

# Administration of charcoal, *Yucca schidigera*, and zinc acetate to reduce malodorous flatulence in dogs

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**Objective**—To determine whether feeding activated charcoal, *Yucca schidigera*, and zinc acetate would ameliorate the frequency and odor characteristics of flatulence in dogs.

**Design**—In vitro screening of active agents followed by a randomized controlled trial.

**Animals**—8 adult dogs.

**Procedure**—A fecal fermentation system was used to assess the effects of activated charcoal, *Yucca schidigera*, and zinc acetate alone and in combination on total gas production and production of hydrogen sulfide, the primary determinant of flatus malodor in dogs. All 3 agents were subsequently incorporated into edible treats that were fed 30 minutes after the dogs ate their daily rations, and the number, frequency, and odor characteristics of flatulence were measured for 5 hours, using a device that sampled rectal gases and monitored hydrogen sulfide concentrations.

**Results**—Total gas production and number and frequency of flatulence episodes were unaffected by any of the agents. Production of hydrogen sulfide in vitro was significantly reduced by charcoal, *Yucca schidigera*, and zinc acetate by 71, 38, and 58%, respectively, and was reduced by 86% by the combination of the 3 agents. Consumption of the 3 agents was associated with a significant decrease (86%) in the percentage of flatulence episodes with bad or unbearable odor and a proportional increase in the percentage of episodes of no or only slightly noticeable odor.

**Conclusions and Clinical Relevance**—Results suggest that activated charcoal, *Yucca schidigera*, and zinc acetate reduce malodor of flatus in dogs by altering the production or availability of hydrogen sulfide in the large intestine. (*J Am Vet Med Assoc* 2001;218:892–896)

Flatulence in dogs is widely recognized as a social nuisance, a source of humor, and an occasional cause of abdominal discomfort. Aside from scatological interest, there has been little research into the origins and nature of rectal gases in dogs, their physiological and clinical importance, or the efficacy of various remedies purported to reduce flatulence in dogs. The only relevant information comes from early experimental studies in which dogs were used to elucidate the role of luminal bacteria in the production of intestinal gases.<sup>1,2</sup>

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Flatulence is a frequent everyday occurrence in humans and is usually associated with no or very mild discomfort. Some patients do seek medical attention because of excessive gas production or increased sensitivity to gastrointestinal distension that is associated with severe pain and is usually relieved by dietary modifications.<sup>3</sup> A number of studies have examined the volume and composition of rectal gases in humans<sup>3-5</sup> and factors that influence the frequency<sup>6</sup> and chemical character of emissions.<sup>7,8</sup> The major rectal gases are nitrogen and oxygen, which are derived from air swallowing and diffusion from the blood, and carbon dioxide, hydrogen, and methane, which are the products of bacterial metabolism and nonbacterial reactions within the bowel.

Odoriferous gases constitute less than 1% by volume of human flatus, and much of the unpleasant odor is attributable to sulfur-containing gases, primarily hydrogen sulfide and methanethiol.<sup>7,8</sup> In a previous study,<sup>9</sup> we found that sulfur-containing gases, particularly hydrogen sulfide, are a major determinant of the malodor of canine flatus, as determined by human organoleptic assessment. The sulfur for reactions that produce these gases is derived from mucin and dietary sources, namely sulfate and sulfur-containing amino acids. Although the latter are usually completely absorbed in the small intestine, sulfate, which is naturally present in high quantities in cruciferous vegetables and nuts and is used during the production of bread and beer, is poorly absorbed in the small intestine. The contribution of dietary components to hydrogen sulfide production was highlighted in a recent study<sup>10</sup> in which production of this gas in rats was reduced 6-fold by withholding food and increased 5-fold by feeding the nonabsorbable sulfur compound carrageenan.

Aside from their odoriferous qualities, sulfur-containing rectal gases are potentially toxic and have been implicated in the pathogenesis of ulcerative colitis in humans.<sup>11</sup> There are, therefore, potential health as well as social benefits to be obtained from reducing the production and availability of sulfur gases in the large intestine. This could be accomplished by reducing dietary sulfate intake or by consuming poorly absorbed divalent cations, such as zinc, iron, and bismuth, and activated charcoal to bind sulfur-containing gases.

The purpose of the present study was to determine whether feeding activated charcoal and zinc acetate as dietary supplements would reduce flatulence in dogs. We also tested the effects of an extract of the yucca plant, as this has been shown to alter the intensity and characteristics of canine fecal odor when included in a

dry dog food.<sup>12</sup> The effects of the 3 agents alone and in combination on total gas production and production of hydrogen sulfide were assessed by use of an in vitro fecal fermentation system. Dogs were then fed dietary supplements containing all 3 agents, and the number, frequency, and odor characteristics of flatulence episodes were measured, using recently described techniques.<sup>9</sup>

## Materials and Methods

**In vitro screening of agents**—An in vitro fecal fermentation system was used to determine the effects of charcoal, *Yucca schidigera*, and zinc acetate on total gas production and production of hydrogen sulfide. Each agent (133 mg of charcoal, 5 mg of *Yucca schidigera*, 7 mg of zinc acetate dihydride) was weighed in duplicate or triplicate into 60-ml glass serum bottles<sup>a</sup> containing 30 ml of fermentation medium.<sup>13</sup> Bottles were then capped with cotton-wool bungs and covered with tin foil. A set of bottles containing all 3 agents was also prepared along with 2 sets of bottles to which none of the agents were added that served as fecal and media controls. All bottles were sterilized in an autoclave (121 C for 15 minutes) and placed in an anaerobic cabinet to maintain anaerobic conditions prior to inoculation with feces.

For fecal inoculation, fresh feces samples were collected from healthy dogs and weighed. Within 15 minutes after defecation, 20 g of feces was added to flasks containing 200 ml of 10 mM sodium phosphate buffer (pH 7.4) that had previously been sterilized and maintained under anaerobic conditions, as described for the fermentation bottles. Flasks were then placed on magnetic stirrers in an anaerobic cabinet for 10 minutes to produce a fecal slurry. Three-milliliter aliquots of each slurry were then added to the test and control fermentation bottles, which were hermetically sealed with gray butyl lyophilization stoppers and aluminium tear-off seals.<sup>4</sup> One milliliter of 10% sodium molybdate dihydride was added to a set of control bottles to inhibit the activity of sulfate-reducing bacteria. All fermentation bottles were incubated anaerobically at 37 C for 24 hours.

At the end of the incubation period, pressure in the head space of each fermentation bottle was measured by use of a manometer connected to a hypodermic syringe needle that was inserted through the butyl rubber stopper. The manometer was set to 0 (ie, atmospheric pressure) before each measurement. Pressure readings (mbar) were converted to volume of gas produced by use of the following equation:

$$\text{Volume of gas produced (ml)} = \text{pressure (mbar)} \times 0.031.$$

This conversion factor was derived for the apparatus used, as described.<sup>14</sup> Total gas production was assessed, using feces from 2 dogs, and results are given as mean  $\pm$  SD of the 2 sets of triplicate results.

Concentration of hydrogen sulfide in the broth in each fermentation bottle was determined colorimetrically, using commercially available reagents.<sup>b</sup> An aliquot (7.5 ml) of broth was decanted from each fermentation bottle into a centrifuge tube to which 22.5 ml of deoxygenated water was added. Tubes were centrifuged at  $13,800 \times g$  for 15 minutes, and 5 aliquots (5 ml each) of the supernatant were removed and stored on ice prior to analysis. Test reagents were added to 3 aliquots, and absorbency was read immediately at 670 nm. An equal volume of deoxygenated water was added to the other 2 aliquots, which served as fecal blanks. A standard curve of absorbency of solutions of sodium sulfide in deoxygenated water ranging from 0 to 50 ppm was constructed for each set of samples. Production of hydrogen sulfide was measured, using feces from 6 dogs; results from each set of duplicate ( $n = 4$ ) or triplicate (2) analyses were averaged. Data are given as the mean  $\pm$  SD of the 6 averages.

**Assessment of flatulence in vivo**—The 3 agents were incorporated into an edible treat, and effects of these treats on flatulence in dogs were evaluated in a double-blind placebo-controlled crossover study. Eight healthy adult dogs were randomly assigned to receive treats containing the active ingredients or wheat starch during the first treatment period. Each treatment arm of the study lasted 5 days, and there was a 9-day washout period between the 2 treatments. Dogs used in the study included 1 Golden Retriever, 5 Labrador Retrievers, and 2 English Mastiffs; there were 3 neutered males, 1 sexually intact female, and 4 neutered females. All dogs were housed individually and fed the same batch of a premium-quality complete dry food<sup>c</sup> for 3 months prior to, and for the duration of, the study. Daily food allowances were determined on the basis of body weight (metabolizable energy in kcal =  $125 \times \text{body weight}^{0.75}$ ) and adjusted to maintain stable body weight and condition during the 3-month pre-trial period. Daily food allowances were then kept constant for the duration of the study.

Dogs were fed their daily ration at 8:30 AM, and the treats were offered 30 minutes later at the rate of 1 treat/5 kg (1 treat/11 lb) of body weight. Treats were in the form of a dry dual component cylindrical biscuit that was 20 mm long and 20 mm in diameter. Each treat contained 320 mg of activated charcoal, 2.5 mg of *Yucca schidigera*, and 17 mg of zinc in the form of zinc acetate dihydride. These ingredients were replaced with wheat starch in the placebo treats.

Production of malodorous flatulence in vivo was assessed by measuring hydrogen sulfide in rectal gases, as described.<sup>9</sup> In brief, rectal gases were collected via a perforated polytetrafluoroethylene tube that was held close to the anus and was attached to a sulfur gas-monitoring pump carried in a jacket over the dogs' shoulders. In the present study, dogs wore the coats for 5 hours continuously between 10:30 AM and 3:30 PM on each of the 5 days of the 2 treatment periods. The monitoring pump was fitted with a hydrogen sulfide sensor that measured hydrogen sulfide concentration in parts per million at 20-second intervals. Data were downloaded to a personal computer at the end of each sampling period, using the manufacturer's software,<sup>d</sup> which allowed direct integration of the data into a spreadsheet.

Three measures of flatulence were calculated from the hydrogen sulfide data: **number of episodes (NOE)**, **mean interval free time (MIFT)**, and human perception of malodor. The NOE was the number of times during the sampling period that pump reading for hydrogen sulfide concentration was  $> 1$  ppm, which is the lower limit of sensory detection for humans in the same room as dogs.<sup>9</sup> The MIFT was a measure of the frequency of flatulence and was calculated as the cumulative sum of flatulence-free intervals (in minutes) divided by the number of flatulence-free intervals.<sup>9</sup> A flatulence-free interval was any 20-second period during which concentration of hydrogen sulfide was  $< 1$  ppm. The human perception of malodor for each episode of flatulence was calculated by use of a previously derived power function,<sup>9</sup> whereby malodor rating (on a scale from 1 to 5) was equal to  $1.51 \times \text{hydrogen sulfide concentration}^{0.28}$ . These odor ratings were categorized as no odor (1), slightly noticeable odor (2), mildly unpleasant odor (3), bad odor (4), and unbearable odor (5).

**Statistical methods**—Data for total gas and hydrogen sulfide production in vitro were analyzed by use of multifactor ANOVA, with dog as a random factor and agent as a fixed factor, to test for significant factor effects. Significant differences among group means were further investigated by use of Tukey HSD intervals. For the in vivo portion of the study, data for MIFT and NOE were log transformed ( $\ln$ ) to ensure that data were normally distributed prior to statistical analysis, as described.<sup>9</sup> The in vivo data were analyzed by use of a general linear model with dog as a random factor and treat-

ment as a fixed factor. Data are given as mean  $\pm$  SD. For all analyses, values of  $P < 0.05$  were considered significant.

## Results

**In vitro screening of agents**—Total gas production was unaltered by charcoal, *Yucca schidigera*, zinc acetate, or the 3 agents in combination (Fig 1). Mean gas production in the fermentation bottles ranged from 17 to 20 ml/100 g feces and was similar to that for the control bottles that contained only medium and feces (18 ml/100 g feces). Total gas production was lower in the control bottles to which molybdate had been added to inhibit sulfate-reducing bacteria (10 ml/100 g feces), but the difference was not statistically significant.

Concentration of hydrogen sulfide in the fermentation broth following incubation was significantly ( $P < 0.001$ ) reduced by all 3 agents, with charcoal, *Yucca schidigera*, and zinc acetate reducing hydrogen sulfide concentration by 71, 38, and 58%, respectively, compared with concentration in the control bottles (Fig 2). Effects appeared additive in that the combination of all 3 agents reduced hydrogen sulfide concentration by 86% ( $P < 0.001$ ); concentrations in bottles to which all 3 agents were added were comparable to concentrations in the control bottle to which molybdate had been added to inhibit the activity of sulfate-reducing bacteria. The combination was significantly ( $P < 0.001$ ) more effective than charcoal, *Yucca schidigera*,

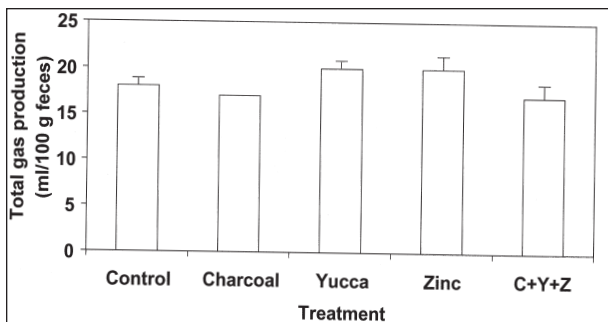


Figure 1—Total gas production during in vitro fermentation of feces from dogs with activated charcoal (charcoal), *Yucca schidigera* (yucca), zinc acetate (zinc), the combination of all 3 agents (C+Y+Z), and none of the agents (control). Data represent mean gas production for 2 experiments. Error bars represent SD (SD for charcoal was 0.2 ml/100 g feces).

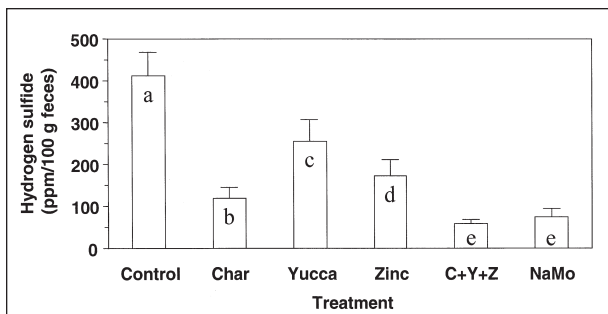


Figure 2—Concentration of hydrogen sulfide in the broth following in vitro fermentation of feces from dogs with activated charcoal (char), *Yucca schidigera* (yucca), zinc acetate (zinc), and the combination of all 3 agents (C+Y+Z). Results are also shown for fermentation without any of the 3 agents (control) and for fermentation without any of the 3 agents but with the addition of sodium molybdate (NaMo). Data represent mean values for 6 experiments. Bars with different letters were significantly ( $P < 0.05$ ) different.

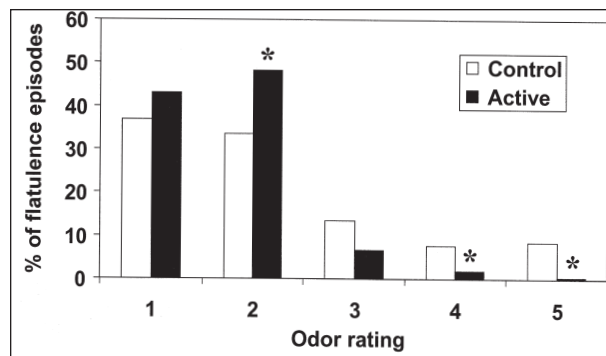


Figure 3—Mean percentages of flatulence episodes scored as 1 (no odor), 2 (slightly noticeable odor), 3 (mildly unpleasant odor), 4 (bad odor), and 5 (unbearable odor) for 8 dogs fed treats containing a combination of activated charcoal, *Yucca schidigera*, and zinc acetate (active) or fed a placebo (control). \*Percentage for active treatment was significantly ( $P < 0.05$ ) different from percentage for control treatment.

or zinc acetate alone. Charcoal alone was significantly more effective than *Yucca schidigera* ( $P < 0.001$ ) and zinc acetate ( $P < 0.05$ ) alone, and zinc acetate alone was significantly ( $P < 0.01$ ) more effective than *Yucca schidigera* alone.

**Assessment of flatulence in vivo**—Feeding treats containing all 3 agents was associated with a small but statistically insignificant decrease in NOE ( $7.77 \pm 2.09$  vs  $11.8 \pm 2.27/5$  hours) and with no difference in MIFT ( $1,900.74 \pm 2.12$  and  $1,339.43 \pm 2.20$  minutes). However, percentages of episodes rated as 4 (bad) or 5 (unbearable) were significantly decreased, and percentage of episodes rated as 2 (slightly noticeable) was significantly increased when dogs were fed treats containing the 3 agents (Fig 3). Consumption of the treats containing the 3 agents reduced the percentage of bad and unbearable episodes by 86%, compared with the placebo treats, so that flatulence episodes rated as 4 or 5 represented only 2.2% of all episodes versus 16.1% when the placebo treats were fed.

## Discussion

Results of the present study suggest that activated charcoal, *Yucca schidigera*, and zinc acetate reduce malodor of canine flatus by altering the production or availability of hydrogen sulfide in the large intestine. In vitro, none of these agents appeared to influence the production of nonodorous gases that make up the bulk of flatus; therefore, it was not surprising that they did not have a significant effect on the number or frequency of flatulence episodes.

Amounts of activated charcoal and *Yucca schidigera* used in the treats tested in the present study were selected on the basis of results of human and farm-animal studies. Daily doses of activated charcoal used in human clinical studies<sup>15,16</sup> have ranged from 1.2 to 4 g. Treats used in the present study contained 0.32 g of activated charcoal and were fed at the rate of 1 treat/5 kg (1 treat/11 lb). This would be equivalent to a daily intake of 3.9 to 5.2 g for a human being that weighed 60 to 80 kg (132 to 176 lb). The treats also contained 2.45 mg of *Yucca schidigera*, which represented approximately 0.0033% (wt/wt) of the daily food ration for a

20-kg (44-lb) dog. This is directly comparable with the amounts of *Yucca schidigera* extract typically included in pig and calf diets (0.003 and 0.0036%, respectively) to reduce ammonia concentrations.<sup>17</sup> Unfortunately, there were no such equivalent data on which to base the amount of zinc acetate used. In the only relevant study,<sup>10</sup> rats were fed a diet containing 1% (wt/wt) zinc acetate, which is an extreme level of incorporation. The American Association of Feed Control Officials specifies a maximum amount of zinc in dog foods of 1,000 mg/kg on a dry-matter basis,<sup>18</sup> and in Europe, current legislation limits zinc concentration in pet foods to 250 mg/kg on an as-fed basis (0.025% wt/wt).<sup>19</sup> The amount of zinc included in the treats was, therefore, calculated so that the daily intake of zinc from diet and treats (each of which contained 17 mg of zinc) did not exceed the maximum permitted by European legislation. The amount of each agent tested in vitro was then calculated from the predicted concentration of each that would be present in the lumen of the colon of the dogs in the study.

Activated charcoal has long been used to treat intestinal gas complaints in humans and is often recommended by veterinarians to reduce the contribution of malodorous gastric compounds to halitosis. Activated carbon compounds are effective adsorbents of many odoriferous gases, including sulfur gases.<sup>20</sup> This adsorbency is a result of their porous structure, which arises from the formation of volatile matter during the charring process and confers a tremendous internal surface area of between 450 and 1,800 m<sup>2</sup>/g. Charcoal did not appear to affect the total amount of gas produced in vitro in the present study, and this is consistent with results of studies in which human fecal homogenates were used.<sup>16</sup> In people, ingestion of activated charcoal (4 g) was found to have no significant effect on the number of episodes of flatulence after ingestion of a gas-producing meal of baked beans,<sup>16</sup> although an earlier study had indicated that this agent did decrease flatus.<sup>15</sup>

Although these data question the benefit of administering charcoal for relief from excessive bloating, results of this and other studies do suggest that charcoal is effective in absorbing the trace quantities of sulfur-containing gases that are primarily responsible for the malodor of flatus. Addition of activated charcoal to human fecal homogenates was previously shown to significantly reduce the release of hydrogen sulfide and methanethiol.<sup>21</sup> Activated charcoal has also been shown to remove virtually all the sulfur-containing gases from human flatus in vitro and to significantly reduce its odor intensity.<sup>8</sup> However, in the only scientific study of oral administration of activated charcoal in humans,<sup>21</sup> ingestion of 0.52 g of activated charcoal 4 times a day was not associated with any significant decrease in fecal release of sulfur-containing gases, total fecal gas release, or self-reported abdominal symptoms. The authors suggested that this lack of efficacy was attributable to saturation of charcoal-binding sites during passage through the gastrointestinal tract, although it is possible that methodologic constraints, particularly the fecal gas release system used, or inadequate dosing was responsible for the lack of effect.

*Yucca schidigera* has been widely used in the livestock industry to bind ammonia and decrease odors from pig and poultry wastes, and there is evidence that this agent may be effective in controlling odors from cattle manure.<sup>15</sup> These benefits appear to be a result of the ammonia-reducing effects of *Yucca schidigera*, which are obtained when the agent is added to feed or applied directly to the litter or slurry depository. Results from the present study indicate that *Yucca schidigera* may also be effective in reducing production or release of hydrogen sulfide in the intestinal tract. Feeding *Yucca schidigera* to dogs and cats has been shown to reduce fecal malodor,<sup>12</sup> although concentrations of sulfur volatiles were not measured.<sup>22</sup> The active components and mode of action of *Yucca schidigera* remain unclear. Saponins are believed to be the active components, and it has been postulated that these reduce fecal odor by inhibiting urease inhibition, binding ammonia, modifying the colonic microflora, or increasing intestinal metabolism or absorption by altering intestinal permeability.<sup>17</sup> In vitro data from the present study suggest that *Yucca schidigera* was binding hydrogen sulfide or decreasing the numbers or activity of sulfate-reducing bacteria that generate hydrogen sulfide.<sup>23</sup>

The effects of zinc acetate in the present study can be attributed to the binding of hydrogen sulfide. Poorly absorbed divalent cations (eg, zinc, iron, and bismuth) bind sulphhydryl compounds such as hydrogen sulfide and methanethiol to form insoluble salts, and this effectively prevents the liberation of these gases. Treatment of human fecal suspensions with zinc sulfate in vitro reduced the concentrations of hydrogen sulfide and methanethiol but not diethyl sulfide and reduced the intensity of malodor.<sup>8</sup> These effects were less pronounced than those observed with activated charcoal, similar to results of the present study, although in both instances, this could simply reflect the relative amounts of each agent used. To our knowledge, there have been no in vivo studies of the effects of dietary zinc sulfate supplementation in humans. Addition of zinc acetate to the diet did reduce cecal hydrogen sulfide concentrations by 5-fold in rats fed carrageenan, a nonabsorbable sulfur compound.<sup>10</sup> However, intake of zinc sulfate in that study (1% of diet wt/wt) was considerably higher than intake in the present study in which zinc acetate intake was approximately 0.01% (wt/wt) of daily food intake. Bismuth, another divalent cation, has been shown to reduce hydrogen sulfide release from rat and human feces in vitro and decrease hydrogen sulfide release in the colon of rats, and treatment of humans with bismuth subsalicylate (524 mg, q 6 h) resulted in a > 95% reduction in fecal release of this gas.<sup>24</sup>

The toxicity of hydrogen sulfide is of the same order as that of cyanide, and there is evidence that excessive sulfide production plays a role in the pathogenesis of ulcerative colitis. Colonic sulfides inhibit oxidation of *n*-butyrate,<sup>25</sup> which is required for maintenance of the epithelial cell barrier. Numbers and activity of sulfate-reducing bacteria are greater in human patients with active, rather than quiescent, ulcerative colitis and relate to the clinical severity grade.<sup>26</sup> Although 1 study indicated that there was no

difference in stool sulfide concentrations between patients with ulcerative colitis and control patients,<sup>27</sup> another indicated that release of hydrogen sulfide is increased in these patients.<sup>28</sup> It has also been recently suggested that defects in colonic detoxification of toxic sulfur gases may play a role in diseases like ulcerative colitis.<sup>29</sup>

Agents that reduce exposure of the colon to hydrogen sulfide may protect against its injurious effects in humans and animals. Results of the present study provide evidence that activated charcoal, *Yucca schidigera*, and zinc acetate will reduce concentration of hydrogen sulfide in the large intestine in dogs. Although we are unaware of any research into the role of hydrogen sulfide in the pathogenesis of ulcerative colitis in dogs, it is clear that idiopathic colitis in dogs is responsive to administration of easily assimilated diets that contain a restricted number of protein sources.<sup>30,31</sup> Although the effects of these diets can be explained by their hypoallergenicity, it is possible that they are associated with more complete absorption of sulfur-containing amino acids, leading to less sulfur in the colon for hydrogen sulfide production. Of course, this is only speculation, but there is evidence that humans with ulcerative colitis consume more proteins than control patients<sup>32</sup> and that withdrawal of milk, eggs, and cheese, which are rich in sulfur-containing amino acids, is beneficial.<sup>33</sup>

In conclusion, results of the present study indicate that activated charcoal, *Yucca schidigera*, and zinc acetate reduce the production or release of hydrogen sulfide in the large intestine in dogs and that this effect is accentuated when the agents are used in combination. Dietary supplementation with these agents was associated with a decrease in the percentage of flatulence episodes that were bad or unbearable, which could decrease the social nuisance of flatulence in dogs. The effect of dietary supplementation with these agents on intestinal health in dogs warrants further investigation.

<sup>a</sup>Wheaton Industries, New York, NY.

<sup>b</sup>Camlab Ltd, Cambridge, UK.

<sup>c</sup>Pedigree Formula Advance Adult Supreme, Pedigree Masterfoods, Melton Mowbray, UK.

<sup>d</sup>ProRAE 50, RAE Systems Inc, Sunnyvale, Calif.

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