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Review of Scientific Publications in the International Literature

- Studies on *Lactobacillus acidophilus* D2/CSL
- Studies on antimicrobial peptides
- Studies on Florentero® ACT

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Florentero® ACT
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Companion or pet animals

Veterinary Record Open

OPEN ACCESS

Effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation on healthy cat performance

Eleonora Fusi,[1] Rita Rizzi,[2] Michele Polli,[3] Simona Cannas,[3] Alberto Giardini,[4] Natascia Bruni,[5] Stefano Paolo Marelli [3]

ABSTRACT

Background The present study aimed to evaluate the effects of the probiotic strain *Lactobacillus acidophilus* D2/CSL (CECT 4529) on nutritional condition and faecal quality in cats.

Methods Ten healthy adult cats from the same cattery were included (aged >9 months; male:female sex ratio=3:7). The animals were randomly assigned to a control group (CTR; n=5; male:female=1:4; room 1: 16 m^2) and to a treated group (LACTO; n=5; male:female=2:3; room 2: 16 m^2) receiving the same commercial dry diet. The LACTO group diet was supplemented with the probiotic (5 x 10^9 cfu/kg feed at least). A five-week experimental period was applied, and nutritional status was monitored by bodyweight (BW) and body condition score (BCS). Faecal quality was evaluated using faecal score (FS) and faecal moisture (FM) parameters. Plate counts of some faecal bacteria species were carried out. The data obtained were analysed using MIXED, GLM and NPAR1WAY procedures (SAS V.9.4; P≤0.05).

Results The two groups did not show differences in BW and BCS data. A clear effect of the probiotic supplementation on FM was recorded (LACTO 44 per cent v CTR group 46 per cent; P=0.04). FS in the LACTO group (3.35) was close to ideal values (2–3) in comparison with the CTR group (3.75). Positive effects of *L. acidophilus* D2/CSL have been recorded in terms of increase in faecal lactobacilli counts and reduction in faecal coli counts.

Conclusions This study's preliminary results describe how inclusion of *L. acidophilus* D2/CSL (CECT 4529) probiotic strain in cats' diets could effectively improve faecal quality parameters and consequently gut health in adult healthy cats.

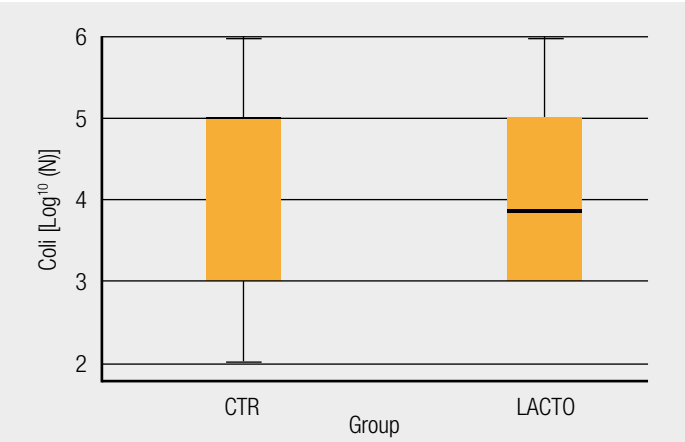

Figure 1. Box plot showing the effect of *Lactobacillus acidophilus* D2/CSL addition to diet on total coliform (Coli) in the overall period (P>0.10; Kruskal-Wallis test). CTR, control group; LACTO, treated group. *Reproduced from Figure 2 of the original publication.*

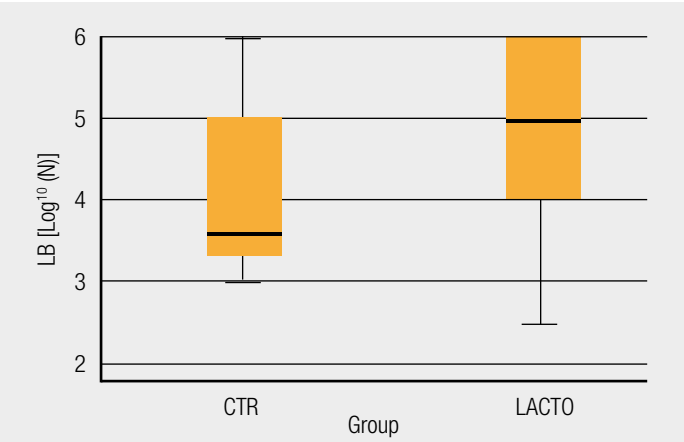

Figure 2. Box plot showing the effect of *Lactobacillus acidophilus* D2/CSL addition to diet on lactobacilli (LB) in the overall period (P>0.10; Kruskal-Wallis test). CTR, control group; LACTO, treated group. *Reproduced from Figure 3 of the original publication.*

KEY POINTS

Administration of *L. acidophilus* in cats induces:

- Improved faecal consistency
- Increase in faecal lactobacilli
- Reduction in faecal coliforms

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ORIGINAL RESEARCH

Effects of probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) on the nutritional and health status of boxer dogs

Stefano Paolo Marelli [1], Eleonora Fusi,[2] Alberto Giardini,[3] Piera Anna Martino,[1] Michele Polli,[1] Natascia Bruni,[4] Rita Rizzi[1]

ABSTRACT

Background The aim of the present study was to investigate the effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) probiotic strain on nutritional status and faecal and microbiological parameters in a group of purebred boxers.

Methods Forty healthy adult boxer dogs were randomly assigned to a treated (LACTO) group receiving a commercial diet supplemented with *L. acidophilus* D2/CSL (CECT 4529) to a final concentration of 5.0 x 10^9 colony-forming unit/kg of food, and a control (CTR) group receiving the same diet but without the probiotic (placebo). Nutritional status (body weight, skinfold thickness, body condition score) and faecal quality parameters were analysed.

Results No differences in body weight and skinfold thickness were found during the whole experimental period. Dogs in the LACTO group showed a significantly higher body condition score than those in the CTR group (4.86 ± 0.55 v 4.65 ± 0.65), and no significant differences were recorded in body weight and skinfold thickness. The LACTO group showed a significantly lower faecal moisture (in per cent) compared with the CTR group (0.67 ± 0.007 v 0.69 ± 0.007). Faecal hardness (in kg) was higher in the LACTO group than in the CTR group (0.86 ± 0.047 v 0.70 ± 0.051), and faecal score also improved in the LACTO group (3.78 ± 0.95 v 4.25 ± 0.91). A significant difference in total *Escherichia coli* counts as well as in lactobacilli counts between the CTR and LACTO groups was only detected at 28 days.

Conclusions Supplementation of *L. acidophilus* D2/CSL (CECT 4529) significantly improved the nutritional status and faecal parameters of dogs.

Table 1. Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on dog performance: least square means (±se) relative to CTR and LACTO groups for FM and FH. *Reproduced from Table 3 of the original publication.*

	Groups		
	CTR	LACTO	p-value
FM%			
Total period	0.69 ± 0.007	0.67 ± 0.007	0.0198
0 day	0.66 ± 0.013	0.68 ± 0.013	0.5169
7 days	0.71 ± 0.016	0.72 ± 0.012	0.3354
14 days	0.66 ± 0.012	0.63 ± 0.012	0.0756
21 days	0.70 ± 0.013	0.65 ± 0.012	0.0010
28 days	0.73 ± 0.013	0.68 ± 0.012	0.0040
35 days	0.69 ± 0.013	0.68 ± 0.012	0.7295
FH (kg)*			
Total period	0.70 ± 0.051	0.86 ± 0.047	0.0035
0 day	0.62 ± 0.066	0.69 ± 0.057	0.2958
7 days	0.49 ± 0.066	0.57 ± 0.057	0.2741
21 days	0.88 ± 0.066	1.11 ± 0.057	0.0024
35 days	0.82 ± 0.066	1.09 ± 0.057	0.0002

*Pressure related to a 4 x 4 cm plate.
CTR, control group; FH, faecal hardness; FM, faecal moisture; LACTO, treated group.

Table 2. Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on dog performance: least square means (±se) relative to faecal total coliform (coli) and lactobacilli (LB) counts. *Reproduced from Table 5 of the original publication.*

	Groups		
	CTR	LACTO	p-value
Coli [$\log^{10}$(N)]			
Total period	4.54 ± 0.24	4.71 ± 0.15	0.3053
7 days	4.16 ± 0.17	3.84 ± 0.17	0.1227
28 days	4.92 ± 0.16	5.59 ± 0.17	0.0023
LB [$\log^{10}$ (N)]			
28 days	4.50 ± 0.22	5.64 ± 0.26	0.0005

CTR, control group; LACTO, treated group

KEY POINTS

Administration of *L. acidophilus* in dogs induces:

- Improved nutritional status
- Improved faecal parameters
- Increased faecal lactobacilli
- Reduction of faecal coliforms

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Study of faecal parameters and body condition in dogs with a diet supplemented with *Lactobacillus acidophilus* D2/CSL (CECT 4529)

Natascia Bruni , Elisa Martello , Eleonora Fusi , Giorgia Meineri & Alberto Giardini

ABSTRACT

Aim of our case-control study is to evaluate the effects of a diet integrated with the probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) on the nutritional status and faecal consistency of healthy dogs belonging to the English Cocker Spaniel (ECS) and Labrador Retriever (LR) breeds. A total of 30 dogs were enrolled in this study, and they were randomly assigned to a Control (CTR, *n* = 14) and a Treated group (LACTO, *n* = 16). The trial consisted in a 7-days adaptation period where all the animals received the same commercial food, followed by a 35-days of data collection period where the LACTO group received the food supplemented with *Lactobacillus acidophilus* D2/CSL. We evaluated Body weight (BW), Body Condition Score (BCS) and Skinfold thickness, Faecal Score (FS) and Faecal Moisture (FM). All dogs in the LACTO group maintained an ideal BCS score during the whole experimental period compared to the CTR group. A significant decrease in skinfold thickness was found throughout the trial in the LACTO group. A significant improvement of the FM was recorded in the LACTO compared to the CTR group in the overall period for both dog breeds, and the FS significantly decreased in the LACTO group. Our results showed good maintenance of the nutritional conditions in dogs that are prone to overweight and a significant improvement of faecal parameters, meaning that even in healthy dogs with no gastrointestinal disorder the addition of this supplement to the diet helps to maintain the optimal balance of their intestinal microbiota.

Table 1. Effect of the addition of *Lactobacillus acidophilus* D2/CSL to diet on body weight (BW), skinfold thickness measured at the 4th cervical vertebra (NECK) and at the 7th/8th rib (THORAX), and faecal moisture (FM). *Reproduced from Table 1 of the original publication.*

	Labrador Retriever (n=15)			English Cocker Spaniel (n=15)		
	CTR	LACTO	p-value	CTR	LACTO	p-value
BW (kg)						
Overall period	29.90 ± 0.68	28.83 ± 0.56	0.2416	12.57 ± 0.96	12.61 ± 0.80	0.9780
T0	30.41 ± 0.84	28.70 ± 0.69	0.1154	12.62 ± 0.99	13.19 ± 0.82	0.6567
T1	30.41 ± 0.84	28.80 ± 0.69	0.1376	12.52 ± 0.99	12.46 ± 0.82	0.9646
T2	29.40 ± 0.84	28.91 ± 0.69	0.6518	12.52 ± 0.99	12.46 ± 0.82	0.9646
T3	29.40 ± 0.84	28.91 ± 0.69	0.6518	12.63 ± 0.99	12.31 ± 0.82	0.8056
Neck (mm)						
Overall period	15.09 ± 0.58	13.15 ± 0.48	0.0270*	5.33 ± 0.44	4.62 ± 0.37	0.2509
T0	15.09 ± 0.82	13.65 ± 0.67	0.1890	5.67 ± 0.62	4.85 ± 0.51	0.3283
T3	15.09 ± 0.82	12.65 ± 0.67	0.0355*	5.00 ± 0.62	4.40 ± 0.51	0.4719
Thorax (mm)						
Overall period	14.68 ± 0.56	12.96 ± 0.46	0.0374*	6.17 ± 0.42	4.96 ± 0.35	0.0592*
T0	15.02 ± 0.78	13.63 ± 0.64	0.1875	6.50 ± 0.60	5.29 ± 0.49	0.1429
T3	14.35 ± 0.78	12.29 ± 0.64	0.0601*	5.83 ± 0.60	4.63 ± 0.49	0.1429
FM (%)						
Overall period	0.68 ± 0.012	0.64 ± 0.010	0.0365*	0.66 ± 0.004	0.63 ± 0.004	0.0084*
T0	0.68 ± 0.019	0.65 ± 0.015	0.2157	0.65 ± 0.012	0.65 ± 0.010	0.9108
T1	0.64 ± 0.019	0.59 ± 0.015	0.0345*	0.60 ± 0.012	0.60 ± 0.010	0.7490
T2	0.72 ± 0.019	0.68 ± 0.015	0.0495*	0.71 ± 0.012	0.62 ± 0.010	<0.0001*
T3	0.67 ± 0.019	0.66 ± 0.015	0.5328	0.67 ± 0.012	0.66 ± 0.010	0.6372

Least square means ± SE from the performed mixed model in control (CTR) and treated (LACTO) groups. Results are presented for the entire trial period and for each experimental time. T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35). *p-values <.10 were considered significant.

Table 2. Effect of the addition of *Lactobacillus acidophilus* D2/CSL to diet on body condition score (BCS). Descriptive statistics and results from the Kruskall-Wallis test. *Reproduce from Table 2 of the original publication.*

				Median
Experimental period	Group	Mean	DS	25°;75°
Labrador retriever (n=15)				
Overall period	CTR	4.63	0.58	5.5[a] (4.0;5.0)
	LACTO	4.94	0.53	5.0[b] (5.0;5.0)
T0	CTR	4.83	0.41	5.0 (5.0;5.0)
	LACTO	4.89	0.33	5.0 (5.0;5.0)
T1	CTR	4.33	0.82	4.5[a] (4.0;5.0)
	LACTO	4.89	0.33	5.0[b] (5.0;5.0)
T2	CTR	4.67	0.52	5.0 (4.0;5.0)
	LACTO	5.00	0.71	5.0 (5.0;5.0)
T3	CTR	4.67	0.52	5.0 (4.0;5.0)
	LACTO	5.00	0.71	5.0 (5.0;5.0)
English Cocker Spaniel (n=15)				
Overall period	CTR	5.67	0.76	5.5[a] (5.0;6.0)
	LACTO	5.28	0.85	5.0[b] (5.0;6.0)
T0	CTR	5.50	0.55	5.5 (5.0;6.0)
	LACTO	5.44	0.53	5.0 (5.0;6.0)
T1	CTR	5.50	0.55	5.5 (5.0;6.0)
	LACTO	5.44	0.53	5.0 (5.0;6.0)
T2	CTR	6.50	0.84	7.0 (6.0;7.0)
	LACTO	5.55	1.33	5.0 (5.0;7.0)
T3	CTR	5.17	0.41	5.0[a] (5.0;5.0)
	LACTO	4.67	0.50	5.0[b] (4.0;5.0)

CTR: control group; LACTO: treated group; T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35). [a,b]Within each period, medians different if superscript differ (p<.10)

KEY POINTS

Administration of *L. acidophilus* in dogs induces:

- Improved faecal consistency
- Improved nutritional status
- Reduction in skinfold thickness

Focus

Alberto Giardini e Antonella Bonioli -CSL.- Centro Sperimentale del Latte Srl

A brief review of bacteriocins

Bacteriocins (BTCs), once called "colicins" (since they were discovered in *E. coli* strains), are bioactive peptides - antimicrobial agents - produced by particular strains within the same bacterial species.

The production of antimicrobial peptides is a first line of defense common to many species of living organisms - microbial, plant and animal - that is part of the so-called "innate" (or nonspecific) immunity. Antimicrobial peptides are relatively short (12-100 amino acids), positively charged (net charge +2 to +9) and amphiphilic.

BTC activity

BTCs are cationic and amphiphilic peptides: they can therefore interact with both the aqueous and lipid phases when they bind to the membrane surface of a susceptible bacterial cell, causing its functional destabilization, and subsequent death.

They normally have biocidal activity against susceptible cells, which are usually strains of Gram-positive bacteria. Cell death occurs rapidly and at low concentrations of the molecule.

The bactericidal property of a BTC on susceptible strains:

- can occur even at very low concentrations (example: Minimum lnhibitory Concentration, MIC = approximately 0.01 µg/mL for pediocin AcH vs. *Listeria monocytogenes*);
- increases at low pH, high temperature, in the presence of a surfactant, towards exponentially growing sensitive cells.

It should be noted that:

- BTC-resistant variants may occur in a population of a strain that is highly sensitive to a BTC, but this resistance is usually reversible after treatment is discontinued;
- stress conditions also render Gram-positive resistant strains and Gram-negative bacteria themselves susceptible to BTC;
- bacterial spores are resistant to BTC, but their vegetative cells may be sensitive (and germination may then be inhibited);
- the presence of high concentration anions in the medium may reduce the effectiveness of cationic compounds;
- various proteolytic enzymes can hydrolyze these peptides, causing them to lose their activity.

KEY POINTS ON BACTERIOCINES

- Antimicrobial peptides produced by certain bacterial strains
- Antimicrobial activity exerted by pore formation and disruption of the microorganism's cell membrane
- Efficacy demonstrated against many pathogenic Gram+ and some Gram- bacteria
- Very little action on eukaryotic cells and thus non toxic

molecules

MDPI

Review

Antimicrobial Activity of Lactoferrin-Related Peptides and Applications in Human and Veterinary Medicine

Natascia Bruni [1], Maria Teresa Capucchio [2], Elena Biasibetti [2], Enrica Pessione [3], Simona Cirrincione [3], Leonardo Giraudo [1], Antonio Corona [4] and Franco Dosio [5,*]

ABSTRACT

Antimicrobial peptides (AMPs) represent a vast array of molecules produced by virtually all living organisms as natural barriers against infection.

Among AMP sources, an interesting class regards the food-derived bioactive agents. The whey protein lactoferrin (Lf) is an iron-binding glycoprotein that plays a significant role in the innate immune system, and is considered as an important host defense molecule.

In search for novel antimicrobial agents, Lf offers a new source with potential pharmaceutical applications. The Lf-derived peptides Lf(1–11), lactoferricin (Lfcin) and lactoferrampin exhibit interesting and more potent antimicrobial actions than intact protein.

Particularly, Lfcin has demonstrated strong antibacterial, anti-fungal and antiparasitic activity with promising applications both in human and veterinary diseases (from ocular infections to osteo-articular, gastrointestinal and dermatological diseases).

Table 1. Synergistic effects of Lf AMPs. Edited from *Table 4 of the original publication.*

Lf Peptide	Drug	Bacterial, Fungal, Parasite Species
Lf (1-11)	Fluconazole	*Candida* sp.
bLfcin	Clotrimazole, ketoconazole, itraconazole, fluconazole	*C. albicans*
	Cecropin A, aureomycin	*E. coli*
	Aureomycin	*S. aureus*
	Fluconazole, itraconazole	*C. albicans*
	Erythromycin	*E. coli*
	Ciprofloxacin, ceftazidime, gentamicin	*S. aureus, E. coli*
	Ciprofloxacin, norfloxacin	*E. coli*
	Minocycline, acid cholic, cysteine, various acylglycerols, β-cyclodextrin	*S. aureus*
	Metronidazole	*Entamoeba histolytica*
	Nisin, Lf	*E. coli, S. epidermidis*
Lfampin	Ampicillin	*S. aureus*

KEY POINTS ON ANTIMICROBIAL PEPTIDES

- Antimicrobial activity exerted by pore formation and disruption of the microorganism's cell membrane
- Proven efficacy on many pathogenic Gram+ and Gram- bacteria, fungi and parasites
- Very little action on eukaryotic cells and therefore no toxicity
- Synergistic action with antibiotics and antifungals
- Proven applications in ocular, orthopedic, dermatological and gastroenterological fields

BACTERIOCINS AND ANTIMICROBIAL PEPTIDES:

RECENT ADVANCES AND NEW HORIZONS FOR THE INTESTINAL WELL-BEING OF DOGS AND CATS

Prof. Giorgia Meineri
Department of Veterinary Sciences - University of Turin

BACTERIOCINS AND ANTIMICROBIAL PEPTIDES

Beneficial gut bacteria are capable to activate an extraordinary variety of defense mechanisms. These systems include the by-products of their metabolism known as "bacteriocins."

This "biological arsenal" is impressive not only for its diversity, but also for its natural abundance ...

... Bacteriocins are an ideal first line of defense against pathogens because of their rapid production and action. The most widely used bacteriocins are those produced by lactic acid bacteria because they are considered safe for human health by the Food and Drug Administration (FDA) ...

NISIN

Nisin acts against pathogenic bacteria through two mechanisms of action: it promotes the formation of pores and ion channels in the cytoplasmic membrane of the attacked cell (permeabilization of the cell membrane) and interferes with the synthesis of the cellular part ...

... Nisin is harmless toward the intestinal cells of the animal organism. Due to the maximum stability of bacteriocins under acidic conditions, the activity of nisin is unaffected by the acidic pH of the stomach, thus performing its function at the intestinal level ...

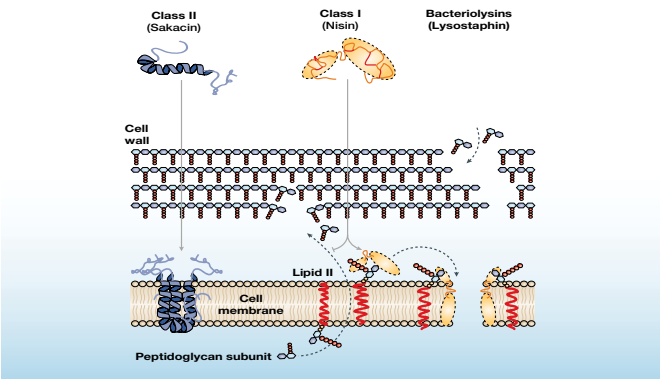


Figure 1. Mode of action of three different bacteriocins. Nisin, class I, has a dual mode of action, being able to bind to lipid II or form pores in the cell membrane. Class II bacteriocins such as sakacin, with a helical structure, insert into the membrane leading to depolarization and cell death. Class III bacteriorolysins can act directly on the cell wall, leading to lysis and cell death. *Reproduced from Figure 2 of the original publication.*

LACTOFERRICIN (LFCIN)

Lactoferrin is the source of Lactoferricin (Lfcin), a peptide metabolite derived from the digestion of Lactoferrin by gastric proteolytic enzymes...

... Lfcin is an even more potent antibacterial and antifungal agent than Lactoferrin. The electrically charged portion of Lfcin interacts with the lipophilic portion of the bacterial cell membrane by incorporating into it and destabilizing its phospholipid structure ...

... Lfcin also increases the release of interleukin-8 from polymorphonuclear leukocytes, suggesting its role in suppressing the inflammatory effects caused by bacteria and a marked immunomodulatory function ...

... Lfcin could be used to replace antibiotic therapy by exploiting its selective antimicrobial action and probiotic effect as an immune modulator.

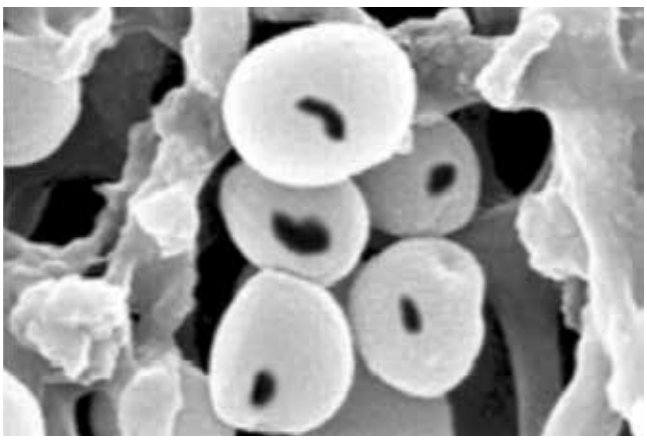


Figure 2. Bacterial wall damage after 1 min contact with product containing *Lactoferricin B.* Reproduced from *Figure 4 of the original publication.*

Study on Florentero® ACT

RESEARCH ARTICLE Open Access

Effects of a synbiotic on fecal quality, short-chain fatty acid concentrations, and the microbiome of healthy sled dogs

Jason W Gagné[1], Joseph J Wakshlag[1,5*], Kenneth W Simpson[1], Scot E Dowd[2], Shalini Latchman[1], Dawn A Brown[3], Kit Brown[3], Kelly S Swanson[4] and George C Fahey Jr[4]

ABSTRACT

Background Sled dogs commonly suffer from diarrhea. Although multiple etiologies exist there are limited field studies using synbiotics as a supplement to prevent or treat diarrhea. The objective of this study was to examine alterations in fecal quality, short-chain fatty acids (SCFA), and the fecal microbiome in two groups of training sled dogs fed a synbiotic or microcrystalline cellulose placebo. Twenty clinically healthy training sled dogs randomized into two cohorts (9 synbiotic-fed, 8 placebo-fed) for a 6 week prospective study were examined. Fecal pH and fecal short chain fatty acid (SCFA) concentrations were measured and tag-encoded FLX 16S rDNA amplicon pyrosequencing (bTEFAP) and quantitative real-time PCR were performed at baseline (10 d prior to the study) and after 2 weeks of treatment with a total treatment time of 6 weeks. Fecal scores for all dogs were assessed at baseline and every day for 6 wk after initiation of treatment.

Results Alterations in the fecal microbiome were observed with a significant rise in *Lactobacillaceae* in the synbiotic group (P = 0.004) after 2 wk of treatment. A positive correlation was found between *Lactobacillaceae* and overall butyrate concentration (R = 0.62, p = 0.011) in all dogs. After 5 wk of treatment, there was an improved fecal score and fewer days of diarrhea (X^2 = 5.482, P = 0.019) in the dogs given synbiotic, which coincided with a presumed contagious outbreak shared by all dogs in the study.

Conclusions Use of this synbiotic results in an increase in presumed beneficial bacterial flora of the host colon which was associated with a decrease in the prevalence of diarrhea in training sled dogs.

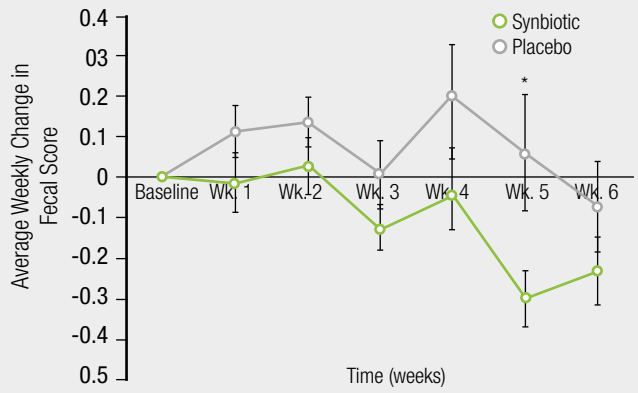


Figure 1. Mean weekly change and standard deviation in group fecal scores from baseline for 6 weeks after initiation of placebo or synbiotic treatment. Initial mean score for placebo was 2.91 and 3.06 for synbiotic. *indicates a $p < 0.05$ for the indicated time point. *Reproduced from Figure 1 of the original publication.*

Table 1. Quantitative real-time PCR results expressed as percent (medians and ranges) of microbial genera in the feces of synbiotic-fed dogs at baseline and after 2 wk of treatment. *Reproduced from Table 3 of the original publication.*

Genera	Synbiotic group (n=9) % at baseline	Synbiotic group (n=9) % after 2 wk of treatment	Synbiotic group (n=9) p-value
Lactobacillus spp.	3.71 (0.47-20.15)	16.04 (0.42-26.82)	0.02
Bifidobacterium spp.	0.02 (0.00-0.12)	0.31 (0.02-1.27)	0.01
Enterococcus spp.	0.05 (0.00-0.79)	0.12 (0.03-0.43)	0.95

Table 2. Quantitative real-time PCR results expressed as percent (medians and ranges) of microbial genera in the feces of placebo-fed dogs at baseline and after 2 weeks of treatment. *Reproduced from Table 4 of the original publication.*

Genera	Placebo group (n=8) % at baseline	Placebo group (n=8) % after 2 wk of treatment	Placebo group (n=8) p-value
Lactobacillus spp.	10.07 (0.30-23.18)	10.78 (1.97-28.64)	0.84
Bifidobacterium spp.	0.10 (0.00-0.33)	0.09 (0.00-0.95)	0.47
Enterococcus spp.	0.02 (0.00-0.40)	0.01 (0.00-0.04)	0.19

KEY POINTS

- Treated dogs have a shorter duration of diarrhea than untreated dogs
- The population of lactobacilli and bifidobacteria in the intestines of treated dogs increases significantly after 2 weeks of administration

Study on Florentero® ACT

Year 31, n° 3, June 2017
VETERINARIA

Evaluation of the efficacy of a commercial probiotic formulation containing *Enterococcus faecium* in cases of acute diarrhea

Enrico Bottero, Giuseppe Innella, Simona Astorina, Elena Benvenuti, Piero Ruggiero, Nicola Di Girolamo, Mauro Bigliati, Natascia Bruni, Francesca Tosti

ABSTRACT

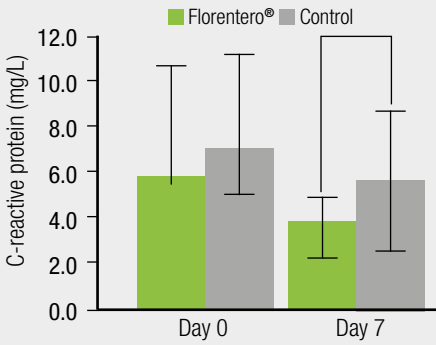
Introduction and aim of the study Acute diarrhea is a common clinical finding in the outpatient setting, but is often not attributable to a specific cause. The aims of the present study was to evaluate the efficacy of a commercial probiotic formulation consisting of *Enterococcus faecium* during acute diarrhea.

Materials and methods Sixty-two owned dogs with acute diarrhea of less than 5 days duration. Report, history and clinical examination data were collected with special reference to the characteristics and frequency of diarrhea, presence of hematochezia and flatulence/meteorism. In addition, coprological examination, urinalysis, protidogram, blood-biochemical profile including C-reactive protein evaluation, and radiographic and/or ultrasound examination of the abdomen were performed. Fecal cytology was performed for all included cases. Thirty-four subjects received probiotic therapy combined with a hyperdigestible diet while 28 subjects received a hyperdigestible diet alone. Subjects were monitored for 7 days.

Results and discussion Complete resolution of the clinical signs was observed in 79% of probiotic-treated patients within a mean time of 4 days, while in the control group this occurred in only 43% of cases; serum C-reactive protein concentrations were lower than in patients treated with hyperdigestible diet alone. Therefore, the use of probiotics has been shown to be useful for rapid improvement of clinical signs in acute diarrhea.

Table 1. Clinical data of patients at baseline and after 7 days. For continuous variables, mean and SD are reported; for categorical variables, the number of positives and the percentage in the total group are reported. *Reproduced from Table 2 of the original publication.*

		Florentero®	Control
Frequency stools/day	Day 0	5 ± 1.8	4 ± 1.4
	Day 7	2 ± 0.7	2 ± 1.2
Fecal Score	Day 0	4 ± 0.5	4 ± 0.68
	Day 7	3.14 ± 0.85	2.6 ± 0.7
Hematochezia (Number of positives and percentage of total treated subjects)	Day 0	11 (32.4%)	10 (35.7%)
	Day 7	0 (0%)	1 (3.6%)
Meteorism/Flatulence (Number of positives and percentage of total treated subjects)	Day 0	25 (73%)	20 (71%)
	Day 7	1 (2.9%)	3 (11.1%)
Days required for clinical resolution		4 ± 1	6 ± 2.6



Mean difference: 3.7 mg/L (CI 95%: 0.3-7.0; p=0.04)

Figure 1. Changes in blood concentration of C-reactive protein on day 0 and on day 7. *Reproduced from Figure 1 of the original publication.*

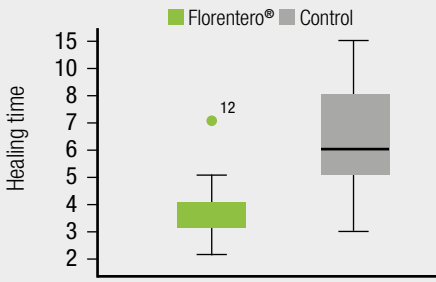


Figure 2. Boxplots of healing times in dogs treated with probiotic and dogs treated with hyperdigestible diet. *Reproduced from Figure 2 of the original publication.*

KEY POINTS

- Treated dogs show clinically recover in a shorter time
- They also show marked improvement in fecal parameters and serum concentration of C-reactive protein
- Reduction in fecal neutrophilia supports the beneficial effect on the gut microbiota

Study on Florentero® ACT

frontiers in Veterinary Science

ORIGINAL RESEARCH
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Pre- and Probiotics to Increase the Immune Power of Colostrum in Dogs

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ABSTRACT

Wide differences in Ig concentration in canine colostrum have been reported. Thus, some litters can be at risk of passive immune transfer failure. Present study evaluated if supplementation with MOS, FOS, *E. faecium* and *L. acidophilus* along pregnancy increases colostrum quality. Twenty Great Dane bitches were divided into 4 groups. Control group (CG) received standard diet, only. Diet was supplemented with pre- and probiotics in other 3 study groups during: the last (1WG), last 2 (2WG), and last 4 (4WG) weeks of pregnancy, until parturition. Serum samples were collected at estrous (T0), supplementation beginning (T1), and parturition (T2). Colostrum was collected at C-section end. The IgG, IgM, and IgA were assayed on both matrices. In serum, IgG were higher at T0 than at parturition in all study groups and they significantly lowered from T0 to T1 in all groups. In colostrum, IgG and IgM were significantly higher in 4WG, while IgA already increased in 2WG group.

Conclusions Four-week pre- and probiotic supplementation resulted in the best immune properties of colostrum, as by the higher IgG, IgM, and IgA colostrum levels found in 4WG. Further studies would verify the exact mechanisms involved: pre-partum IgG mammary accumulation and B-cells GALT proliferation and mammary transfer.

Table 1. Ig concentration in serum of bitches enrolled in the 4 study groups at T0, T1, and T2. *Reproduced from Table 2 of the original publication.*

mg/dl Time	T0	T1	T2
IgG			
Control group	538.53 ± 169.54^{a}		302.19 ± 128.26^{b}
1WG	558.78 ± 138.59^{a}	414.45 ± 266.65^{ab}	320.40 ± 193.75^{b}
2WG	519.03 ± 61.06^{a}	411.93 ± 221.52^{ab}	318.05 ± 127.44^{b}
4WG	588.25 ± 55.82^{a}	443.10 ± 277.14^{ab}	366.50 ± 184.31^{b}
IgM			
Control group	131.78 ± 24.67^{a}		130.71 ± 21.92^{a}
1WG	132.05 ± 18.75^{a}	127.08 ± 20.68^{a}	154.37 ± 12.23^{a}
2WG	128.75 ± 20.15^{a}	135.87 ± 8.61^{a}	148.35 ± 23.89^{a}
4WG	133.70 ± 22.63^{a}	127.33 ± 28.86^{a}	143.46 ± 16.43^{a}
IgA			
Control group	32.15 ± 7.31^{a}		33.35 ± 2.98^{a}
1WG	30.75 ± 1.45^{a}	31.33 ± 0.58^{a}	30.88 ± 1.87^{a}
2WG	31.40 ± 2.65^{a}	32.51 ± 3.94^{a}	31.12 ± 3.23^{a}
4WG	30.45 ± 4.05^{a}	33.84 ± 2.95^{a}	27.06 ± 9.03^{a}

T0, mating time; T1, diet supplementation beginning; T2, parturition. Different superscript letters denote statistical differences within rows for each Ig category. No statistically significant differences were found within columns for any Ig category.
WG, weeks of pregnancy.

Table 2. Ig concentration in colostrum of bitches enrolled in the 4 study groups. *Reproduced from Table 3 of the original publication.*

mg/ml (g/l)	IgG	IgM	IgA
Control group	21.06 ± 5.83^{a}	1.21 ± 0.18^{a}	10.84 ± 3.17^{a}
1WG	21.90 ± 3.88^{a}	1.23 ± 0.04^{ab}	11.24 ± 2.47^{ab}
2WG	29.20 ± 18.36^{ab}	1.23 ± 0.08^{ab}	15.1 ± 1.33^{b}
4WG	46.51 ± 17.85^{b}	1.43 ± 0.09^{b}	15.07 ± 1.57^{b}

Different superscripts denote statistical differences within columns.
WG, weeks of pregnancy.

KEY POINTS

- Administration of FLORENTERO® ACT during pregnancy induces a significant increase in the content of immunoglobulins A, M and G in the colostrum of bitches
- IgA significantly increases in the 2WG (2 weeks of treatment) and 4WG (4 weeks of treatment) groups
- IgM and IgG significantly increase in the 4WG group (4 weeks of treatment)

Abstract EVIDENCE POINTS

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BRIEF RESEARCH REPORT
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A Biotic Support During Pregnancy to Strengthen the Gastrointestinal Performance in Puppies

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ABSTRACT

Up to 60% of neonates can be affected by gastroenteritis due to specific pathogens or aspecific polymicrobial interactions. The present study evaluated if a dietary supplementation with MOS, FOS, *E. faecium* and *L. acidophilus* in pregnancy may reduce gastroenteritis in puppies. Fifteen Great Danes were divided in 3 groups. The control group (CG) ate a standard diet. In 2 study groups, the diet was supplemented with pre- and probiotics during the last (1WG) and the last 4 pregnancy weeks (4WG). Up to 9 weeks, puppies were checked daily to identify first- or second- presentation gastroenteritis. Data were processed by χ^2 ($P < 0.05$). First-presentation gastroenteritis was more frequent in CG than in 1WG than in 4WG. Second-presentation gastroenteritis was more frequent in CG than in 1 and 4WG. Puppies from pre- and probiotics supplemented bitches were less prone to gastroenteritis. 1 or 4WG equally reduced second-presentation gastroenteritis in puppies, but 4WG was better than 1WG on first-presentation gastroenteritis. By entero-mammary link, supplemented bitches produced higher immune quality colostrum, thus puppies faced immunitary challenges better; moreover, maternal microbiota, positively altered by supplementation, was transferred to newborns, becoming more resistant to gastroenteritis. This information can be useful in clinical practice with the goal of preventing gastroenteritis in puppies and reducing its prevalence and severity.

Table 1. Number of puppies in each litter in the 3 study groups. *Reproduced from Table 1 of the original publication.*

Litters	1	2	3	4	5	N° total	Mean±SD
Control group (CG)	6	7	12	4	2	31	6.2 ± 3.77*
1 week (1WG)	1	10	13	2	4	30	6 ± 5.24*
4 week (4WG)	7	5	9	4	7	32	6.4 ± 1.95*

Equal superscripts (*) denote the absence of any statistical difference within the mean number of puppies in the 3 study groups ($p > 0.05$).

Table 2. Intestinal episodes in the 3 study groups. *Reproduced from Table 2 of the original publication.*

Intestinal episodes	1 N (%)	2 N (%)
Control group (CG)	23/31(74.2)*	10/31 (32.3)*
1 week (1WG)	12/30 (40.0)**	0/30 (0)**
4 week (4WG)	5/32 (15.6)***	1/32 (3.1)**

Different superscripts (*, **, ***) denote statistical differences within columns ($p < 0.05$)

KEY POINTS

- Administration of FLORENTERO® ACT during pregnancy induces a significant reduction in gastroenteritis episodes in puppies
- This occurs due to improved immune quality of colostrum and from the transfer of maternal microbiota modified by dietary supplementation

Florentero® ACT

Florentero® Act *is a dietetic complementary feed for particular nutritional purposes for dogs and cats*

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