Impact of Irradiation and Immunoglobulin G Concentration on Absorption of Protein and Immunoglobulin G in Calves Fed Colostrum Replacer

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ABSTRACT

The objective of this study was first to evaluate whether irradiation treatment of a commercial colostrum replacer (CR) affected acquisition of passive immunity. If the irradiation treatment negatively affected the acquisition of passive immunity, the second objective was to evaluate whether an increased total IgG mass, in a single feeding of CR derived from bovine serum fractions, could compensate for this effect. Acquisition of passive immunity was assessed by 24-h serum IgG levels, serum protein levels, apparent efficiency of absorption (AEA) of IgG, and the ability to prevent failure of passive transfer (FPT) in day-old dairy calves fed a single feeding of CR. Single-dose packs of CR were sent to a commercial irradiation facility for electron-beam irradiation at 3 to 7 kGy (low irradiation) or 15 to 20 kGy (high irradiation). Fifty-six Holstein, Jersey, or crossbred calves were randomly assigned to 1 of 5 treatments: 1) 130 g of IgG (460 g of CR), no irradiation; 2) 130 g of IgG (460 g of CR), low irradiation; 3) 160 g of IgG (518 g of CR), low irradiation; 4) 190 g of IgG (575.4 g of CR), low irradiation; and 5) 130 g of IgG (460 g of CR), high irradiation. All CR were reconstituted in water and mixed in a household blender to a constant solids concentration of 18.7%. Increasing doses of irradiation (130 g of Ig with no, low, or high irradiation) resulted in a linear decrease in 24-h serum IgG and AEA of IgG, and increased the percentage of calves with FPT. Increasing the IgG mass in the CR (130, 160, and 190 g of Ig with low irradiation) resulted in a linear increase in 24-h serum IgG and serum total protein levels, and a linear decrease in AEA of IgG. There was no effect of increasing the mass of IgG fed on the per-

centage of calves with FPT. The correlation between serum IgG and serum total protein at 24 h was positive; however, at 24 h the irradiation treatments reduced the serum IgG-to-serum total protein ratio. In this study, CR isolated from bovine serum, providing 130 g of IgG in the first feeding and receiving either no irradiation or a low irradiation treatment, was sufficient to prevent FPT in calves.

Key words: calf, immunoglobulin, colostrum replacer, irradiation

INTRODUCTION

Calf health and survival affect the economics of a dairy operation. Feeding a sufficient quantity of high-quality colostrum within the first few hours of birth plays a vital role in calf health, survival, and subsequent performance (Robison et al., 1988; DeNise et al., 1989; Wittum and Perino, 1995; Faber et al., 2005). Failure of passive transfer (FPT) is generally defined as serum IgG levels of <10 g/L in calves at 24 h of age (Bovine Alliance on Management and Nutrition, 1995; Davis and Drackley, 1998). A number of factors have been identified that influence IgG absorption by the young calf, including the quantity and quality of colostrum, time of first feeding, metabolic status of the calf, and colostrum management practices (Stott et al., 1979a,b; Garry et al., 1996; Morin et al., 1997; Quigley et al., 1998, 2001). Calves with FPT are more likely to die or become chronically ill, which increases medical costs and requires additional labor (Robison et al., 1988; National Animal Health Monitoring System, 1993; Donovan et al., 1998). In addition, fresh maternal colostrum can be contaminated with pathogens (*Salmonella* spp., *Mycobacterium paratuberculosis*, *Escherichia coli*, *Mycoplasma bovis* mastitis, *Staphylococcus aureus* mastitis, bovine viral diarrhea virus, and bovine leukemia virus), resulting in increased risk of disease transmission (McGuirk and Collins, 2004).

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Protein fractions isolated from bovine serum high in IgG (~50% IgG), blended with other nutrients and fed to calves, have been shown to prevent FPT (Quigley et al., 2001; Hammer et al., 2004; Jones et al., 2004). Time of feeding, IgG mass, and total nutrient mass have been shown to affect apparent efficiency of absorption (AEA) of IgG (Quigley et al., 2001; Hammer et al., 2004). Combined use of commercially available bovine serum-based products providing 125 and 50 g of IgG fed at 1 and 6 h after birth, respectively, have been shown to be as effective as high-quality maternal colostrum in preventing FPT (Poulsen et al., 2003). However, Swan et al. (2007) reported that 93% of calves experienced FPT when fed 125 g of IgG from a commercially available colostrum replacer (CR). The optimal mass of IgG derived from bovine serum protein fractions necessary to consistently prevent FPT has not been reported.

Irradiation effectively reduces microbial contamination (Carpenter, 1963). Bender et al. (1989) reported that mycoplasma in calf serum is inactivated when irradiated with 3 kGy of gamma irradiation. However, irradiation has also been shown to reduce vitamin levels, oxidize fat, and alter protein structure (El-Moneim et al., 1988; Leeson and Marcotte, 1993). Tran et al. (2004) reported negligible changes in IgG structure after treatment of an Ig-rich paste with a virucidal dose of irradiation (50 kGy). The effects of irradiation treatment on AEA of IgG from bovine serum protein fractions are unknown.

Therefore, the objectives of this study were first, to evaluate whether irradiation treatment of a commercial CR derived from bovine serum fractions affected acquisition of passive immunity. Second, if irradiation treatment negatively affected acquisition of passive immunity, whether increased total IgG mass in a single feeding could compensate for this effect. Acquisition of passive immunity was assessed by 24-h serum IgG levels, serum protein levels, AEA, and the ability to prevent FPT in day-old dairy calves fed a single feeding.

MATERIALS AND METHODS

Animals and Treatments

Bovine blood was collected from animals slaughtered at a USDA-inspected abattoir into a stainless-steel container containing anticoagulant. Plasma was separated by centrifugation, chilled (approximately 4°C), and transported to a commercial facility for further processing by centrifugation, ultrafiltration, and spray-drying to produce an IgG concentrate. The IgG concentrate contained >90% CP and >50% IgG and was mixed with other ingredients (carbohydrate, fat, vitamins, and minerals) to produce a CR packaged in single-dose units

Table 1. Calculated chemical analysis of colostrum replacers (as-fed basis)

Item	Colostrum replacer		
	130 g of IgG	160 g of IgG	190 g of IgG
IgG, %	28.3	30.9	33.0
CP, %	50.8	55.2	58.7
Fat, %	15.2	13.5	12.1
Ca, %	0.99	0.89	0.82
P, %	0.94	0.92	0.89
Vitamin A, IU/g	109.0	96.5	86.9
Vitamin D, IU/g	21.7	19.3	17.4
Vitamin E, IU/g	0.33	0.29	0.26

(Table 1). When irradiated, single-dose packs of CR were sent to a commercial irradiation facility (Sadex Corporation, Sioux City, IA) and treated with electron-beam (e-beam) irradiation at 3 to 7 kGy (low irradiation; **L**) or 15 to 20 kGy (high irradiation; **H**).

Fifty-six Holstein, Jersey, or crossbred bull calves on a large California dairy were used. Breeds were randomly distributed across treatments. Calves were immediately removed from their dams at birth (<5 min after birth) and moved to a calf housing area, where they were weighed, identified by individual number, navel dipped, fitted with an elastic castrator, and placed in elevated individual stalls fitted with a heat lamp. Calves were randomly assigned to 1 of 5 treatments: 1) 130 g of IgG (460 g of CR), no irradiation (**130NR**); 2) 130 g of IgG (460 g of CR), low irradiation (**130L**); 3) 160 g of IgG (518 g of CR), low irradiation (**160L**); 4) 190 g of IgG (575.4 g of CR), low irradiation (**190L**); and 5) 130 g of IgG (460 g of CR), high irradiation (**130H**). Colostrum replacers containing 130 g of IgG mass were treated with zero, low, or high levels of irradiation to determine whether irradiation treatments affected acquisition of passive immunity. Colostrum replacers treated with low irradiation were formulated to provide 130, 160, or 190 g of IgG mass to determine the amount of IgG mass required to achieve passive immunity, with the presumption that irradiation may adversely affect passive immunity. The CR providing 130 g of IgG and treated with low irradiation was a common treatment in both titrations. All CR were reconstituted in water (41°C) and mixed in a household blender to a solids concentration of 18.7%. Treatments were administered at 1 h of age with an esophageal feeder, followed by a second feeding of 1.9 L of commercial milk replacer (18% protein, 20% fat) at 12 h of age.

At 0.5 and 24 h of age, blood samples were collected from all calves via jugular venipuncture into evacuated red-topped tubes. Serum was collected following centrifugation and frozen (−20°C) for subsequent analysis of serum total protein and IgG levels.

Table 2. Means of treatment parameters for calves fed colostrum replacers containing graded levels of IgG concentration processed with no, low, or high irradiation

Item	Treatment ¹					SEM	Contrasts ² (<i>P</i> =)	
	130NR	130L	130H	160L	190L		1	2
Calves, no.	11	11	11	11	12			
Birth BW, kg	37.3	35.6	37.0	38.6	35.6			
IgG intake, g	130	130	130	160	190			
Serum IgG, ³ g/L								
0 h	0.4	0.2	0.3	0.6	0.7	0.2	0.6987	0.1174
24 h	13.0	11.6	9.7	13.3	14.3	0.8	0.0053	0.0164
Change 0 to 24 h	12.5	11.4	9.4	12.7	13.6	0.8	0.0060	0.0452
Serum total protein, ³ g/dL								
0 h	4.0	3.9	3.8	4.0	3.9	0.1	0.3817	0.7832
24 h	4.9	4.8	4.5	5.0	5.1	0.1	0.0638	0.0860
Change 0 to 24 h	0.9	0.8	0.7	1.0	1.1	0.1	0.2015	0.1118
AEA, ⁴ %	34.3	31.5	25.8	29.8	26.6	1.8	0.0015	0.0562
Failure of passive transfer, %	18.2	18.2	54.6	9.1	8.3	11.8	0.0193	0.5496
IgG:protein ratio, ³ mg/g	259.4	242.7	210.6	262.9	279.3	12.3	0.0063	0.0358

¹Treatments: 130NR = 130 g of IgG with no irradiation; 130L = 130 g of IgG with low irradiation; 160L = 160 g of IgG with low irradiation; 190L = 190 g of IgG with low irradiation; and 130H = 130 g of IgG with high irradiation.

²Contrast: 1 = linear effect of irradiation dose with 130 g of IgG; 2 = linear effect of IgG mass with a low dose of irradiation.

³Least squares means adjusted by BW as a covariate.

⁴Apparent efficiency of IgG absorption, calculated as serum IgG at 24 h × BW at birth, kg × 0.09 (serum volume as a percentage of BW), divided by IgG intake (g).

Analytical Procedures

Serum total protein was determined by the biuret method (Pointe Scientific Inc., Canton, MI). Immunoglobulin G was determined by a commercially available radial immunodiffusion kit (Triple J Farms, Redmond, WA).

Statistical Analysis

Experimental data were analyzed as a completely randomized design by ANOVA, using PROC GLM of SAS (SAS Institute, Cary, NC; Table 2). Independent variables consisted of treatment, with BW as a covariate when appropriate. Dependent variables were serum total protein and IgG at 0 and 24 h and the change from 0 to 24 h, AEA, and the IgG-to-protein ratio. Body weight at birth was used as a covariate in the model of serum total protein and serum IgG (0 and 24 h) to account for breed differences. When BW at birth was included as a covariate, it explained a significant amount of variation in the model ($P < 0.05$); thus, least squares means are reported for serum total protein and serum IgG. Unadjusted means are reported for all other data. The percentage of calves with FPT was analyzed by chi-squared analysis (SAS Institute). Preplanned nonorthogonal contrasts were used to make treatment comparisons. Contrast one was the linear effect of irradiation treatment, which included treatments 130NR, 130L, and 130H, and contrast two was the linear effect

of IgG mass, which included treatments 130L, 160L, and 190L. Linear contrast statements were used to produce the respective contrast; however, for unequally spaced treatments (irradiation treatments) PROC IML (SAS Institute) was used to produce appropriate coefficients for linear contrast statements. Simple regression analysis was used to explain the serum IgG-to-protein relationship attributed to either IgG concentration or irradiation (Figures 1 and 2). Significant values are stated within the text.

RESULTS AND DISCUSSION

Commercial irradiation is used to reduce microbial contamination of feed ingredients or finished products. Several studies have shown that up to 60 kGy of gamma irradiation will reduce bacterial concentrations without damaging protein quality (Metta and Johnson, 1959; Carpenter, 1963; Farag, 1998). Ford (1976) reported no differences in protein quality of rat diets treated with gamma irradiation up to 100 kGy. DeRouchey et al. (2003) reported that irradiation of spray-dried plasma (8.5 kGy from e-beam) used in pig starter feeds resulted in an improved rate and efficiency of growth, whereas irradiation of the complete feed containing spray-dried plasma did not improve nursery pig performance. Additionally, Tran et al. (2004) reported that intravenous Ig preparations could be treated with 50 kGy of gamma irradiation without causing damage to the IgG mole-

Figure 1. Regression of serum IgG and serum total protein at 24 h of age from calves fed a colostrum replacer irradiated with a low dose (3 to 7 kGy) of irradiation and containing 130 (▲), 160 (■), or 190 (×) g of IgG/dose.

Figure 2. Regression of serum IgG and serum total protein at 24 h of age from calves fed a colostrum replacer containing 130 g of IgG/dose and treated with no (0 kGy; ■), low (3 to 7 kGy; ▲), or high (15 to 20 kGy; ●) irradiation.

cule. Leeson and Marcotte (1993) reported no effects on dietary nutrient composition of feed, growth rate, or feed efficiency of poultry when feed was irradiated up to 25 kGy, whereas increasing irradiation greater than 25 kGy resulted in reduced potency of vitamins A and D. In the current study, a commercial irradiation facility was used to irradiate the treatments. Commercial facilities have limitations on dosing ranges because of the density of the material being irradiated. Based on the density of the CR, the irradiation dose was selected to minimize the range applied while still being able to irradiate treatments as commercially packaged, with density similar to the finished product. In this trial, increasing e-beam irradiation to a high of 15 to 20 kGy resulted in a linear decrease ($P < 0.01$) in 24-h serum IgG and AEA of IgG (Table 2). More than 50% of the calves fed the CR irradiated with the high irradiation dose experienced FPT. Similar to the level reported by Bender et al. (1989) to inactivate mycoplasma, the low irradiation dose (3 to 7 kGy) had a minimal effect on the AEA of IgG and the percentage of calves experiencing FPT compared with calves fed CR that was not irradiated (130NR).

Increasing the IgG mass in the CR (130L, 160L, and 190L) resulted in a linear increase in 24-h serum IgG ($P < 0.02$) and serum total protein levels ($P < 0.09$), and a linear decrease in the AEA of IgG ($P < 0.06$; Table 2). There was no effect ($P > 0.10$) of increasing the mass of IgG being fed on the percentage of calves experiencing FPT (18.2, 9.1, and 8.3%, respectively). These data are consistent with other reports indicating that feeding calves between 100 and 250 g of bovine serum-derived IgG was sufficient to prevent FPT in 82 to 100% of the calves (Quigley et al., 2001; Hammer et al., 2004; Jones et al., 2004). However, Swan et al. (2007) recently reported a high incidence of FPT in calves fed bovine serum-based CR. Results of the current study indicated that 130 g of IgG isolated from bovine serum and treated with either a low dose or no irradiation was sufficient to prevent FPT in more than 80% of the calves receiving CR, whereas a higher irradiation dose resulted in more than 50% of the calves experiencing FPT. Increasing the mass of IgG increased the average 24-h serum IgG and serum total protein levels, but did not further reduce the incidence of FPT.

Twenty-four-hour serum total protein and serum IgG concentrations are positively correlated (McBeath et al., 1971; Naylor and Kronfeld, 1977; Calloway et al., 2002; Quigley et al., 2002; Foster et al., 2006). In the current study, increasing the IgG mass (130L, 160L, and 190L) resulted in a linear increase ($P < 0.05$) in the 24-h serum IgG-to-serum total protein ratio (Table 2 and Figure 1). However, increasing doses of irradiation

linearly reduced ($P < 0.01$) the 24-h serum IgG-to-serum total protein ratio (Table 2 and Figure 2).

The results of the current study indicate that irradiation affects the AEA of IgG from bovine serum and that this effect may be dose related. The effects of irradiation are not clearly understood. These data suggest that irradiation affects the protein structure or functionality, resulting in a reduced AEA of IgG. Early studies of serum-based CR did not include an irradiation step in the production of the CR. In recent reports in which IgG isolated from bovine serum was not effective in preventing FPT, the serum-based CR used may have been irradiated at a high level. This could explain the increased incidence of 24-h serum IgG of less than 10 g/L (Swan et al., 2007). Based on the current study, results indicate that either a low irradiation dose or a greater mass of IgG was sufficient to reduce the incidence of FPT. If higher doses of irradiation are used, then a greater mass of IgG should be fed to reduce FPT.

CONCLUSIONS

A low dose of irradiation (3 to 7 kGy) did not reduce the AEA of IgG enough to increase the incidence of FPT in calves fed 130 g of IgG. A high irradiation dose (15 to 20 kGy) reduced 24-h serum IgG, serum protein, and the AEA of IgG and increased the incidence of FPT. A greater mass of IgG fed to calves linearly increased the 24-h serum IgG and serum total protein, and decreased the AEA of IgG. Further studies are needed to determine the changes in IgG that occur with irradiation and the influence of the irradiation level on intestinal IgG absorption in the neonatal calf. In this study, CR isolated from bovine serum and receiving either no irradiation or a low level of irradiation, and providing 130 g of IgG in the first feeding, were sufficient to prevent FPT in at least 80% of the calves.

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