Colostrum immunoglobulin G concentration of multiparous Jersey cows at first and second milking is associated with parity, colostrum yield, and time of first milking, and can be estimated with Brix refractometry

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ABSTRACT

The objective of this study was to evaluate colostrum IgG concentration harvested at first and second milking from multiparous Jersey cows, the dam's lactation number, colostrum yield, and time of first milking. In addition, we validated the use of a Brix refractometer to estimate IgG concentration in colostrum from multiparous Jersey cows using radial immunodiffusion as the reference method. Colostrum samples and total weight of colostrum harvested at first ($n = 134$) and second ($n = 68$) milking were collected from 134 multiparous Jersey cows housed in a California herd. Fresh colostrum samples were analyzed for IgG concentration with Brix refractometry and frozen samples by radial immunodiffusion. A total of 90.4 and 42.7% of the samples from first and second milking met industry standards of quality for IgG concentration (>50 g/L). Second and third lactation cows had similar colostrum IgG concentration but lower than cows on their fourth and greater lactation. At second milking, 56.4% of cows on their fourth or greater lactation had colostrum IgG concentrations >50 g/L. When colostrum yield increased from low (<3 kg), medium (3 to 6 kg), to high (>6 kg), IgG concentration decreased. Higher IgG concentration was observed on colostrum harvested at <6 h (short) versus 6 to 11 h (medium) after calving. However, IgG concentration in colostrum harvested after 11 h (long) was similar to that harvested at short and medium time. Readings of %Brix were highly correlated with IgG at first ($r = 0.81$) and second ($r = 0.77$) milking. The best

Brix threshold to identify colostrum from first milking with >50 IgG g/L was 20.9% based on logit equations with Youden's index criterion and 18.0% based on accuracy criterion. For colostrum harvested at second milking, similar Brix thresholds were obtained, 19.2 and 19.0%, regardless of whether Youden's index or accuracy was used as the selection criterion. Our results indicate that the dam's lactation number, colostrum yield, and time of first milking relative to calving are associated with IgG concentration in colostrum from multiparous Jersey cows. Second milking colostrum from mature Jersey cows should be evaluated to extend colostrum supply on dairies especially during times of shortage. Readings of %Brix can be used to rapidly estimate IgG concentration in Jersey colostrum harvested at first and second milking.

Key words: colostrum, Jersey, radial immunodiffusion, Brix refractometry

INTRODUCTION

Providing newborn calves with adequate IgG supply from colostrum is generally recognized as an essential management practice in calf rearing. Calves that fail to reach serum IgG levels above 10 g/L within the first 2 d of life are considered to undergo failure of passive transfer (FPT; Godden, 2008). Evidence indicates that FPT is a prevalent problem in US dairy herds (Beam et al., 2009). The negative effect of FPT on calf health and production is not limited to a higher mortality and morbidity risk during the pre-weaning period, and detrimental effects have also been shown on feed efficiency and milk yield of mature dams (DeNise et al., 1989; Faber et al., 2005). Economic losses associated with FPT have been estimated to average \$65 per calf when accounting for calf mortality, morbidity, and decrease in average daily weight gain (Raboisson et al., 2016).

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Industry standards define colostrum as high quality when IgG concentration is greater than 50 g/L (Godden, 2008). In a recent survey conducted in 67 herds from 12 states, almost 30% of maternal colostrum failed to reach IgG concentrations above 50 g/L (Morrill et al., 2012). Moreover, in a recent meta-analysis, colostrum IgG concentrations were highly variable within and across studies (Buczinski and Vandeweerd, 2016). Parity, pre-partum diet, season, breed, dry-period length, vaccination of the dam, and delayed colostrum collection are factors associated with colostrum quality that have been previously reviewed (Godden, 2008). Thus, to prevent FPT, it is essential to know the IgG concentration of colostrum and to restrict the first feeding to colostrum that meets the standard of quality.

Radial immunodiffusion (RID) assay is used as a standard method to determine colostral IgG concentration. Specific gravity can be used on the farm to estimate colostral IgG concentration with colostrometers. However, even though colostrometers are relatively inexpensive, their fragility and sensitivity to temperature has limited their adoption on the farm (Quigley et al., 2013). Studies conducted over the last decade, mostly with Holstein colostrum, have shown that %Brix refractometry can be successfully used to estimate IgG concentration. Suggested Brix readings thresholds range widely across studies, from 18% (Morrill et al., 2012) to 22% (Bielmann et al., 2010).

The objectives of this study were to evaluate if IgG concentration in colostrum harvested at first and second milking from multiparous Jersey cows was associated with parity, colostrum yield, and time of first milking relative to calving. Another objective was to validate the use of a Brix refractometer to estimate IgG concentration in colostrum harvested at first and second milking.

MATERIALS AND METHODS

Study Population

The study was conducted on a California commercial dairy farm housing 3,500 Jersey dairy cows from January to February 2016. The study herd had an average daily milk production of 23.8 kg/d. During the close-up period, cows were fed once a day with a corn silage-based TMR prepartum diet with a nutrient composition on a DM basis of 16.3% CP, 26.3% ADF, 1.38 Mcal/kg of NE_M, and -17.6 mEq/100 g of DCAD. Twice a day, at noon and at midnight, postpartum cows were moved from the maternity pen to the colostrum pen, where they remained for 4 d. Cows housed in the colostrum pen were milked twice a day at 1500 and at 0300 h in a double 35-stall herringbone parlor.

Data Collection and Colostrum Storage Process

Colostrum samples were collected from 134 Jersey cows. Sixty-eight cows were sampled at first and second colostrum milking; 66 cows were sampled only at first milking. Colostrum was harvested according to standard practices at the dairy in individual milking buckets. After each cow was milked, harvested colostrum was transferred into plastic containers that were weighed immediately using a portable scale with a readability of 0 to 50 kg $\pm$ 2 g (Ship-Elite, American Weight Scales Inc., Cumming, GA). Colostrum samples were collected directly from the plastic bucket and transferred into 20-mL plastic vials and transported to the laboratory within 30 min after collection. Parity records of enrolled cows were obtained from the herd management software DairyComp305 (Valley Ag Software, Tulare, CA). During the study period, records of calving time were collected every 6 h at the maternity pen from maternity record sheets filled out by on-farm employees as part of the dairy management.

Colostrum Evaluation Process

Upon arrival at the laboratory, fresh colostrum samples were evaluated for %Brix with a handheld electronic refractometer (Reichert Inc., Depew, NY). Approximately 50 μ L were placed on the refractometer well for each reading. In between samples, the refractometer was rinsed with distilled water and dry-cleaned with wipes.

Fresh colostrum samples were aliquoted into 5-mL vials and frozen (-20°C). At the end of the study collection period, frozen samples were shipped to the California Animal Health Food Safety Laboratory (University of California–Davis) for IgG analysis with the RID kit (Triple J Farms kit, Bellingham, WA).

Categorical Variables

Lactation Number. Cows were grouped as second, third, and fourth or greater parity. The proportion of cows in each category was 23.1, 27.6, and 49.3% at first milking, and 19.1, 23.5, and 57.4% at second milking, respectively.

Colostrum Yield. Colostrum yield was classified as low (<3 kg), medium (3 to 6 kg), and high (>6 kg). The proportion of cows in each category was 37.0, 41.5, and 21.5% at first milking, and 32.4, 50.7, and 16.9% at second milking, respectively.

Time from Calving to First Milking. Time from calving to first milking was classified as short (<6 h), medium (6 to 11 h), or long (>11 h). The proportion

Table 1. Descriptive statistics for first and second milking colostrum collected from multiparous Jersey cows on a dairy farm in California

Milking	Item ¹	n	Mean	SD	Minimum	Maximum
First	Colostrum collected (kg)	134	4.1	2.6	0.1	13.7
	Brix (%)	134	25.4	4.4	16.2	37.1
	IgG by RID (g/L)	134	83.8	29.4	23.7	172.9
	Time to milking	125	9 h, 25 min	3 h, 50 min	2 h, 15 min	15 h, 35 min
Second	Colostrum collected (kg)	68	4.3	2.1	0.4	11.5
	Brix (%)	68	18.7	3.0	13.4	29.3
	IgG by RID (g/L)	68	46.9	21.6	6.2	100.0
	Time to milking	65	21 h, 0 min	3 h, 40 min	14 h, 15 min	26 h, 45 min

¹RID = radial immunodiffusion.

of cows in each category was 25.8, 36.3, and 37.9%, respectively.

Statistical Analysis

Descriptive statistics were calculated with the MEANS procedures of SAS (version 9.4, SAS Institute Inc., Cary, NC). The 50th percentile (median, Q_2), 25th percentile (Q_1), and 75th percentile (Q_3) were computed using the PCTLDEF = 4 option in the output statement of the UNIVARIATE procedure. The association between IgG concentration with each of the categorical variables (lactation number, colostrum yield, and time from calving to first milking) was evaluated using the GLM procedure of SAS with the LSD option of the means statement. To evaluate the association between %Brix, IgG, and colostrum yield at first and second milking, Pearson correlation coefficients were calculated with the CORR procedure of SAS.

Data were evaluated for normal distribution using the UNIVARIATE procedure of SAS, including the histogram and normal kernel options. To describe colostrum with at least 50 g/L, diagnostic test characteristics of Brix refractometry, including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy (samples correctly identified as having adequate or inadequate IgG concentrations by RID), were obtained using the FREQ procedure of SAS for Brix refractometer readings of 18, 19, 20, 21, and 22%.

To identify the optimal threshold to differentiate colostrum samples with adequate IgG concentration (>50 g/L) at first and second milking using Brix refractometry, receiver operating characteristic analyses were conducted using the OUTROC option of the MODEL statement from the LOGISTIC procedure of SAS. The maximum potential of effectiveness of the Brix refractometer was calculated based on Youden's index (J) criterion (Youden, 1950) from the OUTROC option output as $J = (\text{sensitivity} + \text{specificity} - 1)$. The probability that resulted in the highest Youden's index was used to identify the threshold that maximized the correct classification of colostrum samples.

RESULTS AND DISCUSSION

Descriptive statistics of first and second colostrum milking including colostrum yield, %Brix, IgG by radial immune diffusion, and time from calving to first and second milking are presented in Table 1.

IgG Concentration in Colostrum

First and Second Colostrum. At first milking, colostrum IgG concentration averaged 83.8 g/L (range: 23.7 to 172.9 g/L). Most colostrum samples (90.4%) met industry standards for IgG concentration (>50 g/L). Prior studies reporting colostrum IgG concentrations in multiparous and primiparous Jersey cows averaged 65.8 g/L (Quigley et al., 1994), 66.5 g/L (Muller and Ellinger, 1981), 84.5 g/L (Quigley et al., 1995), and 72.9 g/L (Morrill et al., 2015). Multiple factors including breed, age, dry period length, prepartum diet, and time to colostrum collection are associated with colostrum IgG concentration. However, the relatively elevated IgG concentrations observed in the present work compared with previous reports can be partially explained because only multiparous cows were enrolled in our study. At second milking, colostrum IgG concentrations were lower ($P < 0.001$) than at first milking, averaging 46.9 g/L (range: 6.2 to 100 g/L). This was expected, as previous studies reported IgG concentrations decreased by 78 and 47% at second and a third milking relative to the first milking (Foley and Otterby, 1978). In our study, nearly half of the second milking colostrum samples (42.7%) met industry standards for desirable IgG concentrations.

Lactation Number. The box plot distribution of IgG concentration by lactation number is shown in Figure 1. The mean IgG concentration in colostrum from first milking was similar for cows on their second (77.3 g/L) and third lactation (74.9 g/L) but lower ($P < 0.01$) than for cows on their fourth and greater lactation (98.4 g/L). Similar results were observed at second milking: IgG concentration was higher ($P < 0.01$) for cows on their fourth and greater parity (55.8

g/L) compared with cows on their second (39.0 g/L) and third parity (38.7 g/L).

Previous studies have reported increasing IgG concentration with parity in Norwegian, Guernsey, and Holstein breeds (Tyler et al., 1999; Moore et al., 2005; Gulliksen et al., 2008; Morrill et al., 2012). Accordingly, prior researchers have also reported an increase in IgG concentrations that was most obvious when comparing cows on their first and second lactation with cows with 4 or more lactations (Moore et al., 2005; Gulliksen et al., 2008). In contrast, Quigley et al. (1994) reported lower colostrum IgG in Jersey cows on their second lactation compared with primiparous cows or cows on their third or greater lactation.

It has been hypothesized that the higher colostrum IgG concentration observed in more mature cows could be explained by their longer exposure to antigens (Godden, 2008). Although in our study IgG concentrations were lower in colostrum harvested at second milking, the proportion of samples that met quality standards, especially in multiparous cows on their fourth or greater lactations (56.4%), warrants measurements of IgG on second milking colostrum from mature cows as a viable strategy to extend colostrum supply on dairies with limited availability.

Colostrum Yield. The box plot distribution of IgG concentration by colostrum yield is shown in Figure 2. Colostrum yield was negatively associated ($P < 0.01$) with IgG concentration at first ($r = -0.37$) and at second ($r = -0.36$) milking. Likewise, other researchers had reported a significant but weak association between IgG concentration and colostrum yield in Holstein cows (Maunsell et al., 1999; Morin et al., 2010). Concentration of IgG in colostrum decreased as yield increased with 97.7, 85.3, and 71.6 g/L at first milking and with 58.5, 47.2, and 34.0 g/L at second milking for low, medium, and high production levels, respectively. Pritchett et al. (1991) proposed 8.5 kg of colostrum as a critical level of production in Holstein cows. In the aforementioned study, cows producing below this threshold point had a greater likelihood of meeting the standards of quality based on colostrum IgG concentration. Other authors have found quantity of colostrum produced at the initial milking is negatively correlated with IgG concentration (Cabral et al., 2016).

Differences in colostrum yield may result in diverse critical levels of production across breeds. Because very few samples failed to meet the standards of quality in our study, we did not try to identify a critical level of production. Nevertheless, at first milking only 25% of the cows with IgG >50 g/L produced >4.9 kg of colostrum, whereas 75% of the cows with IgG <50 g/L produced >4.3 kg of colostrum.

Figure 1. Box plot distribution of colostrum IgG concentration from Jersey cows on their second, third, or >third lactation. Colostrum was harvested at first (A; $n = 134$) and second (B; $n = 68$) milkings. The box plot shows the 50th percentile (median, line within the box), 25th and 75th percentile (box), 10th and 90th percentiles (whiskers), and outliers (dots).

Time from Calving to First Milking. Colostrum was harvested at 9 h, 25 min (SD = 3 h 50 min) and at 21 h, 0 min (SD = 3 h 40 min) after calving for first and second milking respectively. An effect was observed of milking time relative to calving on colostrum IgG

Figure 2. Box plot distribution of colostrum IgG concentration from multiparous Jersey cows producing low (<3 kg), medium (3 to 6 kg), or high (>6 kg) colostrum. Colostrum was harvested at first (A; $n = 134$) and second (B; $n = 68$) milkings. The box plot shows the 50th percentile (median, line within the box), 25th and 75th percentile (box), 10th and 90th percentiles (whiskers), and outliers (dots).

concentration. As expected, a higher IgG concentration was observed when time to harvest colostrum was short (96.7 g/L) versus medium (82.1 g/L). However, when milking time relative to calving was long (84.1 g/L), IgG concentration was not statistically different from short or medium time.

Morin et al. (2010) found no association between milking time relative to calving on 81 Holstein cows. In the aforementioned study, cows were milked as soon as 20 min after calving or as late as 23 h, 50 min. Similar results were obtained by Cabral et al. (2016) on 111 colostrum samples harvested from 1 h to 14 h, 30 min after calving. However, Moore et al. (2005) reported a significant effect of milking time relative to calving on 13 cows from which colostrum was harvested from the same quarter exactly at 2 h (113 g/L), 6 h (94 g/L), 10 h (82 g/L), and 14 h (76 g/L) after calving. Conneely et al. (2013) studied colostrum quality on 704 samples and reported no changes in quality until colostrum was harvested after 9 to 12 h after calving.

In our study, management at the dairy resulted in first colostrum always being harvested not earlier than

2 h and up to 15 h after calving. Based in our field experience, most commercial operations in California lack adequate management to harvest colostrum shortly after calving. Some operations even limit first colostrum collection to once a day. Thus, time from calving to first milking might be a management practice to re-evaluate on dairies that have IgG concentration in colostrum below desirable levels.

IgG and %Brix

A significant ($P < 0.001$) association was observed between %Brix and IgG concentration of colostrum at first ($r = 0.81$) and second ($r = 0.77$) milking (Figure 3) from multiparous cows. Brix readings at first colostrum were $Q_1 = 22.3\%$, $Q_2 = 25.4\%$, and $Q_3 = 28.3\%$, whereas at second colostrum the readings were $Q_1 = 17.0\%$, $Q_2 = 18.4\%$, and $Q_3 = 19.8\%$.

Diagnostic test characteristics for 18, 19, 20, 21, and 22%Brix readings are shown in Table 2. Logit functions were obtained for first milking [$\text{logit}(p) = -20.32 (\pm 6.18) - 1.06 (\pm 0.31) \times \text{Brix}$] and second milking

Figure 3. Association between %Brix readings and IgG concentration by radial immunodiffusion assay from multiparous Jersey colostrum at first (diamonds; $n = 134$) and second milking (circles; $n = 68$). A significant association (<0.001) was observed between IgG (g/L) and Brix (%): $Y [\text{IgG (g/L) at first milking}] = -52.4 (\pm 5.4) + 10.4 (\pm 0.4) \times \text{Brix at first milking}$ ($r = 0.81$); $Y [\text{IgG (g/L) at second milking}] = -49.9 (\pm 10.1) + 5.2 (\pm 0.5) \times \text{Brix at second milking}$ ($r = 0.77$); $Y [\text{IgG (g/L) at first and second milking}] = -53.3 (\pm 5.2) + 5.5 (\pm 0.2) \times \text{Brix at first and second milking}$ ($r = 0.76$). Horizontal line indicates IgG = 50 g/L. Color version available online.

Figure 4. Receiver operating characteristics curves for %Brix readings of colostrum harvested at first (dashed line; $n = 134$) and second milking (solid line; $n = 68$) for diagnosis of poor colostrum quality based on IgG concentrations with radial immunodiffusion. The selected threshold based on Youden's index is represented in gray.

$[\logit(p) = -19.80 (\pm 5.11) - 1.04 (\pm 0.27) \times \text{Brix}]$. Based on the predicted probabilities obtained from the Youden's index criterion (Youden, 1950) for first ($P = 0.13$) and second milking ($P = 0.45$), the best %Brix thresholds to predict colostrum with IgG >50 g/L were 20.9% (area under the curve = 0.977) and 19.2% (area under the curve = 0.897) for colostrum harvested at first and second milking, respectively. Receiver operating characteristics are presented in Figure 4.

In a prior study validating Brix readings with Jersey colostrum from primiparous and multiparous cows, 18% was deemed the most adequate threshold based on accuracy (Morrill et al., 2015). This proposed threshold for Jersey cows is lower than our suggested threshold. Moreover, it is also at the lower end of previous sug-

gested thresholds for Holstein breeds, which ranged from 18% (Morrill et al., 2012) to 22% (Bielmann et al., 2010). However, in our study, when the best threshold for Brix reading was selected based on accuracy criterion, 18.0% at first milking (accuracy = 92.5%) and 19.0% at second milking (accuracy = 79.4%); results were similar to what Morrill et al. (2015) had previously proposed (Table 2). Nevertheless, results should be interpreted carefully as our data included only multiparous cows from a single dairy operation.

In our study the observed differences in Brix thresholds based on the diagnostic measurements chosen are most likely explained by the low number of colostrum samples that fail to meet the standards of quality at first milking. Accuracy is affected by the prevalence of poor colostrum quality; it increases as the prevalence decreases. Youden's index, a global measurement of a test performance based on maximizing the sensitivity and specificity, is not affected by the prevalence of the disease or proportion of poor quality colostrum samples. Thus, the implications of using different diagnostic test characteristics should be taken into account when trying to identify the optimum threshold for Brix readings.

Buczinski and Vandeweerd (2016) recently conducted a meta-analysis to evaluate the diagnostic accuracy of refractometry to predict colostrum quality. The study included 11 research studies with various breeds: Holsteins ($n = 4$), Jerseys ($n = 1$), mixed breeds ($n = 1$), beef breeds ($n = 1$), or nonidentified breeds ($n = 3$). However, the low number of studies with known breeds other than Holstein did not allow the authors to explore the effect of breed on %Brix reading. Results from this meta-analysis showed that Brix readings of $<18\%$ were indicative of poor colostrum quality, whereas Brix readings of $\geq 22\%$ were indicative of good colostrum quality. Buczinski and Vandeweerd (2016) suggested

Table 2. Diagnostic test characteristics for a digital Brix refractometer (18, 19, 20, 21, and 22% thresholds) to predict colostrum that contains at least 50 g/L of IgG measured by radial immunodiffusion¹

Milking	Brix threshold (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Test high/low ²
First	18	96.8	45.5	95.2	55.6	92.5	125/9
	19	94.3	54.6	95.9	46.2	91.0	121/13
	20	93.5	72.7	97.5	50.0	91.8	118/16
	21	87.8	100.0	100.0	42.3	88.8	108/26
	22	83.7	100.0	100.0	35.5	85.1	103/31
Second	18	86.2	66.7	65.8	86.7	75.0	38/30
	19	75.9	82.1	75.9	82.1	79.4	29/39
	20	48.3	97.4	93.3	71.7	76.5	15/53
	21	41.4	100.0	100.0	69.7	75.0	12/56
	22	31.0	100.0	100.0	57.4	70.6	9/59

¹Colostrum was harvested from multiparous Jersey cows at first ($n = 134$) and second ($n = 68$) milking. PPV = positive predictive value; NPV = negative predictive value. Brix refractometer, Reichert Inc. (Depew, NY).

²Number of samples declared high or low (IgG >50 g/L) based on %Brix readings.

that between 18 and 22%Brix colostrum management should be used to ensure that adequate colostrum volume is fed, to reduce the time between birth and first feeding so absorption is maximized (Michanek et al., 1989), to minimize microbial contamination to prevent bacterial sequestration of IgG (Johnson et al., 2007), or to incorporate colostrum supplements into the first feeding. Although classifying colostrum as good, poor, or suspect eliminates the uncertainty associated with readings slightly above or below a yes/no threshold, it will add complexity to the on-farm colostrum management program and may hinder colostrum protocol compliance on poorly managed dairies.

CONCLUSIONS

In this study, the prevalence of good colostrum quality (>50 g/L of IgG) was elevated at first milking and moderate-to-low at second milking. The dam's lactation number, colostrum yield, and time of first milking relative to calving were associated with IgG concentration in colostrum from multiparous Jersey cows. Over half of the second milking colostrum samples from cows on their fourth or greater parity met industry standards for desirable IgG concentrations. This warrants routine evaluation of second milking colostrum in mature cows as an strategy to extend colostrum on dairies with limited supply. Readings of %Brix can be used to rapidly estimate IgG concentration in Jersey colostrum harvested at first and at second milking from multiparous cows. The best %Brix reading threshold to identify colostrum with >50 IgG g/L on first milking colostrum from multiparous Jerseys cows was 20.9% based on Youden's index criterion, challenging the previously proposed 18% Brix reading obtained from primiparous and multiparous cows. When accuracy was used as the selection criterion, results (18.0% Brix) were the same as previous reports. Nevertheless, Youden's index is a more desirable selection criterion as it is not affected by the prevalence of poor quality colostrum.

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