

# Immunoglobulin Concentrations in Serum in Response to Injectable Immunoglobulin in Neonatal Dairy Calves

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## ABSTRACT

Thirty Holstein bull calves were used to investigate the use of an injectable solution of purified Ig to increase Ig in serum of neonatal calves. The Ig were from abattoir blood purified by column chromatography. Calves were blocked by date of birth and assigned randomly to treatment within block. Treatments were s.c. injections of Ig solution (30 or 60 mg of Ig/ml) or .9% NaCl with or without colostrum (41.1 g of IgG/L) feeding. Calves were injected by 24 h of age. The mass of Ig injected was 1.05 g/kg of BW. Calves received 2 L of pooled colostrum or commercial milk replacer at 0, 12, 24, and 36 h. Blood was sampled at 0 and 48 h postinjection and at 28 d of age, and serum was analyzed for IgG and IgM by radial immunodiffusion. Mean IgG and IgM in serum of calves injected with Ig were 4.2 and .7 g/L, respectively, and were higher than in calves receiving no Ig. Mean IgG (14.6 g/L) and IgM (1.0 g/L) concentrations in serum of calves fed colostrum were higher than in other calves. Subcutaneous Ig provided moderate amounts of Ig in serum.

**(Key words):** calves, immunoglobulins, colostrum)

**Abbreviation key:** CF = s.c. injection of .9% NaCl without (-) or with (+) colostrum feeding, L30 = s.c. injection of Ig (30 g/L) in lactated Ringer's injection, S30 = s.c. injection of Ig (30 g/L) in .9% NaCl, S60 = s.c. injection of Ig (60 g/L) in .9% NaCl.

Received June 29, 1994.

Accepted March 3, 1995.

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## INTRODUCTION

Morbidity and mortality of the neonatal calf are of significant economic concern to beef and dairy producers. A recent national dairy heifer survey (5) reported that preweaning mortality of heifer calves born alive or moved onto an operation was 8.4% (SE = .4). Economic losses to producers include the cost of the calf and investments in feed, housing, health care, and labor.

Calves are born hypogammaglobulinemic; therefore, consumption of colostrum is required to provide passive immunity until calf immunity is established (4). For approximately 24 h after parturition, intestinal epithelial cells absorb macromolecules, such as Ig (9, 16, 17), which are transported through the cell to the lymphatic system and then to the general circulation (4, 16). Thereafter, absorption of macromolecules ceases, and chances of acquiring passive immunity are terminated. If calves do not ingest a sufficient mass of Ig prior to cessation of macromolecular transport, increased morbidity and mortality may occur (7, 13). Klaus et al. (10) reported that 30% of calves consuming colostrum may remain hypogammaglobulinemic. More recently, a USDA survey (5) reported that 41% of 2177 calves sampled between 24 and 48 h of age failed to attain serum IgG concentration of 10 g/L. Furthermore, 53.6% of mortality risk among calves with IgG concentration in serum <10 g/L was associated with inadequate Ig transfer and colostrum intake. The degree of acquisition of passive immunity is dependent on several factors, including age at first colostrum feeding and mass of Ig consumed (11, 17). Therefore, techniques are needed to provide Ig to hypogammaglobulinemic calves after cessation of macromolecular transport by intestinal epithelium. Techniques such as serum transfusions are possible but difficult, time-consuming, and costly. Purified Ig preparations (3,

6) have the potential to provide Ig via injection with lower cost. Our objectives were to evaluate the effect of an injectable solution of purified Ig on serum Ig concentrations, growth, and intake in hypogammaglobulinemic calves.

## MATERIALS AND METHODS

### Experimental Design

Holstein bull calves ( $n = 30$ ) were blocked by date of birth and assigned randomly within block to one of five treatments. Treatments were s.c. injection of .9% NaCl with (+) or without (-) colostrum feeding (CF), 30 mg of Ig/ml in lactated Ringer's injection (L30), 30 mg of Ig/ml in .9% NaCl (S30), or 60 mg of Ig/ml in .9% NaCl (S60). Calves on CF-, L30, S30, and S60 treatments were fed 4 L of milk replacer by 24 h of age, and calves on CF+ treatment were fed 4 L of pooled colostrum by 24 h of age. Calves were injected by 24 h of age. The volume of solution administered was calculated to provide serum Ig concentration of 7.5 g/L. Serum volume was assumed to be 7% of BW (14). The volume of solution administered was calculated as the mass of Ig to inject divided by Ig concentration in the solution.

The Ig solution (American Protein Corp., Ames, IA) was from bovine abattoir blood, separated and purified by ion-exchange chromatography (6) and suspended in .9% NaCl. The solution was obtained in three lots and averaged 6.2% total protein (SE = .05) and 6.1% IgG (SE = .04). The Ig solution was diluted in .9% NaCl or lactated Ringer's injection and delivered by s.c. injection. Large amounts of solution (>.5 L) were delivered by multiple injections. Calves on CF+ and CF- treatments were injected with .9% NaCl using volumes for treatments of 30 mg/ml. The s.c. route of administration was chosen because it would be more readily incorporated into the management of calf growers. Many calf raisers routinely provide electrolytes s.c. to neonatal calves purchased from sale barns and transported long distances. The NaCl and lactated Ringer's injection were used to compare the effect of diluent on absorption of fluid and Ig by the calf.

As soon as possible after birth and prior to nursing the dam, calves were moved to the

experimental facility and placed in an individual pen (2 m<sup>2</sup>) bedded with shavings. Calves on L30, CF-, S30, and S60 treatments were fed 2 L of milk replacer (Maxi-Lac®; Tennessee Farmers Cooperative, LaVergne, TN) upon arrival and at 12-h intervals until 48 h of age. Calves on CF+ treatment were fed 2 L of pooled colostrum (mean IgG = 41.1 g/L; SE = 8.2) upon arrival and at 12-h intervals until 48 h of age. Mean age at first feeding was 1.5 h (SE = .2). Thereafter, all calves received milk replacer reconstituted to 14% DM at 10% of BW in two equal feedings at approximately 0800 and 1600 h. Amount of milk replacer offered was adjusted weekly. Commercial calf starter (Co-op Starter/Grower Course-Dec; Tennessee Farmers Cooperative) was offered for ad libitum consumption once daily from 4 d of age. Amounts of milk replacer and starter consumed were measured at each feeding. Water was available at all times.

### Sampling and Analysis

Calves were weighed upon arrival at the experimental facility and every 7 d until 28 d. Samples of milk replacer and calf starter were collected weekly, composited by month, and analyzed for DM, CP, ash, ether extract (1), NDF (8), Ca, P, Mg, and K by atomic absorption spectrophotometry (Table 1). Fecal consistency was scored at the a.m. feeding by the

TABLE 1. Chemical composition of milk replacer and calf starter.

Item	Milk replacer <sup>1</sup>		Calf starter <sup>2</sup>	
	$\bar{X}$	SE	$\bar{X}$	SE
DM, %	95.3	.2	89.1	.5
	( % of DM )			
CP	20.0	.5	17.8	.1
Ash	9.6	.1	7.6	.2
NDF	ND <sup>3</sup>	...	22.6	.6
Ether extract	ND	...	3.8	.2
Ca	.56	.02	1.23	.05
P	.71	.01	.73	.02
K	2.30	.02	1.29	.03
Mg	.17	.01	.36	.01

<sup>1</sup> $n = 4$ .

<sup>2</sup> $n = 3$ .

<sup>3</sup>Not determined.

method of Larson et al. (12). Rectal temperatures and respiration rates were measured once daily at the a.m. feeding.

Jugular blood (approximately 10 ml) was collected into evacuated containers without anticoagulant at 0 and 48 h postinjection and 28 d of age. Serum was separated by centrifugation (3000 × g) and stored (-20°C) prior to duplicate analysis of IgG and IgM by radial immunodiffusion (VMRD Inc., Pullman, WA). Samples of pooled colostrum (100 ml) were collected and stored (-20°C) until duplicate analysis of IgG and IgM by radial immunodiffusion (VMRD Inc.).

**Statistical Analysis**

The Ig concentrations in serum at 48 h postinjection and at 28 d were analyzed as a randomized complete block experimental design by analysis of covariance, IgG, and IgM at 0 h as covariables. Weekly intake of starter and milk replacer, BW, feed efficiency, mean rectal temperatures, fecal scores, and respira-

tion rates were analyzed by repeated measures ANOVA (15) using a randomized complete block design. Significance was *P* < .05 unless otherwise indicated.

**RESULTS AND DISCUSSION**

**Morbidity and Mortality**

Two calves died during the study. In both incidences, death occurred <1 wk of age. Necropsy determined that death was caused by septicemia. Calves were from treatments L30 and CF- and were not replaced. Therefore, least squares means are presented.

Respiration rates tended to be affected by an interaction of age by block (*P* < .10), which may have been due to changes in ambient temperature during the study. Mean respiration rate was 31 (SE = 1). Rectal temperatures tended to be affected by age of calf (*P* < .10) but were unaffected by treatment. Mean rectal temperature was 38.6°C.

Calves injected with Ig tended to experience less severe scouring than calves on CF- treat-

TABLE 2. Least squares means of DMI, BW gain, and feed efficiency.

Item	Treatment <sup>1</sup>					SE	Contrast <sup>2</sup>			
	CF-	L30	S30	S60	CF+		1	2	3	4
BW at birth, kg	42.3	42.8	44.0	43.0	39.2	1.8	NS <sup>3</sup>	†	NS	NS
BW at 28 d, kg	51.4	50.8	51.9	53.1	48.2	2.0 <sup>4</sup>	NS	NS	NS	NS
BW Gain, g/d	247	299	283	360	324	60	NS	NS	NS	NS
DMI, g/d										
Total	588	602	578	617	571	32	NS	NS	NS	NS
Starter	117	145	104	155	142	27	NS	NS	NS	NS
Replacer	471	457	474	462	429	15	NS	*	NS	NS
CP Intake, g/d										
Total	115	117	114	121	111	6	NS	NS	NS	NS
Starter	21	26	19	28	25	5	NS	NS	NS	NS
Replacer	94	91	95	93	86	3	NS	*	NS	NS
BW Gain:DMI, g/kg	406	502	496	584	567	87	NS	NS	NS	NS
BW Gain:CP intake, g/kg	2077	2571	2524	2999	2911	452	NS	NS	NS	NS

<sup>1</sup>Treatments were s.c. injection of .9% NaCl with (+) or without (-) colostrum feeding (CF), 30 g of Ig/L in lactated Ringer's injection (L30), 30 g of Ig/L in .9% NaCl (S30), or 60 g of Ig/L in .9% NaCl (S60).

<sup>2</sup>Contrasts: 1 = Ig injections versus CF-, 2 = Ig injections versus CF+, 3 = 30 g of Ig/L versus 60 g of Ig/L, and 4 = saline versus lactated Ringer's injection at 30 g of Ig/L.

<sup>3</sup> *P* > .10.

<sup>4</sup>SE for L30 and CF- (n = 5): BW at 28 d = 2.2, BW gain = 63, total DMI = 36, starter DMI = 30, replacer DMI = 17, total CP intake = 7, starter CP intake = 5, replacer CP intake = 3, BW gain:DMI = 98, BW gain:CP intake = 506.

†*P* < .10.

\**P* < .05.

ment ( $P < .10$ ), but scours scores did not differ from calves on CF+ treatment. Mean scours scores were 1.3 and 1.6 for calves injected with Ig and CF- treatments, respectively. Mean days scouring (fecal score  $\geq 3$ ), 3.7 d (SE = .9), did not differ by treatment.

Absorption of fluid and Ig from the injection site normally required approximately 10 h from the beginning of the first injection until all solution was absorbed (no visible swelling in the injection area). No obvious difference existed between diluents or concentration of Ig solution on rate of absorption. Occasionally, the injected solution appeared to migrate ventrally along the side of the calf, possibly as a result of the density of the solution. Injections made near the shoulder tended to allow accumulation of material near the joint, producing mild discomfort to the calf when walking. This effect was transient and usually lasted <2 d.

#### BW, Intake, and Feed Efficiency

No effects of treatment were observed on BW at 28 d, average daily BW gain, intake of calf starter, DM, CP, or feed efficiency (Table 2). However, calves on CF+ treatment tended

to have lower initial BW ( $P < .10$ ) than other calves, which reduced consumption of DM and CP from milk replacer. Changes in intake and BW with increasing age were unaffected by treatment and were indicative of normal intake and growth for calves during mo 1 of life. Calves deprived of colostrum during the first 24 h postpartum often experience increased morbidity and mortality (5, 7, 14, 17) and reduced BW gain and intake (13). These data suggest that depressions in BW gain and intake in calves deprived of colostrum may be a function of disease exposure rather than a function of one or more components of colostrum. Our calves were isolated from other calves, and strict hygiene practices were followed. Intake of feed and BW gain were similar among calves fed colostrum, calves injected with Ig solution, and those remaining hypogammaglobulinemic.

#### Serum Ig

Serum IgG and IgM concentrations at 0 h were consistently below the minimum limits of the radial immunodiffusion assay and indicated that calves had not suckled the dam prior to being transported to the experimental facility.

TABLE 3. Least squares means of serum IgG and IgM concentrations in hypogammaglobulinemic calves given s.c. injections of Ig.

Item <sup>3</sup>	Treatment <sup>1</sup>						Contrast <sup>2</sup>			
	CF-	L30	S30	S60	CF+	SE	1	2	3	4
Serum IgG, g/L										
48 h <sup>4</sup>	1.1	4.2	4.4	4.0	14.6	.9	**	**	NS <sup>5</sup>	NS
28 d	5.3	6.0	5.7	5.3	12.4	.9 <sup>6</sup>	NS	**	NS	NS
Serum IgM, g/L										
48 h	.4	.7	.8	.6	1.0	.1	†	†	NS	NS
28 d	.8	.6	.7	.6	.5	.1	*	†	NS	NS

<sup>1</sup>Treatments were s.c. injection of .9% NaCl with (+) or without (-) colostrum feeding (CF), 30 g of Ig/L in lactated Ringer's injection (L30), 30 g of Ig/L in .9% NaCl (S30), or 60 g of Ig/L in .9% NaCl (S60).

<sup>2</sup>Contrasts: 1 = Ig injections versus CF-, 2 = Ig injections versus CF+, 3 = 30 g of Ig/L versus 60 g of Ig/L, and 4 = saline versus lactated Ringer's injection at 30 g of Ig/L.

<sup>3</sup>Means are covariately adjusted for serum IgG at 0 h (serum IgG) and IgM at 0 h (serum IgM).

<sup>4</sup>Serum Ig 48 h after administration of Ig and 28 d of age.

<sup>5</sup> $P > .10$ .

<sup>6</sup>Standard error = 1.0 for L30 and CF+; n = 5.

† $P < .10$ .

\* $P < .05$ .

\*\* $P < .01$ .

Serum IgG concentration at 48 h among calves receiving Ig treatments was greater than serum IgG in calves on CF- treatment ( $P < .01$ ; Table 3). However, concentrations were not different at 28 d. Calves deprived of Ig begin development of active immunity earlier, usually by 10 d of age (14). Serum IgG concentrations were higher for calves on CF+ treatment than for calves on Ig treatments at 48 h and 28 d. Higher intake of Ig by calves fed colostrum is probably responsible for this difference. Mean consumption of IgG and IgM by calves on CF+ treatment at 24 h of age was 164 and 7 g, respectively. Calves receiving Ig obtained approximately 45 g of IgG and 5 g of IgM. Calculated efficiency of absorption of IgG was 24.4 and 28.1% when calves were fed CF+ and injected with Ig, respectively, indicating that the difference in serum IgG in calves was primarily due to amount of Ig administered to the calves.

Serum IgM at 48 h postinjection tended ( $P < .10$ ) to be higher for calves injected with Ig than for calves on CF- treatment (Table 3). Concentrations of IgM at 28 d were higher for calves on the CF- treatment than for calves on Ig treatments, which may have been because of earlier endogenous IgM synthesis in calves receiving no Ig. The first Ig isotype to be synthesized by the calf is IgM (14). Serum IgM concentrations were also higher among calves on CF+ treatment than for calves on Ig treatments for samples at 48 h and 28 d. As with IgG, increased concentrations might have been due to increased amounts of Ig consumed by calves receiving colostrum.

Serum IgG and IgM concentrations may have been slightly higher for calves on CF+ treatment than for calves on Ig treatments because of lower mean birth BW, which were 39.2 and 43.3 kg, respectively. In addition, blood volumes were not measured and may have been underestimated (2).

Concentrations of IgG in serum of calves injected with Ig did not reach 10 g/L, which is considered successful transfer of passive immunity (7). It is probable that the target concentration of 7.5 g of IgG/L of serum was not achieved because insufficient Ig was administered. Differences in expected and actual concentration of Ig solution accounted for differences in expected and actual Ig administered.

The Ig in maternal colostrum reflect the disease exposure of the cow and is an excellent source of Ig for the calf, which may be exposed to similar antigens. The Ig provided by s.c injection do not necessarily reflect the "disease history" of the farm and should not be considered a replacement for maternal colostrum. Instead, sources of Ig other than maternal colostrum should be utilized only when maternal colostrum is unavailable or is of poor Ig composition.

### CONCLUSIONS

Subcutaneous injection of bovine Ig provided moderate circulating concentrations of Ig and appears to be a viable option for hypogammaglobulinemic calves that are older than 24 h. Concentrations to 60 g of Ig/L of injectable solution in .9% NaCl or lactated Ringer's injection were satisfactory. Increasing the amount of Ig injected may increase circulating concentrations to provide greater protection for hypogammaglobulinemic calves. Further study is required to determine the optimal route and amount of administration and efficacy in maintaining calf health.

### ACKNOWLEDGMENTS

The authors thank Kim Lawler, Kris Krishnamoorthy, Alison Brown, and Geoffrey Strickland for calf care and sampling, and American Protein Corp. for partial financial support and for providing Ig solution.

### REFERENCES

- 1 Association of Official Analytical Chemists. 1990. Official Methods of Analysis. Vol. I. 15th ed. AOAC, Arlington, VA.
- 2 Besser, T. E., A. E. Garmedia, T. C. McGuire, and C. C. Gay. 1985. Effect of colostrum immunoglobulin G<sub>1</sub> and immunoglobulin M concentrations on immunoglobulin absorption in calves. *J. Dairy Sci.* 68: 2033.
- 3 Besser, T. E., C. C. Gay, T. C. McGuire, and J. F. Evermann. 1988. Passive immunity to bovine rotavirus infection associated with transfer of serum antibody into the intestinal lumen. *J. Virol.* 62:2238.
- 4 Brambell, F.W.R. 1970. The Transmission of Passive Immunity from Mother to Young. *Frontiers of Biology*. Vol. 18. A. Neuberger and E. L. Tatum, ed. North Holland Publ. Co., Amsterdam, The Netherlands.

- 5 Center for Animal Health Monitoring. 1993. Dairy herd management practices focusing on preweaned heifers. USDA, Anim. Plant Health Inspect. Serv. Vet. Serv. Fort Collins, CO.
- 6 Crowley, M. L., L. J. Fisher, and B. D. Owen. 1994. Blood-derived immunoglobulins in milk replacer, or by injection, for improved performance of colostrum-deprived neonatal calves. *Anim. Feed Sci. Technol.* 47:245.
- 7 Gay, C. C. 1983. Failure of passive transfer of colostrum immunoglobulins and neonatal disease in calves: a review. Page 346 in *Proc. 4th Int. Symp. Neonatal Diarrhea. Vet. Infect. Dis. Organ*, Saskatoon, SK, Canada.
- 8 Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). *Agric. Handbook No. 379*. ARS-USDA, Washington, DC.
- 9 Husband, A. J., M. R. Brandon, and A. K. Lascelles. 1972. Absorption and endogenous production of immunoglobulins in calves. *Aust. J. Exp. Biol. Med. Sci.* 50:491.
- 10 Klaus, G.G.B., A. Bennett, and E. W. Jones. 1969. A quantitative study of the transfer of colostrum immunoglobulins to the newborn calf. *Immunology* 16: 293.
- 11 Kruse, V. 1970. Absorption of immunoglobulins from colostrum in newborn calves. *Anim. Prod.* 12:627
- 12 Larson, L. L., F. G. Owen, J. L. Albright, R. D. Appleman, R. C. Lamb, and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci.* 60:989.
- 13 Nocek, J. E., D. G. Braund, and R. G. Warner. 1984. Influence of neonatal colostrum administration, immunoglobulin, and continued feeding of colostrum on calf gain, health, and serum protein. *J. Dairy Sci.* 67: 319.
- 14 Roy, J.H.B. 1990. *The Calf*. 5th ed. Butterworths, Boston, MA.
- 15 SAS/STAT® User's Guide: Statistics, Version 6, 4th Edition. 1989. SAS Inst., Inc., Cary, NC.
- 16 Staley, T. E., and L. J. Bush. 1985. Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. *J. Dairy Sci.* 68:184.
- 17 Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. I. Period of absorption. *J. Dairy Sci.* 62: 1632.