Addition of Soybean Trypsin Inhibitor to Bovine Colostrum: Effects on Serum Immunoglobulin Concentrations in Jersey Calves

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ABSTRACT

Secretion of trypsin by the neonate may reduce absorption of Ig from colostrum and increase failure of transfer of passive immunity, particularly for calves fed after 12 h of age. Jersey calves (n = 48) were used in a 2 × 2 factorial arrangement of soybean trypsin inhibitor (0 or 1 g) added to 1 L of maternal colostrum at the first two feedings and time of initial feeding (.65 or 12 h of age). A second colostrum feeding was offered 12 h after the initial feeding. Maternal colostrum was analyzed for Ig by radial immunodiffusion and trypsin inhibitor by radial protease diffusion. Jugular blood was sampled at 0, 12, 24, and 48 h after initial feeding, and serum was analyzed for IgG and IgM. Addition of trypsin inhibitor increased serum Ig and total serum protein concentrations, regardless of age at first feeding. Concentrations of serum IgG and IgM were increased 16 and 30%, respectively, when trypsin inhibitor was fed. Age of first feeding did not affect concentrations of IgG or IgM in serum. Supplementation of colostrum with trypsin inhibitor improved transfer of passive immunity to neonatal Jersey calves.
(**Key words**: colostrum, trypsin inhibitor, immunoglobulin, Jersey)

Abbreviation key: **TI** = trypsin inhibitor.

INTRODUCTION

Bovine colostrum contains a large amount of trypsin inhibitor (**TI**), which may assist in transfer of immunity to the neonate (10, 16). Colostrum from the cow, as well as colostrum from the sow, mare, and ewe, contains markedly more TI activity than does milk from the same species (8, 16). Conversely, TI is generally low in colostrum from species that do not rely on colostral transmission of Ig [e.g., human; (16)]. Weström et al. (21) reported that addition of protease inhibitors from soybeans or sow colostrum increased absorption of BSA and dextran labeled with fluorescein-isothiocyanate in pigs. Intestinal absorption of Ig was improved for neonatal pigs when sow milk was supplemented with porcine colostral TI; inactivated TI had no effect on intestinal absorption (10). Carlsson et al. (4) reported reduced absorption of total protein, IgG, and albumin by piglets fed colostrum treated to eliminate naturally occurring TI.

Trypsin secretion by the neonate has not been documented thoroughly. Huber et al. (7) reported that pancreatic protease activity was detectable by 1 d of age and was 29% of the activity measured at 8 d of age. Brown and Perry (3) reported no trypsin activity in newborn lambs, but increased activity from 1 through 14 d of age. Considerable degradation of Ig has been reported in the intestine of pigs <20 h of age, which was reduced by addition of TI (6); therefore, increasing secretion of pancreatic proteases may reduce Ig absorption as the pig ages.

Whether the addition of TI to bovine colostrum improves absorption of Ig from neonatal calves is not clear, particularly if calves are fed at an age when trypsin secretion may be increasing. Therefore, our objective was to determine the effect of added TI on concentrations of Ig and protein in the serum of neonatal Jersey calves fed maternal colostrum at varying ages.

MATERIALS AND METHODS

Calf Assignments and Experimental Design

Jersey calves (n = 48; 24 heifers) were blocked by sex and date of birth and assigned

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randomly within block to a 2 × 2 factorial arrangement of added soybean TI (Type II-S; crude soluble powder; Sigma Chemical Co., St. Louis, MO) and age at first feeding. The soybean TI was obtained in two lots: specific activities were 1.5 and 1.6 mg of trypsin inhibited/mg of TI. Cows were moved to a 2-ha mixed grass pasture approximately 2 wk prior to parturition. Calf and dam were separated as soon as possible after parturition, and colostrum was collected from the dam. Calves were not allowed to nurse the dam. A 50-ml sample of colostrum was frozen (–20°C) and later analyzed for IgG, IgM, and IgA by single radial immunodiffusion (VMRD, Inc., Pullman, WA) and colostral TI activity by a modification of the method of Sandholm et al. (17) using radial protease diffusion. Diffusion plates were prepared using protease gel tablets (Bio-Rad Laboratories, Inc., Melville, NY). When reconstituted in distilled water, each tablet produced a 1% agar gel containing a bovine casein preparation in Tris-buffered NaCl saline (pH = 7.2). The gel was poured onto each plate and allowed to cool, and 4-mm wells were bored using a pipette. Frozen colostrum was warmed to 37°C and mixed, and a 1-ml sample was diluted 1:20 (vol/vol) with PBS in a test tube. Diluted colostrum (1 ml) was added to 1 ml of trypsin solution [300 mg of trypsin (Sigma Chemical Co.) dissolved in 100 ml of 1m*M* HCl and diluted 1:100 (vol/vol) in PBS] and mixed gently. Following equilibration for 5 min at approximately 25°C, 15 μl of colostrum-trypsin mixture were added to each well. Plates were allowed to incubate for 22 h at approximately 25°C and were then overlaid with 3% acetic acid (vol/vol) for 10 min to improve resolution. Ring diameters were measured with a stereomicroscope (10×) with an ocular micrometer. The assay was repeated with multiple dilutions to determine the dilution at which the added trypsin was no longer inhibited by TI (i.e., a measurable ring was observed).

Calves were fed 1 L of colostrum containing 0 or approximately 1 g of added TI as soon as possible after birth or 12 h of age and again 12 h later. Calves were housed in individual fiberglass hutches throughout the 28-d study. On d 2 and 3, calves were fed 1 L of colostrum from second and third milkings of the dam on an a.m.-p.m. schedule.

Commercial calf starter (Tennessee Farmers Cooperative, LaVergne, TN) was offered for ad libitum consumption from 4 to 28 d of age. Commercial milk replacer (Land O'Lakes, Inc., Ft. Dodge, IA) was reconstituted to 12% DM and fed at .95 L/d to 28 d of age. Intakes of starter and milk replacer were recorded daily. Second-cutting alfalfa hay was offered to all calves from 14 d of age. Water was available at all times.

Sampling and Data Collection

Calves were weighed at birth and every week thereafter to 28 d. Incidence of scours, respiratory problems, and signs of illness were recorded for each calf daily. Fecal consistency was estimated at the a.m. feeding by the method of Larson et al. (11).

Approximately 10 ml of jugular blood were collected just prior to the first feeding and at 12, 24, and 48 h thereafter. Blood was allowed to clot, and serum was separated by centrifugation (3000 × *g*). Serum was frozen (–20°C) until transported to the laboratory and analyzed for IgG and IgM (VMRD, Inc.) and for total protein (Sigma Chemical Co.). Samples of milk replacer, hay, and calf starter were taken monthly and analyzed for DM and CP (1), NDF (5), and minerals (atomic absorbance spectrophotometry).

Statistical Analysis

Serum Ig concentrations at 12, 24, and 48 h after initial feeding were initially analyzed as a randomized complete block experimental design using repeated measures analysis of covariance (18). However, neither block nor sex of calf were significant in the model; therefore, data were reanalyzed as a completely randomized design using repeated measures analysis of covariance. Intake of colostral Ig and TI were included in the model as covariants. Orthogonal contrasts were used to determine effects of soybean TI addition, age at first feeding, and interaction. Scours scores, BW at 28 d, and mean BW gain and intake for the 28-d study were analyzed as a randomized complete block experimental design using ANOVA. When significant, BW at birth was included as a covariant. Significance was at $P < .05$ unless otherwise noted.

RESULTS AND DISCUSSION

Calves were generally healthy throughout the study. One calf died at 12 d of age and was not replaced. Forty calves scoured for ≥1 d during the study. Mean scours score was 2.5 (SE = .1) for calves with scours (i.e., scours score ≥2) during the study and was unrelated to treatment.

Serum Ig and Protein

Colostrum contained a large quantity of IgG, IgM, and TI. Mean consumption of IgG and IgM in the first two feedings was 173.7 and 7.2 g, respectively (Table 1). Amount of colostral TI in the first two feedings was equivalent to 1.14 g of trypsin inhibited (SE = .04). Mean age at first feeding of calves fed as soon as possible after birth was .65 h (SE = .08).

Concentrations of IgG and IgM in serum at 0 h were uniformly below the minimum standards of the assay. Serum IgG and IgM increased to 24 h; thereafter, serum IgG remained constant, and IgM declined slightly (Table 2). Concentrations of serum IgG and IgM were increased when soybean TI was fed, indicating that TI, or reduction in availability of trypsin, affected absorption of both IgG and IgM. This result supported the data of Jensen and Pedersen (10), who reported increased serum IgG and IgA in piglets fed sow milk supplemented with porcine Ig and sow colostral TI. Also, Carlsson et al. (4) reported reduced Ig absorption when TI was removed from sow colostrum by addition of porcine trypsin. Our data suggested that supplementation of TI in bovine colostrum might improve Ig absorption in calves. However, TI in colostrum or secretion of trypsin by the pancreas was not likely to have been primarily responsible for cessation of intestinal Ig absorption. Carlsson et al. (4) reported that large quantities of protein were absorbed by piglets when no TI was fed and concluded that other factors were involved in intestinal protein absorption. Absorption of Ig from calves fed no added TI was excellent; serum IgG at 24 h exceeded 27 g/L.

Age at first feeding did not affect serum IgG or IgM concentrations in this study, nor were interactions of age × TI significant. Michanek et al. (13) reported no reduction in transmission of Ig when colostrum feeding was

TABLE 1. Descriptive statistics of variables used in analyses.

Item[1]	Minimum	Maximum	$\overline{X}$	SE
Serum IgG, g/L				
12 h[2]	5.5	48.1	22.4	1.3
24 h	6.2	55.4	32.4	1.5
48 h	7.7	51.2	31.2	1.5
Serum IgM, g/L				
12 h	.5	4.0	2.2	.1
24 h	.7	8.3	3.2	.3
48 h	.7	5.6	2.6	.2
Ig Intake, g				
IgG				
First feeding	25.7	168.7	86.9	4.3
Second feeding	51.4	337.4	173.7	8.6
IgM				
First feeding	1.3	8.2	3.6	.2
Second feeding	2.5	16.3	7.2	.5
TI Intake[3]				
First feeding	.24	.84	.57	.02
Second feeding	.48	1.68	1.14	.04

[1]n = 48 (except serum IgG at 24 h, n = 47).

[2]Hours after first feeding.

[3]Grams of trypsin inhibited by trypsin inhibitor (TI) in colostrum.

delayed up to 8 h after birth. Conversely, Stott et al. (19) reported that increasing age at first feeding reduced the amount of Ig absorbed by calves as early as 4 h after birth.

Total serum protein concentration tended ($P < .10$) to increase with addition of soybean TI (Table 2). Addition of 2 g of soybean TI to colostrum of calves increased total protein intake by 4.5% in the first 24 h. The trend for increased concentration of total protein in serum might have resulted from increased absorption of Ig and soybean TI proteins, particularly at 24 and 48 h after the first feeding. Mean total serum protein concentration at 24 h after feeding was similar to that reported by Tennant et al. (20) for Jersey calves and higher than that for Holstein calves.

Jensen and Pedersen (10) reported increased concentration of IgG and IgA in pigs fed TI, but differences were not significant for serum IgM. Brock et al. (2) reported that IgG was more sensitive to proteolysis by trypsin than IgM, which was relatively resistant to tryptic digestion. However, chymotrypsin markedly reduced bactericidal activity of IgM, but did not affect IgG. Because chymotrypsin is not inhibited by bovine colostral TI (14), the degree of destruction of IgM may be increased. Brown and Perry (3) reported that chymotrypsin was measurable in the small intestine of lambs at 1 d of age. Large amounts of soybean TI added to colostrum in this study (2 g in two feedings) may have reduced available chymotrypsin, thereby increasing concentration of IgM in serum. Soybean TI is capable of inactivating bovine chymotrypsin and several other enzymes (12), although the degree of inhibition is less than other chymotrypsin inhibitors.

Regression analysis was used to determine more completely the relationship between serum IgG and consumption of colostral IgG and TI. Independent variables used in the regression were intake of colostral TI and IgG, added soybean TI, and time of sampling (12, 24, and 48 h after the first feeding). Variables were transformed to natural logarithms, and two-way interactions were included in the model to

TABLE 2. Concentration of serum IgG, IgM, and total protein in Jersey calves fed colostrum with or without additional soybean trypsin inhibitor (TI).

	Treatment[1]					Contrast[2]		
Item[3]	–TI, 0 h	+TI, 0 h	–TI, 12 h	+TI, 12 h	SE	1	2	3
Serum IgG, g/L						NS[4]	*	NS
12 h[5]	19.2	23.4	21.7	25.2	1.9			
24 h	27.9	34.4	32.3	32.3	2.4			
48 h	29.1	35.3	27.7	33.1	2.4			
Serum IgM, g/L						NS	*	NS
12 h	1.9	2.5	1.9	2.2	.2			
24 h	2.7	4.2	2.9	3.1	.5			
48 h	2.1	3.3	2.3	2.6	.4			
Serum protein, g/L						NS	†	NS
12 h	62.1	64.7	63.5	63.1	2.4			
24 h	73.2	75.2	70.8	77.5	2.6			
48 h	70.8	76.5	68.4	74.4	2.5			

[1]Treatment: –TI = 0 g of TI in the first two feedings of colostrum, +TI = 1 g of TI in each feeding of colostrum; 0 h = initial feeding at .65 h of age, 12 h = initial feeding at 12 h of age.

[2]Contrasts: 1 = effect of age of first feeding, 2 = effect of TI addition, and 3 = interaction.

[3]Means were covariantly adjusted for intake of colostral Ig and TI.

[4]$P > .10$.

[5]Hours after first feeding.

†$P < .10$.

*$P < .05$.

improve regression r^2. Significant independent variables in the regression included intakes of colostral TI and IgG, intake of soybean TI, and time of sampling. Interactions of colostral TI intake × colostral IgG intake and colostral TI × time were significant (r^2 = .71); predicted serum concentrations of IgG are in Figure 1.

Serum IgG concentration increased as intake of colostral TI and IgG increased at 12 and 24 h after first feeding (Figure 1). A positive relationship between serum IgG and colostral IgG and TI concentrations has also been reported for swine (9). The curvilinear increase in serum IgG as colostral TI increased at 100 and 150 g of IgG intake indicated reduced efficiency of Ig absorption. Colostral TI might have fully inhibited trypsin in these calves, or serum IgG absorption might have been maximized. However, addition of 1.5 g of soybean TI (g of trypsin inhibited) increased serum IgG concentration from 1 to 6 g/L, depending on the amount of IgG consumed and the time of sampling. These data suggest that the mode of action of soybean TI might have differed from that of colostral TI and that absorption of IgG was not maximized at the intake observed in this study. Soybean TI might have acted by inhibiting chymotrypsin, and colostral TI was primarily involved with inhibition of trypsin. Addition of 3 g of soybean TI had no effect on serum IgG concentration greater than that observed with the addition of 1.5 g (data not shown). Also, serum IgG concentrations and effects of added soybean TI did not differ from 24 to 48 h after first feeding.

Efficacy of addition of soybean TI to increase serum IgG was lower than colostral TI. For example, consumption of 100 g of IgG and 1.5 g of colostral TI resulted in a serum IgG concentration of 33.2 g/L at 12 h (Figure 1a) or 22.1 g/L per gram of colostral TI consumed. Addition of 1.5 g of soybean TI increased serum IgG to 38.3 g/L, or an additional 3.4 g/L per gram of soybean TI consumed. Across all colostral Ig and TI intakes, the serum concentration of IgG was increased from 1.5 to 3.5 g/L per gram of soybean TI consumed. Therefore, soybean TI was only 6 to 24% as effective as colostral TI in increasing serum IgG concentration. Differences may partially have been due to the reduced partial efficiency of soybean TI when added to colostrum already containing maternal TI.

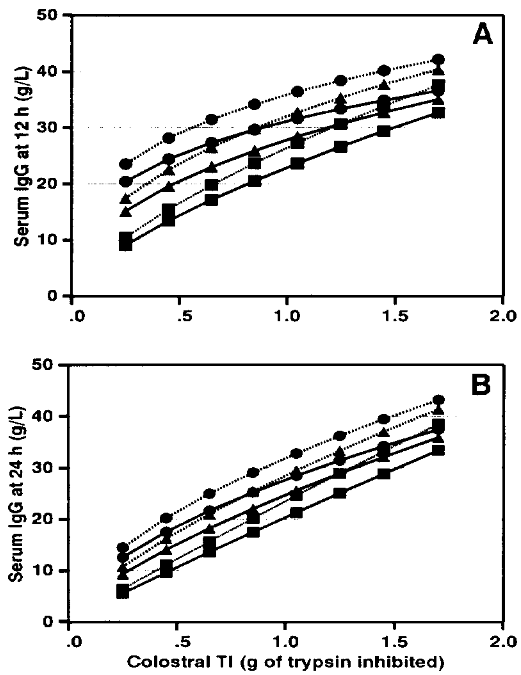

Figure 1. Predicted concentration of serum IgG at 12 h (A) and 24 h (B) after first feeding of colostrum as affected by consumption of colostral trypsin inhibitor (TI) and IgG. Consumption of 50 (■), 100 (▲), and 150 (●) g of IgG and addition of 0 (—) and 1.5 (---) g of soybean TI (g of trypsin inhibited).

A positive regression coefficient for colostral TI × time of sampling indicated that colostral TI was more effective in increasing serum IgG when serum was sampled at later ages (24 or 48 h after first feeding), as indicated by slopes and intercepts in Figure 1. Serum IgG concentrations at 12 h were generally greater than IgG concentrations at 24 or 48 h at the same IgG intake. However, serum IgG concentrations at 150 g of IgG intake and 1.7 g of colostral TI intake, which resulted in the highest observed serum IgG, did not differ by time of sampling and averaged 37.0 g/L. The more linear slopes at 24 h across all IgG intakes suggest that colostral TI was more important to promoting adequate transfer of passive immunity at the second feeding than at the first feeding. Secretion of pancreatic trypsin might be initiated by the first feeding; by 12 h after the first feeding, concentrations of pancreatic proteases might be sufficient to affect IgG absorption.

Intake and BW Gain

Milk replacer CP was 17.6% of DM. Calf starter CP and NDF were 18.8 and 24.4%, respectively, and hay averaged 11.2% CP and 78.3% NDF.

Although calves were assigned randomly to treatment within block, BW at birth tended ($P < .10$) to be affected by an interaction of TI × time of feeding (Table 3). The BW at 28 d, average daily BW gain, and feed efficiency were greater when calves were fed soybean TI at 0 h of age or were fed at 12 h of age with no added soybean TI (Table 3). Improvements in BW gain and feed efficiency were unaffected by intake of nutrients; no intake measure differed by treatment. The number of days scouring was lower in calves fed soybean TI at 0 h and not fed soybean TI at 12 h, which might have contributed to improved BW gain and feed efficiency.

Colostrum contains several nonspecific antimicrobial compounds, including complement, lactoferrin, lysozyme, and components of the lactoperoxidase system (15). Many of these nonspecific antimicrobial factors are susceptible to proteolytic degradation by trypsin (2). Addition of soybean TI may be beneficial not only by protecting Ig, but also by protecting these nonspecific antimicrobial factors from proteolytic degradation.

CONCLUSIONS

Addition of 1 g of soybean TI to 1 L of colostrum at each of the first two feedings improved absorption of IgG and IgM in Jersey calves, as indicated by greater serum Ig concentrations at 12, 24, and 48 h of age. However, soybean TI was not as effective as colostral TI in increasing concentration of Ig in serum. The expense of commercially available soybean TI ($29/g) precludes widespread adoption of this practice, although increases in concentrations of IgG and IgM in serum were significant.

TABLE 3. Least squares means of BW, average daily BW gain (ADG), intake, and feed efficiency in Jersey calves fed colostrum with or without additional soybean trypsin inhibitor (TI).

Item	Treatment[1] –TI, 0 h	+TI, 0 h	–TI, 12 h	+TI, 12 h	SE	Contrasts[2] 1	2	3
BW at birth, kg	22.5	24.5	23.5	22.3	.9	NS[3]	NS	†
BW at 28 d,[4] kg	25.0	26.6	26.4	25.1	.6	NS	NS	*
ADG 0 to 28 d,[4] g	64	121	113	67	22	NS	NS	*
Days scouring[5]	5.9	3.4	4.8	6.9	.9	NS	NS	*
Scours score[6]	2.5	2.5	2.7	2.3	.1	NS	NS	NS
Intake,[4] g/d								
Starter DM	65	94	64	70	19	NS	NS	NS
Replacer DM	220	220	220	220	0	NS	NS	NS
Hay DM	75	75	78	90	5	NS	NS	NS
Total DM	359	389	362	380	18	NS	NS	NS
CP	59	65	60	62	3	NS	NS	NS
ADG:DMI,[4] g/kg	181	305	302	164	50	NS	NS	*

[1]Treatment: –TI = 0 g of TI in the first two feedings of colostrum, +TI = 1 g of TI in each feeding of colostrum; 0 h = initial feeding at .65 h of age, 12 h = initial feeding at 12 h of age.

[2]Contrasts: 1 = effect of age of first feeding, 2 = effect of TI addition, and 3 = interaction.

[3]$P > .10$.

[4]Covariantly adjusted for BW at birth.

[5]Number of days with scours score ≥2, n = 47.

[6]Mean scours score (2 = slight scours, 3 = moderate scours, and 4 = severe scours) for 40 calves with score ≥2 for ≥1 d.

†$P < .10$.

*$P < .05$.

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