Concentrations of Trypsin Inhibitor and Immunoglobulins in Colostrum of Jersey Cows

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ABSTRACT

Colostrum samples from 49 Jersey cows were analyzed for concentrations of trypsin inhibitor, IgG, IgM, IgA, TS, fat, specific gravity, and N fractions. Colostrum (100 ml) was sampled from each cow as soon as possible after parturition. Mean concentrations of IgG, IgM, and IgA were 84.6, 3.4, and 4.5 g/L, respectively. Mean concentration of trypsin inhibitor was 56 mg of trypsin inhibited/dl of colostrum. Concentration of trypsin inhibitor was unaffected by lactation number and averaged 60, 53, and 54 mg of trypsin inhibited/dl of colostrum for cows in first, second, and third or later lactations, respectively. Colostral trypsin inhibitor and IgG were correlated (.54), although correlations between trypsin inhibitor and IgM and IgA were not significant. Trypsin inhibitor in colostrum was also positively correlated with fat, total N, protein N, noncasein N, and TS in colostrum. Variation in concentration of trypsin inhibitor from first-milking colostrum was closely related to colostral IgG concentration and may serve to protect IgG and other proteins from proteolytic degradation in the intestine of the neonatal calf.

(**Key words**: colostrum, immunoglobulin, trypsin inhibitor, Jersey)

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

INTRODUCTION

The neonatal calf must consume an adequate mass of colostral Ig prior to cessation of intestinal transport of macromolecules to ensure the acquisition of passive immunity (4). Colostrums of animal species that rely on colostral transfer of immunity contain protease inhibitors (7, 10, 17), presumably to protect Ig from proteolytic cleavage and to allow absorption of the intact molecule. Bovine colostrum contains large amounts of trypsin inhibitor (TI); colostrum from first milking contains nearly 100 times the amount of TI in milk (5, 17).

Bovine colostral TI is most active against bovine and rat trypsin but is inactive against chymotrypsin and several other proteolytic enzymes (10). Bovine IgG is most sensitive to tryptic digestion (2), so TI may serve to protect IgG without completely inhibiting proteolysis.

Concentration of Ig in bovine colostrum from the first milking is variable (11, 13). The relationship between porcine colostral Ig and TI is positive (6); Piñeiro et al. (10) reported a close relationship between bovine Ig and TI in colostral whey that was taken from cows during the first eight milkings postpartum. Whether variation in Ig content of bovine colostrum is related to similar variation in colostral TI activity, particularly in colostrum from the first milking, is unknown; therefore, our objective was to measure TI and Ig in colostrum from the first milking of Jersey cows and to determine relationships between TI and other colostral constituents.

MATERIALS AND METHODS

Experimental Design

Colostrum from Jersey cows (n = 49) was sampled as soon as possible after parturition. Jersey cows from the Dairy Experiment Station (Lewisburg, TN) were housed on pasture until shortly after parturition. Calves were separated from the dam prior to nursing, and the cows were completely hand-milked. Volume of colostrum collected was measured.

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Cows were observed to ensure that calves did not suckle prior to sample collection. A sample of colostrum (100 ml) was collected and transported to the laboratory.

Sampling and Analysis

Colostrum was allowed to cool to 20°C, and specific gravity was measured using a hydrometer. Colostrum was then divided into two 50-ml samples. One sample was refrigerated prior to analysis of fat by the Babcock method (1). The second sample was frozen (–20°C) prior to analysis of IgG, IgM, and IgA by single radial immunodiffusion (VMRD, Inc., Pullman, WA), total N (1), NPN, casein N (15), and TS. Samples were warmed to 30°C in a water bath and then dried at 100°C overnight to measure TS. Samples were cooled to room temperature (25°C) in a desiccator and weighed to the nearest .1 mg. Concentration of Ig in colostrum was determined on whole colostrum (IgM and IgA) or colostrum diluted 1:10 (vol/vol) in PBS (IgG). Whole colostrum was used instead of whey samples to reduce the variability in the assay (3). Colostral TI activity was measured by a modification of the method of Sandholm et al. (18) 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; 4-mm wells were bored using a pipette. Frozen colostrum was warmed to 37°C and mixed, and 1-ml samples were diluted 1:5, 1:10, and 1:20 (vol/vol) with PBS. One milliliter of diluted colostrum was added to 1 ml of trypsin solution [300 mg of trypsin (Sigma Chemical Co., St. Louis, MO) dissolved in 100 ml of 1 m*M* HCl and diluted 1:100 (vol/vol) in PBS] and mixed gently. Following equilibration for 5 min at room temperature, 15 μl of colostrum and trypsin mixture were added to each well. Plates were allowed to incubate for 22 h at room temperature and then were overlaid with 3% (vol/vol) acetic acid 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 (concentrations of subsequent dilutions were dependent upon results from initial dilutions) to determine the dilution at which the added trypsin was no longer inhibited by TI in colostrum (i.e., a measurable ring was observed).

TABLE 1. Constituents of colostrum from Jersey cows.

Item[1]	Minimum	Maximum	$\bar{X}$	SD
Ig, g/L				
Total	27.9	177.8	92.5	31.2
IgG	25.7	168.7	84.6	29.6
IgM	1.3	8.2	3.4	1.5
IgA	.6	13.4	4.5	2.8
TI[2]	24	84	56	2
TS, %	14.3	31.4	22.6	4.1
Specific gravity	1.044	1.080	1.057	.009
Fat, %	.30	9.50	4.00	2.31
N, g/L				
Total	9.44	33.40	22.79	4.58
NPN	.46	1.02	.67	.12
Protein[3]	8.94	32.38	22.12	4.55
Casein	3.88	10.43	7.39	1.59
Noncasein[4]	5.56	25.93	15.40	4.16

[1]n = 49; except fat, n = 47.

[2]TI = Trypsin inhibitor, milligrams of trypsin inhibited per deciliter of colostrum.

[3]Total N minus NPN.

[4]Total N minus casein N.

Statistical Analysis

Relationships between colostral Ig concentration and colostral components were determined by correlation and regression analyses.

RESULTS AND DISCUSSION

Concentration of total Ig in colostrum averaged 92.5 g/L and ranged from 27.9 to 177.8 g/L (Table 1). This concentration is higher than the mean Ig concentration we (13) recently reported in colostrum from cows in the same herd, but similar to data reported by Muller and Ellinger (8). Increased concentration of total Ig was due to higher concentrations of IgG and IgA and may have resulted from initiation of a vaccination program during the dry period of cows in the current study (16).

Concentrations of other colostral constituents, including TS and specific gravity (Table 1), were similar to our previous report (13); however, total N and noncasein N were somewhat higher in the present study, indicating higher Ig concentration.

Mean TI in colostrum was 56 mg of trypsin inhibited/dl of colostrum (Table 1). This mean compares favorably with data of Honkanen-Buzalski and Sandholm (5) and Sandholm and Honkanen-Buzalski (17), who reported that colostrum from the first milking contained approximately 14 to 100 mg of trypsin inhibited/dl.

Mean TI concentrations were unaffected by lactation number in this study and were 60, 53, and 54 (SE = 4) mg of trypsin inhibited/dl of colostrum from cows in their first, second, or third and later lactations, respectively. Volume of colostrum produced did not influence the concentration of TI in colostrum; volume of colostrum produced ranged from 1 to 10 L; mean was 4.1 L (SE = 1.7).

Colostral TI was positively related to colostral total Ig, total N, protein N, noncasein N, fat, and TS (Table 2). The correlations were highest with total N and protein N (r = .70) and TS (r = .66). Apparently, most TI in colostrum is associated with the noncasein fraction, because the correlation of TI and casein N was lower than that of TI and noncasein N.

Colostrum contains at least two distinct TI. The first TI fraction has a molecular weight of approximately 13,000 and is unique to colostrum (5). The larger TI fraction (molecular weight, 70,000) is also present in mastitic milk and probably protects mammary tissue and milk proteins from proteolysis by proteins of leukocytes (5). The TI content of milk, which is highly correlated with indices of intramammary infection, has been proposed as a test for mastitis (5). In a study by Honkanen-Buzalski and Sandholm (5), the total TI in colostrum from 3 cows was approximately 50% from each TI fraction; however, the degree of mastitis was not reported. In the current study, the proportion of each TI fraction to total TI in colostrum was not determined. To estimate whether the variation in colostral TI concentration was related to incidence of mastitis, samples of transition milk were collected from each quarter of cows at 3 d postpartum and cultured in duplicate as described previously (9). Most cows (n = 36) were treated 14 d prior to expected parturition with a commercially available intramammary antibiotic. Six of 172 quarter samples collected (samples from 6 cows were not collected) were positive for culturable organisms (9). Concentration of TI in colostrum from these cows was somewhat higher than from most other cows (Figure 1), although the correlation of bacterial counts and TI in colostrum was not significant (r = .25; $P < .09$). Milk samples from 28 cows were devoid of culturable bacteria, so numbers of cows with mastitis were small. The relationship between TI and IgG in colostrum from

TABLE 2. Correlation coefficients[1] of trypsin inhibitor (TI) and colostral Ig, N fractions, TS, specific gravity, and volume of colostrum produced.

	TI
IgG	.54*
IgM	.20
IgA	.12
Total Ig	.54*
Fat	.37*
Total N	.70*
Protein N	.70*
NPN	−.02
Casein N	.33*
Noncasein N	.64*
TS	.66*
Specific gravity	.45*
Volume	−.21

[1]n = 49; except fat, n = 47.

*$P < .05$.

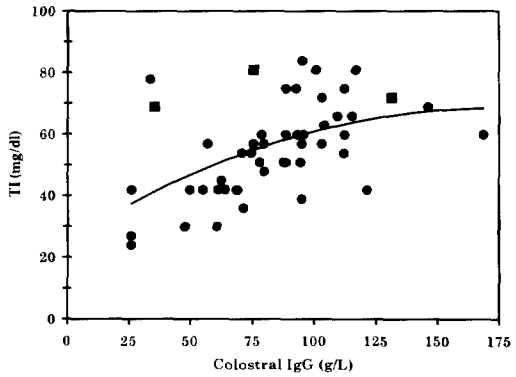

Figure 1. Relationship of IgG and trypsin inhibitor (TI) in colostrum from the first milking of Jersey cows. Squares indicate cows with bacterial counts ≥7500/ml in milk taken 3 d postpartum. Regression equation was TI = 17.7 + .65(IgG) − .002(IgG^2); R^2 = .33.

mastitic cows may differ from that of cows without mastitis.

The significant relationship between colostral TI and IgG (Figure 1) suggests that cows producing colostrum with larger amounts of IgG also increase the amount of TI to protect IgG from proteolytic degradation. The curvilinear relationship between IgG and TI in colostrum indicates that poor quality colostrum (IgG ≤50 g/L) contains increasingly small amounts of TI. Feeding of poor quality colostrum to neonatal calves may compromise the acquisition of passive immunity in two ways; not only does a fixed amount of poor quality colostrum provide a smaller mass of Ig, but the immune components in colostrum may be less well protected from proteolytic damage because of smaller amounts of colostral TI. It is not known whether the amount of TI provided in poor quality colostrum is adequate to protect the IgG in colostrum fully, but addition of soybean TI to colostrum did improve absorption of IgG and IgM (12). Colostrum also contains a number of nonspecific antimicrobial factors, including lysozyme, lactoferrin, components of the lactoperoxidase system, and others (14). Colostral TI may also protect these proteins from proteolytic degradation caused by trypsin secretion.

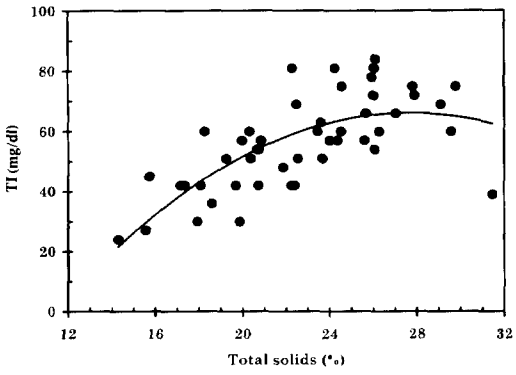

Figure 2. Relationship of TS and trypsin inhibitor (TI) in colostrum from the first milking of Jesey cows. Regression equation was TI = −124.84 + 13.81(TS) − .25(TS^2); R^2 = .53.

At colostral IgG concentrations ≥100 g/L, little increase in TI concentration occurred, suggesting a limited capacity for TI transfer into colostrum.

Concentration of TI in colostrum was most highly related to the amount of colostral TS (Figure 2). As the amount of TS increased, the amount of TI increased to approximately 28% TS. However, the number of observations >28% TS were small, so care must be used in interpreting this observation.

CONCLUSIONS

Trypsin inhibitor in colostrum from first milking of Jersey cows is positively related to the concentration of IgG and TS. Colostrum containing large amounts of Ig, N, and TS generally contains more TI, which probably serves to protect immune components from proteolytic degradation. Conversely, poor quality colostrum not only contains less IgG, but also less TI, which may further reduce transfer of passive immunity to neonatal calves.

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