Diarrhea is defined as an increase in fecal water content, which usually leads to changes in fecal volume, fluidity, and frequency of defecation. Kenneling increases the likelihood of dogs developing diarrhea, and diarrhea is reported as the most common disease of dogs housed in animal shelters. Diarrhea has a direct negative impact on the welfare of kennelled dogs and also may lead to extended time periods in the animal shelter as affected animals cannot be rehomed until they are producing normal feces. In addition, there is a financial burden on the animal shelter resulting from delayed rehoming, veterinary interventions, and additional cleaning.

Probiotics are live microorganisms that, when delivered in adequate amounts, confer a health benefit to the host. Prebiotics are ingredients selectively fermented in the gastrointestinal tract to allow specific changes, in the composition, activity, or both in the gastrointestinal microbiota, conferring benefits upon host well-being and health. When a probiotic and a prebiotic are given together, the combination is called a synbiotic, suggesting a synergistic relationship between the 2. There are several proposed mechanisms to the underlying antagonistic effects of probiotics on various microorganisms, including the following: modification of the gut microbiota, competitive adherence to the epithelial mucosa, strengthening of the gut epithelial barrier, and modulation of the immune system to convey an advantage to the host. These proposed probiotic mechanisms of action could have a clinically relevant impact on treatment and prevention of acute diarrhea.

Two previous clinical trials have investigated the use of probiotic supplementation for the prevention of diarrhea in kenneled dogs. In 1 trial that used different doses of probiotic, significantly fewer supplemented dogs passed unacceptable (loose or diarrheic) feces in the first week of relocation to a kennel facility and during their total stay, when compared to the control group. Another study failed to obtain statistically significant evidence to accept their hypothesis that kenneled dogs fed a probiotic would be less likely to have diarrhea of 2 days duration than those given placebo, and this study frequently is referenced in review articles about probiotics when discussing the efficacy of probiotics on gastrointestinal disease in dogs. Yet of the 182 dogs recruited in the trial, only 2 (1 in each group) had diarrhea of 2 days’ duration. With this incidence of diarrhea in the placebo group (1.25% of dogs), 18,608 dogs would have been required to detect a 33% reduction in ≥2 days’ diarrhea, with a significance level of 0.05 and a power of 80%. Therefore, it is not possible to draw conclusions about probiotic efficacy in kenneled dogs from this trial because of the low incidence of diarrhea.

Diarrhea is the most frequent morbidity affecting kenneled dogs in animal shelters. Diarrhea impacts animal welfare and the finances of the shelter as they must treat, clean, and house affected animals until recovered. 

Hypothesis/Objectives: Supplementing dogs entering an animal shelter with a probiotic-prebiotic, known as a synbiotic, will decrease the incidence of diarrhea. 

Animals: Seven hundred and seventy-three dogs entering an animal shelter in the United Kingdom. 

Methods: A prospective double-blind, randomized, placebo-controlled trial. 

Results: Statistical difference was found between the groups across 3 measures of diarrhea incidence. First, the mean percentage of scored days per dog that were scored as diarrhea throughout their stay was 2.0% in the synbiotic group and 3.2% in the placebo group (P = .0022). Second, the occurrence of diarrhea within the first 14 days’ stay was 18.8% in the synbiotic product group and 27.2% in the placebo group (P = .0008). Third, the occurrence of ≥2 consecutive days of diarrhea within the first 14 days’ stay was 4.6% in the synbiotic product group and 8.0% in the placebo group (P = .0300).

Conclusions and clinical importance: Supplementing healthy dogs entering an animal shelter with a synbiotic supplement significantly decreased the incidence of diarrhea in this trial. Animal shelters can use synbiotic supplements to improve animal welfare and decrease costs involved in cleaning and housing animals as well as potentially decreasing veterinary intervention.

Key words: Canine; Diarrhea and vomiting; Enterococcus faecium; Gastrointestinal; Gastroenterology; Nutrition.
The aim of our study was to test the hypothesis that prophylactic treatment of dogs in an animal shelter with a synbiotic supplement would decrease the incidence of diarrhea compared with a control group.

Materials and Methods

A prospective, randomized, double-blind placebo-controlled trial was designed for dogs entering Wood Green, The Animals Charity, Godmanchester, for which ethical approval was obtained from the University of Cambridge Ethics and Welfare Committee (reference CR93).

All dogs entering the animal shelter were considered for inclusion in the trial. Animals were excluded if they left the shelter before kennel assignment and randomization (i.e., strays that were reclaimed by their owners). All dogs entering the shelter were examined by a veterinarian, and all dogs with abnormal clinical findings suggesting preexisting disease or with a history of chronic or recurrent diarrhea were excluded from the trial (see Fig 1).

On admission to the animal shelter, dogs were housed in an isolation block for 7–10 days before being moved to a main kenneling site that could hold a maximum of 100 dogs. Dogs were fed twice daily by a variety of donated foods or a prescribed diet if the veterinarian deemed doing so necessary. During kennel assignment, dogs were randomized to receive either synbiotic or placebo capsules by taking a randomly selected colored plastic disc from an opaque bag. The bag contained fifty identical plastic discs with equal numbers of 2 different colors; each color assigned to a specific group. Once a counter was selected, it was placed back in the bag. When 2 dogs entered the shelter as a pair, they shared a kennel and both animals were assigned to the same treatment group.

The synbiotic and placebo capsules were produced by Protexin Veterinary and matched for color, size, texture, and packaging. They were differentiated only by capsule A or capsule B printed on the blister foil. The active capsule was a commercially available product containing Enterococcus faecium NCIMB 10415 4b1707, $2 \times 10^{9} \text{ colony-forming units (cfu)/capsule, and 46.4 mg/capsule Preplex^a prebiotic, a combination of fructo-oligosaccharide (FOS) and acacia (gum arabic)^9.}$ The placebo contained 180 mg maltoolxtrin, a complex carbohydrate commonly used as an inert additive. One capsule was opened and sprinkled on each dog’s food once daily, at the evening meal. Supplementation began at the first evening meal after randomization and continued throughout the dog’s stay. All staff at the animal shelter and the monitor were blinded to capsule contents. The unblinding key was held by a third party with no other role in the trial.

Dogs had their fecal consistency score recorded on a daily health sheet throughout the duration of their stay at the shelter. Any results from the day of randomization were not included in analysis because it was not possible to know whether these feces were passed before or after the first dose in the evening meal. Before the trial started, staff received training on how to score feces either: hard, normal, soft, diarrhea, or no feces passed. The score was attained by comparing the feces passed by the dog to a scoring system with a descriptor and a representative picture:

<table>
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<tbody>
<tr>
<td>Normal</td>
<td>Well-formed. Firm but not hard. Easy to pick up.</td>
</tr>
<tr>
<td>Soft</td>
<td>Shaped but wet feces. Lose their shape as they are picked up.</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Watery feces with no shape.</td>
</tr>
</tbody>
</table>

The softest fecal reading of the day’s feces was used as the fecal score for the day for each animal. When dogs shared a kennel, a

![Fig 1](image-url)  
**Fig 1.** Flow diagram of dogs through the trial phases.
single fecal score of the most liquid feces was assigned to both dogs when defecation was not witnessed. Frequency of defecation was not recorded because kennelled animals were not under constant supervision. Dogs that had <1 day of fecal scoring were excluded from analysis because no data were available. Dogs left the trial if they were adopted, fostered, euthanized, or at the request of the veterinarian. The dog’s date of entry into the trial, date of exit from the trial, sex, breed, group allocation, and daily fecal score all were recorded. The average number of days with fecal scores recorded for dogs in each group was statistically compared by a Mann-Whitney U-test. A chi-square test was used to evaluate distribution of sexes to each group which was not statistically different.

Samples of both synbiotic and placebo capsules were removed from the trial site at the end of the trial and sent to a United Kingdom Accreditation Services (UKAS)-accredited laboratory for culture. The primary outcome measure, the incidence of diarrhea, was analyzed by comparing the synbiotic and placebo groups’ mean percentage of days scored as diarrhea out of the total number of days with fecal scores recorded throughout each dog’s stay by a Wilcoxon rank sum test. The secondary outcome measure, the occurrence rate of ≥1 day of diarrhea and ≥2 days of diarrhea by day 14 of the animal’s stay, was plotted on a Kaplan-Meier survival curve and analyzed by log rank tests. Statistical analyses were performed by an independent medical statistician by a commercially available software package.

A power calculation was performed before the trial. Animal shelter staff estimated that 30% of the population of dogs experienced at least 1 episode of diarrhea during their stay. To detect a change in incidence of diarrhea from 30% with the placebo to 20% for the treatment group with a significance level of 0.05 and power of 80%, 582 dogs were required for the trial. To detect a change in the incidence of diarrhea from 30% with the placebo to 20% for the treatment group with a significance level of 0.05 and power of 80%, 582 dogs were required for the trial.

Fecal scores were recorded on each dog’s daily health sheets, and these sheets could not be collected for analysis until after the animal had left the shelter. Therefore, it was not possible to count dogs as they completed the trial. For this reason, a year of recruitment was estimated, based on previous dog intake numbers, to be sufficient to obtain the 582 dogs required by the power calculation.

Results

A total of 773 dogs completed the trial to analysis. Figure 1 includes a description of the dogs’ inclusion or exclusion from the trial. There were 399 dogs with 8,904 days of fecal scores recorded in the synbiotic product group and 374 dogs with 8,411 days of fecal scores recorded in the placebo group (Table 1). Forty-two dogs entered as part of a pair and shared a kennel; 13 pairs were assigned to the synbiotic group and 8 pairs to the placebo group. The number of days with a fecal score recorded for a single dog ranged from 1 to 168 days (median, 16.5 days) for the synbiotic group and 1–148 days (median, 16 days) for the placebo group. By a Mann-Whitney U-test to compare the median number of days with recorded fecal scores, the distribution of the synbiotic and placebo groups did not differ significantly (Z score, 0.3123; P = .7565).

Overall, 55.37%, 428 of 773 dogs were male. The synbiotic group was comprised of 53.13%, 212 of 399 male dogs, and in the placebo group, 57.75%, 216 of 374 dogs were male. The difference between sexes in groups was compared by a 2-tailed chi-square test and was found not to be statistically significant (P = .1965). The 7 most common breeds recorded for both groups were the same, with the 4 most common appearing in the same order of occurrence: crossbreeds (124 synbiotic group, 120 placebo group), Staffordshire Bull Terriers (40 synbiotic group, 54 placebo group), Jack Russell Terriers (39 synbiotic group, 32 placebo group), and Lurchers (26 synbiotic group, 30 placebo group).

Seven dogs in each group received novel protein diets for food-related dermatology conditions, whereas 3 dogs in the synbiotic group and 4 in the placebo group received weight loss diets.

The mean percentage of days scored as diarrhea of the total number of scored days during each dog’s stay was 2.0% in the synbiotic group and 3.2% in the placebo group (P = .0008). Table 1 shows an overview of each day’s fecal score for dogs in the synbiotic group and placebo group. Table 2 shows a summary of the number of dogs with ≥1 day scored as diarrhea throughout their overall stay at the shelter, and by week for the first 4 weeks.

Kaplan-Meier survival curves were used to compare the temporal pattern of first appearance of diarrhea between the intervention groups (Fig 2), evaluated by the log rank test. By day 14, 18.8% of dogs in the synbiotic group and 27.2% in the placebo group had ≥1 day of diarrhea and ≥2 days scored as diarrhea, throughout their overall stay at the shelter, and by week for the first 4 weeks.

Table 1. Overview of each day’s fecal score for dogs in the synbiotic group and the placebo group throughout their shelter stay

<table>
<thead>
<tr>
<th></th>
<th>Synbiotic</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>8904 (100.0%)</td>
<td>8411 (100.0%)</td>
</tr>
<tr>
<td>Normal</td>
<td>7872 (88.4%)</td>
<td>7152 (85.0%)</td>
</tr>
<tr>
<td>Hard</td>
<td>34 (0.4%)</td>
<td>42 (0.5%)</td>
</tr>
<tr>
<td>Soft</td>
<td>820 (9.2%)</td>
<td>959 (11.4%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>178 (2.0%)</td>
<td>258 (3.1%)</td>
</tr>
</tbody>
</table>

Table 2. Summary of the number of dogs with ≥1 day scored as diarrhea, throughout their overall stay at the shelter and by week, for the first 4 weeks

<table>
<thead>
<tr>
<th></th>
<th>Synbiotic</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>Number of dogs with data</td>
<td>399</td>
</tr>
<tr>
<td>%</td>
<td>23%</td>
<td>33%</td>
</tr>
<tr>
<td>Week 1</td>
<td>Number of dogs with data</td>
<td>399</td>
</tr>
<tr>
<td>%</td>
<td>40%</td>
<td>65%</td>
</tr>
<tr>
<td>Week 2</td>
<td>Number of dogs with data</td>
<td>328</td>
</tr>
<tr>
<td>%</td>
<td>10%</td>
<td>13%</td>
</tr>
<tr>
<td>Week 3</td>
<td>Number of dogs with data</td>
<td>226</td>
</tr>
<tr>
<td>%</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td>Week 4</td>
<td>Number of dogs with data</td>
<td>147</td>
</tr>
<tr>
<td>%</td>
<td>6%</td>
<td>16%</td>
</tr>
</tbody>
</table>
experienced at least 1 episode of diarrhea ($P = .0022$). By day 14, 4.6% of dogs in the synbiotic group had experienced diarrhea lasting ≥2 days, compared with 8.0% in the placebo group ($P = .0300$).

No adverse events or effects were reported in either intervention group. The UKAS-accredited laboratory reported the synbiotic capsules to contain $1.58 \times 10^{10}$ cfu/capsule and placebo capsules <18 cfu/capsule of bacteria in the *Enterococcus* genus.

**Discussion**

The objective of our trial was to test the effects of an orally administered prophylactic synbiotic supplement on the incidence of diarrhea in dogs entering an animal shelter. The results indicated that supplementation with the synbiotic significantly decreased diarrhea incidence across all 3 measures in the trial. The first measure of incidence used mean percentage of days per dog that were scored as diarrhea throughout their stay instead of the overall group percentage of days scored as diarrhea. This approach was used to prevent data from being skewed in the case of a few long-stay animals with persistent diarrhea being assigned to the same group. Survival analysis, a collection of statistical procedures for data analysis in which the outcome variable of interest is time until an event occurs, was used for the remaining 2 measures of diarrhea incidence. Fourteen days was chosen before commencing the trial as the time point for Kaplan-Meier survival analysis because it was the median number of days stay at the shelter in the year preceding data collection. Sixteen was the median number of days stay at the shelter for our sample. Kaplan-Meier survival analysis method was used to compare the percentage of dogs that had experienced at least 1 episode of diarrhea in each group within the first 14 days. The percentage of dogs in each group that had had diarrhea lasting ≥2 days by day 14 also was compared. This number was of interest because when diarrhea lasted ≥2 consecutive days, the dogs would be scheduled for a veterinary evaluation and treatment would be prescribed.

A trial that used the probiotic species *Bifidobacterium animalis*, which is commercially available in the United States of America, also found that significantly fewer probiotic-supplemented dogs passed unacceptable feces (loose or diarrheic) during the first week of relocation, and during their overall stay at a kennel facility, when compared to controls. This trial randomized 134 young Labrador Retrievers or Labrador-Golden Retrievers to 4 groups: 3 different doses of probiotic and a control group. The dogs in the *Bifidobacterium animalis* study began their probiotic supplementation 5 weeks before entering the kennels. *Enterococcus faecium* was selected for the current trial because it currently is the only probiotic species licensed in the European Union for dogs. The population of dogs in our study was a mix of ages and breeds, typical of a shelter in the United Kingdom, and were not supplemented before kenneling because it was not realistic for dogs entering an animal shelter. It would be interesting to investigate the effect of synbiotic supplementation in other stressful situations, such as competitions and planned holiday kenneling, to assess the efficacy of the product in these scenarios.

A previously performed trial using the probiotic species *Enterococcus faecium* on dogs in an animal shelter did not find statistical significance to support the hypothesis that the probiotic would decrease the incidence of diarrhea of ≥2 days' duration compared with placebo. This trial had a very low incidence of diarrhea lasting ≥2 days (2 dogs in a study population of 182), which was unexpected and possibly a result of short-stay durations, a consistent diet for all kenneled dogs and lack of stress due to the kenneling facilities. We found a statistically significant effect of *Enterococcus faecium* in our trial, using the same outcome measure (incidence of diarrhea ≥2 days’ duration), in an adequately powered study with a higher incidence of diarrhea. When the same trial was performed in cats, where the incidence of diarrhea ≥2 days’ duration in the placebo group was higher (20.7%), a statistically significant decrease in diarrhea ≥2 days’ duration was found in the probiotic group (7.4%; $P = .0297$).
Limitations to our trial include a lack of medical history for the majority of animals because of the nature of being an animal shelter. This may mean that a predisposition to diarrhea was more prevalent in certain individuals. We believe that this effect was minimized by the randomization process and large sample size. Age was not recorded because of a lack of information given about dogs entering the shelter. Previously, females have been found to have lower odds of developing diarrhea compared with males. There were more males than females in our trial, but this difference was not statistically significant (P = .1965). Diet was not kept constant throughout the trial for all dogs, which could have played a role in the development of diarrhea, but this was meant to reflect normal working practice for animal shelters, the majority of which rely on donations to feed their dogs. Again, the large sample size and randomization process should have minimized the effect on our trial. Forty-two dogs were housed as pairs in our trial and assigned to the same group to decrease the risk of contaminant contamination. Given that the trial was carried out in a working animal shelter, it was not possible to alter normal working practices. One hundred and ninety-one more dogs completed the trial than required by the power calculation (582), and this overage was caused by a specific date as an endpoint rather than counting dogs, which was a more practical solution in a working shelter.

A simple 4-category fecal scoring system (hard, normal, soft, diarrhea) was created for our trial, adapted from the Nestle Purina and Waltham feces scoring systems after staff at the animal shelter expressed concerns over the complexity of the original systems. All staff received training in the 4-category fecal scoring system and posted posters in all dog kitchens and admission rooms as a prompt. Fecal scoring is subjective and there is likely to be some intra-observer and interobserver variability despite having descriptors and representative picture available, but there is evidence to suggest fecal scoring is superior to measuring fecal moisture content and fecal dry matter.

Animals with a known history of chronic or recurrent diarrhea or abnormalities on clinical examination were excluded from the trial. Therefore, conclusions can only be made about healthy animals entering an animal shelter. It is also not possible to draw any conclusions regarding the efficacy of other potential probiotic strains or prebiotics beyond those used in our trial. Samples of both capsules were cultured at the end of the trial to ensure that no cross-contamination of the placebo occurred during manufacture or storage, but also to ensure the active capsule contained at least the specified quantity of probiotic, 2.3 × 10^9 cfu/capsule. The excess quantity of probiotic was due to overage added by the manufacturer in order to guarantee live bacteria numbers at the end of a 2-year shelf life.

In conclusion, the results of our trial support the hypothesis that supplementing dogs entering an animal shelter with a synbiotic decreases the incidence of diarrhea and provides evidence of the beneficial effect of the prophylactic use of this synbiotic supplement. Decreased diarrhea rates would directly improve the welfare of the dogs and decrease the associated costs of cleaning, treating, and extended stays in animal shelters. The results from our study could be included in a cost-benefit analysis to establish the net cost of routinely supplementing dogs entering a shelter. There may be an advantage to the use of this synbiotic preparation in decreasing diarrhea triggered by other stressful events such as holiday kenneling and during competitions, but this hypothesis is yet to be tested.

Footnotes

b Synbiotic D-C, Proxin Veterinary, Probiotics International Ltd., Lopen Head, Somerset, UK
c Wilkinson Associates, Medical Statistics, Radnage, Bucks, UK

Acknowledgments

The authors thank all staff at Wood Green, The Animals Charity, Godmanchester, for their work on this trial. This trial was conducted under the University of Cambridge, Department of Veterinary Medicine, Cambridge Clinical Research Outreach Program. Thanks also to Proxin Veterinary for manufacturing and donating both the active product and placebo product.

Conflict of Interest Declaration: Lindsay Rose is an employee of Probiotics International Ltd. Jeremy Hugh Rose is married to Lindsay Rose. A donation was given to Wood Green, The Animals Charity, Godmanchester, by Probiotics International Ltd. after the trial had been completed. Conflict of interest was managed by a randomized, double-blind, placebo-controlled study design. Case recruitment, data collection, data input, and statistical analysis were performed by persons independent of Probiotics International Ltd.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.
References


