



Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum

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ABSTRACT

Refractometry using a Brix refractometer has been proposed as a means to estimate IgG concentration in bovine maternal colostrum (MC). The refractometer has advantages over other methods of estimating IgG concentration in that the Brix refractometer is inexpensive, readily available, less fragile, and less sensitive to variation in colostrum temperature, season of the year and other factors. Samples of first-milking MC were collected from 7 dairy farms in Maine, New Hampshire, Vermont, and Connecticut ($n = 84$) and 1 dairy farm in California ($n = 99$). The MC was milked from the cow at 6.1 ± 5.6 h postparturition and a sample was evaluated for Brix percentage by using an optical refractometer. Two additional samples (30 mL) were collected from the milk bucket, placed in vials, and frozen before analysis of total IgG by radial immunodiffusion (RID) using commercially available plates and by turbidimetric immunoassay (TIA). The second sample was analyzed for total bacterial counts and coliform counts at laboratories in New York (Northeast samples) and California (California samples). The Brix percentage (mean \pm SD) was 23.8 ± 3.5 , IgG concentration measured by RID was 73.4 ± 26.2 g/L, and IgG concentration measured by TIA was 67.5 ± 25.0 g/L. The Brix percentage was highly correlated ($r = 0.75$) with IgG analyzed by RID. The Brix percentage cut point to define high- or low-quality colostrum (50 g of IgG/L measured by RID) that classified more samples correctly given the proportion of high- (86%) and low-quality (14%) samples in this study was 21%, which is slightly lower than other recent estimates of Brix measurements. At this cut point, the test sensitivity, specificity, positive and negative predictive values, and accuracy were 92.9, 65.5, 93.5, 63.3, and 88.5%,

respectively. Measurement of IgG by TIA correlated with Brix ($r = 0.63$) and RID ($r = 0.87$); however, TIA and RID methods of IgG measurement were not consistent throughout the range of samples tested. We conclude that Brix measurement of total solids in fresh MC is an inexpensive, rapid, and satisfactorily accurate method of estimating IgG concentration. A cut point of 21% Brix to estimate samples of MC >50 g/L was most appropriate for our data. Measurement of IgG in MC by TIA differed from measurement by RID.

Key words: colostrum, immunoglobulin, refractometer, validation

INTRODUCTION

Provision of an adequate mass of absorbable Ig from maternal colostrum (MC), particularly IgG, is essential to minimize the risk of failure of passive transfer of immunity in newborn ungulates, defined as a circulating IgG concentration <10 g/L in newborn calves after 24 h of age (Godden, 2008). The mass of IgG ingested is determined by both the volume of MC fed and IgG concentration. Other factors affecting circulating IgG concentration include the ability of the calf to absorb ingested IgG into the circulation, the movement of absorbed IgG from the circulation into extravascular compartments, and the metabolism of circulating IgG. These various factors are collectively calculated as apparent efficiency of absorption, which is an index of the mass of circulating IgG divided by the mass of ingested IgG (Quigley and Drewry, 1998).

Concentration of IgG in MC significantly affects the acquisition of passive immunity; thus, accurate measurement is essential to proper on-farm MC management. Concentration of IgG in MC is also highly variable (Foley and Otterby, 1978; Quigley et al., 1994; Gulliksen et al., 2008) and is not easily predicted on farm. According to the National Animal Health Monitoring System (2007) study of the US Department of Agriculture, only 13% of producers routinely evaluate the quality of MC, and 56% of those who estimate quality do so with methods such as visual inspection, which are prone to error.

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Measurement of specific gravity has been used to estimate colostrum IgG concentration (Fleenor and Stott, 1980); however, subsequent research has shown that this method of analysis, although rapid and inexpensive, depends on the temperature of MC (Mechor et al., 1991, 1992), breed, and season of the year (Morin et al., 2001). This increases the complexity of its use as an easy, rapid on-farm tool to estimate MC quality.

Brix percentage is a measure of sucrose concentrations in liquids such as fruit juice, molasses, and wine. When used in non-sucrose-containing liquids, Brix percentage approximates TS percentage. Moore et al. (2009) used a Brix refractometer to estimate TS in waste milk. Refractometry using the Brix refractometer has been evaluated as a means of estimating IgG concentration in the MC of sheep (Harker, 1978), horses (Chavatte et al., 1998; Cash, 1999), and cattle (Chigerwe et al., 2008; Biemann et al., 2010; Morrill et al., 2012b).

Radial immunodiffusion (**RID**) is a common method for analysis of IgG in MC and serum. However, RID is time consuming, expensive, and prone to errors in analysis, particularly with bovine MC (Fleenor and Stott, 1981). Turbidimetric immunoassay (**TIA**) has been proposed as a more rapid, lower cost method of measuring IgG in serum and plasma (Etzel et al., 1997). The TIA method was evaluated by others (Davis et al., 2005; McCue, 2007) and found to be an accurate method of analyzing IgG in serum. However, to our knowledge, reports on the use of TIA to measure IgG in MC are lacking. Our objectives were to evaluate the use of an inexpensive Brix refractometer to estimate IgG content of bovine MC and to compare RID and TIA methods of IgG analysis.

MATERIALS AND METHODS

Holstein cows on 7 dairies in the Northeast region of the United States (4 in New Hampshire, 1 each in Connecticut, Maine, and Vermont) ranging from 100 to 1,000 cows and on one 3,800-cow dairy in California were enrolled in the study between June and August 2011. Colostrum was collected from all primiparous and multiparous cattle from observed calvings on dairies in the Northeast. In California, MC was not collected from first-lactation animals or from cows calving between 1800 and 0400 h.

First-milking MC was collected at 6.1 ± 5.6 h after calving into a sanitized milk bucket, and 2 samples (30 mL each) were collected using a sterile syringe. Samples were placed into plastic vials labeled with a cow identification number and collection date and were frozen (-20°C) immediately. These samples were later used for bacterial culture and IgG determination. Time from birth to milking MC was recorded on all farms.

Volume of MC produced on the California farm was also recorded.

Percentage of solids in MC was measured from a third sample taken from the milk bucket by farm personnel by using an optical Brix refractometer (Model 300001; Sper Scientific, Scottsdale, AZ), with a range of 0 to 32% Brix. Frozen samples were shipped to the laboratory (APC Inc., Boone, IA) and IgG was analyzed by using a commercially available RID kit (Triple J Farms, Redmond, WA). Samples were diluted with 0.85% saline before analysis to ensure that sample ring diameters were within the range of standards. The kit used goat anti-bovine IgG against whole-molecule bovine IgG. Typical dilutions were 1:2 to 1:6. A sample was similarly diluted and analyzed for bovine IgG by TIA according to the method of Etzel et al. (1997). A Bovine Serum Calibrator (Midland Bioproducts Corporation, Boone, IA) was used as a standard for TIA. The calibrator (32.33 g of IgG/L) was serially diluted with 0.85% saline to obtain a standard curve. Total bacteria and coliform counts in colostrum were determined at Quality Milk Production Services (Ithaca, NY) for samples in the Northeast and at Sierra Dairy Labs (Tulare, CA) for samples in California. After MC samples were thawed and thoroughly mixed, serial 10-fold dilutions were made and a volume was pipetted onto agar plates for total plate count (**TPC**) and MacConkey agar for coliform counts (**COL**). Plates were incubated for 48 and 24 h for TPC and COL counts, respectively, and colonies were counted.

Data were collected and edited before analysis. Records with missing values were deleted. Two Brix values measured on farm above the maximum value for the instrument (32%) were deleted. Thus, a total of 183 records were included in the final data set. Pearson correlation coefficients were calculated between colostrum IgG by RID, colostrum IgG by TIA and Brix percentage, contamination measures, time of milking, and volume collected (California only).

Analysis of the Brix \times IgG concentration analyzed by RID and TIA was conducted using the MIXED procedure of SAS (Version 8; SAS Institute Inc., Cary, NC), with farm included in the model as a random effect to account for clustering of samples within farm. Break points for determining high-quality MC (assumed to be ≥ 50 g of IgG/L as measured by RID) using Brix percentage were evaluated by calculating sensitivity, specificity, accuracy, positive predictive value, and negative predictive value.

RESULTS AND DISCUSSION

Descriptive statistics of MC measurements are presented in Table 1. Mean time to first milking was 6.1

Table 1. Descriptive statistics of colostrum data used in analyses

Item	n	Mean	SD	Minimum	Maximum
Volume collected ¹ (L)	99	9.5	3.2	3.8	15.1
Time to milking ² (h)	176	6.1	5.6	0.1	24.0
Brix ³ (%)	183	23.8	3.5	12.0	32.0
IgG by RID ⁴ (g/L)	183	73.4	26.2	7.1	159.0
IgG by TIA ⁵ (g/L)	183	67.5	25.0	6.9	139.9
TPC ⁶ (log ₁₀ cfu/mL)	179	5.00	0.78	2.34	6.94
Coliforms ⁷ (log ₁₀ cfu/mL)	179	2.34	1.03	0.00	4.87

¹Volume of colostrum collected was measured only on samples collected at the California dairy.

²Time from delivery of calf to collection of colostrum.

³Colostrum Brix reading.

⁴Colostrum IgG by radial immunodiffusion (RID).

⁵Colostrum IgG by turbidimetric immunoassay (TIA).

⁶Total plate count (TPC) in colostrum.

⁷Coliform count in colostrum.

h and ranged from <1 h to 24 h. Mean IgG (measured by RID) in first-milking MC was similar to data reported by others (Gulliksen et al., 2008; Biemann et al., 2010; Morrill et al., 2012a). The range in observed IgG (measured by RID) was from 7.1 to 159 g/L, indicating the tremendous variability in IgG concentration of first-milking MC. The TPC and COL were similar to other reports in the literature for first-milking MC from commercial dairy farms (Houser et al., 2008; Elizondo-Salazar and Heinrichs, 2009; Morrill et al., 2012a) but were lower than others (Fecteau et al., 2002).

Frequency distributions of colostrum IgG measured by RID and TIA are presented in Figure 1. Both distributions appeared normally distributed, with the greatest number of samples between 50 and 89 g/L. Few samples (16% of observations) were <50 g of IgG/L. Others reported a greater proportion of samples containing <50 g of IgG/L (Gulliksen et al., 2008; Morrill et al., 2012a).

Fewer samples contained <50 g of IgG/L when measured by RID (n = 29) compared with TIA (n = 49). This contributed to a lower ($P < 0.01$) overall mean IgG concentration as measured by TIA.

Pearson product-moment correlation coefficients are shown in Table 2. Significant correlations included volume of MC produced and bacterial contamination, Brix and IgG (both RID and TIA), RID and TIA, and TPC counts and coliforms.

The frequency distribution of Brix percentages is shown in Figure 2. Only 13 samples measured <20% Brix, suggesting the data were not normally distributed. Biemann et al. (2010) also reported few samples <18% Brix, and Kehoe et al. (2007) reported a minimum TS of 18.3% in 55 samples of first-milking MC from dairies in Pennsylvania.

Mixed-model ANOVA using IgG (measured by RID) as the dependent variable with percentage of Brix and location as random effects indicated a highly significant

relationship. The solution for fixed effects was -61.896 ± 8.962 ($P < 0.001$) + $5.666 \pm 0.369 \times$ Brix percentage ($P < 0.001$). A graphical representation of the data, adjusted for random effects of location, is shown in Figure 3.

Calculation of the r^2 statistics in a linear mixed model is more complex than in a linear univariate model. The r^2 for a linear mixed model must account for the proportion of variation in the response explained by the fixed effects and the proportion explained by the random effects (Edwards et al., 2008). Several methods can be used to calculate an r^2 statistic; we used the likelihood ratio test method of Kramer (2005) to calculate the r^2 (0.81) of IgG (RID) compared with Brix.

Other researchers evaluating the relationship between IgG and Brix in MC have generally used linear regression, often ignoring the effects of clustering of data within herds. For the sake of comparison with these studies, we compared the r^2 calculated by linear regression, not adjusted for the random effect of location. This r^2 (0.56) was similar to the reports of Biemann et al. (2010) for optical and digital Brix refractometers (0.51 and 0.53, respectively) but was higher than that of Chigerwe et al. (2008), who reported an r^2 of 0.41. Other published estimates of the relationship between Brix and IgG are shown in Table 3. Morrill et al. (2012b) reported an r^2 of 0.53 between the refractive index (**nD**) of whole MC and IgG. The prediction of Brix percentage based on nD is polynomial (International Commission for Uniform Methods of Sugar Analysis, 2009); however, within the range of 0 to 32% Brix, the relationship is almost linear (r^2 of linear regression of nD and predicted Brix = 0.9995). Thus, the regression of nD and IgG reported by Morrill et al. (2012b) is similar to the reports of others, including our data.

The mixed-model analysis using IgG measured by TIA as the dependent variable and Brix percentage and location as random variables was also highly significant

Table 2. Pearson correlation coefficients for selected variables¹

Variable	Time	Brix	RID	TIA	TPC	COL
Volume	-0.02	-0.00	-0.05	-0.04	-0.24**	-0.19*
Time		-0.04	-0.08	-0.08	0.14	-0.01
Brix			0.75**	0.63**	-0.14	-0.03
RID				0.87**	-0.09	-0.02
TIA					-0.07	-0.02
TPC						0.54**

¹Variable: volume = volume of colostrum collected (California cows only; n = 99); time = time (h) from calving to colostrum collection (n = 176); Brix = colostral Brix (%; n = 183); RID = colostral IgG by radial immunodiffusion (g/L; n = 183); TIA = colostral IgG by turbidimetric immunoassay (g/L; n = 183); TPC = total plate count in colostrum (log₁₀ cfu/mL; n = 179); COL = colostral coliform count (log₁₀ cfu/mL; n = 179).

* $P < 0.05$; ** $P < 0.01$.

($P < 0.0001$). The model was as follows: $-42.033 \pm 10.323 + 4.575 \pm 0.409 \times \text{Brix}$. Both parameters were significant ($P < 0.001$). A graphical representation of the data, adjusted for the random effect of location is presented in Figure 4. The estimated r^2 of the linear mixed model for IgG (measured by TIA) and Brix was 0.65 when using the method of Kramer (2005). This was lower than the r^2 of regression of IgG RID and Brix (0.81), suggesting a less significant linear relationship. The intercept of the regression between RID and TIA (Figure 5) differed from zero ($P < 0.01$) and the slope differed from one ($P < 0.01$), indicating a difference between methods in prediction of IgG.

Figure 6 contains regression lines for published estimates of colostral IgG (measured by RID) \times Brix. Our data are corrected for the random effect of location. Slopes of most linear regressions were similar, except for the regression by Biemann et al. (2010), which appears to have a greater slope and more negative intercept than other measures. Visual evaluation of Figure 6 suggests that the predicted IgG concentration based on Brix percentage is variable. For example, at 50 g of IgG/L, the predicted Brix percentage (indicative of a break point for high-quality MC) ranges from 18% (Morrill et al., 2012b) to 22% (Biemann et al., 2010). It is noteworthy that Biemann et al. (2010) reported

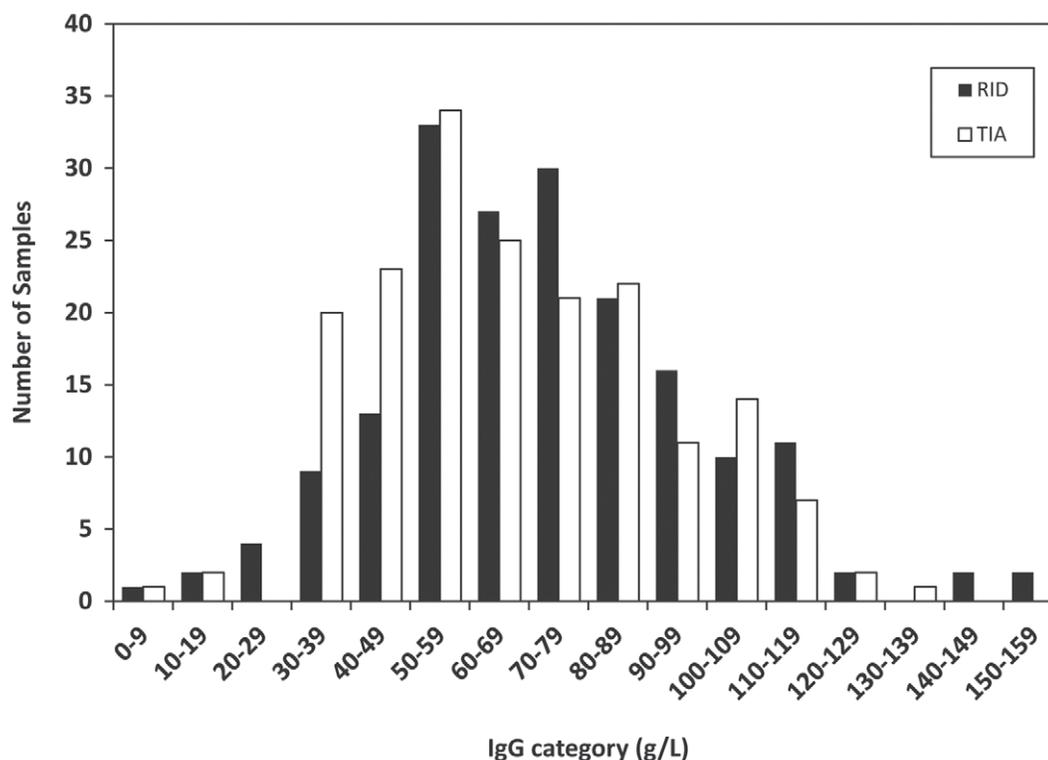


Figure 1. Frequency distribution of IgG concentration in first-milking colostrum from Holstein cattle (n = 183) measured by radial immunodiffusion (RID) or turbidimetric immunoassay (TIA).

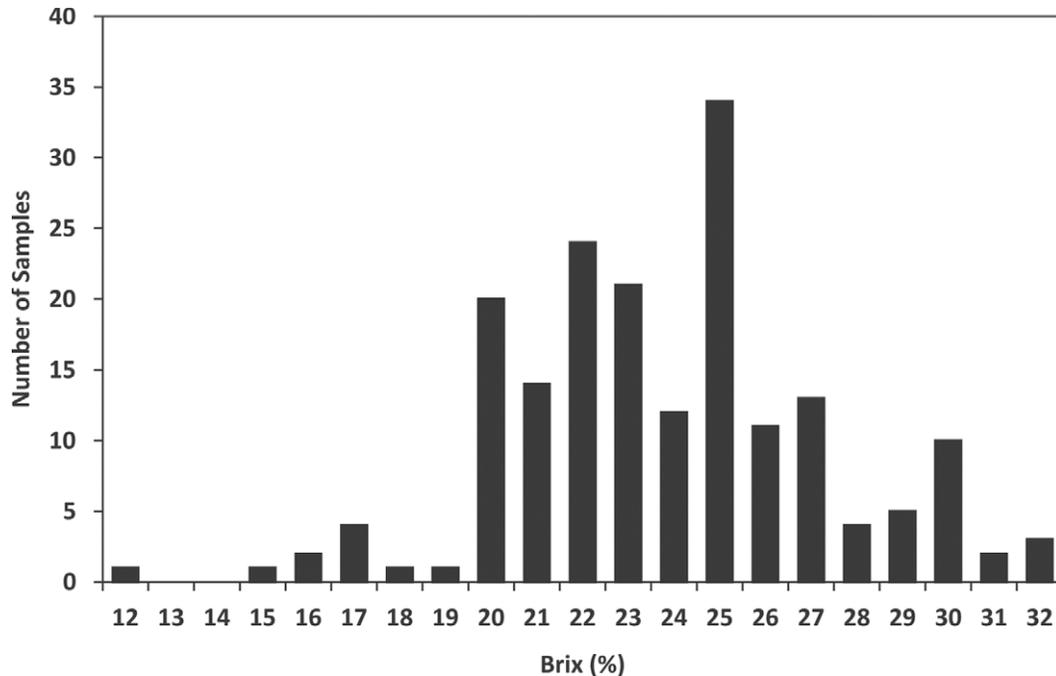


Figure 2. Frequency distribution of TS in maternal colostrum estimated by Brix refractometry (n = 183).

that only 8% of their samples had colostrum IgG <50 g/L. Data sets from the other studies contained 29 to 32% of observations with <50 g of IgG/L. These studies were relatively similar and predicted the break point for good-quality colostrum (50 g of IgG/L), which ranged from 18 to 20%.

Other methods used to estimate IgG concentration of MC include volume of MC produced (Pritchett et al.,

1994), color of MC (Argüello et al., 2005), and specific gravity (Fleener and Stott, 1980). The colostrometer is a widely used hydrometer to estimate IgG in first-milking MC. However, several studies (Pritchett et al., 1994; Quigley et al., 1994; Morin et al., 2001) reported a low r^2 when IgG (measured by RID) is regressed on colostrum specific gravity. The potential for MC color (measured by chromameter) to estimate IgG was evaluated by Argüello et al. (2005). They measured first- and second-milking samples from 1,084 dairy goats on 4 dairy farms in the Canary Islands. The IgG concentration of samples (measured by RID) ranged from <1 to approximately 42 g/L. The r^2 of linear regression

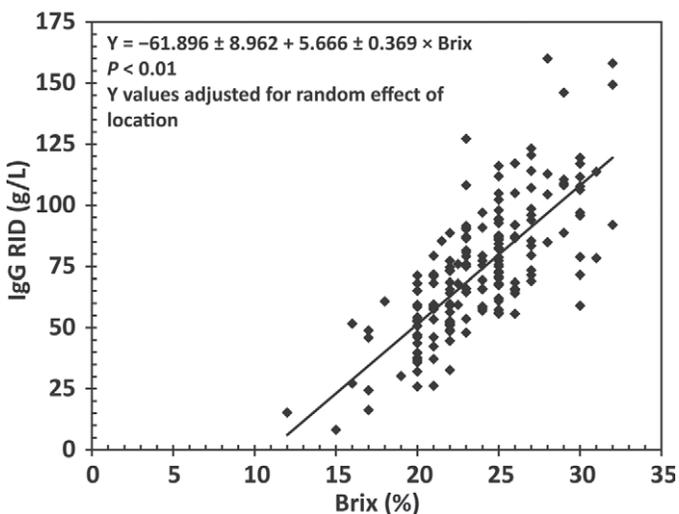


Figure 3. Relationship between Brix and IgG measurement [by radial immunodiffusion (RID)] of first-milking colostrum from Holstein cows on 8 farms (n = 183).

Table 3. Published comparisons of Brix refractometry and measurement of IgG by radial immunodiffusion

Reference	Species	n	r
Harker (1978) ¹	Ovine	NA ²	0.79
Molla (1980)	Bovine	NA	0.89
Chavatte et al. (1998)	Equine	20	0.85
Cash (1999)	Equine	66	0.94
Chigerwe et al. (2008)	Bovine	171	0.64
Bielmann et al. (2010)	Bovine	288	0.71
Morrill et al. (2012b) ³	Bovine	823	0.73
Current study ⁴	Bovine	183	0.75

¹Measured globulin protein by zinc sulfate turbidity.

²Not available.

³Morrill et al. (2012b) measured index of refraction (nD). Brix percentage was estimated using standard predictions based on nD.

⁴Correlation coefficient unadjusted for random effect of location.

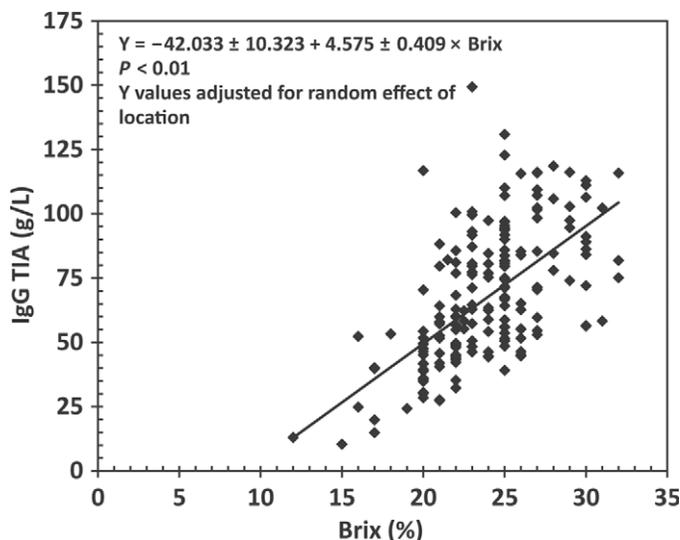


Figure 4. Relationship between Brix and IgG measurement [by turbidimetric immunoassay (TIA)] of first-milking colostrum from Holstein cows on 8 farms ($n = 183$).

between IgG and chroma of each sample was 0.70. To our knowledge, however, no evaluation of MC color and IgG has been undertaken with dairy cow MC.

Moore et al. (2005) reported that delaying collection of MC resulted in lower concentrations of IgG in first-milking MC. We evaluated the effect of time after calving when MC was collected (Figure 7). The IgG concentration of MC, measured by RID, was regressed on time after calving when MC was collected. The model included linear, quadratic, and cubic terms. Both the linear and quadratic terms were significant (P

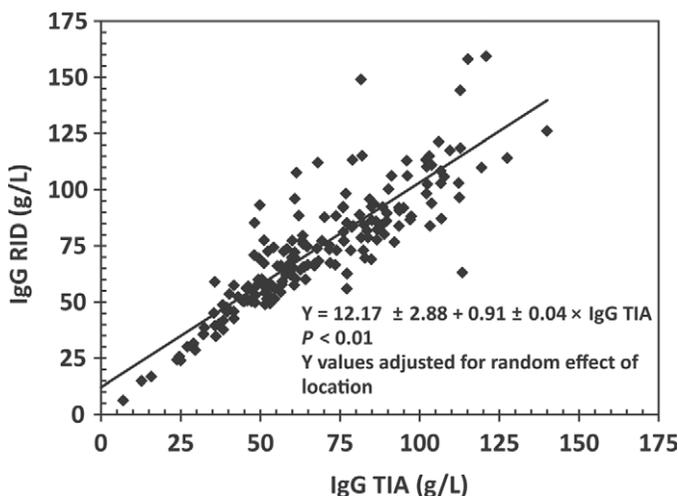


Figure 5. Regression of colostral IgG in first-milking bovine colostrum by radial immunodiffusion (RID) and turbidimetric immunoassay (TIA); $n = 183$.

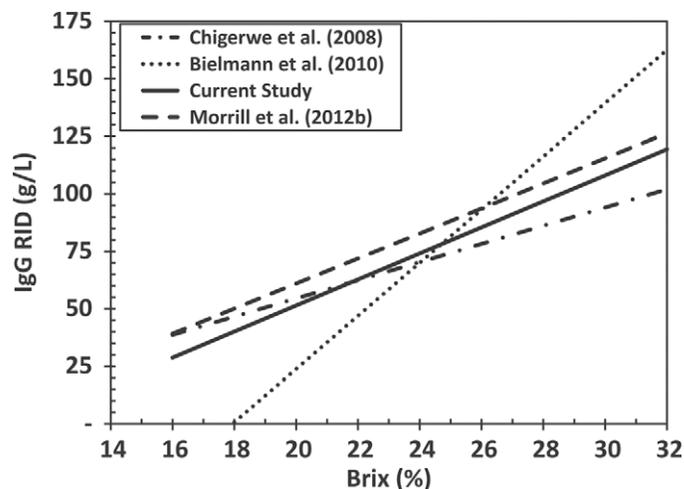


Figure 6. Comparison of regression lines for the current study and other published studies in cattle comparing IgG and Brix in first-milking bovine colostrum. RID = radial immunodiffusion.

< 0.02), indicating that time after calving was related to IgG concentration of MC. Figure 7 indicates that colostral IgG concentration was lower when collected after approximately 8 h postcalving. This differs from the result by Moore et al. (2005), who reported a linear decline in IgG concentration by 2 h of age. However, our study was observational in nature; therefore, a causal relationship between time after calving and IgG concentration may have been confounded by factors not measured in our study. We found no relationship between time after calving and volume of MC produced (California cows only; $n = 99$; $P > 0.10$). Cows in California produced an average of 9.5 L of MC in the first milking. Moore et al. (2005) did not report the volume of MC produced but did indicate no significant effect of time of collection and volume of MC produced.

Diagnostic test characteristics for using the Brix refractometer with different test break points are presented in Table 4. The highest accuracy was at 21% Brix, which is slightly lower than that reported by Biemann et al. (2010). Thus, we recommend that the break point of 21% Brix be used as a standard for determining when MC is > 50 g of IgG/L.

The TIA method of measuring IgG in bovine colostrum was highly correlated with the RID method, but differences were apparent between the 2 techniques, particularly in samples containing > 50 g of IgG/L. Because TIA measures turbidity of the solution, it is possible that the content of non-IgG components of MC, such as fat, may interfere with the assay. Morrill et al. (2012a) reported that fat content of MC from 531 first-milking MC samples collected from dairy farms in 12 US states contained an average of $5.6 \pm 3.2\%$ fat,

Table 4. Diagnostic test characteristics [mean and 95% CI (in parentheses)] for use of a Brix refractometer to predict that colostrum contains at least 50 g/L of IgG as measured by radial immunodiffusion

Cutoff value	Sensitivity (%)	Specificity (%)	PPV ¹ (%)	NPV ¹ (%)	Accuracy (%)	Test high/low ²
23	72.1 (65.0–79.2)	96.6 (89.9–103.2)	99.1 (97.4–100.8)	39.4 (28.1–50.8)	76.0 (69.8–82.1)	112/71
22	85.7 (80.2–91.2)	79.3 (64.6–94.1)	95.7 (92.2–99.1)	51.1 (36.5–65.7)	84.7 (79.5–89.9)	138/45
21	92.9 (88.8–96.9)	65.5 (48.2–82.8)	93.5 (89.5–97.4)	63.3 (46.1–80.6)	88.5 (83.9–93.1)	153/30
20	98.7 (96.9–100.5)	27.6 (11.3–43.9)	87.9 (83.0–92.7)	80.0 (55.2–104.8)	87.4 (82.6–92.2)	173/10
19	98.7 (96.9–100.5)	24.1 (8.6–39.7)	87.4 (82.4–92.3)	77.8 (50.6–104.9)	86.9 (82.0–91.8)	174/9
18	99.4 (98.1–100.6)	24.1 (8.6–39.7)	87.4 (82.4–92.3)	87.5 (64.6–110.4)	87.4 (82.6–92.2)	175/8

¹PPV = positive predictive value; NPV = negative predictive value.

²Number of samples declared high (≥ 50 g of IgG/L) or low (< 50 g/L) by Brix refractometry. Analysis by radial immunodiffusion indicated 154 and 29 samples were high and low, respectively.

with a range of 1.0 to 21.7%. The potential interference of fat may have been exacerbated in our study because dilutions of the initial sample of MC before TIA analysis were 1:2 or 1:4 with 0.85% saline. Effects of lipid on turbidity in first-milking MC are unknown but are potentially significant. Collin et al. (2002) reported the use of a nephelometric immunoassay for bovine milk and noted 2 potential disadvantages of light-scattering assays (including TIA) for analysis of bovine milk IgG concentration. These included the presence of lipid and CN micelles in milk, both of which may interfere with the assay. It is possible that the presence of large amounts of lipid, non-Ig proteins, or both could interfere with the formation of immune complexes, which would, in turn, cause underestimation of total IgG content in a sample. Their method used a 10- μ L sample of defatted milk diluted to 1,000 μ L. Thus, the contribution of lipid to overall error in their procedure would be much less than in the current study. However, the absolute

contribution of fat or non-Ig protein in our TIA method was not determined.

Fleenor and Stott (1981) recommended that whole MC, rather than fat-free MC or the whey fraction of MC, should be used in RID analyses. They further recommended that MC should not be diluted or that reference protein, standards, and unknowns should be diluted with identical volumes of solvent. One criticism of most RID and TIA assays for MC (including in this research) is that standards provided with the commercial kit are typically used. Most standards are derived from serum IgG, which contains a different ratio of IgG₁:IgG₂ than MC. Differences in subclasses may cause erroneous IgG quantification (Gapper et al., 2007). Generally, standards are not diluted with the same diluents used with unknown samples of MC. The extent to which analysis of MC may be affected by these analytical issues is currently unknown.

CONCLUSIONS

Brix refractometry provides an acceptable estimate of IgG in first-milking bovine colostrum. This method is inexpensive and rapid and requires minimal equipment and training. Evaluation of MC can be done at cow side, thereby improving the likelihood that producers can implement management decisions based on the test results. We recommend that when Brix refractometry is used, 21% Brix be considered the break point for high-quality (> 50 g of IgG/L) MC. The IgG concentration in first-milking MC was lower when collected at later time points after calving, consistent with other trials. Consistent with other studies, the volume of colostrum produced was not increased when collected at later time points after calving. Finally, using TIA to measure IgG in MC was related to RID, but regression estimates differed.

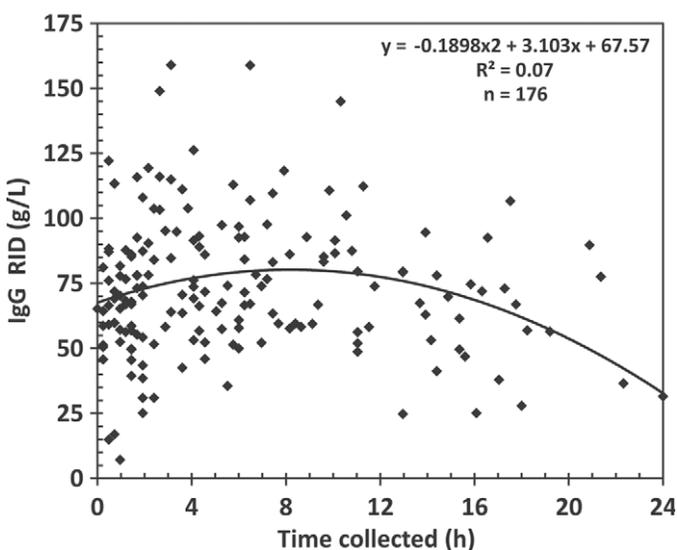


Figure 7. Concentration of IgG in first-milking maternal colostrum with time collected (time elapsed from calving until collection of colostrum). RID = radial immunodiffusion.

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