

## Effect of Suckling Cow's Milk or Milk Replacer on Abomasal Luminal pH in Dairy Calves

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Abomasal ulceration occurs commonly in suckling calves, and the cause for the high prevalence of abomasal ulceration is unknown. We hypothesized that diet may play a role in the etiopathogenesis of abomasal ulceration. Six male dairy calves with an abomasal body cannula suckled fresh Holstein cow's milk, all milk-protein milk replacer, or combined milk- and soy-protein milk replacer twice daily at 12% of body weight/d. Abomasal luminal pH was measured every second for 24 hours by using a miniature glass pH electrode. Mean 24-hour abomasal luminal pH for all milk-protein milk replacer (3.22) and combined milk- and soy-protein milk replacer (3.27) were similar but significantly ( $P < .05$ ) higher than that for cow's milk (2.77; standard error = 0.08). Both milk-replacer formulations failed to clot after the addition of chymosin, whereas cow's milk clotted within 2 minutes. The in vitro titration curve of cow's milk and all milk-protein milk replacer were similar, but different to that of combined milk- and soy-protein milk replacer. The osmolalities of all milk-protein milk replacer (375 mOsm/kg) and combined milk- and soy-protein milk replacer (410 mOsm/kg) were greater than that of cow's milk (278 mOsm/kg). The slightly lower mean abomasal luminal pH in calves suckling cow's milk, compared to milk replacer, was probably due to clotting of cow's milk, with extrusion of low pH whey, and a slower rate of abomasal emptying caused by the hyperosmolality of milk replacer. Examination of our results suggests that suckling cow's milk may increase the prevalence of abomasal ulceration by decreasing mean luminal pH, although this remains to be determined.

**Key words:** Abomasal ulcer; Buffering characteristics; Clotting; Coagulation; Osmolality.

Abomasal ulceration occurs commonly in suckling calves, with reported prevalences of 5–76%,<sup>1</sup> 32%,<sup>2</sup> 45%,<sup>3</sup> 57%,<sup>4</sup> 75%,<sup>5</sup> and 76%.<sup>6</sup> Suckling beef calves aged 1 to 2 months can die as a result of abomasal ulceration and perforation,<sup>7–9</sup> especially after a period of inclement weather.<sup>8–10</sup> The cause for the high prevalence of abomasal ulceration in veal calves, and the occurrence of ulceration and perforation in beef calves, is unknown,<sup>11</sup> although low abomasal pH and diet are suspected to play important roles in the etiopathogenesis.<sup>1,12–16</sup>

Many factors influence abomasal luminal pH in the suckling calf; the most important factors are meal volume, suckling frequency,<sup>16</sup> and abomasal emptying rate, as well as the extent of coagulation (formation of curd and whey)<sup>17,18</sup> and buffering characteristics of the ingested meal.<sup>19–25</sup> A remarkable feature in the suckling calf is that cow's milk clots within 10 minutes of entering the abomasum.<sup>17,26</sup> Cow's milk clots when chymosin (formerly called rennin or rennet) interacts with casein to form a curd,<sup>27–29</sup> trapping casein and fat globules within the coagulum. Whey is extruded during the clotting process, and whey (which contains carbohydrates and electrolytes)<sup>25,30</sup> has a lower pH than uncoagulated cow's milk or milk replacer.<sup>22–24,31</sup> Because whey is the principal fluid emptied from the abomasum during the first 3 hours after suckling cow's milk,<sup>25,30–33</sup> abomasal effluent pH is lower for the 1st few hours after suckling cow's milk, compared to suckling milk replacer that does

not clot.<sup>25,34,35</sup> We therefore hypothesized that abomasal luminal pH, like abomasal effluent pH, would be lower in calves suckling cow's milk, relative to milk replacer. If true, this could provide an explanation for the high prevalence of abomasal ulceration in suckling calves.

Most of the studies evaluating the effects of diet on abomasal luminal pH were completed more than 25 years ago and intermittently measured pH at 30-minute or 1-hour intervals,<sup>22,24,25,31,35,36</sup> with the majority of studies measuring abomasal effluent pH via a duodenal reentrant cannula. We have recently developed a method for continuously measuring abomasal luminal pH in the suckling calf, and have used this methodology to evaluate the effect of oral cimetidine, ranitidine,  $Mg(OH)_2/Al(OH)_3$ , and feeding frequency on abomasal luminal pH in calves fed milk replacer.<sup>14–16</sup> Milk-replacer formulations in the United States have changed considerably over the last 25 years,<sup>37</sup> and milk replacers currently available in North America do not coagulate in the calf's abomasum<sup>38,39</sup> because they have undergone high-temperature heating during processing or because they contain predominantly whey protein and very little casein.<sup>40</sup> The 1st aim of this study was therefore to compare the effect of suckling cow's milk with that of 2 commercially available milk-replacer formulations on 24-hour abomasal luminal pH in suckling dairy calves. The 2nd aim was to compare the clotting ability, buffering characteristics, and osmolality of cow's milk with that of 2 milk-replacer formulations.

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## Materials and Methods

### Animals

The University of Illinois Laboratory Animal Care and Use Committee approved this study. Six male, colostrum-fed, healthy dairy calves (5 Holstein-Friesians and 1 Ayrshire) were surgically instrumented with an abomasal body cannula at 3 days of age, as previously described.<sup>14</sup> Calves were housed in a moveable calf stall, weighed every other day, and fed an all milk-protein milk replacer<sup>®</sup> (12% of body weight/d) at 12-hour intervals. Calves had access to fresh water at all times but were not fed a calf starter ration.

### Experimental Design

Beginning on day 17 of life (mean body weight 46 kg, range 40–51 kg), each calf was administered 1 of the following 3 treatments twice daily at 12% of body weight/d (6% of body weight per feeding): cow's milk, all milk-protein milk replacer, or combined milk- and soy-protein milk replacer. Abomasal luminal pH was monitored continuously for at least 24 hours starting at 7:15 AM, and the test solution was fed at 7:30 AM and 7:30 PM. Treatment order was randomized by using a modified Latin square design. A 24-hour washout period was used between treatments; during this washout period, calves suckled the all milk-protein milk replacer.

Fresh cow's milk was obtained from mature midlactation Holstein-Friesian cows (approximate composition: fat, 3.5%; protein, 3.4%; and total solids, 12.7%). The all milk-protein milk replacer<sup>a</sup> contained the following: crude protein,  $\geq 22\%$ ; crude fat,  $\geq 20\%$ ; crude fiber,  $\leq 0.15\%$ ; calcium,  $\geq 0.50\%$ ; phosphorus,  $\geq 1.00\%$ ; and decoquinatate, 45.4 g/ton, equivalent to a daily ingestion of approximately 0.5 mg of decoquinatate per kilogram of body weight. This milk-replacer formulation meets current guidelines for suckling calves.<sup>41,42</sup> The protein sources were stated as dried whey, dried whey product, dried milk protein, and dried skim milk of unknown relative proportions; however, milk replacers in the United States currently contain relatively little skim milk.<sup>37</sup> Combined milk- and soy-protein milk replacer<sup>b</sup> contained the following: crude protein,  $\geq 20\%$ ; crude fat,  $\geq 20\%$ ; crude fiber,  $\leq 0.50\%$ ; calcium,  $\geq 0.50\%$  and  $\leq 1.00\%$ ; phosphorus,  $\geq 0.60\%$ ; oxytetracycline, 100 g/ton; and neomycin base, 200 g/ton. The protein sources were dried whey, dried whey product, dried milk protein, dried skim milk, soy protein isolate, and soybean flour. Milk-replacer powder was dissolved in water at approximately 37°C at 120 g/L (12%; 4 oz/qt), as directed by the manufacturer.

### Abomasal pH Measurement

A miniature glass pH electrode<sup>c</sup> was advanced through the cannula to protrude 5 mm into the abomasal lumen, secured to the cannula, and connected to a pH meter.<sup>d</sup> The pH meter was connected to an analog-to-digital board,<sup>e</sup> digitized at 1 Hz, and the 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 of pH 2.0 and 7.0 at 20°C.

During off-line data analysis, abomasal pH was smoothed by using 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 due to changes in the calf's position or contraction of the abomasum.

### Abomasal Emptying Rate

The time taken for postprandial pH to decrease to a pH value related to the preprandial value provides an index of abomasal emptying rate in calves fed a standard meal of similar volume and pH.<sup>f</sup> Accordingly, the mean preprandial pH was determined from the pH values for the 15-minute period before calves suckled the test solution. The time taken after suckling for luminal pH to decrease to within 1 pH unit of preprandial pH was then determined.

### Clotting Time, Buffering Characteristics, and Osmolality

Clotting times of milk replacers and cow's milk were determined by using bovine chymosin<sup>g</sup> and previously described techniques.<sup>18,29</sup> Briefly, this involved the addition of 10 units of chymosin to 100 mL of test solution at 38.5°C (pH adjusted to 6.1 by adding 0.1 M HCl or NaOH). Clotting time was determined by dipping a microscope glass slide into the solution every 15 seconds and examining the slide against a dark background for the presence of flakes. The clotting time

was defined as the time when flakes were 1st obvious against a dark background.<sup>18</sup> Samples were observed for clotting for only 20 minutes.

In vitro studies of buffer capacity were conducted on the 2 milk-replacer formulations and cow's milk. Titration curves were developed by adding 2 mL of 1 M HCl to 18 mL of test solution (1:9) to acidify the solution to a pH < 2. The acidified solution was maintained at 38.5°C and back titrated with 0.1 M NaOH (in 1-mL increments) while being continuously stirred. In vitro solution pH during titration was measured by using the same equipment as in vivo abomasal luminal pH. Titration curves were performed in triplicate and the mean value was graphically depicted by plotting pH (0–12) against milliliters of 0.1 M NaOH added (0–30 mL). The buffer capacity of the solution at the fed pH was calculated from the reciprocal of the slope relating pH to milliliters of 0.1 M NaOH added. Osmolality of the fed solution was measured in triplicate by freezing point depression.<sup>h</sup>

### Statistical Analysis

A  $P < .05$  was regarded as significant, and data were expressed as least squares means and SE. Data were analyzed by using repeated-measures analysis of variance and a mixed-effects model with calf declared as a random effect; a compound symmetry covariance structure was used for all analyses except comparing the change in pH over time, which was analyzed by using an autoregressive covariance structure within animal and a random effect between animals.<sup>43</sup> The mixed-effects method of statistical analysis allows for heterogeneous variance and within-subject covariability, and is currently the preferred method for analyzing studies involving repeated measures. Bonferroni adjusted  $P$ -values were calculated for the appropriate posttests. The 3 main variables of interest in determining the effect of treatment on abomasal luminal acidity were mean 24-hour pH and the percent of each 24-hour period that pH > 3.0 and > 4.0. The 2 pH cutpoints (3.0 and 4.0) were selected because an abomasal luminal pH > 3.0 and > 4.0 will be accompanied by a slower activation rate of pepsinogen and prochymosin, respectively, thereby markedly decreasing the proteolytic activity of abomasal secretions and protecting against abomasal ulceration.<sup>16</sup>

## Results

### Animals

Abomasal cannulae were well tolerated by all calves, with maintenance of appetite, a mean increase in body weight of 2.0 kg (range, 1.0–3.6 kg), and a rectal temperature within the reference range during the study period. The mean time taken to suckle the allotted volume was 3.5 minutes (range 3–4 minutes), 4.0 minutes (range 3–10 minutes), and 3.5 minutes (range 3–5 minutes) for cow's milk, all milk-protein milk replacer, and combined milk- and soy-protein milk replacer, respectively.

### Abomasal pH Measurement

Electrode drift during the 24-hour recording period was +0.02 (range -0.08 to +0.09) for buffer pH 2.00 and +0.06 (range -0.05 to +0.26) for buffer pH 7.00. Raw pH values were used for analysis because of the minimal drift.

No difference was found in mean preprandial pH, maximal pH, and minimum pH when calves suckled the 2 milk-replacer formulations or cow's milk (Table 1; Fig 1). When all milk-protein or combined milk- and soy-protein milk replacers were suckled, abomasal luminal pH increased from a baseline value of 1.4 to 6.0 within 3 minutes, remained constant for 2 hours, then decreased to the pre-

**Table 1.** Abomasal luminal pH indices in dairy calves (n = 6) suckling cow's milk, an all milk-protein milk replacer, or a combined milk- and soy-protein milk replacer (60 mL/kg body weight) at 0 hours and 12 hours.

Factor	Cow's milk	All milk-protein milk replacer	Combined milk- and soy-protein milk replacer	SE
Mean 24-hour pH	2.77 <sup>a</sup>	3.22 <sup>b</sup>	3.27 <sup>b</sup>	0.08
% of 24 hours that pH > 3.0	37.7 <sup>a</sup>	49.5 <sup>b</sup>	51.6 <sup>b</sup>	4.7
% of 24 hours that pH > 4.0	26.8 <sup>a</sup>	41.8 <sup>b</sup>	38.9 <sup>b</sup>	4.7
Mean preprandial pH	1.36 <sup>a</sup>	1.34 <sup>a</sup>	1.27 <sup>a</sup>	0.08
Minimum postprandial pH	1.23 <sup>a</sup>	1.02 <sup>a</sup>	1.06 <sup>a</sup>	0.09
Maximum postprandial pH	6.07 <sup>a</sup>	6.09 <sup>a</sup>	6.08 <sup>a</sup>	0.06
Time for pH to return to within 1.0 pH units of preprandial pH (minutes)	320 <sup>a</sup>	383 <sup>b</sup>	399 <sup>b</sup>	25

<sup>a,b</sup> Means with different superscripts were significantly ( $P < .05$ ) different.

prandial value by 8 hours after feeding. Abomasal luminal pH was constant from 8 to 12 hours, and increased again after the 2nd feeding of milk replacer at 12 hours (Fig 1).

Mean 24-hour abomasal luminal pH values for the 2 milk-replacer formulations were similar but higher than that of cow's milk (Table 1). The percentage of the 24-hour period that abomasal pH exceeded 3.0 and 4.0 was higher with both formulations of milk replacer than for cow's milk (Table 1). No difference was found in the percentage of the 24-hour period that abomasal luminal pH exceeded 3.0 or 4.0 between all milk-protein and combined milk- and soy-protein milk replacers.

#### Abomasal Emptying Rate

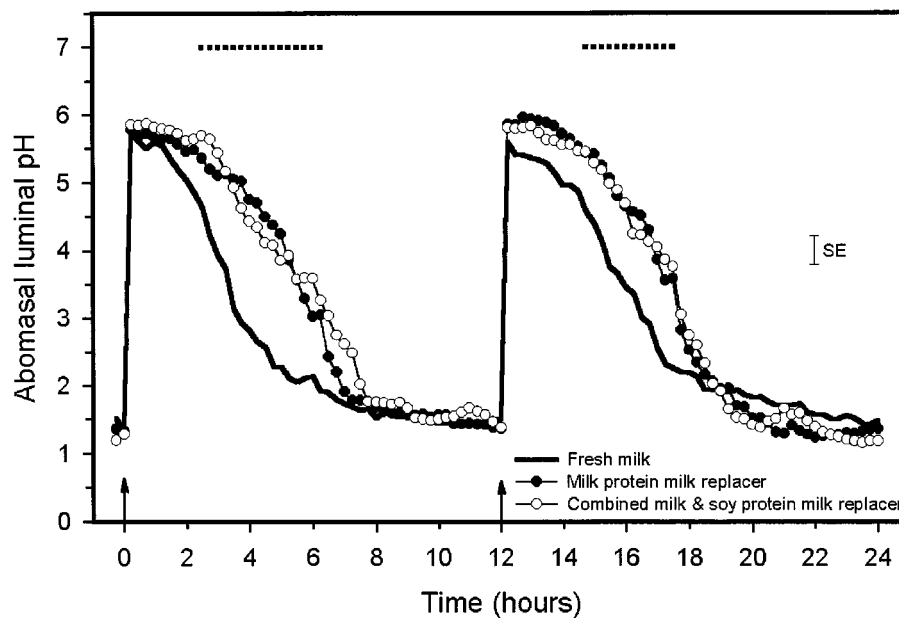
Mean abomasal luminal pH decreased faster after suckling cow's milk, compared to suckling milk replacer (Fig 1). The time taken for luminal pH to return to 1 pH unit above the mean preprandial pH was shorter when calves suckled cow's milk (Table 1).

#### Clotting Time, Buffering Characteristics, and Osmolality

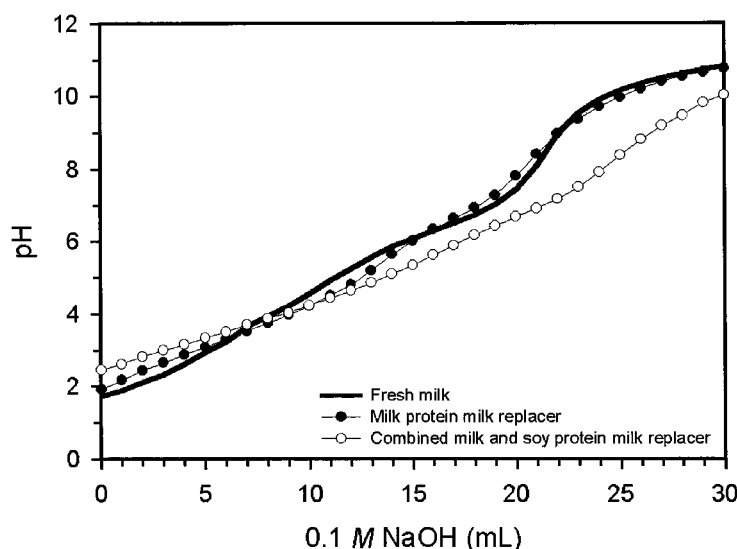
Cow's milk clotted within 2 minutes of the addition of chymosin, whereas milk-protein milk replacer and combined milk- and soy-protein milk replacer failed to clot within 20 minutes.

The titration curve of fresh cow's milk and milk-protein milk replacer were similar, but different from that of combined milk- and soy-protein milk replacer (Fig 2). The buffer capacity of fresh cow's milk at the suckled pH (6.42) was 0.46 mEq/L per pH unit, whereas the buffer capacities of all milk-protein milk replacer (suckled pH = 6.40) and combined milk- and soy-protein milk replacer (suckled pH = 5.81) at the suckled pH were 0.33 mEq/L per pH unit and 0.37 mEq/L per pH unit, respectively.

The osmolality of fresh cow's milk was 278 mOsm/kg. In contrast, the osmolality of milk-protein milk replacer was 375 mOsm/kg, and the osmolality of combined milk- and soy-protein milk replacer was 410 mOsm/kg.



**Fig 1.** Least squares mean abomasal luminal pH in male dairy calves (n = 6) that suckled cow's milk, all milk-protein milk replacer, or combined milk- and soy-protein milk replacer (60 mL/kg body weight) at 12-hour intervals (arrows). Closed squares at the top of graph represent mean values (every 15 minutes) for cow's milk that were significantly ( $P < .05$ ) different from that of all milk-protein milk replacer or combined milk- and soy-protein milk replacer. SE, standard error.



**Fig 2.** Buffering characteristics of fresh cow's milk, milk-protein milk replacer, and combined milk- and soy-protein milk replacer. The solution was acidified with 1 M HCl, then back titrated by addition of 1-mL increments of 0.1 M NaOH.

## Discussion

Major findings of this study were that abomasal luminal acidity was similar when calves suckled an all milk-protein or combined milk- and soy-protein milk replacer, but was lower when calves suckled cow's milk; the 2 milk-replacer formulations did not coagulate after addition of chymosin; the *in vitro* titration curve of an all milk-protein milk replacer was similar to that of cow's milk, but different from that of a combined milk- and soy-protein milk replacer; and the 2 milk-replacer formulations were hyperosmotic.

The lower mean luminal pH when calves suckled cow's milk most likely resulted from clotting of suckled cow's milk, with extrusion of lower pH whey or a faster rate of abomasal emptying after suckling cow's milk. We found, in contrast to cow's milk, that 2 commercially available milk-replacer formulations failed to clot in the presence of chymosin. The clinical significance of this finding is unknown. It has been widely assumed that the calf requires normal curd formation in the abomasum for normal growth and health,<sup>18</sup> although direct evidence is lacking.<sup>27,39,44</sup> Whether curd formation provides a physiologic advantage or protective effect is unknown, because clotting and non-clotting milk-replacer solutions have similar digestibilities in the calf,<sup>45</sup> although clotting fresh bovine milk has a higher digestibility than nonclotting milk.<sup>40</sup> However, curd formation does prevent flooding of the proximal small intestine with a higher than normal pH and concentration of protein and fat that may facilitate growth of pathogenic organisms or induce diarrhea.<sup>23,31,33,45</sup>

The lower mean luminal pH after suckling cow's milk also could be partially due to a faster rate of emptying. The volume of an ingested fluid meal is the most important determinant of emptying rate in monogastric animals<sup>21,46</sup> and suckling calves,<sup>47</sup> because emptying follows an exponential pattern. Another important determinant of emptying rate is the energy density (caloric content) of a meal,<sup>48,49</sup> which is monitored partly by osmoreceptors in the duodenum<sup>48-51</sup>; isocaloric isovolumic milk-replacer formulas are

emptied at the same rate despite differences in protein, fat, and carbohydrate concentrations.<sup>52</sup> Another important determinant of gastric emptying rate is the type of protein or fat. Human breast milk is emptied faster than bovine milk-derived formulas in human infants,<sup>53-55</sup> solutions containing short- and medium-chain volatile fatty acids are emptied faster than solutions containing long-chain fatty acids in dogs,<sup>56,57</sup> and tryptophan delays abomasal emptying rate in calves.<sup>58</sup> A physiologically less important determinant of gastric emptying is duodenal pH, with luminal pH < 2.0 or > 10.0 decreasing the abomasal emptying rate in suckling calves.<sup>59</sup> Osmolality also effects emptying rate, both hypo-osmolar (<300 mOsm/kg) and hyperosmolar (>300 mOsm/kg) solutions decrease emptying rate, relative to iso-osmotic electrolyte solutions,<sup>49,51,60-62</sup> with profound inhibition of emptying occurring when osmolality  $\geq$  600 mOsm/kg. Because the 3 solutions were fed at the same volume and had similar energy density, and because we found that the osmolality of an all milk-protein milk replacer (375 mOsm/kg) and a combined milk- and soy-protein milk replacer (410 mOsm/kg) were higher than that of cow's milk (278 mOsm/kg), it is possible the hyperosmolality of milk replacer delayed abomasal emptying rate, relative to cow's milk.

Preprandial abomasal luminal osmolality in the milk-fed calf is 241–265 mOsm/kg, and increases to 309 mOsm/kg after suckling cow's milk, presumably because of proteolysis of the ingested cow's milk.<sup>47</sup> A US study published in 1982 reported that the osmolality of 6 calf milk-replacer formulations ranged from 335 to 922 mOsm/kg, with a mean of 484 mOsm/kg.<sup>63</sup> For comparison, the osmolalities of US human infant formula solutions were reported in 1977 to range from 237 to 590 mOsm/kg<sup>64</sup> and in 1982 to range from 227 to 622 mOsm/kg.<sup>55</sup> It therefore appears that most calf milk-replacer and human infant formulations are slightly to moderately hyperosmotic. The mild hyperosmolality of the 2 milk-replacer formulations used in this study therefore may have slowed the abomasal emptying

rate and led to a higher luminal pH because hyperosmolar solutions are emptied more slowly than are iso-osmotic solutions.<sup>49,51,59,60</sup>

Examination of the results of the study reported here indicated that suckling cow's milk leads to a slightly lower mean abomasal luminal pH. Because we have previously shown that fasting or infrequent suckling of milk replacer results in sustained periods of low luminal pH (pH < 2),<sup>16</sup> our results provide a potential explanation for the occurrence of abomasal ulceration and perforation in suckling beef calves, in that the frequency of suckling and volume suckled may decrease during a period of inclement weather. The combined effects of decreased suckling frequency, decreased suckled volume, and suckling cow's milk might therefore facilitate the development of abomasal ulceration; this is consistent with the results of epidemiologic studies that indicate that abomasal ulceration and perforation in beef calves is associated with inclement weather.<sup>8-10</sup> However, the release of corticosteroids in response to stress and the presence of unidentified microorganisms may play an accompanying or greater role in the development of abomasal ulcers in suckling calves, although bacteria and fungi associated with ulcers are currently thought to be opportunistic secondary infective agents that invade after injury to the abomasal mucosa.<sup>16</sup> Obviously, additional studies are required to help determine the relative contribution of lower luminal pH to the development of abomasal ulcers in suckling calves.

In conclusion, abomasal luminal pH was lower when calves suckled cow's milk, compared to suckling 2 milk-replacer formulations. We attributed this result primarily to clotting of cow's milk with extrusion of low pH whey, although a slower rate of abomasal emptying after suckling moderately hyperosmolar milk replacer may have played a contributory role.

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### Footnotes

<sup>a</sup> Super Supreme All Milk, AGRIMASTER, Janesville, WI

<sup>b</sup> Supreme All Milk, AGRIMASTER, Janesville, WI

<sup>c</sup> M3 internal reference glass pH electrode, Medical Instruments Corporation, Solothurn, Switzerland

<sup>d</sup> Cole-Parmer pH/mV/Rel mV/°C Benchtop Meter, Cole-Parmer Instrument Co, Vernon Hills, IL

<sup>e</sup> CODAS, Dataq Instruments, Inc, Akron, OH

<sup>f</sup> Marshall TM, Constable PD, Wittek T, Crochik S. Ability of the abomasal luminal pH-time relationship to predict the abomasal emptying rate in Holstein bull calves. Proceedings of the 23rd World Buiatrics Congress, Quebec City, Quebec, Canada, July 2004, 22 (poster abstract).

<sup>g</sup> Sigma Chemical Co, St Louis, MO

<sup>h</sup> Micro-Osmette, Precision Systems Inc, Natick, MA

<sup>i</sup> Proc Mixed, SAS 8e, SAS Inc, Cary, NC

<sup>j</sup> Constable PD, Ahmed AF, Misk NA. Effect of suckling fresh cow's milk and two milk replacer formulations on abomasal luminal pH in dairy calves. XXII World Buiatrics Congress, Hannover, Germany 2002:167-168 (abstract 523-179).

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