Addition of Casein or Whey Protein to Colostrum or a Colostrum Supplement Product on Absorption of IgG in Neonatal Calves

D. F. Davenport,* 1 J. D. Quigley, III,* 2 J. E. Martin,* 3 J. A. Holt,* and J. D. Arthington† 4

*Institute of Agriculture
University of Tennessee
Knoxville 37901

†APC Company, Inc.
1 VisionAire Place, Suite 2
Ames, IA 50010

ABSTRACT

The effects of the addition of nonimmunoglobulin protein on absorption of immunoglobulin G (IgG) from colostrum or colostrum supplement products were determined in two experiments. In experiment 1, 48 Holstein calves were fed 4 L of pooled maternal colostrum or 4 L of reconstituted colostrum supplement with 0, 200, or 400 g of added whey protein concentrate or casein. In experiment 2, 38 Jersey calves were fed 2 L of pooled maternal colostrum with 100 or 200 g of whey protein concentrate or casein added immediately before feeding. Blood was collected at 24 h of age and plasma IgG concentration, total protein, hematocrit (experiment 1 only), and plasma urea N were determined. In experiment 1, blood samples were also collected at 4, 8, 12, 16, and 20 h to evaluate absorption of IgG and protein and urea N concentrations. The addition of 400 g of casein to colostrum supplement in experiment 1 reduced plasma IgG from 5.66 g/L (0 g of casein addition) to 3.88 g/L, increased plasma urea N at 24 h, and reduced the change in plasma total protein from 0 to 24 h. Hourly plasma IgG concentrations increased with the consumption of colostrum or supplements but increased more rapidly in calves fed whey protein concentrate and more slowly in calves fed casein. The addition of 200 g of casein or whey protein concentrate to colostrum supplements had no effect on plasma IgG concentration at 24 h of age. The addition of 100 or 200 g of casein or whey protein concentrate to maternal colostrum had no effect on plasma urea N, total protein, or plasma IgG in experiment 2. The addition of nonimmunoglobulin protein to colostrum supplements or maternal colostrum did not affect IgG absorption from the intestine of newborn calves unless the amount of total protein exceeded 500 g of protein.

(Key words: calves, colostrum, immunoglobulin)

Abbreviation key: AEA = apparent efficiency of IgG absorption, CS = colostrum supplement, C-0 = 302 g of CS in 4 L of water, C-200 = 302 g of CS + 200 g of casein, C-400 = 302 g of CS + 400 g of casein, FITC = fluorescein isothiocyanate, MC = maternal colostrum, MC-0 = 2 L of MC, MC-100 = MC + 100 g of casein, MC-200 = MC + 200 g of casein, MW-100 = MC + 100 g WPC, MW-200 = MC + 200 g WPC, WPC = whey protein concentrate, W-200 = 302 g of CS + 200 g of WPC, W-400 = 302 g of CS + 400 g of WPC.

INTRODUCTION

Absorption of Ig from colostrum during the first 24 h of life has been considered nonspecific, and absorption of non-Ig proteins by the intestinal lumen is well documented (Balfour and Comline, 1959; Hardy, 1969; Pierce, 1961). However, the potential interaction among non-Ig proteins and absorption of IgG during the period of macromolecular transport is less clear. Besser and Osbourn (1993) reported that the addition of BSA (37 mg/ml) to colostrum impaired the absorption of IgG and reduced apparent efficiency of absorption (AEA) of IgG in neonatal calves. Quigley et al. (1998) reported that feeding large amounts (750 g) of a colostrum supplement (CS) product impaired IgG absorption and AEA, compared with smaller amounts of CS, which were absorbed with an efficiency similar to that of maternal colostrum.

Colostral supplement products are designed to provide supplemental IgG (typically 25 to 45 g/dose) to neonatal animals during the period of macromolecular transport. However, due to the nature of IgG sources (lacteal secretions or bovine serum) used, significant amounts of non-Ig protein are fed with these products.
Non-Ig proteins may include α-lactoglobin, β-lactalbumin, casein, serum albumin, and others. We hypothesized that the poor AEA in some CS (Garry et al., 1996) might be due to the presence of excessive amounts of non-IgG protein that compete with macromolecular binding sites in the intestine.

The objectives of this study were to determine whether the addition of non-Ig proteins as whey protein concentrate (WPC) and casein affected the absorption in neonatal calves of IgG from maternal colostrum or a CS product.

**MATERIALS AND METHODS**

**Experiment 1**

Holstein calves (n = 48; 24 bulls) at the University of Tennessee Agricultural Experiment Station born between September 5, 1997, and January 11, 1998, were assigned at birth to receive pooled maternal colostrum (MC), or 302 g of a CS product (APC Company, Inc., Ames, IA) reconstituted in 4 L of water (C-0), or 302 g of CS with the addition of 200 or 400 g of either WPC (C-200 and W-200, respectively) or casein (C-200 and C-400, respectively). The CS contained 57.4% of DM as protein and 21.4% of DM as IgG. Whey protein concentrate (75% CP) was edible grade material and was obtained from commercial sources (Hillmar Cheese Co., Hillmar, CA). Casein (88% CP) was obtained commercially (Sigma Chemical Co., St. Louis, MO) and was prepared by acid precipitation of bovine milk. Calves were blocked by sequence of birth to account for potential differences in season on IgG absorption.

Calvings were monitored throughout the study; any calf not observed at birth was not used to eliminate the possibility of nursing. Calves were removed from the dam within 10 min of birth, moved to the calf facility, weighed, and placed in an individual stall bedded with shavings. All calves were treated with a commercial rotavirus and coronavirus vaccine (CalfGuard, Norden) and navels were dipped with iodine.

Before animals were assigned to treatment, 28 L of colostrum was collected, frozen, thawed, pooled, and refrozen into bottles or bags to provide seven calves with 4 L of colostrum each. A second pool of 4 L was collected during the experiment. First and second milking colostrum was used to make the pools. Two 50-ml samples of each pool were collected and frozen before analysis for IgG by radial immunodiffusion (RID kits, VMRD, Pullman, WA).

Pooled MC was thawed in warm water before feeding and 4 L was administered in one feeding at approximately 1.5 h of age. All CS was reconstituted in 2 L of warm tap water and mixed thoroughly. Additional protein (if any) was added and mixed. A small amount of NaOH was added to bottles containing casein to increase the solubility of the casein. Then, the total volume of solution was brought to 4 L with tap water. All calves were fed by esophageal feeder to eliminate variation in IgG intake.

Blood was collected before feeding and at 24 h of age by jugular venipuncture into evacuated tubes containing EDTA. A sample of blood was also collected to determine hematocrit by microhematocrit centrifuge. Plasma was separated by centrifugation and stored (−20°C) before IgG was determined by radial immunodiffusion (RID kits, VMRD), plasma urea N (urea nitrogen kit, Sigma Chemical Co., St. Louis, MO), and total protein (Sigma Chemical Co.). Blood from calves (n = 18) in blocks 2, 4, and 6 was sampled every 4 h up to 24 h to evaluate changes in blood metabolites with time.

Data were analyzed as a randomized complete block experimental design using GLM procedure of SAS (1989). Variables in the model included block and treatment. Sex of calf and birth BW were evaluated as covariants, but neither explained a significant amount of variation in the model (P > 0.05). Apparent efficiency of IgG absorption was estimated with the data of Quigley et al. (1998) as AEA = \(\frac{\text{Plasma IgG at 24 h (g/L) \times BW (kg) \times 0.092}}{\text{IgG intake (g)}}\). Hourly data (urea N, total protein, hematocrit, and IgG) were analyzed as a repeated measures ANOVA using the MIXED procedure of SAS (1989). Block and block × treatment interaction were random effects and treatment, hour, and hour × treatment were fixed effects. Metabolite concentration at 0 h was included in each model as a covariant. Single degree of freedom contrasts were used to determine effects of CS versus MC, effects of addition of casein and WPC, and level of casein and WPC addition (200 vs. 400 g). Significance was declared at P < 0.05 unless otherwise noted.

**Experiment 2**

Forty Jersey bull (n = 19) and heifer (n = 21) calves born between September 8, 1997, and November 6, 1997, at the Dairy Experiment Station in Lewisburg, Tennessee, were used. Calves were blocked by date of calving and pool of colostrum. Before animals were assigned to treatment, 10 L of colostrum was collected, frozen, thawed, pooled, and refrozen into bottles or bags to provide all calves in a block with 2 L of colostrum. First and second milking colostrum was used to make the pools. Two 50-ml samples of each pool were collected and frozen before analysis for IgG as in experiment 1. Pooled colostral IgG was measured by radial immunodiffusion following dilution with 0.9% NaCl.

All calvings were supervised to assure that calves had no opportunity to nurse the dam. Any calf born...
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unobserved was not used in the study. The time and date of birth were recorded; calves were moved to the calf facility, weighed, identified; and their navels were dipped with iodine. Calves were fed 2 L of colostrum with no added protein (MC-0), 100 or 200 g of added WPC (MW-100 and MW-200, respectively), or 100 or 200 g of added casein (MC-100 and MC-200, respectively). All MC was slightly heated in warm water, mixed with appropriate treatment, and then fed via nipple bottle as soon as possible after birth. Colostrum not consumed voluntarily was administered by esophageal feeder. The amount of colostrum delivered by the esophageal feeder was recorded.

A sample of blood was collected by jugular puncture at 24 h of age into evacuated containers with EDTA. Blood was centrifuged and plasma separated and frozen (−20°C). Concentrations of IgG, total protein, and urea N were measured as in experiment 1.

Data were analyzed by ANOVA using a randomized complete block design. Variables in the model included block and treatment. Dependent variables were serum IgG concentration at 24 h, AEA, plasma urea N, total protein, and age at first feeding. Sex of calf was included as a covariant in the model. Body weight of calf was evaluated in each model, but did not contribute significantly to the model (P > 0.05). Single degree of freedom contrasts were used to determine the effects of the addition of added protein, casein versus WPC, and level of casein and WPC addition. Significance was declared at P < 0.05 unless otherwise noted.

RESULTS

Experiment 1

Calves were healthy during the study, and there was no mortality. Body weights of calves (Table 1) at birth were similar among treatments; mean BW was 39.4 kg. Mean age at first blood sampling was 1.21 h and did not differ by treatment. Calves fed C-200 and C-400 were fed later than other calves due to the time required to solubilize the casein and properly mix the material. However, all animals were fed by 1.5 h of age; therefore, the biological significance of this difference is probably minimal.

Plasma IgG concentrations before feeding were below detectable concentrations of the assay and did not produce rings on the radial immunodiffusion plates. Therefore, they were assumed to be zero. At 24 h of age, plasma IgG increased for all calves and were significantly higher for calves fed MC versus all CS (Table 1). In addition, plasma IgG in calves fed C-400 tended (P < 0.11) to be lower than calves fed C-200. Apparent efficiency of IgG absorption was similar among treatments, except for calves fed C-400. Mean plasma IgG was reduced by 37% when 400 g of casein was added to CS.

Hematocrits at 0 and 24 h were generally unaffected by treatment; hematocrits declined from 0 to 24 h as plasma volume expanded with colostrum intake (Table 1). Change in hematocrit from 0 to 24 h was greater in calves fed casein, but particularly in calves fed C-400 compared to calves fed C-0 (Table 1).

Mean plasma total protein at 0 or 24 h did not differ among treatments and were 4.04 and 4.57 g/dl, respectively (Table 1). Change in total protein from 0 to 24 h differed by treatment, however, and was greater in calves fed MC versus CS and was lower in calves fed C-0 compared to other treatments, except those fed C-400. Change in total protein in calves fed C-400 was 0.08 g/dl compared to 0.21 g/dl in calves fed C-0.

Urea N concentrations differed among treatments at 0 h and were highest in calves fed C-0 compared to other calves fed CS. However, by 24 h, differences among treatments were only significant between calves fed C-200 and C-400. Change in urea N concentration also reflected the greater increase in urea N in calves fed C-400 compared to those fed C-200.

Hematocrit in calves in blocks 2, 4, and 6 declined linearly from birth to 24 h (P < 0.0001; data not shown), in response to liquid absorption and plasma expansion with colostrum intake. Dietary treatment had no effect on changes in hematocrit, suggesting that the amount of protein in solution did not markedly influence liquid absorption from MC or CS.

The hourly profile of plasma IgG (Figure 1) indicated increasing IgG in plasma of all calves to approximately 8 to 12 h; thereafter, IgG concentrations did not increase markedly or were constant. Apparently, absorption of ingested IgG was complete or movement of circulating IgG to extravascular pools had equilibrated with absorption of IgG by approximately 12 h. Changes in plasma IgG hourly profiles to 24 h in calves fed WPC were greater (P < 0.006) than those in calves fed C-0 (Figure 2). Calves fed W-200 and W-400 consumed 6 and 12 g of IgG from WPC, respectively, in addition to IgG from CS. Assuming an AEA of 30% for IgG from CS (Table 1), it is possible to estimate AEA of IgG from WPC. For calves fed 6 and 12 g of IgG, the AEA of additional IgG from WPC were 58 and 45%, respectively. These AEA are higher than other AEA data in this experiment; therefore, the absorption of IgG from the added WPC was not impaired by the additional protein.

Hourly least squares means of plasma IgG in calves fed C-400 tended (P < 0.06) to be lower than IgG in calves fed C-200, suggesting that the increased mass of casein impaired IgG absorption. The concentration of IgG in calves fed C-400 was particularly low at 4 to
Table 1. Least squares means of BW, age at feeding and blood sampling, and concentrations of blood metabolites, experiment 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments1</th>
<th>Contrasts2</th>
<th>SE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td></td>
<td>MC</td>
<td>C-0</td>
<td>C-200</td>
<td>C-400</td>
<td>W-200</td>
<td>W-400</td>
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<td>39.4</td>
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<td>1.54</td>
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<td>69</td>
<td>69</td>
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<td>80.5</td>
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<td>-3.44</td>
<td>-4.88</td>
<td>-5.35</td>
<td>0.95</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<td>4.29</td>
<td>3.90</td>
<td>4.04</td>
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</tr>
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<td>4.88</td>
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<td>Urea N, mM/L</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>0 h</td>
<td>4.18</td>
<td>5.87</td>
<td>3.34</td>
<td>3.64</td>
<td>4.50</td>
<td>3.79</td>
<td>0.8</td>
<td>NS</td>
</tr>
<tr>
<td>24 h</td>
<td>5.10</td>
<td>5.89</td>
<td>5.29</td>
<td>7.97</td>
<td>5.04</td>
<td>4.58</td>
<td>0.58</td>
<td>NS</td>
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<tr>
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<td>0.02</td>
<td>1.95</td>
<td>4.33</td>
<td>0.54</td>
<td>0.58</td>
<td>0.80</td>
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<td>AEA5, %</td>
<td>31</td>
<td>30</td>
<td>29</td>
<td>19</td>
<td>34</td>
<td>32</td>
<td>3</td>
<td>NS</td>
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</table>

1Treatments: MC = 4 L of maternal colostrum; C-0 = 4 L of colostral supplement (CS); C-200 = 4 L of CS + 200 g of casein; C-400 = 4 L of CS + 400 g of casein; W-200 = 4 L of CS + 200 g of whey protein concentrate (WPC); W-400 = 4 L of CS + 400 g of WPC.

2Contrasts: 1 = MC vs. all CS; 2 = C-0 vs. (C-200 + C-400); 3 = C-0 vs. (W-200 + W-400); 4 = C-200 vs. C-400; 5 = W-200 vs. W-400.

3P > 0.10.

4Not determined.

5AEA = [Plasma IgG at 24 h (g/L) × BW (kg) × 0.092] ÷ IgG intake (g).

Figure 1. Plasma IgG in calves fed pooled maternal colostrum (♦), colostral supplement (CS) (○), CS plus 200 g of whey protein concentrate (WPC) (○), CS plus 400 g of WPC (●), CS plus 200 g of casein (●) or CS plus 400 g of casein (■). Standard error of the mean = 0.7 g/L.

Figure 2. Plasma total protein in calves fed pooled maternal colostrum (♦), colostral supplement (CS) (○), CS plus 200 g of whey protein concentrate (WPC) (○), CS plus 400 g of WPC (●), CS plus 200 g of casein (●) or CS plus 400 g of casein (■). Means are covariately adjusted for total protein at 0 h. Standard error of the mean = 0.23 g/dl.
8 h, which may have been due to delays in outflow of protein from the abomasum. The profile of hourly total protein concentrations (Figure 2) generally followed the profile of IgG concentrations. Total protein increased most in calves fed W-400 and least in calves fed C-400.

The profile of plasma urea N (Figure 3) indicated that urea N gradually increased over the 24-h experimental period and generally with little effect of treatment. However, at 20 and 24 h, urea N increased markedly in calves fed C-400 and approached 9 mM by 24 h.

**Experiment 2**

All calves were healthy throughout the study, and there was no mortality during the 24-h study. Two blood samples were inadvertently damaged in transit and were unusable. Therefore, least squares means from 38 calves are reported. Blood samples at birth were not collected in this study; therefore, initial protein and IgG concentrations and changes in protein and IgG concentrations are not reported.

Mean age at first feeding did not differ among treatments and was 1.0 h. Plasma IgG, urea N, and total protein concentrations at 24 h were also unaffected by treatment (Table 2), indicating that 100 or 200 g of casein or WPC did not influence IgG concentrations. Plasma IgG concentrations were substantially higher in this study than in experiment 1, reflecting the greater IgG concentration of the colostrum used in this experiment. The calculated AEA averaged 21% and was similar among all treatments (Table 2).

**DISCUSSION**

Saturation of macromolecular transport may influence absorption of IgG. Presumably, intake of protein or IgG above maximal molecular transport would not be absorbed, and circulating IgG as a proportion of IgG intake would decrease. Besser and Osbourn (1993) reported impaired absorption of IgG with the addition of 37 mg of BSA/ml of colostrum, whereas, the addition of 37 mg of hydrolyzed casein/ml of colostrum had no effect on concentration of IgG or AEA. The authors also suggested that the presence of varying amounts of casein in normal bovine colostrum might increase the variation in AEA in calves fed colostrum with similar IgG content. Our data do not support this hypothesis. Calves in experiment 1 consumed 296 (C-0), 496 (C-200, W-200), or 696 (C-400, W-400) g of DM in the experiment. Protein intakes were 184, 360, 334, 535 and 483 g, respectively. Only at 400 g of casein (535 g of protein intake) was plasma IgG concentration or calculated AEA impaired. The marked increase in total protein in calves fed WPC in experiment 1, coupled with the small increase in plasma urea-N suggests that much of the WPC was absorbed into the circulation but was not metabolized to urea N. Conversely, total plasma protein increased less and urea N increased more when calves were fed casein, but particularly 400 g of casein.

Besser and Osbourn (1993) fed calves 36 to 149 g of protein. The authors hypothesized that increasing protein intake to 149 g depressed AEA and serum IgG concentration. In experiment 1 of the current study, calves were fed 184 g (C-0) to 483 g (W-400) of protein without significant depression of plasma IgG or AEA. Only when protein intake was 535 g (C-400) were IgG absorption and AEA depressed. In the study by Quigley et al. (1998), calves were fed CS that provided 600 and 213 g of protein to newborn calves. Only at 600 g of protein intake was plasma IgG and AEA depressed. Calves in experiment 2 of the current study were fed 94 to 162 g of IgG from MC in addition to 75 to 176 g of additional protein from casein or whey without effect on plasma IgG or AEA.

The AEA reported by Besser and Osbourn (1993) was 59% and is higher than other estimates of AEA (reviewed by Quigley and Drewry, 1998). Possibly, the processing of colostrum to remove casein and produce colostral whey may have inflated the AEA. Estimates of AEA from MC are typically 30 to 35% (Quigley and Drewry, 1998) and are related to absorption of IgG from...
the gut as well as equilibration of circulating IgG with nonvascular liquid pools.

Other researchers have fed varying amounts of protein to neonatal calves without apparent depression of plasma concentration or AEA. Hopkins and Quigley (1997) reported a linear increase in serum IgG concentration with increasing mass of IgG intake from maternal colostrum to 450 g of IgG intake. Although protein intake was not reported in this study (Hopkins and Quigley, 1997), presumably, intake of total protein exerts an influence on the acquisition of passive immunity in newborn calves without apparent depression of plasma protein concentration.

Intake of protein as WPC or casein may influence digestion and absorption of protein and IgG and affect urea N concentrations. Efficiency of protein absorption was calculated by estimating the plasma volume at 24 h as 9.2% of BW (Quigley et al., 1998), and assuming that the plasma volume at 0 h equaled plasma volume at 24 h minus change in hematocrit from 0 to 24 h. Grams of protein at 0 and 24 h were then estimated, and absorption of protein was estimated by difference. Efficiency of protein absorption was estimated as protein absorbed divided by protein intake. Least squares means of calculated efficiency of protein absorption were 9, 7, 1, 12, and 7% (SE = 2%) for calves fed C-0, C-200, C-400, W-200, and W-400, respectively. Differences were significant for calves fed C-0 versus calves fed casein and C-200 versus C-400. These data indicate that 400 g of casein was not associated with significant increases in plasma protein concentration.

Total protein in plasma, particularly when estimated by refractometry, has commonly been used to estimate the acquisition of passive immunity in newborn calves fed colostrum (Naylor and Kronfeld, 1977; Naylor et al., 1977; Tyler et al., 1999). The correlation between plasma total protein and plasma IgG in neonates is usually sufficient to allow adequate estimation of status of passive transfer. However, in experiment 1, the correlation (r) between plasma total protein and IgG at 24 h of age was 0.20 (P > 0.10), indicating that the relationship between protein and IgG in plasma depended on intake of non-Ig proteins, which may vary when exogenous CS are fed. In experiment 2, correlation (r) between plasma protein and IgG at 24 h was 0.49, which
was significant \( P < 0.001 \). These data suggest that the correlation may be dependent on source of IgG and protein and relationships may differ for MC and CS, or the addition of exogenous protein such as casein or WPC can influence the relationship between IgG and total protein.

**CONCLUSIONS**

The addition of WPC to CS or MC had little effect on absorption of IgG in neonatal calves. Large amounts of casein, however, reduced absorption of IgG from CS product. We conclude that the presence of non-Ig protein in CS had little effect on the absorption of IgG unless the amounts of non-Ig protein provide an excessive mass of DM to the intestine.

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