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Evaluation of colostrum quality using digital brix refractometry and correlation with passive transfer of immunity in neonatal calves

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Abstract

Colostrum quality directly determines whether a newborn calf acquires adequate passive immunity, yet on-farm assessment methods remain inconsistent and often subjective. Digital Brix refractometry has been proposed as a rapid, inexpensive proxy for colostrum immunoglobulin G concentration, but the strength of its correlation with actual calf serum IgG-the definitive measure of successful passive transfer-has not been evaluated in a large Dutch dairy herd context. This research assessed the relationship between first-milking colostrum Brix readings and calf serum IgG concentrations at 24-48 hours of life in 157 Holstein-Friesian cow-calf pairs on four commercial dairy farms in the central Netherlands between September 2023 and August 2024. Colostrum was tested within one hour of collection using a digital Brix refractometer (MISCO PA201), and a sample was concurrently submitted for radial immunodiffusion IgG quantification. Calf serum IgG was measured by the same method at 24-48 hours post-ingestion. Mean colostrum Brix reading was 24.1 ± 4.8 %, corresponding to a mean IgG concentration of 68.4 ± 28.7 g L⁻¹. The Pearson correlation between Brix reading and colostrum IgG was 0.84 ($p < 0.001$). When a Brix threshold of ≥ 22 % was applied to classify colostrum as adequate, sensitivity for predicting successful passive transfer (calf serum IgG ≥ 10 g L⁻¹ at 24-48 h) was 89.3 % and specificity was 78.6 %. Multiparous cows produced higher-Brix colostrum than primiparous cows (25.7 vs. 21.3 %; $p < 0.001$), while first-milking volume was negatively correlated with Brix reading ($r = -0.52$; $p < 0.001$). These results confirm that digital Brix refractometry is a reliable on-farm tool for colostrum quality screening in Dutch dairy herds and that the ≥ 22 % threshold provides clinically useful predictive accuracy for passive transfer outcomes.

Keywords: Colostrum, Brix refractometry, passive transfer, immunoglobulin G, dairy calves, Holstein-Friesian, radial immunodiffusion, neonatal immunity, colostrum quality, parity

Introduction

In contrast to many mammalian neonates that receive maternal antibodies across the placenta during gestation, the bovine calf is born essentially agammaglobulinaemic and depends entirely on colostral immunoglobulin absorption during the first 24 hours of life ^[1]. This peculiarity of ruminant placentation-the syndesmochorial placenta permits no transplacental IgG transfer-makes the quality and timely delivery of first-milking colostrum arguably the single most consequential management event in a calf's life ^[2]. Failure of passive transfer (FPT), defined as calf serum IgG below 10 g L⁻¹ at 24-48 hours, has been associated with a two- to fivefold increase in pre-weaning morbidity and mortality across multiple epidemiological studies ^[3].

Despite this well-established importance, on-farm colostrum quality assessment remains haphazard on many dairy operations. A colostrometer-a weighted hydrometer-was the traditional tool, but it is temperature-sensitive, fragile, and imprecise at the extremes of IgG concentration ^[4]. Digital Brix refractometers measure the refractive index of a liquid, which in colostrum correlates with total solids including immunoglobulins, and have gained popularity because they are fast (< 30 seconds), affordable, temperature-compensated, and require only a single drop of sample ^[5]. Several North American studies have validated Brix

refractometry against radial immunodiffusion (RID) and reported correlation coefficients of 0.71-0.93, but data from European herds-particularly Dutch herds with their distinctive Holstein-Friesian genetics and management systems-are limited [6].

Equally important is the downstream question: does a high Brix reading actually predict successful passive transfer in the calf? A colostrum may test as high quality, but if it is fed too late, in insufficient volume, or to a calf with compromised intestinal absorptive capacity, serum IgG may still fall below the FPT threshold [7]. Linking colostrum Brix readings directly to calf serum IgG concentrations-rather than just to colostrum IgG-provides a more clinically meaningful validation.

This research pursued two objectives: first, to quantify the correlation between digital Brix refractometry readings and RID-measured IgG concentrations in first-milking colostrum from Dutch dairy cows; and second, to evaluate the sensitivity and specificity of a ≥ 22 % Brix threshold for predicting successful passive transfer in the corresponding calves. Additional analyses explored the effects of parity and first-milking volume on colostrum quality.

Material and Methods

Material

One hundred and fifty-seven Holstein-Friesian cow-calf pairs were enrolled from four commercial dairy farms in the provinces of Gelderland and Utrecht, central Netherlands (52°00'N, 5°30'E; altitude 2-8 m; maritime temperate climate, Cfb, Köppen), between September 2023 and August 2024. Farms ranged from 120 to 340 milking cows and operated year-round calving systems. Inclusion required a live singleton calf born without dystocia assistance beyond mild traction, and collection of first-milking colostrum within six hours of calving. Cows with clinical mastitis at calving, twins, and stillborn calves were excluded.

Results

Table 1: Diagnostic performance of the ≥ 22 % Brix threshold for predicting successful passive transfer (calf serum IgG ≥ 10 g/L at 24-48 h)

		Calf IgG ≥ 10 g/L	Calf IgG < 10 g/L
Brix $\geq 22\%$	n	109 (TP)	8 (FP)
Brix $< 22\%$	n	13 (FN)	27 (TN)
Sensitivity	89.3%	Specificity	77.1%
PPV	93.2%	NPV	67.5%

TP = true positive; FP = false positive; FN = false negative; TN = true negative; PPV = positive predictive value; NPV = negative predictive value

Table 2: Colostrum quality and calf serum IgG by dam parity category

Parity	n	Brix (%)	Colostrum IgG (g/L)	Calf serum IgG (g/L)
Primiparous	41	21.3 $\pm$ 3.9 ^a	52.1 $\pm$ 22.4 ^a	13.8 $\pm$ 6.2 ^a
2nd-4th parity	78	25.1 $\pm$ 4.2 ^b	74.3 $\pm$ 26.8 ^b	18.7 $\pm$ 7.4 ^b
5th+ parity	38	26.4 $\pm$ 5.1 ^b	78.9 $\pm$ 31.2 ^b	19.4 $\pm$ 8.1 ^b

Values are mean $\pm$ SD. Different superscript letters indicate significant differences between parity groups ($p < 0.05$, Tukey HSD)

Overall, 122 of 157 calves (77.7 %) achieved successful passive transfer. The Pearson correlation between colostrum Brix reading and colostrum IgG measured by RID was 0.84 ($p < 0.001$). The correlation between colostrum Brix and calf serum IgG at 24-48 hours was 0.78 ($p < 0.001$). First-milking colostrum volume ranged from 1.8 to 11.4 L (mean 5.3 ± 2.4 L) and was negatively correlated with Brix reading ($r = -0.52$; $p < 0.001$), confirming the dilution effect observed in high-

Methods

First-milking colostrum was collected by machine or hand-milking and its total volume recorded. A 50 mL aliquot was immediately tested with a digital Brix refractometer (MISCO PA201, temperature-compensated) by placing two drops on the prism and recording the digital readout to 0.1 % resolution. A second 10 mL aliquot was frozen at -20 °C and later submitted to the Wageningen University Veterinary Diagnostic Laboratory for IgG quantification by single radial immunodiffusion (SRID; Triple J Farms, Bellingham, WA, USA) [8]. Calves were fed 3 L of their dam's colostrum within two hours of birth by oesophageal tube or bottle, followed by a second 2 L feeding at 6-8 hours.

Jugular blood samples (5 mL, serum separator tubes) were collected from calves at 24-48 hours of age, centrifuged, and submitted for serum IgG by SRID. Successful passive transfer was defined as serum IgG ≥ 10 g L⁻¹ [3]. Data were analysed using Pearson correlation, receiver operating characteristic (ROC) curves, and multivariable linear regression with colostrum IgG or calf serum IgG as dependent variables and Brix reading, parity (primiparous vs. multiparous), first-milking volume, and season as independent variables. Sensitivity and specificity of the ≥ 22 % Brix threshold were calculated from a 2×2 contingency table. All analyses used R v4.3.2; significance was set at $p < 0.05$.

Ethical considerations

The research was conducted under the authority of the Dutch Experiments on Animals Act (Wet op de dierproeven) and was reviewed by the Wageningen University Animal Welfare Body (Protocol No. WUR-AWB-2023-0047). Blood sampling was performed as part of routine neonatal health monitoring and required no additional ethical approval for experimental procedures. All farmers provided written informed consent.

yielding periparturient cows. Primiparous cows produced significantly lower-Brix colostrum than multiparous cows (21.3 vs. 25.7 %; $p < 0.001$) and their calves had correspondingly lower serum IgG concentrations.

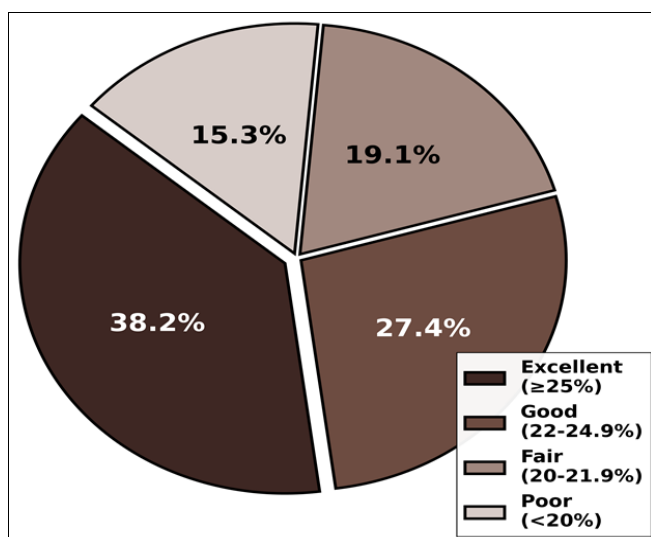


Fig 1: Distribution of colostrum quality categories based on digital Brix refractometer readings across 157 first-milking samples

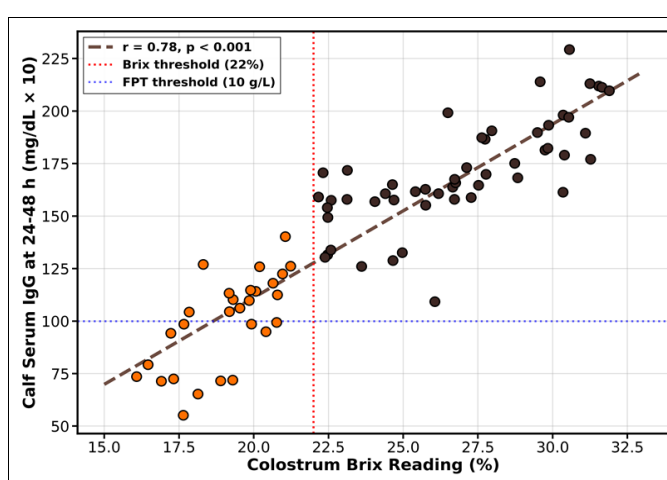


Fig 2: Colostrum Brix reading versus calf serum IgG concentration at 24-48 hours. Dark points indicate colostrum above the 22 % threshold; orange points indicate below threshold. Dashed lines mark the Brix (22 %) and FPT (10 g/L) thresholds

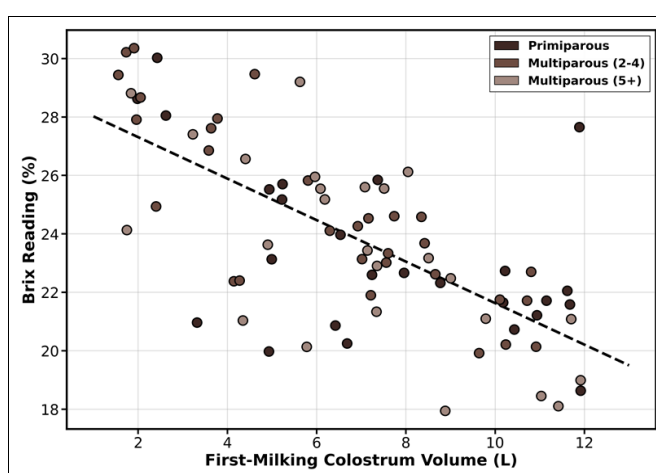


Fig 3: Relationship between first-milking colostrum volume and Brix reading, stratified by dam parity. The negative correlation reflects the dilution of immunoglobulins in higher-volume colostrum

Comprehensive Interpretation

Taken together, the data confirm that digital Brix refractometry reliably identifies high-quality colostrum in Dutch dairy herds, with a correlation of 0.84 against the gold-

standard RID method. The $\geq 22\%$ threshold correctly predicted adequate passive transfer in nearly nine out of ten calves, though the 77 % specificity indicates that some calves with sub-threshold Brix colostrum still achieved adequate IgG levels-likely through compensatory absorptive efficiency or larger-than-average colostrum volumes. The parity and volume effects underscore the importance of managing colostrum from primiparous cows and high-yield calvings with particular care.

Discussion

The correlation of 0.84 between Brix reading and colostrum IgG is at the upper end of values reported internationally, exceeding the 0.71 found by Biemann *et al.* [5] in Canadian herds and matching the 0.85 reported by Quigley *et al.* [9] in a large US dataset. The consistency of this relationship across North American and now European herds suggests that Brix refractometry is robust to breed and management variation within Holstein populations.

The more clinically relevant correlation-between colostrum Brix and calf serum IgG ($r = 0.78$)-was necessarily lower, because calf-level factors such as absorptive window, feeding volume and timing, and individual intestinal permeability introduce additional variance. Still, a correlation of 0.78 is strong enough to justify using the Brix reading as a field-level screening decision: colostrum reading below 22 % should trigger either discarding the sample or supplementing with a stored high-quality alternative [10].

The parity effect-primiparous cows producing colostrum roughly 4 Brix percentage points lower than multiparous cows-is well documented and biologically expected. First-calf heifers have had fewer antigenic exposures and a shorter period of mammary IgG transport during the final weeks of gestation [11]. From a management perspective, this means that colostrum from heifers should be tested rather than assumed adequate, and many farms would benefit from maintaining a frozen colostrum bank sourced from older cows as a backup [12].

The negative correlation between milking volume and Brix reading deserves practical attention. Cows producing more than 8 L at first milking frequently yielded colostrum below 22 % Brix, likely because the immunoglobulin mass was diluted across a larger fluid volume. Farmers should not interpret high colostrum yield as a sign of quality; indeed, the opposite is more often true [4].

Conclusion

This research set out to validate digital Brix refractometry as an on-farm colostrum quality tool in Dutch dairy herds and to link Brix readings to calf passive transfer outcomes. The principal finding is that the $\geq 22\%$ Brix threshold provides a sensitivity of 89 % and specificity of 77 % for predicting successful passive transfer, confirming its clinical utility for rapid decision-making at the point of collection.

Two secondary observations add practical depth. First, primiparous cows consistently produced lower-quality colostrum, reinforcing the need for universal Brix testing rather than selective testing based on perceived cow health. Second, first-milking volume was inversely related to quality, cautioning against equating abundant colostrum with adequate immunoglobulin content.

Future investigations should explore whether combining Brix readings with other rapid tests-such as specific gravity or colostrum cell count-could improve predictive accuracy beyond what Brix alone achieves. Longitudinal tracking of calves that

received marginal-Brix colostrum through the pre-weaning period would clarify whether small differences in passive transfer translate into meaningful differences in health outcomes. And extending the validation to other European breeds and management systems would broaden the evidence base for continent-wide colostrum management guidelines.

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Contributions not qualifying for authorship

Mr. Dirk Hendriks assisted with farm-level sample collection, and Ms. Eva Kuiper performed the radial immunodiffusion assays at the Wageningen diagnostic laboratory.

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