



Evaluation of the agreement between Brix refractometry and serum immunoglobulin concentration in neonatal piglets



A. Schoos^{a,*}, W. De Spiegelaere^b, A. Cools^d, B. Pardon^c, E. Van Audenhove^a, E. Bernaerdts^a, G.P.J. Janssens^d, D. Maes^a

^a Department of Reproduction, Obstetrics and Herd Health, Unit of Porcine Health Management, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

^b Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

^c Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

^d Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium

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ABSTRACT

Neonatal piglets lack immunoglobulins at birth. Sufficient colostrum intake (**CI**) and immunoglobulin absorption are essential for an appropriate passive transfer of immunity via the colostrum. Most methods to measure immunoglobulins in serum of piglets are labour-intensive, expensive or imprecise and not designed for on-farm use. The present diagnostic test study evaluated digital Brix refractometry to measure immunoglobulins in serum of neonatal piglets and to suggest thresholds for different serum immunoglobulin concentration. Additionally, agreements between Brix refractometry and optical refractometer (serum total protein, **STP**) and between Brix refractometry and ELISA (immunoglobulin G, **IgG**) were also investigated. Forty-five sows and 269 piglets from three different farms were enrolled in the study. Piglets were weighed at birth and 24 h later to calculate the CI. Serum was collected at 24 h after birth and analysed for STP, γ -globulins (electrophoresis), % Brix and IgG. In piglets, median (interquartile range, **IQR**) CI was 412 (196) g per piglet. Median (IQR) STP, γ -globulin and % Brix concentrations in piglet serum were 60 (11) g/L, 35 (10) g/L and 8 (2) %, respectively. Average (\pm SD) IgG concentration was 49 ± 23 g/L. Passing-Bablok regression revealed a strong concordance between % Brix and STP (Kendall's tau (**T**): 0.620, $P < 0.0001$, $n = 267$) and % Brix and γ -globulin concentration (Kendall's T: 0.575, $P < 0.0001$, $n = 267$). The agreement between the Brix refractometer and IgG concentration was poor (Kendall's T: 0.267, $P < 0.0001$, $n = 269$). Receiver operating characteristic curves were performed to evaluate test characteristics of Brix refractometry for three γ -globulin cut-off values, i.e. 10, 20 and 30 g/L. The % Brix cut-off values resulting in the optimal combination of sensitivity and specificity were 5.4 (100 and 98.5%), 7.0 (100 and 89.3%) and 7.9 (90.1 and 80.6%), respectively. In conclusion, digital Brix refractometry is a sufficiently fast and practical method to assess serum γ -globulin concentrations in neonatal piglets on-farm and to evaluate them by considering the thresholds found in this study. Further studies are needed to validate those thresholds regarding piglet's survival in the pre-weaning period.

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Implications

As neonatal piglets lack antibodies at birth, sufficient colostrum intake is essential for an appropriate acquirement of immunity shortly after birth. We found that digital Brix refractometry is a practical and fast method, which allows veterinarians to estimate antibody concentration in serum of piglets on-farm. Suggested Brix cut-off values could help the veterinarian to evaluate if there is a lack of antibodies in piglets immediately during a herd visit. Hence, it allows the farmer and the veterinarian to implement proper corrective measures to

improve colostrum intake, instantly limiting pre-weaning mortality and economic losses.

Introduction

Pre-weaning losses are important not only from an economic point of view but also for animal welfare reasons. Piglet mortality during the lactation period varies from 10 to 20% in most pig farms and depends on many factors. Sufficient colostrum intake (**CI**) is essential to support the pre-weaning survival of piglets (Le Dividich et al., 2005). Devillers et al. (2011) described that a minimum of 200 g CI within the first 24 h is necessary for pre-weaning survival. This has been confirmed by Decaluwé et al. (2014) showing that 160–250 g CI per kg birth weight is associated with a 3% mortality rate. However, Hasan et al. (2019)

* Corresponding author.

E-mail address: alexandra.schoos@ugent.be (A. Schoos).

showed that colostrum yield and CI per piglet diminished with increasing litter size, longer farrowing duration and lower birthweights of piglets.

Colostrum provides piglets with the energy essential for the metabolic response to the drastic temperature drop at the moment of birth (Le Dividich et al., 1997). Additionally, it is an important source of immunoglobulins. Because of the epitheliochorial placenta, piglets are deprived of immunoglobulins at birth. Only within the first 24 h after farrowing, immunoglobulins are actively transported from the sow blood to the colostrum (Klobasa et al., 1987). On the other hand, the piglets' gut barrier closes within 24 to 36 h after birth, after which no immunoglobulin absorption is guaranteed anymore (Porter, 1969; Klobasa et al., 1981). Therefore, it is imperative for piglets to get sufficient colostrum that is rich in immunoglobulins, within the first 24 h of life. A good acquisition of passive immunity is necessary to reduce the susceptibility to infections during the first weeks of life and even until after weaning (Varley et al., 1986; Drew and Owen, 1988; Declerck et al., 2016). In other animals, e.g. in calves and foals, a failure of passive transfer (FPT) at 24 h of age is determined by serum immunoglobulin G (IgG) concentrations <10 g/L (Weaver et al., 2000; Godden, 2008) and <4–8 g/L (Tyler-McGowan et al., 1997), respectively. In pigs so far, no such threshold value of FPT has been determined. Hendrix et al. (1978) reported that piglets surviving until 21 days showed a higher average serum γ -globulin concentration at 12 h after birth (40 g/L), determined by electrophoresis and radial immunodiffusion (RID), compared to their litter mates that did not survive the first 3 weeks of life (28 g/L). Devillers et al. (2011) investigated serum IgG concentration of piglets at 24 h of age and their survival rate. Results showed that piglets that died within the first three days had an IgG concentration of 17 ± 2 g/L (mean \pm SEM), whereas piglets dying later on had an IgG concentration of 21 ± 2 g/L. Piglets still alive at weaning had an average serum IgG level of 24 ± 1 g/L 24 h after birth. Cabrera et al. (2012) showed that piglets seemed to have 91% chance of survival until weaning (16–20 days), when their serum IgG concentration was between 23 and 25 g/L at 48–72 h of life. Thus, depending on when the blood sample is taken, different values need to be considered, but up-to-date, no clear threshold values have been documented for FPT in piglets.

Several methods can be used to measure immunoglobulins in serum of pigs, e.g. ELISA, RID, immunocrit and electrophoresis, but most of them are time consuming and expensive. Digital Brix refractometry is currently already used in other animal species (calves, foals) to estimate the serum immunoglobulin concentration on the farm (Deelen et al., 2014; Elsohaby et al., 2019). It measures in non-sucrose-liquids the total solid percentage in a solution (Quigley et al., 2013). Brix refractometry thus provides an approximation of the serum immunoglobulin concentration, as immunoglobulins represent the major fraction (>50%) of total protein in neonatal piglet serum after CI (Cabrera et al., 2012; Huang et al., 2012).

The present study investigated Brix refractometry for measuring immunoglobulin concentration in serum of neonatal piglets and compared the results with those obtained by a traditional optical refractometer (serum total protein, STP), electrophoresis (γ -globulins) and ELISA (IgG). In addition, we calculated thresholds for Brix refractometry based on three plausible γ -globulin cut-off concentrations for pre-weaning survival, which are based on literature (Hendrix et al., 1978; Devillers et al., 2011; Cabrera et al., 2012).

Material and methods

Study population

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC2018–44). The study was performed on three different commercial farrow-to-finish farms (A, B and C) in Belgium from December 2018 until February

2019. In each farm, 15 sows of different parities (4.6 ± 2.7 (\pm SD), range: 1–11) and breed (Danbred, TN70) were included ($n = 45$). In farm A, sows were injected with prostaglandin F₂ α on day 114 of gestation to induce parturition. In farms B and C, sows farrowed naturally. Sows were housed in conventional farrowing crates during the lactation period in accordance with the Council Directive 2001/88/EC laying down minimum standards for the protection of pigs. From each sow, six piglets were enrolled in the study. The time of birth for each piglet was recorded. Piglets were excluded from the study when the exact time point of birth was missed and when the piglet was already suckling colostrum. In that case, the next piglet was enrolled until six piglets in total per sow were included. As the parturitions were intensively surveyed, the six included piglets corresponded in most of the cases to the first six piglets born alive. Piglets were not allowed to be cross-fostered and had to remain with the sow during the first 24 h after birth.

Animal handling, sampling and calculations

Parturitions were supervised from 0600 am until midnight. Within 0–3 h after onset of parturition, 5 mL colostrum was collected in total, from different teats (pectoral, abdominal and inguinal) from each sow and stored at 2–8 °C until transport to the laboratory within 24 h after sampling. At the lab, the samples were aliquoted and stored at –20 °C until further analysis.

After birth, piglets were weighed (BW at birth, **BWB**) and received an individual eartag. Twenty-four hours (23–25 h) after birth, they were weighed again (BW at 24 h after birth, **BW24**) and 7 mL blood was taken from the *vena cava cranialis* on serum clot activator tubes. Blood samples were stored at the farm at 2–8 °C until transport to the laboratory, where they were centrifuged at 2600 \times g for 11 min. Serum was aliquoted into Eppendorf tubes and stored at –20 °C until further analyses.

Colostrum intake was estimated based on the mechanistic model as described by Theil et al. (2014), which includes BWB (kg), weight gain within the first 24 h after birth (**WG**; g) and duration of CI, e.g. 24 h (**D**; minutes). The equation is as follows: $CI = -106 + 2.26 * WG + 200 * BWB + 0.111 * D - 1.414 * WG/D + 0.0182 * WG/BWB$. In case negative values were obtained, CI was considered to be 0 (Devillers et al., 2007; Declerck et al., 2015).

Colostrum and serum analyses

Capillary electrophoresis

Serum proteins were characterised by capillary electrophoresis (MiniCap Flex Piercing, Sebia, Lisses, France), which allowed the separation of proteins into six fractions (albumin, α -globulins 1 & 2, β -globulins 1 & 2 and γ -globulins) (Animal Health Service Flanders (DGZ Vlaanderen), Torhout, Belgium; ISO/IEC 17025:2005). The proteins were separated in two fused-silica capillaries (effective length 15.5 cm, internal diameter 25 μ m, optical cell 100 μ m), applying 9000 V for 4 min at 35.10 °C (Peltier device) and proteins were directly detected by their absorbance at 200 nm (deuterium lamp). The software programme (Phoresis, Sebia) recorded in real time the variation of absorbance due to the flow of protein through the spectrophotometer and produced typical electrophoretic peaks. The thresholds among fractions were selected manually after visual inspection of the curves and identification of the inflection points, based on a reference electrophoretogram for pigs. To obtain protein fraction concentrations, the percentage of each fraction was multiplied by the STP concentration of the respective sample measured with the optical refractometer. Hence, those outcome values are not fully independent from each other. According to the manufacturer's manual, the mean variation coefficient is 2.1% on the percentage of each protein fraction. A human control serum (SEBIA, PN 4785) was used to perform a regular internal quality control.

Immunoglobulin G enzyme-linked immunosorbent analysis

For measurement of porcine IgG in serum and colostrum, a commercially available ELISA (Pig IgG ELISA Core Kit, Pink-ONE, Komabiotech, Seoul, Korea) was used according to manufacturer's instructions. Serum samples were diluted 1:100,000, 1:200,000 or 1:500,000 and colostrum samples 1:500,000. The intra-assay coefficient of variation was $2.3\% \pm 1.6$ and $3.6\% \pm 0.7$ (mean \pm SD) for serum and colostrum samples, respectively. Colostrum samples were analysed on two ELISA plates with an inter-assay variation of 1.8% (1.5–2.1%) based on two reference colostrum samples. In total, eight ELISA plates were used for serum samples, resulting in an inter-assay variation of 1.8% (0.5–2.9%) based on four reference serum samples. All samples were analysed in duplicates and calculation of the IgG value based on calibration curves from eight standard concentrations was performed using the DeltaSOFT program (BioMetallics Inc., Princeton, USA).

Traditional optical refractometer and digital Brix refractometry

In order to measure the STP, always the same operator added 0.5 mL serum on an optical refractometer (Bausch & Lomb Optical Co., Rochester, NY, USA) and read the STP value from the integrated graduated scale (grams total solid in 100 mL solution). Samples were measured in singlicates. The result of this measurement was also taken for calculation of the γ -globulin concentration. Another operator added 0.5 mL serum or colostrum on a digital Brix refractometer (Brix refractometer, Milwaukee Electronics kft. Szeged, Hungary) and read the total protein value from the display (% Brix). Samples were measured in duplicates (coefficient of variation within piglets: $3.7\% \pm 4.3$, mean \pm SD; median: 2.0%; Supplementary Fig. S1), and the average of both values was used for further analyses. In between each sample, the refractometers were cleaned with tap water.

Statistical analyses

Statistical analysis was mainly performed using IBM® SPSS® Statistics Version 24 (IBM, Chicago, IL, USA). The Deming regression (Supplementary Fig. S1) was performed in Excel using XLSTAT (Addinsoft 2020, NY, USA). The multilevel analyses (Supplementary Tables S1–S4) were performed using the lmer function in the lmerTest package (Kuznetsova et al., 2017), and the Bland Altman analysis was performed using the MethComp package (v1.30.0) in R, using R Studio (R-Core-Team, 2019). $P \leq 0.05$ was considered as statistically significant. Normality and homogeneity of variance of continuous variables (live born piglets, parity of sows, % Brix and IgG in colostrum, BWB, BW24 and CI of piglets, STP, % Brix and γ -globulins and IgG in serum) were analysed graphically via histograms and Q-Q plots and were further tested using the Kolmogorov–Smirnov and Shapiro–Wilk test. Equality of variances was analysed using the Levene's test.

Normally distributed values (live born piglets, parity of sows, % Brix and IgG in colostrum, BWB and BW24 of piglets and IgG in serum) were reported as mean \pm SD, and differences between farms were analyzed using a one-way ANOVA followed by a post-hoc Tukey test. The non-normally distributed data (CI of piglets, STP, % Brix and γ -globulins in serum) were represented as median and interquartile range (IQR), and potential differences between farms were analysed with a non-parametric Kruskal–Wallis test followed by a Mann–Whitney–U test. Farm was considered as independent variable in both models.

For agreement analyses, data were pooled, and farm effect was not taken into account. Agreements between Brix values in serum of neonatal piglets and values of STP, γ -globulin concentration and IgG concentrations were analysed by Passing–Bablok regression (Passing and Bablok, 1983). The estimates of the Passing–Bablok regression were compared to those obtained with the Bland Altman analysis for non-constant bias, which regresses the differences between the methods on the averages as proposed by Carstensen (2010). Linearity was verified by a cumulative sum test. The strength of the agreement between

methods (degree of concordance) was evaluated using the non-parametric Kendall's tau (T) correlation coefficient.

Test characteristics (sensitivity and specificity) and receiver operating characteristic (ROC) curves were calculated in Excel (Microsoft Office Professional Plus 2016). As reference standard, electrophoresis (γ -globulin concentration) was used. Sensitivity was defined as the probability of a test result suggestive for FPT for a sample with γ -globulin concentration ≤ 10 g/L, ≤ 20 g/L and ≤ 30 g/L. Specificity was defined as the probability of a test result suggestive for adequate passive transfer for a sample with γ -globulin concentration > 10 g/L, > 20 g/L and > 30 g/L. Three ROC curves were generated to plot the true positive rate against the false-positive rate at 0.1% – unit Brix intervals from 3.5 to 12.2% Brix.

Results

Descriptive results

Sow and litter parameters

The average parity of the sows in all three herds ($n = 45$; 15 sows per farm) was 4.6 with a range from first (gilts) to 11th parity (Table 1). The number of live born piglets per litter on farms A, B and C was on average (\pm SD) 15.7 (± 2.9), 14.6 (± 2.2) and 15.0 (± 1.9), respectively. Results in colostrum of the Brix refractometry yielded $24.7 \pm 1.8\%$ Brix, $26.5 \pm 3.9\%$ Brix and $25.4 \pm 3.5\%$ Brix in farms A, B and C, respectively. Colostrum concentrations of IgG averaged 71 ± 15 g/L, 70 ± 19 g/L and 77 ± 28 g/L in farms A, B and C, respectively. The overall average percentage Brix and IgG concentration in colostrum was $25 \pm 3\%$ and 73 ± 22 g/L, respectively. There was no conclusive difference between the farms. The variation of Brix values and IgG concentration in colostrum between sows is represented in the Supplementary Fig. S2.

Piglet weights and colostrum intake

From each sow, six piglets were enrolled in the study, except for one sow of farm A of which only five piglets were enrolled ($n = 269$). Bodyweight of piglets averaged (\pm SD) 1.26 ± 0.28 kg at birth and 1.34 ± 0.32 kg 24 h after birth. The difference between the three farms was inconclusive (Table 2).

Colostrum intake differed significantly in farm A compared to farms B and C. Piglets on farm A had a CI of 447 (196) g (median (IQR)) compared to piglets of farms B and C, where they had a CI of 406 (203) g and 379 (212) g, respectively. Overall, the CI was 412 (196) g and ranged from 0 to 1103 g. Twenty-three piglets had a CI ≤ 200 g (8.6%), whereof eight piglets even had a CI ≤ 100 g (3.0%). Most piglets had a CI between 400 and 500 g (74 piglets, 27.5%), followed by piglets with a CI between 300 and 400 g (56 piglets, 20.8%), between 500 and 600 g (45 piglets, 16.7%) and between 200 and 300 g (43 piglets, 16.0%). The remaining 28 piglets (10.4%) had a CI between 600 and 1200 g with 23 piglets (8.6%) having a CI between 600 and 700 g. Four piglets (1.5%) had negative CI values. Colostrum intake for these piglets was considered as being equal to 0.

Total protein, γ -globulin, percentage Brix and immunoglobulin G in serum of neonatal piglets

Serum total protein, γ -globulin, % Brix and IgG concentrations were measured in serum of 24 h old piglets. There was insufficient serum left in two piglets of farm A to measure STP by optical refractometer and therefore γ -globulin could not be calculated (Table 3).

Serum total protein differed significantly between the three farms with an overall range from 21 to 80 g/L. Farms A, B and C had a STP of 62 (9) g/L (median (IQR)), 57 (11) g/L and 61 (12) g/L, respectively. Farms A and C showed a significantly higher γ -globulin concentration compared to farm B. The overall median was 35 (10) g/L with a range from 0 to 54 g/L. Percentage Brix values were significantly different

Table 1

Average parity and live born piglets of sows per farm, and % Brix and immunoglobulin G (IgG) concentration in colostrum within 0 to 3 h after birth of first piglet. There is no conclusive difference among the farms ($P = 0.336$ and 0.592 for % Brix and IgG, respectively).

Farm	n	Parity	Live born piglets	% Brix		IgG (g/L)			
		(range)	Mean \pm SD	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
A	15	4 (1–8)	15.7 \pm 2.9	24.7 \pm 1.8	21.2	27.6	71 \pm 15	46	96
B	15	5 (1–11)	14.6 \pm 2.2	26.5 \pm 3.9	19.6	37.4	70 \pm 19	35	110
C	15	4 (1–8)	15.0 \pm 1.9	25.4 \pm 3.5	19.3	31.3	77 \pm 28	26	154
A + B + C	45	5 (1–11)	15.1 \pm 2.4	25.4 \pm 3.3	19.3	37.4	73 \pm 22	26	154

Abbreviations: Min = minimum; Max = maximum.

Table 2

Bodyweight at birth (BWB), bodyweight at 24 h of life (BW24) and colostrum intake (CI) for 269 piglets from three different farms. Negative CI was assumed to be 0 (Devillers et al., 2007; Declerck et al., 2015).

Farm	n	BWB (kg)			BW24 (kg)			CI (g)		
		Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Median (IQR)	Min	Max
A	89	1.28 ^a \pm 0.32	0.45	1.92	1.38 ^a \pm 0.36	0.56	2.14	447 ^a (196)	0	759
B	90	1.26 ^a \pm 0.25	0.67	1.91	1.33 ^a \pm 0.30	0.66	2.06	406 ^b (203)	75	694
C	90	1.24 ^a \pm 0.26	0.67	1.85	1.31 ^a \pm 0.29	0.73	2.03	379 ^b (212)	0	1103
A + B + C	269	1.26 \pm 0.28	0.45	1.92	1.34 \pm 0.32	0.56	2.14	412 (196)	0	1103

Abbreviations: IQR = interquartile range; Min = minimum; Max = maximum.

^{a,b} Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Table 3

Serum total protein (STP) and γ -globulin concentrations in serum of 267 neonatal piglets and of percentage Brix (% Brix) and immunoglobulin G (IgG) concentrations in serum of 269 neonatal piglets (24 h old).

Farm	STP ¹ (g/L)			γ -globulin ¹ (g/L)			% Brix ²			IgG ² (g/L)		
	Median (IQR)	Min	Max	Median (IQR)	Min	Max	Median (IQR)	Min	Max	Mean \pm SD	Min	Max
A	62 ^a (9)	43	77	38 ^a (8)	19	50	8.6 ^a (1.6)	4.1	12.1	63 ^a \pm 19	23	115
B	57 ^b (11)	21	78	32 ^b (11)	0	50	8.2 ^b (1.6)	3.5	11.1	35 ^b \pm 20	0	77
C	61 ^c (12)	38	80	36 ^a (11)	8	54	8.4 ^{ab} (1.8)	5.1	11.3	50 ^c \pm 20	11	98
A + B + C	60 (11)	21	80	35 (10)	0	54	8.4 (1.7)	3.5	12.1	49 \pm 23	0	115

Abbreviations: IQR = interquartile range; Min = minimum; Max = maximum.

^{a,c} Values within a column with different superscripts differ significantly at $P \leq 0.05$.

¹ Farm A: n = 87.

² Farm A: n = 89.

between farms A and B, but not between farms A and C, and farms B and C. Brix values ranged from 3.5 to 12.2 with a median of 8.4 (1.7) % Brix. Immunoglobulin G concentration varied significantly between all three farms with 63 \pm 19 g/L (mean \pm SD), 35 \pm 20 g/L and 50 \pm 23 g/L in farms A, B and C, respectively. The overall IgG concentration averaged 49 \pm 23 g/L with a range from 0 to 115 g/L. The variation of Brix values and IgG concentration in serum between piglets of the same litter, and in relation to the Brix values and IgG concentration in colostrum of their corresponding mother, is represented in the Supplementary Fig. S2.

The serum IgG concentrations showed a normal distribution, whereas distributions of STP, γ -globulin concentrations and % Brix in serum were skewed to the left (Fig. 1). The result of nine samples was ≤ 10 g/L γ -globulins (3.4%), 24 samples ≤ 20 g/L γ -globulins (9.0%) and 66 samples ≤ 30 g/L γ -globulins (24.7%). In 201 samples (75.3%), γ -globulin concentration was > 30 g/L.

Validation of the Brix refractometer in serum of piglets and its test characteristics

Initial regression of the differences on the averages showed that the slopes of each of the comparisons: Brix vs. STP, Brix vs. γ -globulin and Brix vs. IgG were significantly different from 1 ($P < 0.001$), indicating a non-constant agreement between the methods. Subsequent regression of the residuals on the averages indicated that the assumption of

constant variation was met for Brix vs. STP ($P = 0.911$) and for Brix vs. γ -globulin ($P = 0.828$), but not for Brix vs. IgG ($P = 0.001$) (Supplementary Table S5). Because this assumption was not met for all comparisons, we used the Passing–Bablok regression method, which uses a non-parametric method to estimate agreement. The results of the Passing–Bablok regression are shown in Fig. 2. The comparison of Passing–Bablok regression estimates with the Bland Altman analysis for non-constant bias showed no large differences between both estimates. The results of Brix refractometry were significantly correlated to all three different methods, namely the optical refractometer (STP), the electrophoresis (γ -globulins) and ELISA (IgG) ($P < 0.0001$). The strongest concordance was with STP (optical refractometer, Kendall's T = 0.620), followed by γ -globulins (electrophoresis, Kendall's T = 0.575) and IgG concentrations (ELISA, Kendall's T = 0.267).

Test characteristics of sensitivity and specificity for % Brix were assessed. Therefore, three different γ -globulin cut-off concentrations (10, 20 and 30 g/L γ -globulin) were chosen in this study, based mainly on two studies (Devillers et al., 2011; Cabrera et al., 2012), and ROC curves were created (Fig. 3). For a cut-off of 10 g/L γ -globulin, the best combination of sensitivity (100%) and specificity (98.5%) was at 5.4% Brix. The best combination of sensitivity (100%) and specificity (89.3%) for a cut-off of 20 g/L γ -globulin was at 7.0% Brix and for 30 g/L γ -globulin at 7.9% Brix with a sensitivity and specificity of 90.1% and 80.6%.

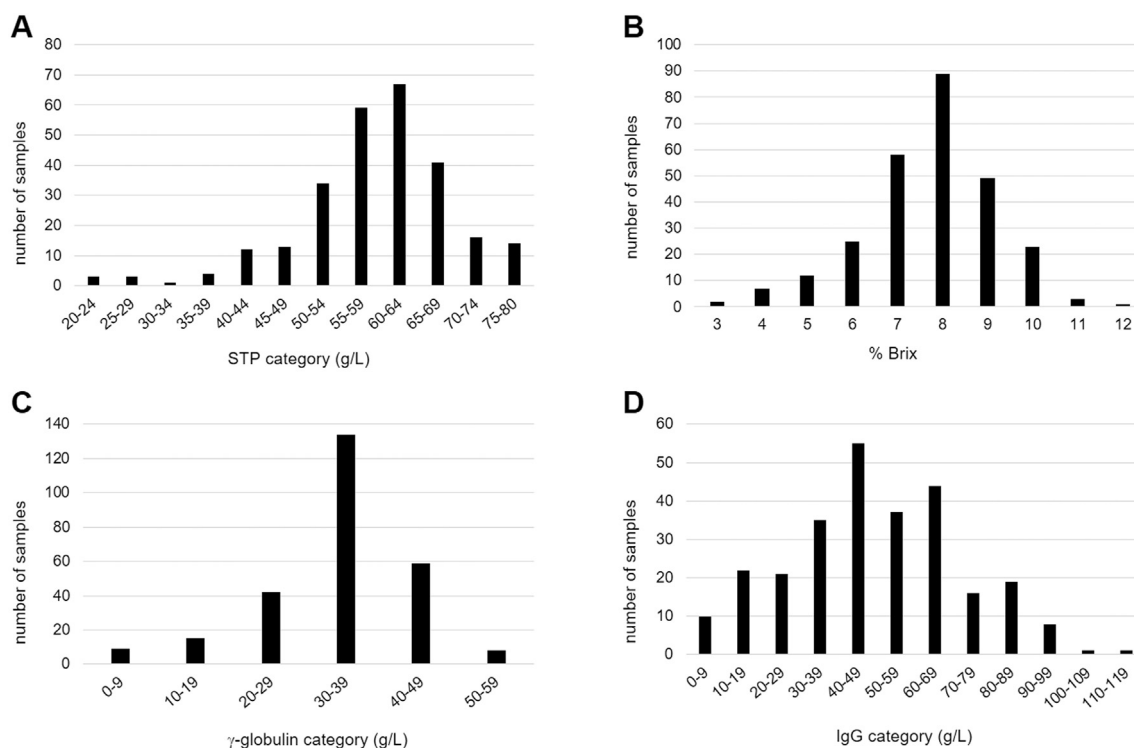


Fig. 1. Frequency distributions of A) serum total protein (STP), B) Brix (% Brix), C) serum γ -globulin, and D) serum immunoglobulin G (IgG) concentration for 269 (B + D) or 267 (A + C) neonatal piglets.

Discussion

The study evaluated Brix refractometry for measurement of immunoglobulins in serum of neonatal piglets (24 h old). Brix refractometry results showed a significant agreement to the optical refractometer (STP) and the electrophoresis (γ -globulin). Threshold values for % Brix with a high sensitivity and specificity could be determined, allowing Brix refractometry to be used as an appropriate diagnostic tool for measuring immunoglobulin concentrations in serum of neonatal piglets. However, is it until now not possible to evaluate if those threshold values are accurate regarding FPT or piglet survival as the clinical outcome of piglets was not investigated in this study.

As colostrum IgG concentration can account for 6% variation in serum IgG levels in piglets (Cabrera et al., 2012), it was important for us to know, where to situate the selected farms in terms of colostrum quality (IgG). In this study, average colostrum quality was good in each farm, and no conclusive difference was found between the three farms. The average colostrum IgG concentrations (73 ± 22 g/L) and colostrum Brix values (25% Brix) are comparable with those of previous studies, namely IgG: 92 ± 73 g/L ($n = 37$, mean \pm SD) (Decaluwé et al., 2014), 62 ± 169 g/L ($n = 72$) (Quesnel, 2011), 54 g/L ($n = 62$) (Kielland et al., 2015) and 52 ± 380 g/L (Hasan et al., 2016) and Brix: 25.0% (Hasan et al., 2016). In our study, the variation (SD) is lower. As described in other publications, colostrum quality can vary significantly between sows and is influenced by many factors (Farmer and Quesnel, 2009; Declerck et al., 2015); therefore, a variation between different studies is not surprising. Moreover, time point of colostrum collection needs to be kept in mind, as well as colostrum IgG decrease is time-dependent.

In order to compare estimated CI between studies, it is important to verify that same equations have been used for the calculation. In this study, we used the equation based on the mechanistic model as described by Theil et al. (2014), who used the same number of piglets per litter and approximately the same number of litters ($n = 40$). In

their study, an estimated average (\pm SD) CI of 443 g (± 151) per piglet was calculated compared to the median (IQR) CI of 412 (196) g per piglet in this study. These CIs are slightly higher than the ones found by Declerck et al. (2016) (367 ± 148 g; mean \pm SD, $n = 1374$) and Kielland et al. (2015) (353 g; mean, $n = 876$). As farrowing was constantly monitored in the present study, the six enrolled piglets per sow corresponded to piglets within the first half of the litter. According to Cabrera et al. (2012), birth order can account for 4% of variation in serum IgG in piglets. It is possible that underachievers or hypoxic piglets were less included in the study. This may also explain why there were only a few piglets with low CI in this study. Nevertheless, in all these studies, the average CI was above the recommended CI by Devillers et al. (2011) and Decaluwé et al. (2014) of approximately 200 g per piglet.

Studies about STP, γ -globulin and IgG concentrations in serum of neonatal piglets are scarce. Also, studies have often used different time points of serum collection, which makes it difficult to compare results. The 24-h time point was chosen in this study, as at that time, the gut closure of piglets is almost complete (Le Dividich et al., 2005). In several studies, it has been shown that serum immunoglobulin concentration reaches a peak at 12 to 16 h after first suckling of colostrum, after which it gradually and slowly decreases (Klobasa et al., 1981; Bland et al., 2003; Klobasa et al., 2004). In those studies, IgG concentrations were approximately 36 g/L 24 h after first suckling. Those values correspond to the γ -globulin levels (35 g/L) rather than to the IgG concentration (49 g/L) in the present study. This may be due to different ELISA or other methods used to measure IgG, or to different colostrum quality and CI. The STP values in the present study (60 g/L) are in the range of total protein values (49–67 g/L) described for growing pigs (50–60 kg) (Klem et al., 2010).

Percentage Brix corresponds to the total solids in non-sucrose liquids, which thus provides an approximation of the STP and, in such young piglets, to γ -globulin levels in serum. In a study in neonatal calves (Deelen et al., 2014), correlation coefficients between % Brix and STP

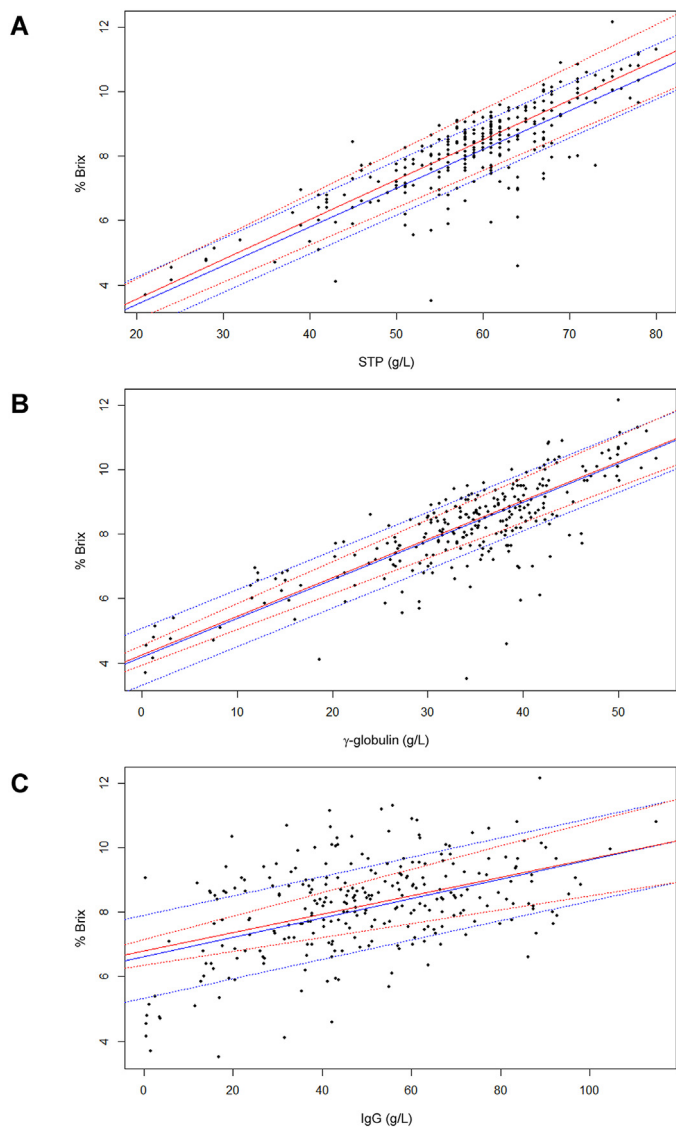


Fig. 2. Passing-Bablok estimates (red) and the Bland Altman analysis for non-constant difference (blue), which regresses the differences between the methods with the averages. The upper and lower 95% limits of agreement are indicated in blue and red dotted lines for each method. A) agreement between Brix (% Brix) and serum total protein (STP) (n = 267 piglets), B) Brix and serum γ -globulin concentration (n = 267 piglets), C) Brix and serum immunoglobulin G (IgG) (n = 269 piglets).

was $r = 1$, and $r = 0.930$ between % Brix and IgG, which is much higher than in this study. Beside a different species and different methods for IgG measurement (ELISA vs. RID), it seems to be noteworthy that also different regression analyses and statistical tests were used in that study. The relevance of the correlation coefficient from the above mentioned study might be questioned, as 1) the use of correlation coefficients is a poor measurement of agreement between two methods (Altman and Bland, 1983; Bland and Altman, 1986; Carstensen, 2011) and 2) the same refractometer was used to measure % Brix and STP and therefore independence of samples, outcome and reproducibility are questionable.

The authors chose three cut-off values for FPT in piglets based on results describing survival rate of neonatal piglets and their serum immunoglobulin concentration (Hendrix et al., 1978; Devillers et al., 2011; Cabrera et al., 2012). Brix refractometry showed a high sensitivity for all three thresholds; however, specificity lowered fast with increasing γ -globulin cut-off value. A percentage Brix of 5.4 yielded the best values

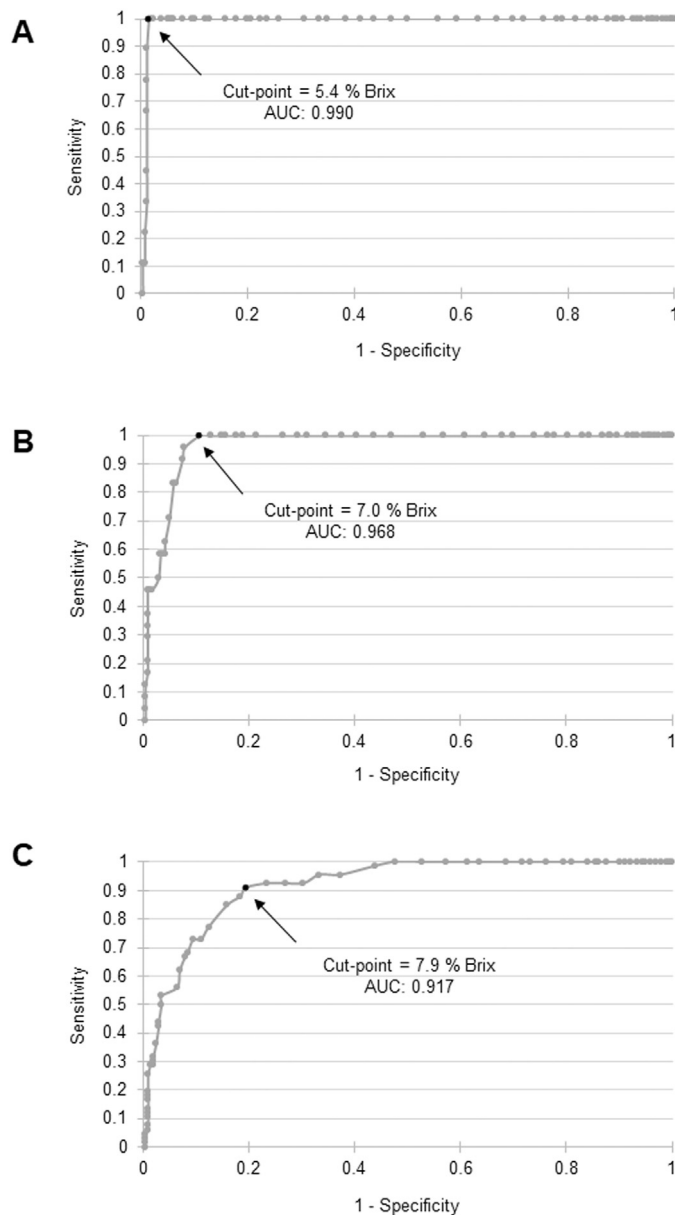


Fig. 3. Receiver operating characteristic curves of the true positive rate (Sensitivity) against the false positive rate (1 - Specificity) for the different % Brix values of the γ -globulin values (percentage of γ -globulin fraction determined by electrophoresis was multiplied by the serum total protein concentration of the respective sample measured with the optical refractometer). Cut-points and the area under the curve (AUC) are indicated in the graphs. A) cut-off of 10 g/L γ -globulin, B) cut-off of 20 g/L γ -globulin, C) cut-off of 30 g/L γ -globulin. (n = 267 piglets).

for sensitivity and specificity indicating an average γ -globulin concentration ≤ 10 g/L. Serum γ -globulin concentration ≤ 10 g/L in neonatal piglets represents already a very low concentration and goes along with only 67% chance of survival according to Cabrera et al. (2012), when measured in 2 to 3 days old piglets. As mentioned earlier, in other animals, i.e. calves and foals, IgG concentrations ≤ 10 g/L at 24 h of age indicate a FPT. In piglets, values of ≥ 20 g/L, preferably ≥ 30 g/L, serum γ -globulin concentration should thus be reached for an adequate transfer of immunity in 24 h old piglets, i.e. 7.0 and 7.9% Brix, respectively. Thus, different % Brix cut-off values need to be considered for evaluation of adequate serum immunoglobulin concentration, depending on the age of the piglet when the blood sample is taken on the farm. However, no further investigations were performed in this study about the clinical outcome of the piglets. Therefore, it is important for the practitioner to

investigate each case individually, as those cut-off values might depend on several parameters, e.g. health status, farm management, colostrum quality. Further research needs to investigate the evaluation and validation of these cut-off values based on clinical outcome of the piglets, i.e. survival, health and performance.

The limitation of this study is the low sample size in the lower range values, i.e. γ -globulin concentrations ≤ 10 g/L. Therefore, the ROC of that threshold (Fig. 3A) needs to be interpreted with caution, and further studies are needed with special focus on piglets with poor STP concentrations to verify and validate the results of this study. Based on the Deming regression (Supplementary Fig. S1), reproducibility and influencing factors of Brix measurements of porcine neonatal serum have to be further investigated in future studies. Moreover, the multi-level analysis reveals that the correlation of piglets between sows within a farm for γ -globulin concentration measurement is good (45.8%, Supplementary Table S2), however, rather poor for Brix refractometry (28.2%, Supplementary Table S3). This might be explainable, as the Brix refractometry is only an indirect measurement of the immunoglobulin determination and thus more imprecise than direct measurements (electrophoresis or ELISA). Almost no correlation exists between farms for γ -globulin concentration nor Brix refractometry measurements (3.6 and 2.4%, respectively). Additional influencing effects of γ -globulin concentration measurement and Brix refractometry are the CI of the piglet, the quality of the colostrum, the breed and the interactions between those parameters. However, the results of the multilevel analysis need to be interpreted carefully, as the number of samples is limited and large-scale testing is necessary to give reliable and solid hierarchical predictions. Further studies would thus need to include more farms, a higher sow sample size and less piglets per sow to verify and validate the results found in this study.

To conclude, this study suggests that Brix refractometry is an appropriate, practical and fast diagnostic tool to provide veterinarians in the field with an approximation of serum immunoglobulin concentration in neonatal piglets. However, further studies are inevitable to evaluate reproducibility, reliability and validation of the cut-off values and their respective sensitivity and specificity. By measuring serum immunoglobulins, it is possible to have a crude estimation of an adequate CI and absorption in terms of immunoglobulins. However, no statement can be done about an adequate intake of other nutrients in colostrum, essential for piglet survival during the lactation period. Therefore, practitioners need to evaluate the whole on-farm situation (colostrum management, farrowing management, health status of the farm, handling of piglets) and should consider results found by Brix refractometry as an additional support in order to implement corrective measures around farrowing after discussion and agreement with the farmer.

Supplementary materials

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2020.100041>

Ethics approval

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC2018–44).

Data and model availability statement

None of the data were deposited in an official repository.

Author ORCIDs

Alexandra Schoos: 0000-0001-7320-9233
Ward De Spiegelaere: 0000-0003-2097-8439
Bart Pardon: 0000-0003-1026-8433
Elise Bernaerd: 0000-0002-4919-7663

Geert Janssens: 0000-0002-5191-3657
Dominiek Maes: 0000-0003-1149-9348

Author contributions

AS contributed to the sample collection, sample analysis, statistical analysis and wrote the manuscript. WS and AC contributed to statistical analysis and edited the manuscript. EA and EB contributed to sample collection and sample analysis. BP, GJ and DM contributed to study design, data analysis and manuscript editing. All authors contributed to manuscript revision, read and approved the submitted version.

Declaration of interest

None of the authors has any conflict of interest to declare.

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