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Evaluation of colostrum quality in the Czech Republic using radial immunodiffusion and different types of refractometers

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Abstract: The objectives of this study were to determine the immunoglobulin G concentration of colostrum in Czech dairy cows, to compare refractometer results with results achieved using the radial immunodiffusion method and to evaluate the reliability of three types of refractometers and recommend the best solution for the evaluation of colostrum quality. Colostrum samples (n = 1522) were collected from 38 herds between 2015 and 2017. The immunological quality of colostrum was estimated using Brix refractometers (optical, simple digital, digital Misco) and compared with the immunoglobulin G concentration assessed using radial immunodiffusion. We found high variability in the quality of colostrum. The minimum, maximum and median of individual measurements were the following: radial immunodiffusion immunoglobulin G – 5.2, 199.1, 76.9 g/l; optical refractometer – 9.5, 32.0, 23.1% Brix; simple digital refractometer – 5.4, 35.0, 19.1% Brix; digital refractometer Misco – 9.8, 37.4, 23.2% Brix. On the basis of immunoglobulin G concentration assessed using radial immunodiffusion, 20.9% of colostrum samples were of low quality (immunoglobulin G < 50 g/l). The Spearman correlation coefficients between radial immunodiffusion and the Brix refractometer readings were 0.62–0.67 (P < 0.001) according to the type of refractometer. The cut-off evaluation of the readings from optical and Misco digital refractometers both showed 20% Brix, with sensitivities of 89.4% and 88.2%, specificities of 73.2% and 74.5% and accuracies of 86.0% and 85.4%, respectively. The cut-off level for the simple digital refractometer showed 17% Brix with a sensitivity of 77.5%, specificity of 80.4% and an insufficient accuracy of 78.1%. For optical and Misco refractometers we recommend the use of two cut-off levels for the evaluation of colostrum: 23% Brix for the selection of good quality colostrum suitable for freezing and 19% Brix to discard poor quality colostrum. The different cut-off levels obtained by measuring with different types of refractometers indicate the need to check the quality of the instruments prior to their use in practice and, where appropriate, to determine their cut-off levels by comparison with results obtained using the reference method.

Keywords: accuracy; Brix refractometers; colostrum; cut-off levels; dairy cow; immunoglobulin G concentration

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Colostrum, the initial secretion from the mammary gland after parturition, is an important source of immunity and nutrition for the neonate (Bielmann et al. 2010). The quality of colostrum varies considerably. The published data report immunoglobulin G (IgG) concentrations ranging from 4 up to 235 g/l assessed by the method of radial immunodiffusion (RID) (Gulliksen et al. 2008).

There are many factors that influence colostrum quality, e.g. parity (Tyler et al. 1999), dry period length (Rastani et al. 2005), the amount of first colostrum (Pritchett et al. 1991), season of calving (Godden 2008), time from calving to the first milking (Moore et al. 2005), hygiene measures during the collection of colostrum (Stewart et al. 2005) etc.

Calves are born agammaglobulinaemic due to their type of placenta which does not allow the transfer of IgG from mother to the foetus (Godden 2008; Beam et al. 2009; Morrill et al. 2015). Therefore, the most important factor influencing calf health and future production is adequate intake of high-quality colostrum as soon as possible (Bielmann et al. 2010). If the amount, or immunological or microbiological quality of consumed colostrum is insufficient, or the administration is too late, failure of passive transfer occurs, resulting in an increased morbidity and mortality of calves (McGuirk and Collins 2004; Bielmann et al. 2010). A concentration of IgG < 10 g/l in blood serum is the threshold for failure of passive transfer diagnosis (Weaver et al. 2000; Calloway et al. 2002; Godden 2008). Therefore, easy and accurate on-farm assessment of colostrum quality is essential (Morill et al. 2015). Progress has been made in this area in recent times. According to the National Animal Health Monitoring system study of the US Department of Agriculture (2014), 53.3% of producers evaluate colostrum quality before feeding to calves, which is four times more than in 2007 but is still not enough. Previous studies revealed that colostrum quality was controlled on 44.1% of farms in the Czech Republic (Stanek et al. 2014) and only on 20.8% of farms in Austria (Klein-Jobstl et al. 2015).

The most accurate method for colostrum quality measurement is the RID assay (Bielmann et al. 2010). However, it is a laboratory method and results are only available after 24 h. It is also expensive. Routine on-farm monitoring of colostrum quality is performed using a colostrometer or an optical or digital refractometer, which measures the refractive index of liquids using a Brix score (Bartens et al. 2016). The colostrometer was still the most used tool for on-farm monitoring of colostrum quality (Stanek et al. 2014; USDA 2016) despite its disadvantages (temperature sensitivity, fragility). Recent research findings show that the Brix refractometer is a suitable tool for colostrum quality assessment because it is rapid, accurate, user-friendly and functions independently of colostrum temperature (Quigley et al. 2013; Bartier et al. 2015; Morill et al. 2015; Bartens et al. 2016). Slightly different cut-off levels ranging from 20% to 23% Brix are recommended for the determination of Holstein colostrum of good quality which corresponds to an IgG concentration of ≥ 50 g/l (Chigerwe et al. 2008; Bielmann et al. 2010; Quigley et al. 2013; Elsohaby et al. 2017).

We hypothesised that on-farm methods for colostrum quality estimation might be quite inaccurate in comparison with the exact RID laboratory method and that individual types of refractometers may show significant differences in measurement accuracy because of their different technical designs. The objectives of this study were to determine the IgG content of colostrum in Czech dairy herds, to compare the accuracy of measurement of colostrum IgG concentrations using three different types of refractometers and the RID method and to recommend the best solution for the evaluation of colostrum quality for routine use on farms.

MATERIAL AND METHODS

Colostrum sample collection. Individual colostrum samples (n = 1522) were collected from 38 dairy cattle herds during the period from autumn 2015 to spring 2017. The samples originated from Holstein cattle (44.2%) and from Czech Fleckvieh cattle (55.8%). Colostrum was collected by farm staff from the first milking until 12 h after calving into prepared sterile vessels (volume of 10 to 20 ml) and labelled with cow identification number and date of collection. Immediately after collection, the samples were frozen and stored on the farm at –20°C. Frozen samples were transported to the laboratory of the Veterinary Research Institute Brno, Czech Republic, and stored there at –20°C pending analysis.

Laboratory analysis. All measurements were made after thawing of samples at room tempera-

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ture in the laboratory over the course of one day to avoid repeated freezing and thawing.

Refractometer readings. For the evaluation of colostrum quality, we used three different types of instruments: an optical refractometer with a scale from 0 to 32% Brix (type RBR32-ATC), a simple digital refractometer with a scale from 0 to 85% Brix (type RDBR85-ATC; 128 detector elements; resolution 400 pixels per inch – PPI) and a Misco digital refractometer with a scale range from 0 to 85% Brix (type Misco DD-3 Digital Dairy^{TM}; 1024 detector elements; resolution 3256 PPI).

Colostrum samples were vortexed for 10 s and then tested using individual refractometers. For the optical refractometer measurement, approximately 50 µl of colostrum were used. The upper limit on the scale of this device was 32% Brix, samples where values exceeded 32% were assigned a value of 32%. For the simple digital and Misco refractometer measurements, approximately 150 µl of colostrum were used. The simple digital refractometer and the Misco refractometer were used differently during measurement. The simple digital refractometer was used to evaluate samples without any protective evaporation cover, and for the Misco refractometer the colostrum was placed on the prism well and the sample was protected by a cover.

Radial immunodiffusion assay to estimate IgG concentrations. Colostral IgG concentrations were determined using the radial immunodiffusion reference method as described by Krejci et al. (2016). Radial immunodiffusion plates were prepared by dissolving 1.5% agarose in phosphate-buffered saline and heating in a water bath. Rabbit anti-bovine IgG (1%) was added to the agarose solution tempered to 56°C, and 22 ml of the final solution was added to 20-cm-diameter Petri dishes. After the agarose had solidified, 2.5-mm-diameter wells were cut in the agar. Thawed colostrum samples were vortexed for 10 s and diluted 1 : 20 with deionised sterile water. Then, 5 µl of each sample were placed in a well. The diameter of the zone of precipitation was recorded after 24 hours of incubation at 23°C. Sample IgG concentrations were determined by comparing diameters of zones of precipitation with a standard curve generated with serial dilutions of bovine IgG standard.

Statistical analysis. Descriptive statistics of all obtained data were calculated. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Because the data for IgG concentrations in colostrum and data measured using the optical refractometer were not normally distributed, nonparametric tests were used for the statistical evaluation. A Spearman rank coefficient of correlation was calculated to determine the level of relationship between RID and individual types of refractometers and also between all three types of refractometers. Colostrum samples with IgG concentrations < 50 g/l were classified as diagnostically positive and samples ≥ 50 g/l were classified as diagnostically negative. Sensitivity was defined as the proportion of poor-quality colostrum samples (< 50 g/l) that were correctly identified using the tested cut-off level (true positive rate); specificity was defined as the proportion of good-quality colostrum samples (≥ 50 g/l) that were correctly identified using the tested cut-off level (true negative rate). Positive predictive value was defined as the probability that poor-quality colostrum was present when the test was positive. Negative predictive value was defined as the probability that poor-quality colostrum was not present when the test was negative. Accuracy was defined as the proportion of colostrum samples that were correctly identified. Sensitivity, specificity, accuracy, positive and negative predictive values are expressed as percentages. Confidence intervals for sensitivity and specificity are "exact" Clopper-Pearson confidence intervals. Confidence intervals for the predictive values are the standard logit confidence intervals given by Mercaldo et al. (2007). For all three types of refractometers, seven cut-off values were considered and epidemiological characteristics were computed. Optimised cut-off values were defined based on the maximum Youden index, calculated as sensitivity plus specificity minus one (Ruopp et al. 2008). In the case of the same Youden index for two cut-off levels, the one with the better accuracy was chosen. Statistical analyses were performed using MedCalc for Windows, version 18.6 (MedCalc Software, Ostend, Belgium, www.medcalc.org).

RESULTS

Evaluation of colostrum quality

The quality of 1522 frozen colostrum samples was evaluated using RID and refractometry. The descriptive statistics of the results obtained using all four methods are shown in Table 1. The RID IgG

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Table 1. Descriptive statistics of the evaluation of colostrum quality using different methods

	Radial	Refractometer		
	imm	opt	sim	mis
No. of samples	1522	1522	1522	1522
Mean	82.3	23.1	18.9	23.0
Median	76.9	23.1	19.1	23.2
SD	40.3	4.5	4.9	4.6
Quartile 25%	52.2	20.1	15.5	20.0
Quartile 75%	108.2	26.2	22.2	26.1
Minimum	5.2	9.5	5.4	9.8
Maximum	199.1	32.0	35.0	37.4

imm = immunodiffusion (IgG, g/l); mis = Misco digital (Brix, %); opt = optical (Brix, %); SD = standard deviation; sim = simple digital (Brix, %)

concentrations in colostrum samples ranged from 5.2 to 199.1 g/l. The mean and median of the RID IgG concentrations were 82.3 and 76.9 g/l, respectively. On the basis of IgG concentrations assessed by RID, 20.9% of colostrum samples were of low quality (IgG < 50 g/l).

The data for IgG concentrations and optical refractometer readings were not normally distributed. However, the readings of both digital refractometers were normally distributed (tested by the Kolmogorov-Smirnov test). Frequency distributions of data measured by different methods are shown in Figure 1. The samples of colostrum had a mean and median Brix score of 23.1 and 23.1% determined using the optical refractometer, 18.9 and 19.1% using the simple digital refractometer and 23.0 and 23.2% using the Misco digital refractometer.

Correlations between RID and refractometry

Correlations between all three types of refractometers and the gold standard – laboratory assay of IgG

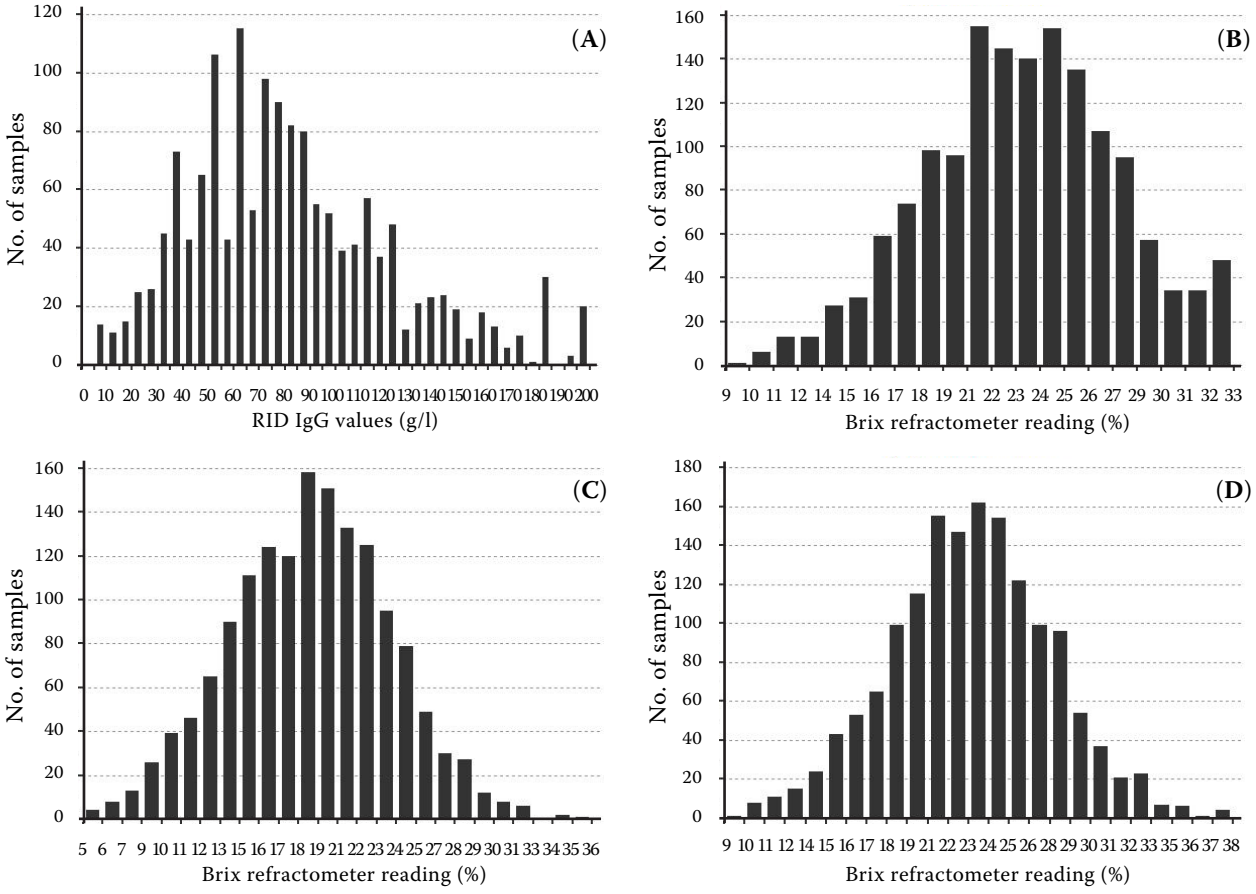

Figure 1. Frequency distributions of (**A**) IgG in colostrum determined by radial immunodiffusion (RID), (**B**) percentage Brix determined by optical refractometer, (**C**) percentage Brix determined by simple digital refractometer and (**D**) percentage Brix determined by Misco digital refractometer

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Table 2. Spearman correlation coefficients between radial immunodiffusion (RID) and refractometry (n = 1522)

	RID	Optical refractometer	Simple digital refractometer
Optical refractometer	0.669*		
Simple digital refractometer	0.623*	0.901*	
Misco digital refractometer	0.666*	0.991*	0.900*

*P < 0.001

concentrations by RID in colostrum – were calculated using Spearman's rank coefficient of correlation. The correlations between RID values and Brix scores from the optical, simple digital and Misco digital refractometers were 0.67, 0.62 and 0.67, respectively (P < 0.001; n = 1552; Table 2). The correlation between Brix scores from the Misco digital and optical refractometers was 0.99, between simple digital and optical refractometers 0.90 and between the simple digital and Misco digital refractometer 0.90. The scatter plots of the relationships between RID and refractometry are shown in Figure 2.

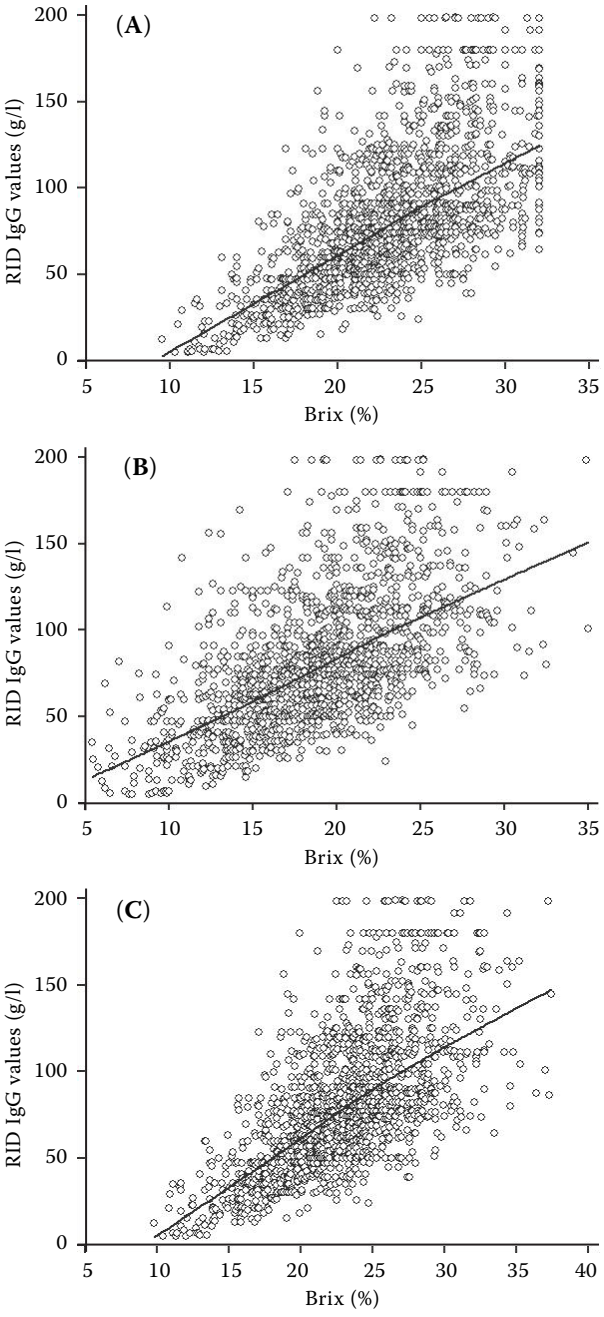

Figure 2. (**A**) The concentration of IgG in colostrum determined by radial immunodiffusion (RID) compared with percentage Brix determined by optical refractometer; (**B**) the concentration of IgG in colostrum determined by RID compared with percentage Brix determined by simple digital refractometer; (**C**) the concentration of IgG in colostrum determined by RID compared with percentage Brix determined by Misco digital refractometer

Diagnostic test characteristics

The test characteristics of the refractometry performed using different instruments were determined for the assessment of poor-quality colostrum (IgG < 50 g/l). The sensitivity (Se), specificity (Sp), accuracy, positive (PPV) and negative predictive values (NPV) were calculated for seven cut-off levels (ranging from 17 to 23% Brix). The diagnostic test characteristics are shown in Table 3. The best cut-off values for the detection of poor-quality colostrum were assessed with the help of the Youden index and accuracy was as follows: for optical refractometer, 20% Brix (Se 74.1%; Sp 88.1%); for simple digital refractometer, 17% Brix (Se 80.4%; Sp 76.9%); for the Misco digital refractometer, 20% Brix (Se 74.8%; Sp 87.9%).

DISCUSSION

Our results show a lower prevalence (20.9% of 1522 samples) of poor-quality colostrum (< 50 g/l IgG) than in most other published works. The lowest prevalence (7.7% of 288 Holstein colostrum samples) was found by Bielmann et al. (2010). Higher values, i.e., 29.1% of 460 colostrum samples

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Table 3. Diagnostic test characteristics for refractometry performed using different types of refractometers (optical, simple digital, Misco digital) for assessment of poor-quality colostrum (IgG < 50 g/l) using seven different cut-off values for individual instruments

	Cut-off value (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)	Youden index
Optical refractometer (Brix)	17	40.4 (34.9–46.0)	98.2 (97.2–98.9)	85.3 (79.0–90.0)	86.2 (85.1–87.3)	86.1 (84.3–87.8)	0.39
	18	51.1 (45.5–56.7)	96.1 (94.8–97.1)	77.5 (71.9–82.3)	88.2 (87.0–89.3)	86.8 (85.0–88.5)	0.47
	19	64.0 (58.5–69.3)	93.0 (91.4–94.4)	70.7 (65.9–75.1)	90.8 (89.5–91.9)	87.0 (85.2–88.6)	0.57
	20	74.1 (68.9–78.9)	88.1 (86.2–89.9)	62.2 (58.2–66.0)	92.8 (91.5–94.0)	86.0 (84.2–87.7)	0.62
	21	81.4 (76.7–85.5)	80.9 (78.6–83.1)	52.9 (49.7–56.0)	94.3 (92.9–95.4)	81.9 (79.9–83.8)	0.62
	22	88.0 (83.9–91.4)	70.9 (68.2–73.4)	44.3 (41.9–46.7)	95.7 (94.3–96.8)	75.6 (73.3–77.7)	0.59
	23	92.7 (89.3–95.3)	61.6 (58.8–64.3)	38.8 (37.0–40.7)	97.0 (95.6–98.0)	69.1 (66.7–71.4)	0.54
Simple digital refractometer (Brix)	17	80.4 (75.6–84.7)	76.9 (74.4–79.3)	47.8 (44.9–50.8)	93.7 (92.3–94.9)	78.1 (76.0–80.2)	0.57
	18	87.1 (82.9–90.6)	69.7 (67.0–72.3)	43.1 (40.7–45.4)	95.3 (93.9–96.5)	73.9 (71.6–76.1)	0.57
	19	91.5 (87.8–94.3)	61.0 (58.2–63.8)	38.2 (36.3–40.0)	96.5 (95.0–97.5)	68.3 (65.9–70.6)	0.53
	20	94.3 (91.2–96.6)	50.0 (47.2–52.9)	33.2 (31.8–34.6)	97.1 (95.5–98.1)	60.2 (57.7–62.7)	0.44
	21	96.5 (93.9–98.3)	42.3 (39.5–45.2)	30.6 (29.5–31.7)	97.9 (96.3–98.8)	53.9 (51.3–56.4)	0.39
	22	98.4 (96.4–99.5)	32.7 (30.1–35.4)	27.8 (27.0–28.6)	98.7 (97.1–99.5)	46.7 (44.1–49.2)	0.31
	23	99.7 (98.3–100.0)	25.3 (22.9–27.9)	26.0 (25.3–26.6)	99.7 (97.7–100)	41.0 (38.5–43.5)	0.25
Misco digital refractometer (Brix)	17	40.7 (35.2–46.3)	98.4 (97.5–99.0)	87.2 (81.0–91.5)	86.3 (85.2–87.4)	86.1 (84.2–87.8)	0.39
	18	51.4 (45.8–57.0)	96.5 (95.3–97.5)	79.5 (73.9–84.2)	88.3 (87.1–89.4)	87.0 (85.2–88.6)	0.48
	19	63.7 (58.2–69.0)	93.1 (91.5–94.5)	70.9 (66.1–75.3)	90.7 (89.4–91.9)	86.9 (85.1–88.6)	0.57
	20	74.8 (69.6–79.5)	87.9 (85.9–89.7)	61.9 (57.9–65.7)	93.0 (91.6–94.1)	85.4 (83.5–87.1)	0.63
	21	81.4 (76.7–85.5)	80.8 (78.4–82.9)	52.7 (49.5–55.8)	94.3 (92.9–95.4)	81.7 (79.7–83.7)	0.62
	22	88.6 (84.6–91.9)	71.0 (68.3–73.5)	44.5 (42.2–46.9)	96.0 (94.6–97.0)	75.5 (73.3–77.6)	0.60
	23	92.4 (88.9–95.1)	62.1 (59.3–64.8)	39.1 (37.2–41.0)	96.9 (95.5–97.9)	69.3 (66.9–71.6)	0.55

of unknown breed, 32% of 171 Holstein colostrum samples, 34.7% of 193 Holstein colostrum samples and 48% of 258 Holstein samples were reported by Bartier et al. (2015), Chigerwe et al. (2008), Bartens et al. (2016) and Elsohaby et al. (2017), respectively. The highest prevalence of poor-quality colostrum was reported by Gulliksen et al. (2008): 57.8% of 1250 Norwegian dairy cow samples. Dos Santos et al. (2017) described the effect of farm characteristics on colostrum quality. On farms where the daily milk production was lower than 200 l/day almost 60% of colostrum samples showed IgG concentrations < 50 g/l, and on farms with daily milk production > 700 l/d only 28% colostrum samples had IgG < 50 g/l, which nearly corresponds with what we found exclusively on large farms (> 700 l milk/day). Similarly, large differences were also reported in the average detected concentrations of IgG in colostrum. Many authors found lower mean concentrations of IgG compared to our work (82.3 g/l), namely between 60 and 69 g/l (Morrill et al. 2012a; Bartier et al. 2015; Lokke et al. 2016; Elsohaby et al. 2017). Similar to our values, Quigley et al. (2013) found 73.4 g/l, but some other authors (Bielmann et al. 2010; Kehoe et al. 2011) reported even higher values ranging from 94 to 96 g/l. The lowest values of 51.7 and 48.1 g/l were found by dos Santos et al. (2017) on farms with daily milk production of < 200 l and 201–700 l, respectively. The great variability between the results of individual studies is probably caused by breed, differing numbers of animals and farms included, selection of the animals and variability in environmental and management aspects.

Both the RBR32-ATC optical refractometer and the RDBR85-ATC simple digital refractometer were chosen because of their easy availability and very favourable prices for the breeding community, which allows easy acquisition and potentially efficient use of the instruments on each farm.

In our study, the correlation coefficients between RID-measured IgG concentrations and the Brix re-

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fractometers data ranged from 0.62 to 0.67 according to the type of refractometer (optical, simple digital and Misco digital). The data reported in the literature differ. A lower correlation coefficient (0.61, n = 171) was reported in one earlier study (Chigerwe et al. 2008), but a similar correlation coefficient of 0.64 was reported by Bartier et al. (2015). These authors evaluated a large number of samples (n = 569). However, this number is still much lower than we evaluated in our study (n = 1522). Other authors mostly found closer relationships, but they evaluated much lower numbers of colostrum samples. Elsohaby et al. (2017) reported 0.72 for digital and 0.71 for optical refractometers (n = 255); similarly, Bielman et al. (2010) found 0.71 (n = 288), Quigley et al. (2013) 0.75 (n = 183) and Lokke et al. (2016) 0.81 (n = 126). These data show that the authors who worked with smaller datasets found better correlations. We suggest that the reason for this phenomenon might be more homogeneous groups of samples. In our dataset, we included herds with different levels of milk production and the samples were collected in different seasons over the course of the whole year. The composition of colostrum could influence the results of the refractometry. Lokke et al. (2016) evaluated the relationship between colostrum composition and immunological quality. IgG and total protein were correlated (r^2 = 0.70), whereas the fat concentration varied independently of the IgG or protein concentrations. Both fat and protein significantly influenced the results of Brix refractometry. We suppose that the differences in protein and fat concentrations in our colostrum samples contributed to the worse correlations between IgG and Brix. Concerning other factors, the study of Morrill et al. (2012b) suggests that breed, lactation number, nutrient composition and bacterial contamination had minimal effects on the relationship between the refractive index and IgG concentration. However, Bartier et al. (2015) suggested that the nutrient content of the colostrum may have contributed to variation in the correlation coefficients (IgG vs % Brix), because the Brix refractometer measures total dissolved solids and not IgG specifically.

The relationships between Brix scores measured using different devices are close. A very high Spearmann correlation coefficient of 0.991 was found between the optical and Misco digital refractometer. However, the correlations between the simple digital refractometer and the optical or Misco digital refractometer were lower (0.901 and 0.900, respectively). These lower correlations show that the simple digital refractometer did not measure precisely and explain the lower correlation coefficients with IgG found in comparison with the other two devices. On the basis of these results, we can declare that the simple digital refractometer did not measure properly and the use of this type of refractometer represents a risk of incorrect evaluation of colostrum quality. The cut-off levels for refractometry, assessed with the help of the Youden index, differed between individual devices. Evaluation of the readings from the optical refractometer and the digital Misco refractometer both showed 20% Brix. However, the cut-off was only 17% Brix for the simple digital refractometer. At 20% Brix, the optical and Misco digital refractometer had sensitivities of 74.1% and 74.8%, specificities of 88.1% and 87.9% and accuracies of 86.0% and 85.4%, respectively. These values demonstrated that the two types of refractometers give similar quality measurements. Previous studies have suggested that the appropriate cut-off level for the evaluation of poor-quality colostrum using a Brix refractometer is between 18% and 23% Brix. Morrill et al. (2015) recommended a cut-off level of 18% Brix for Jersey colostrum and 21% Brix for Holstein colostrum (Quigley et al. 2013; Morrill et al. 2015). Bielmann et al. (2010) recommended a higher value (22% Brix), while Bartier et al. (2015) and Elsohaby et al. (2017) actually recommended 23% Brix.

Buczinski and Vandeweerd (2016) performed a systematic review and meta-analysis of the diagnostic accuracy of refractometry and recommended two cut-off levels for the evaluation of colostrum: 22% Brix for the selection of good-quality colostrum suitable for freezing and < 18% Brix for the discarding of poor-quality colostrum. Colostrum with ≥ 18% and < 22% Brix was considered as suspect, and adding frozen colostrum or colostrum supplement was recommended. We hope that this solution is interesting and useful for practitioners, because this approach allows the optimal use of colostrum, while maintaining an adequate intake of antibodies by calves. If we use a similar approach on our set of data and choose values with sensitivity ≥ 90% as a limit for exclusion of poor-quality colostrum and a specificity of ≥ 90% as a limit for good-quality colostrum suitable for the first feeding or freezing, the recommended range is the same for the optical and Misco digital refractometers, i.e. 19–23% Brix.

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These values are slightly higher than those suggested by Buczinski and Vandeweerd (2016).

The cut-off level for a simple digital refractometer is only 17% Brix with a sensitivity of 80.4%, specificity of 76.9% and accuracy of 78.1%. An accuracy of at least 80% is recommended for the method used in practice. On the basis of these results, this type of refractometer underestimates the concentration of IgG and has lower accuracy than the two other devices. We did not find in the available literature any comparison of different types of digital refractometers; nevertheless, some authors have compared digital and optical refractometers. Similar results for both types of refractometers were found by Elsohaby et al. (2016) and Bielamann et al. (2010), but Bartens et al. (2016) determined higher cut-off values for optical (27% Brix) than for digital (23.4% Brix) refractometers. Different cut-off levels obtained by measuring with different types of refractometers indicate the need for checking the technical quality of the instruments (e.g., number of detector elements and pixels per inch) prior to their use in practice and, where appropriate, to determine their cut-off levels on the basis of comparison of the results with the reference method.

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