Short Communication: Evaluation of a Color Method for Testing Immunoglobulin G Concentration in Goat Colostrum

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ABSTRACT

Colostrum samples (n = 1084) of first and second milking from Majorera goats were taken. The immunoglobulin (Ig) G concentrations estimated by measurement of the color of goat colostrum and by the radial immunodiffusion technique were compared. Least squares analysis of the relationship between the color measurement method and IgG concentration resulted in a significant linear relationship. Using 20 mg of IgG/mL of colostrum as the cut-off point for colostrum selection, the sensitivity, specificity, and negative predictive value of the color method as a test of IgG concentration in goat colostrum were 93.03, 71.43, and 78.12%, respectively.

(**Key words:** goat, colostrum, color, immunoglobulin G)

Abbreviation key: NPV = negative predictive value, SRID = single radial immunodiffusion.

In ruminants, the placenta impedes the transfer of Ig from the dam to the fetus. As a result, kids are agammaglobulinemic at birth (Constant et al., 1994; Argüello et al., 2004). Consequently, consumption of colostrum by the progeny of these species has a fundamental role in the acquisition of passive immunity. Therefore, kids need colostrum during the first hours of life as the principal IgG source during the first month of life (Vihan, 1988; Argüello et al., 2004). Indeed, the survival of newborn kids is correlated with the colostrum fed in the first 2 d postpartum (Morand-Fehr, 1989). In artificial rearing management practices, the kid must be removed after parturition to minimize or terminate the mother-kid link that is established in the first hours after birth (Ramírez et al., 1996). This practice facilitates the acceptance of artificial teats by the kid, thus encouraging the practice of hand-feeding

colostrum to kids. The colostrum may be fresh (obtained directly from the mother) or preserved (Argüello et al., 2004).

Fleenor and Stott (1980) described a hydrometer test to estimate Ig concentration in bovine colostrum from the specific gravity of fresh whole colostrum. Fleenor and Stott (1980) and Quigley et al. (1994) observed a greater correlation between colostrum specific gravity and colostrum protein concentration than with Ig concentration; and colostrum temperature can affect this relationship (Mechor et al., 1991; 1992). Morin et al. (2001) demonstrated that colostrum specific gravity reflects colostrum protein concentration more closely than IgG₁ concentration and is markedly influenced by month of calving, thereby limiting the predictive accuracy of this test.

Espada and Vijverberg (2002) reported that the differences in average color and color pattern are useful tools for developing algorithms for the identification and automatic separation of abnormal milk, and Argüello (2000) observed a correlation between color coordinates and IgG colostrum concentration. The objective of this study was to compare IgG concentrations measured in the laboratory by the single radial immunodiffusion (SRID) technique with IgG concentration predictions using colostrum color, and to evaluate the sensitivity, specificity, and negative predictive value (NPV) of colostrum color as a method for measuring IgG concentration in goat colostrum.

From January 1997 to April 2003, 1084 samples of first- and second-milking colostrum were obtained from Majorera goats on 4 dairy farms in the Canary Isles, Spain. The first and second milking of the dam were by machine, and afterwards, colostrum CIE L* a* b* color was measured using a Minolta CR200 Chroma-meter (Aquatecnics, Madrid, Spain), where L* depicts relative lightness, a* indicates relative redness, and b* represents relative yellowness. Chroma were calculated using a* and b* values [$\text{Chroma} = (a^* + b^*)^{1/2}$] according to Wyszecki and Stiles (1982). Chroma-meter measurements were done 3 times on fresh colostrum at 25°C. Afterwards, approximately 25-mL colostrum samples from a bucket were stored in 30-mL polypropylene bottles at –20°C until analyzed for IgG concentration. Se-

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Figure 1. Scatter plot with linear regression curve (solid line) for the relationship between colostral Chroma and radial immunodiffusion (RID) IgG concentration for 1084 samples of goat colostrum. The linear regression equation was: Colostral IgG (mg/mL) = $-7.263 + (1.565 \times \text{Chroma value})$, $R^2 = 0.695$.

rum IgG concentration was determined using an immunodiffusion method (Mancini et al., 1965). The standard curve was prepared in accordance with Catty and Raykundalia (1988) using pure goat IgG (Sigma-Aldrich, St. Louis, MO).

The data were analyzed initially by least squares linear regression. Estimates of the relationship between SRID and Chroma were calculated; SRID being the dependent variable and Chroma the regressor. No effect of milking number was observed. To calculate sensitivity, specificity, and predictive value of the color method as a test of IgG concentration, 2-way frequency tables were constructed in accordance with Pritchett et al. (1994). For these calculations, the color method was considered the diagnostic test value and SRID was considered the true value. The cut-off point between high- and low-quality colostrum for both methods was 20 mg of IgG/mL of colostrum. Sensitivity was defined as the probability of colostrum with an IgG concentration <20 mg/mL being classified as low by the color method. Specificity was defined as the probability of colostrum with an IgG concentration ≥ 20 mg/mL being classified as high by the color method. The NPV was defined as the probability of colostrum classified as high by color method being truly high.

The linear relationship between the color method and SRID was significant (Figure 1); model $r^2 = 0.695$. The least squares estimates of linear relationship resulted in the regression equation for goat colostrum: $\text{SRID} = -7.263 + (1.565 \times \text{Chroma})$. The color method r^2 was higher than that reported for specific gravity by Pritchett et al. (1994; $r^2 = 0.4692$) and Morin et al. (2001; $r^2 = 0.28$) in cow colostrum. The sensitivity, specificity, and NPV of the color method as a test to classify colostrum with IgG concentrations <20 or ≥ 20 mg/mL were 93.03, 71.43, and 78.12%, respectively (Table 1). In our

Table 1. Two-way frequency table to calculate sensitivity,¹ specificity,² and negative predictive value³ of the color method⁴ to classify colostrum with IgG concentrations <20 or ≥ 20 mg/mL.

	Single radial immunodiffusion ⁵		Total
	Low (<20 mg/mL)	High (≥ 20 mg/mL)	
Color method			
Low	748 ^a	80 ^b	828
High	56 ^c	200 ^d	256
Total	804	280	1084

¹Sensitivity = $a/(a + c) \times 100 = 748/804 \times 100 = 93.03\%$.

²Specificity = $d/(b + d) \times 100 = 200/280 \times 100 = 71.43\%$.

³Negative predictive value = $d/(c + d) \times 100 = 200/256 \times 100 = 78.12\%$.

⁴The color method estimated IgG concentration in goat colostrum from the CIE L* a* b* color coordinates of fresh colostrum. The color method estimated IgG concentration in milligrams of IgG per milliliter of colostrum. Low color method is <20 mg/mL, and high is ≥ 20 mg/mL.

⁵IgG concentration in milligrams of IgG/mL of colostrum.

study, the color method, using a cut-off point of 20 mg/mL for classifying colostrum as high or low, was satisfactory because a high sensitivity was shown (Table 1). Low sensitivity values were reported by Pritchett et al. (1994) using the specific gravity of colostrum as a predictor for IgG colostrum concentration.

The color method, using a Chroma value for predicting IgG in goat colostrum concentration, is a new method with high sensitivity and good specificity that may replace specific gravity as the IgG colostrum predictor. Farmers could easily use the color method by using a plastic color fan with each color representing a different Chroma value (and corresponding IgG concentration). The operator would be able to introduce the different strips into the colostrum to decide which is closest to the colostrum color, and hence, determine the IgG concentration.

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