial substances production (Jones et Versalovic, 2009), increasing of immune response (Paganini et al., 2010) and metabolites regulation (Soo et al., 2008). Because those mechanisms of action are complex, the probiotics are considered to have a systemic impact on the host. In this way, some usually assessed parameters can be influenced by

a probiotic treatment. Thus, it is important to know if those parameters variations are dependent or independent to the probiotic treatment.

Our aim was to assess the potential effect of a probiotic formula containing *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* (Fidospore®, Microbiome Labs, LLC) on the serum activity of the main ions when administered 30 days consecutively on healthy dogs and dogs with apparent GI dysbiosis. Moreover, we wanted to find out if this treatment may influence the ions, in order to establish if a confusion can be made when the ions are dosed in different pathologies.

Materials and method

Study design

The study was conducted at the Faculty of Veterinary Medicine Cluj-Napoca in the departments of animal physiology and internal medicine.

The cases were taken from the clinics and were represented by real clinical cases. 11 dogs of different ages were enrolled in the study based on inclusion and exclusion criteria. The study population was divided in 2 groups: group 1 (n=5) - healthy dogs and group 2 (n=6)- dogs with GI manifestations. All dogs were enrolled after the owners were fully informed about all the procedures and voluntarily signed an informed consent. The study was approved by the Institutional Bioethics committee (decision no 130/December 2018).

On day 0 and day 31 blood samples without coagulant were collected in order to separate blood serum. Between day 1 and 30 the probiotic product was administered to the dogs by the owners.

Study population

11 dogs, divided in 2 groups formed the study population. They were enrolled in the study after a full clinical exam performed in order to establish if the inclusion/exclusion criteria are met for each group (Table 1).

Table 1.
Inclusion and Exclusion criteria for study population groups

	Inclusion criteria	**Exclusion criteria**
Group 1- Healthy dogs	Absence of GI manifestation (vomiturition/diarrhea) No antibiotic treatment in the last 14 days Current on vaccination and deworming Clinically healthy One meal per day	Diarrhea Vomiturition Current on antibiotic treatment
Group 2- Dogs with GI manifestations	One meal per day Diarrhea Vomiturition Antibiotic treatment	Acute/Chronic renal failure Acute/Chronic hepatic failure Intestinal parasites

Treatment

Each dog received the probiotic treatment for 30 consecutively days. The product was administered by the owner, together with dog's regular food. The probiotic product was composed by two bacterial strains: *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* (Fidospore®, Microbiome Labs, LLC).

Ions assessment

The ions were assessed using VetScan machine, Comprehensive test kit. The samples used were represented by serum samples (Table 2).

Table 2.
Assessed ions

No	Parameter	Indication	Principle of method
1.	**Calcium**	Parathyroid, bone and chronic renal disease; tetany	Calcium in sample binds with arsenazo III to form a calcium-dye complex. Absorbance is measured.
2.	**Phosphorus**	Kidney disease, hypoparathyroidism and nutritional disorders.	The method uses sucrose phosphorylase (SP) coupled with the phosphoglucomutase (PGM) and glucose- 6-phosphate dehydrogenase (G-6-PDH) reactions. Absorbance is measured.
3.	**Potassium**	Malnutrition and renal disease. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms.	Enzymatic method is based on the activation of pyruvate kinase (PK) with potassium. Absorbance is measured.
4.	**Sodium**	Dehydration, and diabetes. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms.	β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose. Absorbance is measured.

Statistics

Statistical analysis was performed using GraphPad Prism 8 software. Statistics tests realized were represented by descriptive statistics and T test for comparation between results pre-administration and after probiotic administration, $p<0.05$.

Results and discussion

The results obtained show a physiological variance of values before and after the treatment (Table 3, Table 4, Figure 1).

Table 3.
Descriptive statistics of ions results before and after the probiotic treatment for Group 1 - healthy dogs

Group 1	Descriprive statistics						T test	
Parameter	Min	Max	Mean	St Dev	St error of mean	CV (%)	p value	Sign
Ca pre-A	2.33	2.64	2.482	0.1219	0.0545	4.913	0.3074	ns
Ca post-A	2.03	2.65	2.404	0.2543	0.1137	10.580		
P pre-A	1.20	2.10	1.612	0.3560	0.1592	22.090	0.9512	ns
P post-A	1.18	1.99	1.618	0.2988	0.1336	18.470		

K pre-A	4.60	8.50	5.540	1.6710	0.7474	30.170	0.3151	ns
K post-A	4.70	12.00	6.340	3.1720	1.4190	50.040		
Na pre-A	145.00	149.00	146.400	1.6730	0.7483	1.143	0.0647	ns
Na post-A	139.00	146.00	142.400	2.5100	1.1220	1.763		

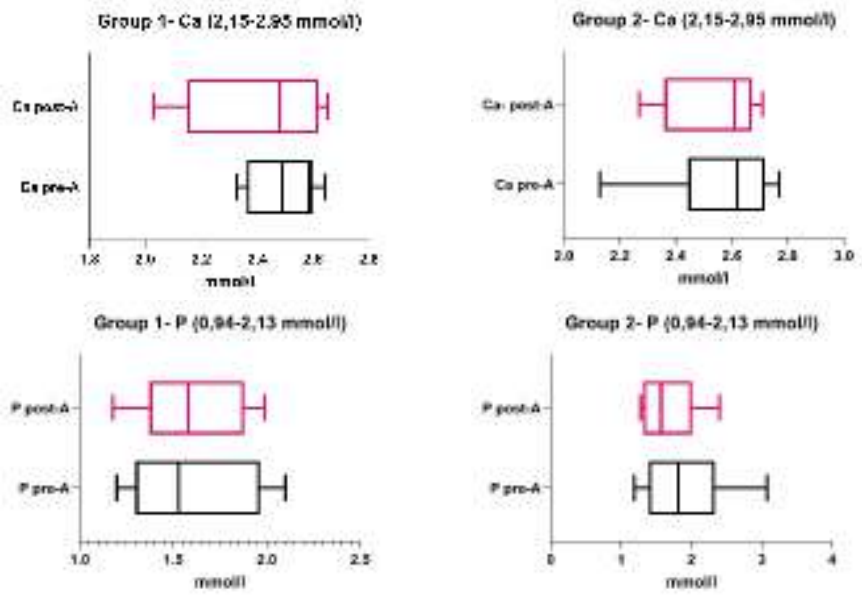
Ca- calcium; P- phosphorus; K- potassium; Na- natrium; pre-A- measurement before treatment (day 0); post-A- measurement after treatment (day 31); ns- without statistical significance

Table 4.

Descriptive statistics of ions results before and after the probiotic treatment for Group 2- dogs with GI manifestations

Group 2	Descriprive statistics						T test	
Parameter	Min	Max	Mean	St Dev	St error of mean	CV (%)	p value	Sign
Ca pre-A	2.13	2.77	2.565	0.2284	0.0932	8.903	0.6211	ns
Ca post-A	2.27	2.71	2.542	0.1728	0.0705	6.798		
P pre-A	1.19	3.08	1.908	0.6555	0.2676	34.350	0.0800	ns
P post-A	1.28	2.40	1.672	0.4347	0.1775	26.000		
K pre-A	3.90	4.90	4.650	0.3886	0.1586	8.357	0.0648	ns
K post-A	4.00	4.70	4.417	0.2927	0.1195	6.627		
Na pre-A	144.00	149.00	145.800	1.7220	0.7032	1.181	0.6203	ns
Na post-A	141.00	149.00	145.000	2.8280	1.1550	1.951		

Ca- calcium; P- phosphorus; K- potassium; Na- natrium; pre-A- measurement before treatment (day 0); post-A- measurement after treatment (day 31); ns- without statistical significance



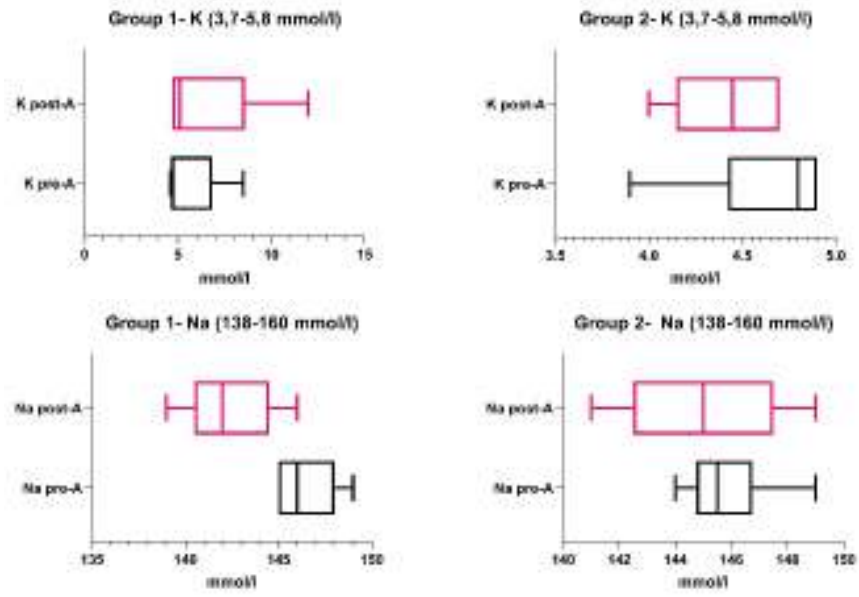


Fig. 1. Graphical representations of ions results before and after the treatment

The probiotic potential of *Bacillus* spp. was discovered starting with 1958, being a component of a therapeutic formula used as nutritive supplement in Italy. However, the real potential of those bacteria was discovered only in the last 20 years (Cutting, 2011). Studies show that those type of bacteria are able to survive into canine GI tract, regardless the hard environmental conditions, even if they are not present anymore in feces after 3 days from the end of the treatment (German et al., 2000).

B. subtilis, B. licheniformis and *P. acidilactici* combination did not induce any significant change in calcium, phosphorus, potassium or natrium serum values. Moreover, this combination had no clinically observable adverse effects neither on the healthy dogs' group, nor in the group of dogs with GI manifestations.

Ions values on the first assessment showed normal values for all the parameters. At the second examination, normal variations were registered, but all the values remained in the physiological interval. This variation can be considered normal and linked with every individual, being a physiological observation. Moreover, this variance between the values cannot be attributed to the probiotic treatment as long as there is no evidence on the literature that can prove that a probiotic treatment is able to influence one way or another the serum ions values.

The electrolyte and ions equilibrium are affected by a several number of conditions. Every pathology that involve water loss or a heavy loss of body fluids this equilibrium is evidentially affected. GI manifestations like diarrhea and vomiturition are two conditions that evolve with the loss of water and other body fluids. As a mechanism of action, if the electrolyte balance is affected, this fact can be revealed as clinical signs (i.e. dehydration) and also on ions profiles (Wirth, 1967).

On the other hand, ions values are modified in other important pathologies, thus they can be used as an important indicator. Calcium as an assessed parameter usually is representative for tumoral process or endocrinological diseases. Phosphorus can be modified in some endocrinal diseases, bones diseases or renal diseases. Potassium in physiological limits translate a good

functioning of the heart and muscle. Sodium is representative of the kidney function (Poli G., 2016). Because all those parameters have an important clinical significance, all the factors that are able to influence their values, regardless the disease, should be known as much as possible. This pilot study suggests that *B. subtillis, B. licheniformis* and *P. acidilactici* are safe to use when the ions are assessed, without any significant influence on their values.

Conclusion

The present pilot study suggests that the probiotic combination of *B. subtilis, B. licheniformis* and *P. acidilactici* administered to healthy dogs and dogs with GI manifestation for 30 days induced no significant variation in serum ions. In conclusion, this combination is safe to use in conditions when serum ions are assessed, without inducing any bias in the interpretation of results.

ACKNOWLEDGMENT: This work was supported by 1 funding source:
MICROBIOME LABS PHYSICIANS EXCLUSIVE, LLC, Agreement no 30130/18 December 2018.

Bibliography

1. *** Poli G**.,** 2016. Mini vet guide- Companion animal medicine, p 34-39.
2. BARKO P.C., M.A. MICHAEL, K.S. SWANSON, D.A. WILLIAMS, 2018, The gastrointestinal microbiome: a review, Journal of Veterinary Internal Medicine, Vol 32, 9-25;
3. BIOURGE V., VALLET C., LEVESQUE A., SERGHERAERT R., CHEVALIER S., ROBERTON J.L., 1998, The use of probiotics in the diet of dogs, Journal of Nutrition, Vol 128 (12 Suppl.), 2730S–2732S;
4. CUTTING SIMON M., 2011, Bacillus probiotics, Food Microbiology, vol 28, 214- 220;
5. GERMAN A.J., HELPS C.R., HALL E.J., DAY M.J., 2000, Cytokine mRNA expression in mucosal biopsies from German shepherd dogs with small intestinal enteropathies, Digestive Diseases and Sciences, Vol 45, 7–17;
6. JONES S.E., VERSALOVIC J., 2009, Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors, BMC Microbiology, Vol 9, 35;
7. LEE Y.-K., PUONG K.-Y., OUWEHAND A.C., SALMINEN S., 2003, Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli, Journal of Medical Microbiology, Vol 52 (Pt 10), 925–930;
8. PAGNINI C., SAEED R., BAMIAS G., ARSENEAU K.O., PIZARRO T.T., COMINELLI F., 2010, Probiotics promote gut health through stimulation of epithelial innate immunity, Proceedings of the National Academy of Sciences of the United States of America, Vol 107, 454–459;
9. ROSARIO LUCENA, MANUEL NOVALES, BEATRIZ BLANCO, EDUARDO HERNÁNDEZ, PEDRO JOSÉ GINEL, 2019, Effect of probiotic Enterococcus faecium SF68 on liver function in healthy dogs, J Vet Intern Med. 2019;1–8.
10. SAUTER SN, BENYACOUB J, ALLENSPACH K, et al. Effects of probiotic bacteria in dogs with food responsive diarrhea treated with an elimination diet. J Anim Physiol Anim Nutr. 2006;90:269-277.
11. SCHMITZ SILKE, J. SUCHODOLSKI, 2016, Understanding the canine intestinal microbiota and its modification bt pro-, pre- and synbiotics- what is the evidence?, Veterinary Medicine and Scienc, vol 2, 71- 94;
12. SOO I., MADSEN K.L., TEJPAR Q., SYDORA B.C., SHERBANIUK R., CINQUE B. et al., 2008, VSL#3 probiotic upregulates intestinal mucosal alkaline sphingomyelinase and reduces inflammation, Canadian Journal of Gastroenterology, Vol 22, 237–242;
13. W. WIRTH, 1967, Elektrolythaushalt und parenterale Fliissigkeitstherapie beim Hund Klinische und experimentelle Untersuchungen am Serumionograrnm, Zbl. Vet. Med., Reihe A, Bd. 14, Heft 8.