J. Dairy Sci. 102:7459–7463
https://doi.org/10.3168/jds.2019-16467

Short communication: Use of a digital refractometer in assessing immunoglobulin G concentrations in colostrum and the first 5 transition milkings in an Irish dairy herd

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ABSTRACT

Transition milk is a source of immunoglobulin G (IgG) and could potentially be used to provide calves with passive immunity, when the IgG concentration is ≥50 g/L. Assessment of IgG concentrations in transition milk would be required before feeding and could be conducted using cow-side tests such as refractometers. Currently, limited information is available on the ability of refractometers to assess transition milk quality. We hypothesized that digital refractometry could be used to provide an accurate cow-side assessment of IgG concentrations in colostrum and transition milk, and IgG concentration in colostrum and one or more transition milking in an Irish herd is >50 g/L. The objectives of this study were to determine the IgG concentrations in colostrum and first, second, third, fourth, and fifth transition milk, and determine the utility of a digital refractometer in assessing quality of colostrum and transition milk produced by cows in a pasture-based dairy production system. A convenient sample of 75 dairy cows were enrolled. Colostrum and transition milk IgG concentrations were determined by radial immunodiffusion and refractometry. Sensitivity and specificity of the refractometer were determined and cut-off points that maximized sensitivity and specificity were determined using receiver operating characteristic curves. Median (range) IgG concentrations in colostrum and first, second, third, fourth, and fifth milking were 99.6, 43.5, 12.5, 5.3, 1.9, and 1.8 g/L, respectively. The sensitivity (0.8–1) of digital refractometry in identifying samples with low IgG concentrations in colostrum, first, second, and third transition milk was acceptable. In contrast, digital refractometry was not useful for assessing IgG concentrations in the fourth and fifth milking due to low IgG concentrations.

Key words: dairy cow, transition, immunoglobulin, refractometer, milk

Short Communication

Adequate passive immunity achieved through ingestion and absorption of colostral immunoglobulins reduces the risk of morbidity and mortality in dairy calves (Weaver et al., 2000). Factors affecting passive immunity in calves include colostral immunoglobulin concentration, and the age of the calf when colostrum is received (Osaka et al., 2014). To ensure achievement of adequate passive transfer (**APT**) of immunity, feeding a total IgG mass of 150 to 200 g has been recommended (Chigerwe et al., 2008b). Consequently, colostrum samples with IgG concentrations of >50 g/L are recommended as they are likely to achieve APT of immunity (McGuirk and Collins, 2004). These recommendations can present a challenge as large variation exists in colostrum quality (Shivley et al., 2018) and quantity produced (Denholm et al., 2018) among cows.

In a study by Oyeniyi and Hunter (1978), first and second transition milk from Holstein cows was reported to have IgG concentrates equivalent to 78.3 and 47.5% of colostrum. In another study, average IgG concentrations of the first 5 transition milkings were 41, 17, 8.9, 5.2, and 2.6 g/L, respectively (Stott et al., 1981). In Irish dairy herds, fed predominantly grass-based diets, a mean colostrum IgG concentration of 112 g/L was reported (Conneely et al., 2013), which is considerably higher than the recommended cut-off point of 50 g/L (McGuirk and Collins, 2004). The high IgG concentration in colostrum from Irish dairy herds suggests that transition milk could also potentially contain >50 g/L of IgG, making it suitable to provide adequate passive immunity. However, as IgG concentrations in transition milk are lower than that of colostrum (Stott et al., 1981), assessment of IgG content of transition milk

Received February 11, 2019.
Accepted April 23, 2019.
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would be required. Cow-side tests such as refractometry, which have been used to assess colostrum quality (Silva-Del-Río et al., 2017), could also be used to assess the suitability of transition milk for first feeding.

Several on-farm methods with acceptable diagnostic sensitivity and specificity for estimating colostral IgG concentrations include hydrometers (Chigerwe et al., 2008a) and digital refractometers (Elsohaby et al., 2017; Silva-Del-Río et al., 2017). To the authors' knowledge, no studies have reported the utility of refractometers in assessing IgG content of transition milk produced by cows in a pasture-based dairy production system, and IgG concentrations in transition milk in Irish herds. We hypothesized that digital refractometry could be used to provide an accurate cow-side assessment of IgG concentrations in colostrum and transition milk, and IgG concentration in colostrum and one or more transition milking in an Irish herd is >50 g/L. The objective was to determine utility of a commercially available digital Brix refractometer in assessing quality of colostrum and transition milk produced by cows in a pasture-based dairy production system, and to determine the IgG concentrations in colostrum and first (**T1**), second (**T2**), third (**T3**), fourth (**T4**), and fifth (**T5**) transition milk.

A convenient sampling technique was used to enroll cows from a dairy research facility in Teagasc, Fermoy, Co. Cork, Ireland. Seventy-five Holstein-Friesian cows were dried off at least 8 wk before parturition and housed in cubicle sheds. Cows were visually assessed to determine BCS (Edmonson et al., 1989). Cows with the desired BCS of 3.25 were fed a diet of grass silage. Cows with BCS <3.25 were fed a TMR consisting of 7.1 kg of maize, 7.15 kg of silage, and 8.3 kg/DM concentrates. All cows were fed a pre-calving mineral supplementation at 100 to 120 g/head per d (Immunoboost Elite Precalver, Nutribio Ltd., Tivoli Industrial Estate, Cork, Ireland) 6 to 8 wk before parturition. Calving was supervised by trained and experienced personnel. Immediately after birth, calves were separated from their dams, and cows were milked either with a single portable milking unit (DeLaval Mobile Milking Unit, Carlow, Ireland) in the calving pens or in the milking parlor (Dairymaster, Causeway, Co. Kerry, Ireland) at the next scheduled milking (0700 or 1430 h). A 250-mL sample of colostrum or transition milk was collected at this time. Colostral samples with evidence of blood or mastitis were excluded.

Quality of colostrum, T1, T2, T3, T4, and T5 samples were assessed using a digital Brix refractometer (Refractometer MA871, Milwaukee Electronics, Milwaukee, WI). The refractometer was temperature compensating (range 10–40°C). An aliquot (0.2–0.5 mL) of deionized water was placed on the prism well of the refractometer to obtain a standardized reading as per the manufacturer's recommendations. Following removal of the deionized water, the refractometer was set to determine colostral quality. An aliquot (0.2–0.5 mL) of the colostrum was placed in the prism well of the refractometer and the result displayed within a minute. The test result from the refractometer was reported as Brix %. Colostrum and transition milk samples were frozen at −80°C until subsequent IgG concentration determination.

Colostrum and transition milk samples were analyzed for IgG concentrations using a commercial single radial immunodiffusion (**SRID**) kit (Radial Immunudiffusion Test for Quantitation of Bovine IgG in Serum or Plasma, Triple J Farms, Bellingham, WA) according to the manufacturer's recommendations. The SRID was considered the reference method for determination of colostral and transition milk IgG concentrations. The SRID plates were stored at 4°C and contained specific anti-bovine IgG, agarose gel, 0.1 *M* phosphate buffer pH 7.0, 0.1% sodium azide as a bacteriostatic agent, and 1 μg/mL of amphotericin B as an antifungal agent. Serum IgG concentration determination range of the kit was 196 to 2,803 mg/dL. Colostrum and transition milk samples were thawed at room temperature (approximately 20°C) for 24 h. Phosphate-buffered saline was used as a diluent; colostrum samples were diluted at 1:4 or 1:6, whereas T1 samples were diluted at 1:2 or 1:4. The T2, T3, T4, and T5 samples were not diluted unless IgG concentrations were determined to be out of the kit's measurement range, at which point they were diluted at 1:2 and retested.

Test kits were incubated at room temperature for 30 min before inoculation with colostrum or transition milk, at 5 μL per well. Once all samples had been added, plates were incubated at room temperature (20–24°C) for 24 h. Zones of precipitation in the plate were then measured using an SRID plate reader (Digital RID Plate Reader, The Binding Site, San Diego, CA). Concentration of IgG in the samples was determined by comparing the diameter of the precipitated ring to the standard curve produced by the reference sera (196, 1,402, and 2,803 mg/dL) provided with the kit. Coefficient of determination values for the standard curves were between 0.96 and 0.99 for the regression equations produced, indicating accurate prediction of the IgG concentrations of the inoculum.

Data analyses were performed using the commercial statistical software JMP Pro14 (SAS Institute, Cary, NC; SAS version 9.4, SAS Institute Inc.). Only samples with both IgG concentrations and corresponding Brix refractometer measurements were used for analysis. De-

Table 1. Summary of median (range) of colostral and transition milk IgG concentrations in pasture-based dairy cows

Sample[1]	No. of samples	Median (range) Brix refractometer value (%)	Median (range) IgG concentration (g/L)
Colostrum	66	25.6 (15.9–36.6)	99.6 (11.4–164.9)
T1	63	17.8 (8.7–31.1)	43.5 (9.7–106.2)
T2	61	12.6 (8.7–22.3)	12.5 (1.3–42.2)
T3	59	11.8 (9.2–26.4)	5.3 (1.8–24.9)
T4	53	11.4 (8.6–16.1)	1.9 (1.8–10.6)
T5	41	11.2 (9.2–16.8)	1.8 (1.8–9.8)

[1]T1 = first transition milk; T2 = second transition milk; T3 = third transition milk; T4 = fourth transition milk; T5 = fifth transition milk.

scriptive statistics for IgG concentrations in colostrum and transition milk were calculated. The distribution of the data was assessed using the Shapiro-Wilk test, and mean (±SD) values were reported when data were normally distributed. For data that did not follow a normal distribution pattern, median (range) values were reported. Colostrum and T1 samples with IgG concentrations <50 g/L were considered inadequate. Second milking samples with IgG concentrations <25 g/L were considered inadequate, whereas T3, T4, and T5 samples with <10 g/L were considered inadequate. The 25 and 10 g/L endpoints were arbitrarily chosen based on the distribution of the IgG concentrations. Using these cut-off points, sensitivity and specificity of the refractometer for colostrum and transition milk were measured, using SRID data as the reference test result. Sensitivity of the refractometer was defined as the probability of a test result indicative of an inadequate IgG concentration, using SRID data as the reference test result. Specificity of the refractometer was defined as the probability of a test result indicative of an adequate colostral IgG concentration using SRID data as the test result. An endpoint that maximized both sensitivity and specificity was then determined using receiver operating characteristic (**ROC**) curve. The area under the ROC curve and 95% confidence intervals were also determined.

Colostral and Brix values were not normally distributed, thus median (range) were reported. Median (range) of IgG concentrations and Brix refractometer values in colostrum and transition milk are summarized in Table 1. Cows in their third or greater lactation had higher ($P = 0.029$) colostral IgG concentrations (median 107.1 g/L; 95% CI, 90.2, 123.1) compared with cows in their first or second lactation (median 93.0 g/L; 95% CI, 82.4, 102.2). Proportions of colostrum and T1 samples with IgG concentrations >50 g/L were 95.5 and 36.5%, respectively. The proportion of T2 samples with IgG concentrations >25 g/L was 13.1%. Proportions of T3 and T4 samples with >10 g/L of IgG were 23.7 and 3.8%, respectively. Brix refractometer endpoints maximizing sensitivity and specificity for colostrum and transition milk are summarized in Table 2. Sensitivity and specificity for T5 samples were not calculated because all samples had IgG concentrations of <10 g/L.

Table 2. Summary of sensitivity and specificity of the refractometer at different cut-off points for colostrum and milk samples in pasture-based dairy cows[1]

Sample[2]	IgG concentration cut-off point (g/L)	Refractometer cut-off point (Brix %)	Sensitivity (95% CI)	Specificity (95% CI)	AUC[3] (95% CI)
Colostrum	<50	24.3	1.0 (1.0)	0.65 (0.59, 0.71)	0.88 (0.66, 1)
T1	<50	19.3	0.83 (0.77, 0.89)	0.51 (0.46, 0.56)	0.81 (0.68, 0.93)
T2	<25	14.0	0.83 (0.79, 0.87)	0.71 (0.61, 0.81)	0.81 (0.57, 1)
T3	<10	12.3	0.80 (0.75, 0.85)	0.51 (0.44, 0.58)	0.79 (0.66, 0.91)
T4	<10	11.3	0.51 (0.47, 0.55)	0.51 (0.33, 0.69)	0.69 (0.33, 0.99)
T5	—	—	—	—	—

[1]The sensitivity and specificity for T5 samples was not calculated because none of the samples had IgG concentrations >10 g/L.
[2]T1 = first transition milk; T2 = second transition milk; T3 = third transition milk; T4 = fourth transition milk; T5 = fifth transition milk.
[3]AUC = area under the receiver operating characteristic curve.

Our study findings indicated that T1 samples had median IgG concentrations <50 g/L, contrary to our hypothesis. Although median T1 concentrations were <50 g/L, feeding ≥4 L of T1 samples (43.5 g of IgG/L × 4 L = 174 g) will deliver the recommended total IgG mass of 150 to 200 g that must be fed to achieve APT of immunity in calves (Chigerwe et al., 2008b). The T2, T3, T4, and T5 samples contained low colostral IgG concentrations and are unlikely to confer APT of immunity in calves. Colostral IgG concentrations reported in this study were consistent with previous studies in Irish herds, which reported mean IgG concentrations of 112 g/L (Conneely et al., 2013). Cows in their third or greater lactation had higher IgG concentration compared with cows in their first or second lactation consistent with studies performed in Irish dairies (Conneely et al., 2013). Transition milk IgG concentrations in this study are consistent with previous studies (Oyeniyi and Hunter, 1978) performed in North America.

Digital refractometer was an acceptable cow-side test for assessing quality of colostrum, T1, T2, and T3 samples based on the test sensitivity (0.8–1) and the area under the ROC curves. The Brix % threshold value of 24.3 as the cut-off point for 50 g/L of colostral IgG concentrations in this study is consistent with previous studies that recommended values of 20 to 23% (Chigerwe et al., 2008a; Elsohaby et al., 2017). The Brix % threshold value of 19.3% as the cut-off for 50 g/L IgG concentrations in T1 samples is consistent with previous studies that recommended values of 19.0 to 19.2% (Silva-Del-Río et al., 2017). When T2 samples are considered, a Brix threshold value of 14.0% was identified as the cut-off point for an IgG concentration of 25 g/L, whereas a Brix threshold of 12.3% was identified as the cut-off point of 10 g/L in T3 samples. The digital refractometer is not reliable for assessing IgG concentrations in T4 samples because of low sensitivity (0.51) and a low area under the curve (0.51) for the ROC curves constructed. The digital refractometer is also not useful for assessing T5 samples because of the nearly undetectable (<10 g/L) IgG concentrations.

Based on these findings, producers raising pasture-fed dairy cows should consider assessing IgG concentrations in T1, T2, and T3 samples using a digital Brix refractometer. At least 4 L of T1 with >50 g/L IgG should be considered for feeding to calves after assessment with a digital refractometer to achieve APT of immunity when colostrum is unavailable. Although T2 and T3 are unlikely to confer adequate transfer of passive immunity, they should be considered for feeding to calves following ingestion of colostrum. Ingestion of IgG in calves older than 24 h has been reported to confer localized immunity in the gut, thereby reducing morbidity in neonatal calves (Berge et al., 2009).

This study has several limitations because results are based on a single pasture-based dairy farm and the recommendations could have limited external validity. The recommendations might be relevant to farms which feed transition milk to calves and manage dairy cows that produce colostrum and transition milk with high IgG concentrations. Future studies should focus on comparing the associated effect of feeding colostrum and transition milk, with similar IgG concentrations, on calf morbidity and mortality in the neonatal period.

Immunoglobulin G concentrations in T1 samples were relatively high and could provide adequate passive immunity to calves when fed in appropriate quantities. The sensitivity of the digital refractometer in identifying samples with low IgG concentrations in colostrum, first (0.83), second (0.83), and third (0.80) transition milk was acceptable. In contrast, digital refractometry is not useful for assessing IgG concentrations in the fourth and fifth milking due to low IgG concentrations (>10 g/L).

ACKNOWLEDGMENTS

Funding was provided by the University of California–Davis Global Programs Seed Grants.

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