Validating a refractometer to evaluate immunoglobulin G concentration in Jersey colostrum and the effect of multiple freeze–thaw cycles on evaluating colostrum quality

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ABSTRACT

The objectives of this study were to (1) validate a method using refractometry to rapidly and accurately determine immunoglobulin (IgG) concentration in Jersey colostrum, (2) determine whether there should be different refractive index (nD) and %Brix cut points for Jersey colostrum, and (3) evaluate the effect of multiple freeze–thaw (FT) cycles on radial immunodiffusion (RID) and a digital refractometer to determine IgG concentration in Jersey colostrum. Samples (n = 58; 3 L) of colostrum were collected from a dairy in northwestern Iowa. Samples were analyzed within 2 h of collection for IgG concentration by RID, %Brix, and nD by refractometer and an estimate of IgG by colostrometer. Samples were frozen, placed on dry ice, and transported to the laboratory at Iowa State University (Ames). Samples arrived frozen and were placed in a −20°C manual-defrost freezer until further analysis. On d 7 (1FT), d 14 (2FT), and 1 yr (3FT) all samples were thawed, analyzed for IgG by RID, %Brix, nD by refractometer, and IgG estimate by colostrometer, and frozen until reanalysis at the next time point. Fresh colostrum had a mean (±SD) IgG concentration of 72.91 (±33.53) mg/mL, 21.24% (±4.43) Brix, and nD 1.3669 (±0.0074). Multiple FT cycles did affect IgG as determined by RID and colostrometer reading. The IgG concentrations were greater in fresh and 1FT samples as compared with 2FT and 3FT samples (72.91, 75.38, 67.20, and 67.31 mg of IgG/mL, respectively). The colostrometer reading was lower in 1FT samples compared with fresh and 2FT samples. Multiple FT cycles had no effect on nD or %Brix reading. In fresh samples, IgG concentration was moderately correlated with nD (r = 0.79), %Brix (r = 0.79), and colostrometer reading (r = 0.79). Diagnostic test characteristics using the recommended cut point of 1.35966 nD resulted in similar sensitivities for 1FT and 2 FT samples (94.87 and 94.74%, respectively). Cut points of 18 and 19% Brix resulted in the greatest sensitivities (92.31 and 84.62%) and specificity (94.74 and 94.74%, respectively). The 18% Brix cut point resulted in 94.83% of the samples being correctly classified based on IgG concentration. These data support the use of digital refractometer to accurately and rapidly determine IgG concentration in fresh Jersey colostrum. Additionally, these data suggest that IgG concentration determined by RID is affected by multiple FT cycles, whereas estimates obtained by refractometer are not affected by multiple FT cycles.
Key words: colostrum, immunoglobulin, refractometer, Jersey, Brix

INTRODUCTION

Newborn animals of many species rely on maternal colostrum to provide them with the nutrients needed to sustain life. Because Ig cannot cross the placental structure of cattle, calves are born agammaglobulinemic with no measurable circulating IgG or IgM. Provision of high-quality colostrum (>50 mg of IgG/mL and <100,000 cfu/mL total plate count; McGuirk and Collins, 2004) fed within the first hours of life provides the calf with sufficient amounts of Ig to reduce the risk of failure of passive transfer of immunity (serum IgG <10 mg/mL at 24 h or age; Godden, 2008) and provide passive immunity for the first 30 to 90 d of life (Guy et al., 1994) until the immune system of the calf is better equipped to respond to pathogens on its own. The total mass of IgG received by the calf is determined by the volume of colostrum fed and IgG concentration of colostrum. Concentration of IgG in colostrum affects acquisition of passive transfer, and therefore, accurate, on-farm assessment of colostrum quality is essential to achieve passive transfer in calves.

It has been well documented that IgG concentration in colostrum has a wide range (Gulliksen, et al., 2008; Morrill et al., 2012a) and can be influenced by breed, parity (Muller and Ellinger, 1981; Kume and Tanabe, 1993; Gulliksen et al., 2008; Morrill et al., 2012a), dry-period length, time of milking postpartum, individual

farm (Gulliksen et al., 2008), whether the colostrum was pooled before feeding, and region of the country (Morrill et al., 2012a). Because of the variation in colostrum IgG concentration, it is important to evaluate the quality before feeding or storage; unfortunately, only 13% of US dairy producers routinely evaluate colostrum quality (USDA, 2007b).

Until recently, dairy farmers had to rely on colostrometers as the primary on-farm method to evaluate colostrum quality. The quality readings on the colostrometer are based on the specific gravity of normal milk and provide an estimate of relative quality, not actual IgG quantity (Fleenor and Stott, 1980). Colostrometer readings are affected by both the temperature of colostrum and total solids content of colostrum (Mechor et al., 1992; Morin et al., 2001), which can lead to on-farm inaccuracies. Currently, laboratory techniques exist that allow for the quantification of IgG concentration; however, these can take up to 48 h to complete.

Refractometers, either optical or digital, can be used to estimate the IgG concentration in bovine colostrum (Bielmann et al., 2010; Morrill et al., 2012b; Quigley et al., 2013) and are gaining popularity on-farm. Protein solutions refract light, and refractometers use this property to estimate total protein in a solution (Chavatte et al., 1998). Previous research has documented a strong correlation between IgG concentration of colostrum and refractive index (**nD**; r = 0.79, n = 823; Morrill et al., 2012b) and, more popularly, a strong correlation between IgG concentration and %Brix (r = 0.64, Chigerwe et al., 2008; r = 0.71, Bielmann et al., 2010; r = 0.75, Quigley et al., 2013). With the exception of 85 Jersey and 14 crossbreds in the Morrill et al. (2012b) data set, the majority of colostrum samples used to validate refractometers to evaluate colostrum quality, and develop industry-recognized cut points, came from Holsteins (n = 1,366). Currently Jersey cattle represent 5.3% of the dairy cattle population in the United States (USDA, 2007a) and continue to gain popularity because of greater heat-stress tolerance (Smith et al., 2013) and smaller carbon footprint (Capper and Cady, 2012) as compared with Holstein cattle.

The objectives of this study were to (1) validate a digital refractometer to rapidly and accurately determine IgG concentration in Jersey colostrum, (2) determine whether there should be different nD and %Brix cut points for Jersey colostrum, and (3) evaluate the effect of multiple freeze–thaw (**FT**) cycles on radial immunodiffusion (**RID**) analysis and a digital refractometer to determine IgG concentration in Jersey colostrum.

MATERIALS AND METHODS

Samples of colostrum (3 L) were collected from the first milking after calving from Jersey dairy cattle (n = 58) on one northwestern Iowa dairy in June 2012. Samples were analyzed within 2 h of collection for IgG concentration by RID, %Brix, and nD by refractometer and an estimate of IgG concentration by colostrometer. Samples were then frozen, placed on dry ice, and transported to the laboratory at Iowa State University (Ames). All samples arrived frozen and were placed in a −20°C manual-defrost freezer until further analysis. On d 7, d 14, and 1 yr, colostrum samples were thawed in a warm water bath and brought to room temperature (20°C). Temperature of each sample was recorded before analysis by RID, refractometer, and colostrometer. Analysis of colostrum was completed and the sample frozen. Samples went through a total of 3 FT cycles.

Refractometer Readings

Approximately 50 µL of colostrum was placed on the refractometer prism (model 300034; SPER Scientific, Scottsdale, AZ), and nD readings and %Brix (sugar content) were recorded. The nD is the refractive index of a solution measured at the wavelength of the sodium D line (589.3 nm) at 20°C. The %Brix value can be obtained from the polynomial fit to the ICUMSA (2009) table: Brix = ({[(11,758.74 × nD − 88,885.21) × nD + 270,177.93] × nD − 413,145.80} × nD + 318,417.95) × nD − 99,127.4536. The refractometer was calibrated with distilled water at the start of each set of analyses.

RID Analysis

Colostrum samples were thawed in a warm water bath and thoroughly mixed before RID analysis. One milliliter of colostrum was added to 4 mL of distilled water and mixed well. A total of 5 µL of diluted colostrum solution was added to each well of a bovine IgG RID test plate (Triple J Farms, Bellingham, WA). Radial immunodiffusion plates were incubated for 24 h, and the diameter of the precipitin ring was measured. The diameter of the precipitin ring was compared with a standard curve created by the internal test standards to determine IgG concentration. All samples were run in duplicate. Samples with a precipitin ring greater than that of the highest internal standard (26.25 mg/mL) were further diluted and reanalyzed. Samples with a precipitin ring smaller than that of the lowest internal standard (1.84 mg/mL) were reanalyzed in an undiluted form.