Relationship between Milking Fraction and Immunoglobulin G Concentration in First Milking Colostrum from Holstein Cows

Sandra M. Godden, *DVM, DVSc;* **Amber Hazel,** *BS*
Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota,
St. Paul, MN 55108
Corresponding author: Sandra M. Godden, Tel: 612-625-8177; Fax: 612-625-6241;
E-mail address: godde002@umn.edu

Abstract

The objective of this study was to describe immunoglobulin G (IgG) concentrations in various milking fractions (0% fraction [cisternal], 25% fraction, 50% fraction, 75% fraction, 100% fraction [alveolar], or composite sample) in first milking colostrum from Holstein dairy cows. First milking colostrum was collected from 26 primiparous and multiparous Holstein cows on a commercial dairy farm in western Wisconsin. For each cow, 10-mL colostrum samples were collected representing the following milking fractions: cisternal colostrum (first 10-15 seconds), 25%, 50%, 75%, 100% (alveolar), and a composite sample (entire milking), and tested for IgG concentration (mg/mL).

Contrast analysis showed that IgG concentrations were significantly higher in cisternal colostrum samples (LSmean ± SE = 75.3 ± 6.06 mg/mL) as compared to IgG concentrations in the 25% fraction (70.1 ± 6.06), the 50% fraction (71.1 ± 6.06), the 75% fraction (70.2 ± 6.06), and the composite sample (72.2 ± 6.06) ($P < 0.05$). Levels of IgG in cisternal samples were not different than levels in the 100% (alveolar) fraction (73.1 ± 6.06) ($P = 0.15$). Apart from the cisternal samples, IgG concentrations were not different among the other milking fractions tested.

Producers or researchers wishing to measure colostrum quality should avoid using forestripping samples for testing purposes, as these samples may overpredict the IgG concentration in what will be fed to the calf. The current study found no difference in IgG concentration among the other milking fractions. However, when considering the effect of milking fraction on other milk components that may be of importance to the neonatal calf, including fat, producers should completely milk out the cow at the time of first colostrum harvest.

Keywords: colostrum, immunoglobulin, milking fraction

Résumé

L'objectif de cette étude était de décrire la concentration des immunoglobulines G (IgG) dans plusieurs types de fraction laitière (0% [citerne], 25%, 50%, 75%, 100% [alvéolaire], ou échantillon composite) dans le colostrum de la première traite recueilli chez des vaches laitières Holstein. Le colostrum de la première traite a été recueilli chez 26 vaches Holstein primipares et multipares dans une ferme laitière commerciale de l'ouest du Wisconsin. Pour chaque vache, des échantillons de 10 ml de colostrum ont été recueillis représentant les diverses fractions suivantes : colostrum de la citerne (premières 10-15 secondes), 25%, 50%, 75%, 100% (alvéolaire), un échantillon composite (traite entière). Tous ont été testés pour les concentrations d'IgG (mg/ml).

Une analyse de contrastes révéla que les concentrations d'IgG étaient significativement plus élevées dans les échantillons de colostrum de la citerne (Moyenne ajustée ± ET = 75.3 ± 6.06 mg/ml) que dans les échantillons contenant 25% de colostrum alvéolaire (70.1 ± 6.06), 50% (71.1 ± 6.06), 75% (70.2 ± 6.06) et l'échantillon composite (72.2 ± 6.06) ($P < 0.05$). Les concentrations d'IgG dans les échantillons de colostrum de la citerne ne différaient pas de celles dans l'échantillon contenant 100% de colostrum alvéolaire (73.1 ± 6.06) ($P = 0.15$). Mis à part les échantillons de colostrum de la citerne, les concentrations d'IgG ne variaient pas entre les différentes fractions laitières.

Les producteurs et les chercheurs qui désirent évaluer la qualité du colostrum devraient éviter d'utiliser les échantillons provenant du colostrum de la citerne car ces échantillons peuvent surestimer la concentration des IgG dans ce qui sera donné au veau. Nous n'avons pas détecté de différence au niveau de la concentration des IgG entre les diverses fractions laitières. Toutefois, en prenant en ligne de compte l'effet de la fraction sur les autres composants du lait qui peuvent être importants

pour le veau nouveau-né, comme le gras, les producteurs devrait extraire tout le lait de la vache lors de la cueillette du premier colostrum.

Introduction

Achieving early and adequate intake of high quality colostrum is widely recognized as the single most important management factor in determining health and survival of the neonatal calf.[5,23,11] A recent national study survey reported that 7.8% of live-born heifer calves on US dairies die prior to weaning. The same study reported that 19% of 1,816 calves tested from 394 herds in 17 states had failure of passive transfer (FPT), with FPT defined as having a serum immunoglobulin G (IgG) concentration < 10 mg/mL between one and seven days of age.[16] This is evidence that there is still ample room for the average US dairy producer to make improvements in colostrum management.

One of the factors necessary for a successful colostrum management program is to harvest and feed high quality, clean colostrum. While colostrum is known to contain a large number of nutrients, non-specific immune factors, and growth factors important to the calf, experts have typically focused on describing IgG concentration when defining colostrum quality, with high quality colostrum having ≥ 50 mg/mL of IgG. Factors identified as affecting colostrum IgG concentration include, but are not limited to, breed, parity of the dam, season of calving, preparturient vaccination of the dam, dry period length, prepartum maternal nutrition, and time to colostrum collection after calving.[1,3,4,12,13,14,15,18,20,22] One additional possible factor that has received limited study considers whether colostral IgG concentrations may differ by milking fraction (e.g. cisternal , 25%, 50%, 75%, alveolar, or composite samples). Discovering the answer to this question may be of practical importance if the answer 1) guides producers in determining what sample to collect for testing colostrum quality or 2) guides producers in determining what milking fractions are best to collect for feeding to the calf.

Previous studies have reported on the compositional differences of whole, saleable milk during the course of milkout.[6,17] However, these characteristics could conceivably be different for colostrum because some colostrum and milk components are secreted by different mechanisms.[17] During the dry period, IgG, and IgG_1 in particular, is transported into the alveolar space by transcellular mechanisms.[9] Stott et al proposed that the Ig diluting effect that occurs with the onset of lactation at parturition would be expected to initiate at the alveolar level where secretion occurs, leaving the teat and gland cistern with higher residual Ig concentrations.[21] However, in a study of 12 Holstein

cows these authors reported there was no difference in IgG concentration (mg/mL) between cisternal samples (63.2 ± 25.1) as compared to composite (bucket) samples (73.4 ± 42.2).[21] In a more recent study, Hostetler et al reported that, though concentrations of IgG (g/L) in cisternal samples (86 ± 51) and composite (bucket) samples (81 ± 42) were not different, IgG levels in both of the aforementioned sample types were higher than for alveolar samples (67 ± 36).[8]

Hostetler et al indicated the need for future studies to examine changes in IgG concentrations in the temporal fractions of the first milking.[8] However, to date only one study has been reported. Eight Red Holstein x Simmental cows in Switzerland were used to describe concentrations of dry matter, fat, protein, lactose, somatic cell count (SCC), Na, K, Cl, osmolarity, conductivity, IgF-1, insulin, prolactin, γ-glutamyltransferase, and IgG in the cisternal fraction, 25% milk fraction, 50% milk fraction, 75% milk fraction, and alveolar milk (100% fraction) when cows were sampled at the third milking after calving.[17] While this study described large numeric differences, it detected no statistically significant differences in IgG levels among the various milking fractions tested. However, producers should be cautious extrapolating results of this study to North American Holstein herds, given breed and production differences, and considering that the aforementioned Swiss study tested third milking, and not first milking, colostrum. Also, the aforementioned study did not describe IgG concentrations in the composite milk sample (all fractions represented) that would be fed to the calf. The objective of the current study was to describe the IgG concentration in various milking fractions (0% [cisternal], 25%, 50%, 75%, 100% [alveolar], and composite sample) in first milking colostrum from Holstein dairy cows.

Methods

Study Herd

This observational study was conducted in a transition management facility (Emerald Dairy II) in western Wisconsin in late May, 2006. This is a 400-cow freestall barn that manages dry and transition cows, from dry-off to approximately 14 days-in-milk, for two large Holstein dairies. Close-up dry cows and heifers are housed in freestall pens that are walked on an hourly basis by herd staff to detect cows in labor. When calving is imminent, cows are moved into individual straw-bedded calving pens where calving can be observed and assistance provided if needed. Within 30 to 60 minutes post-calving, the calf is routinely removed from the maternity pen, prior to suckling, and cows are brought to a chute where the udder is prepped and first milking colostrum collected into a clean, sanitized milking bucket.

Cow Inclusion Criteria and Sample Size Estimates

Primiparous or multiparous cows were eligible for inclusion in the study if they had an observed calving in the previous 60 minutes, the cow had four functional quarters, and there was no evidence of clinical mastitis at time of first milking after calving. It was our original intent to enroll at least 23 cows into the study, as this would allow us to detect a 10 mg/mL difference in colostral IgG between different milking fractions with a power of 80% and 95% confidence (assumptions: SD = 12 mg/mL; two-tailed test).

Sample Collection and Testing

The cow or heifer was brought to the colostrum collection chute within 30 to 60 minutes post-calving, and the udder was cleaned and disinfected using the herd's routine udder preparation procedure. The milking unit was then attached and the process of colostrum collection began. Between 0 to 15 seconds after the onset of milking, and repeating every 30 seconds thereafter until milking was completed, a 10 mL sample of colostrum was collected into a clean syringe through a sampling port that had been placed in the milk line immediately below the claw.

The time of milking onset and completion was recorded for each cow, and the total duration of milking calculated. Using the calculated total milking time, the study technician selected the following six 10-mL samples to keep for testing: 1) 0 time sample (cisternal); 2) 25% time sample, 3) 50% time sample, 4) 75% time sample, and 5) 100% time sample (alveolar). All other samples not needed were discharged back into the milking bucket. After mixing the contents of the milking bucket thoroughly, the bucket contents were weighed and a 10-mL composite colostrum sample was collected from the milking bucket. All samples were labeled (date, cow ID, sample type) and frozen at –4°F (–20°C). Information recorded for each cow included cow ID, calving date, colostrum collection start time, colostrum collection stop time, total milking time (minutes), and quantity of colostrum harvested (lb/kg). After each cow was milked, all sampling equipment was pulled apart, rinsed three times with warm water, washed with hot water and detergent, rinsed with hot water and acid, then dried prior to reuse. Frozen colostrum samples were submitted (on ice) to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, for IgG testing using a turbidometric immunoassay (TIA).

Statistical Analysis

Data describing cow characteristics (ID, calving date, parity, milking duration (min)), colostrum quantity harvested (lb/kg), and IgG concentration (mg/mL) for the 0% (cisternal), 25%, 50%, 75%, 100% (alveolar), and composite colostrum samples were entered into an Excel spreadsheet.[a] Descriptive statistics were calculated describing the aforementioned cow and sample characteristics (mean, SD, minimum, maximum) in SAS.[b] Proc MIXED[b] and contrast analysis was used to describe the relationship between sample type (explanatory variable of interest: cisternal, 25%, 50%, 75%, alveolar, or composite) and colostrum IgG concentration (dependent variable of interest, mg/mL). Additional covariates offered into the model included milking duration (min), colostrum quantity harvested (lb/kg), and parity. Cow was included as a random effect in the model to account for the clustering of multiple samples collected for each cow. Final significance was set at $P < 0.05$.

Results

Twenty-six Holsteins were included in the study. The mean (median; range) parity was 2.3 (2.0; 1 to 8). The mean (SD; range) milking duration and quantity of colostrum harvested was 4.7 (1.5; 2 to 8) minutes and 12.3 (8.49 ± 2.0 to 30) lb (5.6 (3.85; 0.91 to 13.6) kg), respectively. The mean (SD; range) quality of colostrum harvested, according to composite samples collected from the milking bucket, was 72.2 (30.8; 22.4 to 139.6) mg/mL. The unadjusted mean (SD; range) IgG concentration (mg/mL) for the various milking fractions are as follows: cisternal (0%) fraction: 75.3 ± 30.6, 20.9 to 137.3; 25% fraction: 70.1 ± 30.1, 21.7 to 132.6; 50% fraction: 71.1 ± 32.6, 21.5 to 144.9; 75% fraction: 70.2 ± 29.3, 19.1 to 125.9; alveolar (100%) fraction: 73.1 ± 31.8, 21.0 to 141.3; composite sample: 72.2 ± 30.8, 22.4 to 139.6 (Figure 1).

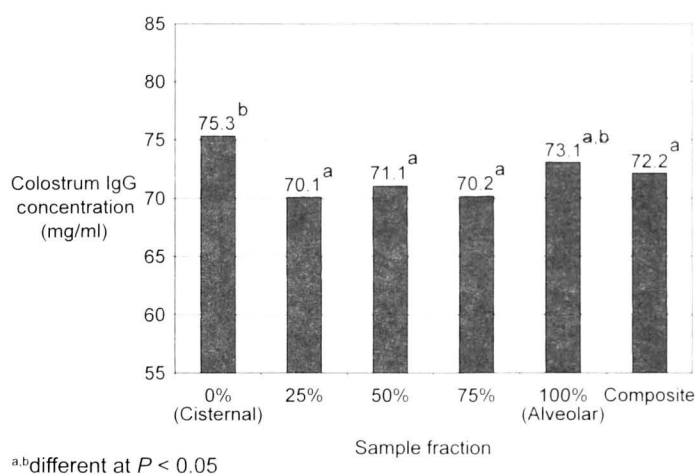In regression analysis, variables describing parity, milking duration (min) and colostrum quantity

a,b different at $P < 0.05$

Figure 1. LSmean immunoglobulin G (IgG) concentrations in milking fractions for first milking colostrum from 26 Holstein dairy cows.

harvested (lb/kg) were not associated with colostrum IgG concentration ($P > 0.05$), and so were excluded from the final model. The final regression model indicated that colostrum IgG concentration was associated with milking fraction (Type 3 P value = 0.0078). However, the only important difference related to zero time (cisternal) colostrum samples, which had significantly higher IgG concentrations (LSmean ± SE = 75.3 ± 6.06 mg/mL) as compared to IgG concentrations in the 25% fraction (70.1 ± 6.06), the 50% fraction (71.1 ± 6.06), the 75% fraction (70.2 ± 6.06), and the composite sample (72.2 ± 6.06) ($P < 0.05$) (Figure 1). Concentrations of IgG in cisternal samples were not different than levels in the 100% (alveolar) fraction (73.1 ± 6.06) ($P = 0.15$). Apart from differences in cisternal samples, IgG concentrations were not different among the other milking fractions tested (Figure 1).

Discussion

Previous studies investigating the relationship between milking fraction and IgG concentrations in colostrum are extremely limited. In a small study of 12 Holstein cows, Stott et al reported no difference in IgG concentrations between cisternal and composite (bucket) samples.[21] Similarly, Hostetler et al reported no difference in IgG concentrations between cisternal and composite samples, but reported lower IgG concentrations in alveolar samples.[8] Ontsouka et al described levels of IgG, as well as several other constituents, in milking fractions of third milking (second day) colostrum in eight Swiss Red Holstein x Simmental cows.[17] In that study, while there were large numeric differences in IgG concentrations among some milking fractions (cisternal = 38.3 ± 8.6; 25% = 20.6 ± 3.1; 50% = 24.3 ± 3.9; 75% = 34.5 ± 7.7; 100% = 36.2 ± 11.3), the authors reported no statistically significant difference in IgG concentrations among these fractions. One possible explanation for the aforementioned study in failing to detect an association, despite large numeric differences, may be the relatively small sample size of eight cows (lack of power). The current study found a similar pattern in IgG concentrations among the milking fractions tested, with IgG levels being highest in the 0% (cisternal) samples. However, IgG concentrations were not different among the other milking fractions tested. The overall quality of first milking colostrum in this study of 26 Holstein cows was relatively high (72.2 mg/mL) as compared to values reported by Ontsouka et al.[17] These differences in overall colostrum quality may be attributed to breed differences, the fact that the current study tested first milking colostrum and not third milking colostrum, or other herd or study factors.

Results of the current study may be useful in helping guide producers or researchers as to the most appropriate sample to collect for cow-side or laboratory testing of colostrum quality. In North American dairy herds, producers are increasingly interested in estimating IgG concentrations in colostrum quality at cow side by using such instruments as a hydrometer to measure specific gravity or, more recently, a Brix refractometer to measure total solids content.[2,4,19] Bielmann et al recently reported that a total solids cutpoint of 22 g/dl had very good accuracy in identifying high quality colostrum (IgG $\geq$ 50 mg/mL).[2] The Brix refractometer instrument may have advantages over the traditionally used hydrometer, including improved accuracy, a lack of dependence upon temperature, and that it requires only a very small volume of colostrum for testing (< 1 mL). In the current study, cisternal colostrum, collected within the first 0 to 15 seconds of milking, had higher IgG concentrations than most other fractions tested. As such, producers requiring only a very small volume of colostrum in order to estimate IgG concentration (e.g. when using a Brix refractometer) should avoid the temptation to collect a forestripping sample in order to take this measurement, as a reading from a forestripping sample may overestimate the colostral IgG concentration in what will be fed to the calf. Instead, producers should test the composite pool of colostrum, after harvest, as this will more accurately represent the quality of what will be fed to the calf.

Results of the current study may be also be useful in helping to answer the question of whether or not it matters if producers completely strip out the cow at first milking, or if they only harvest enough colostrum to feed the calf, leaving all remaining colostrum in the udder until the second milking. The current study found no difference in IgG fractions among 25%, 50%, 75%, 100% or composite samples. As such, whether a producer only partially versus fully milks out a cow at first milking is probably not of importance if one only considers the IgG concentration in the colostrum harvested. However, there may be other factors affecting colostrum quality and related to milkout fraction that should not be overlooked. While the current study described only IgG concentrations in the various milking fractions, a previous study reported that there are significantly higher concentrations of dry matter, fat, and lactose (g/L) in later milking fractions as compared to early milking fractions in third milking colostrum.[17] In that study, mean fat levels (g/L) in cisternal, 25%, 50%, 75%, and 100% fractions of third milking colostrum from eight cows were 25.9 ± 7.9, 44.5 ± 10.8, 69.9 ± 7.7, 93.2 ± 11.6, and 124.5 ± 12.9, respectively. As such, when considering the effect of milking fraction on other milk components that may be of importance to the neonatal calf, including fat, producers are advised to completely milk out the cow at the time of first colostrum harvest.

While it was not a stated study objective, it is interesting to note that the current study did not detect an association between colostral IgG concentration (mg/mL) and variables such as cow parity or quantity of colostrum harvested. While not all studies report similar findings, most studies report higher IgG concentrations in colostrum produced by multiparous cows as compared to primparous heifers, presumably due to older animals having had a longer period of exposure to farm-specific pathogens.[4,13,15,18] Though not reported in the results, the current study found a positive but non-significant correlation between parity and colostral IgG concentration ($R^2 = 0.21$, $P = 0.29$). It may be that the current study would have required a larger sample size in order to describe this relationship as significant. Similarly some, but not all, studies have described a negative relationship between the quantity of colostrum harvested and the colostral IgG concentration in that colostrum. Pritchett *et al* observed that cows producing < 18.7 lb (8.5 kg) of colostrum at first milking were more likely to produce high quality (> 50 mg/mL) colostrum than higher producing cows, presumably due to dilutional effects.[18] However, two recent studies report that there is no predictable relationship between colostrum IgG concentration and weight of colostrum produced at first milking.[7,10] Chigerwe *et al* reported a negative correlation between weight of first milking and colostral IgG concentration, but concluded that use of weight of the first milking as a screening test to identify bovine colostrum with inadequate IgG concentration could not be justified because of the low sensitivity.[4] Though not reported in the results, the current study did find a negative but non-significant correlation between quantity of colostrum harvested and IgG concentration ($R^2 = -0.29$, $P = 0.16$). It may be that the current study would have required a larger sample size in order to describe this relationship as significant. However, the sample size for the current study was originally derived to investigate our primary question, that being to describe the relationship between milking fraction and IgG concentration in first milking colostrum in North American Holstein cows.

Conclusions

Immunoglobulin G concentrations in first milking Holstein colostrum were higher in cisternal colostrum harvested between 0 to 15 seconds after the start of milkout, as compared to the 25%, 50%, 75% milking fractions or a composite colostrum sample. As such, producers or researchers wishing to measure colostrum quality should avoid using forestripping samples for this testing, as these samples may overpredict the IgG concentration in what will be fed to the calf. The current study found no difference in IgG concentration among the other milking fractions (25%, 50%, 75%, 100%, composite samples). However, when considering the effect of milking fraction on other milk components that may be of importance to the neonatal calf, including fat, producers should completely milk out the cow at the time of first colostrum harvest.

Endnotes

[a]Excel, Microsoft Corp., Redmond, WA
[b]SAS version 9.2, SAS Institute, Cary, NC

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