

A pilot study on the effect of fat loading on the gastrointestinal tract of healthy dogs

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OBJECTIVE: To assess the effect of a high fat meal (fat loading) on gastrointestinal motility and the appearance of intestinal villi using video capsule endoscopy and ultrasound.

Materials and Methods: Four healthy staff-owned dogs were included in a prospective blinded crossover study. Dogs had initial baseline video capsule endoscopy to measure gastrointestinal transit times and allow for visual assessment of intestinal mucosa. Abdominal ultrasound was also performed to obtain intestinal wall measurements and assess for the presence of mucosal hyperechoic speckling. All dogs had diagnostics repeated twice between one and two hours after ingestion of either corn oil or dairy cream for a total of four control and 16 fat loaded studies.

Results: Dogs in the corn oil group had greater mean gastric emptying times (740.3 ± 187.6 minutes vs. 237.9 \pm 155 minutes) and total transit times (54.50 \pm 22.2 hours vs. 23.25 \pm 6.1 hours) than controls. Feeding of a fatty meal resulted in substantial retention of the capsules (10 of 16) within the stomach. While intestinal wall thickness of fat loaded dogs did not differ from control dogs, mucosal hyperechoic speckling scores of the duodenum of dairy cream dogs were greater when compared to control dogs (1.625 \pm 0.518 vs. 0.500 \pm 0.577).

CLINICAL SIGNIFICANCE: Data from this pilot study provides further evidence that feeding of a small high fat meal results in ultrasonographic as well as visual changes to the intestinal mucosa of healthy dogs. This data suggests that previous recommendations to feed fatty meals to dogs with lymphangiectasia might not allow differentiation with healthy individuals. In addition, due to the marked effect on gastric emptying time, video capsule endoscopy should be avoided in dogs fed a high fat meal.

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INTRODUCTION

Intestinal lymphangiectasia is a common cause of protein-losing enteropathies in veterinary medicine. Though this disease can be congenital in origin, it most commonly occurs secondary to inflammatory or neoplastic intestinal disease (Larson et al. 2012). In the presence of these conditions, the intestinal lymphatics become obstructed, resulting in villous lacteal dilation and rup-

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ture into the intestinal lumen. The engorged villous lacteals can be seen endoscopically and ultrasonographically and are considered a hallmark of this disease (Kull et al. 2001, Larson et al. 2012).

While the gold standard for diagnosis of lymphangiectasia is the histopathological demonstration of distension of the mucosal and submucosal lymphatics with lipid, the clinical utility of adjunct imaging modalities, such as endoscopy and ultrasonography, has been investigated. Visual endoscopic scoring alone has been shown to lack specificity and sensitivity, though the latter can be significantly improved when combined with clinicopatho-

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logic changes, namely hypoalbuminemia, hypocholesterolaemia and lymphopenia (Larson *et al.* 2012). Likewise, ultrasonographic abnormalities such as hyperechoic speckling of the intestinal mucosa and thickening of the intestinal wall, have been reported in association with lymphangiectasia in dogs but their true sensitivity and specificity remain unclear (Kull *et al.* 2001, Sutherland-Smith *et al.* 2007).

Since most alimentary lipids are absorbed through the enteric lymphatic system, the feeding of a high fat meal (fat loading) before endoscopic or ultrasonographic imaging has been recommended by some clinicians in order to increase the conspicuity of the engorged lymphatics (Peterson & Willard 2003). Feeding of corn oil (CO) to both healthy dogs and dogs with lymphangiectasia has been shown to cause increased hyperechogenicity of the intestinal mucosa as assessed by ultrasound (Pollard et al. 2013). However, no significant difference was found in the degree of hyperechoic changes between healthy dogs and dogs with lymphangiectasia. In contrast, the only two case reports of fat loading in people with suspected lymphangiectasia employed dairy cream (DC) as a lipid source for fat loading and this form of lipid was shown to be effective in increasing lacteal conspicuity (Veldhuyzen van Zanten et al. 1986, Lee & Kong 2008). The effect of other fat sources on the visual or ultrasonographic mucosal appearance has not been evaluated in veterinary medicine.

The recent availability of video capsule endoscopy (VCE) in veterinary medicine offers many possibilities for the visual assessment of the intestinal tract of dogs in a safe and minimally invasive manner without requiring anaesthesia. In experimental settings, VCE has been shown to be an accurate tool for the identification of intestinal parasites in dogs and has been shown to compare favourably with post-morten examination in its capacity to localise and quantify Ancylostoma caninum and Toxocara canis worms in the small intestines (Lee et al. 2011, 2014, 2015). In contrast, the clinical reliability of VCE in the diagnosis of gastrointestinal disease remains limited to two case reports and two case series of dogs being investigated for suspected gastrointestinal haemorrhage (Davignon et al. 2016, Hardy et al. 2016, Mabry et al. 2019, Grimes et al. 2020).

The objective of this pilot study was to evaluate the effect of two different fat-loading protocols on the intestinal mucosal appearance and gastrointestinal transit times (GTTs), including oesophageal transit time (ETT), gastric emptying time (GET), small intestinal transit time (SITT) and total transit time (TTT) using VCE and abdominal ultrasound. We hypothesized that feeding a small volume high fat meal to healthy dogs would result in slowing of gastrointestinal transit parameters as well as a visible engorgement of the intestinal villi without interfering with visual assessment of the surface of the mucosa. In addition, we hypothesized that these visual changes would parallel concurrent ultrasonographic changes of the intestinal tract.

MATERIALS AND METHODS

Dogs

The study design was approved by the Iowa State University's Institutional Animal Care and Use Committee. Four healthy

dogs belonging to employees and students of the Lloyd Veterinary Medical Centre of Iowa State University were included in the study. Enrolled dogs ranged in age from two years to 13 years, had a weight range of 21.5 to 36.5 kg and a body condition score ranging from 4/9 to 6/9. For inclusion, all dogs were required to have a bodyweight above 10 kg and be free of overt systemic disease as assessed by physical exam and blood work consisting of a complete blood count (CBC), chemistry panel and urinalysis. Dogs were excluded if they had a history of gastrointestinal signs such as vomiting or diarrhoea in the past seven days, had biochemical or haematological evidence of hypoalbuminemia, hypocholesterolaemia or anaemia, or if their weight precluded the use of VCE.

Study design and outcomes

The study design was a prospective blinded crossover study. Each dog underwent VCE five times over the course of the study: one control and four test procedures (two tests for each lipid source) with a minimum time of 48 hours between arms of the study. For control procedures, dogs were administered an endoscopy capsule followed by abdominal imaging between one and two hours afterward. For test procedures, dogs were administered a 2 mL/kg oral dose of either CO or heavy DC (35% milk fat content) followed by administration of an endoscopy capsule between one and two hours afterward in order to prevent the ingesta from obscuring the gastric and intestinal mucosa. As with control procedures, abdominal imaging was performed 1-2 hours after receiving the capsule in order to approximately coincide with the estimated passage of the capsule into the small intestines (Pomrantz et al. 2016). All dogs were fasted 16 hours before and eight hours after each procedure according to ALICAM® manufacturer's recommendations (Infiniti Medical, LLC, Redwood City, CA). Measured outcomes included VCE unit transit times, gastric retention rates, intestinal wall thickness and intestinal mucosal echogenicity as assessed by ultrasound. All dogs were sedated with 0.03 mg/kg IV acepromazine (MWI Animal Health, Chicago, IL) 15 minutes before diagnostic imaging. Each test procedure (CO or DC) was repeated twice in all dogs.

Video capsule endoscopy

Video capsule endoscopy systems used were the ALICAM® system. Capsule endoscopy videos were reviewed in a blinded manner by a board-certified internist. Transit times were recorded based on the first and last images obtained in each organ (oesophageal, gastric and small intestinal). The TTT was defined as the time between administration and defecation of the capsule. The median battery life of the VCE units was 15.4 hours (range 12.1-19 hours). Severity of lacteal dilation and prominence of intestinal villi, as assessed with VCE, was subjectively scored as absent, mild, moderate and severe.

Imaging

All ultrasounds were performed by a board-certified radiologist using a Philips EPIQ 7G ultrasonography system (Philips, Andover, MA). For all studies, both a C 8-5 MHz (curvilinear) transducer and a L 18-5 MHz (linear) transducer were used to obtain images. Ultrasound still images and videos of the duodenum, jejunum and ileum were stored for blinded review by sec-

ond board-certified radiologist. The criteria evaluated included intestinal wall thickness and mucosal speckling for each section of the gastrointestinal tract. Mucosal speckling was subjectively scored on a four-point scale with 0 corresponding to no speckling and 4 corresponding to marked speckling .

Statistical analysis

Statistical analysis was performed using a commercial software (SAS v9.4, Cary, NC). The effect of different treatments on each outcome was analysed by using linear mixed model with treatments being fixed effect and patient being random effect. Multiple comparisons of different treatments with Tukey-Kramer adjustment were also provided. Treatment groups were divided into control dogs and dogs receiving CO or DC. Results were considered statistically significant if P < 0.05.

RESULTS

Capsule transit times

In total, 20 VCE studies were successfully completed. Three VCE studies had to be repeated due to failure to retrieve the capsule in two cases and vomiting of the capsule in one case. The median time between fat loading and VCE administration was 86 minutes (range 55-125 minutes).

ETTs did not differ significantly between groups (Table 1). Mean GET was significantly prolonged in the eight CO studies when compared to four control studies (740.3 and 237.9 minutes, respectively; P=0.018). Likewise, mean TTT differed significantly between CO studies and control studies (54.50 and 23.25 hours, respectively; P=0.028). Gastric retention, defined as retention of the VCE capsule within the stomach for

Group	Mean	Standard deviation	P-value
ETT (seconds)			
Control (4)	10.3	6.0	
DC (8)	8.1	3.4	0.939
CO (8)	13.8	15.2	0.845
DC versus CO			0.533
GET (minutes)			
Control (4)	237.9	155.0	
DC(8)	626.7	344.2	0.069
CO (8)	740.3	187.6	0.018
DC versus CO			0.665
SITT (minutes)			
Control (4)	114.7	51.5	
DC (8)	103.2	29.3	0.907
CO (8)	111.2	_	0.997
DC versus CO			0.982
TTT (hours)			
Control (4)	23.25	6.1	
DC (8)	51.13	29.9	0.05
CO (8)	54.5	22.2	0.028
DC versus CO			0.921

ETT Oesophageal transit time, GET gastric emptying time, SITT small intestinal transit time, TTT total transit time, DC Dairy cream, CO Corn oil; numbers in parentheses indicate the number of studies with recorded values

Table 2. Gastric retention rates of dogs				
Group	Rate of retention (%)	P-value		
Control	0/4 (0%)			
DC	3/8 (37.5%)	0.228		
CO	7/8 (87.5%)	0.003		
DC versus CO		0.033		
DC Dairy cream, CO corn	oil			

the entirety of battery life, occurred in none of the four control studies but occurred in 10 of the 16 fat loading studies. Consequently, SITTs were only recorded in six fat loading studies and no significant differences were noted between groups. The incidence of gastric retention (Table 2) was significantly higher in the CO (7 of 8) group than in the control (0 of 4) or DC (3 of 8) groups (P = 0.003 and 0.033, respectively).

Visual assessment by VCE

Due to gastric retention of the capsules, images of the small intestines were available for 4 of 4 control studies but only 6 of 16 fat loading studies (1 CO, 5 DC). Subjective visual assessment of the small intestinal mucosa revealed that mucosal changes were absent to mild in 4 of 4 studies of the control group and in 1 of 5 DC studies (Fig 1). The remaining five fat loading studies had moderate to marked mucosal changes noted.

Ultrasonographic assessment

The median time between fat loading and administration of abdominal ultrasound imaging was 193 minutes (range 180-240 minutes). Ultrasonographic measurement of intestinal wall thickness failed to identify a significant difference between controls and any of the fat loaded studies at any point along the small intestines (Table 3). However, mean duodenal mucosal speckling scores (Fig 2) were significantly higher in DC studies (mean score = 1.625; n = 8) when compared to controls (mean score = 0.5; n = 4) (P = 0.018).

DISCUSSION

Using VCE in a small population group of healthy client-owned dogs, our study documents that the feeding of a small fatty meal, regardless of specific composition, resulted in visible engorgement of the intestinal lacteals as well as significant slowing of GET and TTT. In addition, we documented changes to the duodenal mucosa that persisted for over 3 hours following fat loading.

The feeding of a high fat meal before endoscopy of suspected lymphangiectasia cases has been previously described in case reports in people (Veldhuyzen van Zanten *et al.* 1986, Lee & Kong 2008) and has been recommended in the veterinary literature (Peterson & Willard 2003). However, controlled studies to identify the optimal protocol or documentation of the visual changes to the intestinal mucosa caused by fat loading have been lacking. Unfortunately, gastric retention of the capsules limited the number of studies where images of the intestinal mucosa

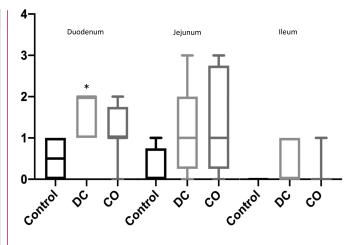


FIG 1. Representative capsule endoscopy images from the duodenal mucosa of control dogs (A, B) and dogs a fed fatty meal (C, D)

Group	Mean (mm)	Standard deviation	P-value
Duodenal			
Control (4)	3.74	0.61	
DC (8)	4.28	0.43	0.2137
CO (8)	4.34	0.50	0.2598
DC versus CO			0.9864
Jejunal			
Control (4)	3.34	0.27	
DC (8)	3.31	0.51	0.9931
CO (8)	3.46	0.46	0.9169
DC versus CO			0.8092
lleal			
Control (4)	2.12	0.21	
DC (6)	2.17	0.32	0.9803
CO (8)	2.61	0.63	0.2989
DC versus CO			0.3065

were obtained. Though subjective evaluation of the images from fat loaded studies suggests noticeable changes to the intestinal mucosa, the low numbers available for review precluded statistical comparison with images from control studies. In addition, our study found that a delay of 60-120 minutes after administration of cream or oil was enough to clear the ingesta from the gastric and small intestinal lumens and allow for adequate visual assessment of the mucosal surface. Given that small volumes of meals were sufficient to incite these changes, it would appear that administration of a small lipid-rich meal 60-120 minutes before anaesthesia for gastroduodenoscopy would be feasible with minimal risk of vomiting of stomach content or impairment of visual assessment of the gastrointestinal lumen.

The effect of food, and specifically the effect of fat content, on the ultrasonographic appearance of the intestinal mucosa has previously been reported in healthy dogs as well as dogs with lymphangiectasia (Pollard *et al.* 2013, Gaschen *et al.* 2016). Similarly to what has been reported in these studies, the fat-loaded dogs showed increased ultrasonographic echogenicity of the intestinal



DC Dairy cream, CO corn oil, *p < 0.05

FIG 2. Intestinal mucosal scores of fat loaded dogs

mucosa when compared to controls. However, the dogs in our study failed to demonstrate any changes in intestinal wall thickness. It is possible that this difference from the previous report is due to differences in the times between feeding and ultrasound imaging. Indeed, while dogs in the Pollard et al. report were imaged at 60, 90 and 120 minutes after feeding, our dogs were imaged up to 240 minutes post-feeding. Such a delay in imaging might have resulted in our missing the periods of peak intestinal blood flow and thus the associated thickening of the intestinal wall (Kircher *et al.* 2003).

The increase in GET associated with feeding has been previously reported in research dogs as assessed by various electronic capsule devices (Sagawa *et al.* 2009, Mahar *et al.* 2012, Koziolek *et al.* 2019). This increase in transit time has been proposed to be due to interruption of the migrating motor complex which occurs after feeding. Gastric retention precluded accurate measurement of transit times in many of our studies and likely underestimated

transit times. In spite of that, the GET reported in our dogs, both fasted and fed, were substantial longer than previously reported using capsule devices. While previous studies reported fasting GET between 0.57 and 1.4 hours, the mean fasting GET in our dogs was almost 4 hours. Similarly, while previously reported postprandial TTT ranged from 2.9 to 20 hours, the mean TTT of dogs fed any of the fatty meals was in excess of 50 hours. Since the population of dogs used in our study was composed of client-owned dogs of varying age, breed and size, it is difficult to compare these results with those obtained with purpose-bred research dogs. Indeed, our transit times more closely resemble those from a study investigating the effect of laparoscopic gastropexy on transit times in client-owned dogs (Balsa et al. 2017). In that study, average capsule GET and TTT before surgery was in excess of 7 and 32 hours, respectively. The time spent in hospital with our dogs might be an important factor in the prolongation of transit times since hospitalisation and the associated stress has been shown to prolong GET over fourfold (Warrit et al. 2017). On study days, our dogs spent approximately 8 hours in hospital in order to undergo the necessary diagnostic testing. Research dogs, being housed and acclimated to their usual settings for the previously referenced studies would likely not be subjected to increased stress. Another possible contributing factor is the effect of sedation on gastrointestinal motility. Multiple studies using barium contrast radiography in dogs and cats have shown that acepromazine failed to have a significant effect on gastrointestinal motility and contraction even when used at doses superior to those used in our protocol (Zontine 1973, Hogan & Aronson 1988, Scrivani et al. 1998). However, these studies employed liquid barium as a marker of gastrointestinal motility and it remains unclear how well liquid and particulate transit times correlate (Lester et al. 1999). The population of dogs used in our study constituted a possible source of limitations as their signalment and husbandry practices were not standardised. However, though the dogs varied greatly in size, it is unlikely that this is a significant influential factor in GTT. Canine studies have shown conflicting data as to the effect of bodyweight on GTT. Though one canine study using a ¹⁴C-octanoic acid breath test to measure GET found a positive correlation with bodyweight (Bourreau et al. 2004), two studies using radio-opaque markers or sulfasalazine found no correlation between body size and GET or SITT in dogs (Weber et al. 2002, 2003). Perhaps more relevant to our study, when gastrointestinal motility was assessed using a capsule similar in size to the device used in our study, no correlation was found between the size of dogs and either GET or SITT (Boillat et al. 2010). There is limited data available assessing the effect of diet on gastrointestinal function and motility. Since the dogs in our study were not fed a standardised diet before inclusion, it is possible that this might account for some differences in their basal gastrointestinal motility. However, one study evaluating GTT in dogs being fed different diets for 4 weeks failed to identify any significant effect of diet on TTT as measured using chromium oxide or plastic beads (Rolfe et al. 2002). Lastly, though the dogs included in our study underwent some preliminary screening for possible underlying gastrointestinal disease, we cannot definitively rule out the presence of occult disease. Full ultrasonographic assessment of the intestinal tract, mesenteric lymph nodes or the pancreas was not performed. Likewise, though VCE was performed in these dogs, histopathologic review of the intestinal mucosa was not performed. Though faecal flotation examinations were not performed and we cannot rule out the presence of protozoal infections, the fact that VCE control studies failed to identify any intestinal worms makes helminthic infections less likely. Indeed, in experimental canine infections VCE has been shown to correlate well with necropsy quantification of worms and counts between these two modalities do not differ significantly (Lee *et al.* 2011, 2015).

The data presented here constitute the results of an exploratory study on the usage of a relatively novel technology, VCE, in comparing two different fat loading protocols. It is unfortunate that a substantial number of VCE studies could not be completed due to gastric retention of the capsules. However, in and of itself, this complication highlights a possible important limitation of VCE that should be considered when designing experimental methods for future studies. Finally, results of this pilot study suggest that the administration of a high fat meal to healthy dogs before endoscopy enhances the visual conspicuity of intestinal lacteals. However, further endoscopic studies involving larger populations of healthy dogs as well as dogs with lymphangiectasia are required to determine if outcomes to fat loading differ between these two populations and if fat loading can be used to aid in the endoscopic diagnosis of gastrointestinal diseases such as lymphangiectasia. Finally, this study highlights some important limitations to the use of VCE in the evaluation of nutritional interventions as a substantial number of studies could not be completed due to high rate of gastric retention.

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AUTHOR CONTRIBUTIONS

Jean-Sebastien Palerme: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; writing-original draft; writing-review and editing. Auri Silverstone: Data curation; formal analysis; investigation; project administration. Elizabeth Riedesel: Data curation; writing-review and editing. Kisrtina Simone: Data curation; methodology; writing-review and editing. Jill Pomrantz: Data curation; methodology; writing-review and editing.

Conflict of Interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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