The Use of Probiotics in the Diet of Dogs[1]

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EXPANDED ABSTRACT

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Probiotics are microorganisms such as bacteria or yeast that can be added to the food with the purpose of regulating the intestinal flora of the host (Parker 1974). Probiotics have been used therapeutically in the treatment of diarrhea or prophylactically in humans and animals to minimize drift in the composition of the intestinal microflora associated with antibiotherapy or traveler's gastroenteritis (Barrows and Deam 1985, Lestradet 1995, Van De Kerkove 1979).

More recently, probiotics have also been found to have beneficial effects on the health of the host (Fuller 1989). The mechanisms are not completely understood, but they could be due to the ability of probiotics to act as regulators of the intestinal microflora as a source of digestive enzymes and/or stimulating factors on the immune system (Lestradet 1994). This is the rationale behind the use of probiotics in the feed of farm animals, i.e., to improve their breeding performance despite all of the stresses (high animal concentration, early weaning or rapid growth) associated with modern husbandry (Lestradet 1994).

Weaning, a new home and dietary changes are all conditions that are known to affect the intestinal microflora of dogs and for which probiotics might be beneficial. Probiotics might also be of benefit to dogs living in a large colony or sold in pet shops in which animal concentration, pressure of infection and stress can significantly affect animal resistance to disease.

Paciflor is a patented strain of *Bacillus* recorded at the Pasteur Institute under the number CIP 5832 and marketed as a feed additive in animal nutrition (Lestradet 1995). The *bacillus* is commercialized in its sporulated form, allowing a better resistance to heat and better stability over time. *Bacillus* CIP 5832 has been shown to have beneficial effects on the survival of mice infected with *Klebsiella pneumoniae* and on the breeding performances of rabbits, pigs, chickens, turkeys, ducks, calves and horses (Lestradet 1995). *Bacillus* CIP 5832 is also the active ingredient in Bactisubtil (Merrell, Neuilly-sur-Seine, France) a drug approved in France in the 1950s and recommended in infants against antibiotic-induced diarrhea (Lestradet 1995).

A series of pilot studies were undertaken to evaluate the feasibility of including a probiotic in dry dog foods, to determine the kinetics of *Bacillus* CIP 5832 in vivo in dogs and to assess its effects on diet digestibility.

Materials and methods. *Bacillus* CIP 5832 is commercially available in its sporulated form as a powder (Paciflor, Prodeta, Vannes, France) at the concentration of 10^{10} colony forming units (CFU)/g. The recommended dosage in the food is 10^6 CFU/g.

A first set of experiments was planned to assess the feasibility of including *Bacillus* CIP 5832 in a dry dog food. In the first experiment, the probiotic was added to the meal of a commercial diet [RCCI M25 (protein 250 g/kg, fat 120 g/kg, minerals 70 g/kg and dietary fibers 65 g/kg), Royal Canin, Aimargues, France] before expansion-extrusion at a dose of 10^6 CFU/g of meal. Spore survival was evaluated in the meal and the expanded diet. In a second experiment, survival was evaluated with the probiotic added to a powder (500 g/kg brewers yeast-500 g/kg lactalbumen) to obtain a final concentration 10^8 CFU/g of powder. This powder was then coated (10 g/kg of diet) on four batches of a pilot diet (protein 320 g/kg, fat 240 g/kg, minerals 69 g/kg and dietary fiber 55 g/kg) after expansion-extrusion and drying. Samples of these four batches were then followed over 1 y to evaluate *Bacillus* survival over time. *Bacillus* CIP 5832 concentration on five samples of each diet was determined in 10 g of ground diet as described in Michard and Levesque (1989). For the survival study, samples of the batches were kept in commercial packaging at room temperature in a dry, well-ventilated warehouse. Spore survival was evaluated after 0, 6, 9 and 12 mo.

In the second set of experiments, the kinetics of *Bacillus* CIP 5832 in dogs was evaluated. Five female spayed dogs (2 German Pointers and 3 German Shepherds, age 5–10 y, weight 24 ± 3 kg) belonging to the dog colony of the Royal Canin Research Center were used. The colony is approved and regularly inspected by veterinarians of the French "Direction des Services Vétérinaires."

For all of the studies, *Bacillus* CIP 5832 was mixed at a concentration of 1.5×10^8 CFU/g to a powder made by grinding the same commercial diet as the one used for feeding

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TABLE 1

Effects of Process and Time on the Survival of Bacillus CIP 5832 Added to Four Batches of Commercial Dry Dog Food as a Powder Coating

	Expected concentration in the batch[1]	Concentration found in the diet in 10^6 colony forming units/ g of diet[2] after			
		0 mo	6 mo	9 mo	12 mo
Batch 1	1.3	1.1 ± 0.3	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.4
Batch 2	1.6	0.8 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
Batch 3	1.2	0.8 ± 0.1	0.7 ± 0.3	0.5 ± 0.1	0.6 ± 0.1
Batch 4	1.5	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
Mean loss (%)		46*	17**	23**	25**

1 Calculated from Bacillus CIP 5832 concentration in the coating powder
2 Results are the mean ± SEM for 5 samples.
*,** Compared with expected concentration and mo 0, respectively.

the dogs (RCCI M25, Royal Canin, Aimargues, France). *Bacillus* concentrations in the mix were checked before, during and after the study as described above. To evaluate the delay of appearance and disappearance of *Bacillus* CIP 5832 in the feces, 5 g of the mix was added or no longer added to the daily meal (493 ± 28 g of kibbles/d, thus ± 1.5×10^6 CFU/g diet). All feces were collected 1 d before and 7 d after *Bacillus* CIP 5832 supplementation or removal. Fecal samples were individually frozen (−18°C) pending analysis, and *Bacillus* concentration was determined before (spores + vegetative forms) and after heat treatment (80°C for 10 min, spores only) as described in Michard and Levesque (1989). Before the disappearance study, dogs had been supplemented for 3 wk with the *Bacillus* CIP 5832. For the *Bacillus* CIP 5832 balance study, the diet of the dogs was supplemented with 5 g of the probiotic mix as described above, all feces were collected and pooled for each dogs over a 5-d period and frozen pending analysis. Dogs had been supplemented with *Bacillus* CIP 5832 for 2 wk before the study.

Dry matter, protein, lipid and energy digestibility studies were conducted according to the American Association of Feed Control Officials (AAFCO 1997) protocol without or with the daily addition of 7.5×10^8 CFU of *Bacillus* CIP 5832.

All results are expressed as the mean ± standard error (SEM). Digestibility results were compared using a paired Student's *t* test; $P < 0.05$ was considered to be significant.

Results and discussion. The first set of experiments was intended to prove that *Bacillus* CIP5832 could be added to a dry dog food and to evaluate the loss of viable spores over a 12-mo shelf life.

Resistance of Bacillus CIP 5832 to expansion-extrusion and drying. *Bacillus* CIP 5832 concentration in the meal and in the expanded product was 1.10 ± 0.04 × 10^6 and 0.02 ± 0.05 × 10^6 CFU/g, respectively. The extrusion-expansion and drying process resulted thus in the loss of >99 % of the spores. *Bacillus* CIP 5832 should thus not be included in the diet before the extrusion- expansion and drying process.

Resistance of Bacillus CIP 5832 when applied as a powder coating. After powder coating, the observed level of spores was ±60% of the expected levels in four different batches (**Table 1**). These relatively high losses may be due in part to spores trapped within the lipid fraction of the diet when the ground food is mixed in 0.2% sodium hydroxide as the first step of the bacteriological count. However, despite these apparent losses, powder coating remains an efficient and convenient way to add *Bacillus* CIP 5832 to the diet.

Spore survival over time. Follow-up of four different batches over a 12-mo period was associated with a loss of spores of <25% compared with the bacteriological count just after processing (Table 1). These three pilot studies confirmed that addition of *Bacillus* CIP 5832 to a dry dog food is feasible.

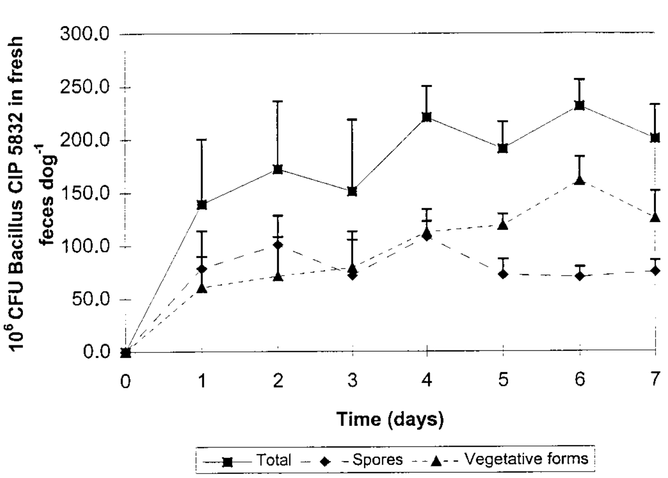

FIGURE 1 Total *Bacillus* CIP 5832 count in the fresh feces of five dogs after dietary supplementation [d 0, 7.5 x 10^8 CFU/(dog · d)]. Results are expressed as a mean ± SEM.

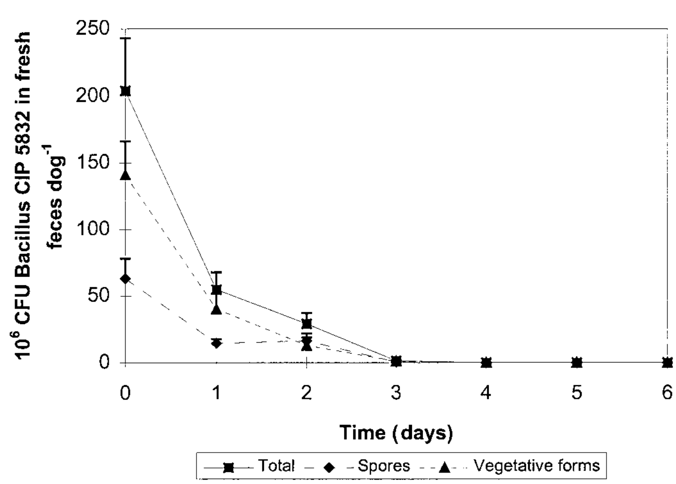

FIGURE 2 Total *Bacillus* CIP 5832 count in the feces of five dogs after stopping dietary supplementation (d 0). Results are expressed as a mean ± SEM.

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TABLE 2

Dry Matter, Protein, Lipid and Energy Digestibilities in Five Dogs Supplemented or Not with 7.5 × 10^{6} *CFU of Bacillus* CIP 5832

	Bacillus CIP 5832	
	−	+
	Digestibility in %[1]	
Dry matter	86.4 ± 0.4	86.9 ± 0.3
Protein	83.4 ± 0.8	84.2 ± 0.6
Lipids	95.9 ± 0.2	95.6 ± 0.3
Energy	83.9 ± 0.3	84.1 ± 0.2

[1] Results are expressed as means ± SEM. There was no significant difference due to *Bacillus* CIP 5832.

The second set of experiments was intended to prove that *Bacillus* CIP 5832 could survive in the gastrointestinal tract of dogs as well as to determine the time required for its appearance and disappearance in the feces. Dogs consumed all of their food as well as the mix containing the spores in all studies.

Delay of appearance of Bacillus CIP 5832 in the feces. When the probiotic was added to the diet, spores and vegetative forms were detected in the feces within 24 h and reached a plateau within 2 and 4 d, respectively (**Fig. 1**).

Delay of disappearance of Bacillus CIP 5832 from the feces. When the probiotic was withdrawn from the diet, spores and vegetative forms could not longer be detected after 3 d (**Fig. 2**).

Bacillus CIP 5832 balance. The balance study showed that 29.6 ± 5.6% of the *Bacillus* CIP 5832 ingested was found in the feces of the five dogs; 69.9 ± 3.5% was in vegetative form. As expected from studies in other species, *Bacillus* CIP 5832 will thus survive and germinate in the gastrointestinal tract of dogs but will not persist if not fed continuously (Lestradet 1995).

Effect of Bacillus CIP 5832 on dry matter, protein, lipid and metabolizable energy digestibility. Although the digestibilities appeared slightly improved with the probiotic, the difference were not significant (**Table 2**).

In summary, these studies demonstrated that the addition of *Bacillus* CIP 5832 to a dry dog food was feasible under certain circumstances and that *Bacillus* CIP 5832 will survive and germinate in the gastrointestinal tract of dogs.

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