

## Formulation of Colostrum Supplements, Colostrum Replacers and Acquisition of Passive Immunity in Neonatal Calves<sup>1</sup>

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### ABSTRACT

Provision of an adequate mass of IgG from maternal colostrum is essential to health and survival of neonatal calves. Colostrum supplements (CS) have been developed to provide supplemental immunoglobulin when maternal colostrum is of poor quality. However, colostrum replacers (CR) that provide  $\geq 100$  g of IgG have not been formulated. Our objective was to determine the absorption of IgG in newborn calves fed CS derived from bovine serum or CR derived from bovine immunoglobulin concentrate. The CS were prepared by collecting, processing, and spray drying bovine serum and blending with other ingredients to provide 45 to 50 g of IgG per dose. The CR were prepared by further processing bovine serum to increase IgG concentration to  $>50\%$  IgG and blending with other ingredients to provide 100 to 122 g of IgG per dose. Holstein calves ( $n = 160$ ) were fed 90 to 244 g of IgG from CS or CR in 1 or 2 feedings in two experiments. Blood was collected from each calf by jugular venipuncture at 0 and 24 h of age and plasma IgG was determined by turbidimetric immunoassay. Apparent efficiency of IgG absorption was calculated. Plasma IgG concentrations at 24 h of age were indicative of IgG intake and averaged 5.5 to 14.1 g/L in calves fed CS and CR. Mean apparent efficiency of IgG absorption in calves fed CS was 25 and 28% in experiments 1 and 2, respectively. Mean apparent efficiency of IgG absorption in calves fed CR ranged from 19 to 32% and were affected by method of processing and number of times fed. Treatment of plasma with polyethylene glycol reduced the efficiency of IgG absorption in experiment 1. The addition of animal fat to CR had no effect on IgG absorption. A second feeding of CR increased plasma IgG, but efficiency of absorption was reduced. Mean body weights at 60 d of

age were not affected by treatment and ranged from 64.3 to 78.2 kg. Plasma IgG concentration in calves fed  $\geq 122$  g of IgG from Ig concentrate approached (9.9 g/L) or exceeded 10 g/L, indicating successful transfer of passive immunity. Provision of IgG to prevent failure of passive transfer is possible with CR containing  $>20\%$  IgG when fed at 454 g per dose.

**(Key words:** calves, colostrum, immunoglobulin G, colostrum supplements)

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

### INTRODUCTION

Passive immunity is provided when maternal colostrum (MC) containing large amounts of IgG are fed to neonatal calves within the first few hours of birth. Unfortunately, several studies indicate that the acquisition of passive immunity is often inadequate in young calves in the United States (Besser and Gay, 1994; Donovan et al., 1998; Virtala et al., 1999). Failure of passive transfer may be attributed to colostrum containing inadequate mass of IgG, poor colostrum feeding methods, and poor efficiency of IgG absorption in calves (Abel Francisco and Quigley, 1993; Lee et al., 1983; Rea et al., 1996; Quigley and Drewry, 1998). Colostrum supplement (CS) products have been developed to provide exogenous IgG to calves when colostrum is of low IgG concentration. Many producers also use these products to replace colostrum when it is unavailable due to maternal agalactia, acute mastitis, or other causes.

Typically, Ig in CS are derived from lacteal secretions (milk or colostrum), bovine serum extracts, or chicken eggs. Absorption of IgG from CS derived from lacteal secretions have been reported to be poor (Abel Francisco and Quigley, 1993; Garry et al., 1996; Ikemori et al., 1997; Mee et al., 1996; Morin et al., 1997; Zaremba et al., 1993), although the reasons for poor IgG absorption have not been defined clearly. Conversely, absorption of CS derived from serum protein generally has been reported to be similar to that of MC (Arthington et al., 2000; Quigley et al., 1998). Most CS provide 25 to 45 g

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**Table 1.** Formulation of experimental colostrum supplements (CS) and colostrum replacers (CR).

Ingredient	Experiment 1			Experiment 2		
	CS-1	CR-1	CR-2	CS-2	CR-3	CR-4
	% of formula					
Bovine serum, dried	55.0	0.0	0.0	50.0	0.0	0.0
Lactose	26.5	25.9	43.1	37.5	37.5	32.5
Ig concentrate method 1 <sup>1</sup>	0.0	55.6	0.0	0.0	50.0	50.0
Ig concentrate method 2	0.0	0.0	38.4	0.0	0.0	0.0
Dry fat blend <sup>2</sup>	5.0	5.0	5.0	0.0	0.0	5.0
Other ingredients <sup>3</sup>	13.5	13.5	13.5	12.5	12.5	12.5

<sup>1</sup>Lipid was removed from plasma prior to manufacturing Ig concentrate in experiment 2.

<sup>2</sup>Dry fat blend containing 7% CP and 60% crude fat.

<sup>3</sup>Other ingredients included dextrose, glycine, salt, emulsifier, lecithin, vitamin/mineral premix, potassium chloride, magnesium sulfate, and flavor.

of IgG/dose (typically 454 g), which is reconstituted in 2 L of water. Therefore, feeding one or two doses of CS will not provide a sufficient mass of IgG to prevent failure of passive transfer in young calves. Most veterinarians recommend consumption of at least 100 g of IgG within the first 12 h of birth. Colostrum replacer (CR) products should provide a minimum of 100 g of IgG in a form that is readily absorbable by the calf. Our objective was to compare the plasma IgG concentration and efficiency of IgG absorption in calves fed CS derived from bovine serum or with bovine IgG concentrates formulated into CR.

## MATERIALS AND METHODS

### Preparation of Experimental Ingredients and Feeds

Bovine blood was collected from abattoirs under USDA inspection. Blood was collected into stainless steel containers, 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. Bovine serum powder was prepared by removing fibrin by addition of excess Ca, and resulting serum was concentrated by filtration and spray-dried to produce a light tan powder containing approximately 20% IgG and 75% CP. Bovine serum powder was mixed with other ingredients (Table 1) to produce test products containing 11 (CS-1) or 10% (CS-2) IgG.

Many methods have been published for fractionating blood and isolating the IgG fraction. In experiment 1, we modified two existing methods (IG-1 and IG-2) to produce a bovine IgG concentrate from bovine plasma by removing bovine serum albumin. In experiment 2, we utilized method IG-1, except that lipid was removed from the plasma before fractionation. The resulting Ig fractions were separated by centrifugation, concen-

trated by ultrafiltration, and spray dried. The resulting Ig concentrate powder contained >90% CP and >50% IgG. The Ig concentrate powders were mixed with other ingredients (Table 1) to produce experimental products (CR-1, CR-2) that provided 100 g of IgG per 454-g dose. In experiment 2, the CR were prepared without (CR-3) or with (CR-4) 5% animal fat replacing lactose and provided 122 g of IgG per dose (Table 1). All products were weighed into 454-g doses and stored at room temperature until fed. Individual doses were mixed with 1.9 L of warm (approximately 40°C) water and mixed with a wire whip or blender.

### Experimental Procedure

Experiments 1 and 2 utilized 60 and 100 Holstein calves, respectively, at a large dairy farm in California; due to the large size of the dairy, all calvings were observed. Experiment 1 was conducted from April 10 to 12, 1999, and experiment 2 was conducted from July 9 to 16, 1999. Calves were enrolled in the study if they had no obvious congenital defects and were clinically healthy. Each calf was removed from the dam within 10 min of birth and moved to the calf housing area. The calf was processed (weighed, identified with an ear tag, its navel was dipped with tincture of iodine, bulls were fitted with an elastic castrator, and extra teats were removed from heifers), stimulated with burlap sacks and moved to an individual elevated metal stall fitted with a heat lamp. Calves were not weighed in experiment 2 due to a malfunction of the scale during the experiment. Calves in experiment 1 were assigned randomly to receive one of four treatments: 3.9 L of MC as soon as possible after birth and an additional 1.9 to 3.9 L at approximately 8 h of age; 454 g of CS-1 as soon as possible after birth and at 8 h; 454 g of CR-1 as soon as possible after birth; or 454 g of CR-2 as soon as possible after birth. Calves fed CR-1 and CR-2 did not

receive a second feeding. All calves in experiment 1 were fed by nipple bottle. Any liquid refused was offered in another feeding approximately 20 min after the first. If the liquid was refused a second time, it was administered by esophageal feeder. In experiment 2, calves were assigned randomly to receive 454 g of CS-2 as soon as possible after birth and approximately 8 h of age; CR-3 as soon as possible after birth (no second feeding), or as soon as possible after birth and approximately 8 h of age (CR-3 2×), CR-4 as soon as possible after birth (no second feeding), or as soon as possible after birth and approximately 8 h of age (CR-4 2×). All liquids were administered by esophageal feeder in experiment 2.

Pooled MC was used in experiment 1 as a positive control treatment. Colostrum was collected from the dam or other cows, pooled, and stored in 20-L plastic containers before use. Colostrum was collected at the dairy, tested with a colostrometer, and only colostrum >50 g of IgG/L was used. Samples of colostrum (25 ml) were collected and frozen (−20°C) before measurement of IgG by radial immunodiffusion (Triple J Farms, Redmond, WA) after dilution with saline (1:10).

At approximately 0.5 and 24 h of age, a blood sample was collected from each calf by jugular venipuncture. Plasma was collected by centrifugation and frozen (−20°C) before analysis of IgG by turbidimetric immunoassay (Etzel et al., 1997). Calves were housed and fed in the calf barn for approximately 24 h, then were moved to a separate calf rearing facility at approximately 0600 h. Calves remained in individual calf hutches until 60 d of age. The number of treatments with antibiotics was determined daily to d 60 and BW on d 60 was estimated with a heart girth tape.

### Statistical Analyses

Body weight at birth and 60 d, plasma IgG at 0 h, 24 h, change in IgG, calculated apparent efficiency of absorption (AEA; Quigley and Drewry, 1998) were analyzed as completely randomized experimental design using Proc GLM of SAS (SAS, 1989). Body weight at birth was evaluated as a covariable in experiment 1, but did not contribute significantly to any model; therefore, unadjusted means are presented. Significance was declared at  $P < 0.05$  unless otherwise noted, and trends toward statistical significance were considered at  $P > 0.05$  to  $P < 0.10$ .

## RESULTS

### Experiment 1

Due to concerns related to acquisition of calves and timing of the study, heifer calves were fed MC and bull

calves were fed CS or CR. Mean birth BW for all calves did not differ significantly by treatment and was 37.1 kg.

Administration of MC, CS and CR were uneventful, and no negative effects were observed during the experimental period. The proportion of calves that required administration of CS and CR by esophageal feeder was unrelated to treatment and was approximately 10%. Intake of MC was variable among calves, as intake of the second colostrum feeding was not reliably measured. Also, refusals of MC were typically not administered by esophageal feeder. Therefore, intake of MC is only an estimate, based on amount of MC offered and estimated MC refusal. Five pools of MC were used, and IgG concentration ranged from 76.8 to 133.8 g/L. Age at first feeding was not significantly affected by treatment, although calves fed CS-1 tended ( $P < 0.10$ ) to be fed later than other calves. This difference was due to attempts to nipple feed as many calves as possible, and slight delays in consumption of CS-1 due to palatability differences.

Mean plasma IgG at 0 h was not measurable in 48 calves but was greater than 0 g/L in 12 calves. Mean plasma IgG concentrations at 24 h were significantly higher in calves fed MC compared with calves fed CR and CS-1 (Table 2); however, the greater intake of IgG from MC was responsible for this difference. Mean plasma IgG concentrations at 24 h tended ( $P < 0.10$ ) to be greater in calves fed CS-1 versus CR and were 8.35 and 6.62 g/L, respectively. Also, plasma IgG tended ( $P < 0.10$ ) to be higher in calves fed CR-1 versus CR-2. Calculated AEA was lowest in calves fed MC. Also, AEA was higher in calves fed CS-1 and CR-1 versus those fed CR-2 (Table 2). Body weight at 60 d and BW gain were unaffected by treatment, although calves fed MC tended ( $P < 0.10$ ) to have greater BW gain than calves fed CS-1. The number of veterinary treatments required for each calf from d 0 to 60 was unaffected by treatment (Table 2) and was <1, indicating a high overall level of management on the farm. There was no mortality of calves on the experiment; however, several bull calves were sold before 60 d.

### Experiment 2

Peak daytime temperatures during the first 5 d of experiment 2 exceeded 38°C; consequently three calves died within the first 24 h due to complications with delivery attributed to heat stress. Plasma IgG concentrations at 0 h were typically not measurable; however, several readings were 1 to 3 g/L. Plasma from calves with IgG > 0 g/L at 0 h were evaluated for antibody titers against bovine viral diarrhea at the Iowa State

**Table 2.** Least squares means of IgG intake, plasma IgG, BW, and number of veterinary treatments in calves fed maternal colostrum, experimental colostrum supplement (CS), or replacers (CR) experiment 1.

Item	Treatment <sup>1</sup>				SE	Contrasts <sup>2</sup>		
	MC	CS-1	CR-1	CR-2		1	2	3
No. calves at d 0	15	14	14	14	...	...	...	...
No. calves at d 60 <sup>3</sup>	12	13	14	14	...	...	...	...
Birth BW, kg	35.9	36.2	38.9	38.0	1.9	NS <sup>4</sup>	NS	NS
BW at 60 d, kg	78.2	72.1	77.7	75.4	3.0	NS	NS	NS
BW gain, 0–60 d	0.71	0.59	0.65	0.62	0.04	0.10	NS	NS
Age at feeding, h								
First feeding	0.5	1.3	0.4	0.4	0.4	NS	0.10	NS
Second feeding	...	10.7	...	...	1.2	...	...	...
IgG intake, g	429	100	100	100	13.8	0.01	NS	NS
Plasma IgG, g/L								
0 h	0.98	0.75	0.20	0.11	0.29	0.06	0.10	NS
24 h	18.81	8.35	7.64	5.59	0.83	0.01	0.09	0.09
Change, 0–24 h	17.82	7.60	7.44	5.48	0.93	0.01	NS	NS
AEA, % <sup>5</sup>	14	25	26	19	2	0.01	NS	0.01
Treatments, n	0.5	0.7	0.9	0.7	0.3	NS	NS	NS

<sup>1</sup>Treatments: MC = 4 to 6 L of maternal colostrum; CS-1 = 50 g of IgG from CS fed twice; CR-1 = 100 g of IgG from CR fed once; CR-2 = 100 g of IgG from CR fed once.

<sup>2</sup>Contrast: 1 = MC vs. all CS and CR; 2 = CS-1 vs. (CR-1 + CR-2) ÷ 2; 3 = CR-1, vs. CR-2.

<sup>3</sup>Calves not on the farm at 60 d were sold.

<sup>4</sup> $P > 0.10$ .

<sup>5</sup>Apparent efficiency of IgG absorption, calculated as plasma IgG at 24 h × BW at birth, kg × 0.09 (plasma volume as percent of BW).

University Veterinary Diagnostic Laboratory, and one sample was positive.

Concentrations of plasma IgG at 24 h were affected by mass of IgG fed but not by the addition of fat to CR-4 (Table 3). Increasing mass of Ig from 90 to 122 g increased plasma IgG at 24 h by 44% ( $P < 0.0001$ ). A second feeding of CS increased plasma IgG by an additional 27.5% ( $P < 0.0001$ ). Calculated AEA was based on an estimate of birth BW (37.7 kg); estimates of AEA were reduced when calves were fed 244 g of IgG in two feedings compared with intake of 90 or 122 g of IgG in one or two feedings (Table 3).

Body weights at 60 d and number of veterinary treatments per calf were unaffected by method of supplementation and averaged 66.6 kg and 1.0 treatment, respectively (Table 3).

## DISCUSSION

Results of these studies indicate that IgG derived from bovine serum or Ig concentrates are well absorbed in neonatal calves. Several authors (Abel Francisco and Quigley, 1993; Garry et al., 1996; Ikemori et al., 1997; Mee et al., 1996; Morin et al., 1997; Zaremba et al., 1993) have reported poor AEA in calves fed CS derived from lacteal secretions (milk and colostrum), whereas others have reported satisfactory AEA in calves fed CS derived from bovine serum (Arthington et al., 2000;

Davenport et al., 2000; Quigley et al., 1998). Source of IgG and method of processing may influence IgG absorption or half-life. Plasma IgG in calves fed CS-1 and CS-2 was lower than the amount suggested as required to prevent or treat failure of passive transfer (Besser and Gay, 1994; Wells et al., 1996), but AEA was markedly higher than other reports of circulating IgG concentrations in CS derived from lacteal secretions. A factor affecting plasma IgG concentration and AEA is mass of IgG fed. Most CS products provide 25 to 45 g of IgG, which may be difficult to measure in the circulation using conventional methods of analysis. Many veterinarians recommend that calves consume a minimum of 100 g of IgG in the first 24 h after birth. Clearly, providing IgG from CS alone would be insufficient to provide satisfactory transfer of passive immunity.

Fractionation of bovine plasma to manufacture CR influenced absorption of IgG. Method IG-2 utilized polyethylene glycol, which produced an Ig concentrate containing 57% IgG, resulted in 26% lower plasma IgG concentration at 24 h than method IG-1. Polyethylene glycol is used widely as a method for purifying IgG from plasma, lacteal secretions, and eggs (Akita and Nakai, 1993; Svendsen et al., 1995; Uemura et al., 1989). However, our data suggest that polyethylene glycol reduced plasma IgG concentration or may have altered absorption kinetics, equilibration of IgG with nonvascular IgG

pools or metabolism of IgG by the body within the first 24 h.

Feeding additional CR in a second feeding in experiment 2 increased plasma IgG concentrations above those in calves fed one feeding, but the incremental increase due to the second feeding was low. Reduced plasma IgG and AEA may have been due to the presence of protein and IgG in the intestine from the first feeding, cessation of macromolecular transport, or other factors. However, dairy producers attempting to maximize IgG absorption by utilizing a CR may further increase IgG concentration in calves with a second feeding of CR.

The addition of ingredients such as animal fat used in formulation of calf milk replacers had no effect on IgG absorption as indicated by plasma IgG concentration and estimates of AEA in experiment 2. This has important implications to the formulation of products intended to replace colostrum. Colostrum provides significant calories in addition to its role as a source of IgG (Vermorel et al., 1983), therefore, products intended as replacements of colostrum must contain significant fat and carbohydrates. Although the formulations used in this study contained only approximately 10% fat, the fat did not impair IgG absorption. In addition to calories, colostrum is an important source of protein (Yvon et al., 1993), vitamins and minerals (Foley and Otterby, 1978), growth factors and hormones (Hammond et al., 2000), and viable leukocytes (Riedel-Caspari and Schmidt, 1991). Requirements for many of these compounds have not been well defined. Clearly, further

research is required to optimize formulation of CR to maximize neonatal survival and health.

Calculated AEA in experiment 1 was greater in calves fed CS compared with MC; however, calves fed MC consumed >6 L of colostrum in the first 24 h, possibly maximizing binding sites in the intestine and reducing AEA. Davenport et al. (2000) reported that protein intake >500 g may impair IgG absorption. Maternal colostrum used on the farm was higher in IgG concentration than other recent reports of colostrum on dairy farms in the United States (Levieux and Ollier, 1999; Quigley et al., 1995; Tyler et al., 1999). The farm used a colostrometer as part of the normal management, and exclusion of poor colostrum from the pools effectively resulted in pools of very high quality.

The reason for measurable IgG in calves at birth in both experiments is not clear. Several samples were measured by radial immunodiffusion to confirm results of turbidimetric immunoassay; all samples produced measurable rings. Calves in the study were removed from the dam within 10 min of birth, and no calf had the opportunity to stand before removal from the dam. Every birth except two were from cows in maternity stalls; the other two were in the dry lot. One plasma sample from precolostrum calves was positive for bovine diarrhea virus; therefore, it is possible that seroconversion occurred in utero, resulting in measurable IgG titers.

Calves fed MC tended to have greater BW gain from 0 to 60 d than calves fed all CS (Table 2). It is not clear,

**Table 3.** Least squares means of IgG intake, plasma IgG, and number of veterinary treatments in calves fed experimental colostrum supplements (CS) or replacers (CR), experiment 2.

Item	Treatments <sup>1</sup>					SE	Contrasts <sup>2</sup>		
	CS-2	CR-3	CR-3 2×	CR-4	CR-4 2×		1	2	3
Calves at d 0	20	20	20	20	20	...	...	...	...
Calves at d 60	19	18	19	19	20	...	...	...	...
BW at birth, kg	...	...	...	...	...	...	...	...	...
BW at 60 d, kg	69.7	65.8	64.3	66.3	67.1	2.2	NS <sup>3</sup>	NS	NS
Age at feeding, h									
First feeding	0.5	0.5	0.6	0.5	0.5	0.1	NS	NS	NS
Second feeding	9.1	...	8.7	...	8.6	0.4	NS	...	...
IgG intake, g	90	122	244	122	244	...	...	...	...
Plasma IgG, g/L									
0 h	0.21	0.20	0.67	0.16	0.00	0.14	NS	NS	0.01
24 h	7.36	11.18	13.03	10.08	14.08	0.63	0.001	0.001	NS
Change	7.15	11.06	12.42	9.92	14.08	0.63	0.001	0.001	NS
AEA, % <sup>4</sup>	28	32	19	29	20	1	0.02	0.0001	NS
Treatments, n	0.9	0.8	1.1	1.3	1.7	0.3	NS	NS	NS

<sup>1</sup>Treatments: CS-2 = 90 g of IgG from CS fed in 2 feedings; CR-3 = CS containing 27% IgG fed once; CR-3 2× = CR-3 fed twice; CR-4 = CS containing 27% IgG with added fat, fed once; CR-4 2× = CR-4 fed twice.

<sup>2</sup>Contrasts: 1 = CS-2 vs. all CR; 2 = (CR-3 + CR-4) vs. (CR-3 2× vs. CR-4 2×); 3 = effect of added fat.

<sup>3</sup>P > 0.10.

<sup>4</sup>Apparent efficiency of IgG absorption, calculated as plasma IgG at 24 h × BW at birth, kg (37.7 kg) × 0.09 (plasma volume as percent of BW).

however, if the trend in BW gain was associated with absorption of IgG or intake of nutrients in the first 24 h of life. In addition to IgG, colostrum also contains significant amounts of fat, protein, and growth factors. Calves fed colostrum had improved metabolic profile and endocrine status, intestinal absorptive capability, and growth (Kühne et al., 2000). However, the confounding of heifers (fed MC) and bulls (fed all CS) makes interpretation of this observation difficult.

The numbers of veterinary treatments for calves in both experiments in the study were low (0.5 to 1.1 treatments/calf), indicating a high overall level of management on the farm. Additionally, the number of calves with plasma IgG < 10 g/L still indicated a lack of relationship between morbidity, mortality, and plasma IgG concentration.

Colostrum supplement products that contain IgG are regulated in the United States by the USDA Center for Veterinary Biologics. Regulatory approval of products for prevention or treatment of failure of passive transfer requires that products produce an increase in circulating IgG concentration in at least 20 neonatal animals fed a dose of the product above a minimum standard for the specific species (9 CFR 113.499). To date, no approved CS has been shown to increase IgG concentration above the species standard. In Experiment 2, mean plasma IgG exceeded the bovine species standard (10 g/L) in calves fed 122 or 244 g of IgG. Our data suggest that mass of IgG and method of processing are critical to ensure adequate transfer of passive immunity. It is important to recognize that products providing less than 100 g of IgG/dose should not be used to replace colostrum; rather two discrete classes of products (CS and CR) with unique functions and formulation should be recognized.

## CONCLUSION

The composition of CS and CR and methods of IgG processing may affect absorption of IgG from products derived from bovine serum or plasma. Preparation of an IgG concentrate with polyethylene glycol to fractionate the IgG from bovine plasma reduced the concentration of IgG in plasma of calves at 24 h of age; further research is needed to determine the nature of this reduced concentration. Feeding increased doses of IgG from further processed bovine serum can increase circulating IgG in calves.

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