Gastrointestinal volatile fatty acid concentrations and pH in cats

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Objective—To measure volatile fatty acid (VFA) concentrations and pH in the gastrointestinal tracts of healthy adult cats fed a commercial dry cat food.

Animals—14 cats.

Procedure—The gastrointestinal tracts were excised immediately after euthanasia and divided into 6 sections (stomach, duodenum, jejunum, ileum, proximal portion of the colon, and distal portion of the colon). Luminal contents were collected from each segment, pH was measured, and contents were centrifuged. The supernatant was analyzed for acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate concentrations by use of gas chromatography.

Results—Mean total VFA concentrations were lowest in the stomach (20 mmol/L); increased through the duodenum, jejunum, and ileum (30, 29, and 41 mmol/L, respectively); and were greatest in the proximal and distal portions of the colon (109 and 131 mmol/L, respectively). Estimated mean total VFA amounts were low (< 600 µmol) throughout all segments of the gastrointestinal tract; pH values increased from the stomach through the ileum and subsequently decreased in the colon.

Conclusions and Clinical Relevance—Total VFA concentrations in the colon were comparable to values reported for the forestomach of ruminants and large intestines of monogastric animals, whereas values in the small intestine were higher than reported for other species. Total VFA amounts were low, consistent with the short, nonvoluminous gastrointestinal tract of carnivores. Luminal pH varied throughout the gastrointestinal tract in a pattern similar to other monogastric animals. Volatile fatty acids probably contribute minimal metabolic energy in cats but may be important in the maintenance of local mucosal health. (*Am J Vet Res* 2000;61:359–361)

Volatile fatty acids (VFA) are the primary end products of anaerobic microbial fermentation in the mammalian gastrointestinal tract, where they are readily absorbed and used for numerous functions. Mammals lack the enzymatic capability to split certain chemical bonds of plant origin, but microbial fermentation allows the host to utilize otherwise indigestible food sources (eg, plant celluloses, fibers, sugars, pectins, and dextrans). The energy contribution from VFA produced in the gastrointestinal tract can be substantial, ranging from 60 to 70% of the daily **metabolic energy requirement (MER)** from the rumen of sheep and cows to 5 to 10% of MER from the large intestine of dogs and humans.[1-3] Volatile fatty acids also maintain intestinal mucosal integrity and intestinal fluid and electrolyte balance, influence carbohydrate and lipid metabolism, and possibly prevent colon cancer in humans.[2-5] Dietary composition influences the quantities and types of VFA produced by altering the populations and metabolic activities of gastrointestinal microbes. Manipulation of the diet to optimize VFA production has been extensively investigated in several species, particularly ruminants.[3,4,6,7]

Volatile fatty acid concentrations have been measured in numerous species, including cows, horses, pigs, rabbits, rats, dogs, and humans.[1,3,4,6-8] However, there is a paucity of information regarding concentrations of VFA in the gastrointestinal tract of cats. Several investigators have examined the production of VFA by cat fecal flora in vitro and the influence of various dietary fibrous substances on the fermentative process.[9-15] The bacterial population in the proximal portion of the small intestine of healthy cats has also been identified and quantitated.[16,17] To the authors' knowledge, however, there are no reports of VFA concentrations measured in vivo in cats. The purpose of the study reported here was to identify and quantitate VFA in the gastrointestinal tracts of healthy adult cats fed free-choice.

Materials and Methods

The cadavers of 14 random-source, adult domestic shorthaired cats were provided immediately after euthanasia at the conclusion of an unrelated injectable anesthetic trial. Samples were collected 4 to 27 days after the trial, so that the anesthetic drugs (ketamine hydrochloride, butorphanol, and medetomidine hydrochloride) were unlikely to affect the outcome of this study. The 11 male and 3 female cats were neutered and weighed 2.5 to 5.0 kg. All cats had been vaccinated[a] and dewormed (ivermectin, 2.5 mg [body weight > 4 kg] or 2.0 mg [body weight < 4 kg], SC). For all cats, PCV, serum total protein concentration, and WBC counts were within reference ranges, and results of fecal analyses for parasites were negative.

Cats were fed a free-choice balanced commercial laboratory dry diet[b] with a guaranteed analysis of 32% (minimum) crude protein, 12% (minimum) crude fat, and 3% (maximum) crude fiber. Percentage metabolizable energy provided by the major nutrients in this diet were estimated at 33% protein, 30% fat, and 37% carbohydrate. The major ingredients, listed by weight, were ground corn, poultry by-product meal, soybean meal, animal fat (preserved with butylated hydroxyanisole), corn gluten meal, ground wheat, wheat middlings, fish meal, dried skim milk, poultry digest, and dried whey. Fresh water was provided ad libitum.

Euthanasia was performed, using a commercial euthanasia solution[c] (1 ml/4.5 kg of body weight), and the gastrointestinal tract was removed en bloc, divided with hemostats, and transected into 6 sections: stomach, duodenum, jejunum,

Received Jul 26, 1999.
Accepted Dec 20, 1999.
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Supported by the Veterinary Medical Teaching Hospital, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610-0126.

Table 1—Concentrations (mmol/L; mean ± SD [range]) of volatile fatty acids (VFA) in various segments of the gastrointestinal tract in cats

VFA	Stomach	Duodenum	Jejunum	Ileum	Colon (proximal)	Colon (distal)
Acetate	11 ± 15[c] (1–50)	19 ± 12[b] (5–43)	23 ± 19[b] (5–74)	29 ± 28[b] (5–104)	52 ± 28[a] (18–106)	57 ± 36[a] (22–146)
Proprionate	3 ± 4[b] (0–17)	2 ± 1[b] (0–4)	2 ± 4[b] (0–16)	4 ± 7[b] (0–24)	21 ± 9 (7–37)	27 ± 17[a] (12–74)
Butyrate	2 ± 2[b] (0–7)	2 ± 0[b] (2–4)	2 ± 1[b] (0–5)	3 ± 4[b] (0–13)	13 ± 6[a] (4–24)	15 ± 6[a] (6–27)
Isobutyrate	2 ± 2[b] (0–6)	2 ± 1[b] (1–4)	1 ± 1[b] (0–2)	2 ± 1[b] (0–2)	8 ± 4[a] (0–13)	9 ± 4[a] (0–14)
Valerate	1 ± 2[b] (0–5)	1 ± 1[b] (0–3)	1 ± 3[b] (0–10)	1 ± 1[b] (0–3)	7 ± 5[a] (0–13)	8 ± 4[a] (0–13)
Isovalerate	1 ± 1[b] (0–5)	2 ± 1[b] (1–4)	1 ± 1[b] (0–2)	1 ± 1[b] (0–3)	7 ± 5[a] (0–12)	7 ± 4[a] (0–11)
Total VFA	**20 ± 25[c] (3–90)**	**30 ± 14[b] (12–52)**	**29 ± 23[b] (9–79)**	**41 ± 39[b] (9–147)**	**109 ± 47[a] (46–190)**	**131 ± 66[a] (49–264)**

[a-c]Within rows, means with different superscript letters are significantly ($P < 0.05$) different.

Table 2—pH Values and estimated total VFA (mean ± SD [range]) in various segments of the gastrointestinal tract in cats

Variable	Stomach	Duodenum	Jejunum	Ileum	Colon (proximal)	Colon (distal)
pH	2.5 ± 0.7[e] (1.5–3.7)	5.7 ± 0.5[a-d] (4.9–6.7)	6.4 ± 0.5[b-d] (5.9–7.6)	6.6 ± 0.8[b-d] (5.1–7.6)	5.8 ± 0.4[a-d] (5.0–6.3)	5.3 ± 0.4[a,b,d] (4.4–5.7)
Total VFA (mmol)	**310 ± 560 (10–1900)**	**40 ± 80 (3–260)**	**120 ± 110 (3–400)**	**60 ± 110 (6–370)**	**560 ± 630 (50–2160)**	**440 ± 320 (30–1010)**

[a-e]Within rows, means with different superscript letters are significantly ($P < 0.05$) different

ileum (distal 10 cm of the small intestine), and colon (divided equally into proximal and distal segments). The luminal contents were gently extruded from each segment into clean, preweighed plastic centrifuge tubes. If the sample was of insufficient quantity, the segment was opened and the contents collected by gently scraping the mucosa with a metal spatula. The pH of the luminal contents of each section was measured by inserting a calibrated micro-pH probe[d] directly into each centrifuge tube. The tubes were reweighed to determine total luminal content weights and centrifuged at 17,540 × g at 6 C for 17 minutes. The supernatant was removed and immediately frozen at –70 C for later analysis. The remaining pellet was weighed; the weight was subtracted from the total luminal content weight to obtain estimated supernatant weight. Supernatant volume was calculated by dividing estimated weight by an estimated fluid specific gravity of 1.000.

On the day of analysis, samples were thawed and filtered through a 0.45-mm filter into glass vials. Colonic samples were diluted 1:4 with ultra pure water. Concentrations of VFA were determined by use of a gas chromatograph[f] with a hydrogen flame ionization detector and fused silica capillary column[g] (30 cm; internal diameter, 0.25 mm; film thickness, 0.25 µm). The carrier gas was helium (117 kPa), and the detector gas was hydrogen (flow rate, 45 ml/min). The oven, injection port, and detector port temperatures were 155, 250, and 180 C, respectively. A 1-µl sample was injected with a split ratio of 1:100.

Statistical analyses—Peak heights were compared with those of external standards run with each test batch. Estimated total VFA amounts were calculated for each segment by multiplying total VFA concentration by supernatant volume. Volatile fatty acid concentrations, amounts, and pH were reported as mean ± SD. For most segments of the intestine, results of the Kolmogorov-Smirnov test indicated these data were not normally distributed. There were insufficient luminal contents to measure VFA concentrations in 8 of 84 (< 10%) intestinal segments. These missing data points were estimated as the mean VFA concentration or pH from each segment. Comparisons were performed on this balanced data set after log transformation by use of an ANOVA procedure,[h] with intestinal location as a single repeated measures factor. Results were similar to those of a Friedman 2-way ANOVA performed on untransformed data. Post-hoc comparisons were performed on the log transformed data set by use of a Tukey Honestly Significant Difference test. A type 1 error of < 0.05 was considered significant.

Results

Six VFA were measured from the gastrointestinal luminal contents: acetate, proprionate, butyrate, isobutyrate, isovalerate, and valerate. Volatile fatty acid concentrations and pH were significantly ($P < 0.001$) different among segments of the gastrointestinal tract. Total and individual VFA concentrations were greatest in the colon, whereas total VFA and acetate concentrations were lowest in the stomach (**Table 1**). Mean total VFA concentrations (mmol/L) were 20 (stomach), 30 (duodenum), 29 (jejunum), 41 (ileum), 109 (proximal colon segment), and 131 (distal colon segment). Estimated total amounts of VFA were low (< 600 µmol) throughout all segments of the gastrointestinal tract (**Table 2**). The pH values increased from the stomach through the ileum and subsequently decreased in the proximal and distal colon segments (Table 2). Mean pH values were 2.5 (stomach), 5.7 (duodenum), 6.4 (jejunum), 6.6 (ileum), 5.8 (proximal colon segment), and 5.3 (distal colon segment).

Discussion

Total VFA concentrations in the proximal and distal colon segments of the cats reported here were comparable to those reported in the forestomach of ruminants and the large intestines of monogastric animals, where values typically range from 60 to 150 mmol/L.[3] Mean small intestinal VFA concentrations in the cats reported here were higher than expected, with values of 30, 29, and 41 mmol/L in the duodenum, jejunum, and ileum, respectively. In contrast, the small intestines of most animal species have been reported to be essentially sterile and produce VFA concentrations not > 10 to 20 mmol/L.[6-8] This finding is consistent with recent reports that clinically normal cats harbor high numbers of small intestinal bacteria such that the established criteria for small intestinal bacterial overgrowth in dogs and humans are fulfilled.[16,17] Acetate, proprionate, and butyrate were the most abundant VFA in these cats, with ratios of approximately 70:20:10 in the stomach, 80:10:10 in the small intestine, and 60:25:15 in the colon, which are similar to those of other nonruminant species.[1,2]

For each species, the metabolic energy provided by VFA is dependent on concentrations, rates of production and absorption, and the relative capacity and surface area of the fermentative segment. Although total VFA concentrations and individual VFA ratios in the cats reported here were comparable to those of ruminants and other monogastrics, estimated total VFA amounts were low in all segments. Carnivores possess short, nonsacculated, nonvoluminous gastrointestinal segments in comparison with animals (such as pigs and horses) with relatively voluminous, sacculated large intestines,[8] so that energy provided by VFA would be expected to be minimal in cats and dogs.

Although VFA probably contribute little metabolic energy in cats, they likely have numerous local benefits. The movement of water out of the colon is osmotically linked to VFA and sodium transport, and production and absorption of VFA has a substantial effect on the normal secretory and absorptive functions of the colon.[3,7] Volatile fatty acids also promote local mucosal health and integrity by stimulating gastrointestinal epithelial blood flow and serving as the primary local energy source for colonocytes.[1,3]

Luminal pH in these cats varied throughout the gastrointestinal tract in a pattern similar to other monogastric species.[6-8,10] Mean pH was lowest in the stomach, which is consistent with the secretion of gastric acid, and highest in the ileum, which is consistent with the secretion of bicarbonate. The pH declined in the proximal and distal colonic segments, which likely reflects the higher VFA concentrations in these segments. The colonic pH values were comparable to fecal pH values reported for cats fed raw starch and disaccharide (ie, fermentable) diets.[18]

Results indicated that VFA were produced throughout the gastrointestinal tracts of the cats reported here, which were fed a free-choice dry diet containing a relatively large amount of carbohydrate and moderately fermentable fiber (compared with most canned commercial diets). Manipulating these factors (eg, limited vs free choice feeding, canned vs dry food, or changing the carbohydrate or fiber concentrations) may alter VFA production in cats.[13,15,18]

[a]Felocell CVR, Pfizer Animal Health, Exton, Penn.
[b]Cat Diet 7770, Harlan Teklad, Madison, Wis.
[c]Beuthanasia-D, Schering Plough Animal Health Corp, Union, NJ.
[d]Microcomputer pH Vision model 05669-20, Cole Parmer Instrument Co, Vernon Hills, Ill.
[e]High Pressure Liquid Chromatography (HPLC) water, Fisher Scientific, Fair Lawn, NJ.
[f]Perkin Elmer Autosystem GC, Perkin-Elmer Corp, Norwalk, Conn.
[g]Fused Silica Capillary Column, Supelco Inc, Bellefonte, Penn.
[h]Statistica 1997 Software, Tulsa, Okla.

References

1. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tracts in various species. *Physiol Rev* 1990;70:567–590.

2. Bugaut M. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Biochem Physiol* 1987;86:439–472.

3. Royall D, Wolever MS, Jeejeebhoy KN. Clinical significance of colonic fermentation. *Am J Gastroenterol* 1990;85:1307–1312.

4. Titus E, Ahearn GA. Vertebrate gastrointestinal fermentation: transport mechanisms for volatile fatty acids. *Am J Physiol* 1992;262:R547–R553.

5. Binder HJ, Sandle GI. Electrolyte transport in the mammalian colon. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. 3rd ed. New York: Raven Press, 1994:2133–2171.

6. Herschel DA, Argenzio RA, Southworth M, et al. Absorption of volatile fatty acid, Na, and H_2O by the colon of the dog. *Am J Vet Res* 1981;42:1118–1124.

7. Stevens, CE. Physiological implications of microbial digestion in the large intestine of mammals: relation to dietary factors. *Am J Clin Nutr* 1978;31:S161–S168.

8. Banta CA, Clemens ET, Krinsky MM, et al. Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of dogs. *J Nutr* 1979;109:1592–1600.

9. Sunvold GD, Fahey GC, Merchan NR, et al. In vitro fermentation of selected fibrous substrates by dog and cat fecal inoculum: influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. *J Anim Sci* 1995;73:1110–1122.

10. Sunvold GD, Fahey GC, Merchan NR, et al. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. *J Anim Sci* 1995;73:1099–1109.

11. Sunvold GD, Fahey GC, Merchan NR, et al. Dietary fiber for cats: in vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. *J Anim Sci* 1995;73:2329–2339.

12. Sunvold GD, Fahey GC, Merchan NR, et al. Fermentability of selected fibrous substrates by dog fecal microflora as influenced by diet. *J Nutr* 1994;124:2719–2720.

13. Sunvold GD, Hussein HS, Fahey GC, et al. In vitro fermentation of cellulose, beet pulp, citrus pulp and citrus pectin using fecal inoculum from cats, dogs, horses, humans and pigs and ruminal fluid from cattle. *J Anim Sci* 1995;73:3639–3648.

14. Sunvold GD, Reinhart GA. Maintaining gastrointestinal health via colonic fermentation, in *Proceedings.* Gastrointest Health Symp, 1997:7–12.

15. Sunvold GD, Titgemeyer EC, Bourquin LD, et al. Fermentability of selected fibrous substrates by cat fecal microflora. *J Nutr* 1994;124:2721S–2722S.

16. Johnston K, Lamport A, Batt RM. An unexpected bacterial flora in the proximal small intestine of normal cats. *Vet Rec* 1993;132: 362–363.

17. Papasouliotis K, Sparkes AH, Werret G, et al. Assessment of the bacterial flora of the proximal part of the small intestine in healthy cats, and the effect of sample collection method. *Am J Vet Res* 1998;59:48–52.

18. Kienzle E. Effect of carbohydrates on digestion in the cat. *J Nutr* 1994;124:2568–2571.