Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves

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Objective—To determine and compare the abomasal emptying rates in calves suckling milk replacer or an isotonic or hypertonic solution of NaHCO₃ or glucose.

Animals—5 male Holstein-Friesian calves that were < 30 days of age.

Procedures—Calves were fed 2 L of milk replacer or isotonic (300 mOsm/L) or hypertonic (600 mOsm/L) solutions of NaHCO₃ or NaCl. Venous blood samples and transabdominal ultrasonographic abomasal dimensions were obtained periodically after feeding, and abomasal luminal pH was continuously monitored by placement of a luminal pH electrode through an abomasal cannula. Abomasal emptying rate was assessed by the time to maximal plasma acetaminophen concentration, ultrasonographic determination of the half-time of abomasal emptying, and the time for luminal pH to return to within 1 pH unit of the preprandial value.

Results—Hypertonic NaHCO₃ solution was emptied slower than an isotonic NaHCO₃ solution, isotonic glucose solution was emptied slower than an isotonic NaHCO₃ solution, and hypertonic glucose solution emptied slower than an isotonic glucose solution.

Conclusions and Clinical Relevance—An electrolyte solution for oral administration with a high osmolality and glucose concentration may lead to a slower resuscitation of dehydrated diarrheic calves because such solutions decrease the abomasal emptying rate and therefore the rate of solution delivery to the small intestine. Whether slowing of the abomasal emptying rate in dehydrated diarrheic calves suckling an oral electrolyte solution is clinically important remains to be determined. (Am J Vet Res 2006;67:1377–1384)

Diarrhea can lead to dehydration, acidemia caused primarily by strong ion (metabolic) acidosis, hyperkalemia, and impaired cardiovascular and renal function. Oral administration of an electrolyte solution provides a practical and inexpensive method for treating dehydration and mild to moderate strong ion acidosis in neonatal calves that have a suckle reflex. However, there is still uncertainty about the electrolyte concentration, type of buffer, and type and amount of energy source as well as the pH and osmolality of the ideal OES. Commercially available OESs in the United States range in osmolarity from 300 to 720 mOsm/L, with osmolality being determined primarily by the concentrations of sodium and glucose. The rate of abomasal emptying influences the rate at which the electrolyte solution is delivered to the small intestine and therefore, the speed of rehydration. The volume and caloric content of an ingested fluid meal are the most important determinants of gastric emptying rate. Other important determinants of emptying rate are the type of protein or fat, osmolality, and duodenal pH, with a luminal pH of < 2.0 or > 10.0 decreasing the abomasal emptying rate in suckling calves.

Hypertonic (> 300 mOsm/L) solutions decrease emptying rate in monogastric animals, relative to isotonic electrolyte solutions, with profound inhibition of emptying occurring when osmolality is ≥ 600 mOsm/L. However, the effect of osmolality on emptying rate in suckling calves appears to have been examined in only 2 studies, with the surprising finding in these studies was that hypertonic solutions of NaHCO₃ or NaCl were emptied faster from the calf abomasum than isotonic solutions, with the optimal rate of emptying occurring with calorically inert solutions containing 400 to 600 mOsm/L. These studies used the serial test meal method of Hunt et al and Hunt and Knox and therefore assumed that salivary and abomasal secretion rates were similar for all test substances. Ignoring the effect of swallowed saliva and abomasal secretion introduces a confounding when different solutions are suckled by calves because of differences in abomasal secretion rates. Accordingly, the first aim of the study reported here was to confirm that a hypertonic NaHCO₃ solution (300 mmol/L, equivalent to 600 mOsm/L) empties slower than an isotonic...
NaHCO₃ solution (150 mmol/L; equivalent to 300 mOsm/L) in neonatal calves, as in adult monogastric animals.

Maintaining a serum glucose concentration within reference range is an important goal in the use of an OES, as calves with naturally acquired diarrhea are usually hypoglycemic and in a catabolic state.⁴⁻¹² An OES with high glucose content provides greater nutritional support than low glucose solutions⁶₃⁻³⁴ and minimizes the body weight loss that occurs when healthy calves are deprived of milk.⁴⁶ Because an increased caloric content of an ingested meal slows gastric emptying rate,¹¹,¹³ the second aim of the study reported here was to determine whether isotonic (300 mmol/L) and hypertonic (600 mmol/L) glucose solutions were emptied slower than isotonic (150 mmol/L) and hypertonic (300 mmol/L) NaHCO₃ solutions, respectively. We tested our objectives by use of 3 methods for assessing abomasal emptying rate (ie determination of acetaminophen absorption, ultrasonography, and measurement of the change in abomasal luminal pH). We expected that results of this study would increase our understanding of the effect of electrolyte solution osmolarity and glucose concentration on abomasal emptying rate in calves, thereby facilitating interpretation of studies that use a commercially available OES.

**Materials and Methods**

**Animals**—Our institutional animal care and use committee approved this study. Five healthy male Holstein calves were obtained from the University of Illinois dairy within the first week of life. Mean body weight of these calves was 44.8 kg (range, 40.8 to 49.9 kg). The calves had been surgically provided with a cannula into the abomasal body, as previously described.⁶⁶ After the calves had recovered from surgery, they were kept unrestrained in individual stalls that were bedded with wood shavings, led a nonmedicated all-milk protein replacer (crude protein, minimum of 20%; crude fat, minimum of 20%; crude fiber; maximum of 0.15%; calcium, minimum of 0.5%; maximum of 1.0%; and phosphorus, minimum of 0.6%) twice a day at 60 mL/kg. Calves had access to fresh water at all times.

**Experimental design**—Beginning after day 5 of life, at least 2 days after surgical cannulation, at least 36 hours after the previous study, and at least 12 hours after the previous feeding of milk replacer, calves were weighed and placed in a movable calf stall that allowed sitting and standing but prevented excessive lateral and forward movement. Abomasal luminal pH was monitored continuously for 15 minutes before administration of the test solution and at least 12 hours after administration of the test solution. Acetaminophen powder (50 mg/kg) was added to each solution and mixed well. Calves suckled 2 L of each of the following treatments in randomized order (Latin square design) from a nipple bottle: all-milk protein milk replacer (control); isotonic NaHCO₃ solution (150 mmol/L); hypertonic NaHCO₃ solution (300 mmol/L); isotonic glucose solution (300 mmol/L; 5.4% solution, equivalent to 2.4 g of glucose/kg); and hypertonic glucose solution (600 mmol/L; 10.6% solution, equivalent to 4.8 g of glucose/kg). Sodium bicarbonate and glucose were obtained as anhydrous powders and dissolved in the appropriate volume of tap water. A 36-hour washout period was used between treatments; during this washout period, calves suckled milk replacer.

**Abomasal pH measurement**—A miniature glass pH electrode¹ was advanced through a 6-cm-long rubber tube (outside diameter, 12 mm; inside diameter, 7 mm) containing a silastic plug with a small central hole at the proximal end to minimize electrode migration. The pH electrode was then advanced through the cannula to protrude 5 mm into the abomasal lumen. The electrode was secured to the cannula by placing the distal end of rubber tube over the outside of the cannula. The glass pH electrode was connected to a pH meter that was connected to an analog to digital board;¹ the electrical signal was digitized at 1 Hz, and data were stored on the hard disk of a personal computer. The pH electrode was calibrated immediately before insertion and after removal against reference buffer solutions with a pH of 2.0 and 7.0 at 20°C.

During offline data analysis, abomasal pH was smoothed by use of a 60-point moving average and the lowest smoothed pH value for each minute was used as the pH value for that minute. The smoothing procedure minimized recording artifacts that occurred when the pH probe transiently contacted the abomasal mucosa as a result of changes in the position of the calf or contraction of the abomasum. The mean preprandial pH (from time –15 to 0 minutes) and maximum pH after sucking, minimum pH after sucking, and mean pH after sucking (all from time 0 minutes to 12 hours) were determined by use of the pH values for each minute of the recording period.

**Abomasal emptying rate as determined by ultrasonographic findings**—Ultrasonographic measurement of abomasal dimensions provides an accurate method of determining abomasal volume and location in suckling calves.⁷⁻²⁰ Evaluation of the change in calculated abomasal volume after ingestion of a standardized meal provides an accurate method for determining the abomasal emptying rate in suckling calves.⁷⁻²⁰ For ultrasonographic evaluation of the abomasum, hair on the ventral aspect of the abdomen of each calf was clipped. Each calf was gently restrained in a standing position, and a 3.5-MHz ultrasound sector probe was applied to the ventral aspect of the abdomen in transverse and sagittal planes as described²² to determine the maximal, ultrasonographically visible abomasal dimensions (length, width, and height). Ultrasonographic measurements were obtained immediately before the start of sucking and at 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after the start of sucking. Abomasal volume was calculated from the ultrasonographically determined measurements by use of the equation for the volume of an ellipsoid (ie, volume = width × length × height × π/6, where π = 3.142). This method has been validated for use in calves.²³ A modified power exponential equation²⁴ was used to calculate the t₅₀ of abomasal emptying from the abomasal volume by use of nonlinear regression,⁴ as described.³⁷ Briefly, a volume-versus-time curve was generated for each experiment by use of the following equation:

\[ y(t) = 1 - \left(1 - e^{-\frac{t}{k}}\right)^{\frac{t}{\beta}} \]

where \( y(t) \) is the proportion of peak volume after sucking at time \( t \) (the time interval from start of sucking in minutes), \( k \) is the slope of the terminal portion of the emptying curve (measured in min⁻¹), and \( \beta \) is the extrapolated y-intercept for the terminal portion of the curve. Values for \( k \) and \( \beta \) obtained from nonlinear regression analysis of experimental data were applied in the calculation as follows:

\[ t_{50} = (-1/k) \times \log \left(1 - 2^{-\beta}\right) \]
Abomasal emptying rate as determined by acetaminophen absorption—Acetaminophen is widely used orally as an analgesic and antipyretic drug in humans, and acetaminophen absorption provides an accurate method of determining the emptying rate of liquid-phase meals in humans, horses, and calves. When administered orally, acetaminophen is absorbed in the small intestine, with the rate-limiting step for absorption being the rate of gastric emptying in animals with normal small intestinal function. Because the apparent rate of absorption is faster than the rate of elimination in suckling calves, the maximal acetaminophen concentration and time to maximal acetaminophen concentration after oral ingestion are primarily dependent on the rate of abomasal emptying.

Venous blood samples for determination of plasma acetaminophen concentrations were obtained at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, and 720 minutes after the start of suckling isotonic NaHCO3 and glucose solutions and at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, and 420 minutes after the start of suckling hypertonic NaHCO3 and glucose solutions and milk replacer. Different sample collection times were selected in an attempt to have at least 6 data points before and after the time of maximal acetaminophen concentration to facilitate nonlinear regression analysis. Blood samples were collected into 6-mL tubes containing sodium fluoride and potassium oxalate and centrifuged at 1,000 g for 15 minutes, and 3 mL of plasma was harvested and stored at –20°C until analysis.

Plasma was thawed at room temperature (approx 20°C) and analyzed spectrophotometrically by use of an colorimetric nitration assay as previously described. The observed Cmax (ie, actual Cmax) and observed Tmax (ie, actual Tmax) were obtained from a plot of the plasma acetaminophen concentration-versus-time data. The first derivative of the modified power exponential formula of Siegel et al was used to model the acetaminophen time curve, as previously described. The equation was derived from the fact that the acetaminophen concentration-versus-time curve represented as a cumulative dose curve is an inverse analogue of the following scintigraphic curve:

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

where C(t) is the acetaminophen concentration in plasma (μg/mL) at time t in minutes and m, k, and β are constants; m is the total cumulative recovery of acetaminophen when time is infinite; k is an estimate of the rate constant for abomasal emptying; and β provides an estimate of the duration of the lag phase before an exponential rate of emptying is reached. Nonlinear regression was used to estimate values for m, k, and β, as previously described.

The time to calculated Cmax (model Tmax) was obtained as follows:

Model Tmax = ln(β)/k

and the calculated value for model Cmax was determined by applying the values for m, k, β, and t = model Tmax to the cumulative dose curve.

Abomasal emptying rate as determined by luminal pH—Preprandial luminal pH in suckling calves is usually < 1.5. Because a large volume of test solution with a pH > 3.0 was suckled by calves in this study, the change in abomasal luminal pH over time must be associated, in some manner, with the abomasal emptying rate. Accordingly, we calculated the pH return time, which was defined as the time required for post suckling luminal pH to return to 1 unit above the mean preprandial pH value. This cut-point has been validated as an index of abomasal emptying rate in suckling calves.

Glucose absorption curve—Plasma glucose concentration was determined by use of an automatic analyzer. The change in mean plasma glucose concentration over time was expressed graphically.

Statistical analysis—Data were expressed as least squares mean and SE, and a value of P < 0.05 was considered significant. The primary variables of interest were ultrasonographic t1/2, acetaminophen absorption model Tmax, and pH return time. Repeated-measures ANOVA (with repeated measures on treatment and time) was used to determine the main effects of treatment and time and the interaction between treatment and time. Variables with non-normal distributions were analyzed using a log-normal transformation. Significant differences were determined using Fisher’s least significant difference method. The threshold for significance were set at \( P < 0.05 \).

### Table 1

<table>
<thead>
<tr>
<th>Methods of assessment</th>
<th>Isotonic NaHCO3 (150 mmol/L)</th>
<th>Hypertonic NaHCO3 (300 mmol/L)</th>
<th>Isotonic glucose (300 mmol/L)</th>
<th>Hypertonic glucose (600 mmol/L)</th>
<th>Milk replacer</th>
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References:

- Siegel et al...
- Preprandial pH value.
- t1/2 = t1/2 of abomasal emptying, β = Extrapolated γ-intercept of the abomasal volume versus time relationship.
- pH return time = Time required for post suckling luminal pH to decrease to 1.0 pH unit above the mean preprandial pH value.
- Values on the same row with different superscripts letters are significantly (\( P < 0.05 \)) different.
were log transformed or ranked before analysis was performed. Appropriate Bonferroni-adjusted post hoc tests were conducted whenever the F-test value was significant. A software program was used for all comparisons.

**Results**

All calves remained healthy during the study period. The mean time to suckle 2 L of the test solutions ranged from 1.6 to 4.0 minutes. Calves that suckled hypertonic glucose solutions appeared to produce a larger volume of urine during the first 4 hours after suckling, although urine volume was not quantified. Most of the calves suckling hypertonic glucose solution produced a loose stool within 4 hours of suckling, although fecal consistency was normal at 12 hours after suckling. A loose stool was not observed in calves suckling isotonic glucose solution, isotonic or hypertonic NaHCO₃ solutions, or milk replacer.

Mean preprandial pH (1.27 to 1.44) was similar for all treatments (Table 1). The maximum luminal pH value occurred immediately after suckling, then gradually decreased toward the preprandial pH value (Figure 1). The maximum postsuckling pH was greatest for hypertonic and isotonic NaHCO₃, intermediate for milk replacer, and lowest for hypertonic and isotonic glucose. The mean postsuckling pH for isotonic and hypertonic glucose was lower than that of hypertonic sodium bicarbonate and milk replacer.

Ultrasonographic findings revealed that the t₁/₂ of abomasal emptying for isotonic NaHCO₃ was shorter than that for all other solutions (Table 1; Figure 2). Hypertonic NaHCO₃ was emptied at a numerically faster rate than hypertonic glucose, although the difference was not significant. Hypertonic glucose emptied slower than isotonic glucose, although the difference was not significant.

Results of acetaminophen absorption revealed that isotonic NaHCO₃ emptied faster than hypertonic glucose and milk replacer on the basis of model Tₘₐₓ (Table 1; Figure 3). Isotonic glucose emptied faster than hypertonic glucose and milk replacer on the basis of model Tₘₐₓ. Hypertonic NaHCO₃ emptied faster than milk replacer on the basis of model Tₘₐₓ.

Return times of abomasal luminal pH were longer for milk replacer than those for the 4 other solutions (Table 1; Figure 1). Glucose absorption curves for calves suckling hypertonic or isotonic glucose solutions were different from those for calves suckling calorically inert solutions such as isotonic or hypertonic NaHCO₃ (Figure 4). As expected, calves suckling hypertonic glucose solution had the highest sustained plasma glucose concentration, with hyperglycemia being sustained for at least 240 minutes. In contrast, plasma glucose concentrations in calves suckling isotonic glucose solution were increased for 120 minutes. Plasma glucose concentration did not change in calves suckling isotonic or hypertonic NaHCO₃ solutions or milk replacer.
The major findings of our study in suckling calves were that a hypertonic NaHCO₃ solution was emptied slower than an isotonic NaHCO₃ solution, an isotonic glucose solution was emptied slower than an isotonic NaHCO₃ solution, and a hypertonic glucose solution emptied slower than an isotonic glucose solution. Taken together, these results indicate that electrolyte solution osmolarity and glucose concentration are inversely related to the abomasal emptying rate and therefore the speed of rehydration in suckling calves. Our results are consistent with the presence of receptors in the duodenum that sense the osmolarity and caloric density of fluid emptied from the abomasum, leading to reflex changes in abomasal emptying rate.

Calves in our study suckled solutions that varied in osmolarity, caloric content, and pH. The difference in emptying rate between isotonic and hypertonic NaHCO₃ was the result of the difference in osmolarity of the solutions and was consistent with the findings in monogastric animals, in which hypertonic calorically inert solutions are emptied slower than isotonic solutions. The previous incorrect conclusion that hypertonic solutions are emptied faster than isotonic solutions in calves appears to have resulted from the use of the serial test meal method of Hunt et al and Hunt and Knox, in which similar salivary and abomasal secretion rates are assumed. The caloric content of hypertonic glucose is higher than that of a similar volume of isotonic glucose, and this was reflected in the longer model tₚmax. Likewise, isotonic glucose was emptied slower than isotonic NaHCO₃, as reflected by the longer t½ for abomasal emptying. The mechanism by which suckled glucose solutions slow the abomasal emptying rate appears not only to be related to an increased intraduodenal glucose and total caloric load and hyperglycemia, but also to independent hyperinsulinemia. The slowing of gastric emptying during pathologic and physiologic hyperglycemia has been well documented for monogastric animals and is associated with suppression of antral waves, changes in the organization of antroduodenal motility, a reduction in proximal gastric tone, and stimulation of phasic and tonic pyloric pressure. The total glucose load rather than the osmolarity of the glucose solution is the more important determinant of emptying rate in monkeys and, presumably, calves. In general, a slower rate of gastric emptying occurs following ingestion of rehydration solutions containing > 4% glucose in humans; this is consistent with our finding that suckling an isotonic glucose solution (5.4% glucose) slowed the abomasal emptying rate in calves, relative to suckling a calorically inert isotonic solution of NaHCO₃.

Discussion

The major findings of our study in suckling calves were that a hypertonic NaHCO₃ solution was emptied slower than an isotonic NaHCO₃ solution, an isotonic glucose solution was emptied slower than an isotonic NaHCO₃ solution, and a hypertonic glucose solution emptied slower than an isotonic glucose solution.
The primary energy source in commercially available formulations of OES is glucose, which usually provides 1 to 3 g of glucose/kg. Suckling a solution that contains glucose at a concentration that provides 3 g of glucose/kg results in a maximal plasma glucose concentration of 150 mg/dL at approximately 90 minutes after suckling, which is similar to the time at which plasma acetaminophen concentration was maximal in the calves suckling isotonic glucose solution (2.4 g of glucose/kg; model T\text{max}=100 minutes) in our study. Other investigators have shown that calves suckling a hypertonic electrolyte solution that provides 2.5 g of glucose/kg have a maximal glucose concentration of 125 mg/dL at approximately 180 minutes after suckling. Glucosuria and diarrhea were not present in these calves. Because the renal threshold for glucose in suckling calves is 140 to 160 mg/dL, providing 2 to 3 g of glucose/kg appears to be the upper limit for glucose content in an OES, as glucosuria and urinary loss of energy and free water would be expected at higher glucose intakes. Maintenance of plasma glucose content at the lower end of the renal threshold from 60 to 150 minutes in calves suckling hypertonic glucose (containing 4.8 g of glucose/kg) supports our speculation that glucosuria was present in these calves.

Suckling the hypertonic glucose solution (providing an equivalent of 4.8 g of glucose/kg) resulted in diarrhea in some calves, which was attributed to the osmotic effect of unabsorbed glucose. Diarrhea was not observed in calves fed the isotonic glucose solution (providing an equivalent of 2.4 g of glucose/kg). Glucose has been previously fed to calves to provide 4.4 g of glucose/kg without causing diarrhea. In halothane-anesthetized calves, glucose was absorbed in healthy and diarrheic calves at a similar rate of 2.4 to 7.2 mg/cm of small intestinal segment/h. Based on a mean small intestinal length of 15.8 to 18.6 m in 1 to 2-week-old Holstein-Friesian calves, the total glucose absorption rate in the small intestine is therefore estimated to range from 3.8 to 13.4 g/h. Assuming a 12-hour feeding interval, calves should be able to absorb up to 161 g of glucose in a given feeding. This calculation suggests that for 45-kg calves with plasma glucose concentrations within reference range and normal gastrointestinal motility, the upper limit of the amount of glucose in an OES may be that which provides 3.6 g/kg. Glucose administration rates above these calculated values run the risk of allowing unabsorbed glucose to carry over into the large intestine, where it may be fermented to short-chain volatile fatty acids, exacerbate fecal water loss, and be manifest as a loose stool.

Calorically inert isotonic fluids, such as isotonic NaHCO₃, are evacuated from the stomach rapidly and in an exponential manner. Ultrasonographic emptying curves reflect the exponential nature of emptying for isotonic NaHCO₃. Caloric fluids, such as isotonic and hypertonic glucose solutions and milk replacer, are emptied in a more linear manner; this is a physiologic response to ensure that nutrients are presented to the small intestine at a relatively constant rate. Our finding that isotonic glucose (5.4% solution) was emptied faster than milk replacer was similar to that obtained previously, whereby the ileum-transit time in calves suckling a 5% glucose solution was shorter than when calves suckled fresh cow's milk.

The difference in pH of the suckled solutions probably had a small or no effect on abomasal emptying rate in the calves in our study. Although duodenal pH does influence abomasal emptying rate, the effect of duodenal pH is observed only at extreme nonphysiologic values for pH (ie, < 2.0 or > 10.0) of ingested liquids.

The speed of rehydration is dependent on the rate of abomasal emptying and the rate of absorption of water and sodium from the small intestine. Results of our study suggest that an OES with a high osmolality (≥ 600 mOsm/L) and glucose concentration (≥ 10.8%) or glucose content (to provide ≥ 2.4 g of glucose/kg) may lead to a slower rate of rehydration as a result of a slower delivery of free water, sodium, and glucose to the small intestine. For instance, the t\text{1/2} of abomasal emptying is 2.3 times longer for hypertonic NaHCO₃ compared with isotonic NaHCO₃, meaning that the rate of delivery of free water to the small intestine is 2.3 times slower for hypertonic NaHCO₃. However, the rate of sodium delivery to the small intestine was similar for both solutions because the hypertonic NaHCO₃ solution contained twice the sodium concentration of the isotonic NaHCO₃ solution. The rate of water uptake from the small intestine is maximized by a solution osmolarity that is slightly hypotonic with respect to plasma. Accordingly, oral administration of a low caloric isotonic electrolyte solution should provide the fastest rate of rehydration in calves.

We do not believe that the presence of an abomasal cannula altered the emptying rate in the calves in our study because placement of a percutaneous endoscopic gastrostomy tube did not slow gastric emptying in cats and the presence of the much more invasive duodenal reentrant cannula did not alter abomasal emptying rate in milk-fed calves.

In conclusion, in contrast to the conclusion of previous studies in calves, our results indicate that the effects of osmolality and glucose concentration on abomasal emptying rate in euhydric calves are similar to those in euhydric monogastric animals. Whether slowing of the abomasal emptying rate in dehydrated diarrheic calves suckling an electrolyte solution is clinically important remains to be determined.

a. Percutaneous endoscopic gastrostomy kit, MILA Instruments Inc, Florence, Ky.
b. Agri Master, Supreme All Milk, Blain Supply, Janesville, Wis.
c. Sigma-Aldrich, St Louis, Mo.
d. M3 internal reference glass pH electrode, Medical Instruments Corp, Solothurn, Switzerland.
e. Cole-Parmer pH/V/mV/Rel mV/C Benchtop meter, Cole-Parmer Instrument Co, Vernon Hills, Ill.
f. CODAS, Datas Instruments Inc, Akron, Ohio.
g. PROC NLIN, SAS, version 8.2, SAS Institute Inc, Cary, NC.
h. Becton-Dickinson Vacutainer Systems, Becton-Dickinson, Franklin, NJ.
j. Hitachi 704 automatic analyzer, Hitachi, Tokyo, Japan.
k. PROC MIXED, SAS, version 8.2, SAS Institute Inc, Cary, NC.
References


