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Calf Note 187 - BRIX Refractometer for serum IgG

Introduction

Monitoring the success or failure of passive immunity is key to a successful colostrum program. Most of us understand that calves absorb immunoglobulins (**Ig**) from the colostrum they consume in the first 24 hours of life. These Ig (known as IgG, IgM and IgA) provide temporary immunity (also called passive immunity) until the calf's active immunity system matures.

Measuring the immunoglobulins in colostrum is generally complex and time consuming and use methods restricted to laboratories. Although calf-side quick and simple assays have been developed, they have not gained wide popularity. Instead, many veterinarians and calf specialists recommend using a total protein refractometer to estimate serum total protein, and, then in turn, estimate serum IgG concentration. Numerous research studies have shown that total protein of 5.2 g/dl to 5.5 g/dl indicates that calves have achieved "successful passive transfer" of immunity – that is, a serum IgG concentration ≥10 g/L.

I would pose the question – if we use a refractometer to estimate serum total protein and from that value estimate serum IgG, why don't we just use the refractometer to estimate serum IgG directly?

Two Refractometers… or One?

A refractometer measures the way light bends when it passes through a solution. The number and types of particles influence this bending, called the "refractive index". (For more information regarding the use of refractometers and colostrum, see Calf Notes #62, #183, and #186. A thorough overview of BRIX measurements and refractometers is also available at Wikipedia.)

The *serum total protein refractometer* measures the refractive index and predicts the serum total protein content of the sample. The prediction is based on the statistical relationship between total protein and refractive index.

Indeed, every refractometer does the same thing – estimates the quantity of something based on the relationship to the refractive index.

The *BRIX refractometer* is commonly used in the brewing industry to measure the sugar concentration of liquids. We have learned that the BRIX refractometer estimates of BRIX % are closely related to colostrum IgG concentration; consequently, we recommend using the BRIX refractometer to estimate colostrum IgG concentration.

Let's consider the question – **can we use the BRIX refractometer (which is usually cheaper than the total protein refractometer) to predict serum IgG in addition to colostrum IgG?** Can we use the BRIX refractometer instead of the total protein refractometer? The short answer… yes.

Three recent research studies have considered this question. Table 1 contains some of the key statistics regarding the relationship between BRIX and serum IgG.

A key statistic in Table 1 is the correlation between BRIX percentage and serum IgG. In all studies, the correlation was very high – from 0.86 to 0.93. These values indicate an excellent and (importantly) a linear relationship) between BRIX and serum IgG. For example, Figure 1 shows the relationship between BRIX measurement of serum and serum IgG concentration.

We can see from Table 1 that the relationship between BRIX and serum IgG is linear through a wide range of values. In each of the three studies, serum IgG ranged from <4 to >45 g/L, which is wide enough to be predictive under most conditions on farm.

It is clear that a linear relationship exists between the two values. Similar relationships were reported by Deelen et al. (2014) and Elsohaby et al. (2015). Indeed, when Deelen et al. (2014) compared BRIX % and serum total protein (i.e., comparing results from two different refractometers), the correlation coefficient was 1.0, indicating a perfect relationship. This is not surprising, as both refractometers were reporting similar information using different scales. But this observation suggests that BRIX % can easily replace total protein percent.

Item	Morrill	Deelen	Elsohaby
No. of samples	185	397	200
Breed	Holstein	Holstein	Holstein
Age of calf, days	1	3 to 6	1 to 11
Min. serum IgG, g/L	3.5	2.1	1.3
Max. serum IgG, g/L	47.0	59.1	60.0
Avg. serum IgG, g/L	19.0	24.1	17.7
Correlation of BRIX to IgG	0.87	0.93	0.79
Cut-point	7.8	8.4	8.3
Sensitivity	90	89	86
Specificity	94	89	83

Table 1. Statistics from studies evaluating BRIX refractometer and serum IgG concentrations.

Studies:

Morrill, et al. 2013. J. Dairy Sci. 96:4535-4541.
Deelen et al. 2014. J. Dairy Sci. 97 :3838–3844.
Elsohaby et al. 2015. J. Vet. Intern. Med. 29:721-726.

Each of the authors determined that "cut-point" at which BRIX values indicated successful passive transfer of immunity (i.e., serum IgG ≥10 g/L). These values varied somewhat, from 7.8 (Morrill) to 8.3 (Deelen) and 8.4 (Elsohaby). It is not clear why the variation occurred, although different refractometers, ages of calves, methods of sampling all could contribute to the slightly different relationship between the three studies.

Predicting Serum IgG

Figure 1, from Morrill et al. (2013) reported the regression equation for BRIX and serum IgG. That equation: 9.12846x -59.2122, allows us to predict the serum IgG at any given BRIX value between 3.5 and 47 g/L.

For example, if we measure the BRIX value of a sample of serum and it reads 7.5% BRIX, the estimated serum IgG concentration = 9.12846 x 7.5 - 59.2122 = 9.25 g/L. In this case, the estimated serum IgG concentration indicates the calf did NOT receive enough passive immunity and is more susceptible to disease.

Neither Deelen et al. nor Elsohaby et al. reported the regression equations of BRIX and serum IgG; however, it is possible to closely estimate the equations using graphical techniques. Figure 2 contains these estimated regression equations along with the regression from Morrill et al. I also calculated an average of the three regression equation (NOTE: the average is the simple arithmetic average of the three equations – it is not adjusted for number of animals or any other factor). The average equation is quite similar to the estimate of Deelen. I then used this equation to create Table 3, which predicts serum IgG concentration at BRIX values from 7.5 to 14%. NOTE: these predictions are based on three published studies, but have not been thoroughly evaluated experimentally.

Predicting serum IgG, whether using a total protein refractometer, BRIX refractometer or other method, is always an on farm estimate. Therefore, the method is best used for multiple animals and to determine if there are weaknesses in on-farm management.

One Caveat

One important note in the use of ANY refractometer – although most modern refractometers have "automatic temperature correction", the refractive index of any solution is strongly affected by temperature. It is best to let the sample to be measured to equilibrate to room temperature. For colostrum, it should be allowed to cool. Remember, we only need to cool the sample we're measuring (a couple of drops on the refractometer), so this will only take a few minutes. Serum will cool during collection and processing, so no adjustment should be necessary. For more information on the effect of temperature on automatic temperature correcting refractometers, see this article from the refractometer manufacturer Misco.

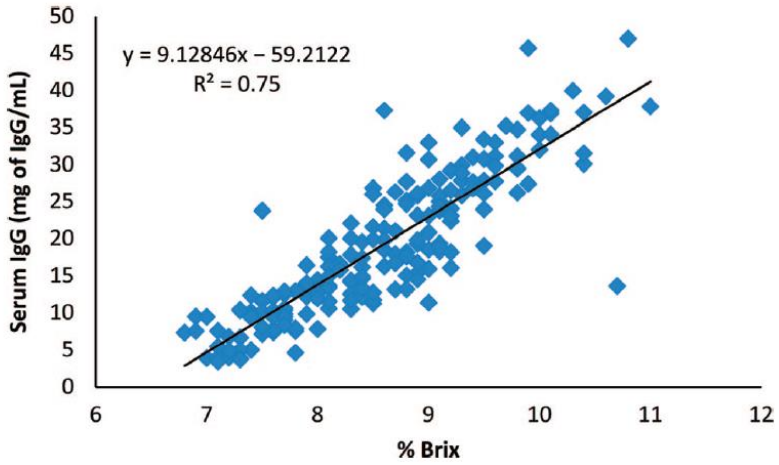

Figure 1. Relationship between serum IgG concentration and % BRIX measured with optical BRIX refractometer. From: Morrill et al., 2013.

Summary

A BRIX refractometer is just as accurate as the total protein refractometer in predicting the success of the colostrum feeding program.

The advantages of the BRIX refractometer are that it is equally accurate; it can predict serum IgG instead of total protein (which must then be used to estimate IgG); and the BRIX refractometer is usually much less expensive. Values in Table 2 may be used as a general guide to determining the effectiveness of the colostrum management program.

References

Deelen S, T. Ollivett, D. Haines, and K. Leslie. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97:3838–3844.

Elsohaby, I., J.T. McClure, and G.P. Keefe. 2015. Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in dairy calves. J. Vet. Intern. Med. 29:721–726.

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

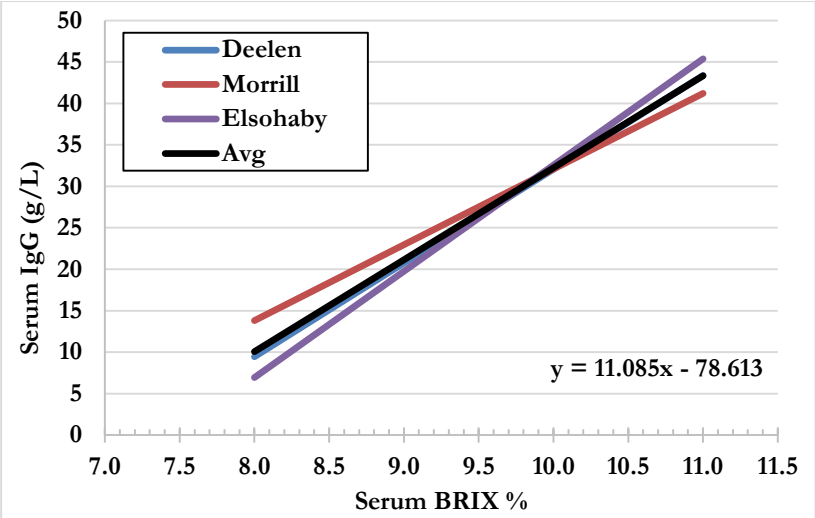

Figure 2. Regression of serum BRIX % and serum IgG from three published studies.

BRIX %	Serum IgG, g/L
7.5	4.5
8.0	10.1
8.5	15.6
9.0	21.2
9.5	26.7
10.0	32.2
10.5	37.8
11.0	43.3
11.5	48.9
12.0	54.4
12.5	59.9
13.0	65.5
13.5	71.0
14.0	76.6

Table 2. Predicted serum IgG from BRIX values using regression from Figure 2.