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Use of clinic refractometer at farm as a tool to estimate the IgG content in goat colostrum

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ABSTRACT

Refractometry has been proposed as a farm technique to estimate the IgG concentration in colostrum. In order to validate the method in goat colostrum using a clinical refractometer, 216 colostrum and milk samples were obtained from 54 dairy goats. Samples were evaluated for protein concentration using a clinical refractometer and IgG concentration was measured using a commercial ELISA. The r^2 for the linear regression between refractometry value and IgG concentration measured by ELISA was 0.79, and the area under the receiver operating characteristics curve was 0.99. The proposed cut-off value using the clinical refractometer was 10 mg/mL. At this point, the sensitivity, specificity, negative predictive value, positive predictive value, Youden's index and accuracy were 100%, 95.19%, 100%, 76.32%, 0.95% and 95.83%, respectively.

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Colostrum; goat; clinical refractometer; IgG; ELISA

Introduction

Goat kids are hipogammaglobulinaemic at birth due to the special characteristic of the synepitheliochorial placenta that did not allow the IgG transport from the goat to the goat kid (Castro et al. 2011). This anatomical and physiological characteristic gives to colostrum an essential role in the neonate immunity. Colostrum feeding will provide enough IgG to the neonate goat kid until his immune system will be able to generate it by himself (Moretti et al. 2013). Also, colostrum has been used as a supplement in other species (Akdemir et al. 2018). An adequate colostrum feed is related with positive repercussions on growth, health and a reduction in mortality rate (Argüello et al. 2004; Furman-Fratczak et al. 2011; Nagyova et al. 2017). The range of colostrum IgG concentration is wide, it was from 7 to 159 mg/mL (Quigley et al. 2013) in the healthy udder of cows, but when inflammation is present, IgG concentration may be affected (Watanabe et al. 2017). In addition, the source of immunoglobulins has an effect on the IgG concentration in blood (Rana et al. 2016). It is important to note the differences in colostrum composition observed between dairy species (Hernandez-Castellano et al. 2016). Due to all of these factors, the colostrum IgG concentration must be checked before to feed the animals in order to reduce the presence of Failure of Passive Transfer cases and assure a good health and growth in the ruminant neonates in the modern dairy farms. The golden reference method for determining the IgG concentration in colostrum is the Radial Immune Diffusion (RID) according to the techniques described by Mancini et al. (1965), also ELISA has been used for the same objectives in cow (Gelsing et al. 2015) and in goat colostrum (Rodríguez et al. 2009). But RID and ELISA are time-



consuming, expensive and laboratory-dependent techniques so that these techniques are not suitable to use on a day-to-day basis in dairy farms. The technique to measure colostrum IgG in the farm must be cheap, user-friendly and with a good correlation with golden techniques. Nowadays some other techniques are also used in farms. Densitometer or colostrometer is used daily in cow dairy farms (Lokke et al. 2016), but colostrometer needs a specific temperature to run it adequately (Bartier et al. 2015), and its use is not extended in dairy goat farm. For the past 10 years, refractometry has been used for determining the IgG concentration in the colostrum in farms (Chigerwe et al., 2008; Quigley et al. 2013) observing a significant correlation between Brix index and colostrum IgG concentration in dairy cows. Although more types have been observed in Brix refractometers (Phipps et al., 2016), clinical ones have been successfully used in cow colostrum (Vandeputte et al., 2011). As the literature is limited in reference to the use of clinical refractometer for measuring colostrum IgG in dairy goats, the present study was conducted.

Materials and methods

The experiment was not under the directive 2010/63/UE due to the practices not caused pain, suffering, distress or lasting equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

Animals

This study was performed at the Faculty of Veterinary Medicine (Universidad de Las Palmas de Gran Canaria, Spain). Two

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hundred and sixteen colostrum and milk samples were obtained from 2010 to 2017 from 54 dairy goats of Majorera Breed from partum to 40 days of lactation. Samples were obtained according to the previous experiments (Capote et al., 2006). Briefly, animals were milked in a milking parlour at 42 KPa of vacuum pressure, 50:50 ratio and 100 pulse per minute. Four samples were obtained from each goat on different dates. One sample was obtained in each of the four periods of 10 days between partum and 40 days of lactation. Samples were aliquoted in 5 mL tubes and frozen at -80° centigrade.

Refractometry and ELISA

Colostrum samples were defrosted in a water bath at 20°C before analysis. Approximately $50\ \mu\text{L}$ of colostrum was placed on the prism and the sample cover was lowered. A clinical refractometer (Atago, USA) with a scale for serum protein ranging from 0 to 12 mg/mL was used after calibration with distilled water. The refractometer was then held up to a light source, and the serum total protein value was read at the line between the light and dark areas that appeared on the scale. The refractometer prism was washed with distilled water between samples. Samples with readings higher than scale were diluted 1:1 with distilled water and measured again, the second reading result was multiply by two. Quantification of IgG in colostrum samples was performed using goat IgG ELISA kits (Bethyl Laboratories, Montgomery, TX) following the procedure described by Capote et al. (2006), Rodriguez et al. (2009) and Dunn et al. (2017).

Statistical analysis

The SAS (Version 9.4., SAS Institute Inc., Cary, NC, USA) program package was used for statistical analysis. The relationship between refractometer and ELISA IgG concentrations was

studied using the PROC REG. Break points using a clinical refractometer, assuming IgG concentration higher than 20 mg/mL measured by ELISA as a good colostrum, were evaluated by sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Youden index was calculated as sensitivity plus specificity minus one according to Ruopp et al., 2007. The receiver operating characteristics (ROC) curve and the area under the curve (AUC) were calculated as a measure of the quality of the cut-off points according to Bartens et al., 2016.

Results and discussion

Two hundred and sixteen colostrum and milk samples were analysed. The IgG concentration mean value, according to the ELISA results, was 7.93 ± 12.37 with a maximum of 54.03 and a minimum of 0.12 mg/mL. Refractometer mean value, for protein concentration, was 6.78 ± 3.16 with a maximum of 18 and a minimum of 3.6 mg/mL. Based on ELISA IgG concentration, 29 samples were of good quality ($\text{IgG} \geq 20\ \text{mg/mL}$) and 187 samples were of poor quality ($\text{IgG} < 20\ \text{mg/mL}$). The aim of the present study was to evaluate the clinical refractometer as an easy and cheap tool to predict the colostrum quality. The variability in the IgG concentration in the colostrum is huge, as it has been reported previously by (Baumrucker et al., 2014), to obtain a wide range of IgG concentrations is crucial to evaluate any predictive method. The present study displays a narrower range than that previously reported by (Bartens et al., 2016) in cow, but similar to other studies in dairy goats (Sanchez-Macias et al., 2014).

Samples were frozen after obtained, but previous studies have demonstrated that one freeze–thaw cycle has little effect in IgG goat colostrum concentration (Arguello et al., 2003) or refractometer reading (Morrill et al., 2015).

The relationship between IgG concentration measured by ELISA and clinical refractometer was established using a linear

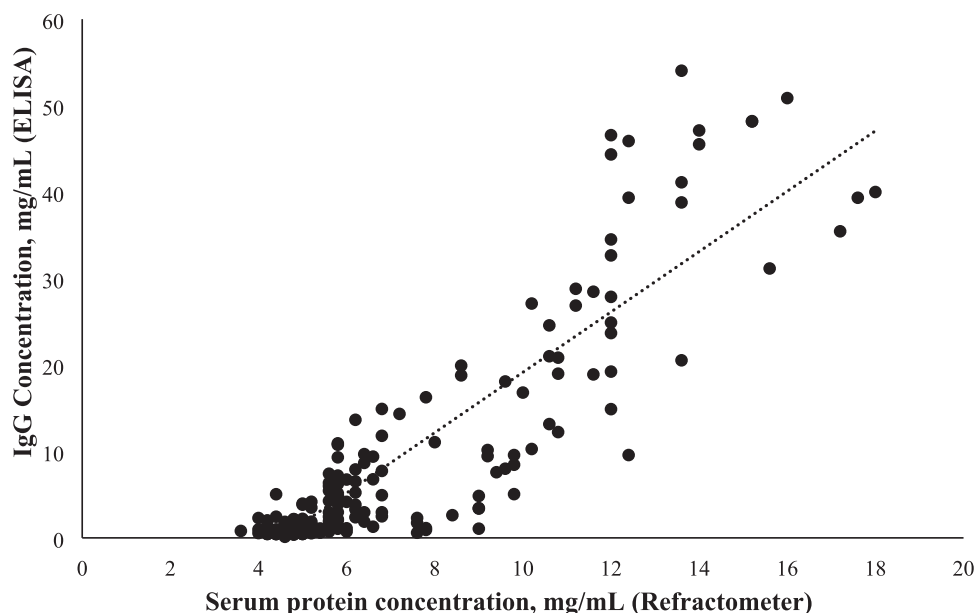


Figure 1. Regression of Colostral IgG concentration by ELISA and clinical refractometer.

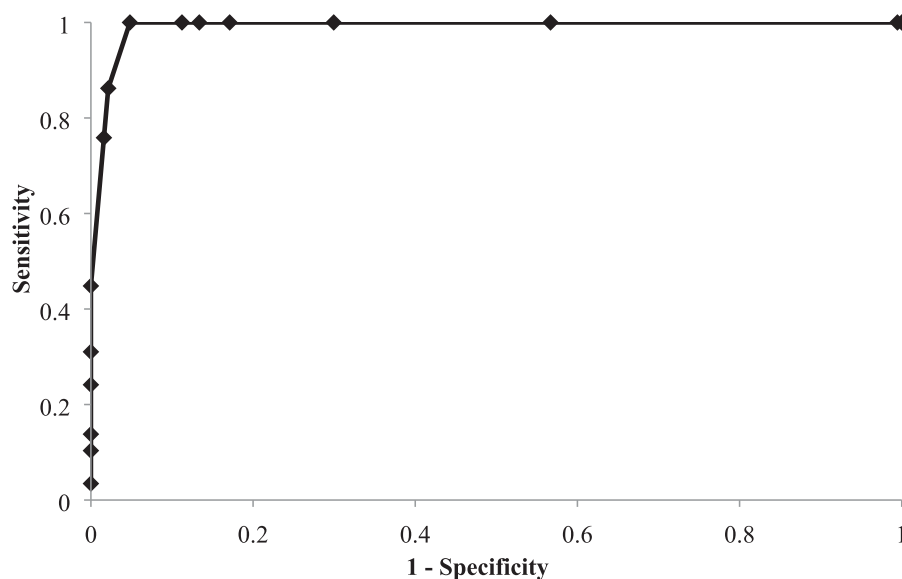


Figure 2. Receiver operating characteristic curve for clinical refractometer.

Table 1. Diagnostic test characteristics to use clinical refractometer to estimate that goat colostrum IgG concentration is higher than 20 mg/mL.

| Cut-off value | Sensitivity (%) | Specificity (%) | NPV (%) | PPV (%) | Youden index | Accuracy (%) |
|---------------|-----------------|-----------------|---------|---------|--------------|--------------|
| 18 | 3.45 | 100 | 86.98 | 100 | 0.03 | 87.04 |
| 17 | 10.34 | 100 | 87.79 | 100 | 0.10 | 87.96 |
| 16 | 13.79 | 100 | 88.21 | 100 | 0.14 | 88.43 |
| 15 | 24.14 | 100 | 89.47 | 100 | 0.24 | 89.81 |
| 14 | 31.03 | 100 | 90.34 | 100 | 0.31 | 90.74 |
| 13 | 44.83 | 100 | 92.12 | 100 | 0.45 | 92.59 |
| 12 | 75.86 | 98.40 | 96.34 | 88.00 | 0.74 | 95.37 |
| 11 | 86.21 | 97.86 | 97.86 | 86.21 | 0.84 | 96.30 |
| 10 | 100 | 95.19 | 100 | 76.32 | 0.95 | 95.83 |
| 9 | 100 | 88.77 | 100 | 58.00 | 0.89 | 90.28 |
| 8 | 100 | 86.63 | 100 | 53.70 | 0.87 | 88.43 |
| 7 | 100 | 82.89 | 100 | 47.54 | 0.83 | 85.19 |
| 6 | 100 | 70.05 | 100 | 34.12 | 0.70 | 74.07 |
| 5 | 100 | 43.32 | 100 | 21.48 | 0.43 | 50.93 |
| 4 | 100 | 0.53 | 100 | 13.49 | 0.01 | 13.89 |
| 3 | 100 | 0 | 100 | 13.43 | 0 | 13.43 |

Note: NPV: negative predictive value; PPV: positive predictive value.

regression shown in Figure 1. No data are available for goat colostrum/milk, but the r^2 in the present study (0.79) is higher than that in the recently published studies in cows (Bielmann et al., 2010, Quigley et al., 2013, Bartier et al., 2015), although r^2 values of cows were obtained using a brix refractometer. Arguello et al. (2005) reported a similar r^2 using the colostrum colour as a predictor of IgG concentration in the goat colostrum. In addition to the results of r^2 , Figure 2 displays an AUC value of 0.99. Referred AUC values for hydrometer and optical and digital brix refractometers ranged from 0.71 to 0.81 in colostrum from dairy cows (Bartens et al., 2016). Higher AUC values in the ROC curves mean a better quality of the cutpoints.

Table 1 shows the cut-off value, sensitivity, specificity, negative predictive value, positive predictive value, Youden index and accuracy for the clinical refractometer as a tool to estimate the IgG concentration in colostrum and milk using the ELISA as a gold technique. The highest accuracy (95.83%) was observed at 10 mg/mL as a cut-off value, in coincidence with the highest sensitivity (100%), negative predictive value (100%) and Youden index (0.95). These values are similar to those reported previously in colostrum from dairy cows using a brix refractometer

(Quigley et al., 2013; Bielmann et al., 2010). The value of AUC observed in the present study (10 mg/mL) can be used as a cut-off value point in goat colostrum using a clinical refractometer as a predictor device. The use of a brix refractometer is an easy, cheap and accurate method to estimate the goat colostrum quality in farm.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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