A Novel Method to Determine Colostrum IgG Concentration

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Kimberley M. Morrill, graduate student;
Erin Conrad, graduate student;
Howard Tyler, professor of animal science

Summary and Implications

Our objectives were to evaluate the use of refractometry as a means of estimating immunoglobulin **(IgG)** concentration of bovine maternal colostrum **(MC). S**amples of MC (n = 827) were collected from 67 farms in 12 states. Colostrum was analyzed for IgG by radial immunodiffusion **(RID)** and refractive index **(nD)** by a digital refractometer. The relationship between nD and IgG was strong (r = 0.73; $P \leq 0.05$; n = 823). Samples analyzed by refractometry fresh and went through 1 freeze/thaw cycle prior to RID analysis resulted in the strongest relationship between IgG and nD (r = 0.93 and 0.90, respectively). The MC samples collected fresh (not refrigerated or frozen) on the farm but frozen two or more times prior to analysis by refractometry and RID had a weak relationship between IgG and nD (r = 0.01). Breed and lactation number did not impact the relationship between RID and nD. These results indicate refractometry is an accurate and rapid method to determine IgG concentration when colostrum is analyzed fresh.

Introduction

Primary factors that influence the acquisition of passive immunity include: the volume of quality maternal colostrum (**MC**); immunoglobulin concentration and time elapsed postpartum prior to feeding. Thus, a rapid, accurate and inexpensive method to estimate immunoglobulin G (**IgG**) concentration in MC is essential for proper colostrum management. Laboratory methods to accurately measure IgG concentration are too complex and expensive for routine use on farm. Currently only 13% of all U.S. dairy operations evaluate MC quality prior to feeding. The most common methods used by farms that measure MC quality were use of a colostrometer and visual appearance (43.7 and 41.6% of farms, respectively). Volume of colostrum and other methods comprise the remaining 14.7%.

Colostrometers estimate total globulin concentration based on measurement of specific gravity. Colostrometer readings are affected by temperature of the MC and total solids **(TS)** content. Refractometers, digital or optical, measure the total protein content of a solution. Protein solutions refract light, and refractometers use this property to estimate TP in a solution. Most of the protein in MC is IgG; thus, measuring TP in MC may be correlated with IgG concentration. Objectives of this study were to validate an on-farm method to determine IgG concentration by refractometry and determine if nD of MC is impacted by breed, parity, or storage method.

Materials and Methods

Dairy farms (n = 67) in 12 states participated in the study between June and October, 2010 (Table 1). Participating farms were required to feed MC that was not supplemented with commercial colostrum supplements nor pasteurized.

Table 1. Colostrum samples collected across region, state, breed, lactation and storage method.

			Breed			Lactation					Stored[1]		
Region State	Farms	Samples	Holstein	Jersey	N/A[2]	1	2	3	4+	N/A[2]	1	2	3
Northeast													
NH	1	18	18	~	~	~	6	3	9	~	18	~	~
NY	5	59	41	3	15	4	11	4	2	38	17	29	13
PA	5	51	42	9	~	~	5	17	22	7	~	23	28
Southeast													
FL	4	35	33	2	~	1	22	~	~	12	17	9	9
GA	2	30	30	~	~	~	30	~	~	~	11	19	~
VA	7	60	22	3	35	~	2	1	~	57	25	20	15
Midwest													
IA	1	40	40	~	~	~		~	~	40	~	~	40
MN	11	97	35	~	62	8	10	4	5	70	2	6	89
WI	6	27	27	~	~	~	5	~	~	22	~	~	27
Southwest													
AZ	2	61	40	~	21	2	7	~	~	52	7	9	45
CA	14	173	161	8	4	34	76	~	~	63	93	34	46
TX	9	176	5	62	109	~		61	~	115	6	3	167
TOTAL	**67**	**827**	**494**	**87**	**246**	**49**	**174**	**90**	**38**	**476**	**196**	**152**	**479**

[1]Location where colostrum was stored prior to sampling. 1 = fresh/not stored, 2 = refrigerator, 3 = freezer.
[2]Breed and lactation information were not available if samples were pooled prior to collection.

Samples of MC were collected based on availability of MC at the time of site visit. Frozen, refrigerated, or freshly milk MC was selected. The MC was sampled from individual cows or from multiple cow pools according to the normal management of the farm. A 50-ml sample of MC was collected using a sterile dipper and divided into three sample vials, frozen, placed on dry ice and shipped to the respective laboratory for analysis of IgG, nutrient composition and bacterial contamination. A third sample was analyzed for nD. Samples were classified based on storage prior to feeding: fresh = no storage, refrigerated = stored in a refrigerator, or frozen = stored in a freezer. Information, including if the sample was from an individual cow or pool, breed, lactation number and number of times the sample was frozen prior to analysis, were recorded for each sample. Samples representing lactations three and greater were combined into one group.

A digital refractometer was used to determine the nD of MC. IgG concentration was determined using radial immunodiffusion kits. Nutrient and bacterial analysis was completed at the DHI laboratory (Dubuque, IA). The PROC CORR procedure of SAS was used to determine the relationship between IgG and nD. Epidemiological diagnostic test characteristics (sensitivity, specificity and predictive values) were calculated to compare nD to RID.

Results

A total of 827 MC samples were analyzed (Table 1). Breed and lactation data were not recorded if samples were from colostral pools or if the farm did not record information prior to storage.A strong relationship between IgG and nD was observed (r = 0.73, $P < 0.0001$; n = 823; Figure 1). Classifying MC samples by storage method prior to collection (Table 2) indicated that fresh samples provided the strongest relationship between nD and IgG.

Table 2. Relationship between IgG and refractive index (nD) of whole colostrum across storage groups and freeze/thaw cycles.

	RID*nD			Times frozen prior to analysis	
Category[1]	n	r	P	nD	RID
Fresh 0	29	0.90	<0.0001	0	1
Fresh 1	128	0.78	<0.0001	1	1
Fresh 2	25	0.01	0.6838	1	2
Refrigerated 0	29	0.41	0.026	0	1
Refrigerated 1	123	0.70	<0.0001	1	1
Frozen 1	11	0.70	<0.0002	1	2
Frozen 2	138	0.80	<0.0003	2	2
Frozen 3	296	0.69	<0.0004	1	3

[1] Category: Fresh = MC not refrigerated or frozen prior to collection; Refrigerated = MC collected from a refrigerator; Frozen = MC collected from previously frozen prior to collection.

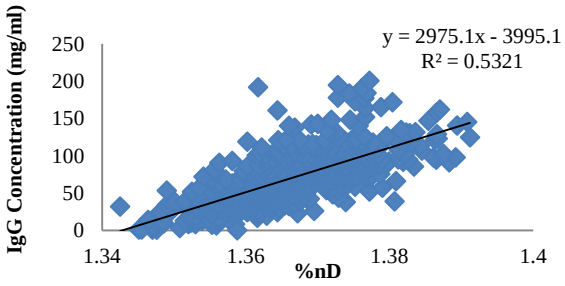

Figure 1. Regression of whole colostrum nD and IgG (n = 827).

Method of on-farm storage and number of times a sample was frozen prior to analysis affected the relationship between IgG and nD. Samples collected fresh (not refrigerated or frozen) and not frozen prior to analysis of nD had the strongest relationship with IgG (r = 0.93 and 0.90, respectively). Multiple freeze / thaw cycles prior to analysis of nD or IgG appeared to reduce predictability of nD to IgG. Correlation differences were unaffected by breed, lactation, nutrient content or bacterial contamination. We predicted a cut-point of 50 mg of IgG per ml of MC based on regression of nD estimates and IgG. Then, sensitivity, specificity, positive and negative predictive values were calculated. Sensitivity of predictions using nD were high across storage groups. When fresh samples, without multiple freeze/thaw cycles, were analyzed the specificity and PPV increased (Table 3).

Table 3. Diagnostic test characteristics for the digital refractometer measuring supernatant from whole colostrum compared with the IgG determined by radial immunodiffusion assay across methods of storage

			Fresh (n = 196)			
Cut-point	Samples	n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1.35966	All Samples	827	93.6	92.2	96.7	85.6
	Fresh	196	93.28	88.41	93.28	88.41
	Refrigerated	152	96.03	86.05	95.28	88.1
	Frozen	479	92.84	94.6	97.94	82.68

[1]Correlates to an IgG concentration of 50 mg/ml using the equation: IgG = 2975.1 * nDw – 3995.1

Discussion

These data presented represent MC available on selected farms from June through October, 2010; it does not necessarily represent all MC produced by all dairy cattle in the United States. Colostrum that was discarded after milking and never entered the feeding pool was not sampled. Storage method was determined by where the MC was stored prior to collection; it does not indicate the temperature or length of storage time.

Providing a tool for producers to rapidly and accurately estimate IgG concentration of MC could potentially improve the health of calves and profitability of producers. Whole MC samples collected fresh and analyzed by refractometry prior to freezing resulted in a stronger relationship than has been previously reported. The relationship between nD and RID was impacted by colostrum storage method ;however breed and lactation number did not impact the accuracy of the refractometer.

The process of freezing, storing, thawing and potentially re-freezing can alter biological samples. It has previously been reported that the temperature at which milk samples are stored and the thawing process are important for accurate analysis of milk components. Storage and handling of dairy products after harvest and prior to consumption can alter physical, nutritional and bacterial characteristics.

To quickly determine if MC is of high quality to feed to calves, nD cut-points were determined to correspond to IgG concentrations > 50 mg/ml. Diagnostic test characteristics were established for MC nD based off of the entire data set, storage method and FT cycles to determine if there should be different cut-points for multiple variants. Maximizing the sensitivity of a test allows for the smallest number of samples being inaccurately identified as greater than 50 mg/ml. This prevents the feeding or storage of MC with insufficient IgG (less than 50 mg/ml) thus making sensitivity of a MC quality test more important than specificity. Use of cut-point values lower than optimal would increase the amount of MC classified as adequate, but would increase the chances that inadequate MC would be classified as adequate. Using cut-points greater then optimal, would decreases the chances of inaccurately classifying poor MC as adequate, however some adequate MC may be incorrectly classified as inadequate.

The sensitivity (93.58%) and specificity (92.24%) for whole MC in this data set, suggests that the 1.35966 cut-point, determined from the relationship between IgG and nD, is highly reliable to identify quality MC. This cut-point provided high sensitivity and specificity across breed, lactations and storage methods. This again suggests that parity and breed do not have an impact on the accuracy of nD on determining if whole MC is adequate.

Conclusion

The objectives of this study were 1) to validate an on-farm method to determine IgG concentration by refractometry and 2) determine if nD of MC is impacted by breed, parity, or storage method. This study concludes that breed, lactation and nutrient composition do not impact the accuracy of a digital refractometer to estimate IgG concentration in MC. The storage of MC does impact the accuracy of the refractometer. Samples analyzed fresh, provide rapid and accurate methods to determine IgG concentration. This study concludes that the nD of fresh MC provides producers with an easy, rapid and accurate method to determine IgG concentration of MC.