

# An Assessment of the Accuracy of Digital and Optical Brix Refractometers for Estimating Passive Immunity in Beef Calves

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## Abstract

This study was aimed at determining and comparing the diagnostic accuracy of digital and optical Brix refractometers (D-Brix and O-Brix, respectively) for the estimation of passive immunity status (<16 and <24 g/L) in beef calves. Blood was sampled from 163 one to seven-day-old calves. Immunoglobulin G (IgG) concentrations were estimated with D-Brix and O-Brix refractometers, and measured by the radial immunodiffusion (RID) assay used as the reference test. Correlation coefficients ( $r$ ) were calculated for the results of each method. Youden's  $J$ -index was used to select optimal refractometer cut-off values for estimating IgG of <16 and <24 g/L. Overall test performance and refractometer agreement were assessed using AUCs, diagnostic test accuracy, Cohen's kappa coefficient ( $\kappa$ ), and Bland-Altman analysis. Positive correlations existed between the RID-IgG concentrations and Brix percentages ( $r=0.903$  for D-Brix,  $r=0.885$  for O-Brix), and between the results of the two refractometers ( $r=0.992$ ). The overall test performances of the refractometers were excellent ( $AUC>0.90$ ). For predicting serum IgG concentrations of <16 and <24 g/L, the optimal cut-off values were  $\leq 8.3\%$  and  $\leq 9.4\%$  for the D-Brix refractometer, and  $\leq 8.4\%$  and  $\leq 9.6\%$  for the O-Brix refractometer, respectively. At the optimal thresholds for estimating serum IgG concentrations of <16 g/L, the sensitivity and specificity were 91.89% and 97.62% for the D-Brix refractometer, and 91.89% and 96.83% for the O-Brix refractometer, respectively. At the optimal thresholds for estimating serum IgG concentrations of <24 g/L, the sensitivity and specificity were 88.14% and 80.77% for the D-Brix refractometer, and 86.44% and 80.77% for the O-Brix refractometer, respectively. Cohen's kappa coefficients suggested an almost perfect agreement between the results of the two refractometers for the estimation of IgG of <16 ( $\kappa=0.90$ ) and <24 g/L ( $\kappa=0.86$ ). In conclusion, digital and optical Brix refractometers could be safely used as monitoring tools for assessing passive immunity status in neonatal beef calves.

## Introduction

Calves lack immunity at birth, as the ruminant placenta does not allow the transplacental transfer of maternal immunoglobulins (Ig). Feeding colostrum to neonatal calves within the first 24 h after birth enables the transfer of passive immunity (TPI). An inadequate absorption of colostrum immunoglobulins by neonatal calves within this period is referred to as inadequate transfer of passive immunity (ITPI). On the other hand, serum IgG concentrations below the 10 g/L threshold, which mainly serves to reduce mortality, are described as failure of transfer of passive immunity (FTPI). The health status, viability, and performance of newborn calves are closely associated with their passive immunity status (Godden *et al.*, 2019; Lombard *et al.*, 2020; Fischer-Tlustos *et al.*, 2021).

In dairy calves, serum IgG concentrations greater than 10 g/L are traditionally defined as indicating adequate TPI (Godden *et al.*, 2019). In a recent study by Lombard *et al.* (2020), the passive immunity statuses of dairy calves were classified under four strata (poor (<10 g/L), fair (10 – 17.9 g/L), good (18 – 24.9 g/L) and excellent ( $\geq 25$  g/L)), based on morbidity, mortality and performance criteria. However, currently, there is no consensus on an adequate passive immunity threshold for dairy and beef calves (Waldner and Rosengren, 2009; Chigerwe *et al.*, 2015). Serum IgG concentrations of <16 g/L, <24 g/L and <27 g/L are associated with mortality, morbidity, and growth performance in beef calves (Wittum and Perino, 1995; Dewell *et al.*, 2006; Waldner and Rosengren, 2009). Serum IgG concentrations above 16 g/L are considered to indicate adequate TPI (Waldner and Rosengren, 2009).

Despite being known as the gold standard for the detection of IgG concentrations (Fleener and Stott, 1981), the radial immunodiffusion (RID) assay has not found common use on the field, given that it is expensive, produces results only after 24 h, is influenced by temperature alterations during incubation, and is required to be performed by experienced staff. Thus, alternative methods have been investigated for the assessment of passive immunity status (Pekcan *et al.*, 2013; Deelen *et al.*, 2014; Elsohaby *et al.*, 2014; 2015; Elsohaby and Keefe, 2015; Hogan *et al.*, 2015; 2016; Drikic *et al.*, 2018; Topal *et al.*, 2018; Akköse *et al.*, 2022a). Digital and optical Brix refractometers are efficient, low-cost and practical devices that can be used on the farm to detect ITPI. The Brix scale refers to the sugar concentration of solutions; such that 1% Brix is equivalent to 1 g/dL of sucrose (Ball, 2006). The Brix percentages of biological fluids are correlated with their total solids concentrations. It is also possible to use Brix refractometers in determining colostrum quality (Buczinski *et al.*, 2016). While a large number of comparative studies are available on the utility and performance of refractometers in diagnosing ITPI in dairy calves (Buczinski *et al.*, 2021; Akköse *et al.*, 2022a:b), there are only very few studies that have evaluated refractometry methods for the assessment of passive immunity status in beef calves (Vandeputte *et al.*, 2011; Todd *et al.*, 2018; Gamsjäger *et al.*, 2021; Pisello *et al.*, 2021; Akköse *et al.*, 2022c). This study aimed at determining and comparing the diagnostic accuracy of digital and optical Brix refractometers for the prediction of different passive immunity statuses in beef calves, and at demonstrating the correlation and agreement level of the results achieved with these refractometers.

## Materials and Methods

### Animal Material and Collection of Serum Samples

The Local Ethics Board for Animal Experiments of Harran University (HRU-HADYEK) approved the conduct of this study.

This study was conducted between April 2018 – March 2019 at two beef farms located in Turkey. A total of 163 healthy calves, aged 1–7 days and of the breeds Aberdeen Angus and Limousine, were used. Each calf was sampled for blood from the jugular vein, and samples were collected into 10-mL plain tubes (Hema Tube, Ankara, Turkey). The blood samples were centrifuged at 3000 x g for 10 min for the extraction of sera. Serum was stored in volumes of 2 mL under freezing at -20°C until being used for RID analyses after refractometer analyses.

### Refractometer Analyses

Refractometer analyses were performed on the farm using fresh serum samples. Brix percentages were measured with a handheld optical Brix (O-Brix) refractometer (ATC HT 113, China) and a handheld digital Brix (D-Brix) refractometer (Atago PAL-1, Tokyo, Japan). The D-Brix refractometer had an automatic

temperature compensation function. The D-Brix and O-Brix refractometers were précised to 0.1% Brix and 0.2% Brix, respectively. The scales of the D-Brix and O-Brix refractometers were 0 to 53 and 0 to 32, respectively. Samples of approximately 200 microliters were used for the refractometer analyses. The D-Brix refractometer used led light, and the results were read on the digital panel of the device. The O-Brix refractometer results were read by looking into the eyepiece, while pointing the refractometer at a source of direct light. The white/blue boundary on scale showed the measured value of the O-Brix refractometer. Between samples, the prism of the refractometer was washed in tap water and dried with paper towel. Prior to each series of analyses, the refractometers were calibrated with distilled water.

### Radial Immunodiffusion (RID) Analyses

After being thawed at room temperature, the serum samples underwent RID analyses performed with commercial test kits (Triple J Farms, Bellingham, WA, USA). The test kits were stored in a refrigerator at +4 °C and removed half an hour before being used. The test kits included 3 reference sera with known IgG concentrations. Each kit had 21 sample wells and 3 control wells. The test procedure was performed as instructed by the manufacturer. Five- $\mu$ l automatic pipettes were used for the transfer of the reference sera and serum samples to the RID plates. Subsequently, the kits were incubated in their locked cases in a room temperature. After 24 h of incubation, the diameters of the precipitin rings were measured with a 10x peak scale loupe precise to 0.1 mm. The diameters were compared to the standard curve, which was constructed using the IgG concentrations of the reference sera. The reference table provided with the test kit was used to determine the IgG concentrations of the serum samples. Serum samples with precipitin ring diameters that did not fall within the range indicated in the reference table were diluted 1:1 with 0.9% NaCl sterile solution for reanalysis.

### Statistical Analyses

The distribution of the D-Brix, O-Brix and RID-IgG results was determined based on skewness and kurtosis values. Values ranging from (-1.5) to (+1.5) indicated normal data distribution. Descriptive statistics were calculated for the IgG concentrations measured by RID assay and Brix percentages obtained by D-Brix and O-Brix refractometers.

The correlation between the RID-IgG concentrations and the refractometer results as well as the correlation between the results of the O-Brix and D-Brix refractometers were determined by calculating Pearson's correlation coefficients.

The accuracy of the refractometers in determining different passive immunity statuses was assessed according to epidemiological diagnostic test characteristics (sensitivity, specificity, positive and negative predictive values), based on receiver operating characteristics curve (ROC) analysis.

Youden's J-index was used for the selection of the optimal thresholds. Sensitivity (Se) was defined as the proportion of calves accurately determined with refractometry as having RID-IgG concentrations of <16 g/L and <24 g/L. Specificity (Sp) was defined as the proportion of calves accurately determined with refractometry as having RID-IgG concentrations of ≥16 g/L and ≥24 g/L. Youden's J-index was calculated using the equation:  $J = Se + Sp - 1$ . The maximization of J minimized the total test misclassifications, including the assumption of an equal effect of false negative and false positive results. The positive predictive value (PPV) was defined as the probability of calves, classified by refractometry as having serum IgG concentrations of <16 g/L and <24 g/L in reality also having IgG concentrations of <16 g/L and <24 g/L, respectively. On the other hand, the negative predictive value (NPV) was defined as the probability of calves, classified by refractometry as having IgG concentrations of ≥16 g/L and ≥ 24 m/L, in reality also having IgG concentrations of ≥16 g/L and ≥24 g/L, respectively. A receiver operating characteristics (ROC) curve was constructed for each refractometer at two passive immunity levels (<16 g/L, <24 g/L).

The discrimination ability of the refractometers to estimate passive immunity statuses in beef calves was determined by assessing areas under the ROC curves (AUCs). AUCs pointed out to either excellent accuracy (AUC = 1; Se = Sp = 1), high level of accuracy (AUC > 0.9), moderate accuracy (AUC = 0.7-0.9) or

poor accuracy (AUC = 0.5-0.7). Inter-rater agreement (Cohen's kappa coefficient,  $\kappa$ ) was calculated to ascertain the agreement level between the results obtained with the two refractometers using the respective cut-off based test dichotomization. Agreement levels were graded as poor agreement ( $\kappa < 0.20$ ), fair agreement ( $0.20 < \kappa \leq 0.40$ ), moderate agreement ( $0.40 < \kappa \leq 0.60$ ), substantial agreement ( $0.60 < \kappa \leq 0.80$ ) and almost perfect agreement ( $\kappa > 0.80$ ). The level of agreement between the D-Brix and O-Brix results was further assessed by a Bland-Altman analysis (Petrie and Watson, 2013).

Statistical significance was set at  $p \leq 0.05$ . The SPSS 24 statistical package program was used for the statistical analyses.

### Results

In total 163 serum samples belonging to Aberdeen Angus (n=144) and Limousine (n=19) calves were subjected to % Brix and RID analyses. Based on skewness and kurtosis coefficients, it was determined that the data were normally distributed. The descriptive statistics of the D-Brix and O-Brix percentages and RID-IgG concentrations are given in Table 1. According to the results of the RID analyses of the blood samples of 163 calves, IgG concentrations were <16 g/L in 37 calves (22.7%), <24 g/L in 59 calves (36.2%), and ≥24 g/L in 104 calves (63.8%).

**Table 1.** Descriptive statistics of % D-Brix, % O-Brix and RID-IgG concentrations of serum samples taken from beef calves.

Variable	N	Min.	Max.	Mean ± SD	Median	Skewness	Kurtosis
D-Brix (%)	163	6.2	13.1	9.5±1.4	9.7	-0.328	-0.525
O-Brix (%)	163	6.1	12.9	9.6±1.5	10.0	-0.413	-0.619
RID-IgG (g/L)	163	0.0	75.6	27.8±16.3	27.1	0.036	-0.263

### Correlations

Positive correlations were determined between the RID-IgG concentrations and D-Brix percentages ( $r=0.903$ ), as well as the RID-IgG concentrations and O-Brix percentages ( $r=0.934$ ). Furthermore, positive correlations were also ascertained between the D-Brix and O-Brix results ( $r=0.993$ ). Scatter-plots showing the correlation between RID-IgG, D-Brix and O-Brix percentages are presented in **Figure 1**.

### Diagnostic test characteristics

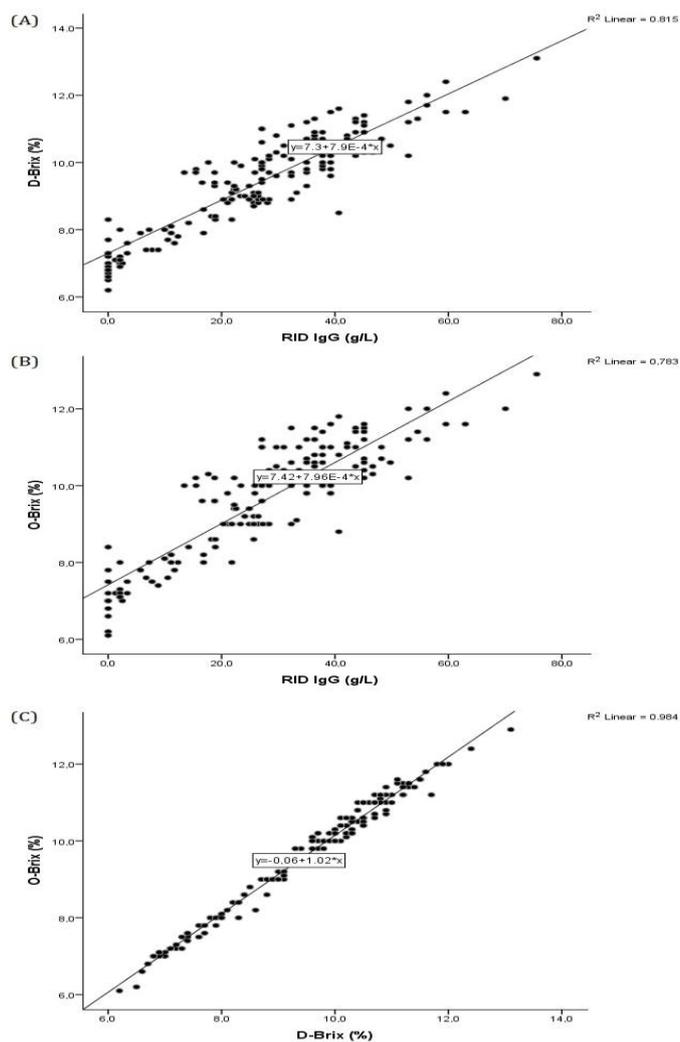
Sp, Se, PPV, NPV and Youden's J-index calculations were made for digital and optical Brix refractometry, which are used for the estimation of passive immunity status.

While Youden's J-index was used to determine optimal refractometer cut-off values, the Se and Sp values were calculated by ROC analysis. Accordingly, the optimal cut-off values for predicting serum IgG concentrations of <16 g/L and <24 g/L were determined as ≤8.3% and ≤9.4%, respectively, for the D-Brix refractometer, and ≤8.4% and ≤9.6%, respectively, for the O-Brix refractometer (**Table 2**).

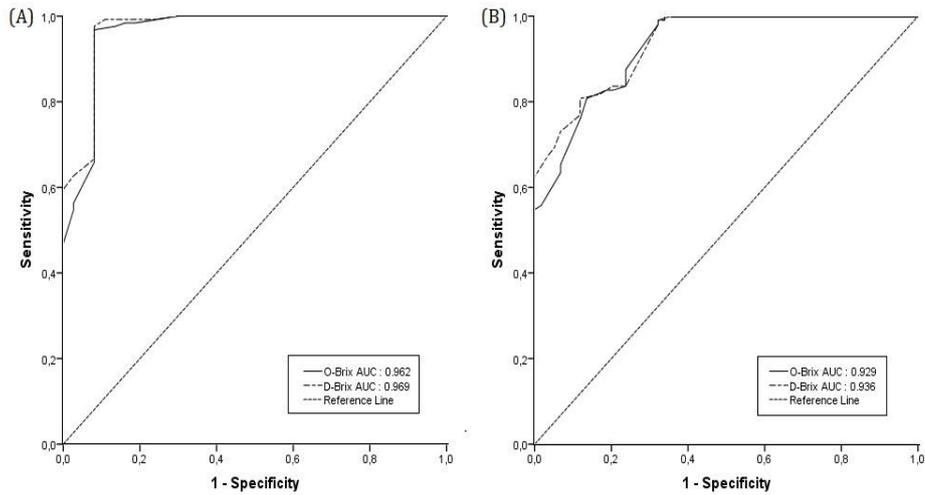
The ROC curves constructed for the passive immunity statuses estimated with the D-Brix and O-Brix refractometers are shown in **Figure 2**. In beef calves, the AUC values of the two refractometers for predicting passive immunity statuses of <16 g/L and <24 g/L were determined to be >0.9.

**Table 2.** Diagnostic test characteristics of the digital and optical Brix refractometers for the estimation of different passive immunity levels.

IgG Conc. (g/L)	Threshold		Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
	(g/L)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
D-Brix	<16	≤8.3	91.89	78.1 – 98.3	97.62	93.2 – 99.5	91.9	78.7-97.2	97,6	93.3-99.2
	<24	≤9.4	88.14	77.1 – 95.1	80.77	71.9 – 87.8	72.2	63.4-79.6	92.3	85.6-96.0
O-Brix	<16	≤8.4	91.89	78.1 – 98.3	96.83	92.1 – 99.1	89.5	76.3-95.7	97.6	93.2-99.2
	<24	≤9.6	86.44	75.0 – 94.0	80.77	71.9 – 87.8	71.8	62.9-79.3	91.3	84.6-95.3



**Figure 1.** (A) Scatter-plot showing the correlation between the D-Brix percentages and RID-IgG concentrations of beef calves. The solid line indicates the fitted regression equation:  $Y = 0.00079 (X) + 7.3$ . (B) Scatter-plot showing the correlation between the O-Brix percentages and RID-IgG concentrations of beef calves. The solid line indicates the fitted regression equation:  $Y = 0.000796 (X) + 7.42$ . (C) Scatter-plot showing the correlation between the D-Brix and O-Brix percentages of beef calves. The solid line indicates the fitted regression equation:  $Y = 1.02 (X) + 0.06$ . D-Brix, digital Brix refractometer; O-Brix, optical Brix refractometer; RID, Radial immunodiffusion; IgG, immunoglobulin G.

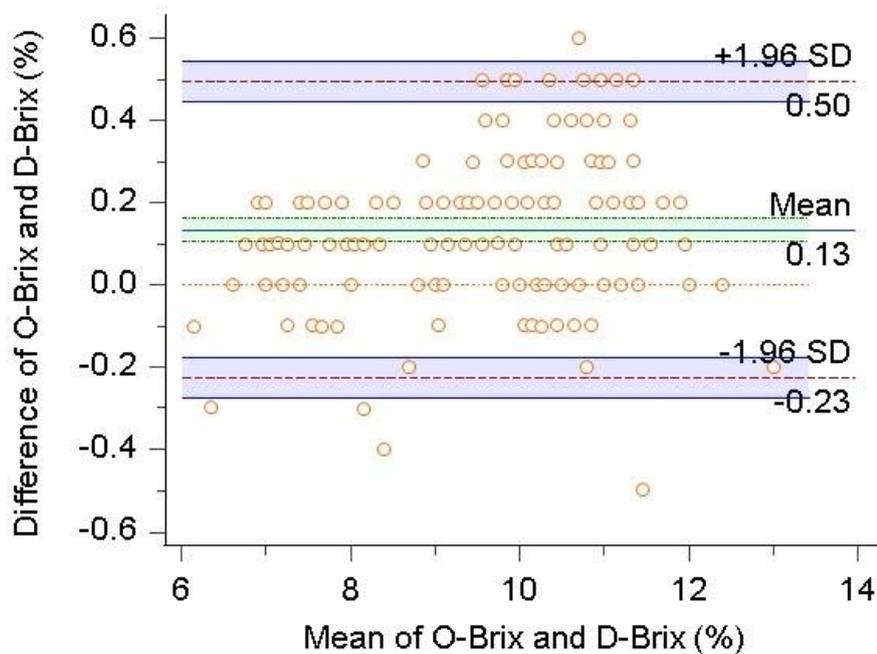


**Figure 2.** Receiver operating characteristic curves (ROCs) constructed and areas under the curve (AUCs) calculated for the prediction of serum IgG concentrations of <16 g/L (A) and <24 g/L (B) by two different refractometers in neonatal beef calves. AUC, area under the ROC curve; ROC, Receiver operating characteristic curve.

**The Agreement between the Refractometers at the selected cut-off values**

The results of the D-Brix and O-Brix refractometers for the prediction of IgG concentrations of <16 g/L ( $\kappa=0.90$ ) and <24 g/L ( $\kappa=0.86$ ) almost perfectly agreed.

This was also evaluated by a Bland-Altman analysis, and it was confirmed that there was no systematic bias, but only a small mean difference of percentage (0.13%) between the D-Brix and O-Brix results (**Figure 3**).



**Figure 3.** Bland–Altman plot graph illustrating the agreement between the Brix percentages measured by the O-Brix and D-Brix refractometers. The solid line indicates mean differences of O-Brix and D-Brix refractometer measures and dotted lines represent 1.96 SD from the mean difference. The top and bottom solid line on each graph represent the 95% limits of agreement. O-Brix, optical Brix refractometer; D-Brix, digital Brix refractometer, SD, Standard deviation.

## Discussion

Farm blindness may falsely normalize calf mortality associated with poor colostrum management (Mee, 2020). There is need for the practical monitoring of the colostral transfer of passive immunity to calves. Our study compared the diagnostic accuracy of optical and digital Brix refractometers in predicting different passive immunity statuses in beef calves. To date, only very few studies have assessed digital Brix percentages indicative of serum IgG of <16 g/L (Gamsjäger *et al.*, 2021; Pisello *et al.*, 2021; Akköse *et al.*, 2022c) or <24 g/L (Gamsjäger *et al.*, 2021; Akköse *et al.*, 2022c). On the other hand, there is no report on optical Brix percentages indicative of serum IgG of <24 g/L.

The optimal cut-off value we ascertained in the present study for the D-Brix refractometer for the estimation of IgG concentrations of <16 g/L was similar to previously reported digital Brix percentages for IgG of <16 g/L (Gamsjäger *et al.*, 2021; Pisello *et al.*, 2021; Akköse *et al.*, 2022c). The cut-off value we determined for the D-Brix refractometer for estimating IgG concentrations of <24 g/L was higher than the digital Brix refractometer cut-off value previously reported by Gamsjäger *et al.* (2021) and lower than the digital Brix refractometer cut-off value previously reported by Akköse *et al.* (2022c). The cut-off value ascertained for the O-Brix refractometer for estimating IgG concentrations of <16 g/L was similar to the optical Brix refractometer cut-off value reported by Pisello *et al.* (2021). The present study proposes, for the first time, a cut-off value for the optical Brix refractometer for estimating serum IgG concentrations of <24 g/L in beef calves. Furthermore, the diagnostic test characteristics at the optimal cut-offs determined for the D-Brix and O-Brix refractometers were similar to those reported in previous studies (Gamsjäger *et al.*, 2021; Akköse *et al.*, 2022c).

Different cut-off values having been reported in studies could be attributed to the use of different refractometers (Elsohaby *et al.*, 2015), calf age (Topal *et al.*, 2018), breed-specific variations (Villarroel *et al.*, 2013), and the hydration status of calves. Other influential factors include the automatic temperature compensation capability of the refractor prism and the precision of the refractometer. The refractive index of a material depends on the temperature of the environment, samples or refractometer prism. The precision of the D-Brix and O-Brix refractometers was set to 0.1% Brix and 0.2% Brix, respectively. On the other hand, finding different cut-off values does not imply that these values are truly different. In fact, the best choice for a cut-off value is also related to the uncertainty around it and, thus, to the prevalence and total number of cases of the target condition (Leeflang *et al.*, 2008).

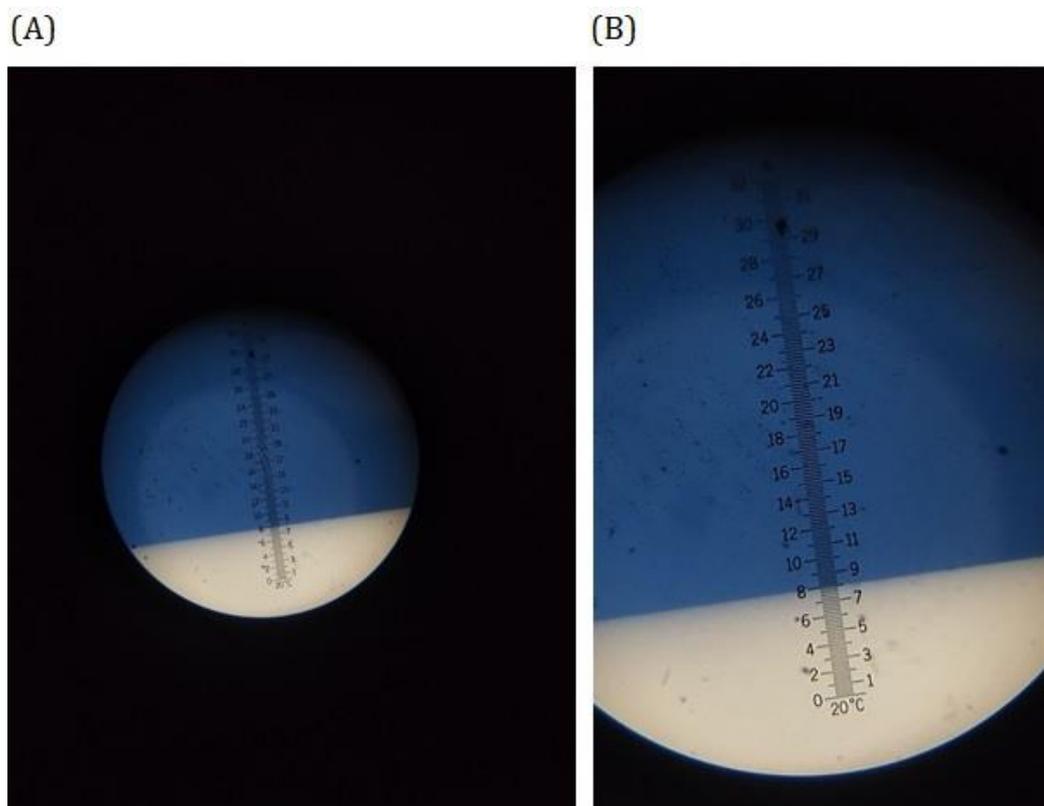
In beef calves, mortality and morbidity rates are related to serum IgG concentrations of <16 g/L or <24 g/L (Wittum and Perino, 1995; Dewell *et al.*, 2006; Waldner and Rosengren, 2009). Serum Brix percentages of  $\leq 8.4\%$

were reported to be associated with significantly higher odds of morbidity and mortality in suckler beef calves (Todd *et al.*, 2018). In a recent study in beef calves, digital Brix refractometer cut-off values of  $\leq 8.4\%$  and  $\leq 8.8\%$  were reported for the estimation of serum IgG concentrations of <16 g/L and <24 g/L, respectively (Gamsjäger *et al.*, 2021). In a study describing ITPI as being associated with a serum IgG concentration of <16 g/L, a cut-off value of  $\leq 8.4\%$  was proposed for the digital Brix refractometer (Pisello *et al.*, 2021). Our research team has recently found digital and optical Brix refractometers cut-off values of <8.5% and <10.1% for the estimation of serum IgG concentrations of <16 g/L and <24 g/L, respectively, (Akköse *et al.*, 2022c). These results agree with our findings for the estimation of serum IgG concentrations of <16 and <24 g/L.

AUC is a criterion used for the assessment of the overall accuracy of diagnostic tests and is presented as the mean Se values for all probable Sp values (Lee *et al.*, 2008). In the present study, the AUC values of the refractometers that were used to predict two of the passive immunity statuses were similar to those previously reported by Gamsjäger *et al.* (2021), but were higher than those reported by Pisello *et al.* (2021), when using a threshold of <16 g/L. The AUCs demonstrated that the two refractometers used to determine calves with IgG concentrations of <16 g/L and <24 g/L did not differ for the results they produced. The AUC value having been determined as >90% suggests that the diagnostic tests used in the present study were successful.

Cohen's kappa coefficient suggested an almost perfect agreement between the D-Brix and O-Brix refractometers for the estimation of IgG concentrations of <16 g/L and <24 g/L. Furthermore, the assessment of the agreement between the serum Brix percentages measured by the D-Brix and O-Brix refractometers using Bland–Altman plots demonstrated that there was no obvious systematic bias between the optical and digital refractometers. This suggests that the two refractometers can be used interchangeably. Our findings agree with those reported by Pisello *et al.* (2021) for beef calves. The small mean of 0.13% Brix between the O-Brix and D-Brix refractometers showed that the O-Brix refractometer measured, on average, 0.1 unit (%) higher than the D-Brix refractometer. This little difference could be attributed to the different precisions of the refractometers (Gamsjäger *et al.*, 2021), which is 0.1% Brix for the D-Brix refractometer and 0.2% Brix for the O-Brix refractometer.

Being readily available for use on dairy farms to determine IgG concentrations in colostrum and calf serum, two different types of Brix refractometers were compared in this study. Brix refractometers are either



**Figure 4.** The reading of the optical Brix results through the eyepiece of the refractometer with a scale of 0-30 does not suffice for the clear distinction of the white/blue boundary, (A) and thus, yields results that may vary among individuals. Results that are more accurate can be achieved by reading the scale of the optical Brix refractometer using a magnifier, such as a mobile phone camera (B).

optical (traditional) or digital, the latter being more appealing since it gives a rapid objective measurement of the Brix percent of the sample. The traditional refractometer produces subjective results, as the reading depends on individual eyesight and two persons may report different readings according to how they read the scale (**Figure 4**). The scale of optic refractometers differs for each model or type of refractometer. The commonly used scales of the traditional refractometer are 0 to 10, 0 to 30, and 0 to 50. Logically, the 0 to 10 scale would be expected to yield more accurate readings (MacKenzie, 2021). However, when performing colostrum measurements, the refractometer's line on the scale can be read only using a 0 to 30 scale. Owing to its automatic temperature compensation function, higher technology and digital sensor, the digital refractometer offers more than double the resolution of the traditional refractometer, and produces far more accurate readings.

#### Conclusion

In conclusion, as they do not require special laboratory equipment, digital and optical Brix refractometers can be safely used on the farm as

inexpensive and reliable management tools for the estimation of passive immunity statuses after colostrum intake in neonatal beef calves. Owing to a greater objectivity of results and ease of use, the digital Brix refractometer can be preferred over the optical refractometer. Veterinarians and producers can identify calves with ITPI using Brix refractometers, and may choose to monitor the health status of calves under risk. Furthermore, they may use the ITPI prevalence to evaluate the efficiency of the colostrum management strategy of the farm. Farms with a high percentage of tested calves with low Brix% may resort to the adjustment of colostrum management strategies such as inquiring prepartum management strategies of dams, the measurement of IgG concentrations in maternal colostrum or additional colostrum supplementation to calf. On the other hand, farms with a low percentage of tested calves with low Brix% or achieving target Brix% may benefit from the motivation of having confirmed that all is running smoothly on the farm. Therefore, the monitoring of the passive immunity status in beef calves using a Brix refractometer can contribute to both reducing morbidity and mortality rates and preventing economic losses.

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## Author contributions

All authors contributed equally to the study.

## Conflicts of interest

The author declare no conflicts of interest.

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