Short communication: Addition of varying amounts of sodium bicarbonate to colostrum replacer: Effects on immunoglobulin G absorption and serum bicarbonate in neonatal calves¹

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ABSTRACT

Fifty-two dairy calves were blocked by birth date and, within each block, randomly assigned to 1 of 4 treatments to investigate the effects of incremental levels of sodium bicarbonate (NaHCO_3) on IgG metabolism. Treatments were (1) colostrum replacer (CR) + 0 g of NaHCO_3 (control); (2) CR + 15 g of NaHCO_3 ; (3) CR + 30 g of NaHCO_3 ; or (4) CR + 45 g of NaHCO_3 . Calves were fed colostrum replacer (>200 g of IgG) in one feeding within 45 min of birth (0 h) and 2 L of milk replacer at 12, 24, 36, and 48 h. Only calves born in calving pens from multiparous cows with no dystocia were used in this study. Blood samples were taken at 0, 6, 12, 24, and 48 h postpartum, and serum was analyzed for IgG using radial immunoassay and bicarbonate using spectrophotometry. Feeding increasing levels of sodium bicarbonate had negative linear effects on IgG concentration, IgG apparent efficiency of absorption, and IgG area under the curve, primarily due to the effect of the highest dose of NaHCO_3 (45 g). Sodium bicarbonate treatments had no effect on serum bicarbonate concentration. However, area under the curve of serum bicarbonate increased linearly with the amount of NaHCO_3 fed.

Key words: calf, colostrum replacer, immunoglobulin G, sodium bicarbonate

Short Communication

Calves are born hypogammaglobulinemic due to the synepithelialchorial placenta that prevents maternal immunoglobulin transfer to the fetus. Colostrum is the first mammary secretion and provides, through passive transfer, immunoglobulins, predominantly from the IgG₁ subclass, which are essential for the health and

well-being of the neonate (Butler, 1969). Serum concentrations of IgG ≥ 10.0 g/L are indicative of adequate passive transfer (NAHMS, 2007). Inadequate passive transfer has resulted in reduced growth rates; increased risks of diseases, death, and early culling; and decreased milk production in the first lactation (Smith and Foster, 2007; Beam et al., 2009). Therefore, it is crucial that calves receive colostrum immediately after birth to maximize the passive transfer of IgG while the small intestine is able to absorb intact Ig. Sodium bicarbonate added as a buffer to fermented colostrum increased the serum IgG concentration in neonatal calves compared with those fed fermented colostrum only (Foley and Otterby, 1978). Feed intake was improved when acidified (Eppard et al., 1982) or fermented (Jenny et al., 1984) colostrum was supplemented with NaHCO_3 . Sodium bicarbonate also has bacteriostatic effects, inhibiting the growth of *Escherichia coli* 0111 in human milk (Bullen et al., 1972) and bovine colostrum (Griffiths and Humphreys, 1977). Recently, Morrill et al. (2010) reported that supplementing colostrum replacer (CR) with NaHCO_3 increased the serum concentration of IgG compared with CR alone. However, the optimum dose of NaHCO_3 that maximizes IgG absorption has not been determined. We aimed to explore the effect of supplementing CR with increasing levels of NaHCO_3 on IgG concentration, and on the apparent efficiency of absorption (AEA) of IgG.

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee. Fifty-two Holstein calves born from multiparous cows were used in this study. Calves were blocked by birth date and, within each block, randomly assigned to 1 of 4 treatments as follows: (1) CR + 0 g of NaHCO_3 (control); (2) CR + 15 g of NaHCO_3 (15-g treatment); (3) CR + 30 g of NaHCO_3 (30-g treatment); or (4) CR + 45 g of NaHCO_3 (45-g treatment). The pH of the reconstituted CR with or without NaHCO_3 was measured using a pH meter (Orion 230A pH Meter, Thermo Fisher Scientific Inc., Beverly, MA).

Calves were removed from their dams within 30 min after birth and weighed on a platform scale (Salter

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Scales, Fairfield, NJ), and their navels were dipped in 7% iodine. Calves were then placed in a naturally ventilated enclosed calf room and individually housed in pens (1 × 2.15 m) bedded with kiln-dried sawdust where they remained for the duration of the study (48 h). Calves were assigned a dystocia score of 1 to 3 based on the difficulty of the calving: 1 = unassisted calving, 2 = assisted easy calving, or 3 = assisted difficult calving (Morrill et al., 2010). Only calves with a dystocia score of 1 or 2 were used in the current study. All calves received 1 feeding of 2 sachets of Land O'Lakes Colostrum Replacement (Saskatoon Colostrum Co., Saskatoon, SK, Canada) containing >100 g of IgG/sachet (total amount of IgG fed >200 g; Table 1) within 45 min of birth (referred to as 0 h) using nipple bottles. If the entire volume was not consumed in the first feeding, the CR was kept warm and reintroduced 1 h later. If the remaining CR was not consumed in the second feeding, it was administered to the calf via esophageal tube. For all subsequent feedings, nonmedicated milk replacer (340 g; Blue Seal Feeds Inc., Londonderry, NH) was reconstituted with 2 L of water and fed to calves at 12, 24, 36, and 48 h after birth. Samples of both CR and milk replacer were submitted to Agri-King (Fulton, IL) for nutrient analysis: CP (method 990.03; AOAC, 2002) and minerals (Ca, P, Mg, K, Na, and Fe; method 985.01; AOAC, 2002). Concentration of total fatty acids was determined by saponification with KOH in ethyl alcohol. Fatty acids were then liberated from the soaps with HCl and extracted with petroleum ether (AOAC, 1995). Lactose was determined using method 984.22 (AOAC, 2002) with the following modifications: flow rates through the HPLC (Beckman, Fullerton, CA) were 1 mL/min, melibiose was the internal standard, and evaporative light scattering detection was used instead of a refractive index. Nutrient analyses of CR and milk replacer are presented in Table 1.

Blood samples were collected into 7-mL Vacutainer tubes (Kendall, Mansfield, MA) via jugular venipuncture using a 22-gauge needle before the initial feeding of CR and at 6, 12, 24, and 48 h after birth. The blood collected was allowed to clot at room temperature for no longer than 1 h followed by centrifugation (CentraMP4R; International Equipment Company, Needham Heights, MA) at 1,310 × *g* at 25°C for 20 min. The serum was harvested and stored at -20°C until analyzed for the concentrations of IgG by RIA (Saskatoon Colostrum Co.) and bicarbonate concentrations (Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, Canada) using a Hitachi analyzer (Hitachi 912 Automatic Analyzer; Roche Diagnostics, Quebec, Canada; Stockham and Scott, 2008).

Apparent efficiency of absorption at 24 h of age was estimated using the equation [plasma IgG (g/L) × BW

(kg) × 0.09/IgG intake] × 100% (Quigley and Drewry, 1998). The area under the curve (AUC) for serum samples at 0, 6, 12, 24, and 48 h was determined using the trapezoidal rule (Phillips and Taylor, 1973) with the equation 0.5 × difference in time × difference in IgG or serum bicarbonate concentrations.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (2001; SAS Institute Inc., Cary, NC). The 24-h serum concentrations of IgG and serum bicarbonate, AEA of IgG, and AUC of serum IgG and serum bicarbonate were fitted to the following model:

$$Y_{ij} = \mu + B_i + S_j + E_{ij},$$

where *Y* = the dependent variable, μ = the overall mean, B_i = the random effect of block *i* (*i* = 1, ..., 13), S_j = the fixed effect of the *j*th NaHCO₃ level (*j* = 0, 15, 30, 45), and E_{ij} = the residual error $\sim N(0, \sigma^2_e)$.

Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS (SAS Institute). Single degree of freedom contrasts for linear and quadratic effects were determined. Significant treatment effects were declared at $P \leq 0.05$ and trends at $0.05 \leq P \leq 0.10$. Block effect was not significant ($P > 0.25$) and was removed from the model for all variables.

Average BW of all calves at birth was 44.2 ± 5.5 kg. The pH of the CR used in the 4 treatments was 6.10 ± 0.34 (control), 6.60 ± 0.09 (15-g treatment), 6.90 ± 0.08 (30-g treatment), and 7.10 ± 0.21 (45-g treatment). At birth, the average serum IgG concentration for all calves was 0.5 ± 0.7 g/L. During the initial feeding 4 calves were fed via esophageal feeder, 1 was given 2 feedings of CR on the control treatment, 1 calf was given 2 feedings of CR on the 15-g treatment, 3 calves

Table 1. Nutrient analysis of colostrum replacer and milk replacer (DM basis)¹

Item	CR	MR
CP, %	55.3	21.4
Fat, %	20.9	20.5
Lactose, %	12.5	41.1
Ca, %	0.95	1.01
P, %	0.80	0.76
K, %	0.68	1.75
Mg, %	0.13	0.15
Na, %	0.28	0.87
Zn, mg/kg	75	113
IgG, g/dose	211.6 ² /216.8 ³	—

¹CR = Land O'Lakes Colostrum Replacer (Saskatoon Colostrum Co., Saskatoon, SK, Canada); MR = Milk Replacer, nonmedicated (Blue Seal Feeds, Londonderry, NH).

²Calves 1–7 were fed this concentration.

³Calves 8–52 were fed this concentration.

were given 2 feedings of CR on the 30-g treatment, and 1 calf on the 45-g treatment was fed CR via esophageal feeder.

Serum IgG concentrations at 24 h showed a negative linear trend ($P < 0.08$; Table 2) with increasing NaHCO_3 in the CR. Of the 52 calves, 51 attained serum IgG concentration ≥ 10.0 g/L, indicating successful passive transfer. Area under the curve ($P = 0.03$) and AEA ($P = 0.05$) for serum concentration of IgG showed negative linear responses to increasing levels of NaHCO_3 from 0 to 45 g (Table 2). These negative linear effects were driven by the 45-g treatment, suggesting that at this level of supplementation calves may be experiencing metabolic alkalosis, decreasing their ability to absorb IgG (Figure 1). Elevated concentrations of serum bicarbonate can be associated with metabolic alkalosis or as a compensation for acidosis (Stockham and Scott, 2008). The AUC for serum concentration of bicarbonate in our study had a positive linear effect with increasing dietary levels of NaHCO_3 (Table 2, Figure 2), which may reduce intestinal IgG uptake.

Eight calves had serum concentrations of IgG ≥ 1 g/L when blood samples were collected at 0 h (0.49 ± 0.73 g/L). Typically, calves are born with lower levels of IgG in their blood. However, abnormal placentation or a damaged placenta can result in transfer of maternal IgG to the fetus (Redman, 1979) or calves may produce IgG after transplacental or transcervical exposure to an infectious agent (Chigerwe et al., 2008). Even though the dams of our calves had no retained placentas, some damage to the placenta may have occurred. Calves exposed to infection in utero have shown increased serum IgG and IgM concentrations (Chigerwe et al., 2008), which may also explain the elevated serum IgG observed in the current study.

Morrill et al. (2010) observed higher ($P < 0.05$) 24-h serum IgG concentrations and higher AEA for calves receiving supplemental NaHCO_3 compared with those that did not (IgG 16.3 vs. 13.2 g/L and AEA 31.2 and 26.1%, respectively). In the present study, AUC for IgG showed a negative linear response ($P = 0.03$) with increasing dietary NaHCO_3 (Table 2). Conversely, Morrill et al. (2010) reported an increase in the AUC in calves supplemented with NaHCO_3 , which may be explained by differences in feeding protocols between studies. Morrill et al. (2010) fed CR in 2 feedings, providing 132 g of IgG $\pm$ 19.5 g of NaHCO_3 at the first feeding and 66 g of IgG $\pm$ 9.75 g of NaHCO_3 in the second feeding 6 h later. In the current experiment, 212 or 217 g of IgG (variation due to 2 lots of CR) was fed with or without NaHCO_3 supplementation at a single feeding (0-h feeding). Jaster (2005) observed that feeding calves 2 feedings consisting of 2 L of colostrum rather than one 4-L feeding resulted in higher AEA levels and increased IgG absorption. The difference in IgG absorption reported by Jaster (2005) may be attributed to saturation of the intestinal absorptive capacity for IgG when feeding 4 L of colostrum at a single feeding. Therefore, it can be hypothesized that the single feeding of CR that provided >200 g of IgG may have exceeded the intestinal capacity to absorb IgG suppressing any beneficial effect of NaHCO_3 on enhancing IgG absorption.

Apparent efficiency of absorption of IgG at 24 h showed a negative linear response ($P = 0.05$) when the dietary levels of NaHCO_3 were increased from 0 to 45 g (Table 2). The AEA of calves on the 45-g treatment was lowest overall, indicating that absorption was compromised in these calves. These results differ from those of Morrill et al. (2010), who observed an increase ($P < 0.05$) in AEA (+5.1%) in calves supplemented

Table 2. Initial BW (mean $\pm$ SD), 24-h serum IgG concentrations, apparent efficiency of absorption (IgG), area under the curve (IgG), 24-h serum bicarbonate concentrations, and area under the curve (serum bicarbonate) of calves fed varying amounts of NaHCO_3

Item	Treatment ¹				SE	Contrast	
	Control	15-g	30-g	45-g		Linear	Quadratic
Initial BW, kg	42.8 $\pm$ 4.4	45.4 $\pm$ 5.4	42.4 $\pm$ 5.0	44.8 $\pm$ 5.9			
Serum IgG, g/L							
24 h	16.9	16.0	17.2	14.5	0.75	0.08	0.22
Lowest ²	13.3	12.0	12.0	7.70			
Highest ³	20.1	18.5	23.1	18.8			
AEA, % ⁴	32.1	30.3	31.3	27.2	1.51	0.05	0.43
AUC IgG, g/L $\times$ h ⁵	680	622	682	562	29.1	0.03	0.29
Serum bicarbonate, mmol/L, 24 h	29.0	31.7	33.0	33.1	4.67	0.28	0.49
AUC serum bicarbonate, mmol/L $\times$ h ⁶	1,277	1,405	1,466	1,475	25.6	<0.0001	0.03

¹Treatments: Control = 0 g of NaHCO_3 ; 15-g = 15 g of NaHCO_3 ; 30-g = 30 g of NaHCO_3 ; 45-g = 45 g of NaHCO_3 .

²Lowest 24-h serum IgG concentration.

³Highest 24-h serum IgG concentration.

⁴Apparent efficiency of absorption: $[\text{plasma IgG (g/L)} \times \text{BW (kg)} \times 0.09/\text{IgG intake}] \times 100\%$ (Quigley and Drewry, 1998).

⁵Area under the curve; $0.5 \times \text{difference in time} \times \text{difference in IgG concentration}$ (Phillips and Taylor, 1973).

⁶Area under the curve; $0.5 \times \text{difference in time} \times \text{difference in serum bicarbonate concentration}$ (Phillips and Taylor, 1973).

Figure 1. Serum IgG concentration for calves receiving 0, 15, 30, or 45 g of NaHCO₃ added to the colostrum replacer.

Figure 2. Serum bicarbonate levels for calves fed 0, 15, 30, or 45 g of NaHCO₃ added to colostrum replacer.

with 30 g of NaHCO_3 compared with those with no supplementation.

The serum concentrations of bicarbonate were similar across treatments (mean = 32 mmol/L), which may be explained by the body's physiological response to maintain homeostasis (Table 2). Our results are corroborated by data from the University of Saskatchewan showing that the physiological serum concentrations of bicarbonate for 3- to 7-yr-old dairy cattle range from 17 to 33 mmol/L (S. Myers, Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, Canada; unpublished data).

In summary, adding incremental levels of NaHCO_3 to CR had negative linear responses on the serum concentration of IgG, IgG AEA, and IgG AUC. Supplementing CR with 45 g of NaHCO_3 resulted in the most detrimental effect on IgG absorption. Area under the curve for serum bicarbonate increased linearly with increasing amounts of dietary NaHCO_3 . Future research in this area should focus on feeding protocols with or without addition of NaHCO_3 and their effects on IgG absorption, which would help to elucidate the efficacy of this supplement in different feeding regimens.

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