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Immunoglobulin concentration in bovine colostrum obtained by indirect methods

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Research Article

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Abstract

The quality of colostrum is essential for the successful transfer of passive immunity and the early development of the immune system in newborns. This research was conducted with the aim of evaluating the agreement between measurements from different equipment: colostrometer, digital Brix refractometer and optical Brix refractometer, using fresh colostrum samples maintained at 21°C. Colostrum samples were collected from two farms ($n_1 = 31$ and $n_2 = 193$). Farm 1 included females from second to ninth lactation, comprising 15 Holstein Friesian (HF), 5 Jersey and 11 Holstein × Jersey crossbreeds. Farm 2 had HF heifers and cows from first to seventh lactation. Immunoglobulin content was assessed indirectly with a colostrometer and both types of Brix refractometers (digital and optical). For the correlation analysis, Pearson's product-moment method was used to assess the linear association between the equipment, followed by a Student's *t*-test and comparison of the obtained values with specific correlation coefficients (50%, 70%, 90% and 99%). There was a 98% correlation between the refractometers on Farm 1, which was significantly higher ($P < 0.0001$) than the commonly used reference values for weak (50%), moderate (70%) and strong (90%) correlations, and statistically equivalent to a 99% correlation. This indicates a very high, positive linear association between the digital and optical Brix refractometers. On Farm 2, a 97% correlation was found between the refractometers, which was significantly higher ($P < 0.0001$) than the specific reference values of 50%, 70% and 90%, but significantly lower than a 99% correlation.

Bovine placentas are synepitheliochorial, consisting of six layers: three from maternal tissues and three from foetal tissues. This structure impairs the transfer of immunoglobulins (Ig) from the cow to the calf (Blum and Baumrucker, 2008; Peter, 2013). As a result, calves are born without Ig and rely entirely on colostrum for their passive Ig supply (Godden *et al.*, 2019). The immunological quality of colostrum is related to its Ig concentration (Godden *et al.*, 2019), making the evaluation of colostrum Ig concentration essential in classifying colostrum based on its effectiveness in the establishment of passive immunity and development of the immune system in newborn calves (Ahmann *et al.*, 2021).

Methods for estimating colostrum immunological quality include direct methods, such as enzyme-linked immunosorbent assay (ELISA) and radial immunodiffusion (RID), and indirect methods, such as the colostrometer and digital and optical Brix refractometers (Ahmann *et al.*, 2021). These methods differ in terms of cost, time to obtain results, techniques and procedures, and the skills required to perform them. These factors may explain the various inconclusive and/or conflicting results shown when comparing these methods (Bartier *et al.*, 2015; Gelsinger *et al.*, 2015; Dunn *et al.*, 2018; Zobel *et al.*, 2020; Röder *et al.*, 2023).

The RID technique is considered the gold standard among the direct methods and shows a moderate to strong correlation with indirect techniques (Bielmann *et al.*, 2010; Elsohaby *et al.*, 2017; Zobel *et al.*, 2020). However, indirect methods are generally faster and more practical for on-farm use, making them more suitable for routine assessments of colostrum quality (Ahmann *et al.*, 2021). The colostrometer is a hydrometer commonly used on dairy farms in which the total Ig concentration is measured through specific gravity (Ahmann *et al.*, 2021), which may be affected by colostrum temperature (Geiger, 2020). The Brix refractometer, on the other hand, is a device used to measure sucrose concentrations in liquids (wine, molasses and fruit juice). When used for milk or colostrum, it is known that there is a correlation between the value obtained and the total solids content of the sample, enabling the indirect assessment of Ig levels (Deelen *et al.*, 2014).

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In general, indirect methods use equipment that is easier to handle and read; they are faster and cheaper, making them widely used in dairy farm routines. Nevertheless, the assessment of colostrum quality varies between them, as the colostrometer determines the specific gravity, while the Brix refractometer uses light refraction to estimate the total solids content (Ahmann *et al.*, 2021). However, the reports found in the literature on the correlation between Ig estimates obtained using indirect techniques are controversial, and it is not known whether estimates from different farms, which may employ varying technologies, are correlated (Bartier *et al.*, 2015; Gelsinger *et al.*, 2015; Dunn *et al.*, 2018; Zobel *et al.*, 2020). Furthermore, there is still a lack of agreement or consensus regarding colostrum quality assessments obtained using different equipment (Elsohaby *et al.*, 2017; Dunn *et al.*, 2018; Zobel *et al.*, 2020).

Therefore, this study aims to quantify the magnitude and direction of the association between indirect measurements of colostrum quality (using colostrometers and digital and optical Brix refractometers), as well as the concordance between the results from these methods. It will also investigate whether these results vary when comparing different types of measuring equipment and farms with different technological levels.

Materials and methods

Location

The study was conducted in two farms. Farm 1, belonging to the Agência Paulista de Tecnologia dos Agronegócios (APTA), was located in Americana, São Paulo, Brazil (22°45'09.0"S 47°16'51.9"W). According to the Köppen classification, the region has a tropical climate, with dry winters and average annual rainfall of 1,478 mm, and average temperatures of 21.7°C. Farm 2, located in Carambeí, Paraná, Brazil (24°47'03.0"S 50°12'31.1"W) and Melkstad is the second-biggest milk producer in Brazil, presenting high technological levels and an intensive production system. According to the Köppen classification, the climate is temperate, with mild summers, average precipitation of 1,547 mm well distributed throughout the year. Annual average temperature is 17°C.

This study followed ethical standards and was approved by the Ethics Committee on the Use of Animals from the Universidade Federal de Mato Grosso do Sul (no. 1.223/2022).

Animals

The animals from Farm 1 were freshly calved multiparous Holstein ($n = 15$), Jersey ($n = 5$) and Holstein × Jersey ($n = 11$) cows, varying from second to ninth lactation, from July to September 2021. Thirty days before the expected calving date, the cows were placed in the pre-partum group, where they received a total mixed ration composed of 60% sorghum silage and 40% concentrate with corn meal, soybean meal and minerals. They had free access to water and a shaded area with a shade screen.

Animals from Farm 2 were freshly calved primiparous and multiparous Holstein cows ($n = 193$), varying from first to seventh lactation, from May to June 2022. Cows were kept in a free-stall barn with sprinklers and free access to feed and water. Before parturition, pregnant heifers and cows were kept in a separate group. The ration was composed of 60% corn silage and 40% concentrate based on corn meal, soybean meal, wheat bran, cottonseed, minerals and vitamins. From the beginning of parturition, heifers and cows were monitored until their end.

Measurements

In both farms, cows and calves were separated after parturition, and cows were taken to the milking parlour to harvest the colostrum within 2 hours. The pre-dipping was performed using chlorhexidine, and the first three fore-strips were discarded during the strip cup test. After the test, teats were sanitized again using the pre-dipping solution and were dried with a paper towel.

All colostrum was harvested using a machine milking system within 2 hours after parturition and was visually evaluated for the presence of blood, pus and somatic cells. When colostrum was altered, it was not used in the experiment. All the colostrum from each cow was homogenized, and a 500 mL sample was taken to be evaluated using different equipment.

Colostrum immunological quality

All samples ($n = 224$; being, $n_1 = 31$ and $n_2 = 193$) were split into duplicate subsamples and were submitted to indirect methods of colostrum immunological quality. Colostrometer analyses were performed using 500 mL of colostrum at 21°C, and the manufacturer's (Colostrometer™) guidelines were followed. At both farms, when the colostrum temperature exceeded 21°C, samples were cooled to 21°C for instrument readings. Sample temperatures were checked with a universal digital thermometer for liquids. Each replicate was homogenized and placed in a beaker containing a colostrometer. Specific gravity was then read using the device's numerical scale.

To evaluate the samples using the digital Brix refractometer, an instrument with a 0%–85% scale was used. First, it was calibrated using two drops of distilled water and dried with a soft paper towel. Then, a homogenized colostrum sample was put in the equipment reader, as described in the manufacturer's guidelines (Palm Abbe, model # PA202x; Misco, Solon, OH).

In the optical Brix refractometer analyses, the calibration was performed using distilled water before putting each homogenized colostrum sample in the prism of the device. Then the refractometer was positioned perpendicular to the light, to read the results, according to the manufacturer's instructions (Bel Engineering, model Brix model RZT; China), on a scale of 0%–32%.

The estimates obtained were used to classify each sample as high or low immunological quality. In the colostrometer, high immunological quality samples were those samples whose readings were equal to or greater than 50 mg/mL; samples whose readings were lower than 50 mg/mL were considered low immunological quality. In digital and optical Brix refractometers, high immunological quality samples were those with estimates equal to or greater than 23%, as recommended by Chigerwe *et al.* (2008), Bartier *et al.* (2015), Elsohaby *et al.* (2017) and Godden *et al.* (2019). Low-quality samples were those with estimates lower than 23%.

Statistical analysis

The calculation of Pearson's product-moment correlation coefficient and the comparisons between the coefficients were conducted using, respectively, the 'cor.test' function and the 'cocor' packages (Diedenhofen and Musch, 2015) using the software R (version

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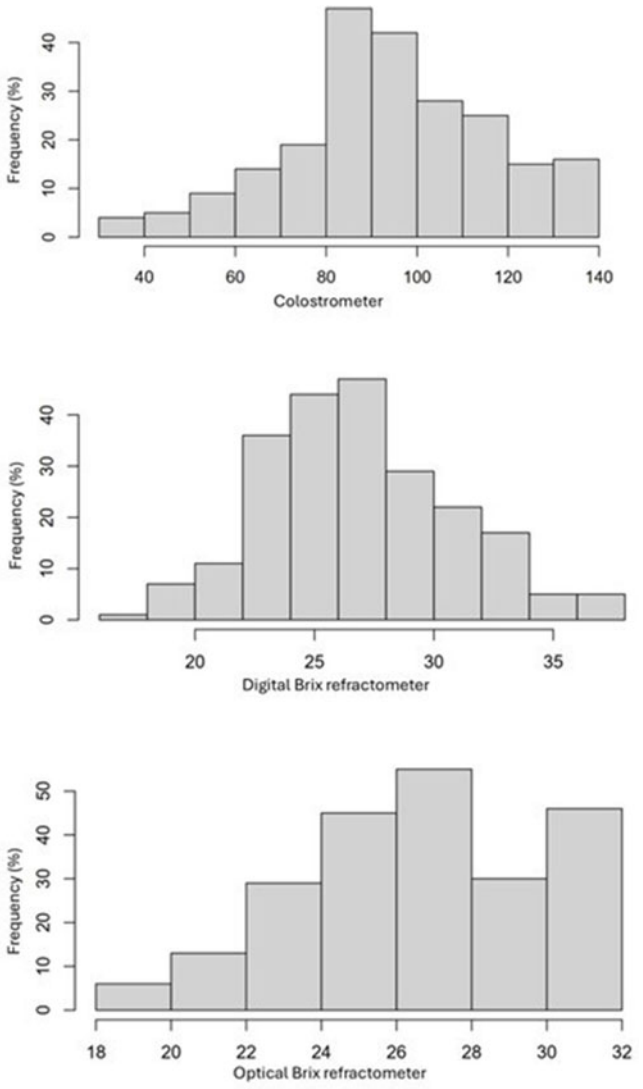


Figure 1. Distribution of Ig concentrations in the 224 bovine colostrum samples from both dairy farms ($n_1 = 31$ and $n_2 = 193$), evaluated using a colostrometer (A), digital Brix refractometer (B) and optical Brix refractometer (C).

4.2.2). Correlation strength was interpreted according to the criteria proposed by Mukaka (2012), where values of the correlation coefficient (*r)* from 0.00 to 0.29 indicate negligible, 0.30–0.49 low, 0.50–0.69 moderate, 0.70–0.89 high and 0.90–1.00 very high. The concordance analysis was performed by calculating Cohen's Kappa calculation using the package '*psych*' (Revelle, 2022). The strength of agreement was interpreted based on the scale proposed by McHugh (2012): values from 0.00 to 0.20 indicate none, 0.21–0.39 minimal, 0.40–0.59 weak, 0.60–0.79 moderate, 0.80–0.90 strong and above 0.90 nearly perfect. Comparisons of correlation coefficients of samples with specific correlation values (50%, 70%, 90% and 99%) were performed by transforming Fisher's Z statistic in SAS® PROC CORR. All analyses were conducted at the 5% significance level.

Results

A greater frequency of high-quality colostrum was observed as estimated above 80 mg/mL immunoglobulin by a colostrometer or above 20% reflection by refractometers (Fig. 1).

In both farms, the average estimates for Ig in colostrum were similar (Table 1), although for digital and optical Brix refractometers, these values were closer. Average estimates were closer to medians, and the maximum and minimum values between farms for each equipment were similar to each other. There was greater variation for the measurements obtained with the colostrometer (CV = 28.91% on Farm 1, and 23.22% on Farm 2), followed by the digital Brix refractometer (CV = 15.80% on Farm 1, and 14.38% on Farm 2) and less variation for the optical Brix refractometer (CV = 13.62% on Farm 1 and 12.12% on Farm 2).

For the total data ($n = 224$), the average estimated immunoglobulin concentrations measured by the colostrometer were 97.19 mg/mL, while the digital and optical Brix refractometers showed mean values of 26.98% and 27.03%, respectively. The variability of the data also followed the same pattern, with the highest coefficient of variation observed in the measurements taken with the colostrometer (CV = 24.26%), followed by the digital refractometer (CV = 14.56%), and the lowest variation in the optical refractometer (CV = 12.30%).

In Farm 1, we observed a positive and very high correlation between the digital and optical Brix refractometer estimates ($r = 0.98$; $P < 0.001$), according to the interpretive criteria proposed by Mukaka (2012), which classify values between 0.90 and 1.00 as very high. The colostrum immunoglobulin concentration estimated by the colostrometer also showed a high and significant correlation with the digital ($r = 0.81$; $P < 0.001$) and optical ($r = 0.83$; $P < 0.001$) Brix refractometer readings, although the magnitude was lower (Table 2). Similarly, on Farm 2, the correlation between digital and optical Brix refractometer readings was again positive and very high ($r = 0.97$; $P < 0.001$). The colostrometer estimates also showed high, albeit slightly lower, correlations with those from the digital ($r = 0.83$) and optical ($r = 0.84$) Brix refractometers (Table 2).

When combining the total data, the strong correlation observed between the digital and optical Brix refractometers was maintained ($r = 0.97$, $P < 0.001$). Similarly, the colostrometer estimates showed a strong correlation with both the digital ($r = 0.81$, $P < 0.001$) and optical ($r = 0.83$, $P < 0.001$) refractometer readings, indicating consistent agreement between the methods across the entire dataset.

When comparing the correlation coefficients with specific reference values (Table 3), the coefficient of 98% between the digital and optical Brix refractometers on Farm 1 was superior to 50%, 70% and 90% ($P < 0.001$) and similar to 99% ($P = 0.179$). According to Mukaka (2012), this indicates a very high correlation, suggesting a high level of agreement and consistency between the refractometers. The correlation between the colostrometer and the digital Brix refractometer ($r = 0.81$) exceeded 50% ($P = 0.003$), was similar to 70% ($P = 0.188$) and 90% ($P = 0.057$), and was lower than 99% ($P < 0.001$). A comparable result was observed for the optical Brix refractometer ($r = 0.83$) with values surpassing 50% ($P = 0.001$), not differing from 70% ($P = 0.107$) and 90% ($P = 0.108$), and remaining below 99% ($P < 0.001$). Based on the same interpretive criteria, these correlations are classified as high. However, the slightly lower magnitude compared to the refractometers may reflect the influence of external factors on the colostrometer readings, such as colostrum fat content and temperature.

Based on the same interpretative criteria, these correlations are classified as high.

The differences between the correlation coefficients for the pairs of equipment were similar between farms. Thus, an analysis was performed to calculate the common correlation coefficient. For

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Table 1. Descriptive statistics of colostrum samples from Farm 1, Farm 2 and total data showing measurements obtained by the colostrometer (Ig concentration, mg/mL), digital Brix refractometer (% Brix) and optical Brix refractometer (% Brix)

Descriptive statistics	Farm 1 ($n = 31$)			Farm 2 ($n = 193$)			Total data ($n = 224$)		
	Colost[a]	Dig[b]	Opt[c]	Colost[a]	Dig[b]	Opt[c]	Colost[a]	Dig[b]	Opt[c]
Average	86.77	27.24	26.93	98.86	26.94	27.04	97.19	26.98	27.03
SD[d]	25.08	4.30	3.67	22.96	3.87	3.28	23.58	3.93	3.33
CV[e] (%)	28.91	15.80	13.62	23.22	14.38	12.12	24.26	14.56	12.30
Median	85.00	27.00	27.00	100.00	26.50	27.00	97.50	26.70	27.00
Maximum	140.00	35.60	32.00	140.00	37.60	32.00	140.00	37.60	32.00
Minimum	30.00	18.80	19.00	35.00	17.30	18.00	30.00	17.30	18.00

[a]Colostrometer.
[b]Digital Brix refractometer.
[c]Optical Brix refractometer.
[d]Standard deviation.
[e]Coefficient of variation.

Table 2. Correlation coefficients among the equipment used to estimate the colostrum immunological quality

Equipment	Correlation coefficient	95% CI[a]	*P*-value
Farm 1 ($n = 31$)			
Colost[b]× Digital[c]	0.81	0.631–0.902	<0.001
Colost[b] × Optical[d]	0.83	0.664–0.912	<0.001
Digital[c] × Optical[d]	0.98	0.966–0.992	<0.001
Farm 2 ($n = 193$)			
Colost[b]× Digital[c]	0.83	0.778–0.868	<0.001
Colost[b] × Optical[d]	0.84	0.795–0.878	<0.001
Digital[c] × Optical[d]	0.97	0.957–0.975	<0.001
Total data ($n = 224$)			
Colost[b] × Digital[c]	0.81	0.756–0.849	<0.001
Colost[b] × Optical[d]	0.83	0.782–0.865	<0.001
Digital[c] × Optical[d]	0.97	0.960–0.976	<0.001

[a]Confidence interval.
[b]Colostrometer.
[c]Digital Brix refractometer.
[d]Optical Brix refractometer.

Farm 2, the common correlation coefficient between the digital and optical Brix refractometer equipment was greater ($r = 0.97$) than the correlation between the colostrometer and optical Brix refractometer ($r = 0.84$), which was greater than the correlation between the colostrometer and digital Brix refractometer ($r = 0.83$).

When pooling the total data, the strong correlation between the digital and optical Brix refractometers was confirmed, with a coefficient of 0.97 that exceeded the reference values of 50%, 70% and 90% ($P < 0.001$), while remaining statistically lower than 99% ($P < 0.001$). Similarly, the correlations between the colostrometer and the digital ($r = 0.81$) and optical ($r = 0.83$) refractometers were higher than 50% ($P < 0.001$), statistically comparable to 70% and 90%, and below 99% ($P < 0.001$). These results indicate very high agreement between the refractometers and high correlations involving the colostrometer. The consistency of these findings across farms reinforces the reliability of Brix refractometers in assessing colostrum quality under diverse herd conditions.

Based on the sample classification for its immunological quality (high or low), the concordance among the equipment was evaluated (Table 4). A low rate of disagreement was observed between the digital and optical Brix refractometers (4.0%) compared with disagreement between the digital Brix refractometer and the colostrometer (9.4%), and between the optical Brix refractometer and the colostrometer (12.5%). According to the interpretive scale proposed by McHugh (2012), the Kappa coefficient indicated strong agreement between the digital and optical Brix refractometers (0.80), and minimal agreement between the colostrometer and both the digital and optical Brix refractometers (0.22 and 0.28, respectively; Table 4). These results suggest lower consistency in classification when using the colostrometer compared to the Brix refractometers. The concordance analysis conducted within each farm followed a similar pattern (Table 4), with greater inconsistencies observed on Farm 1.

Discussion

High-quality colostrum administration to newborn calves is necessary as soon as possible after parturition, since calves are born

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Table 3. Evaluation of the extent of pairwise Pearson correlation coefficients (ρ) between pairs of equipment

Equipment	Correlation coefficient (r)	*P*-value			
		r (H0 = 0.50)	r (H0 = 0.70)	r (H0 = 0.90)	r (H0 = 0.99)
Farm 1 (n = 31)					
Colost[a] × Digital[b]	0.81	$P = 0.003$	$P = 0.188$	$P = 0.057$	$P < 0.001$
Colost[a] × Optical[c]	0.83	$P = 0.001$	$P = 0.107$	$P = 0.108$	$P < 0.001$
Digital[b] × Optical[c]	0.98	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.179$
Farm 2 (n = 193)					
Colost[a] × Digital[b]	0.83	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Colost[a] × Optical[c]	0.84	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$
Digital[b] × Optical[c]	0.97	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Total data (n = 224)					
Colost[a] × Digital[b]	0.81	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Colost[a] × Optical[c]	0.83	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Digital[b] × Optical[c]	0.97	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

[a]Colostrometer.
[b]Digital Brix refractometer.
[c]Optical Brix refractometer.

Table 4. Concordance coefficient for pairs of equipment considering total data (n = 224), Farm 1 (n = 31) and Farm 2 (n = 193)

Equipment	Digital Brix refractometer	Optical Brix refractometer
Total data (n = 224); concordance coefficient		
Optical Brix refractometer	0.80 (0.68 ≤ 0.80 ≤ 0.93)	–
Colostrometer	0.22 (0.03 ≤ 0.22 ≤ 0.40)	0.28 (0.07 ≤ 0.28 ≤ 0.50)
Farm 1 (n = 31);		
Optical Brix refractometer	0.76	–
Colostrometer	0.05	0.05
Farm 2 (n = 193);		
Optical Brix refractometer	0.81	–
Colostrometer	0.26	0.36

without antibodies (Blum and Baumrucker, 2008; Peter, 2013; Godden *et al.*, 2019). In practice, evaluating colostrum quality is essential to ensure the adequate transfer of passive immunity to calves. However, as it would be impractical to carry out direct measurements of immunoglobulin levels through laboratory methods, indirect techniques are commonly employed in dairy farming (Fleenor and Stott, 1980; Chigerwe *et al.*, 2008; Bielmann *et al.*, 2010; Morrill *et al.*, 2012; Quigley *et al.*, 2013).

Previous research has demonstrated moderate to strong correlations between direct and indirect techniques (Johnsen *et al.*, 2019; Zobel *et al.*, 2020). However, even though several studies have been conducted evaluating direct and indirect methods (Bielmann *et al.*, 2010; Gelsinger *et al.*, 2015; Bartens *et al.*, 2016), the evaluation of the association between indirect methods to evaluate bovine colostrum, as well as the agreement between them, has not been satisfactorily explored yet. In the present study, it was demonstrated that the indirect assessment of colostrum immunological quality is strongly associated between the colostrometer and digital and optical Brix refractometers, although the agreement between the equipment is variable, being low between the Brix refractometer equipment (digital and optical) when compared to the colostrometer.

To test the equipment, the average Ig concentration was measured in two farms using the colostrometer, the digital Brix refractometer and the optical Brix refractometer. The results were 86.77 mg/mL of Ig, 27.24% and 26.93%, respectively, for Farm 1; 98.86 mg/mL of Ig, 26.94% and 27.04%, respectively, for Farm 2; and 97.16 Ig mg/mL, 26.98% and 27.03%, respectively, for the total data. These values indicate that the samples used in the experiment presented high quality, according to the cutoff point of 23%, which is equivalent to an Ig concentration greater than 50 mg/mL, commonly recommended in the literature (Chigerwe *et al.*, 2008; Bartier *et al.*, 2015; Elsohaby *et al.*, 2017; Godden *et al.*, 2019), despite the differences between farms.

On the farms, colostrometers presented greater variability compared to Brix refractometers (CV = 28.91 for Farm 1, CV = 23.22

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for Farm 2 and CV = 24.26 to the total data). It can be justified by the varying conditions that can influence the measurements using the colostrometer, such as the sample's fat content and temperature. However, during the experiment, all the samples were measured at 21°C in the analysis, following the manufacturer's recommendations. However, the fat content could be a factor influencing the equipment measurements. Colostrum with a greater solids content or more fat will lead to a greater specific gravity reading (Geiger, 2020).

It is worth mentioning that the need for a specific temperature to ensure the reliability of the estimate is a factor that makes the use difficult in the routine of farms. This fact demands more from the employee responsible for the activity and is certainly a source of errors. Other sources of variation in equipment measurements may be related to difficulty in reading the equipment scale and its cleaning, since the presence of residues may cause changes in the weight of the lactodensitometer. Besides, the colostrometer is made of glass, making it fragile and therefore susceptible to replacement. Therefore, the equipment has several disadvantages regarding its use, which makes its estimates less feasible.

The correlation coefficients obtained in the present study are corroborated by data from Zobel *et al.* (2020). In their research, they found a similar correlation between the measurements made by digital and optical Brix refractometers ($r = 0.99$), evaluating goat colostrum. Bielmann *et al.* (2010) also compared a digital and an optical Brix refractometer and found a high and positive correlation, but they did not compare it to a colostrometer, as in the present study.

The greater variability in the colostrometer estimates leads to weaker correlations between this equipment and the Brix refractometer, which can be observed by the fact that the correlation between this equipment is not superior to 70% in Farms 1 and 2. On the other hand, the correlation between digital and optical Brix refractometers was found to be similar to 99% in Farm 1 and greater than 90% in Farm 2, showing a very high association.

Since the correlation coefficients were not different between Farms 1 and 2, we calculated the common coefficient to represent both farms. It can be observed that higher or lower values observed in the colostrometer will also be reflected in higher or lower values in the digital and optical Brix refractometers. Still, every time the colostrometer is compared to a digital or optical Brix refractometer, the correlation value is lower. On the other hand, the common correlation coefficient between the refractometers is very high.

Using the Altman scale (Kwiecien *et al.*, 2011), we were able to classify the concordance between the Brix refractometers as good (0.80), but the concordance between the colostrometer and the digital Brix refractometer (0.22) and the optical Brix refractometer (0.28) was weak when we evaluated all data taken together. When the data were analysed by farm, the refractometers showed a very high correlation in Farm 1 (0.76) and Farm 2 (0.81), while the comparisons with the colostrometer were very weak (0.05) in Farm 1 and weak in Farm 2 (0.26 and 0.36 to digital and optical Brix refractometer, respectively). These results indicate that the reproducibility between the colostrometer and the digital and optical Brix refractometers is low. In contrast, there is high reproducibility between the digital and optical Brix refractometers.

Besides the lower variability and greater reliability presented by the refractometers, it is a cheap and easy-to-use piece of equipment, and its reading is independent of the sample temperature (Bielmann *et al.*, 2010; Bartens *et al.*, 2016). It can also be used in a dairy farm for other purposes, such as the evaluation of the passive immune transfer (Deelen *et al.*, 2014) and the total solids in milk (Chigerwe and Hagey, 2014).

The digital Brix refractometer is a robust, highly durable instrument, but it is more expensive than the optical one. Its measurement demonstrates precise values, since the device itself displays the exact number on its display, so its reading is more reliable. In addition, the scale ranges from 0% to 85%; therefore, it can detect colostrum that has high concentrations of Ig.

The optical Brix refractometer is more sensitive to handling, cheaper and has a limited range (0%–32%). To analyse the sample, an individual must position the instrument perpendicular to the light and read the Brix percentage through the scale, which can generate doubts and cause bias.

Due to the high variability observed in the colostrometer data and the disadvantages mentioned above, its use may be limited. Owing to the near-perfect agreement between the refractometers observed in this study, digital Brix refractometer models provide more consistent results. However, when resources are limited, the optical Brix refractometer can be a viable alternative, producing results close to those obtained with the digital Brix refractometer, although it is more susceptible to human error during reading. Therefore, the choice of equipment should take into account the conditions of the farm and the availability of resources.

These findings are applicable in large- and small-scale dairy farms since the evaluations in Farms 1 and 2 pointed to a similar result, besides each farm's particularities. It is important to note that n_2 was greater than n_1, and a greater number of observations leads to a more robust result. However, in our analyses, we were able to observe that both in the individual evaluations of the farms and in the evaluation of all the data together, the results followed the same pattern, which is an indication of the reliability of the results obtained.

Despite the consistent results observed, this study presents certain limitations that should be taken into consideration, such as the absence of a gold standard test, which restricted the possibility of directly quantifying immunoglobulin concentration and, consequently, validating the greater accuracy of the results obtained through the indirect methods evaluated. Furthermore, the correlation between indirect methods and the gold standard is not perfect, being approximately 80% for the Brix refractometer and around 60% for the colostrometer (Bielmann *et al.*, 2010; Elsohaby *et al.*, 2017; Zobel *et al.*, 2020).

Conclusions

The indirect assessment of the immunological quality of colostrum is strongly associated with the colostrometer, digital Brix refractometer and optical Brix refractometer equipment, even though the agreement between the equipment is variable, being low between the Brix refractometer equipment (digital and optical) when compared to the colostrometer. Thus, the assessment of the immunological quality of colostrum by indirect methods, although strongly and positively correlated with each other, presents important disagreements which can be better investigated by applying direct methods. However, considering the practical and economic disadvantages of using the colostrometer, as well as greater variability between the measurements performed on the equipment between and within farms, this method seems to be the least desirable as a tool for assessing the immunological quality of colostrum on farms.

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