

Absorption of Protein and IgG in Calves Fed a Colostrum Supplement or Replacer¹

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ABSTRACT

Newborn Holstein bull calves (n = 32) were assigned to receive a colostrum supplement (CS) containing defibrinated bovine plasma or a colostrum replacer (CR) containing an immunoglobulin concentrate obtained by concentrating the immunoglobulin (Ig)G fraction of bovine plasma. The CS and CR contained 11.1 and 21.2% of dry matter as IgG, respectively. Each animal was fed two 454-g feedings at 1 and 8 h of age. The two feedings of CS and CR provided 95 and 187 g of IgG, respectively. Mean plasma IgG at 24 h of age was 8.0 and 13.6 g/L in calves fed CS and CR, respectively, indicating acceptable absorption of Ig from both sources. Mean apparent efficiency of IgG absorption in calves fed CS and CR were 33 and 30%, respectively, and did not differ between treatments. Mean plasma total protein at 24 h in calves fed CS and CR were 4.99 and 4.98 g/dl and did not differ between treatments. Increased plasma protein concentration from 0 to 24 h (4.5 g/L) was lower than the mean increase in plasma IgG concentration during the same period (10.3 g/L), indicating altered protein profile in the blood during the first 24 h of life. Correlation between plasma IgG and total protein at 24 h of age was significant within treatment, but the relationship between IgG and protein in plasma at 24 h varied between treatments. Predicted plasma total protein concentrations at 10 g of IgG/L of plasma at 24 h were 5.4 and 4.2 g/dl, in calves fed CS and CR, respectively. Prediction of plasma IgG concentration using total plasma protein may be inappropriate when calves are fed CS or CR.

(Key words: calf, colostrum, immunoglobulin)

Abbreviation key: **AEA** = apparent efficiency of IgG absorption, **CR** = colostrum replacer, **CS** = colostrum supplement, **MC** = maternal colostrum.

INTRODUCTION

Calves are born hypogammaglobulinemic and require consumption of colostrum as a source of immunoglobulins during the neonatal period. Failure of passive transfer (inadequate circulating IgG concentration) in calves after cessation of macromolecular absorption is a common condition that predisposes calves to increased morbidity and mortality.

Mean apparent efficiency of IgG absorption (**AEA**) from maternal colostrum (**MC**), calculated as grams of IgG in the blood at 24 h divided by grams of IgG intake, typically averages 20 to 35% (Quigley and Drewry, 1998). Inadequate absorption of IgG from colostrum or the lack of high quality colostrum sufficient to meet the minimum IgG intake of newborn calves has prompted the development of colostrum supplements (**CS**), which provide exogenous IgG from bovine lacteal secretions, eggs, or bovine serum. The **AEA** of **CS** varies considerably, depending on the source material, method of processing, and mass of IgG fed (Abel Francisco and Quigley, 1993; Garry et al., 1996; Mee et al., 1996; Morin et al., 1997). Effects of other factors, such as the chemical milieu of the formulation on **AEA** have been evaluated (Hardy, 1969; Quigley et al., 2000). However, **CS** typically do not provide sufficient IgG to completely replace **MC**. Therefore, highly concentrated sources of IgG are required to provide sufficient IgG per dose of product to replace **MC**. Colostrum replacers (**CR**) are products containing a greater mass of IgG to achieve a minimum of 10 g of IgG/L in plasma of calves at 24 h of age. Our objective was to determine absorption of IgG in newborn dairy calves fed **CS** or **CR** as the sole source of Ig.

MATERIALS AND METHODS

Preparation of IgG Products

Defibrinated bovine plasma was manufactured via the following procedure: fresh bovine blood was col-

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lected from abattoirs under USDA inspection. All animals passed veterinary inspection before slaughter. Blood was collected into stainless steel containers containing anticoagulant, inspected, and approved for human consumption, then centrifuged to separate plasma and red blood cells. Plasma was chilled (5°C) and transported to the processing facility. Fibrin was removed from plasma by the addition of excess Ca and resulting defibrinated plasma was concentrated by ultrafiltration and was spray-dried to produce a light tan powder containing approximately 20% IgG and 75% CP.

A commercially available Ig concentrate product (NutraGammmax 40, Proliant, Inc., Ames, IA) was used in the study. The product was obtained by the concentration of bovine plasma by removal of fibrin, albumin, and most of the lipid, followed by ultrafiltration and spray-drying. Samples of defibrinated plasma and Ig concentrate were analyzed for protein and IgG by turbidimetric immunoassay (Etzet et al., 1997).

Formulation of Diets

Experimental CS and CR were produced by a commercial blending facility and packaged into 454-g dose packages. The CS was formulated to contain defibrinated bovine plasma, lactose, glycine, dextrose, salt, potassium chloride, magnesium sulfate, emulsifiers, and flavors. The CR contained Ig concentrate, lactose, salt, high fructose corn syrup, potassium chloride, magnesium sulfate, dry fat blend (7% CP, 60% fat), dextrose, emulsifiers, and flavors. Samples of CS and CR were analyzed for proximate nutrients according to AOAC procedures at a commercial facility (Silliker Laboratories, Cedar Rapids, IA) and IgG (Etzet et al., 1997). Both products and required supplies were then shipped to a dairy in California.

Experimental Procedure

Research was conducted between November 16 and November 20, 2000. Calvings were monitored for a 24-h period and all calvings were observed. Each calf was removed from the dam within 10 min of birth and moved to the calf area. The calf was processed (weighed and identified with an ear tag, and its navel was dipped with tincture of iodine, fitted with an elastic castrator), rubbed with a burlap sacks to dry and stimulate respiration, and moved to an individual elevated metal stall fitted with a heat lamp. Calves were assigned randomly to receive one of the experimental products.

Experimental diets were mixed in a household blender. One dose (454 g) of CS or CR was added to 1 L of warm tap water, and the mixture was blended at medium speed until well mixed. Contents of the blender

Table 1. Chemical and physical composition of experimental diets.

Item, ¹ %	Colostrum supplement	Colostrum replacer
DM	94.30	97.40
CP	46.97	32.40
Fat	3.54	10.32
Ash	3.50	4.86
Carbohydrate ²	45.99	52.42
Ca	0.13	0.35
P	0.07	0.26
Mg	0.02	0.07
K	0.19	0.47
IgG	11.08	21.20

¹Analyses are on a DM basis, except DM.

²Calculated.

were then poured into an esophageal feeder. The blender was rinsed with warm tap water, which was then added to the esophageal feeder to bring the volume to 1.9 L. Reconstituted product was administered at approximately 1 h and, again, at 8 h of age. Esophageal feeders and the blender were carefully washed between uses.

At approximately 0.5 and at 24 h of age, a blood sample was collected from each calf by jugular venipuncture into evacuated tubes containing EDTA. Plasma was collected by centrifugation and total protein was determined with a handheld refractometer (Schuco Clinical Refractometer). The refractometer was corrected for temperature before each use. Remaining plasma was frozen (-20°C) before analysis of IgG by turbidimetric immunoassay (Etzet et al., 1997). Calves were housed in the calf barn for approximately 24 h, then were moved to a separate calf rearing facility at approximately 0600 h.

Experimental data were analyzed as completely randomized experimental design using the model $Y_{ij} = \mu + T_i + \epsilon_{ij}$, where: Y_{ij} = individual observation of dependent variable; μ = overall mean; T_i = effect of i th treatment ($i = 1..2$); ϵ_{ij} = error. Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS

The Ig concentrate product used in CR was 59% IgG and 85% CP. Chemical composition of experimental CS and CR (Table 1) were consistent with formulated values. The CS contained more CP but less fat, ash, carbohydrate, and IgG than CR. The CR was formulated to be a more nutritionally complete feed; therefore, amounts of fat and carbohydrate (52.4% of DM) were increased. The carbohydrate was composed of lactose, dextrose, and fructose in CR, whereas the CS contained only lactose and dextrose.

Table 2. Least squares means of calf BW, calving score, age at administration of experimental treatments, and concentration of IgG and CP in calves fed colostrum supplement (CS) or colostrum replacer (CR).

Item	Treatments			
	CS	CR	SE	P
No. of calves	16	16
BW at birth, kg	42.7	45.5	2.1	NS
Calving score ³	2.3	2.4	0.2	NS
Age at feeding, h				
1st feeding	0.99	0.98	0.01	NS
2nd feeding	8.06	8.03	0.06	NS
IgG intake, g	95	187
Protein intake, g	401	286
Plasma IgG, g/L				
0 h	0.58	0.32	0.27	NS
24 h	7.97	13.57	0.67	0.001
Change	7.39	13.26	0.64	0.001
AEA, ⁴ %	33	30	3	NS
Plasma protein, g/dL				
0 h	4.48	4.58	0.09	NS
24 h	4.99	4.98	0.11	NS
Change	0.51	0.39	0.09	NS

¹Treatments: CS = colostrum supplement; CR = colostrum replacer.

²Probability of a significant treatment effect; NS = $P > 0.10$.

³Calving score: 1 = no assistance to 3 = strong pull required.

⁴AEA = Apparent efficiency of IgG absorption, calculated as plasma IgG (g/L) \times Plasma volume (L) \div IgG intake (g).

Holstein bull calves ($n = 32$) were assigned randomly to treatment. One calf died before the 24-h sample collection due to respiratory failure and was replaced. Administration of CS and CR were uneventful, and all calves were administered treatments at 1.0 and 8.0 h of age. Because CS and CR were administered by esophageal feeder, palatability was not determined.

Mean BW of calves and calving score did not differ between treatments (Table 2) and were 44.2 kg and 2.3, respectively.

Intakes of IgG from CS and CR were 95 and 187 g, respectively. Plasma IgG values at 0 h (Table 2) were generally below the minimum detection limit of the assay, although a few calves had measurable concentrations of IgG. By 24 h, however, plasma IgG had increased to 8.0 and 13.6 g/L in calves fed CS and CR, respectively. Mean AEA (31%) was unaffected by treatment.

Mean plasma total protein at 0 and 24 h were unaffected by treatment and were 4.53 and 4.99 g/dl, respectively (Table 2). Intake of protein was markedly higher in calves fed CS (401 g) versus CR (286 g) due to differences in product formulation. Although calves fed CR had a mean plasma IgG concentration ≥ 10 g/L at 24 h of age, mean plasma total protein was < 5.0 g/dl, which has been used as an indication of failure of passive transfer of immunity (Donovan et al., 1998).

DISCUSSION

Absorption of IgG and protein from CS and CR were acceptable in this study. Mean plasma IgG increased significantly in both groups from birth to 24 h of age, and 15 of 16 calves fed CR achieved a plasma IgG concentration ≥ 10 g/L. Others (Abel Francisco and Quigley, 1993; Mee et al., 1996; Garry et al., 1996; Morin et al., 1997) reported that AEA of CS derived from lacteal secretions was poor. Apparently, the source of IgG, method of processing, and possibly, composition of the CS formula may influence IgG absorption from CS or CR. Calculated AEA of CS in this study was similar to other published studies using serum as a source of IgG (Quigley et al., 1998b; Arthington et al., 2000; Davenport et al., 2000; Quigley et al., 2001) and similar to that reported for maternal colostrum (Quigley and Drewry, 1998).

Increase in IgG concentration from birth to 24 h of age was greater than the increase in plasma protein concentration during the same period (Table 2). Nonimmunoglobulin proteins in plasma at 0 h of age may have moved out of the circulation during the first 24 h, possibly being used for gluconeogenesis and protein synthesis. A similar observation was reported by Davenport et al. (2000) in calves fed a CS. There was no treatment effect on plasma protein at 24 h of age, although calves fed CS consumed nearly twice the protein

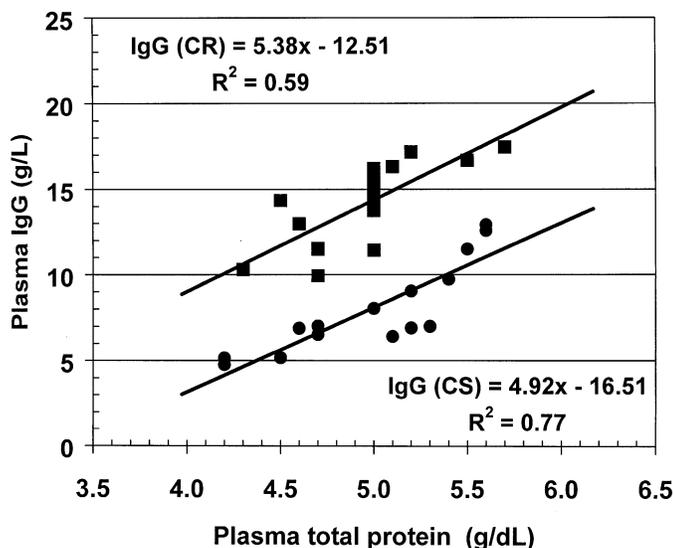


Figure 1. Regression of plasma IgG and plasma total protein at 24 h of age in calves fed colostrum replacer (CR; ■) or colostrum supplement (CS; ●).

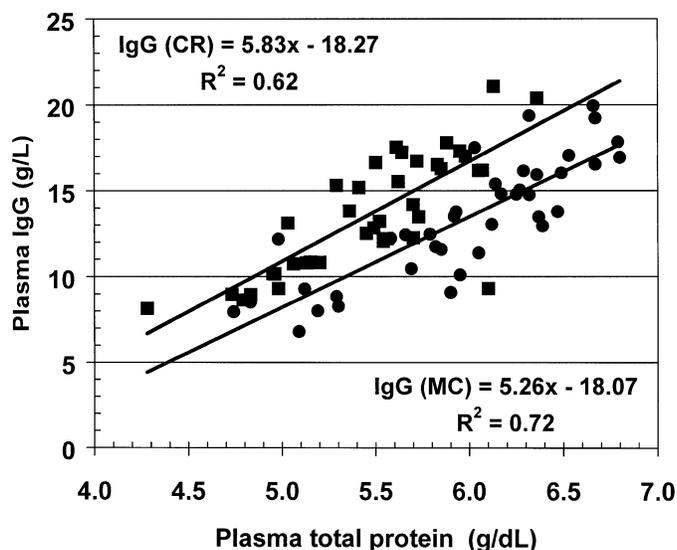


Figure 2. Regression of plasma IgG and plasma total protein at 24 h of age in plasma from calves fed colostrum replacer (CR; ■) or maternal colostrum (MC; ●). Adapted from Mowrey, 2001.

than calves fed CR. Protein in CS and CR were primarily derived from bovine serum (albumin and globulins), and a small amount of glycine was added to each formula. It is likely that non-Ig proteins in CS were metabolized during the first 24 h. There are no data available regarding metabolism of serum-derived proteins in neonates; however, Yvon et al. (1993) reported rapid digestion of α -lactalbumin in neonatal lambs fed MC. Conversely, the composition of CR contained significantly less non-Ig protein, most of which was residual albumin remaining following concentration of the IgG component. The mechanisms responsible for regulation of plasma protein concentration in calves fed CS and CR are not clear from this study.

The variable relationship between circulating plasma IgG and total protein in calves fed CS and CR (Figure 1) may have important implications in neonatal management. Within treatment, calves were administered the same mass of protein and IgG from CS or CR; however, absorption of protein and IgG varied among animals. Variation in absorption of IgG and protein was unrelated to BW ($P > 0.10$) and was probably related to variable AEA due to differences in metabolic state of the animals (Quigley and Drewry, 1998). Within treatment, absorption of IgG and protein were highly correlated, as indicated by the significant linear regression of IgG and protein (Figure 1). However, there was a considerable difference between treatments ($P < 0.01$), as indicated by difference between regression lines. Calves fed CR had nearly twice the plasma IgG concen-

tration at 24 h than calves fed CS at the same protein concentration (Figure 1).

Total plasma protein is often used as an estimate of circulating IgG concentration and as an indicator of susceptibility to neonatal disease (Tyler et al., 1996; Naylor and Kronfeld, 1977; Naylor et al., 1977; 1999; Wheeler et al., 2000). Wheeler et al. (2000) recently concluded that measurement of total protein by refractometer is suitable for herd monitoring and provides a reasonably accurate assessment of passive transfer status. The authors suggested that total protein concentration ≥ 5.2 g/dl is indicative of adequate passive transfer. When this cutoff value is used in our study, estimates of passive transfer are reasonably accurate in calves fed CS; however, these values seriously underestimate the adequacy of passive transfer in calves fed CR. The refractometer measures total plasma protein rather than IgG; therefore, protein serves as a marker for plasma IgG. The ratio of IgG to total protein in MC affects estimates of the adequacy of passive transfer. Differences in the ratio of IgG to total protein in CS and CR were primarily a function of differences in processing and ingredient selection and were 224 and 654 mg/g, respectively.

The relationship between IgG and protein in plasma of calves fed MC was not determined in this study. However, Mowrey (2001) measured the relationship between IgG and plasma protein in calves at 24 h of age when fed MC or a CR similar to that used in the current study. The relationship between plasma protein and IgG was highly significant in both groups (Figure 2),

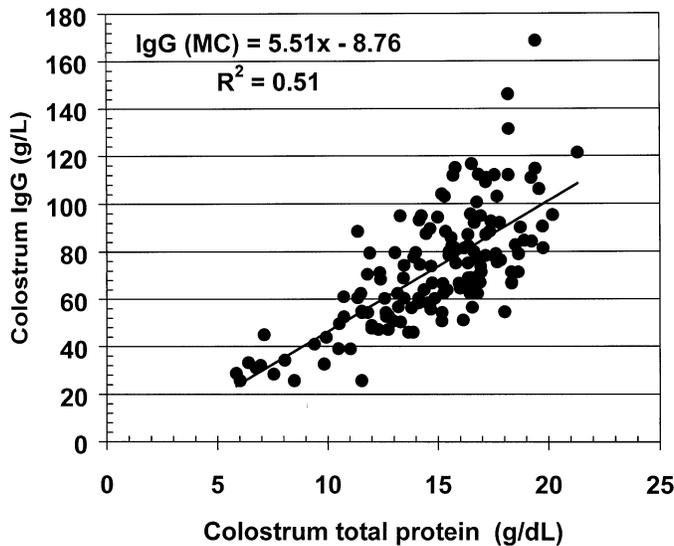


Figure 3. Regression of total protein and IgG in colostrum from 146 Jersey cows. From Quigley et al., 1994 and 1995.

but the plasma protein concentration indicative of successful passive transfer (10 g of IgG/L) varied considerably (Mowrey, 2001). Mean plasma protein concentrations indicative of successful passive transfer were 5.34 and 4.85 g/dl in calves fed MC and CR, respectively. These data suggest that if estimates of successful passive transfer—originally developed from experiments with calves fed MC—are applied to calves fed CS or CR, the proportion of calves determined to have failure of passive transfer will be artificially inflated.

The ratio of IgG to protein in MC varies. Total protein and IgG concentrations in colostrum from 146 Jersey cows used in previous studies (Quigley et al., 1994; 1995) is in Figure 3. Correlation between colostrum protein and IgG was highly significant ($r = 0.71$). Mean ratio of IgG to protein in colostrum ranged from 223 to 869 mg of IgG/g of protein with a mean of 488 (SD = 111). Maunsell et al. (1998) reported that colostrum from 106 multiparous Holstein cows with or without mastitis contained 78 to 80 g of IgG/L and 17.7 to 18.1 g of protein/dl. The ratio of mean IgG1 to mean protein in the four experimental groups ranged from 426 to 441 mg of IgG1/g of protein. Nardone et al. (1997) reported mean IgG of 79.3 and 64.0 g/L and protein of 16.6 and 13.9% in first-milking colostrum from Holstein heifers ($n = 6$) exposed to thermoneutral or high ambient temperatures, respectively. The ratio of IgG to protein in these two groups was 477 and 460 mg of IgG/g of protein, respectively. Based on the normal variability of protein and IgG in colostrum, it seems likely that the relationship between protein and IgG in plasma of calves would be variable, also. However, from the above

studies, the mean ratio of IgG to protein in MC appears to be in the range of 400 to 500 mg/g. Ratios of Ig to protein in CS and CR used in this study were outside this range.

Other methods of estimating adequacy of transfer of passive immunity that are indirect markers of colostrum consumption (e.g., gamma-glutamyl transferase, glutaraldehyde precipitation) may be affected by feeding CR or CS. Therefore, expanded use of CR and CS in the industry will require that estimates of failure of passive transfer be based on direct measurement of IgG rather than indirect measurement of total protein or other markers.

Provision of an adequate mass of IgG during the first 24 h of life is essential to ensure the survival and health of the calf. A common recommendation is for calves to receive a minimum of 100 g of IgG in the first 24 h. However, if AEA from MC, CS, or a CR varies from 20 to 35% (Quigley and Drewry, 1998), then 100 g of IgG intake will be inadequate for many calves. For example, if a 40-kg calf has a plasma volume of approximately 9% of BW (Quigley et al., 1998a), then the expected plasma IgG concentration of a calf consuming 100 g of IgG with an efficiency of 20% is $100 \times 20\% / 3.6 = 5.6$ g/L. Even with AEA of 35%, the predicted IgG concentration of a calf at 24 h of age is 9.7 g/L. A more adequate recommendation is that calves should consume 103 to 180 g of IgG in the first 24 h of life to reach a minimum of 10 g/L with AEA of 20 to 35%. Prediction of an animal's ability to absorb ingested IgG remains difficult; therefore, it seems prudent to be conservative in estimates of AEA. A recommendation of ingestion of 150 to 200 g of IgG in the first 24 h will reduce the risk of failure of passive transfer in most cases.

The terms "colostrum supplements" and "colostrum replacers" are poorly defined in the literature and in the industry. The term "colostrum supplement" should refer to those preparations intended to provide <100 g of IgG/dose and are not formulated to completely replace colostrum. Supplements should be formulated to be fed in conjunction with colostrum and to increase IgG concentration and provide nutrients that are inherently variable in MC (e.g., vitamin E). Additional research is needed to identify components critical to successful CS formulation.

In addition to an adequate mass of IgG (>100 g of IgG/dose), CR must provide nutrients required by the calf. Energy as carbohydrate and lipid is needed to allow the calf to thermoregulate and to establish homeostasis. Digestible protein sources are required as a source of AA for gluconeogenesis and protein synthesis, and vitamins and minerals are essential to successful CR formulation. Colostrum is a highly concentrated source of fat-soluble vitamins, as placental transfer of these vitamins

is limited. Additional research is also required to define requirements for hormones and growth factors that are found in high concentrations in MC. Inclusion of viable leukocytes normally found in MC is not possible presently.

CONCLUSION

The IgG in CS and CR used in this study were readily absorbed by newborn calves. Plasma IgG concentration at 24 h of age was indicative of successful transfer of passive immunity when calves were fed CR. The CR containing 94 g of IgG and administered by esophageal feeder at 1 and 8 h of age provided calves with an adequate level of passive immunity. Measurement of plasma total protein as an estimate of failure of passive transfer may be erroneous when calves are fed CS or CR.

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