Characterization of a Colostrum Replacer Containing IgG Concentrate and Growth Factors

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Summary and Implications

The objective of this study was to characterize absorption of colostrum replacer (CR) or supplement (CS) containing fractions of bovine plasma. Immunoglobulin concentrate (IGC) was prepared from abattoir blood to a final purity of approximately 90% IgG. Blood was also processed to produce a fraction containing elevated concentrations of IGF-I and TGF-β (GF). Both IGC and GF were spray-dried and blended with other ingredients to produce CR (30% IgG) or CS (15% IgG) containing 0 or 5% GF. Forty Holstein bull calves were assigned to one of five treatments: 1) Pooled colostrum (MC) - 1.9 L of pooled colostrum at 1 and 8 h of age; 2) Low supplement (LC) - 1.9 L of CS at 1 and 8 h of age to provide 150 g of IgG; 3) Low supplement + GF (LG) - 1.9 L of a CS with GF at 1 and 8 h of age to provide 150 g of IgG; 4) High supplement (HC) - 1.9 L of CR at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial milk replacer (MR) at 8 h of age; and 5) High supplement + GF (HG) - 1.9 L of a CR with GF at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial MR at 8 h of age. Apparent efficiency of IgG absorption was higher for calves fed HC and HG compared to those fed LC and LG and was lower for calves fed LG and HG compared to those fed LC and HC. IgG concentrations at 24 h were highest in calves fed MC compared to other calves and were higher in calves fed HC and HG compared to LC and LG. Calves fed LG and HG had lower IgG concentrations at 24 h of age compared to those fed LC and HC. Xylose absorption was not influenced by treatment in calves fed HG, HC, and MC. These results indicate that 150 g of IgG provided in one dose soon after birth is superior to 150 g of IgG fed in two doses 7 h apart. Also, addition of a fraction of bovine plasma containing elevated concentrations of IGF-I and TGF-β to the CS and CR formulation decreased IgG absorption in the neonatal calf.

Introduction

Colostrum is vital to the health and survival of the neonatal calf, and 18% of dairy cows provide colostrum yields containing less than 100 g of IgG, the most commonly recommended amount to prevent failure of passive transfer **(FPT)**. Colostrum-deprived calves are 50-75 times more likely to die before 21 d of age than colostrum-fed calves, with most deaths occurring during the first week of life. Therefore, colostrum supplements **(CS)** and colostrum replacers **(CR)** can be provided when colostrum is either of poor quality or unavailable. Colostrum supplements are preparations intended to provide < 100 g of IgG/dose and are not formulated to replace colostrum. On the other hand, colostrum replacers contain > 100 g IgG/dose and usually provide additional nutrients required by the calf.

The ability to absorb IgG intact across the intestinal epithelium diminishes rapidly after birth and ceases by approximately 24 h of age. Current management practices often include feeding a certain volume of colostrum to calves at birth and again 12 h later. When the mass of IgG is held constant, greater amounts are absorbed when the concentration in the colostrum is higher – for example 1 L of colostrum containing 100 mg IgG/ml has is absorbed with greater efficiency than 2 L of colostrum containing 50 mg IgG/ml. Studies in calves comparing colostrum replacer that provide a similar mass of IgG fed in one or divided into two feedings are limited.

Colostrum not only provides passive immunity for the newborn calf, but it can also have profound effects on the development of the neonatal intestine. Villous circumference, area, height and height/crypt depth ratio in the duodenum are higher for calves fed colostrum compared to colostrum-deprived calves. Calves fed colostrum also have higher plasma xylose concentrations after oral administration of xylose compared to calves fed milk replacer **(MR)**, suggesting enhanced absorptive capabilities in colostrum fed animals.

Colostrum contains elevated levels of many growth factors, including insulin-like growth factor-I **(IGF-I)**, which has been shown to enhance intestinal development in piglets. Calves fed colostrum have increased xylose uptake when compared to calves fed formula similar to colostrum but containing only 15% of the IGF-I content of colostrum. In contrast, no enhancement of gut development is observed in colostrum fed calves after oral or subcutaneous injection of IGF-I. The effect of growth factors on immunoglobulin absorption has not been characterized.

Therefore, the objectives of this study were two-fold: (1) to characterize the absorption of colostrum replacer (CR) or supplement (CS) containing a fraction of bovine plasma with elevated concentrations of IGF-I and transforming growth factor-β **(TGF-β)** and (2) to characterize the absorption of a similar mass of IgG fed in one feeding or divided into two feedings.

Procedures

Diet Formulation

Bovine blood was collected from animals in an USDA inspected abattoir. Animals were inspected and approved for human consumption followed by stunning with captive bolt and exsanguination. Blood was collected into a stainless steel container treated with anticoagulant. Plasma was

separated by centrifugation, chilled (approximately 4°C) and transported to the laboratory. Immunoglobulin G was fractionated from plasma using a precipitation procedure. The resulting immunoglobulin concentrate (**IGC**) contained nine-times the percentage of IgG in plasma. Bovine plasma was also processed to produce a fraction containing elevated concentrations of IGF-1 and TGF-β (**GF**). Both IGC and GF were spray-dried and blended with other ingredients (Table 1) to produce CR (30% IgG) or CS (15% IgG) and either containing 0 or 5% GF. The CS was formulated to provide 75 g of IgG/500 g dose; two doses were fed to provide a total of 150 g IgG. The CR was formulated to provide 150 g IgG/500 g dose; one dose was fed to provide 150 g IgG.

Maternal colostrum was collected and stored in 5-gallon buckets prior to feeding. Colostrum was fed within four hours of collection. Samples colostrum and experimental diet were analyzed for IgG content and for proximate nutrients according to AOAC procedures at a commercial facility (Silliker Inc., Cedar Rapids, IA).

Experimental Procedure

Holstein bull calves (n = 40) were removed from their dams immediately after birth and assigned to one of five treatments: 1) Pooled colostrum **(MC)** - 1.9 L of pooled colostrum at 1 and 8 h of age; 2) Low supplement **(LC)** - 1.9 L of CS at 1 and 8 h of age to provide 150 g of IgG; 3) Low supplement + GF **(LG)** - 1.9 L of a CS with GF at 1 and 8 h of age to provide 150 g of IgG; 4) High supplement **(HC)** - 1.9 L of CR at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial MR at 8 h of age; and 5) High supplement + GF **(HG)** - 1.9 L of a CR with GF at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial MR at 8 h of age. Experimental diets were reconstituted in water and mixed in a household blender until well blended. The mixture was then poured into a nipple bottle and offered to the calf. Amounts not voluntarily consumed were provided via an esophageal feeder.

At 0 and 24 h of age, blood was collected from all calves by jugular venipuncture into evacuated tubes containing EDTA. A small sample of blood was used for hematocrit determination using a microhematocrit centrifuge. Plasma was collected by centrifugation and total protein was determined with a handheld refractometer (Schuco Clinical Refractometer). Remaining plasma was frozen (-20°C) for later determination of IgG by turbidimetric immunoassay.

Xylose Absorption

Five calves each from the treatment groups HG and MC, and six calves from group HC were administered an oral xylose solution (0.5 g *d*-xylose/kg body weight) at 2 d of age approximately 4 h after the morning meal. Blood samples were obtained from each calf via jugular venipuncture prior to xylose administration and again at 2 h after xylose ingestion. Plasma was collected by centrifugation and frozen (-20°C) for later spectrophotometric analysis.

Statistical Analysis

Experimental data were analyzed using the general linear models procedure of SAS. Orthogonal contrasts were used to test differences between MC and CR, low IgG dose and high IgG dose, 0 and 5% GF addition, and high IgG dose with and without GF addition. Chi square analysis was used to determine differences in FPT. Significance was declared at $P < 0.05$ unless otherwise noted.

Results and Discussion

Composition of the experimental CS and CR, as well as the MR provided to calves on treatment HC and HG, is provided in Table 2. Crude protein percentage was slightly higher in HC and HG diets; however, total protein consumed was similar between treatments since calves on treatment HC and HG were fed MR at 8 h of age. Composition of GF is in Table 3. Four calves did not survive until the 24 h blood sample; data from these calves were excluded from analyses. Mean BW of calves did not differ among treatments (Table 4). Frequency of feeding via esophageal feeder also did not differ among treatments.

Concentrations of IgG (Table 4) were highest at 24 h of age in calves fed MC compared to calves in the other four treatment groups, which is easily attributable to the difference in IgG intake. Total IgG intake was 282 g for calves fed MC and 150 g for calves fed all other treatments. The GF fraction contributed an additional 4 g of IgG. Concentrations of IgG were higher at 24 h of age in calves fed HC and HG compared to LC and LG, even though the total IgG intake was equal between all groups. Apparent efficiency of IgG absorption **(AEA)**; (Table 4) was also higher in calves fed HC and HG compared to those fed LC and LG. These results contradict previous findings that show no difference in serum IgG concentrations and similar AEA after feeding calves a large amount at birth or the same amount divided into two or three feedings. However, comparisons in previous studies were based on feeding maternal colostrum, whereas calves in the current study received CS or CR. The AEA for CR derived from bovine Ig concentrate has been shown to be higher when fed once compared to feeding twice after birth 8 h apart. The values for AEA observed in this trial are similar to those previously reported for CS and CR prepared from bovine plasma. However, IgG concentrations were higher in calves fed the CS and CR formulations than those previously reported for calves receiving 150 g IgG obtained from bovine serum.

Calves fed LG and HG also had lower IgG concentrations at 24 h of age and lower AEA compared to those fed LC and HC, suggesting that addition of GF to the CS and CR formulation negatively influenced IgG absorption. Concentrations of TGF-β and IGF-1, although concentrated in the serum fraction, were still well below the concentrations found in bovine colostrum. Values for IGF-I and TGF-β_1 in cow colostrum range from 203-1850 ng/ml and 12.4-42.6 ng/ml, respectively. Calves fed a bovine colostral extract that also contained low levels of IGF-I shiowed increases in intestinal villus circumference and

villus height and decreased xylose absorption that was hypothesized to be a result of normal intestinal maturation but reduced absorptive capacity.

The lack of difference in xylose absorption after addition of GF also supports previous reports that show no enhancement of gut development after oral administration of IGF-I. Xylose absorption was not different between calves fed either 0 or 5% GF in the current study, however, there were differences in IgG absorption suggesting a change in absorptive capacity or in IgG metabolism. Xylose is absorbed in a manner similar to glucose, utilizing carrier-facilitated transport. This is in contrast to IgG, which is absorbed non-selectively for approximately the first 24 h after birth. It is possible that the addition of GF to the formulations resulted in increased maturation of the intestine, resulting in a loss of pinocytotic ability, but not affecting carrier-mediated monosaccharide absorption.

Newborn calves readily absorbed the IgG concentrate used in this study, and plasma IgG concentrations at 24 h of age were indicative of successful passive transfer. Results from this study indicate that 150 g of IgG provided in one dose soon after birth is superior to 150 g of IgG fed in two doses 7 h apart. These data also suggest that addition of a fraction of bovine plasma containing elevated concentrations of IGF-I and TGF-_ to the CS and CR formulation decreased IgG absorption in the neonatal calf. Further studies are needed to determine the biological mechanisms by which growth factors influence intestinal immunoglobulin absorption in the neonatal calf.

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

Ingredient (% of formula)	LC[1]	LG	HC	HG
Ig concentrate	16.67	16.67	33.33	33.33
Dry fat blend$_2$	35	35	35	35
Whey protein concentrate (75%)	10	5	5	0
Growth factor fraction	0	5	0	5
Whey	35.83	35.83	24.17	24.17
Premix[3]	2.5	2.5	2.5	2.5

[1]LC = 1.9 L of CS at 1 and 8 h; LG = 1.9 L of CS with GF at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h
[2]Dry fat blend containing 7% and 60% crude fat
[3]Premix contains vitamins and minerals to meet 1998 NRC requirements

Table 2. Composition of experimental colostrum supplements, colostrum replacers, and milk replacer.

Item[1], %	LC[2]	LG	HC	HG	MR[3]
DM	96.8	93.6	95.5	93.6	97.1
CP	31.3	32.1	40.3	41.5	21.3
Ash	5.7	5.5	4.8	4.8	7.1
Fat	19.8	23.6	23.1	23.8	23.6

[1]Items are on a DM basis, except for DM
[2]LC = 1.9 L of CS at 1 and 8 h; LG = 1.9 L of CS with GF at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h
[3]MR = commercial milk replacer provided to calves in treatments HC and HG at 8 h of age

Table 3. Composition and analysis of serum fraction GF.

Item[1]	Value
DM, %	96.6
TP, %	93.24
Albumin, %	56.64
IgG, %	19.7
Ash, %	0.61
TGF-β_1, ng/g	765
TGF-β_2, ng/g	9
IGF-1, ng/g	1368

[1]Items are on a DM basis, except for DM

Table 4. Least squares means of treatment parameters for calves fed LC, LG, HC, HG and MC.

	Treatment[1]						Contrasts[2]			
Item	MC	LC	LG	HC	HG	SE	1	2	3	4
No. calves	7	7	7	8	7	…	…	…	…	…
BW, kg	49.4	47.3	45.4	45.2	49.2	1.8	NS[3]	NS	NS	NS
IgG intake, g	282	150	150	150	150	9	0.001	NS	NS	NS
Protein intake, g	494	303	302	296	299	13	0.001	NS	NS	NS
Plasma IgG, g/L										
0 h	0	0	0	0	0	0	NS	NS	NS	NS
24 h	18.1	10.6	9.1	13.0	10.3	0.9	0.001	0.05	0.02	NS
Change 0-24 h	18.1	10.6	9.1	13.0	10.3	0.9	0.001	0.05	0.02	NS
AEA[4] %	29	30	24	35	30	2	NS	0.02	0.03	0.07
FPT[5], %	0	42	57	0	28	14	0.06	0.02	NS	NS
Plasma protein, g/L										
0 h	4.75	4.62	4.37	4.57	4.51	0.15	NS	NS	NS	NS
24 h	6.12	5.02	5.20	5.24	4.91	0.17	0.001	NS	NS	NS
Change 0-24 h	1.37	0.40	0.86	0.61	0.40	0.15	0.001	NS	NS	0.06
Hematocrit, %										
0 h	40.14	42.57	33.14	37.62	39.28	2.62	NS	NS	0.09	0.06
24 h[6]	33.19	33.65	29.85	34.76	36.21	1.58	NS	0.02	NS	0.01
Change 0-24 h[6]	-5.12	-4.66	-8.45	-3.55	-2.10	1.58	NS	0.02	NS	0.01

[1]MC = 1.9 L of maternal colostrum at 1 and 8 h; LC = 1.9 L of CS at 1 and 8 h; LG = 1.9 L of CS with GF at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h
[2]Contrasts: 1 = MC vs. CR; 2 = Low vs. High; 3 = 5% GF vs. 0% GF; 4 = LG vs. HG
[3]$P > 0.10$
[4]Apparent efficiency of absorption, calculated as plasma IgG (g/L) × BW (kg) × 9% ÷IgG intake (g)
[5]Failure of passive transfer (IgG < 10 g/L)
[6]Covariate adjusted

Table 5. Least squares means for xylose absorption in calves fed HG, HC, and MC.

	Treatment[1]				Contrasts[2]	
Item	MC	HC	HG	SE	1	2
No. calves	5	6	5	…	…	…
Xylose absorption, mg/dl						
0 h	16.56	15.22	15.89	0.56	NS[3]	NS
2 h	71.55	55.97	60.76	9.0	NS	NS
Change 0-2 h	54.98	40.74	44.87	8.9	NS	NS

[1]MC = 1.9 L of maternal colostrum at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h
[2]Contrasts: 1 = MC vs. CR; 2 = 5% GF vs. 0% GF [3]$P > 0.10$