The influence of a probiotic product with *Bacillus subtilis* and *Pediococcus acidilactici* on some hematological and clinical parameters of healthy dogs

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Abstract
The study was conducted at the University of Veterinary Medicine in Cluj-Napoca (Romania) in the animal physiology department, between May 2018 and June 2019. The aim of the study was to assess the influence of a probiotic product with Bacillus subtilis and Pediococcus acidilactici on healthy dogs. The probiotic was administered orally once a day to six healthy adult dogs from 1 year to 7 years old for a period of 30 days. The dogs entering the study were submitted to a general clinical examination. At day 1 and day 31/36 of the study, blood samples was collected and haematological parameters were evaluated. The objectives of the research were multiple. First of all, we observed whether the probiotic influenced the general health of the dogs and changes in clinical parameters. Then, the dynamics of the haematological parameters was identified. The in vivo study showed the safety of the probiotic product on healthy dogs and the minimal impact on hematologic parameters. In fact, no adverse reactions were observed.
Keys words: *Bacillus subtilis, Pediococcus acidilactici, canine*

Introduction

The use of probiotics in human has been reported hundred years ago (Endres et al., 2009). They have worldwide utilization and are widely developed in Asia. As an example, the Japanese fermented soybean dish Natto is famous for its probiotic's properties. Many more natural dishes associated with probiotics are found across the world such as TapaiUbi (Malaysia), Douchi (China), Rabadi (India, Pakistan), Soibum (India), etc... (Elseghabee et al., 2017)

These last decades' probiotics have been described in many ways by a large panel of scientists. In 2014, experts have established a common opinion about probiotics (Bernadeau et al., 2017). Probiotics are usually 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 (Amraii et al., 2014).

Probiotics must fulfil some safety criteria to be used in animals and human health. They should be non-toxic, non-pathogenic and be a normal inhabitant of the gastrointestinal tract (GIT) host. Accurate taxonomic identification is required to avoid the use of unsafe bacteria (Gaggia et al., 2010).

Other criteria such as antagonism towards pathogenic bacteria and be genetically stable to avoid antibiotic resistance or other adverse effects. They should show stability during processing, storage and delivery (Gaggia et al., 2010). More assays must be performed to confirm the safety of probiotics such as antibiotic resistance, hemolytic activity, and production of enzymes, toxins or biogenic amines (Gaggia et al., 2010).

Probiotics related to *Bacillus* species emerged 15 years ago. *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus indicus* are the predominant species (Cutting, 2011).

The objectives of the research were multiple. The dynamics of the haematological parameters in healthy dogs treated with probiotics was identified. In addition the influence on the general health of the dogs was analysed and changes in clinical parameters were observed.

Material and methods

The study was conducted between May 2018 and June 2019 at the University of Veterinary Medicine in Cluj-Napoca (Romania). The investigations were performed in three departments of the university: Animal physiology, Animal Nutrition and Internal medicine.

Study design

A total of 6 adults dogs aged between 1year and 7 years old entered in the study from 10th of April till the 29th of July. The probiotic product composed of *Bacillus subtilis* and *Pediococcus acidilactici* was given to the 6 dogs once a day with the meal during a thirty day period.

In day 0 dogs were clinical examined and biological samples were collected (blood) for para-clinical examinations. Between day 1 and day 30 the probiotic product was administrated to the animals, respecting the manufacturer recommendations. In day 31, all the dogs were clinical examined and biological samples were collected (blood) for the para-clinical examinations.

All the clinical examinations for the animals have the aim to diagnose the general health status and the temperature, cardiac frequency and respiratory frequency registration.

Study population

Inclusion criteria for the **healthy dogs** (table 2.1) were: absence of gastro-enterological manifestations (diarrhea, vomiting), no antibiotic treatment in the last 6 months, clinically healthy, one meal/day. Exclusion criteria for this category of dogs were: gastro-enterological manifestations (diarrhea, vomiting), antibiotic treatment in the last 6 months.

Table 2.1. – Patient description and code

Patient code	Breed	Age	Sex	Observations
P-01	Czechoslovakian wolfdog	4 years	castrated male	All vaccination no specific external or internal treatment
P-02	Swiss White Shepherd	3 years	castrated male	
P-03	Border collie	3 years	female	
P-04	Shetland shepherd	1 year	female	
P-05	Shetland shepherd	7 years	female	
P-06	Mixed dog breed	1.8-year	female	

Treatment

The probiotic product tested is composed of two strains of bacteria: *Bacillus subtilis HU58* and *Pediococcus acidilactici*. The probiotic product is presented as capsules. During thirty 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 taking.

Clinical investigations

To ensure that the dogs entering the study are healthy and for observed the evolution of the principals functions we performed individual clinical investigations. A general clinical examination was performed and some clinical parameters (temperature, cardiac frequency, respiratory rate) were measured in all six dogs. For these investigations we based the normal range values for dogs according to the Merck Veterinary Manual (2016).

Hematological parameters

The blood will be collected in hematologic tubes with an anticoagulant (EDTA). The hematologic samples are analyzed via the device ABACUS JUNIOR VET®. These evaluations were performed ante- (day 0) and post-administration (day 31) of the probiotic (table 2.2).

Table 2.2- Hematologic parameters and their methods

No.	Parameter	The method
1.	Hematocrit (HCT)	Calculated from RBC and MCV, HCT= RBC x MCV x 100
2.	Hemoglobin (HGB)	Measured photometrically
3.	Red blood cell count (RBC)	Number of erythrocytes
4.	Mean corpuscular volume (MCV)	Average volume of individual erythrocytes derived from the RBC histogram
5.	Mean corpuscular hemoglobin (MCH)	MCH= HGB/RBC
6.	Mean corpuscular hemoglobin concentration (MCHC)	MCHC= [HGB / HCT] x 100
7.	Total White blood cell count (WBC)	Number of leucocytes
8.	Differential white cell count	Smear / Microscopy

Statistical analysis

Data were analyzed using a condition (healty dogs) × experiment time (pre administration and 31-day post-administration) analysis of variance (ANOVA). Significance was set at $P < 0.05$. The significance of results was determined using Student-Newman-Keuls Multiple Comparisons Test.

Results and discussion

Clinical investigations

A general clinical examination is performed on all the dogs before to start the probiotic treatment and to the end of the study.

All the dogs presented no significant modifications at general examination. The temperature for all the dogs was normal between 37.9-39.9 °C. The cardiac frequency was without significant importance. All the dogs present a higher value for the respiratory rate between. The normal value at rest for the respiratory frequency is between 18 -34 breaths per minute. The fact that all the dogs had an increased in respiratory rate was not alarming it could be due to the excitement, stress and/or the too high temperature in the room.

The clinical appearance of healthy dogs was not altered by the treatment. No symptoms such as diarrhea or vomiting have been observed. In dog P-03, hair loss and skin lesions at level of the left anterior limb were observed. Problems started during gestation and were maintained after parturition (3 months ago). Probiotic treatment improved the appearance of the hair and induced skin lesions improvement.

Hematological parameters

The following table (table 3.1) represents the hematological results before and after the probiotics treatment for all the six cases.

Table 3.1 - Results for hematological parameters before and after probiotics treatment

Hematologic Parameters	P 01		P 02		P 03		P 04		P 05		P 06	
Day	0	31	0	31	0	31	0	31	0	31	0	31
HCT (37-55%)	46.74	47.01	43.91	39.07	48.00	48.08	42.76	48.94	47.67	47.53	45.25	42.71
HGB (12-18 g/dL)	21.30	21.70	19.80	19.20	20.60	22.00	18.20	23.60	21.10	21.40	21.40	21.70
RBC (5.5-8.5*10^{12}/L)	7.11	7.13	6.70	6.25	6.86	7.30	5.82	6.74	7.05	6.85	7.22	6.84
MCV (60-77 fL)	66.00	66.00	66.00	63.00	70.00	66.00	73.00	73.00	68.00	69.00	63.00	62.00
MCH (19.5-24.5 pg)	30.00	30.50	29.50	30.70	30.00	30.20	31.20	35.00	29.90	31.20	29.60	31.70
MCHC (32-36 g/dL)	45.60	46.20	45.00	49.10	42.90	45.80	42.50	48.20	44.20	45.00	47.20	50.80
WBC (6-17*10^{9}/L)	13.35	12.95	12.13	15.22	16.29	12.43	11.43	17.28	9.75	16.99	18.80	19.69
PCT (%)	0.11	0.21	0.15	0.18	0.15	0.33	0.37	0.45	0.51	0.37	0.25	0.30

The results show for P-01, P-03, P-04 an increase of the hematocrit. For P-02, P-05 and P-06 there is a decrease in hematocrit. However, the differences are not significative. For all the dogs except P-02 we observe a slight increase in hemoglobin production after the treatment. We observe a slight increase of the red blood cells for P-01, P-03, P-04 but a slight decrease for P-02, P-05 and P-06. However, the differences are not significative.

The MCV presents no variation in P-01 and P-04 while we observe a decrease in MCV after probiotics treatment for P-02, P-03 and P-06; P-05 presents an increase of the MCV after the probiotic's treatment. For all dogs we observe an increase of the MCH and the MCHC after the probiotic's treatment. These parameters are in the normal range of value and their variations are not significant.

We observe for P-01 and P-03 a decrease of the white blood cells while for P-02, P-04, P-05 and P-06 there is an increase in white blood cells.

All the dogs except P-05 show an increase in platelets after the probiotic's treatment. Even if some of the results show values outside of the normal range it is not alarming because it can be due to the device. The hematological results are considered in the normal range for dogs and don't represent any signs of diseases.

The hematological parameters for our six dogs are quite heterogenous but are always in the normal range of values for dogs. Nevertheless, we observe a slight increase in hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), plateletcrit (PCT) and white blood cells (WBC) for almost all the dogs after the probiotic treatment.

These results are not significant and confirm the study of Hong et al. (2008). In fact, in their study, they tested the effect of oral administration of *Bacillus subtilis* for 30 days in rabbit. After the probiotic treatment, rabbits don't show any significant changes in hematological parameters (Hong et al., 2008).

The ability of *Bacillus subtilis* to form biofilms, to sporulate anaerobically, to secrete antimicrobials and the adaptation to gastrointestinal tract made authors like Hong et al. (2009) to consider it a gut commensal rather than solely soil microorganism.

The table 3.2 records the percentage of leucocytes before and after the probiotic's treatment for all the six cases.

Table 3.2 - Percentage of leucocytes before and after the probiotic's treatment

Leucocytes	P 01		P 02		P 03		P 04		P 05		P 06	
Day	**0**	**31**	**0**	**31**	**0**	**36**	**0**	**36**	**0**	**36**	**0**	**31**
Neutrophil (58-85%)	43	51	63	40	47	65	62	56	61	57	54	59
Eosinophil (0-9%)	7	7	6	30	5	6	8	5	4	6	6	7
Basophil (0-1%)	0	0	0	0	0	0	0	1	0	0	0	0
Monocyte (2-10%)	17	12	18	13	20	12	10	18	18	16	9	11
Lymphocyte (8-21%)	33	30	13	17	28	17	20	20	17	21	31	23

For P-01, P-03 and P-06 there is an increase of the neutrophils. For P-02, P-04 and P-05 there is a decrease in neutrophils.

P-02 presents an important increase of the eosinophils from 6 to 30% after probiotics treatment. P-03, P-05 and P-06 show a slight increase in eosinophils. While P-04 presents a slight decrease in eosinophils.

Concerning P-02, the important increase in eosinophils can correlates with the second coprological examination confirming the presence of intestinal parasites. Even if the mechanism is not very clear, the probiotics influence and modifies the gastrointestinal environment. The process involves a stimulation of immune system cells to produce cytokines, which play a role in the induction and regulation of the immune response. The environment became less adequate for parasites and a possible release of toxins by dead parasites is a valuable hypothesis. Eosinophils increase in the presence of parasitic toxins and in allergic reactions (Travers et al., 2011).

Usually in dogs, basophils are very rare and that why there are not present in our results. We found one basophil in P-04 after probiotics treatment. However, it is not significative.

For P-01, P-02, P-03 and P-05 there is a decrease of the monocytes while for P-04and P-06 there is an increase in monocytes.

We observe for P-01, P-03 and P-06 a decrease of lymphocytes. For P-02 and P-05 there is an increase in lymphocytes; P-04 show no variation in lymphocytes.

Even if the monocytes and lymphocytes seem a bit elevated, it is not alarming. The leucocytes values before and after probiotic treatment present heterogenous results but are in the normal range of values for dogs. The eosinophils tend to slightly increase in most of the dogs after treatment. Neutrophils, basophils, monocytes and lymphocytes increase or decrease according to the individual. These results confirm the study of Hong et al. (2008) and conduct to no significant changes of the leucocytes after probiotic treatment.

Conclusions

The clinical appearance of dogs is not altered by the treatment however one of the dogs presented improvements of the coat aspect. No symptoms such as diarrhea or vomiting have been observed.

The hematological and biochemical parameters have shown slight changes pre and post administration of probiotics with values in the normal range.

The use of the probiotic with *Bacillus subtilis* and *Pediococcus acidilactici* presented no adverse effects in healthy dogs.

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