Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves

S. M. Deelen,[*1] **T. L. Ollivett,**[*] **D. M. Haines,**[†‡] **and K. E. Leslie**[*]
*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1
†Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B4
‡The Saskatoon Colostrum Co. Ltd., Saskatoon, Saskatchewan, Canada S7K 6A2

ABSTRACT

The objective of this study was to evaluate the utility of a digital Brix refractometer for the assessment of success of passive transfer of maternal immunoglobulin compared with the measurement of serum total protein (STP) by refractometry. Blood samples (n = 400) were collected from calves at 3 to 6 d of age. Serum IgG concentration was determined by radial immunodiffusion (RID), and STP and percentage Brix (%Brix) were determined using a digital refractometer. The mean IgG concentration was 24.1 g/L [standard deviation (SD) ± 10.0] with a range from 2.1 to 59.1 g/L. The mean STP concentration was 6.0 g/dL (SD ± 0.8) with a range from 4.4 to 8.8 g/dL. The mean %Brix concentration was 9.2% (SD ± 0.9) with a range of 7.3 to 12.4%. Brix percentage was highly correlated with IgG (r = 0.93). Test characteristics were calculated to assess failure of passive transfer (FPT; serum IgG <10 g/L). The sensitivity and specificity of STP at 5.5 g/dL were 76.3 and 94.4%, respectively. A receiver operating characteristic curve was created to plot the true positive rate against the false positive rate for consecutive %Brix values. The optimal combination of sensitivity (88.9%) and specificity (88.9%) was at 8.4% Brix. Serum total protein was also positively correlated with %Brix (r = 1.00) and IgG (r = 0.93). Dairy producers can successfully monitor their colostrum management and the overall success of passive transfer using a digital Brix refractometer to estimate IgG concentration of colostrum and calf serum.

Key words: passive transfer, refractometer, immunoglobulin, calf

INTRODUCTION

Newborn calves depend on the success of passive transfer of maternal IgG from colostrum for protection from infectious disease (Smith et al., 1964). Colostrum management is critical in successful calf rearing (Godden, 2008), and failure of passive transfer (**FPT**) of immunoglobulins is associated with increased morbidity and mortality (McEwan et al., 1970; Boyd, 1972; McGuire et al., 1976; Tyler et al., 1999). The radial immunodiffusion (**RID**) assay is the gold standard method for determining passive transfer by measuring the quantity of IgG in the serum; FPT is defined as a neonatal serum IgG concentration <10 g/L (Weaver et al., 2000; Godden, 2008). The RID assay is a laboratory procedure that requires trained laboratory technicians and approximately 18 to 24 h to determine the results. As such, RID is expensive and not conducive to on-farm application. Consequentially, alternative methods for rapid and simple monitoring of passive transfer are needed.

The evaluation of serum total protein (**STP**) by refractometry can be used to determine adequate passive transfer in calves (Calloway et al., 2002; Moore et al., 2009). Bovine colostrum consists of a mixture of lacteal secretions and constituents of blood, most notably immunoglobulins and other serum proteins. Refractometry using either a digital or optical refractometer provides an approximation of the serum immunoglobulin concentration, because immunoglobulins constitute a large proportion of the protein in neonatal calf serum (Calloway et al., 2002). Numerous reports in the literature have documented the relationship between STP and IgG (Weaver et al., 2000; Godden, 2008). Using serum IgG of <10 g/L as the standard for FPT, serum protein levels of <5.2 to 5.5 g/dL provide reasonable predictive value (Naylor and Kronfeld, 1977; Godden, 2008). Furthermore, STP can be successfully measured on samples that have not been previously centrifuged to harvest serum (Wallace et al., 2006). Thus, STP evaluation can be implemented as an on-farm monitoring program for colostrum management and as a predictor of calf health. However, only 2.1% of farms have implemented this approach for routine monitoring according to the 2007 National Animal Health Monitoring System report (NAHMS, 2007), suggesting that dairy produc-

Table 1. Descriptive statistics of serum samples used in analyses (n = 397)

Item	Mean	SD	Minimum	Maximum
Serum total protein (g/dL)	6.0	0.8	4.4	8.8
Brix (%)	9.2	0.9	7.3	12.4
IgG (g/L)	24.1	10.0	2.1	59.1

ers have not embraced this method as a tool to rapidly and accurately identify potential FPT calves.

Digital and optical refractometers can be used to evaluate colostrum quality (Bielmann et al., 2010; Morrill et al., 2012; Quigley et al., 2013). The instrument used is a Brix refractometer, which measures the sucrose concentration in liquids such as fruit juice, molasses, and wine. When used in non-sucrose-containing liquids, percentage Brix (**%Brix**) approximates the total solids percentage. Considerable utility exists in using the same instrument to evaluate colostrum quality and assess passive transfer in the calf. Recent work suggests that Brix refractometry of calf serum provides a strong estimate of IgG concentration. One report suggests that a value of <7.8% Brix may be used to identify FPT in 1-d-old calves (Morrill et al., 2013). However, that study did not compare results of the Brix instrument to measurement of STP on the same samples. As such, the objective of the present study was to evaluate the utility of the digital Brix refractometer for the assessment of success of passive transfer compared with the more commonly used method of assessment of STP.

MATERIALS AND METHODS

Calf Enrollment, Feeding, and Sampling

Holstein calves from 3 commercial dairy herds in southwestern Ontario, 1 herd at the University of Guelph Elora Dairy Research Center, and 1 herd at the University of Guelph Ponsonby Dairy Research Center were sampled between January and September 2012. This study was approved by the University of Guelph Animal Care Committee (Guelph, ON, Canada; Animal Utilization Protocol no. 1537), and performed according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Whole blood was collected from 3- to 6-d-old calves by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ), into a sterile, plastic, Vacutainer tube without anticoagulant (BD Vacutainer, Becton Dickinson and Co.). Samples (6 mL) were stored on ice for transportation to the University of Guelph. Within 4 to 6 h of collection, serum was separated by centrifugation at $1,500 \times g$ for 15 min at ~20°C. Aliquots of serum were collected and stored in triplicate at −80°C. One aliquot of each sample was shipped on ice to the Saskatoon Colostrum Company (Saskatoon, SK, Canada) for IgG testing.

During the sampling period, the postcolostral feeding protocol for 3 of the dairy herds was to provide neonatal calves with 3 L of whole unpasteurized milk twice a day, whereas 1 herd fed 2 L of whole unpasteurized milk 3 times a day, and 1 herd fed 5 L of 22:20 milk replacer (22% CP, 20% crude fat) twice a day.

STP, Brix, and RID Analyses

Samples were stored at −20°C until analyzed. Samples were allowed to thaw at room temperature, and analyses of STP and %Brix were performed using a standard digital refractometry instrument (PA202X-003-105, Misco, Cleveland, OH). Quantitative serum IgG concentration was performed using the RID assay essentially as described by Chelack et al. (1993). The antiserum to bovine IgG was from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). The standard curve was generated with a bovine serum calibrator from Midlands BioProducts Corp. (Boone, IA), and the species standard serum for plate validation was from The Center for Veterinary Biologics of the USDA (Ames, IA). Precipitin rings were measured using a computer-assisted RID plate reader from The Binding Site (Birmingham, UK).

Statistical Analysis

The STP and Brix refractometer results, in grams per deciliter and %Brix units, respectively, were plotted against the measured IgG content in grams per liter. From these distribution plots, correlation coefficients (r-values) were determined. These comparisons were performed between the STP and Brix refractometer scores and IgG determinations for all serum samples. The Brix refractometer scores were also evaluated against the digital refractometer STP measurements. Test characteristics (sensitivity and specificity) were calculated using Excel 2010 (Microsoft Corp., Redmond, WA). Sensitivity was defined as the probability of a test result indicative of FPT for a sample with IgG <10 g/L. Specificity was defined as the probability of a test result indicative of adequate passive transfer for a sample with IgG ≥10 g/L. A receiver operating charac-
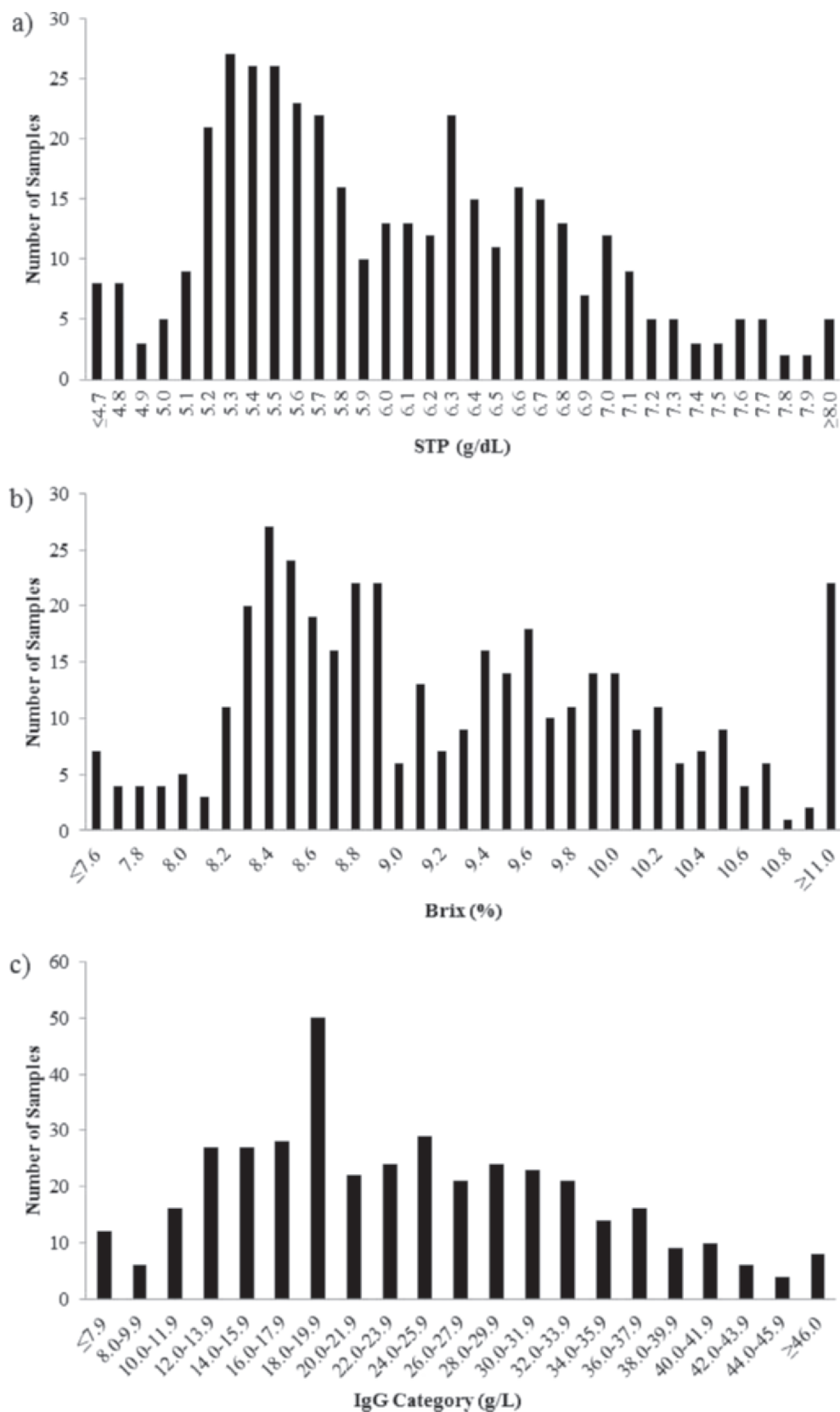
Figure 1. Frequency distributions of (a) serum total protein (STP), (b) percentage Brix (%Brix), and (c) serum IgG concentration measured by radial immunodiffusion assay (RID) for 397 Holstein dairy calves.

teristic curve was created to plot the true positive rate against the false positive rate at 0.1-percentage-unit Brix intervals from 7.3 to 8.8% Brix.

RESULTS AND DISCUSSION

A subset of 400 serum samples was randomly selected from a set of almost 1,000 samples that were collected from Holstein dairy calves between January and September 2012. Due to improper labeling of vials, 3 samples were excluded from the analyses. Descriptive statistics of serum sample measurements are shown in Table 1. The mean STP concentration was 6.0 g/dL (SD ± 0.8) with a range of 4.4 to 8.8 g/dL. The mean %Brix concentration was 9.2% (SD ± 0.9) with a range of 7.3 to 12.4%. The mean IgG concentration was 24.1 g/L (SD ± 10.0) with a range of 2.1 to 59.1 g/L. The frequency distributions of STP, %Brix, and IgG are shown in Figures 1a, 1b, and 1c, respectively. Distributions of STP and IgG appeared normally distributed. However, the distribution of %Brix was skewed to the right, such that only 58 samples measured <8.4%. It is noteworthy that 379 samples had IgG concentrations ≥10 g/L, consistent with adequate passive transfer. Only 18 samples had IgG concentrations <10 g/L, indicating FPT. The percentage of calves in this study with FPT (4.75%) was considerably less than previously reported in the literature (Wallace et al., 2006; NAHMS, 2007; Trotz-Williams, 2008; Windeyer et al., 2014). The calf serum utilized in this study was randomly selected from a serum bank of samples collected as part of a large clinical trial. The farms enrolled in this study included 2 research facilities and 3 well-managed commercial dairy farms. As such, it is clear that calves on these farms generally received an adequate volume of good quality colostrum after birth.

Correlations

The distributions of %Brix versus STP, STP versus IgG, and %Brix versus IgG for the 397 samples analyzed are plotted in Figures 2a, 2b, and 2c, respectively. From these plots, correlation coefficients were calculated. Serum total protein was positively correlated with %Brix (r = 1.00) and IgG (r = 0.93). This finding is different from the results of McBeath et al. (1971), where they found a moderate relationship between STP and IgG as measured by RID (r = 0.72). In the current study, %Brix was also positively correlated with IgG (r = 0.93). This correlation is similar, yet numerically higher, than that obtained by Morrill et al. (2013), who found the correlation between %Brix and serum IgG to be 0.87. The study by Morrill et al. (2013) did not compare results of the Brix instrument to measurement of STP on the same samples.
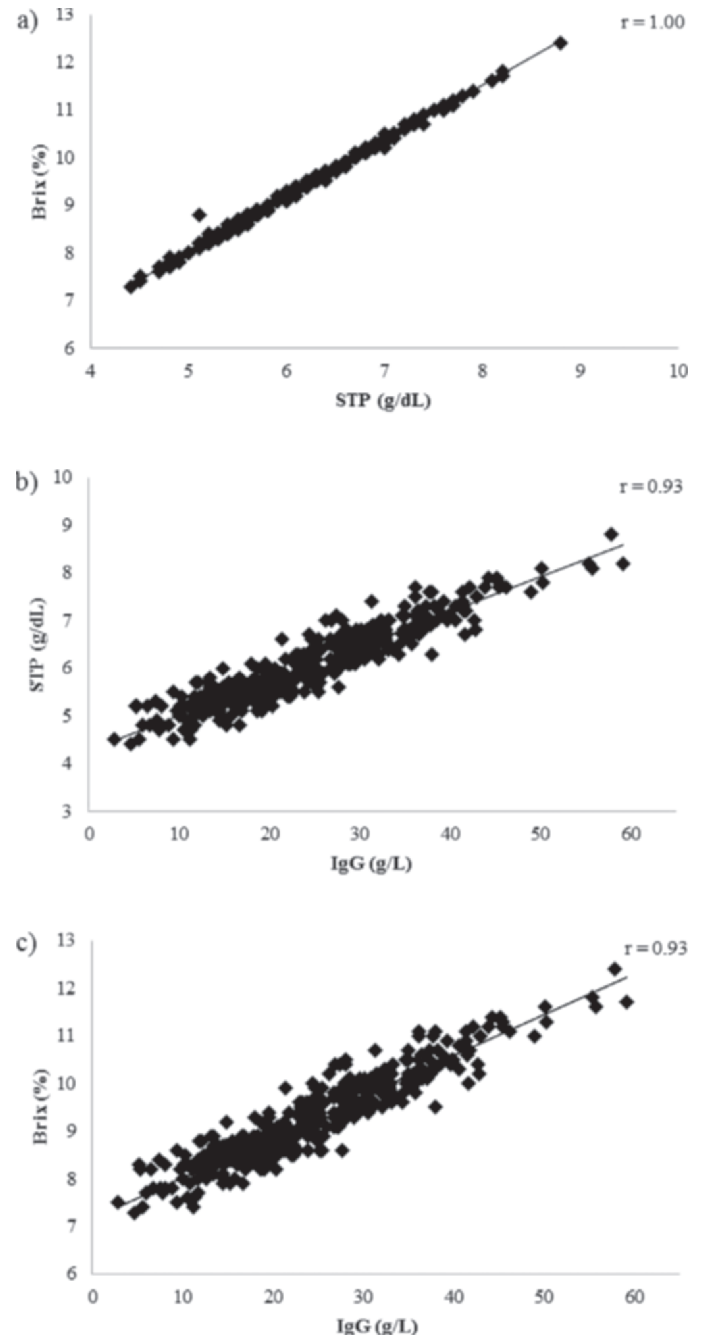
Figure 2. (a) Percentage Brix (%Brix), determined by digital refractometry, compared with serum total protein (STP) of calf serum (n = 397; r = 1.00); (b) serum IgG concentration, determined by radial immunodiffusion assay (RID), compared with STP of calf serum (n = 397; r = 0.93); (c) serum IgG concentration, determined by RID, compared with %Brix of calf serum (n = 397; r = 0.93).

Test Characteristics

Test characteristics of sensitivity and specificity for STP and %Brix were determined for the assessment of FPT (serum IgG <10 g/L). The sensitivity and speci-

Table 2. Test characteristics for serum total protein (STP) and percentage Brix, at previously published values chosen for optimal sensitivity and specificity for identification of samples with failure of passive transfer (IgG <10 g/L)

| Test characteristic (%)	STP (g/dL)			Brix (%)		
	5.2	5.5	5.7	7.8	8.0	8.3
Sensitivity	94.5	76.3	64.6	98.9	97.9	93.7
Specificity	66.7	94.4	100	38.9	61.1	77.8

ficity for STP and %Brix at previously recommended values are shown in Table 2. For example, the sensitivity and specificity of STP at 5.5 g/dL (Godden, 2008) were 76.3 and 94.4%, respectively. The sensitivity and specificity of %Brix at 7.8%, as recently reported by Morrill et al. (2013), were 98.9 and 38.9%, respectively. In comparison, the sensitivity and specificity of %Brix at 8.3% (Wenz, 2011) were 93.7 and 77.8%, respectively.

A receiver operating characteristic curve was created to plot the true positive rate against the false positive rate for %Brix at 0.1-percentage-unit intervals from 7.3 to 8.8% Brix (Figure 3). The best combination of sensitivity (88.9%) and specificity (88.9%) was at 8.4% Brix. The cut-point of 8.5% Brix yielded a sensitivity of 82.1% and a specificity of 94.4%; however, at this level, the probability of a test result indicative of FPT for a sample with IgG <10 g/L was less than that at 8.4% Brix.

Previous reports have evaluated Brix refractometers for estimating IgG in colostrum (Bielmann et al., 2010;

Morrill et al., 2012; Quigley et al., 2013). However, evaluation of adequate passive transfer of immunity in the serum of calves has typically been conducted using a refractometer specifically calibrated for STP. In the current study, the same refractometer was used to measure both %Brix and STP of serum. Both methods were shown to have equal correlation to IgG values, which suggests that the 2 methods are equal in efficacy in the samples tested. As such, it is practical and beneficial for producers to purchase a Brix refractometer, rather than a specific STP refractometer, to estimate IgG concentration of both maternal colostrum and calf serum. This approach would allow producers to monitor both colostrum quality and success of passive transfer at the herd level using one instrument.

Using refractometry of serum to estimate passive transfer has many advantages compared with measuring IgG. The RID assay for IgG has a long incubation time (18 to 24 h) and is time consuming and expensive, all of which limit its on-farm application. Digital re-
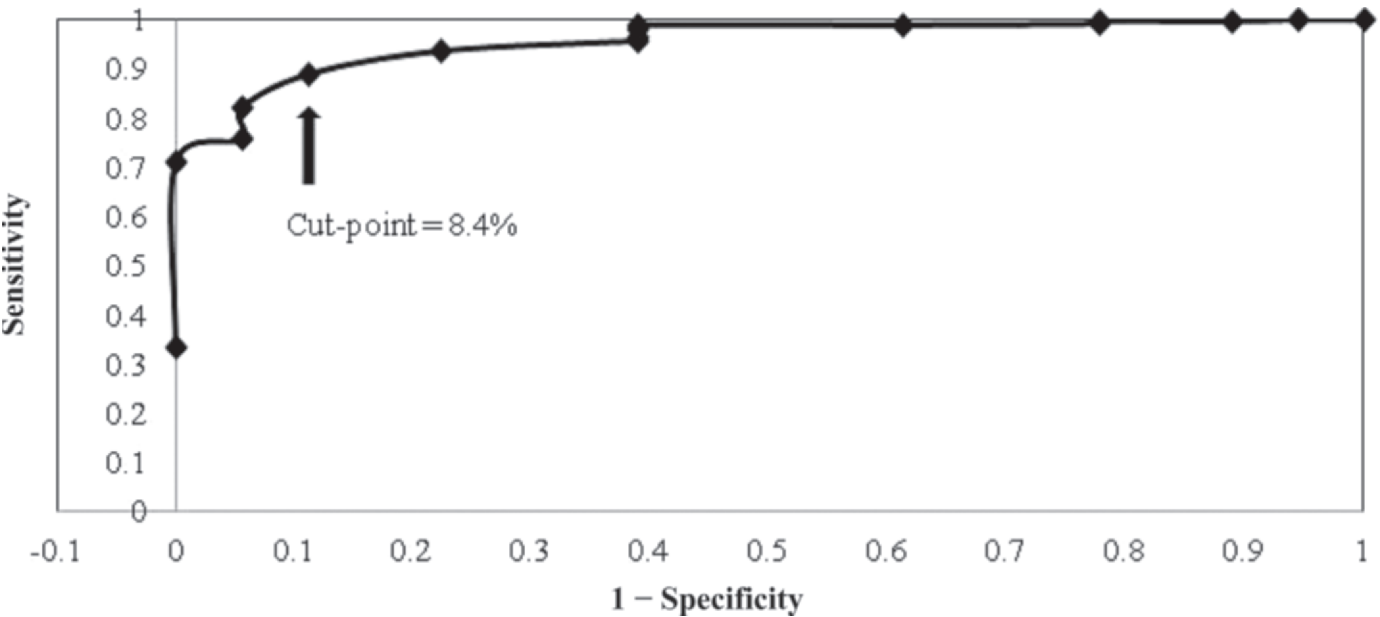
Figure 3. Receiver operating characteristic curve of the true positive rate against the false positive rate for the different percentage Brix (%Brix) values of the radial immunodiffusion assay (RID) test (n = 397).

fractometry produces a reading in less than 15 s once a serum sample is obtained. Very few dairy farms own or have access to a centrifuge to separate serum. However, Wallace et al. (2006) reported that serum collected from blood tubes allowed to clot had an STP content (as determined by a standard digital refractometer) that was highly correlated ($R^2 = 0.95$; n = 234) to the STP content of a duplicate sample that was centrifuged before serum collection. If this finding holds true for the Brix refractometer, it would suggest that producers could take a blood sample from a calf, allow it to clot without centrifugation, and transfer a small amount of serum to the well using a pipette. Confirmation of this finding with the Brix refractometer is warranted. If so, the simplicity of this method to determine the IgG concentration of serum is advantageous and easily allows for on-farm adaptation. Because relatively few samples in the current study had IgG levels <10 g/L, further evaluations of different populations are warranted.

CONCLUSIONS

An important component of a calf-monitoring program is assessment of the success of passive transfer. However, it is critical that the method used be inexpensive and technically simple and can be performed on-farm. The objective of this study was to evaluate the utility of a digital Brix refractometer to assess the success of passive transfer compared with STP by refractometry. Brix refractometer measurements were highly correlated with serum IgG. A value of <8.4% Brix most accurately predicted FPT, providing a reasonable estimate of serum IgG concentration in the majority of calves tested. However, because relatively few samples had IgG levels <10 g/L, further evaluations of different populations are warranted. Serum total protein was also positively correlated with %Brix and IgG concentration measured by RID. Digital Brix refractometry is convenient and affordable, allowing producers to use the same digital refractometer to estimate IgG concentration of maternal colostrum and calf serum, thereby monitoring both colostrum quality and success of passive transfer.

ACKNOWLEDGMENTS

REFERENCES

Bielmann, V., J. Gillan, N. R. Perkins, A. L. Skidmore, S. Godden, and K. E. Leslie. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. J. Dairy Sci. 93:3713–3721.

Boyd, J. W. 1972. The relationship between serum immune globulin deficiency and disease in calves: A farm survey. Vet. Rec. 90:645–649.

Calloway, C. D., J. W. Tyler, R. K. Tessman, D. Hostetler, and J. Holle. 2002. Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves. J. Am. Vet. Med. Assoc. 221:1605–1608.

CCAC (Canadian Council on Animal Care). 2009. CCAC Guidelines On: The Care and Use of Farm Animals in Research, Teaching and Testing. CCAC, Ottawa, ON, Canada.

Chelack, B. J., P. S. Morley, and D. M. Haines. 1993. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves. Can. Vet. J. 34:407–412.

Godden, S. 2008. Colostrum management for dairy calves. Vet. Clin. North Am. Food Anim. Pract. 24:19–39.

McBeath, D. G., W. J. Penhale, and E. F. Logan. 1971. An examination of the influence of husbandry on the plasma immunoglobulin level of the newborn calf using a rapid refractometer rest for assessing immunoglobulin content. Vet. Rec. 88:266–270.

McEwan, A. D., E. W. Fisher, and I. E. Selman. 1970. Observations on the immune globulin levels of neonatal canes and their relationship to disease. J. Comp. Pathol. 80:259–265.

McGuire, T. C., N. E. Pfeiffer, J. M. Weikel, and R. C. Bartsch. 1976. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. J. Am. Vet. Med. Assoc. 169:713–718.

Moore, D. A., J. Taylor, M. L. Hartman, and W. M. Sischo. 2009. Quality assessments of waste milk at a calf ranch. J. Dairy Sci. 92:3503–3509.

Morrill, K. M., E. Conrad, J. Polo, A. Lago, J. Campbell, J. Quigley, and H. Tyler. 2012. Estimate of colostral immunoglobulin G concentration using refractometry without or with caprylic acid fractionation. J. Dairy Sci. 95:3987–3996.

Morrill, K. M., J. Polo, A. Lago, J. Campbell, J. Quigley, and H. Tyler. 2013. Estimate of serum IgG concentration using refractometry with or without caprylic acid fractionation. J. Dairy Sci. 96:4535–4541.

NAHMS (National Animal Health Monitoring System). 2007. Dairy 2007: Heifer calf and management practices on U.S. dairy operations. US Department of Agriculture: Animal and Plant Health Inspection Service: Veterinary Services: Centers for Epidemiology and Animal Health (USDA:APHIS:VS:CEAH), Fort Collins, CO.

Naylor, J. M., and D. S. Kronfeld. 1977. Refractometry as a measure of the immunoglobulin status of the newborn dairy calf: Comparison with zinc sulfate turbidity test and single radial immunodiffusion. Am. J. Vet. Res. 38:1331–1334.

Quigley, J. D., A. Lago, C. Chapman, P. Erickson, and J. Polo. 2013. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. J. Dairy Sci. 96:1148–1155.

Smith, V. R., R. E. Reed, and E. S. Erwin. 1964. Relation of physiological age to intestinal permeability in the bovine. J. Dairy Sci. 47:923–924.

Trotz-Williams, L. A. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91:3840–3849.

Tyler, J. W., B. J. Steevens, and D. E. Hostetler. 1999. Colostral IgG concentrations in Holstein and Guernsey cows. Am. J. Vet. Res. 60:1136–1139.

Wallace, M. M., B. D. Jarvie, N. R. Perkins, and K. E. Leslie. 2006. A comparison of serum harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves. Can. Vet. J. 47:573–575.

Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569–577.

Wenz, J. 2011. Passive transfer of immunity and associated management on WA dairies—Project summary. Winter 2011. Veterinary Medicine Extension, Washington State University, Pullman.

Windeyer, M. C., K. E. Leslie, S. M. Godden, D. C. Hodgins, K. D. Lissemore, and S. J. LeBlanc. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev. Vet. Med. 113:231–240.