Evaluation of a modified acetaminophen absorption test to estimate the abomasal emptying rate in Holstein-Friesian heifers

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Objective—To assess the suitability of the modified acetaminophen absorption test for evaluation of abomasal emptying rate in ruminating cattle.

Animals—7 Holstein-Friesian heifers.

Procedures—In a crossover study design, heifers consecutively underwent an IV infusion of 1 L of saline (0.9% NaCl) solution (control treatment), 1 L of saline solution containing metoclopramide (0.1 mg/kg), and 1 L of saline solution containing atropine (0.1 mg/kg), with an interval of 15 days between treatments. Immediately after each treatment, acetaminophen diluted in ethanol (50 mg/kg) was infused transcutaneously into the abomasum. Blood samples were obtained repeatedly for measurement of plasma acetaminophen concentration, and pharmacokinetic data were obtained.

Results—Maximum plasma acetaminophen concentration was significantly lower after atropine treatment than after control or metoclopramide treatment, whereas no difference was identified between control and metoclopramide treatments. The interval to maximum plasma acetaminophen concentration was significantly longer in atropine-treated versus metoclopramide-treated heifers. The interval to maximum acetaminophen concentration obtained from a pharmacokinetic model was significantly longer for atropine than for control and metoclopramide treatment. Similarly, areas under the plasma acetaminophen concentration-time curves for the first 60, 90, 120, and 240 minutes after administration were significantly lower for atropine versus metoclopramide or control treatment, whereas differences between metoclopramide and control treatments were not identified.

Conclusions and Clinical Relevance—The modified acetaminophen absorption test was a practical, minimally invasive, and reliable method to assess abomasal emptying in cattle. Metoclopramide administered at a dose of 0.1 mg/kg did not increase the abomasal emptying rate. (Am J Vet Res 2011;72:1600–1606)

Slow abomasal motility and delayed abomasal emptying are considered important factors in the etiopathology of abomasal disorders in adult cattle. Abomasal emptying in cattle of all ages has been evaluated through various approaches, including the phenol red dye or polyethylene glycol dilution method, d-xylose absorption test, or cobalt EDTA or chromium EDTA marker test. Furthermore, nuclear scintigraphy, ultrasoundography, measurement of abomasal intraluminal pressure or pH in abomasal fluid, and electromyography have been used for this purpose. With the exception of the d-xylose test, these procedures are technically challenging or require sophisticated equipment and thus are not well suited for use in a field setting. The d-xylose test has successfully been used to determine abomasal emptying rates in healthy and diseased adult cattle. Nonetheless, concerns have been raised because of the increase in osmolarity of the abomasal fluid to > 1,000 mOsmol/L that can occur after abomasal infusion of 50% d-xylose solution at
acetaminophen in alcohol. Therefore, in monogastric species and preruminating calves, the APAT, which involves measurement of plasma acetaminophen concentrations in short intervals after oral administration of an acetaminophen solution, is considered a reliable diagnostic tool to determine the gastric emptying rate. In ruminants, the APAT has been used to evaluate the function of the reticular groove reflex in lambs and calves as well as to determine effects of some presumably prokinetic drugs on abomasal emptying in suckling calves.

To our knowledge, only 1 study has been reported in which an APAT was used in adult cattle. In that study, a watery acetaminophen suspension was infused into the abomasum of cows fitted with a rumen cannula by passing a tube from the rumen into the abomasum. Because of the poor solubility of acetaminophen in water, a large volume of water would be required to entirely dissolve acetaminophen for transcutaneous infusion through a small-bore needle into the abomasum. Because volume is the most important determinant of gastric emptying, a test for measuring gastric emptying rates should not or should only minimally alter the abomasal volume. To overcome this challenge, ethanol could be used instead of water as a solvent for acetaminophen (time 0) as well as every 15 minutes afterward. The needle was deemed properly located when the pH of the fluid was measured. In the study reported here, we hypothesized that transcutaneous infusion of acetaminophen dissolved in a 96% ethanol solution into the abomasum of cattle with a fully developed forestomach system would allow estimation of the abomasal emptying rate. Accordingly, our objective was to assess the suitability of the modified APAT to determine changes in the abomasal emptying rate induced by parenteral administration of drugs known to alter gastrointestinal motility in ruminating cattle such as atropine and metoclopramide.

Materials and Methods

Animals—Seven 12- to 30-month-old nonpregnant Holstein-Friesian heifers weighing between 325 and 385 kg were used in the study. This sample size was calculated by use of the effect size and variation observed in a preliminary, unreported trial. Cattle were determined healthy through performance of a thorough physical examination and serum biochemical and hematologic analyses.

Heifers were fed twice daily at 4 AM and 4 PM. The ration offered consisted of alfalfa hay, corn silage, ground barley grain, wheat bran, and trace mineralized salt and had been formulated to meet the dietary requirements for replacement heifers. Throughout the study period, health status of the heifers was monitored daily by observation of their behavior and feed intake. Heart rate, respiratory rate, and frequency and intensity of rumen contraction as well as rectal temperature were determined. All methods and procedures were in compliance with institutional guidelines for the care and use of agricultural animals in agricultural research and teaching.

Preparation of acetaminophen solution—The acetaminophen solution was prepared by dissolving acetaminophen (50 mg/kg) in a 96% ethanol solution (1 g of acetaminophen/10 mL of 96% ethanol). The solution was then thoroughly mixed to ensure complete dissolution of acetaminophen. The amounts of acetaminophen infused ranged from 16.2 to 29.2 g dissolved in a total volume of 96% ethanol ranging from 162 to 292 mL. The equivalent amounts of ethanol thus ranged from 128 to 230 g of ethanol/treatment.

Procedures—In a crossover design, the heifers received 3 consecutive treatments (control treatment, metoclopramide, and atropine) in the same order, with an interval of 15 days between each. On the morning of each treatment, heifers were weighed. A jugular groove in each heifer was clipped of hair, and a 14-gauge catheter was aseptically fitted and secured in a jugular vein. In the first treatment, a bolus IV infusion of 1 L of saline (0.9% NaCl) solution was administered. In the second treatment, a similarly administered bolus of 1 L of saline solution containing metoclopramide (0.1 mg/kg) was given, and in the third, 1 L of saline solution containing atropine (0.1 mg/kg) was given.

Immediately after IV infusion, each heifer underwent transcutaneous abomasocentesis, which was performed as described elsewhere. Briefly, the puncture site approximately 10 cm caudal to the xyphoid process and 10 cm to the right of the ventral midline was identified, shaved, and prepared aseptically. An 18-gauge spinal needle with a stylette was then forwarded through skin and body wall in a dorsal direction to puncture the abomasum. The proper location of the needle tip in the lumen of the abomasum was confirmed by measuring the pH of fluid draining through the needle by use of litmus paper. The acetaminophen solution was then infused, and the needle location was again verified afterward. The needle was deemed properly located when the pH of the fluid was < 3.0.

Blood samples were obtained from the jugular catheter immediately before administration of acetaminophen (time 0) as well as every 15 minutes afterward for 6 hours. Collected blood samples were immediately transferred into 10-mL tubes containing potassium oxalate as anticoagulant, and tubes were then centrifuged (1,800 g for 15 minutes). Harvested plasma was stored at –20°C until analyzed for plasma acetaminophen by use of a spectrophotometric technique described elsewhere. Puncture sites were monitored daily by visual inspection and palpation to identify signs of inflammation on the morning of each treatment day as well for 5 days following each treatment.

Pharmacokinetic analysis—The Cmax and Tmax were obtained from a plot of the plasma acetaminophen concentration versus time data. A pharmacokinetic model was used to model the acetaminophen absorption curve for each heifer treatment combination. The Cmaxmodel and Tmaxmodel were determined from this modeled curve. The first derivative of a modified power exponential for...
ula was used to describe the plasma acetaminophen concentration-time relationship as follows:

\[ C(t) = m \cdot k \cdot e^{-\beta t} \cdot (1 - e^{-\alpha t})^{-1} \]

in which \( C(t) \) is the acetaminophen concentration in plasma at time \( t \); \( m \), \( k \), and \( \beta \) are constants; and \( m \) is the total cumulative acetaminophen recovery when time is infinite. The modified power exponential model focuses on gastric emptying and subsequent small intestinal absorption of a marker substance and therefore provides a suitable model to assess gastric emptying. This model is reportedly the best method for describing the acetaminophen absorption curve in suckling calves. The modified power exponential model was used to describe the plasma acetaminophen curve. The value for \( C_{\text{max}} \) was determined by applying the values for \( m \), \( k \), \( \beta \), and \( t = T_{\text{max}} \) to the cumulative dose curve. The value for \( \text{AUC}_{\text{acet}} \) was calculated for the first 60, 90, 120, and 240 minutes following acetaminophen administration by use of the trapezoidal rule to provide an index of the amount of acetaminophen absorbed for each heifer treatment combination.

Statistical analysis—Summary data are expressed as mean ± SD. Normal distribution of data was tested by determining the Shapiro-Wilk \( W \) and associated \( P \) value as well as by examining the normal probability plots. Repeated-measures ANOVA performed by use of statistical software was used to determine the main effects of treatments (3 levels: control, atropine, and metoclopramide) and time (25 levels representing 4 samples every 15 minutes for 6 hours in addition to the first sample at time 0) as well as the interaction between treatment and time. The effects of treatment and time or their interaction were considered fixed, and heifer was considered a random effect. Bonferroni-adjusted \( P \) values were used to assess changes within and differences between groups whenever the result of the \( F \) test was significant. Values of \( P < 0.05 \) were considered significant.

Results

Animals—All heifers remained healthy throughout the study and appeared to tolerate the abomasocentesis and jugular catheterization without obvious complications as determined by daily physical examination for 5 days following each treatment.

Pharmacokinetics for the modified APAT—The plasma acetaminophen absorption curves were stratified by treatment (Figure 1), and the \( C_{\text{max}} \), \( T_{\text{max}} \), \( C_{\text{maxmod}} \), and \( T_{\text{maxmod}} \) as well as \( \text{AUC}_{\text{acet}} \) for the first 60, 90, 120, and 240 minutes after acetaminophen administration were summarized (Table 1). Repeated-measures ANOVA of the plasma acetaminophen concentration revealed that only time effects were significant, whereas treatment effects were not significant (\( P = 0.091 \)). Treatment effects were determined for \( C_{\text{max}} \) (\( P < 0.001 \)), \( C_{\text{maxmod}} \) (\( P < 0.001 \)), \( T_{\text{max}} \) (\( P = 0.017 \)), and \( T_{\text{maxmod}} \) (\( P = 0.002 \)).

Whereas \( C_{\text{max}} \) and \( C_{\text{maxmod}} \) were significantly lower after atropine treatment than after control or metoclopramide treatment in the heifers, no differences between control and metoclopramide treatments were identified for \( C_{\text{max}} \) or \( C_{\text{maxmod}} \). For \( T_{\text{max}} \), significant group differences were identified only between atropine and metoclopramide treatments (\( P = 0.005 \)) but not between atropine and control treatments. In contrast, \( T_{\text{maxmod}} \) was significantly higher for the atropine treatment than for the control and metoclopramide treatments (\( P = 0.009 \)) and metoclopramide (\( P = 0.001 \)) treatments. Similarly, the \( \text{AUC}_{\text{acet}} \) for the first 60, 90, 120, and 240 minutes after acetaminophen administration was significantly lower for the atropine than for the metoclopramide or control treatment in the study heifers, whereas differences between metoclopramide and control treatments were not identified.

Discussion

The main objective of the present study was to assess the suitability of a modified APAT to comparatively estimate the abomasal emptying rate in heifers with a completely developed forestomach system under field conditions. The values obtained for \( T_{\text{max}} \) were slightly lower than the range determined in healthy adult cattle.
by means of the d-xylose absorption test (90 to 120 minutes)3,5 but considerably higher than the mean T_max determined in rumen-cannulated cows by means of an APAT (48 minutes).2 The mean C_max reported in that study2 was 55.8 µg/mL, slightly above the C_max determined after the control treatment in the present study. Because abomasal emptying rates for cattle reported here and in the literature were determined by use of different marker substances (d-xylose vs acetaminophen), volumes administered into the abomasum (1 vs 0.5 mL/kg), solvents (water vs ethanol), and routes of administration (transcutaneous vs intra-abomasal infusion with a tube passed through a rumen cannula), we believe that the reported values are only comparable to a limited extent.

D-xylose is commonly administered as a 30% solution with an osmolarity of > 3,000 mOsmol/L. Intra-abomasal infusion of this solution at a dose of 1 mL/kg increases intra-abomasal osmolarity to values > 1,000 mOsmol/L and is therefore likely to decrease the abomasal emptying rate.6 Differences in administered volume might also result in differences in abomasal emptying rates as gastric volume reportedly has a strong impact on gastric emptying rate.2,3,6

Assuming that the abomasal volume of an adult dairy cow is equivalent to 0.33% to 0.38% of its total body weight,3,5,32 abomasal infusion with a volume of 0.5 mL/kg versus 1 mL/kg would result in an increase in abomasal volume of approximately 14% versus 28%. The effect of this difference in abomasal volume on the abomasal emptying rate remains to be determined, but considering findings in goats,31 in which an abomasal volume increase of 40% had only minor effects on the abomasal emptying rate, we deem this effect to be of minor relevance.

In the present study, 96% ethanol was used as a solvent for acetaminophen (1 g of acetaminophen/10 mL of ethanol) because this allowed a considerable reduction in the volume needed to infuse in the abomasum, compared with the volume of aqueous acetaminophen solution typically used. The volume of ethanol could have been further decreased to 5 mL/g of acetaminophen (about half the volume used in our study) by warming the ethanol solution to 30°C.27 Use of water as a cosolvent in an 80% ethanol solution at 30°C would have allowed a further decrease in solvent volume to 4.12 mL of 80% ethanol/g of acetaminophen.39

Use of ethanol as a solvent for acetaminophen as in the present study may have altered the acetaminophen absorption rate because ethanol is readily absorbed through mucosal membranes, making absorption of ethanol-dissolved acetaminophen from the abomasum more likely. It may also have altered the abomasal emptying rate. Little information is available about the effect of ethanol on abomasal emptying rate in ruminants. In humans and cats, oral administration of 4% to 10% ethanol solutions reportedly delays gastric emptying.35,37 On the other hand, gastric emptying in humans is accelerated when an isocaloric, iso-osmotic meal containing ethanol is ingested, compared with the effects of the same meal containing dextrose instead of ethanol.38 Enhancing acetaminophen absorption from the abomasum with ethanol without altering the abomasal emptying rate would lower C_max but would be unlikely to alter T_max whereas delaying abomasal emptying without altering the acetaminophen absorption from the abomasum would delay T_max without affecting C_max. Infusion of an acetaminophen solution into the abomasum by manual introduction of tubing from a rumen cannula through the reticular omasal orifice into the omasum and into the abomasum, as has been described,4 is associated with manipulation of the reticulo-omasal and omaso-abomasal sphincters and therefore is likely to alter the motility of the forestomachs, thereby changing the abomasal emptying rate. Because of the lack of standardization of the various procedures used to estimate the abomasal emptying rate by means of d-xylose or acetaminophen as a marker substance, we would discourage a direct comparison of the results obtained through different approaches. Nonetheless, we believe that use of the same standardized procedure to compare results between treatments or animals is likely to provide a valid estimate of the difference between animals or treatments tested. To determine the absolute validity of the abomasal emptying rates measured with different approaches, the obtained results would need to be compared with results obtained via scintigraphy, which is considered the criterion-referenced technique for measurement of gastric emptying rate.8 To our knowledge, such data are available for calves but not for adult cattle.

In the present study, atropine and metoclopramide were used to modulate the abomasal emptying rate. Atropine, which is an amine antimuscarinic agent, inhibits acetylcholine at parasympathetic neuroeffector sites, thereby decreasing gastrointestinal motility.39 Metoclopramide is a derivate of para-aminobenzoic acid that presumably sensitizes the upper gastrointestinal tract to the effects of acetylcholine, thereby increasing gastric motility.40 As expected, when heifers were treated with atropine in our study, the effect was a significantly lower C_max, T_max, and AUC for the first 60, 90, 120, and 240 minutes after acetaminophen administration as well as a higher T_max compared with control and metoclopramide treatments, suggesting that these parameters were suitable on a comparative basis to identify the effect of atropine. When the heifers received metoclopramide, in contrast, none of the parameter values differed from control values, suggesting that the modified APAT was not sensitive enough to identify the effect of metoclopramide on abomasal emptying rate or that metoclopramide treatment did not result in a prokinetic effect. Although a significant increase in myoelectric activity of the abomasum occurs in goats after parenteral metoclopramide administration at a dose of 0.3 mg/kg,41 several studies34,42 conducted to evaluate the effect of metoclopramide on abomasal emptying rate in adult cattle and calves failed to identify the abomasal prokinetic effect of metoclopramide at doses between 0.1 and 0.5 mg/kg. The absence of these findings suggests that the evaluated parameters between metoclopramide and control treatments is thus in agreement with previous findings and provides further evidence that metoclopramide at a dose of 0.1 mg/kg does not affect the abomasal emptying rate.
abomasal emptying rate in cattle. Because adverse reactions can develop after metoclopramide administration at a dose of 0.3 mg/kg, the dose of metoclopramide cannot unhesitatingly be increased in an attempt to obtain enhanced abomasal motility. Metoclopramide thus does not appear to be a suitable prokinetic drug for use in cattle.

In the present study, C_{max}, T_{maxmodel}, T_{max}, and AUC_{0→240} as well as AUC_{0→60}, AUC_{0→90}, and AUC_{0→120} minutes after acetaminophen administration were determined to estimate the abomasal emptying rate. A previous study explored the suitability of these parameters to estimate the abomasal emptying rate in calves by comparing values of these parameters with values obtained via scintigraphy. In the present study, as in other studies, T_{max} obtained from a pharmacokinetic model (equivalent to T_{maxmodel} in the present study) provided the most accurate estimation of emptying rate. All determined parameters with the exception of T_{max} revealed significant group differences between atropine and control treatments as well as between atropine and metoclopramide treatments, suggesting that T_{max} was the weakest parameter for assessment of abomasal emptying by means of the modified APAT. This finding is in contrast to other findings in dairy calves, in which not only T_{maxmodel} but also T_{max} had the strongest association with the abomasal emptying rate determined by use of scintigraphy. We believe that this discrepancy can be explained at least in part by differences in digestive physiology between preruminating calves and adult cows with a fully developed forestomachs system. More variation in abomasal emptying rate, which depends not only on abomasal volume but also on energy density, osmolarity, and pH of the abomasal content, would be expected in adult cattle as opposed to calves. In preruminating calves, the abomasal content will mainly be governed by the characteristics of the administered solution, whereas in adult cattle, ingesta continuously entering the abomasum from the forestomachs will considerably alter the composition of the abomasal fluid and thus are likely to have an impact on the abomasal emptying rate as well. In contrast, the T_{maxmodel} index of abomasal emptying rate because it is less susceptible to the effects of individual variation.

Animals in the present study received 96% ethanol solution at a dose of 0.5 mL/kg intra-abomasally, which is equivalent to approximately 0.38 g of ethanol/kg. Similar doses (0.4 g/kg) have been administered IV to sheep without causing any clinically obvious adverse effects. Because ethanol is produced in significant amounts during conservation and fermentation of forages, dairy cows are likely to ingest up to 600 g of ethanol/d orally. In ruminants, the bulk of dietary ethanol is metabolized by ruminal microorganisms that convert this alcohol into short-chain fatty acids. Unmetabolized ethanol is absorbed through the mucosa of the gastrointestinal tract and transported to the liver through the venous portal system, where ethanol is metabolized via alcohol and aldehyde dehydrogenases. Feeding typical silage-based rations to dairy cows reportedly results in peak ethanol concentrations > 180 mg/dL in portal venous blood and of approximately 130 mg/dL in arterial blood. We conservatively extrapolated the volume of distribution for ethanol of 0.5 × body weight from data obtained in adult sheep to roughly estimate the maximum blood ethanol concentration possibly reached in heifers in the present study. Assuming immediate distribution throughout the volume of distribution of 50% body weight, an IV bolus infusion of a 0.4 g/kg dose of ethanol could reach a maximum plasma ethanol concentration of 80 mg/dL, which is considerably lower than values measured in ruminants fed yeast-spoiled silages. Therefore, we deem the 1-time intra-abomasal infusion of ethanol at the dose used here to have no risk for cattle. Despite close monitoring of the study cattle after acetaminophen administration to detect possible changes in attitude, mentation, and behavior that could be suggestive of neurologic signs associated with ethanol intoxication, no changes were observed in any heifer.

Orally administered acetaminophen has been associated with liver necrosis in a dose-dependent manner in humans and several other species. To our knowledge the hepatotoxic effect of acetaminophen in ruminants has not been directly investigated. Acetaminophen at up to 50 mg/kg, IV, given to calves did not result in clinically appreciable adverse effects of hepatotoxicity. In sheep, hepatotoxic effects have been reported after 1 IV dose of 400 mg/kg. Other changes observed in the serum biochemical profile of toxic sheep are hypoglycemia, which is associated with interference of carbohydrate metabolism, a decrease in the albumin-to-globulin ratio, and hypercholesterolemia. On the basis of the available information in combination with our clinical experience in using acetaminophen in cattle and small ruminants, we believe an APAT involving 1 dose of 50 mg/kg in ruminants is a safe diagnostic procedure.

A major caveat for use of the APAT in a clinical setting is that acetaminophen is not licensed in the United States for use in food-producing animals and thus can only be used in a research setting. In the European Union, acetaminophen is labeled for oral use in swine and thus can be used in an extralabel manner in cattle when indicated.

In the present study, heifers tolerated repeated transcutaneous abomasal puncture without any clinically obvious complications. Transcutaneous puncture of abomasum performed with or without ultrasonographic guidance by use of a 16- or 18-gauge needle was performed in previous studies without obvious clinical complications and has also been proposed as a diagnostic procedure in cattle. In calves, accidental puncture of the abomasum during collection of peritoneal fluid reportedly does not cause changes in the WBC count, total protein, or fibrinogen concentration in blood, nor does it affect total protein concentration, fibrinogen concentration, or nucleated cell count in peritoneal fluid. Similarly, accidental puncture of the rumen or abomasum during peritoneal fluid collection in adult cattle does not result in clinically relevant complications. For the present study, the general health status of each heifer as well as the clipped puncture site were monitored daily for 5 consecutive days after each treatment by visual inspection as well as by palpation.

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to identify signs of inflammation. No external signs of inflammation in or around the puncture site were identified in any heifer.

Findings reported here suggest the modified APAT, consisting of a transcutaneous intra-abomasal infusion of acetaminophen (50 mg/kg) dissolved in a 96% ethanol solution (1 g of acetaminophen/10 mL of 96% ethanol solution) followed by blood sample collection in 15-minute intervals for 2 hours, is a practical, minimally invasive, and reliable method of assessing abomasal emptying in adult cattle. Our results also provide further evidence that metoclopramide administered at a dose of 0.1 mg/kg does not increase the abomasal emptying rate in cattle.

References


