Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in Chianina beef–suckler calves reared in Umbria

Lorenzo Pisello , Antonio Boccardo , Claudio Forte , Davide Pravettoni , Nicoletta D'Avino , Fabrizio Passamonti & Fabrizio Rueca

To cite this article: Lorenzo Pisello , Antonio Boccardo , Claudio Forte , Davide Pravettoni , Nicoletta D'Avino , Fabrizio Passamonti & Fabrizio Rueca (2021) Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in Chianina beef–suckler calves reared in Umbria, Italian Journal of Animal Science, 20:1, 315-323, DOI: 10.1080/1828051X.2021.1884007

To link to this article: https://doi.org/10.1080/1828051X.2021.1884007

Published online: 15 Feb 2021.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2021, VOL. 20, NO. 1, 315–323
https://doi.org/10.1080/1828051X.2021.1884007

Taylor & Francis
Taylor & Francis Group

PAPER

Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in Chianina beef–suckler calves reared in Umbria

Lorenzo Pisello[a], Antonio Boccardo[b], Claudio Forte[c], Davide Pravettoni[b], Nicoletta D'Avino[d], Fabrizio Passamonti[a] and Fabrizio Rueca[a]

[a]Dipartimento di Medicina Veterinaria, University of Perugia, Perugia, Italy; [b]Dipartimento di Medicina Veterinaria, University of Milan, Milano, Italy; [c]Dipartimento di Scienze Veterinarie, University of Turin, Grugliasco, Italy; [d]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy

ABSTRACT

There are few published data on the accurate on-farm diagnosis of failure of transfer of passive immunity (FTPI) in beef–suckler calves. This observational study aimed to evaluate the diagnostic performance and differences among four types of refractometers for assessing FTPI in Chianina beef–suckler calves. Blood samples were collected from 85 Chianina calves aged 2–7 days. The serum immunoglobulin G (IgG) concentration was measured using radial immunodiffusion (RID), digital and optical serum total protein (STP) refractometers, and digital and optical serum refractometers. The diagnostic performance of the refractometers was determined based on the serum IgG threshold of 16 g/L (measured by RID). A receiver operating characteristic (ROC) curve was used to identify the optimal cutoff values for all refractometers. The RID IgG concentration was positively correlated with all four refractometers (correlation coefficient: 0.75–0.84). ROC analysis yielded optimal cutoff values for predicting FTPI of 51 g/L (sensitivity (Se)=0.63 and specificity (Sp)=0.96) and 52 g/L (Se = 0.69 and Sp = 0.90) for the digital and optical STP refractometers, respectively. At the threshold of 8.3% Brix, the Se and Sp were 0.66 and 0.92 for the optical Brix and 0.77 and 0.92 for the digital Brix refractometer, respectively. All four refractometers were useful for assessing FTPI in Chianina calves. However, the digital Brix had the highest combined diagnostic accuracy for FTPI. The on-farm use of refractometers to assess FTPI can become part of routine monitoring of the colostrum management program in beef–suckler calf herds.

HIGHLIGHTS

- The cutoff values were 51 and 52 g/L for the digital and optical serum total protein refractometers, respectively.
- The digital Brix refractometer was the most accurate for the detection of calves with inadequate transfer of passive immunity.

ARTICLE HISTORY
Received 3 August 2020
Revised 5 November 2020
Accepted 26 January 2021

KEYWORDS
Calves; suckler herds; refractometer; serum total protein; brix

Introduction

Failure of transfer of passive immunity (FTPI) occurs when a calf fails to absorb an adequate quantity of colostral passive immunity. This condition is highly correlated with the health of calves and, consequently, with the productivity of the farm (Raboisson et al. 2016). Several direct and indirect methods have been used to monitor passive immunity transfer in calves. Among the direct methods, radial immunodiffusion (RID) is considered the gold standard for determining the immunoglobulin G (IgG) concentration in calf serum but requires a specialised laboratory, and the results are not available immediately (Morrill et al. 2013). In contrast to direct methods, digital and optical refractometers are frequently used for detecting FTPI because these methods are inexpensive and easy to perform under field conditions (Vandeputte et al. 2011). The tools more commonly used are the Brix refractometer, which approximates the percentage of the total solids (%Brix) in liquids, and the serum total protein refractometer, which is used to evaluate the serum total protein (STP; g/L) concentration.

CONTACT Antonio Boccardo antonio.boccardo@unimi.it Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Via dell'Università, 6, Lodi 26900, Italy

Although the efficacy of refractometers in determining passive immunity has been largely evaluated in dairy calves (Tyler et al. 1996; Deelen et al. 2014; Elsohaby et al. 2015; McCracken et al. 2017; Wilm et al. 2018), there is still a need to evaluate the efficacy of refractometers for use in beef cattle breeds (McGee and Earley 2019).

Recent studies have indicated that the STP concentration and %Brix refractometry for health and growth outcomes in beef calves ranged from 53 to 63 g/L and >8.4%, respectively (Todd et al. 2018). In a mixed population of beef and dairy calves, surveys such as those conducted by Calloway et al. (2002) and Dawes et al. (2002) have shown that serum refractometry test endpoints of 50–52 g/L yielded accurate results in comparison to a cutoff value of 10 g/L serum IgG as tested by RID (Calloway et al. 2002; Dawes et al. 2002). In Belgian Blue beef calves, Vandeputte et al. (2011) observed that a threshold of 54–58 g/L STP assessed by optical and digital refractometers had a high correlation coefficient with the optical serum IgG concentration as measured by high-performance liquid chromatography (HPLC).

Although a serum IgG concentration of 10 g/L measured by RID is a generally accepted threshold for dairy calves, recent trends suggest that there is little consensus regarding IgG cutoff values for FTPI classification in both beef and dairy calves (Waldner and Rosengren 2009; Chigerwe et al. 2015). In beef calves, an RID-IgG threshold value between >16 g/L (Wittum and Perino 1995) and 24 g/L (Dewell et al. 2006) was significantly associated with lower morbidity and mortality rates and better growth outcomes. Therefore, a threshold of >16 g/L can be considered sufficient to achieve an adequate transfer of passive immunity (Waldner and Rosengren 2009). The threshold used can influence the accuracy of the indirect tests used to assess the passive immunity, which demonstrates the need for further research to establish more accurate reference values for monitoring passive immunity transfer using a RID-IgG threshold value adjusted for beef calves.

The objectives of this study were, therefore, to (1) evaluate the diagnostic performance of the four types of refractometers (digital STP refractometer, DSTP; optical STP refractometer, OSTP; digital Brix refractometer, DBRIX; and optical Brix refractometer, OBRIX) in Chianina beef–suckler calves for serum IgG estimation; (2) calculate the appropriate cutoff values with a sensitivity (Se) and specificity (Sp) comparable to IgG measured by RID and based on the threshold of 16 g/L; and (3) compare digital and optical refractometers in terms of FTPI evaluation.

Materials and methods

Study design and sampling

This observational study was designed using beef–-suckler calves selected from Chianina cattle farms, which were regularly checked by our ambulatory clinic. The criteria for selecting the farms were: location of the farms (≤1 h drive from the Veterinary Teaching Hospital [OVUD], of the Department of Veterinary Medicine, University of Perugia), willingness to capture newborn calves to perform blood sampling, and compliance with the traditional methods of calf rearing on the Chianina cattle farms in Central Italy. On these farms, calves feed on colostrum spontaneously from the dams and are housed for up to 15–20 days in a calving pen before entering multiple pens in which they remain with the dams for up to 5–6 months after birth. Each farm was visited once per week between 1 February 2019 and 30 September 2019. Animals eligible for inclusion in this study were healthy male and female Chianina calves aged 2–7 days and born by eutocic delivery. Sample size was calculated using the expected proportion of calves with a serum IgG level of <16.0 g/L of 0.25 using a 95% confidence interval and precision of 10%; a sample size of >72 calves was established as adequate for the study.

Whole blood was collected from the calves by jugular venepuncture using a 20-gauge hypodermic needle (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ, USA) and stored in a sterile Vacutainer tube without anticoagulant (BD Vacutainer, Becton Dickinson and Co. Franklin Lakes, NJ, USA). Samples were transported to the OVUD lab in a portable cooler and then centrifuged (Neya 8, Remi Elektrotechnik Ltd – Instrument division, Vsai, India) at 20 °C for 10 min at 900 g within 4 h of collection. Three aliquots of serum were collected, numerated, and stored at −80 °C in Eppendorf tubes before analysis. The study was approved by the University of Perugia Bioethics Committee (Perugia, Italy; Protocol no. 2019-43).

Digital and optical refractometric measurements

Digital and optical refractometric measurements were performed on fresh samples. To avoid any bias in the results, the readings of the optical refractometers were first performed by the same person (LP). Optical

analyses were performed using two temperature-compensating optical refractometers, one for STP (Rifrattometro Milwuakee mod. MR514ATC, Milwaukee, Gallarate, Italy) and one for Brix (Rifrattometro Milwuakee mod. MR32ATC, Milwaukee, Gallarate, Italy). Before use, both optical refractometers were calibrated with distilled water. The serum was aspirated into a soft plastic Pasteur pipette; approximately 100 μL of serum was placed on the prism. Both refractometers were exposed to a light source, and the STP (g/100 mL; ±0.2 g/100 mL; reading scale range 0–12 g/dL) and %Brix (%; ±0.2%; reading scale range 0–32% Brix) values were read at the line between the light and dark areas that appeared on the scale. The prism of each refractometer was cleaned with distilled water after each reading.

All digital measurements were performed at 22 ± 2 °C after the optical measurements. Brix and STP analysis were performed using a digital refractometer (MISCO Palm Abbe no. PA201, Misco, Solon, OH, USA). According to the manufacturer's recommendations, before use, an aliquot of 0.4 mL of deionised water was placed on the prism well of the digital refractometer to obtain a standardised reading. Subsequently, an aliquot of the serum (0.4 mL) was placed on the refractometer prism. The refractometer reported the STP content in g/dL, with a lower limit of 1.0 and an upper limit of 14.0 (±0.1 g/dL), and the Brix value in % with a lower limit of 0% and an upper limit of 85% (±0.1%). All results regarding STP concentration were then converted to g/L.

For both optical and digital evaluation, each serum sample was analysed in duplicate and the mean value was used for statistical analysis.

RID analysis

After refractometric measurements, the IgG serum concentration was directly evaluated using a commercial RID kit (Bovine Ig Test Kit, Triple J Farms, Bellingham, WA) as the reference method. According to the manufacturer's instructions, 5 μL of undiluted serum samples were pipetted into the plate wells using the reference sera provided by the manufacturer as controls. Plates were incubated for 24 h at 22 ± 2 °C and the diameter of the precipitin rings was measured using a handheld caliper. Serum IgG concentrations were determined by comparing the diameter of the samples to a standard straight line of best fit between the three points corresponding to the standard sera. Serum samples with IgG concentrations greater than the manufacturer's stated performance range for the assay (>3,000 mg/dL) were diluted (1:1) with deionised sterile water and retested. All samples were run in duplicate and the mean value of the results was converted to g/L and used for statistical analysis.

Statistical analysis

Descriptive statistics were calculated for the results of the RID and the digital and optical Brix and STP refractometers, and the normality of the data was assessed using the Shapiro–Wilk test. Results from the digital and optical refractometers, in g/L and Brix units, were plotted against the measured IgG concentration from the RID in g/L. From these plots, correlation coefficients (r) were calculated using Pearson's correlation coefficient, since the data were normally distributed; P values of <0.05 were considered statistically significant. All analyses were performed using R statistical software V 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Epidemiological diagnostic test characteristics – Se, Sp, positive predictive value (PPV), and negative predictive value (NPV) – were calculated to evaluate the clinical applicability of digital and optical refractometers for the diagnosis of FTPI, based on the serum IgG threshold of 16 g/L (measured by RID) for FTPI-positive cases. Sensitivity was defined as the proportion of calves that tested positive for FTPI (serum RID-IgG concentration <16 g/L). Specificity was defined as the proportion of calves that tested negative for FTPI, with a serum IgG concentration of ≥16 g/L. Calves with an STP concentration or a Brix percentage lower than the cutoff values were classified as FTPI-positive cases, and calves with serum protein concentrations or Brix values greater than or equal to the cutoff values were classified as FTPI negative. The accuracy of the four types of refractometers examined was also determined based on three different FTPI prevalence scenarios (5, 15, and 25%).

A receiver operating characteristic curve (ROC) was created to plot the true positive rate against the false positive rate, for both the digital and optical refractometers, at 0.1-%-unit Brix intervals for Brix percentage and 0.1-g/dL-unit intervals for STP. The computed Se and Sp for each of the possible cutoff values were tabulated and the optimal cutoff value was defined as the value that yielded the optimum combination of Se and Sp with a high Youden's Index. The area under the curve (AUC) was calculated for each test to provide an overall summary of the ability of the test to differentiate calves with and without FTPI; an AUC

value of 1 indicates a perfect test, >0.9 indicates high accuracy, 0.7–0.9 indicates moderate accuracy, 0.5–0.7 indicates low accuracy, and <0.5 indicates a chance result (Deelen et al. 2014). The proportion of calves in the study population that were correctly classified by each refractometer and test endpoint was calculated using the following formula: $[Se \times p] + [(Sp) \times (1-p)]$, where p represents the prevalence of FTPI observed in the studied population.

Bland–Altman plots were used to examine the difference and interchangeability between the digital and the optical refractometers for the evaluation of STP and Brix . We calculated the mean difference between the digital and the optical refractometers (DSTP – OSTP and DBRIX – OBRIX), the standard deviation (SD) of the differences, and the limits of agreement (mean ± 1.96 × SD of the difference). The mean difference is the estimated bias, and the SD of the differences represents the random fluctuations around this mean value. The agreement was calculated by Bland–Altman as follows: Agreement (%)=$(A/B) \times 100$ where A is the number of samples located within the limits of agreement and B is the number of total samples.

Results

Descriptive analysis

Thirty-eight (45%) male and 47 female calves (55%), belonging to nine Chianina farms, were enrolled in the study, which yielded a total of 85 calves. The median age of the calves was 4.5 ± 1.9 days. The average number of selected calves per farm was 9 ± 7 (range 4–25 calves). The distributions of the Brix and STP measurements, evaluated with the digital and optical refractometers, were normal, as assessed by the Shapiro–Wilk test. The results of all descriptive analyses are reported in Table 1. A total of 35 (41.2%) calves had an FTPI (RID-IgG <16 g/L).

Correlation coefficients

The RID IgG concentration was positively correlated with the STP concentration ($r = 0.84$, $p < .0001$; Figure 1(A)) and Brix ($r = 0.84$, $p < .0001$; Figure 1(B)) measured by the digital refractometers. Similarly, the RID-IgG concentration was positively correlated with the results obtained from the OSTP ($r = 0.77$, $p < .0001$, Figure 1(C)) and DBRIX ($r = 0.75$, $p < .0001$, Figure 1(D)) refractometers.

Table 1. The mean, standard deviation (SD), the minimum and maximum value measured with digital and optical refractometers and radial immunodiffusion test (RID) in 85 Chianina beef–suckler calves from 9 farms in Umbria.

Item		Mean	SD	Minimum	Maximum
DSTP STP (g/L)	85	55.0	7.8	38.0	72.0
OSTP STP (g/L)	85	54.0	7.9	34.0	74.0
DBRIX Brix (%)	85	8.5	0.9	6.2	10.6
OBRIX Brix (%)	85	8.8	0.9	6.8	11.2
RID IgG (g/L)	85	16.9	9.2	2.0	34.0

DSTP: Digital serum total protein (STP) refractometer; OSTP: Optical STP refractometer; DBRIX: Digital Brix refractometer; OBRIX: Optical Brix refractometer.

Diagnostic test characteristics

The test characteristics of Se and Sp for STP and %Brix measured by optical and digital refractometers were determined for the assessment of FTPI. ROC curves were created to plot the true positive rate against the false positive rate for concentrations of STP and Brix (Figure 2). The AUC was greatest for Brix and STP measured using the digital refractometers (both 0.89), followed by Brix and STP measured using the optical refractometers (both 0.85). All four refractometers exhibited moderate accuracy.

The test characteristics (Se, Sp, PPV, NPV, and the proportion of calves correctly classified) associated with the optimal cutoff values are shown in Table 2 for each refractometer. The optimal STP concentration cutoff values determined from the ROC curves for diagnosing FTPI were <51.0 g/L (digital refractometer) and <52.0 g/L (optical refractometer), with a Se and Sp of 0.63 and 0.96 for the digital refractometer and 0.69 and 0.90 for the optical refractometer, respectively. The optimal cutoff value for Brix 8.3% for both for the digital and the optical refractometers, with a Se and Sp of 0.77 and 0.92 for the digital refractometer and 0.66 and 0.92 for the optical refractometer, respectively. When used with the optimal cutoff value (8.3%), the digital Brix refractometer yielded the highest proportion of correctly classified animals (86%) relative to the other refractometers. Altering the FTPI hypothetical prevalence resulted in no alteration of the NPV was observed for any of the refractometers under examination, while evident worsening of the PPV was observed in the low prevalence scenario (5%) (Table 3).

Agreement between tests

Bland–Altman plots revealed that the mean difference between STP (Figure 3(A)) as evaluated by the digital and optical refractometers was 0.7 g/L and the 95% limits of agreement were −0.69 to 0.83 g/L. The level

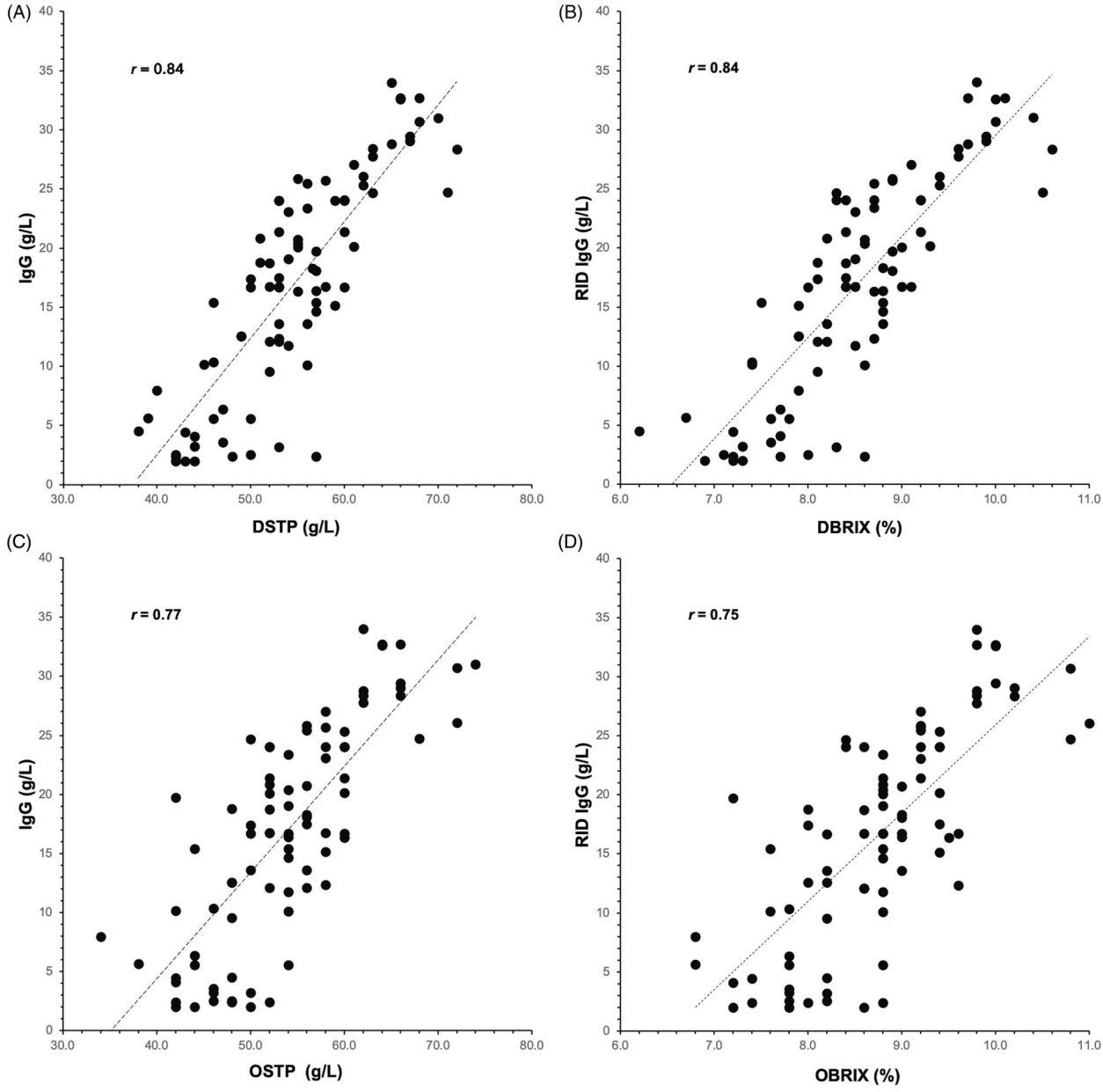

Figure 1. Correlation plot between: (A) serum total protein (g/L), determined by digital refractometry (DSTP), compared with serum IgG concentration (g/L) ($n = 85$; $r = 0.84$); (B) Brix percentage (%), determined by digital refractometry (DBRIX), compared with serum IgG concentration (g/L) ($n = 85$; $r = 0.84$); (C) Serum total protein (g/L), determined by optical refractometry (OSTP), compared with serum IgG concentration (g/L) ($n = 85$; $r = 0.77$); (D) Brix percentage (%), determined by optical refractometry (OBRIX), compared with serum IgG concentration (g/L) ($n = 85$; $r = 0.75$). Serum IgG (g/L) concentration was assessed by radial immunodiffusion (RID).

of agreement between DSTP and OSTP was 94.1%; 5.9% (5/85) were outside the limits of agreement. The mean difference between Brix (Figure 3(B)) as evaluated by the digital and optical refractometers was −0.27% and the 95% limits of agreement were −0.95 to 0.41%. The level of agreement between the DBRIX and OBRIX refractometers was 89.4%; 10.5% (9/85) were outside the limits of agreement.

Discussion

This study examined the diagnostic performance of four types of refractometers used for the diagnosis of FTPI in Chianina beef–suckler calves.

The results of our investigation show that the STP concentration and %Brix as evaluated by the digital refractometers had the highest correlation with IgG obtained by RID in assessing the transfer of passive

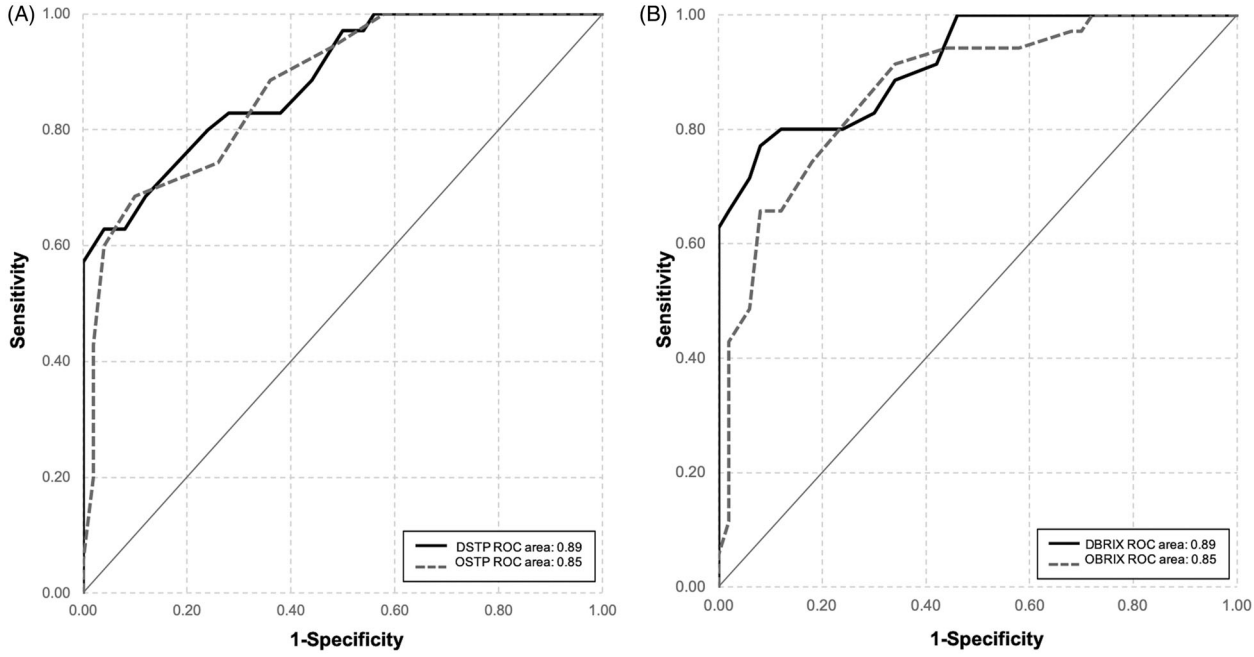

Figure 2. Receiver operating characteristics curves (ROC) used to determine optimal cut points for diagnosing inadequate transfer of passive immunity in Chianina beef–suckler calves (defined as a concentration of IgG in serum <16 g/L) for (A) concentration of serum total protein in serum (g/L; solid line, digital refractometer [DSTP]; dashed line, optical refractometer [OSTP]) and (B) %Brix (%; solid line, digital Brix refractometer [DBRIX]; dashed line, optical Brix refractometer [OBRIX]).

Table 2. Diagnostic test characteristics for the digital and optical refractometers for assessing the failure of transfer of passive immunity (serum immunoglobulin G concentration <16 g/L) in 85 Chianina beef–suckler calves from 9 farms in Umbria.

Refractometer	Cut point	Sensitivity	Specificity	PPV	NPV	Accuracy (%)
DSTP (g/L)	51	0.63	0.96	0.92	0.79	82
OSTP (g/L)	52	0.69	0.90	0.83	0.80	81
DBRIX (%)	8.3	0.77	0.92	0.87	0.85	86
OBRIX (%)	8.3	0.66	0.92	0.85	0.79	81

Optimal criteria (cut point) determined by receiver operating characteristic curves (ROC).
DSTP: Digital serum total protein (STP) refractometer; OSTP: Optical STP refractometer; DBRIX: Digital Brix refractometer; OBRIX: Optical Brix refractometer; PPV: positive predictive value; NPV: negative predictive value.

Table 3. Predicted positive value (PPV) and negative predictive value (NPV) calculated for the digital and optical refractometers at the best cut point using different three different failure of passive transfer prevalence scenarios (5, 15, and 25%) defined as serum immunoglobulin G <16 g/L.

	PPV (%)	NPV (%)
Hypothetical 5% prevalence		
DSTP (51 g/L)	45.3	98.0
OSTP (52 g/L)	26.6	98.2
DBRIX (8.3 %)	33.6	98.7
OBRIX (8.3 %)	30.3	98.1
Hypothetical 15% prevalence		
DSTP (51 g/L)	73.5	93.6
OSTP (52 g/L)	54.9	94.3
DBRIX (8.3 %)	62.9	95.8
OBRIX (8.3 %)	59.3	93.9
Hypothetical 25% prevalence		
DSTP (51 g/L)	84.0	88.6
OSTP (52 g/L)	69.7	89.7
DBRIX (8.3 %)	76.2	92.3
OBRIX (8.3 %)	73.3	89.0

DSTP: Digital serum total protein (STP) refractometer; OSTP: Optical STP refractometer; DBRIX: Digital Brix refractometer; OBRIX: Optical Brix refractometer.

immunity ($r = 0.84$ for both DSTP and DBRIX). These findings are similar to those obtained by Morrill et al. (2013) in dairy calves, where the correlation between serum IgG and %Brix as measured by DBRIX was found to be 0.87. In beef calves, the correlation between DSTP and serum IgG measured by ELISA was 0.64 (Todd et al. 2018) and the correlation between DSTP and serum IgG as measured by HPLC was 0.96 (Vandeputte et al. 2011). However, the variation in the methodologies used to measure the serum IgG concentration makes it difficult to compare our results with those obtained by Todd et al. (2018) and Vandeputte et al. (2011). Moreover, although the ELISA results were significantly correlated with the RID values in dairy calves (Sutter et al. 2020), the IgG concentration was lower when tested with ELISA than when measured with the gold standard RID, making the comparison of ELISA and RID results challenging (Wallace et al. 2006; Gelsinger et al. 2015; Thornhill et al. 2015).

In our study, the measurements with the optical refractometers (OSTP and OBRIX) were positively correlated with the serum IgG concentration tested by RID ($r = 0.77$ for OSTP and 0.75 for OBRIX), although

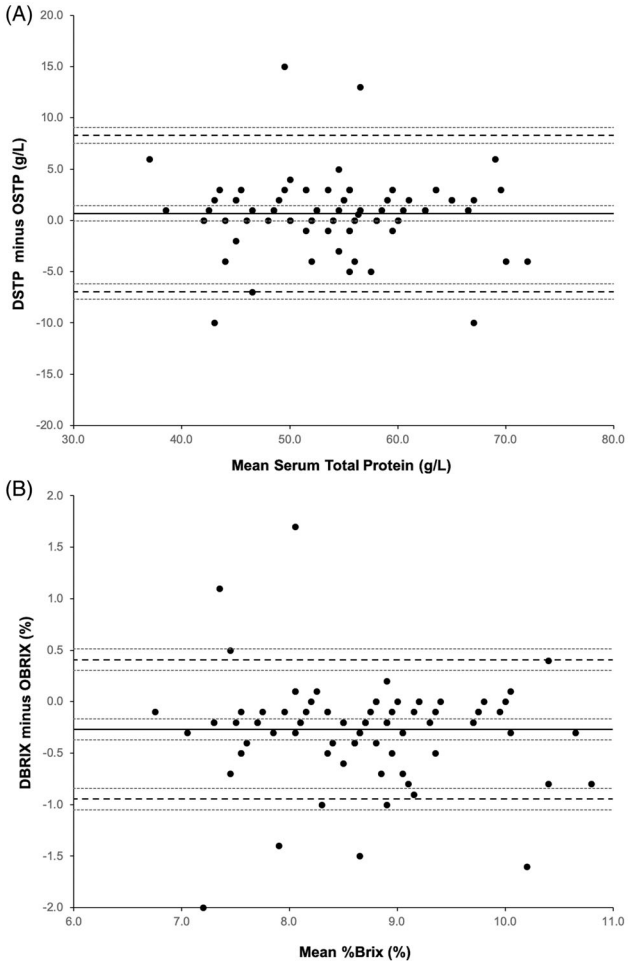

Figure 3. Bland–Altman plots showing the relationship between the difference between (A) serum total protein (STP, g/L) evaluated by digital (DSTP) and optical (OSTP) refractometer and the mean of the two values; (B) Brix percentage (%) evaluated by digital (DBRIX) and optical (OBRIX) refractometer and the mean of the two values. The black solid line indicates the mean difference between the 2 measures and the dotted lines represent 1.96 standard deviation from the mean difference. The top and bottom dashed grey line on each graph represent the 95% limits of agreement.

numerically lower than those obtained with the digital refractometers DSTP and DBRIX. A similar correlation between OSTP and IgG measured by RID ($r = 0.74$) has been reported in dairy calves (Elsohaby et al. 2015). However, Vandeputte et al. (2011) observed correlation coefficients of 0.95–0.96 between the STP assessed with optical refractometers and the IgG concentration measured with HPLC in Belgian Blue beef calves. Similarly, Todd et al. (2018) reported a correlation coefficient of 0.77 between OBRIX and serum IgG measured with ELISA in a mixed population of both beef and dairy calves.

The test characteristics of Se and Sp for all the refractometers under investigation were determined for the diagnosis of FTPI according to a serum IgG threshold of <16 g/L. The optimum threshold in our study for identifying Chianina calves with FTPI using STP was 51 g/L and 52 g/L for the DSTP and OSTP, respectively. Numerous studies have investigated the IgG titre assessed with digital or optical serum refractometers; however, most focussed exclusively on dairy calves. In these animals, the optimal cutoff values for the diagnosis of FTPI ranged from 51 to 58 g/L (Tyler et al. 1996; Elsohaby et al. 2015; Buczinski et al. 2016; Hernandez et al. 2016; Cuttance et al. 2017), although these studies used an IgG threshold of 10 g/L. In our study, using an IgG cutoff value of 16 g/L, as suggested previously (Wittum and Perino 1995; Waldner and Rosengren 2009), the cutoff value of 51 g/L that we established for DSTP had a Se of 0.63, and the cutoff value of 52 g/L that we established for OSTP had a Se of 0.69. Similar Se results were reported in 203 Holstein calves aged 1–11 days (Elsohaby et al. 2015) and in a mixed population of beef and dairy calves (Dawes et al. 2002). Regarding the %Brix measurements, the best combination of Se and Sp was found with cutoff values of ≤8.3% for both DBRIX and OBRIX. A similar cutoff value was reported by Todd et al. (2018), which indicated that the odds of death within 6 months of birth were almost threefold higher in suckler–beef calves with ≤8.4% Brix than in calves with >8.4% Brix. An identical cutoff value was reported by Elsohaby et al. (2015) and Cuttance et al. (2017) in dairy calves. The Se, Sp, NPV, and PPV were 0.77, 0.92, 0.85, and 0.87 for DBRIX and 0.66, 0.92, 0.85, and 0.79 for OBRIX, respectively. In the test population, as in the case of the serum refractometers, the Sp, accuracy, NPV, and PPV of the %Brix refractometers were satisfactory, while the lower Se indicated the reduced ability of the refractometers to correctly identify calves with FTPI. The performance of refractometers depends on both the cutoff value (Buczinski et al. 2018) and the prevalence of FTPI in the study population (Calloway et al. 2002). Although the lower Se could indicate the need to collect more samples in order to determine the prevalence of FTPI in our Chianina calves, the high Sp of the evaluated tools demonstrates their utility as screening tools on farms where colostrum management is already well established and of good quality. Indeed, in a population with a low disease prevalence, it is always desirable to use a test with an excellent Sp to avoid inappropriate classification of healthy animals (Berman et al. 2019). One interesting finding is that the NPVs from all refractometers under examination were not affected by the FTPI prevalence scenarios (Table 3). The optimal combination of PPV (84%) and NPV (88.6%) was

calculated for the DSTP with a 25% hypothetical prevalence. These results have an important practical application in the field, suggesting that the probability that calves with a negative screening test have adequate passive immunity transfer is not affected by the FTPI prevalence. On the other hand, the probability that subjects with a positive screening test truly have an FTPI needs to be interpreted with caution in low prevalence herds.

The agreement between the serum Brix percentage and STP concentrations measured by the digital and optical refractometers assessed using Bland–Altman plots showed no obvious systematic bias between the digital and optical refractometers. Our findings were in agreement with those reported in dairy calves and foals (Elsohaby et al. 2015, Elsohaby et al. 2019). However, the results derived from testing serum with DBRIX and OBRIX showed a slightly lower agreement (89.4%) than that with DSTP and OSTP (94.1%).

There are some limitations to our study. First, this observational study was performed exclusively on Chianina beef–suckler calves in Umbria. We believe our study has external validity because the herd genetics and the management of our convenience sample are typical for all the Chianina beef-farming enterprises of Central Italy. Furthermore, >34% of Italian subjects are bred in Umbria (ANABIC 2020). Second, the evaluation of the %Brix and STP concentration was carried out on blood samples in a laboratory setting. However, Wallace et al. (2006) reported that a strong positive correlation ($r = 0.95$) existed between the serum collected from noncentrifuged blood tubes allowed to clot and the STP content in centrifuged samples. Similarly, although speculative, a strong positive correlation may exist for the %Brix (Deelen et al. 2014). The simplicity of these methods is advantageous and easily allows for on-farm adaptation even when no centrifuge is available. Third, the accuracy of the tools under examination depends on the prevalence of FTPI in the population tested. In our study, the prevalence of FTPI (41.2%) was higher than previously reported (Dewell et al. 2006; Waldner and Rosengren 2009). Although an in-depth evaluation of the FTPI in the Chianina population was beyond the scope of this study due to the low number of samples, the results showed that the calves with a negative screening test were correctly classified (high NPV) by the digital and optical refractometers in different FTPI prevalence scenarios.

We believe that these results have some clinical applications. The use of refractometers to assess FTPI on the farm can become part of the routine monitoring of the colostrum management program in suckler–beef calf herds. We found that simply evaluating STP or %Brix with a digital refractometer had an optimal correlation with IgG obtained by RID. In our study population, the Brix refractometer was the most accurate method for detecting of calves with FTPI. All the devices studied can be used in farms with different FTPI prevalence; however, caution is necessity where the prevalence is exceptionally low because the assessment could result in an increase of false-positive cases.

Conclusions

The results of this observational study indicate that digital and optical refractometers are rapid and accurate tools for diagnosing FTPI in Chianina calves. ROC analysis showed that the optimal cutoff STP values for predicting FTPI were 51 and 52 g/L for the digital and optical STP refractometers, respectively. These results were associated with an Se of 0.63, an Sp of 0.96, a PPV of 0.92, and an NPV of 0.79 for the DSTP and an Se of 0.69, an Sp of 0.90, a PPV 0.80, and an NPV of 0.83 for the OSTP. At the threshold of 8.3%, the Se, Sp, NPV, and PPV were 0.66, 0.92, 0.85, and 0.79 for the OBRIX, and 0.77, 0.92, 0.85, and 0.87 for the DBRIX. The DBRIX was found to be the most accurate refractometer for the assessment of FTPI in our sample.

Disclosure statement

None of the authors has a financial or personal relationship with other people or organisations that could inappropriately influence this publication.

References

ANABIC – Associazione Nazionale Allevatori Bovini Italiani da Carne. 2020. [accessed May 20]. http://www.anabic.it.

Berman J, Francoz D, Dufour S, Buczinski S. 2019. Bayesian estimation of sensitivity and specificity of systematic thoracic ultrasound exam for diagnosis of bovine respiratory disease in pre-weaned calves. Prev Vet Med. 162:38–45.

Buczinski S, Fecteau G, Chigerwe M, Vandeweerd JM. 2016. Diagnostic accuracy of refractometer and Brix refractometer to assess failure of passive transfer in calves: protocol for a systematic review and meta-analysis. Anim Health Res Rev. 17(1):3–8.

Buczinski S, Gicquel E, Fecteau G, Takwoingi Y, Chigerwe M, Vandeweerd JM. 2018. Systematic review and meta-analysis of diagnostic accuracy of serum refractometry and Brix refractometry for the diagnosis of inadequate transfer of passive immunity in calves. J Vet Intern Med. 32(1): 474–483.

Calloway CD, Tyler JW, Tessman RK, Hostetler D, Holle J. 2002. Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves. J Am Vet Med Assoc. 221(11):1605–1608.

Chigerwe M, Hagey J, Aly SS. 2015. Determination of neonatal serum immunoglobulin G concentrations associated with mortality during the first 4 months of life in dairy heifer calves. J Dairy Res. 82(4):400–406.

Cuttance EL, Mason WA, Denholm KS, Laven RA. 2017. Comparison of diagnostic tests for determining the prevalence of failure of passive transfer in New Zealand dairy calves. NZ Vet J. 65(1):6–13.

Dawes ME, Tyler JW, Hostetler D, Lakritz J, Tessman R. 2002. Evaluation of a commercially available immunoassay for assessing adequacy of passive transfer in calves. J Am Vet Med Assoc. 220(6):791–793.

Deelen SM, Ollivett TL, Haines DM, Leslie KE. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J Dairy Sci. 97(6):3838–3844.

Dewell RD, Hungerford LL, Keen JE, Laegreid WW, Griffin DD, Rupp GP, Grotelueschen DM. 2006. Association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves. J Am Vet Med Assoc. 228(6):914–921.

Elsohaby I, Mcclure JT, Keefe GP. 2015. Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in dairy calves. J Vet Intern Med. 29(2):721–726.

Elsohaby I, Riley CB, McClure JT. 2019. Usefulness of digital and optical refractometers for the diagnosis of failure of transfer of passive immunity in neonatal foals. Equine Vet J. 51(4):451–457.

Gelsinger SL, Smith AM, Jones CM, Heinrichs A. 2015. Technical note: Comparison of radial immunodiffusion and ELISA for quantification of bovine immunoglobulin G in colostrum and plasma. J Dairy Sci. 98(6):4084–4089.

Hernandez D, Nydam DV, Godden SM, Bristol LS, Kryzer A, Ranum J, Schaefer D. 2016. Brix refractometry in serum as a measure of failure of passive transfer compared to measured immunoglobulin G and total protein by refractometry in serum from dairy calves. Vet J. 211:82–87.

McCracken MM, Morrill KM, Fordyce AL, Tyler HD. 2017. Technical note: Evaluation of digital refractometers to estimate serum immunoglobulin G concentration and passive transfer in Jersey calves. J Dairy Sci. 100(10):8438–8442.

McGee M, Earley B. 2019. Passive immunity in beef-suckler calves. Animal. 13(4): 810–825.

Morrill KM, Polo J, Lago A, Campbell J, Quigley J, Tyler H. 2013. Estimate of serum immunoglobulin G concentration using refractometry with or without caprylic acid fractionation. J Dairy Sci. 96(7):4535–4541.

Raboisson D, Trillat P, Cahuzac C. 2016. Failure of passive immune transfer in calves: a meta-analysis on the consequences and assessment of the economic impact. PLoS One. 11(3):e0150452.

Sutter F, Rauch E, Erhard M, Sargent R, Weber C, Heuwieser W, Borchardt S. 2020. Evaluation of different analytical methods to assess failure of passive transfer in neonatal calves. J Dairy Sci. 103(6):5387–5397.

Thornhill J, Krebs G, Petzel C. 2015. Evaluation of the Brix refractometer as an on-farm tool for the detection of passive transfer of immunity in dairy calves. Aust Vet J. 93(1–2):26–30.

Todd CG, McGee M, Tiernan K, Crosson P, O'Riordan E, McClure J, Lorenz I, Earley B. 2018. An observational study on passive immunity in Irish suckler beef and dairy calves: tests for failure of passive transfer of immunity and associations with health and performance. Prev Vet Med. 159: 182–195.

Tyler JW, Hancock DD, Parish SM, Rea DE, Besser TE, Sanders SG, Wilson LK. 1996. Evaluation of 3 assays for failure of passive transfer in calves. J Vet Intern Med. 10(5):304–307.

Vandeputte S, Detilleux J, Rollin F. 2011. Comparison of four refractometers for the investigation of the passive transfer in beef calves. J Vet Intern Med. 25(6):1465–1469.

Waldner CL, Rosengren LB. 2009. Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. Can Vet J. 50(3):275–281.

Wallace MM, Jarvie BD, Perkins NR, Leslie KE. 2006. A comparison of serum harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves. Can Vet J. 47(6):573–575.

Wilm J, Costa JHC, Neave HW, Weary DM, von Keyserlingk MAG. 2018. Technical note: Serum total protein and immunoglobulin G concentrations in neonatal dairy calves over the first 10 days of age. J Dairy Sci. 101(7): 6430–6436.

Wittum TE, Perino LJ. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. Am. J. Vet. Res. 56:1149–1154.