Short communication: Addition of sodium bicarbonate to maternal colostrum: Effects on immunoglobulin G absorption and hematocrit in neonatal calves¹

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ABSTRACT

Twenty-six Holstein bull calves born from primiparous and multiparous cows without dystocia were assigned in a randomized complete block design to 1 of 2 treatments: pooled maternal colostrum (PMC) or PMC supplemented with 30 g of sodium bicarbonate (NaHCO_3). Calves were fed PMC from 9 different batches containing (mean $\pm$ SD) 82.05 ± 8.45 g/L of IgG. Calves were fed 2.68 L of PMC at birth (referred to as 0 h) and 1.32 L of PMC 6 h later. The total amount of IgG fed was 329.89 ± 34.56 g. Calves were fed 2 L of milk replacer at 24, 36, and 48 h postpartum. The addition of NaHCO_3 had no effect on IgG absorption. Serum IgG concentrations at 0, 6, 12, 24, and 48 h postpartum were not different between calves supplemented with or without 30 g of NaHCO_3 to colostrum. Area under the curve, apparent efficiency of absorption, and hematocrit were not affected by the NaHCO_3 treatment.

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

Short Communication

Successful calf management starts with the first feeding of colostrum. Due to the synepitheliochorial placenta of the cow, negligible transfer of immunoglobulin occurs from the dam to the fetus. As a result, the calf is born hypogammaglobulinemic. Calves depend on consumption of colostrum to provide passive immunity against pathogens. Colostrum is the initial mammary secretion that provides a highly concentrated source of immunoglobulin, especially IgG₁, which is the main contributor to passive immunity in the neonate. Colostrum also contains other nutrients to provide a complete diet for the neonate, support growth of the intestinal mucosa, enhance intestinal absorptive capacity, and stimulate

digestive functions of the small intestine (Butler, 1969; Hammon and Blum, 2002). The calf's gastrointestinal tract evolved to temporarily allow the absorption of macromolecules, including immunoglobulin, during the first 12 to 24 h of life (Stott et al., 1979). Achievement of passive transfer is indicated by serum IgG concentrations of ≥ 10 g/L at 24 h postpartum and is primarily affected by colostrum quality and quantity, as well as age at first feeding of colostrum (Nocek et al., 1984; NAHMS, 2007). Therefore, high-quality colostrum should be fed soon after birth to optimize immunoglobulin absorption.

Sodium bicarbonate (NaHCO_3) has been added to acidified and fermented colostrum to act as a buffer. Foley et al. (1978) showed that adding NaHCO_3 to fermented colostrum enhanced absorption of immunoglobulin in the newborn calf. Sodium bicarbonate has also been observed to have a bacteriostatic effect on certain bacteria species such as *Escherichia coli* 0111 found in bovine colostrum (Griffiths and Humphreys, 1977). Recently, our group (Morrill et al., 2010) reported an increase in IgG uptake of 3.1 g/L at 24 h when 29.25 g of NaHCO_3 was added to colostrum replacer (CR) in 2 feedings. However, we observed discrepant results when calves were fed CR supplemented with incremental levels (0 to 45 g) of NaHCO_3 (Cabral et al., 2011). Specifically, we found that 45 g of NaHCO_3 resulted in the lowest intestinal absorption of IgG and a negative linear trend ($P = 0.08$) was observed for the serum IgG concentrations at 24 h after birth. However, little research exists evaluating supplementation of pooled maternal colostrum (PMC) with NaHCO_3 . The objectives of this study were to determine the effects of adding NaHCO_3 to PMC on absorption of IgG and hematocrit in the neonatal calf.

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Approval #100503). Twenty-six Holstein bull calves born from primiparous and multiparous cows were used in this study. Calves were blocked by birth date and randomly assigned to 1 of 2 treatments within each block: (1) PMC + 0 g of NaHCO_3 (control, CON) or (2) PMC + 30 g of NaHCO_3 .

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Calves were removed from their dam before nursing within 30 min after birth. Calves were then weighed on a platform scale (Salter Housewares USA Inc., Fairfield, NJ) and had their navels dipped in 7% iodine. Calves were placed in a naturally ventilated, enclosed calf room and housed in individual 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 calving: 1 = unassisted calving, 2 = assisted easy calving, and 3 = assisted difficult calving. Calves used for this study had a calving score of a 1 or 2.

Nine batches of maternal colostrum (MC) that tested greater than 50 g/L of IgG with a colostrometer were collected, pooled, placed in freezer bags, and stored at -20°C until needed. A 5-mL sample of MC from each batch was collected and stored at -20°C until analyzed for IgG by radial immunoassay (Triple J Farms, Bellingham, WA). Calves on the C treatment received 2.68 L of PMC + 0 g of NaHCO_3 at 0 h (within 75 min of birth) and 1.32 L of PMC + 0 g of NaHCO_3 at 6 h after birth. Calves on the 30-g treatment received 2.68 L of PMC + 20 g of NaHCO_3 at 0 h (within 75 min of birth) and 1.32 L of PMC + 10 g of NaHCO_3 at 6 h after birth. Another 5-mL sample of PMC was taken before each feeding and stored at -20°C until analyzed for IgG by radial immunoassay. The pH of the PMC was measured using a pH meter (Orion 230A pH meter; Thermo Fisher Scientific Inc., Beverly, MA) before it was fed to the calves. If the calves did not consume the feedings from the bottle within 30 min, they were fed via esophageal tube.

Three hundred and forty grams of nonmedicated milk replacer (Blue Seal Feed Inc., Londonderry, NH) was reconstituted with 2 L of warm water and fed to calves at 24, 36, and 48 h after birth. A sample of milk replacer was sent to Agri-King (Fulton, IL) for nutrient analysis (Table 1). The sample was analyzed for CP (method 99.03; AOAC 2002) and minerals (Ca, P, Mg, K, Na, Fe, and Zn; method 985.01; AOAC, 2002). Concentrations of total FA were determined by saponification with KOH in ethyl alcohol. Fatty acids were then released from the soaps with HCl and extracted with petroleum ether (AOAC, 1995). Lactose was determined using the method 984.22 (AOAC, 2002) with these modifications: flow rates through 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.

Blood samples were collected in 7-mL blood-collection tubes (Kendall Healthcare Products Co., Mansfield, MA) via jugular venipuncture using a 22-gauge needle before the first feeding of colostrum (within 45 min of birth, referred to as 0 h) and before feeding at

6, 12, 24, and 48 h after birth. Three capillary tubes of blood were subsampled and centrifuged (Haematokrit 210; Andreas Hettich GmbH & Co. KG, Germany) at $16,060 \times g$ at 25°C for 5 min. The remaining blood was allowed to clot at room temperature then centrifuged (CentraMP4R; International Equipment Co., Needham Heights, MA) at $1,310 \times g$ at 25°C for 20 min. Serum was collected and stored at -20°C until analyzed for IgG by radial immunoassay.

Apparent efficiency of IgG absorption (AEA) at 24 h of age was estimated using the following equation: $[\text{plasma IgG (g/L)} \times \text{BW (kg)} \times 0.092/\text{IgG (g) intake}] \times 100\%$ (Quigley et al., 1998). The IgG concentrations at 6, 12, 24, and 48 h were also analyzed. The area under the curve (AUC) was determined for serum samples at 0, 6, 12, 24, and 48 h using the trapezoidal rule with the equation: $0.5 \times \text{difference in time} \times \text{difference in IgG concentration}$ (Phillips and Taylor, 1973).

Calf serum IgG concentrations and hematocrit were analyzed as a randomized complete block design using the repeated measures determined in the MIXED procedure of SAS (SAS Institute, 2001) according to the following model:

$$Y_{ijk} = \mu + B_i + S_j + \beta X_{ij} + H_k + E_{ijk},$$

where Y_{ijk} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 13$), S_j = the fixed effect of the j th NaHCO_3 level ($j = 0$ or 30), β = the regression (covariate coefficient), X_{ij} = the covariate measurement, H_k = is the fixed effect of hour of the experiment ($k = 0, 6, 12, 24, \text{ or } 48$), and E_{ijk} = the residual error $\sim N(0, \sigma_e^2)$.

In this model, the random effect of calf within treatment subclass was used as the error term for the effect of NaHCO_3 . Residual errors, which refer to errors within calf across time and represent errors from repeated measurement in the experimental units (calf) were modeled using an unstructured covariance structure. Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS (SAS

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

Item	MR ¹
CP, %	21.4
Fat, %	20.5
Lactose, %	41.1
Ca, %	1.01
P, %	0.76
K, %	1.75
Mg, %	0.15
Na, %	0.87
Zn, mg/kg	113

¹Milk replacer, nonmedicated (Blue Seal Feeds, Londonderry, NH).

Institute, 2001). A covariate term was included in the model to reduce the variance due to calf within treatment subclasses. The covariate variable was initial BW. Block was found to be not significant ($P > 0.25$) and removed from the model for all variables. Least squares means were determined for treatment. Treatment responses with $P \leq 0.05$ were considered to be statistically significant. The PDIF option in SAS was used to test treatment differences among least squares means.

Apparent efficiency of absorption and AUC were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, 2001) according to the following model:

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

where Y_{ijk} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 13$), S_j = the fixed effect of the j th NaHCO_3 level ($j = 0$ or 30), and E_{ijk} = the residual error $\sim N(0, \sigma_e^2)$.

In this model, the random effect of calf within treatment subclasses was used as the error term for the effect of NaHCO_3 . Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS (SAS Institute, 2001). Block was found to be not significant ($P > 0.25$) and removed from the model for all variables. Least squares means were determined for treatment. Treatment responses with $P \leq 0.05$ were considered to be statistically significant. Results are reported as means $\pm$ standard deviations.

Average initial BW of all calves at birth was 43.8 ± 6.1 kg. The average IgG concentration of the 9 PMC batches was 82.1 ± 8.5 g/L and the average amount of IgG fed amount was 330 ± 34.6 g. The pH of the PMC treatments was 5.97 ± 0.27 (CON) and 6.64 ± 0.20 (30 g). At birth, the average serum IgG concentration for all calves was 0.49 ± 0.96 g/L. Of the 26 calves, all attained passive transfer of ≥ 10 g/L at 24 h. During the

initial feeding, 4 calves were fed via esophageal feeder with 2 calves being on the CON and 2 calves on the 30-g treatment. These 4 calves and 6 others were fed via esophageal feeder at the 6 h feeding with 2 calves being on treatment CON and 4 calves on treatment 30 g.

Five calves had unexpected 0-h serum IgG levels of ≥ 1 g/L in our study (0.49 ± 0.96 g/L), which ranged from 0.00 to 4.18 g/L. Although the mechanisms associated with this premature immunological response are not well understood, both damaged placenta and in utero exposition to pathogens may have triggered fetal production of IgG (Chigerwe et al. 2008).

Serum IgG concentrations at 24 h after birth were similar between treatments (Table 2). Our results differed from Morrill et al. (2010) who reported that calves fed CR supplemented with 30 g of NaHCO_3 had higher serum IgG concentrations at 24 h ($P < 0.05$) compared with control calves (16.3 vs. 13.2 g/L, respectively). These discrepancies between results from our laboratory were likely caused by the use of PMC in the current study and CR in Morrill et al. (2010). Colostrum replacer has been found to be an acceptable alternative to MC; however, absorption of IgG is lower in CR-fed calves compared with MC-fed calves (Smith and Foster, 2007). Researchers have studied how the source of IgG, method of IgG fractionation, amount and type of non-IgG protein, and the presence of fat and lactose in CR products and supplements affect IgG absorption in calves (Arthington et al., 2000; Davenport et al., 2000). Davenport et al. (2000) observed that large amounts of casein in colostrum supplements significantly reduced the efficiency of IgG absorption and Hopkins and Quigley (1997) found that the addition of some colostrum supplements derived from bovine serum reduced the absorption of IgG from MC.

The results from the current study were similar to those of Cabral et al. (2011) who found that calves

Table 2. Initial BW, serum IgG concentrations, area under the curve (IgG), apparent efficiency of absorption (IgG), and 24-h hematocrit of calves with or without the addition of NaHCO_3

Item	Treatment ¹		SE	P-value ²
	CON	30 g		
Initial BW, kg $\pm$ SD	45.3 $\pm$ 5.70	42.2 $\pm$ 6.15		
Serum (24 h), g of IgG/L	32.59	31.73	1.42	0.55
AUC ³ , g of IgG/L $\times$ h	1,270.04	1,188.41	45.21	0.29
AEA ⁴ , %	35.02	32.33	2.24	0.20
Hematocrit, %	34.32	32.44	2.00	0.36

¹CON = 0 g of sodium bicarbonate; 30 g = 30 g of sodium bicarbonate.

²The probability that the CON treatment is different than the 30-g treatment.

³Area under the curve: $0.5 \times \text{difference in time} \times \text{difference in IgG concentration}$ (Phillips and Taylor, 1973).

⁴Apparent efficiency of absorption: $[\text{plasma IgG (g/L)} \times \text{BW (kg)} \times 0.092/\text{IgG intake}] \times 100\%$ (Quigley et al., 1998).

fed CR supplemented with 30 g of NaHCO_3 had similar serum IgG concentrations at 24 h compared with calves fed CR without NaHCO_3 (17.2 vs. 16.9 g/L, respectively). The researchers hypothesized that the lack of response after supplementing CR with NaHCO_3 could be a result of the large amount of IgG fed at the initial feeding. Cabral et al. (2011) also observed a linear reduction in IgG absorption with increasing levels of NaHCO_3 , likely caused by the 45 g of NaHCO_3 negatively affecting IgG absorption (14.5 g/L). The discrepant results between Morrill et al. (2010) and Cabral et al. (2011) were possibly related to differences in their feeding protocols. Whereas Morrill et al. (2010) fed CR (198 g of IgG) in 2 feedings (0 and 6 h) with or without NaHCO_3 , Cabral et al. (2011) fed, on average, 214 g of IgG with or without NaHCO_3 at a single feeding.

Only calves with a dystocia score of 1 (no assist) or 2 (slight assist) were used in the current experiment and 2 other experiments conducted by this laboratory (Cabral et al., 2011, 2012). However, Morrill et al. (2010) used 40 calves, 11 of which were from a difficult calving (dystocia score of 3). In that experiment, all calves with a dystocia score of 3 attained passive transfer, regardless of treatment. Vermorel et al. (1983) indicated that 1 calf experiencing dystocia had lower blood pH than calves born with little difficulty. Therefore, calves in the current study may not have responded to the supplemental NaHCO_3 because they were not dystocial or potentially acidotic. Using nondystocial calves, Cabral et al. (2012) observed an interaction for higher 24-h IgG concentration with supplemental NaHCO_3 added to CR fed in 1 feeding or no supplemental NaHCO_3 with the CR split into 2 feedings compared with calves fed 1 feeding of CR or 2 feedings of CR with supplemental NaHCO_3 . These data suggest that the effect of supplemental NaHCO_3 may not be limited to dystocial calves. The lack of NaHCO_3 effect in our study may also be explained by the amount of IgG fed (329.89 ± 34.56 g) between 0 and 6 h. Researchers recommend feeding newborn calves 4 L of colostrum with greater than 50 g/L of IgG (~200 g of IgG) within the first 6 to 8 h of life (McGuirk and Collins, 2004). In the current study, calves were fed, on average, more than 100 g of IgG above what is considered the minimum recommended amount, which may have caused a saturation of the macromolecular transport mechanism across the calf intestinal epithelium or the serum IgG concentration reached a threshold level (Besser et al., 1985).

It has been observed that the greater the amount of immunoglobulin fed at the initial feeding of colostrum, the higher the serum immunoglobulin concentrations at 24 h in calves (Stott et al., 1979; Besser et al., 1985). Stott et al. (1979) found that calves fed 2 L of colostrum at birth had mean serum concentrations of IgG

almost double those fed 1 L (14.9 vs. 8.5 g/L). Calves in the present study received 2.68 L of high-quality MC at the initial feeding and another 1.32 L at 6 h. Also, these researchers (Stott et al., 1979; Besser et al., 1985), were providing lower amounts of IgG (~100 g of IgG), whereas in the current study, the mean IgG fed amount was 330 ± 34.6 g, which may have offset the effect of NaHCO_3 . At 6 h postpartum, the serum IgG concentrations averaged 12.0 and 12.2 g/L for CON and 30 g treatments, respectively indicating that calves reached passive transfer before the second feeding of colostrum.

When a high amount of IgG is fed, the serum IgG concentrations are increased, but AEA decreases (Besser et al., 1985; Jaster, 2005). Apparent efficiency of absorption of IgG at 24 h was not improved by addition of NaHCO_3 , which disagrees with data from Morrill et al. (2010). Morrill et al. (2010) observed an increase in AEA in calves supplemented with 30 g of NaHCO_3 . However, Cabral et al. (2011) showed a negative linear response, with the 45 g treatment showing the lowest AEA. The AEA measured in the current study averaged 35.0% for calves receiving the CON treatment and 32.3% for those in treatment 30 g, indicating values greater than those in previous studies (Morrill et al., 2010; Cabral et al., 2011) but agrees with the mean AEA (20 to 35%) from MC (Quigley and Drewry, 1998). Although the current study's AEA values were higher than previous studies with NaHCO_3 , the discrepancy could be due to the use of PMC instead of CR. Also, the serum IgG concentrations at 24 h for calves receiving 30 g of NaHCO_3 was twice as high for the current study than for Morrill et al. (2010) and Cabral et al. (2011), which can be explained by the larger amount of IgG fed and PMC was fed instead of MC.

The area under the curve for IgG was also not affected by NaHCO_3 , which contradicts data from Morrill et al. (2010) who observed an increase in AUC with calves fed NaHCO_3 . Cabral et al. (2011) found a negative linear response with increasing levels of NaHCO_3 . The discrepancies between these studies may be caused by the amount of IgG fed at the first feeding and CR having a lower absorption of IgG compared with MC.

Hematocrit data showed no effects of NaHCO_3 treatment. All calves showed a numerical decrease over the 48-h period, which indicated that the calves were becoming more hydrated.

The addition of 30 g of NaHCO_3 had no effect on hematocrit and IgG absorption in calves, which may be explained by the large amount of IgG fed, causing a saturation of the macromolecular transport mechanism across the calf intestinal epithelium. The AUC for IgG and the AEA for IgG were also not affected by supplementing PMC with NaHCO_3 . More research needs to

be conducted regarding the interactions of supplemental NaHCO_3 and MC, as well as the amount of IgG fed and its effects on IgG absorption in neonatal calves. Studying the different components in maternal bovine colostrum that affect intestinal development and absorption of IgG is needed to help explain the discrepant results obtained using NaHCO_3 in our laboratory.

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