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Abstract: Objectives: To investigate the accuracy of both the optical and digital Brix refractometers compared with radial immunodiffusion (RID) for determining the immunoglobulin G (IgG) concentrations in dairy calf serum. Design: The experiment design was a cross-sectional survey of four dairy farms. Serum was sampled from 12 calves from each farm at approximately 48 hours of age. Methods: Serum IgG concentrations of 48 calves were measured using RID and both types of Brix refractometer. Results: IgG co ...

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An evaluation of the Brix refractometer as an on-farm tool for the detection of passive transfer of immunity in dairy calves

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Objectives
To investigate the accuracy of both the optical and digital Brix refractometers compared to radial immunodiffusion (RID) for determining the immunoglobulin G (IgG) concentrations in dairy calf serum.

Design
The experiment design was a cross sectional survey of 4 dairy farms. Serum was sampled from 12 calves from each farm at approximately 48 hours of age.

Methods
Serum IgG concentrations of 48 calves were measured using RID and both the optical and digital Brix refractometers.

Results
IgG concentrations measured by Brix refractometer scores were correlated with RID, 0.74 and 0.71 for the digital and optical devices, respectively. The minimum Brix score that identified calf serum with success of passive immunity (>1000 mg/dL RID IgG) with 100% accuracy was 10% for both devices. The optical and digital devices performed similarly at identifying IgG concentrations in calf serum with a concordance of 87%.

Conclusion
The results indicate that Brix refractometer scores of 10% and above can be used to classify calves with success of passive immunity and are sufficiently accurate for use as a simple, inexpensive on-farm tool for the monitoring of neonatal dairy calf immunity levels.

Key words: dairy calf health; passive immunity transfer; Brix refractometer; immunoglobulin; on-farm tool; serum.

Abbreviations
RID, Radial Immunodiffusion.

Introduction
Calves are born hypogammaglobulinaemic and depend entirely on the absorption of maternal immunoglobulins from colostrum within 24 h of birth to establish a functional immune system. For a calf to mount an immune response to pathogen challenge it requires a minimum of 1000 mg/dL of immunoglobulin G (IgG) in serum. IgG accounts for 90% of the total immunoglobulins present in bovine colostrum. Hypogammaglobulinaemic calves or failure of passive transfer of immunity is a highly prevalent issue as it increases the risk of mortality and morbidity and negatively impacts on the productive abilities of the animal. It has been identified that 19.2% of calves in the United States fail to acquire the minimum threshold level of IgG concentration in serum which results in failure of passive immunity transfer. There is currently only information from one study assessing the prominence of this issue in Australia. The study identified that 38% of calves measured from Victorian dairy farms had failure of passive immunity transfer.

The most accurate method for measuring IgG concentration is radial immunodiffusion (RID). This method is a laboratory procedure that requires in excess of 24 h to produce results. This time frame is not suitable for utilising the 24 h window of opportunity of immunoglobulin absorption in the calf. There is a need by the dairy industry to be able to rapidly determine the immune status of a neonatal calf. Several methods have been assessed for their effectiveness of estimating IgG concentrations in calf serum including: total protein by refractometry, whole-blood glutaraldehyde coagulation test, sodium sulphite turbidity test and zinc sulphate turbidity tests. However, none of the techniques currently available for measuring serum IgG concentrations are appropriate for on-farm use. Farmers often have no way of identifying if calves have consumed colostrum post calving upon removal from the cow; consequently, over 40% of calves in the US fail to absorb an adequate amount of immunoglobulins when left to suckle from the cow. If farmers had a rapid method of identifying calves that fail to absorb immunoglobulins, it could be possible to alter this status by supplementary feeding high quality colostrum before the 24 h post-partum window of opportunity for absorption closes. Such a technology would reduce the proportion of calves at increased risk of mortality and morbidity due to being hypogammaglobulinaemic.

An on-farm tool is required to be rapid, reliable, inexpensive and simple to use. One such tool is the Brix refractometer. The Brix refractometer has been promoted by the Australian dairy industry as a suitable cow-side test for colostrum quality. The device has demonstrated potential for identifying IgG concentrations in colostrum when compared to radial immunodiffusion (RID) with correlations with a range of $r^2 = 0.64$ to 0.75. These results indicate the Brix refractometer may be an effective on–farm tool to estimate the immunoglobulin component of calf serum.

The hypotheses tested were; i) that the correlation of the Brix refractometer scores with RID IgG concentrations in calf serum will be similar to those published for colostrum; and ii) that there will be concordance between the optical and digital Brix refractometers for classifying calf serum IgG concentrations.
Materials and methods

Calf serum source and collection

Approval from the Animal Care and Ethics Committee of Charles Sturt University (protocol number 11/064) was obtained prior to commencement of the experiment. The first 12 calves born after 1 April 2012, on each of four commercial dairy farms located in the Riverina region of New South Wales were recruited for the experiment (n=48). The calves comprised of 24 Holstein Friesian, 12 Holstein Friesian x Jersey and 12 Brown Swiss x Holstein Friesian.

Samples of blood were collected when the calves were approximately 48 h of age. The blood was collected from the jugular vein by venipuncture into vacutainers free of anticoagulant. The samples were immediately put on ice and centrifuged at 3,500 rpm for 15 minutes within 2 h of collection. The serum was decanted from the cell pellet and was stored at -20 °C until all were analysed.

Radial immunodiffusion analysis of serum IgG concentration

All samples were thawed at room temperature prior to analysis. The IgG concentration of serum was determined using immunodiffusion test plates (Triple J Farms 777 Jorgensen Place Bellingham, WA USA). The plates were stored at 4°C prior to use and the directions detailed by the manufacturer were followed. Included in the test kits were reference sera of pooled bovine serum of known IgG concentration (2748 mg/dL, 1402 mg/dL and 196 mg/dL); these three standard solutions were included in all of the test plates (24 wells). Into three wells of each plate, 5µL of each standard solution were pipetted. Calf serum was pipetted into the remaining 21 wells. The plates were incubated in an incubator at 22°C for 24 h. Serum samples with a diffusion ring that exceeded the diameter of the standard solutions were appropriately diluted with physiological saline and pipetted into new plates with the standard solutions and incubated at 22°C for 24 h. Each plate was treated as a separate test, with a standard graph (IgG concentration of the standard solutions (Y axis) and size of the diffusion ring [mm] (X axis)) developed for each test plate. The line of best fit was determined for each standard graph and, from this, the IgG concentration (mg/dL) of the serum samples was determined. The appropriate dilution factor was applied to determine the final IgG concentration for each of the serum samples that required dilution.

Brix refractometer analysis

The serum samples were tested using both an optical Brix refractometer (E_Line Optical Refractometer 44-803, 0 to 32% scale) and a digital Brix refractometer (Hanna Instruments Digital Refractometer HI 96801, Rhode Island, USA). Both the digital and optical devices measure the refractive index of liquids on a Brix scale. The refractive index of the serum estimates the protein concentration which is an indication of the concentration of
immunoglobulins. The optical device measurement was determined first to prevent any bias of the interpretation of the Brix scale. Both devices were cleaned with deionised water to ensure contamination did not occur between each sample and to facilitate proper calibration of the devices.

**Statistical analysis**

Descriptive statistics were used to compare the optical and digital Brix refractometer scores for serum to the RID IgG values for the same serum sample. Statistical analyses of the data were performed using the statistical software S-Plus. Analysis of variance (ANOVA) and simple linear regression were used to determine the probability (ρ) values, ρ values of <0.05 were considered to be significant. The correlation between both refractometer instruments and the serum RID IgG concentrations were determined using simple linear regression. Concordance of the optical and digital Brix refractometers was determined using Lin’s concordance correlation coefficient (ρc = ρCb). Diagnostic test characteristics were determined to assess the Brix refractometers effectiveness at detecting serum IgG concentrations. The diagnostic test characteristics, sensitivity and positive and negative predictive values, were calculated using the RID IgG concentrations as the reference standard. The sensitivity test is the probability that a truly positive sample will test positive. Serum samples with IgG concentrations >1000 mg/dL were classified as positive and samples < 1001 mg/dL were classified as negative. The performance of the devices were also tested by calculating the positive predictive value (PPV), which is the probability that those samples testing positive truly have IgG concentrations >1000 mg/dL, and the negative predictive value (NPV), which is the probability that those samples testing negative truly have IgG concentrations < 1001 mg/dL. Six different Brix refractometer scores were tested; 7, 8, 9, 10, 11 and 12% (Table 1), and the diagnostic test characteristics were calculated for each score. The diagnostic test characteristics were used to evaluate which Brix score was the most accurate at classifying IgG concentrations.

**Results**

**Descriptive analysis**

A total of 48 serum samples were collected from 48 calves between April 1 and May 31, 2012. The RID IgG concentration range for the calf serum was 120 mg/dL to 3900 mg/dL. The mean, median and standard deviation of the RID IgG concentrations were 2550, 2550 and 1909 mg/dL, respectively. Of the calf serum samples, 23 % were below the 1000 mg/dL IgG threshold for successful passive transfer of immunity. The Brix scores ranged from 7.2 to 12.5 for the optical device and from 7.1 to 12.0 for the digital device. The optical Brix refractometer had a mean, median and standard deviation of 10.45, 10.45 and 2.89, respectively. The digital Brix refractometer had a mean, median and standard deviation of 10.2, 10.2 and 2.54, respectively.
**Correlation coefficients**

The correlation and prediction equations between both refractometer devices and the serum RID IgG concentrations are presented in Figures 1 and 2. The standard error of the prediction equations was ± 789 mg/dL and ± 695 mg/dL for the optical and digital devices, respectively. The correlation between the IgG concentration measured by RID and the optical Brix refractometer scores (Figure 1) was $r^2 = 0.71$ (P < 0.001, n = 48). Similarly the correlation between the RID IgG concentration and the digital Brix scores (Figure 2) was $r^2 = 0.74$ (P < 0.001, n = 48).

**Concordance correlation coefficient**

There was concordance between the two devices, 87% (Figure 3). The concordance was more accurate, 97%, than precise, 89%.

**Diagnostic test characteristics**

The optical and digital Brix refractometer devices were tested using diagnostic test characteristics to evaluate their effectiveness as a diagnostic tool for detecting IgG concentrations in calf serum. For both the optical and digital Brix refractometers the highest sensitivity was found at scores above 10% which produced a sensitivity of 100% for detecting calf serum samples with IgG concentrations >1000 mg/dL.

**Discussion**

The objective of this experiment was to validate both the optical and digital Brix refractometers ability to accurately identify passive transfer of immunity in dairy calves. The finding of this study support the first hypothesis; which states the correlation of the Brix refractometer scores with RID IgG concentrations in calf serum will be similar to those published for colostrum (range of 0.64 to 0.75). The Brix refractometer scores were correlated to the IgG concentrations in calf serum determined by RID. The Brix refractometers in the current experiment produced scores which described the IgG concentration of calf serum with correlation values 0.74 for the digital device and 0.71 for the optical device. This is supported by recent research from the US which identified a correlation of 0.75 for the digital Brix refractometer compared with calf serum RID IgG concentrations. The Brix refractometer has been accepted by the Australian dairy industry as a tool for the measurement of colostrum quality. Based on the findings of the current research and the consistency of the correlations between the literature, there is evidence to support the use of the Brix refractometer in the detection of neonatal dairy calf immunity levels. The Brix
Refractometers are effective at measuring IgG concentrations in dairy calf serum and could be used as an on-farm support tool for the management and monitoring of dairy calf health.

The optical and digital Brix refractometers performed similarly in this experiment, supporting the second hypothesis; which states there will be concordance between the optical and digital devices. Both refractometer devices use the same mode of action to measure the refractive index of liquids. However, the devices require different operating methods to produce the measurement. The optical Brix refractometer requires the operator to look into the instrument’s eye piece to interpret the Brix scale displayed on the screen. The device requires a light source, either natural or artificial, to determine the refractive index as a percentage on a scale of 0 – 32%. This percentage reading is identified by a blue line across the scale. The interpretation of the blue line on the scale can be interpreted by individuals differently because the visibility of the line can be affected by the size of milk fat globules and casein micelles causing it to appear unfocussed. The digital Brix refractometer removes the subjective nature associated with the optical device. The digital device, provided that it is properly calibrated with deionised water and cleaned, produces consistent results between operators. The digital device presents the reading in Brix units on a digital screen. There was concordance (Figure 3) between the two devices (87%). The concordance was more accurate (97%), than it was precise (89%). This concordance indicates that the subjective nature of the optical devices interpretation only slightly influenced the precision of the device. The optical device can therefore be used with similar confidence as the digital device. This finding is particularly important to farmers as the optical device is a fraction of the cost of the digital device. In addition to the refractometers being inexpensive and easy to use, the blood does not need to be centrifuged for the devices to produce reliable results. The published correlation between the results produced by the refractometers from centrifuged and non-centrifuged calf serum is 0.95. This indicates that farmers will not need access to a centrifuge and can allow blood to clot naturally to perform the test without compromising the accuracy of the results.

The diagnostic test characteristics determined that the Brix refractometer devices can identify calf serum with IgG concentrations >1000 mg/dL, or success of passive transfer of immunity with high sensitivity. The Brix refractometer score that identified calf serum with IgG concentrations >1000 mg/dL with 100% sensitivity was 10% for both devices (Table 1). The positive and negative predictive values support this result by determining the probability that the serum samples were correctly classified as either > 1000, or < 1000 mg/dL IgG concentration. The cutpoint identified by the current study can be compared to recent work in the US which identified a cutpoint of 7.8% with a sensitivity of 90% for correctly classifying serum samples with IgG concentrations >1000 mg/dL. The cutpoint identified by the current study is a more conservative estimate of the predictability of the Brix percentage as representation of the IgG concentration in calf serum. The cutpoint recommended by the
current study is higher than previously published but it also produced a 100% accuracy. As a practical implication, having a cutpoint that is higher with a greater degree of accuracy may lead to more calves being correctly classified as having insufficient immunoglobulins. Alternatively, the current study identified the cutpoint of 9% with a sensitivity of 90.9% can also be used but to a lesser degree of accuracy compared with the 10% cutpoint. Therefore, Brix scores below 10% can be assumed that the calf has inadequate immunoglobulin's to mount an effective immune response to pathogen challenge when they enter the calf rearing environment. Within 24 hours of birth, supplement colostrum can be fed to the calf which may increase the likelihood that the IgG concentration in the blood will increase, and therefore increase its survivability. The high accuracy of the Brix refractometers at identifying success of passive immunity transfer indicates that this tool could be successfully incorporated into calf management practices using the Brix refractometer readings of 10% and above to identify neonatal calves that have received adequate colostrum.

There are some limitations associated with the practical use of the Brix refractometers on-farm. This device requires farm staff to obtain blood samples from neonatal calves which requires training and the correct sterile blood sampling equipment. There is also the issue of utilising the 24 hour window of opportunity, as results from supplementary feeding colostrum outside this window would have limited success. Considering these limitations the device still has the potential for other uses in the livestock industries, for example in the bobby calf industry it may be useful to determine the health status of a calf by identifying immunity levels before deciding to sell or purchase the animal. It may also have implications in the beef and lamb industries as mismothering and neonatal deaths contribute to significant losses in these industries.26, 27

Conclusion

This experiment validated the use of the Brix refractometer as an effective decision making tool for determining the health status and estimating the survivability of neonatal dairy calves. The Brix device values from calf serum of 10% and above allow dairy farmers to identify calves with adequate immunity levels and to introduce them to the calf rearing environment with a higher chance of overcoming pathogen challenge. Due to the Brix refractometers ease of use and reliability of identifying success of passive transfer of immunity it can be used as an on-farm tool for managing the immunity levels of the neonatal calf population for preventing failure of passive immunity transfer.

Acknowledgements

The authors appreciate the participation of the dairy producers and technical assistants involved in the sample collection. The contribution of Dairy New South Wales for the funding of the RID laboratory analysis test kits is acknowledged.
References


Figure 1. The linear relationship, Pearson’s correlation coefficient, prediction equation and p-value for the optical Brix refractometer scores for serum and serum RID IgG concentration.

Figure 2. The linear relationship, Pearson’s correlation coefficient, prediction equation and p-value for the digital Brix refractometer scores for serum and serum RID IgG concentration.
Table 1. Diagnostic test characteristics of the Brix refractometers for measuring calf serum IgG concentrations using RID IgG concentrations as the reference standard.

<table>
<thead>
<tr>
<th>Brix Refractometer</th>
<th>Brix scores (%)</th>
<th>Sensitivity (%)</th>
<th>PPV (%)*</th>
<th>NPV (%)*</th>
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* PPV = Positive predictive value, NPV = Negative predictive value.

Figure 3. The Lin’s concordance correlation coefficient ($\rho_c$), the accuracy ($C_v$), and the precision ($\rho$) between the optical and digital Brix refractometer scores for serum.