Clinical reports

A CLINICAL RESEARCH
FOR THE VETERINARIAN
FROM AFFINITY PETCARE

DIET, MICROBIOTA AND NATURAL DEFENSES

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The microorganisms in the intestine play an essential role in vital functions, such as nutrition, digestion, defences and even the mood of dogs and cats.

Diet is one of the most important factors that affect the composition of the intestinal microbiota. For this reason, Advance has been studying the role of nutrition in the canine and feline microbiota and its role in the health of dogs and cats for over 10 years.

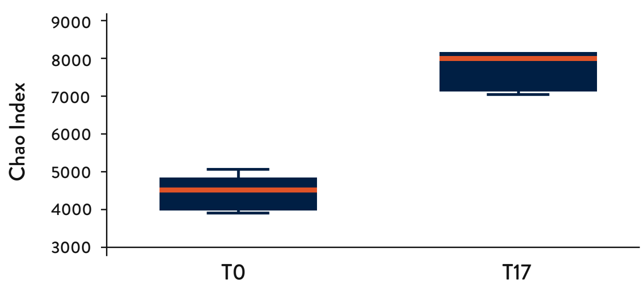
Preliminary studies focused on **how to modify the microbiota of obese dogs through diet,** to obtain a bacteria profile that was healthier and rich in species that would contribute to weight loss, maintenance of ideal weight once achieved and a better body composition.

The **latest studies** presented here aim **to test the effect of various selected functional ingredients** on the intestinal microbiota and the natural defences in healthy dogs.

ADVANCE STUDIES ON MICROBIOTA

Advance has carried out previous studies to evaluate changes in the intestinal microbiota and body composition of dogs and cats.[1,2] Advance researchers demonstrated that restricted feeding with a diet low in fat and high in fibre successfully modifies intestinal microbiota in obese dogs. These studies showed that after a 17-week body weight loss programme, lean dogs presented a greater bacterial richness, which is generally associated with a healthier microbiota[2] (**Figure 1**). In addition, obese animals showed a greater representation of types of bacteria with a high energy extraction capacity from complex carbohydrates.[3] This predominant presence of a specific type of bacteria is what is known as an altered microbiota pattern or intestinal dysbiosis.[4] Similar results were obtained in lean and overweight cats.[1]

FIGURE 1 Bacterial diversity after a weight loss program (17 weeks) in dogs.



Chao Index (species richness) at the beginning and end of the study.

Two recent studies have been carried out with the aim of creating the impact of various functional ingredients in the microbiota and the natural defences in **healthy dogs.**

STUDY 1

In the **first study**, 34 healthy adult sterilised dogs, both male and female, were adapted to a control diet for one month (T0), and then randomly assigned to one of two groups. The control group received the control diet for 6 weeks, and the prebiotic group received the same diet supplemented with 0.5% fructans (inulin) for 3 weeks, and then 1% inulin for the following 3 weeks.

In this study, supplementation with 1% inulin resulted in a tendency ($P<0.1$) to increase total short-chain fatty acids (SCFA) in the faeces (+98%), mainly represented by propionate (+18%) and valerate (+80%). In addition to this effect on the microbiota fermentation product, the CD4+/CD8+ ratio increased (54%) in dogs supplemented with 1% inulin upon completion of the study, which seems to indicate an improvement in the immune status of the animal. Regarding the microbiota global analysis, beta diversity analyses showed differences in the structure and composition of the microbiota between the control and inulin groups upon completion of the study ($P=0.038$). The microbiota taxonomy after the study was completed

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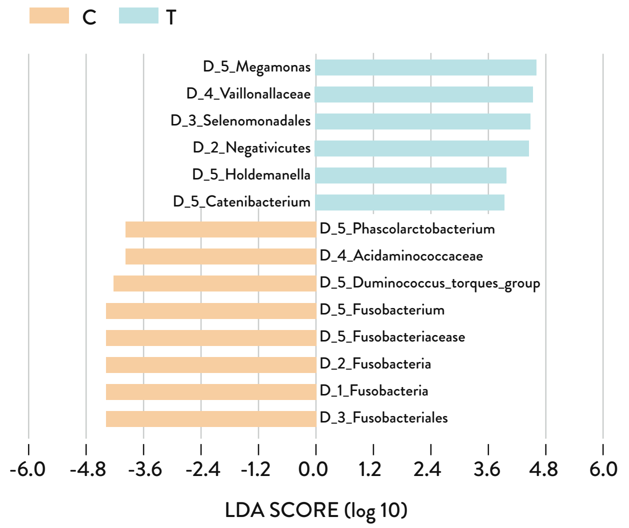
revealed significant differences between the control group and the group supplemented with inulin (**Figure 2**).

Among the most interesting differences, we found the increase in the levels of the Firmicutes taxon, with a contribution mainly from the genus *Megamonas* and *Catenibacterium*, and a decrease of the Fusobacterium genus that affected each taxon level up to the phylum Fusobacteria. *Megamonas* are a type of propionate-producing bacteria with anti-inflammatory properties whose decrease has been described in cats with diarrhoea[5]. *Catenibacterium* is a bacterial genus that helps to digest carbohydrates using glucose to produce SCFA[6]. Given that *Fusobacterium* has been linked in the literature to diarrhoea[7], its decrease could be an indicator of good intestinal health. The decrease of *Ruminococcus* torques in dogs supplemented with inulin, a mucin-degrading bacteria, could imply that it is an important factor in maintaining the integrity of the intestinal epithelial barrier[8].

FIGURE 2 Microbiota taxonomy differences in dogs' supplemented with 1% fructan fibers (i.e. inulin)



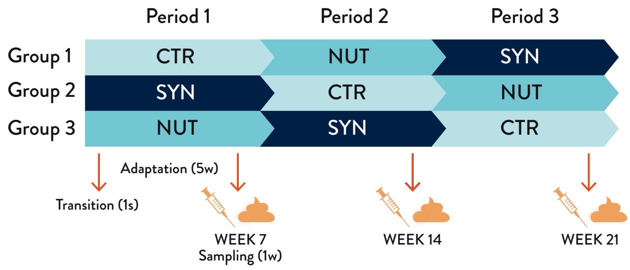
This first study showed that a 1% inulin supplementation in dogs is able to modify intestinal microbiota and their functional metabolites, and generate a healthier composition, in addition to improving blood immune biomarkers, which helps the natural defences of dogs.

STUDY 2

A **second study** was carried out to demonstrate the effect of a combination of functional ingredients on the microbiota of dogs and their natural defences. This specific combination was composed of 1% inulin (PRE), different sources of fibre (FIB), such as sugar beet pulp, wheat bran and pea fibre and the probiotic *Bacillus velezensis* (PRO): a strain, approved by the EFSA, which has been shown in previous studies to provide various benefits at a dose of 1 x 10^9 CFU/kg of food, such as an increase in faecal consistency and a decrease in ammonia content, in addition to a lower C-reactive protein in serum and a greater presence of IgA in the faeces.[9,10]

A study was carried out (**Figure 3**) in 12 adult Beagle dogs, who were fed for 3 periods with 3 different diets for a total of 7 weeks: a control diet (CON), a diet with the combination of PRE, PRO and FIB described above (SYN), and a third diet (NUT), which consisted of the SYN diet supplemented with porcine plasma, a source of immunoglobulin G.

FIGURE 3 Experimental design of Study 2 in dogs



The SYN and NUT treatments showed a higher faecal concentration of total SCFAs compared to the CON treatment (p contrast = 0.011) and a significant increase in acetate concentration (110 vs. 94 µmol/g FM) in addition to numerical increases in butyrate (19.0 vs. 16.8 µmol/g FM). However, the concentration of branched-chain fatty acids (BCFA), associated with undesired proteolytic fermentation, was reduced in these diets (5.53 vs. 7.34 µmol/g FM). Regarding the microbiota global analysis (**Figure 4**), dogs on SYN and NUT diets showed drastic statistical differences in beta diversity compared to dogs on the CON diet, implying that there were changes in the structure and composition of the microbiota.

In addition, the microbiota taxonomy showed significant differences between dogs fed with the SYN and NUT diets, as they promoted the presence of the Tyzzerella and Phocaeiola plebeius genus while the presence of the Catenisphaera genus decreased (**Figure 5**).[11] The Tyzzerella genus has been associated with healthy dogs, being found with less abundance in dogs with acute diarrhoea, which is why it has been suggested as an indicator of intestinal health.[12]

Phocaeiola plebeius is a type of bacteria that has been described in humans as contributing to polysaccharide degradation, and producing SCFA[13], but it has also been associated with dogs fed with diets high in fibre.[14]

FIGURE 4 Beta diversity differences among diets

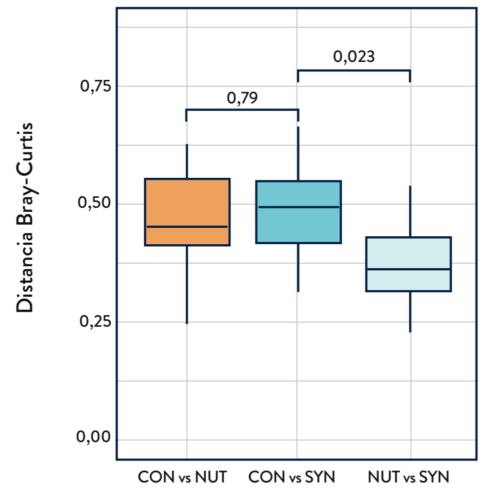
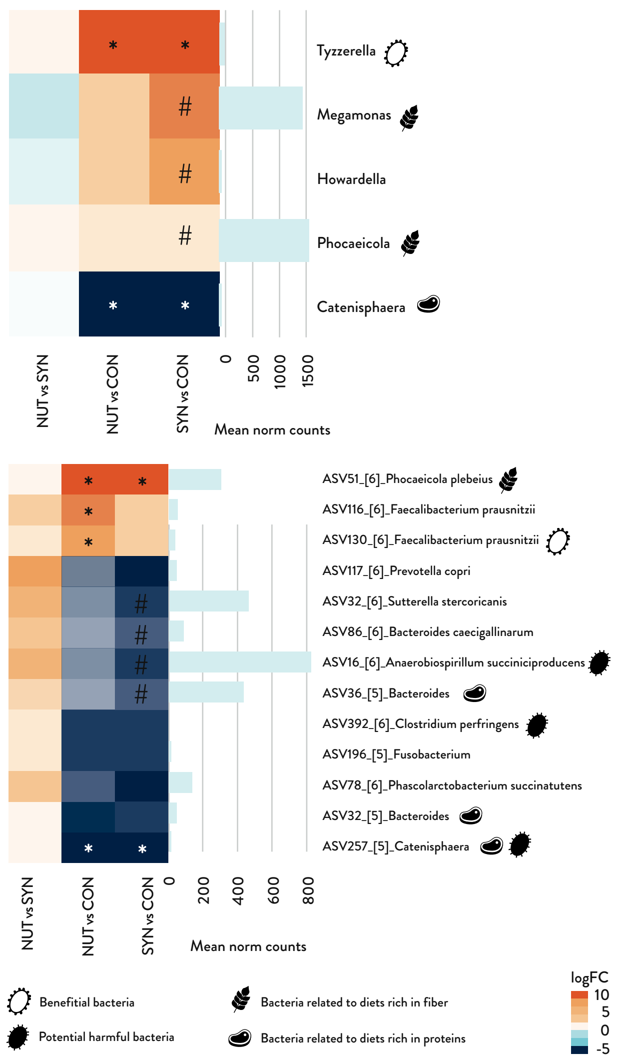


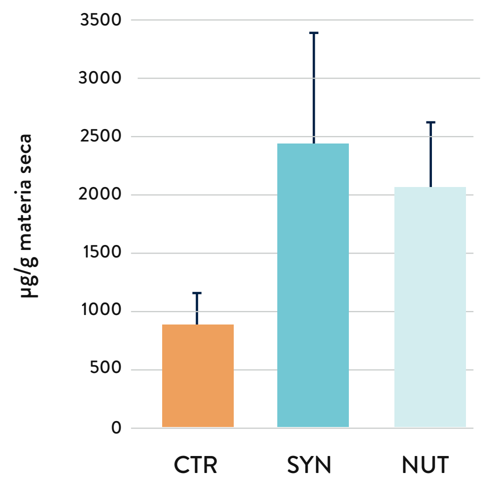
FIGURE 5 Microbiota taxonomy among diets. A=CON; B=NUT; C=SYN



The presence of the Catenisphaera genus has been associated with a physiological stress model in rodents.[15] Additionally, the SYN diet also showed a significant increase in Faecalibacterium prausnitzii (**Figure 5**), a type of bacteria that has recently received greater attention for its potential protective role against inflammatory bowel disease in humans.[16]

In relation to the immune parameters analysed, on the one hand, dogs supplemented with SYN and NUT showed a tendency to increase the level of IgA in the faeces (p = 0.069; **Figure 6**), a marker of intestinal mucosal immunity;[17] on the other hand, dogs supplemented with NUT showed a decrease in serum haptoglobin (0.90 vs. 1.30 mg/dL; p = 0.056), suggesting an improvement in the inflammatory status of the dogs.[18]

FIGURE 6 Fecal IgA



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