The Use of Chromic Oxide as a Marker for Measuring Small Intestinal Digestibility in Cannulated Dogs

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ABSTRACT: Small intestinal digestibility can be measured by comparing feed with effluent collected from an ileal T-cannula. Nevertheless, a nondigestible, nonabsorbable marker, such as chromic oxide (Cr2O3), must be included in the diet because simple T-cannulae do not divert chyme completely. This study was conducted to evaluate the excretion pattern of Cr2O3 in cannulated dogs because the kinetics of Cr2O3 has not been previously investigated in this nonruminant species. Chromic oxide was added to four diets fed to eight cannulated mixed-breed dogs in a Latin-square design. The four diets contained reciprocal proportions of protein from texturized vegetable protein (0% to 57%) and from beef (100% to 43%), so protein and carbohydrate digestibility varied among diets. All feces were collected during wk 2 and all ileal effluent during wk 3 of each diet period. Ileal recovery of Cr2O3 was almost complete (94%) and was greater than fecal recovery (87%) (P ≤ .03). Recovery was not different among diet groups. Ileal DM digestibility was approximately 2 percentage units lower on d 1 (P ≤ .007) than on d 2 to 4. Nevertheless, ileal DM digestibility varied little on these subsequent days so single-day collections should be accurate. Chromic oxide concentration in chyme varied widely during each collection but increased at the start and declined towards the end of each collection. Spot sampling may therefore result in inaccurate estimates of nutrient digestibility. In conclusion, Cr2O3 may be used as a marker to measure digestibility in dogs with simple T-cannulae, but total collections should be attempted for greater accuracy.

Key Words: Chromic Oxide, Digestibility, Cannulae, Dogs


Introduction

Digestibility determined by comparing feed with feces does not distinguish small intestinal digestion from large intestinal fermentation. Small intestinal digestibility can be measured in isolation, however, by comparing feed with effluent collected from an ileal cannula. Simple T-cannulae are widely used to measure digestibility because they are easy to implant and have little effect on the passage of ingesta or on digestibility (Rohr et al., 1984; Brass and Schünemann, 1989). Nevertheless, simple T-cannulae do not divert chyme completely, so a nondigestible, nonabsorbable marker, such as chromic oxide (Cr2O3), must be included in the diet.

The cannulation of dogs to measure small intestinal digestibility is becoming more common. Pet food manufacturers require accurate measurement of nutrient digestibility to formulate diets that provide optimum nutrition at minimum cost. Nevertheless, the use of Cr2O3 as a marker to measure digestibility in cannulated dogs has not been investigated. In ruminants, Cr2O3 does not mark liquid and particulate phases accurately (Beever et al., 1978). Conflicting reports have suggested that intermittent (spot) collections may or may not be accurate (Prigge et al., 1981; Rohr et al., 1984). The purpose of this study therefore was to measure the recovery and excretion pattern of Cr2O3 from the canine ileum.

Materials and Methods

Eight adult castrated male hound dogs, weighing 20 to 30 kg, were obtained from a commercial source. They were housed in 1.4 × 1.9 m cages in a room with a 12-h light:dark cycle at 24°C. During ileal collec-
tions, dogs were kept in a lighted 28-m² laboratory. Each dog was walked three times daily during the week and once daily during weekends. Each dog was fed to maintain constant body weight once daily between 0800 and 0900; water was available at all times. This study was approved by the Institutional Animal Care and Use Committee of the University of Florida, and all dogs were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals (NRC, 1985a).

The dogs were in good overall health as ascertained by normal physical examination and by the results of a complete blood count and blood chemistry panel. The urine analysis was within normal limits, and fecal examination was negative for parasites in all dogs. Anthelmintics were administered throughout the study to prevent parasite infection: fenbendazole (Panacur® Granules 22.2% for Dogs, Hoechst Rousell Agrivet, N. Somerville, NJ; 50 mg/kg orally daily for 3 d) at admission and then ivermectin (Ivomec® Injection for Cattle 1% Sterile Solution, Merck & Co., Rathway, NJ; 200 μg/kg subcutaneously) every 3 wk. Each dog was implanted with a simple T-cannula similar to that described by Thomas (1941) and Hudock et al. (1966) using a surgical method similar to that described by Markowicz et al. (1964).

The recovery and excretion patterns of Cr2O3 were measured using experimental diets designed to compare the digestibility of texturized vegetable protein (TVP) with that of lean beef. Texturized vegetable protein is an extruded form of soybean flour that is widely used as an economical source of protein in pet foods. Beef contains protein, fat, water and no carbohydrate, whereas TVP contains protein, carbohydrate, and almost no fat or water. Beef tallow and water were added to TVP, and cornstarch was added to beef, to produce two mixtures that were identical as measured by proximate analysis. One mixture contained TVP protein, and the other contained beef protein. A third low-protein mixture composed of cornstarch, beef tallow and water was also produced. Vitamins and minerals were added to each mixture in accordance with NRC (1985b) recommendations. All three mixtures were canned in a still retort at 120°C for 80 min with an initial temperature of 70°C as a single batch at Alpo Petfoods.

These three mixtures were combined, as shown in Table 1, to make four experimental diets (A, B, C, and D) that were fed to each dog. The TVP and beef mixtures were included in reciprocal amounts. The low-protein mixture was included to lower the final protein concentration. Diets A, B, C, and D all contained approximately 23% crude protein, 32% fat (ether extract), 31% nitrogen free extract (by difference), 13% ash, and 1% crude fiber on a DM basis (AOAC, 1990). Soybean flour contains carbohydrate that is poorly digested in the small intestine of nonruminants whereas cornstarch is easily digested, so DM digestibility was expected to decrease as TVP increased in the diets.

Dogs were fed to maintain body weight. They were weighed once a week after defecation but before feeding. Dogs weighed 23 ± 2.2 kg at the start of the study and 23.7 ± 2 kg at the end. The amount of food necessary to maintain body weight was determined during a 4-wk acclimatization period. Each dog was offered an excess of Diet B for 30 min once daily throughout the acclimatization period. The weight of food consumed daily was measured. Dogs were then fed the average weight of food consumed daily during the acclimatization period (1,000 to 1,600 g of food wet weight) throughout the rest of the study. The moisture content and gross energy density of the experimental diets were almost identical (=77% and 24 MJ/kg, respectively), so gross energy and DM intake were constant.

Each of eight dogs was fed each of four diets (A, B, C, or D) in a four by four Latin-square design with two dogs randomly assigned to each block. Each diet was fed for 3 wk: wk 1 for adaptation to the new diet; wk 2 for fecal collection; and, wk 3 for ileal collection. Chromic sesqui-oxide (.5 g; Fisher Scientific, Pittsburgh, PA) was mixed thoroughly with each meal, and a representative sample (approximately 100 g) of mixed diet was retained, weighed, and frozen at −20°C for subsequent analysis.

During the fecal collection week, all feces passed from noon on d 1 till noon on d 5 were collected in 500-g Whirl-Pak bags (Fisher Scientific), mostly by direct catch. During the ileal collection week, ileal effluent was collected on d 1 through 5, and any feces passed from noon on d 1 to noon on d 6 were collected.

### Table 1. Composition of the four experimental diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Canned diets</th>
<th>Source of protein</th>
<th>Source of carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef mix, %</td>
<td>TVP mix, %</td>
<td>% from TVP</td>
</tr>
<tr>
<td>A</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

*TVP = texturized vegetable protein.*
Table 2. Chromic oxide recovery (%)

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal (n = 8)</td>
<td>92.0</td>
<td>82.1</td>
<td>85.0</td>
<td>90.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Ileal (n = 8)</td>
<td>94.9</td>
<td>94.1</td>
<td>93.3</td>
<td>98.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

All feces were weighed and frozen at −20°C for subsequent analysis.

On each ileal collection day, collections were begun immediately after feeding. The screw-cap used to close the cannula between collections was replaced by a nipple. This nipple was screwed into the neck of the cannula, which allowed a 180-g capacity Whirl-Pak bag to be attached using the bag’s wire twist closure. Effluent then drained easily by gravity. The bags were well tolerated but an Elizabethan collar was used to prevent interference with the bags during collections. Dogs were thus able to walk around the laboratory or to rest with minimal supervision. Each bag was changed every 45 min, weighed, and frozen at −20°C for subsequent analysis. Collections were continued until no more effluent was produced (15 h after feeding).

Food, ileal, and fecal samples were lyophilized and then reweighed to determine DM weight. Samples from d 1 to 4 of fecal collection and d 1 to 3 of ileal collection were pooled and then homogenized by grinding in a blender to a powder. Each pooled sample was then passed through a 1-mm sieve. The Cr2O3 concentration in food varied among dogs, which made comparison of Cr2O3 in the ileal effluent among dogs difficult. The concentration of Cr2O3 (DM basis) in the ileal effluent was therefore normalized to allow comparison among dogs. The average Cr2O3 concentration for each sample was calculated as a percentage of this average. This percentage of average Cr2O3 concentration was then plotted against time (Figure 1). Preliminary analysis of this percentage over time revealed no effect of dogs or diets, so all dogs and diets were analyzed together using random coefficient repeated measures regression (Gumpertz and Pantula, 1989).

Results

Ileal collections were very successful; 50 to 100 g of effluent was collected every 45 min from the time of feeding (0800 to 0900) until no more effluent was produced (2300 to 2400). The production of effluent declined towards the end of the day until no effluent was produced for 60 to 90 min. A final large pulse of effluent was produced following this hiatus, which probably represented the product of the first migrating myoelectric complex of the interdigestive period (Szurszewski, 1969). No more effluent was produced when these collections were continued for 2 h after this final pulse, so collections were subsequently stopped after this final pulse.

Recovery of Cr2O3 was shown in Table 2. Recovery of Cr2O3 was not different between the four diet treatments, but ileal recovery (94%) was significantly lower than the pooled recovery.
greater than fecal recovery (87%) (P < .03, n = 8). There was no interaction between diet and the collection method.

Day to day variation of DM digestibility is shown in Table 3. Collections were not always possible on consecutive days, so a balanced analysis of variance was only possible for three diets using six dogs over four sequential collection days. Ileal DM digestibility was approximately two percentage units lower on the first day than on d 2 to 4 of a collection week (P ≤ .007). Ileal DM digestibility decreased (P ≤ .001) as the amount of TVP increased in the diet, but there was no interaction between amount of TVP and day.

Changes in Cr₂O₃ concentration relative to DM during a day’s collection are illustrated in Figure 1. The rate of appearance in the bags was pulsatile, and the Cr₂O₃ concentration varied widely during any one collection. Nevertheless, there was an overall trend in Cr₂O₃ concentration so that Cr₂O₃ concentration tended to increase rapidly for 3 h, then remained constant through most of the collection period before declining from 10 to 15 h after feeding. This trend is represented by the regression line:

\[
\% \text{ Cr}_2\text{O}_3 = 67.3 + 11.6 \times \text{TIME} - 0.778 \times \text{TIME}^2
\]

where TIME is the time (h) from feeding.

**Discussion**

This study shows that almost complete collection of Cr₂O₃ is possible from the ileum of a dog using gravity drainage from a simple T-cannula. Chromic oxide recovery approached 100%, though carbohydrate and DM digestibility varied widely among diets. Complete deflection of the ileal stream was not achieved because some Cr₂O₃ (1% to 5%) was detected in feces passed during ileal collections. Little or no feces was passed during ileal collections, however, which suggests that very little substrate passed the ileum to act as substrate for bacterial growth in the colon. It is unlikely therefore that Cr₂O₃ did not mark chyme representatively or passed out of the cannula preferentially.

Studies in ruminants suggest that Cr₂O₃ leaves the duodenum early with the fluid compartment, does not mark particulate matter effectively, and may be unsuitable when spot collections are used with a simple T-cannula to measure digestibility (Drennan et al., 1970; Faichney, 1972; Beever et al., 1978). It has been advocated therefore that two markers, such as chromium EDTA and a rare earth metal, should be used to mark the fluid and particulate compartments, respectively (Faichney, 1972). A rare earth metal was not used as a marker in this study because all diets contained very little cell wall material or insoluble fiber to act as a substrate. Rare earth metals tend to form suspensions of insoluble hydroxides with characteristics similar to those of Cr₂O₃ in the absence of a substrate (Kotb and Luckey, 1972). These rare earths would therefore probably carry no advantage over Cr₂O₃ except in diets containing more insoluble fiber.
A particulate marker in the form of 1-mm long pieces of a 5 French gauge (1.65 mm diameter) radiopaque angio graphic catheter (Cook Inc., Bloomingon, IN) was included in the original study design, but recovery of catheter pieces in the ileal effluent was so poor that meaningful interpretation was impossible. Gastric emptying of particulate markers in the presence of food is greatly affected by size and density of the particles. Particles greater than 1.6 mm in diameter are retained and not emptied until after a meal, so it is possible that the catheter pieces were retained in the stomach (Meyer et al., 1985; Gruber et al., 1987; Devereux et al., 1990).

Some doubt has been raised as to whether digestibility can be measured accurately when Cr2O3 is used as a marker and ileal effluent is collected intermittently in spot collections. Prigge et al. (1981) found wide variation in the DM concentration of Cr2O3 in the feces of ruminants and concluded that spot collections were not necessarily accurate. Rohr et al. (1984), however, found little difference between estimates of digestibility in cows if spot collections using Cr2O3 as a marker were compared with total collections.

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In the study reported here, an attempt was made to collect all ileal chyme. Collections were started immediately after feeding and continued until after a final pulse of effluent. This final pulse was thought to be associated with the first migrating myoelectric complex (Szurszewski, 1969) and seemed to be a discrete endpoint because no more effluent appeared if the collection was continued. This last pulse contained less Cr2O3 and was especially large (50 to 100 g wet matter) when TVP-based diets were fed. Premature cessation of the collection would therefore have markededly influenced apparent digestibility.

Meyer and Schüinemann (1989) reported measuring ileal digestibility using spot collections in cannu lated dogs starting 3 h after feeding. In the present study, Cr2O3 was excreted continuously throughout the day but its concentration relative to DM varied widely. This almost pulsatile pattern of excretion is similar to that observed in ruminants by Prigge et al. (1981) and suggests that spot sampling of ileal effluent may not measure ileal digestibility accurately if Cr2O3 is used as a marker unless many samples are obtained. Furthermore, the DM concentration of Cr2O3 was below the daily average at the beginning and end of each collection and was 105% of the daily average for most of the day. Intermittent sampling that did not include the start and finish of a day's collection would therefore have given a high estimate of the average Cr2O3 concentration and of digestibility.

The effect of total ileal collections on day to day variation of DM digestibility was unexpected. Dry matter digestibility was two percentage units less on the first day than on subsequent days of ileal collection. This difference was of little physiological importance but accounted for some of the variability observed. The colon contained fecal material on the first day but not on subsequent days, so some feedback effect from the colon may have been responsible. Dry matter digestibility did not vary on d 2 to 4, so single day collections of ileal effluent may be sufficient to obtain an accurate measure of digestibility. Nevertheless, when collections from more than 1 d must be pooled to produce enough DM for multiple analyses, it may be prudent to evaluate only material collected on d 2 to 4.

Fecal recovery of Cr2O3 showed much greater variation despite collections that lasted for 4 d. Defecation was irregular and often less than once a day when dogs were fed digestible diets. Fecal recovery of Cr2O3 was also less than ileal recovery, so some Cr2O3 may have been retained by the large intestine. It is likely therefore that the fecal Cr2O3 concentration did not reach equilibrium during the fecal collection periods.

Implications

This study describes the behavior of chromic oxide when used as a marker for measuring digestibility in cannulated dogs. It shows that almost total collection of chromic oxide is possible from simple ileal T-cannulae in dogs using gravity drainage. Day to day variation of ileal dry matter digestibility was small, so single day collections should be satisfactory. There was wide variation in chromic oxide concentration during a collection; hence spot collections may result in inaccurate estimates of nutrient digestibility unless many samples are obtained throughout each day. Ideally, therefore, complete collections should be attempted for accurate determination of nutrient digestibility.

Literature Cited


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