Validation of a handheld refractometer to assess Merino ewe colostrum and transition milk quality

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ABSTRACT

Colostrum quality is generally defined by the IgG concentration in colostrum, and many methods have been used to assess it. Methods to measure colostrum quality both in the laboratory and in the field have been validated in cattle; however, this is only a recent topic of interest for sheep colostrum. Laboratory-based methods are often time consuming and require trained personnel compared with new handheld evaluation tools such as the digital Brix refractometer, which gives real-time results. The aims of this study were to (1) evaluate the relationship between the digital Brix refractometer and constituents indicative of quality (IgG, protein, fat, and lactose) in colostrum and transition milk, and (2) determine an appropriate Brix % cut-off value for the Brix refractometer in sheep colostrum and transition milk. The study used 50 colostrum samples (collected at 0 h postpartum, before lambs’ sucking) and 169 transitional milk samples (collected at 4 and 24 h postpartum, after lambs had sucked) collected over 6 lambing trials in 2 years (2019 and 2020). We concluded that the Brix refractometer results correlated weakly with IgG concentration determined by radial immunodiffusion assay in colostrum collected at 0 h postpartum (r = 0.11) and in transition milk collected at 4 h postpartum (r = 0.12); however, a moderate to strong correlation was shown in transition milk samples collected at 24 h (r = 0.66). Brix % was significantly correlated with fat %, lactose %, and protein % at all timepoints. To determine an appropriate Brix % cut-off value indicating an IgG concentration of 20 mg/mL, we analyzed sensitivity and specificity of the Brix refractometer at 0, 4, and 24 h. In samples collected at 0 and 4 h, the highest combination of sensitivity, specificity, and accuracy was achieved at a Brix % cut-off value of 27%; in samples collected at 24 h postpartum, a Brix % cut-off value of 27% gave the highest sensitivity, specificity, and accuracy. Overall, the Brix refractometer has potential as a useful in-field tool for researchers and producers in both extensively and intensively managed flocks to measure and determine the quality of sheep colostrum and transition milk.

Key words: sheep, IgG concentration, refractometer, colostrum quality, lactation

INTRODUCTION

Sheep have a syndesmochorial placenta, which prevents immunoglobulins and other immune factors from crossing the placental barrier; therefore, lambs are born agammaglobulinemic and require colostrum to facilitate passive transfer of immunity (Logan and Pearson, 1978; Stelwagen et al., 2009; Hernández-Castellano et al., 2014; Agenbag et al., 2021). Colostrum contains essential proteins, fats, hormones, vitamins, and antibodies that facilitate immunity, growth, and development (Castro et al., 2011; Hernández-Castellano et al., 2014); therefore, it is important that colostrum is of high quality to ensure the best start to life for offspring. Although ewe colostrum composition has been investigated and extensively reviewed (Banchero et al., 2004a,b, 2015; Hart et al., 2006), colostrum quality has only recently been a topic of interest within the sheep industry (Agenbag et al., 2021). Colostrum contains essential proteins, fats, hormones, vitamins, and antibodies that facilitate immunity, growth, and development (Castro et al., 2011; Hernández-Castellano et al., 2014); therefore, it is important that colostrum is of high quality to ensure the best start to life for offspring. Although ewe colostrum composition has been investigated and extensively reviewed (Banchero et al., 2004a,b, 2015; Hart et al., 2006), colostrum quality has only recently been a topic of interest within the sheep industry (Agenbag et al., 2021). In contrast, colostrum quality has been studied intensively in cattle, where “quality” is defined as a colostrum IgG concentration of 50 mg/mL (Bielmann et al., 2010; Quigley et al., 2013; Bartier et al., 2015; Silva-Del-Río et al., 2017; Kessler et al., 2021). In this article, colostrum is defined as the secretion collected from the first milking (0 h postpartum), whereas secretions collected from subsequent milkings (4 and 24 h postpartum) are referred to as transition milk.

Colostrum quality is mainly measured using technical laboratory assays where the IgG content is determined using either an ELISA or, more commonly, a radial immunodiffusion (RID) assay (Gelsinger et al., 2015).
Although these laboratory methods are considered the gold standard for determining IgG concentration, they require specialist equipment and are technical and time consuming (24- to 48-h incubation period; Bielmann et al., 2010; Gelsinger et al., 2015; Elsohaby et al., 2017). One tool for quantification is a handheld digital refractometer, the Brix refractometer, which allows for rapid, real-time estimation of colostrum quality by measuring the percentage of total milk solids (Brix %) within a sample. In dairy cows, moderate to high correlation coefficients have been found between Brix % and colostral IgG (r = 0.74, Bielmann et al., 2010; r = 0.75, Quigley et al., 2013; r = 0.77, Bartier et al., 2015). Although the Brix refractometer has been extensively used and validated for dairy cattle (Bielmann et al., 2010; Quigley et al., 2013; Silva-Del-Río et al., 2017; Puppel et al., 2019; Kessler et al., 2021), it has only recently been used to assess colostrum quality of ewes (Alves et al., 2015; Torres-Rovira et al., 2017; Santiago et al., 2020; Kessler et al., 2021), where “quality” is also defined by the IgG concentration within colostrum. Colostrum quality is also influenced by composition constituents such as fat, lactose, and protein; however, few studies have shown the relationship between the Brix refractometer and these factors (Santiago et al., 2020; Kessler et al., 2021).

Consumption of high-quality colostrum improves neonatal survival and increases reproductive potential and future productivity in pigs (DeNise et al., 1989, Bartol et al., 2017), dairy cattle (Faber et al., 2005), and sheep (Banchero et al., 2004a). Although the current management strategy for breeding ewes (i.e., extensive grazing environments) limits the collection of fresh colostrum samples for analysis, there are demonstrated benefits for access to and consumption of quality colostrum, which not only affects lamb survival but can also determine the reproductive potential and productivity of replacement breeding ewes.

Another area in which the Brix refractometer could be used is in assessing transition milk, the secretion available to offspring as colostrum transitions into mature milk, and it is characterized by a decrease of fat and protein and an increase in lactose (Tsioulpas et al., 2007; Abd El-Fattah et al., 2012). Although transition milk is lower in component concentrations, it contains essential immunoglobulins and other bioactive factors required by offspring to develop and grow. Measuring transition milk can provide information on the quality of colostrum that offspring have consumed. Colostrum is defined as the first secretion; once it is removed by offspring, the secretion is then known as transition milk; therefore, colostrum quality can only be measured within a short period of time. This may not be practical, especially in extensive settings where it is not always evident when offspring were born. Transition milk provides an opportunity to extend this period of time to measure quality, and the results obtained could be extrapolated to determine what the colostrum quality is or was. The Brix refractometer could be used to provide immediate results, especially if a Brix % cut-off value were determined for transition milk. As such, the validation of a reliable, accurate, and easy-to-use tool, such as the Brix refractometer, would benefit sheep researchers and producers alike, specifically those who manage their high-value flocks intensively.

We hypothesized that strong correlation coefficients between the Brix refractometer (Brix %), colostrum and transition milk quality and composition constituents would enable us to quantify both high-quality colostrum and transition milk in ewes. The aims of this study were to (1) determine the relationship between the Brix refractometer, IgG, and fat %, lactose %, and protein % determined by Milkoscan (Foss Analytical) in ewe colostrum and transition milk; (2) determine an appropriate Brix % cut-off value to identify high- or low-quality colostrum; and (3) determine an appropriate colostrum IgG concentration cut-off value to determine high- or low-quality colostrum.

MATERIALS AND METHODS

Animal Management and Experimental Design

In 2019 and 2020, colostrum and transition milk samples were collected from mature primiparous and multiparous South Australian Merino “control” ewes during 3 intensive (under 24-h surveillance in an indoor animal facility; Brougham et al., 2020, McCarthy et al., 2021; Murdoch et al., 2021) lambing trials (2019) and 3 extensive (outdoor paddock-based) lambing trials (2020) as part of a large-scale collaborative research project between the University of Adelaide, South Australian Research and Development Institute (SARDI) and Meat and Livestock Australia. Ewes were housed and managed at SARDI’s Turretfield Research Centre, Roseau, South Australia (34°33′05.4″ S 138°50′02.5″ E). Animal ethics approval was granted by the Department of Primary Industries and Regions South Australia (PIRSA) animal ethics committee (#06/18; #16/18; #06/19; #10/20).

Sample Collection and Analysis

Within each trial, colostrum was collected at 0 h postpartum and transition milk at 4 and 24 h postpartum. Aseptic techniques were used when collecting colostrum and transition milk, with 70% ethanol sprayed on the teats before collection and gloves worn during collec-
 tion. To ensure no blockages or environmental debris, such as straw or dirt, was included in the samples, both teats were stripped 2 to 3 times before sample collection. Approximately 10 mL of colostrum/transition milk was then manually extracted across both teats (~5 mL per teat) directly into a 10-mL polypropylene conical tube (Sarstedt AG & Co.) labeled with the ewe ID and timepoint of collection. Approximately 10 mL of colostrum/transition milk was then manually extracted across both teats (~5 mL per teat) directly into a 10-mL polypropylene conical tube (Sarstedt AG & Co.) labeled with the ewe ID and timepoint of collection. Samples collected at 4 and 24 h postpartum were only included in this study if the lamb(s) had sucked from the udder to ensure it was transition milk that was collected, which was achieved by using surveillance footage collected during the trials.

**Brix Refractometer**

Within 1 h of sample collection, the Brix % was determined using an IC-PAL-1 digital handheld pocket refractometer (Instrument Choice Dry Creek) following the manufacturer’s instructions. The refractometer allows for automatic temperature compensation to ensure accurate measurements without recalibration at different ambient temperatures. The accuracy of the Brix refractometer was ±0.2% Brix at 20°C. Briefly, at room temperature, the device was first zeroed using reverse osmosis water; then, approximately 0.3 mL of sample was transferred into the device’s reading well and Brix % recorded 3 consecutive times. The 10-mL colostrum or transition milk sample was then divided into 5 × 1-mL aliquots and 1 × 5-mL aliquot, and aliquots were frozen at −20°C and stored long-term at −80°C until further analysis could be conducted.

**Immunoglobulin G**

Colostrum IgG analysis was conducted using an in-house RID assay based on techniques described by Mancini et al. (1965), modified and validated for ewe colostrum (Murdock et al., 2021; Swinbourne et al., 2022). Colostrum samples were diluted with Milli-Q water (Merck) to 1:100. Transition milk samples were diluted with Milli-Q water 1:80 for 4-h and 1:40 for 24-h samples. Immunoglobulin G standards of 0.125, 0.25, and 0.50 mg/mL were used for analysis.

**Fat, Lactose, and Protein**

Colostrum and transition milk fat %, lactose %, and protein % analysis was conducted by Milkoscan analysis by National Herd Development Laboratory (Cohuna, Victoria, Australia). Briefly, 5 mL of colostrum or 5 mL of transition milk was thawed from −80°C to −4°C and diluted with 14.8 mL of PBS. The samples were kept refrigerated at 4°C and transported to the National Herd Development Laboratory for analysis the following day. Once the samples arrived at the laboratory, 0.2 mL of preservative was added to ensure no microbial growth while undergoing liquid infrared (Milkoscan) analysis.

**Specificity and Sensitivity of the Brix Refractometer**

The Brix refractometer was analyzed for specificity and sensitivity against IgG concentration determined by RID. An IgG concentration cut-off value of 20 mg/mL (as previously used by Kessler et al., 2021) was used across all timepoints; 0, 4, and 24 h. For assessment of the Brix refractometer, Brix % cut-off levels from 26 to 29% were used, based on previously published values (Torres-Rovira et al., 2017; Kessler et al., 2021).

**Statistical Analysis**

All statistical analyses were conducted using SPSS (version 28; IBM Corp.) and the Excel Real Statistic Package (Xrealstats: Microsoft Office Suite 365). Paired samples from ewes were analyzed using linear regression and Pearson correlations to determine the direction and strength of the relationship, Lin’s concordance correlation coefficient ($LCCC$) was used to determine reproducibility, and Bland-Altman plots were conducted to determine the number of outliers. At each timepoint (0, 4, and 24 h), variables (IgG, fat %, lactose %, and protein %) were measured against Brix %. Brix % and IgG were also analyzed as a repeated measure to determine differences between timepoints. Descriptive data are presented as means ± standard errors of the mean (SEM), and significance differences were accepted at $P \leq 0.05$, with tendencies reported at $P < 0.1$. Last, the sensitivity and specificity, as well as the positive predictive value, negative predictive value, and accuracy value of the Brix refractometer (Brix %) were measured to further define an acceptable IgG cut-off value (20 mg/mL) in Merino ewe colostrum and transition milk.

**RESULTS AND DISCUSSION**

This study aimed to investigate the relationship between the digital Brix refractometer (Brix %) and colostrum and transition milk constituents such as IgG, fat, protein, and lactose in Southern Australian Merino ewes across varying timepoints. As mentioned above, traditional methods of measuring colostrum and transition milk constituents to determine quality can be time consuming and require trained laboratory staff, whereas the Brix refractometer allows for rapid, real-time results that can be used to estimate colostrum and transition milk quality.
A total of 50 fresh colostrum samples and 169 fresh transitional milk samples were collected throughout 2019 and 2020 at 0 (n = 50), 4 (n = 54), and 24 (n = 115) h postpartum. The digital Brix refractometer was used to measure fresh colostrum and milk samples soon after collection. Brix % ranged from 21.6 to 44.7% at 0 h, 15.1 to 45.3% at 4 h, and 12.0 to 40.4% at 24 h postpartum (Figure 1A), with average levels shown in Figure 2A. The colostral Brix % (range: 12.0 to 45.3%) in this study was similar to previously published ranges of 15.4 to 40.0% (Kessler et al., 2021) and higher than that (<8.0 to >38.0%) in Torres-Rovira et al. (2017). Concentrations of IgG varied from 8.7 to 77.5 mg/mL at 0 h, 11.4 to 77.9 mg/mL at 4 h, and 1.3 to 58.1 mg/mL at 24 h postpartum (Figure 1B). The colostral IgG concentrations in this study were slightly higher than previously published ranges of 1.2 to 60.7 mg/mL (Alves et al., 2015) and 6.2 to 65.4 mg/mL (Kessler et al., 2019). However, the average IgG concentration (45.2 mg/mL) was similar to those previously published for sheep; for example, in the meat breeds: Scottish blackface 45.1 mg/mL (Dwyer and Morgan, 2006), Suffolk 54.1 mg/mL (Dwyer and Morgan, 2006), and Rasa Aragonesa 64.2 mg/mL (Loste et al., 2008); and in the dairy breeds: Awassi 60.9 mg/mL (Higaki et al., 2013).

Several factors could explain the differences between this study and previous studies, such as the timing of colostrum collection (at 0 h postpartum vs. later), ewe BCS from conception through to lactation, nutritional status, animal health, environmental factors, and breed (Banchero et al., 2004a,b; Hernández-Castellano et al., 2015; Kessler et al., 2019). Sample size also affects the data displayed, with larger sample sizes representing a higher proportion of the population and encompassing differences in management, nutritional, and environmental factors, all of which affect colostrum and colos- trum quality (Godden, 2008). The number of colostrum samples collected at 0 h postpartum in the current study was half the sample size reported in Kessler et al. (2021) (n = 100) and approximately one-tenth of the sample size reported in Torres-Rovira et al. (2017) (n = 536). During the first 24 h after parturition, colostrum composition changed significantly as it transitioned into milk, with high IgG concentrations declining over time (Figure 1B), which coincides with gut closure in lambs (Bush and Staley, 1980). It is important that lambs are able to suck and consume colostrum early because the marked decrease in colostral IgG concentration coincides with gut closure in the lamb and may result in failure of passive transfer of immunity (Sawyer et al., 1977; Alves et al., 2015).

Average fat %, protein %, and lactose % were determined at 0, 4, and 24 h. Fat % and protein % decreased slightly across each of the timepoints: fat % was 14.9, 13.3, and 12.1% (Figure 1C) and protein % was 20.9, 19.6, and 11.7% (Figure 1D) at 0, 4, and 24 h postpartum, respectively. Lactose % increased at each timepoint: 1.7, 1.9, and 4.1% at 0, 4, and 24 h postpartum, respectively (Figure 1E). These values were similar to previously published ranges, in which mature ewe colostrum contained 7 to 13% fat, 2 to 5% lactose, and 7 to 10% non-Ig protein (Hadjipanayiotou, 1995). Fat, protein, and lactose are important colostral components as they contribute to energy requirements, growth, and muscle development and further assist in passive immune transfer in neonates (Hernández-Castellano et al., 2014).

The correlations between Brix %, IgG concentration, fat %, protein %, and lactose % across the various timepoints are presented in Table 1. Data from Bland-Altman plots and LCCC are also given in Table 1. Bland-Altman plots showed that all data had fewer than 10% outliers across all timepoints (Supplemental Material, https://doi.org/10.25909/6266855e9d8b8), and LCCC indicated low to medium reproducibility across all relationships. Correlations between Brix % and IgG concentration across the 24-h period differed from those previously reported. In the current study, IgG concentrations in colostrum samples collected at 0 h and transitional milk samples collected at 4 h postpartum were weakly correlated with Brix %, whereas previous work reporting results from 10 sheep species reported a strong correlation between Brix % and IgG concentration for colostrum samples collected within 390 min postpartum (r = 0.75; Kessler et al., 2021). This is the expected relationship between the Brix refractometer and IgG concentration; however, our data only demonstrated a moderately strong positive correlation between Brix % and IgG levels in transition milk collected at 24 h postpartum (r = 0.66). Weak correlation coefficients between Brix % and IgG concentration at 0 and 4 h postpartum may be due to the aforementioned differences in ewe breeds and management before par- turation or differences in sample analysis techniques. Analysis techniques were identified as a potential confounding factor in Swinbourne et al. (2022), where the integrity of fresh versus frozen-thawed samples may result in different or lower correlation coefficients than those previously published. Although one study in dairy cattle reported no differences in colostrum quality from analysis of fresh versus frozen-thawed colostrum samples using both an optical and a digital refractometer (Biølmann et al., 2010), a similar study has yet to be conducted in sheep colostrum and milk. A suggestion for future analysis would be to use either fresh or frozen-thawed samples for all analyses, including determining Brix % and IgG concentration by RID assay. An additional contributor to the lower correla-
tion coefficients observed in our study may have been the concentration of total solids present in the samples. Due to the thick and viscous nature of early colostrum, the Brix refractometer may not be able to accurately read the total solids present within it. Viscosity was not measured during this study; however, a suggestion for future analysis would be to measure viscosity and potentially dilute 0-h colostrum and 4-h transition milk. However, as the Brix refractometer is intended to be used as a simple, in-field diagnostic tool, diluting colostrum may complicate the process too much (extra materials will be needed). These suggestions warrant further investigation because the effectiveness of the Brix refractometer to evaluate colostrum and transition milk quality in Merino ewes depends on the accuracy of these results.

The correlations between Brix % and fat %, lactose %, and protein % were significant \((P < 0.05)\) at all timepoints. Fat % had moderate positive correlations with Brix % at all timepoints \((0 \text{ h: } r = 0.41, 4 \text{ h: } r = 0.41, 24 \text{ h: } r = 0.38)\). Protein % had a low positive correlation with Brix % at 0 h postpartum \((r = 0.28)\),

Figure 1. (A) Brix %, (B) IgG concentration (mg/mL), (C) fat %, (D) protein %, and (E) lactose % in colostrum and transitional milk collected at 0, 4, and 24 h postpartum. The box indicates the 25th to 75th percentile, the solid line within the box represents the median, and the whiskers show the 10th to 90th percentile distribution of the data.
a moderate positive correlation at 4 h \((r = 0.39)\), and a strong positive correlation at 24 h \((r = 0.72)\). Lactose % was negatively correlated at all timepoints \((0 \text{ h}: r = -0.69, 4 \text{ h}: r = -0.64, 24 \text{ h}: r = -0.52)\), with moderate to strong relationships. Correlations for the 0-h samples were similar to those of a previous study, in which fat % showed a significant positive correlation \((r = 0.55)\) and lactose % a significant negative correlation \((r = -0.75)\). Protein % was also significantly correlated but with a much stronger correlation \((r = 0.80; \text{Kessler et al., 2021})\). We are demonstrating the correlations between each of these variables by using the Brix refractometer as a tool to evaluate colostrum and transition milk quality. Subsequent research is under way to provide an in-depth analysis of the composition of Merino ewe colostrum and transitional milk, which will include a detailed discussion of the importance of these components within colostrum.

To ensure that offspring receive high-quality colostrum and for the Brix refractometer to be used to its full potential, there is a need to determine a Brix % cut-off value that is highly correlated with an IgG concentration at or over a certain concentration. To determine this Brix % cut-off value, the Brix refractometer results must be analyzed for specificity and sensitivity. For example, the cut-off value for quality colostrum in dairy cattle is 50 mg/mL IgG concentration, which equates to 21 to 23% Brix \((\text{Bielmann et al., 2010; Quigley et al., 2013; Bartier et al., 2015; Buczinski and Vandeweerd, 2016})\). For sheep, 50 mg/mL IgG may not be

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**Table 1.** Comparative analysis of Brix % against IgG concentration, fat %, protein %, and lactose % in Merino colostrum at 0 h postpartum and transitional milk at 4 and 24 h postpartum

| Data analysis | n  | Regression | B-A plot | LCCC | r value | P-value |
|---------------|----|------------|----------|------|---------|---------|
| 0 h Brix % vs. IgG | 50 | 0.51 | 0.887 | 0.022 | 0.105 | 0.470 |
| Brix % vs Fat % | 50 | -0.76 | 0.043 | 0.058 | 0.413 | 0.002 |
| Brix % vs Protein % | 50 | -0.32 | 0.235 | 0.098 | 0.276 | 0.048 |
| Brix % vs Lactose % | 50 | -1.84 | 0.925 | -0.006 | -0.694 | 0.001 |
| 4 h Brix % vs. IgG | 54 | 0.69 | 0.858 | 0.037 | 0.123 | 0.374 |
| Brix % vs Fat % | 27 | -0.72 | 0.015 | 0.058 | 0.431 | 0.022 |
| Brix % vs Protein % | 27 | -0.47 | 0.002 | 0.169 | 0.393 | 0.039 |
| Brix % vs Lactose % | 27 | -1.76 | 0.929 | -0.008 | -0.639 | 0.001 |
| 24 h Brix % vs. IgG | 87 | 0.21 | 0.492 | 0.358 | 0.659 | 0.001 |
| Brix % vs Fat % | 56 | -1.99 | 0.994 | 0.126 | 0.379 | 0.004 |
| Brix % vs Protein % | 56 | -0.55 | 0.050 | 0.246 | 0.723 | 0.001 |
| Brix % vs Lactose % | 56 | -1.35 | 0.802 | -0.023 | -0.523 | 0.001 |

Regression analysis, Bland-Altman (B-A) plots, Lin’s concordance correlation coefficient (LCCC), and Pearson’s correlation coefficient \(r\) value. \(P\)-value indicates significance \((P < 0.05)\) of using the Brix refractometer (measured in Brix %) to measure IgG, fat %, protein %, and lactose %.

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**Figure 2.** Mean values and differences in Merino ewe colostrum at 0, 4, and 24 h postpartum for (A) Brix value (%) \(P < 0.001\) and (B) IgG concentration (mg/mL) \(P < 0.001\). Letters (a, b) indicate significant \((P < 0.05)\) differences between timepoints. Data presented as mean ± SEM.
an appropriate concentration because the majority of samples were below this threshold. In the current study, 68% (34/50 samples) of 0-h samples, 68.5% (37/54 samples) of 4-h samples, and 93.9% (108/115 samples) of 24-h samples had an IgG concentration <50 mg/mL. Previous literature has suggested an IgG concentration of 20 mg/mL as an appropriate IgG cut-off for sheep colostrum (Torres-Rovira et al., 2017; Kessler et al., 2021). Using this IgG cut-off level, a Brix % cut-off value between 26 and 29% can then be implemented (Torres-Rovira et al., 2017; Kessler et al., 2021).

For the assessment of the Brix refractometer in this study, Brix % cut-off levels from 26 to 29% were used (Tables 2, 3, and 4). A combination of high sensitivity and specificity, a high positive predictive value, and a low negative predictive value are necessary to ensure that when the Brix refractometer is used, the user can be confident that it correlates strongly with an IgG concentration (e.g., 21% Brix = 50 mg/mL IgG concentration in dairy cattle colostrum). The sensitivity value is used to determine the probability of a low-quality sample, and specificity determines the probability of a good-quality sample. The positive and negative predictive values confirm the probability that the sample is of low or high quality (Trevethan, 2017). In this study, specificity of the refractometer was very poor for colostrum samples collected at 0 h postpartum and for transitional milk samples collected at 4 h postpartum. Sensitivity remained high throughout the analysis of the 0- and 4-h samples; however, specificity was 0.00 for almost all Brix % cut-off points. This indicates that the probability of a good-quality sample in our data set was not very likely. The ideal Brix % cut-off value for colostrum samples collected at 0 h postpartum and for transitional milk collected at 4 h postpartum at 20 mg/mL IgG was 29% because it had the highest sensitivity and specificity combination, with 90% and 80% positive and negative predictive values, respectively. For transitional milk samples collected at 24 h postpartum, the Brix % cut-off value was 27% because this value had the highest combination of sensitivity and specificity and an accuracy of 50%. A previous study (Kessler et al., 2021) that compared cut-off values between species found that 26.5% was a suitable Brix % cut-off value for sheep colostrum: it resulted in 75% sensitivity, 91% specificity, 96% positive predictive value, and 51% negative predictive value. Although the current study showed sensitivity and specificity values lower than reported by Kessler et al. (2021), depending on the time of collection, the test parameters followed a similar trend. The poor to moderate correlation coefficients between Brix % and IgG concentration at the varying timepoints may be a contributing factor for these differences, or as previously stated, the differences may be due to differences in sample size. Although this is one of the first studies to determine an appropriate IgG concentration cut-off value in sheep colostrum and transition milk with the use of the Brix refractometer, further research, in which samples are collected at varying timepoints, is warranted.

**CONCLUSIONS**

The digital Brix refractometer is a promising and easy-to-use tool to measure colostrum quality in sheep, allowing for rapid, real-time results that can be correlated with colostrum constituents such as IgG to measure and define quality. Our findings demonstrated a weak correlation between Brix % and IgG concentration in colostrum (0 h) and transitional milk (4 h),

| Brix % cut-off | Se (%) (95% CI) | Sp (%) | PPV (%) | NPV (%) | Acc (%) |
|---------------|----------------|--------|---------|---------|---------|
| 26            | 89.74 (79.69–99.79) | 0.00   | 87.50   | 0.00    | 79.55   |
| 27            | 89.48 (79.16–99.79) | 0.00   | 87.18   | 0.00    | 79.07   |
| 28            | 86.49 (74.64–98.33) | 0.00   | 86.49   | 0.00    | 76.19   |
| 29            | 83.33 (70.00–96.67) | 17.67 (0.00–89.71) | 85.71 | 14.29 | 73.81 |

| Brix % cut-off | Se (%) (95% CI) | Sp (%) | PPV (%) | NPV (%) | Acc (%) |
|---------------|----------------|--------|---------|---------|---------|
| 26            | 84.09 (72.31–95.88) | 0.00   | 94.87   | 0.00    | 80.44   |
| 27            | 77.27 (63.19–91.36) | 0.00   | 94.44   | 0.00    | 73.91   |
| 28            | 75.00 (60.23–89.77) | 0.00   | 94.29   | 0.00    | 71.74   |
| 29            | 75.00 (60.23–89.77) | 0.00   | 94.29   | 0.00    | 71.74   |
but a moderate to strong correlation with 24-h transitional milk samples. Based on the highest combination of sensitivity and specificity of the Brix refractometer when 20 mg/mL IgG was used, a Brix % cut-off value of 29% for samples collected at 0 and 4 h postpartum and 27% for transitional milk collected at 24 h postpartum would be the most appropriate for identifying quality colostrum and transitional milk in Merino ewes. Although the analysis techniques of measuring and defining colostrum quality must be refined, this study provides valuable information regarding the use of the Brix refractometer, especially in Merino ewes, and its potential as a valuable tool for researchers or producers managing lambing ewes intensively or extensively.

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