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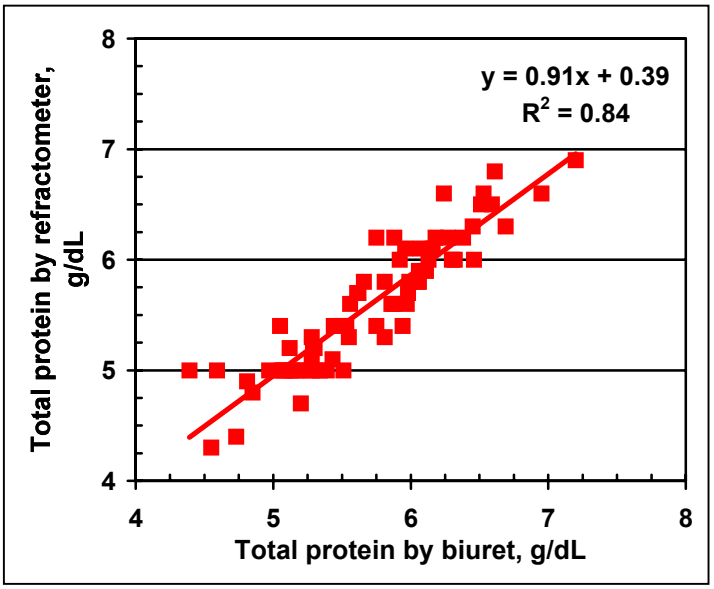
Calf Note #62 – Calf Age, Total Protein and FPT in Calves

Introduction. Measuring the degree of transfer of passive immunity is very important to proper management of young calves. Passive immunity is determined by the amount of colostrum consumed by calves within the first 24 hours after birth, the quality of the colostrum and the calf's ability to absorb IgG from the intestine. Unfortunately, determination of failure of passive transfer (FPT) on the farm is difficult. Most veterinarians agree that FPT occurs when serum IgG concentration in calves is less than 10 grams of IgG per liter of serum (or, 1,000 mg/dl of serum).

Measuring FPT on the farm can be a challenge. Tests available for measuring FPT are time consuming, difficult or expensive. One method, measuring total protein with a device called a refractometer is a reasonable alternative to other methods of measuring FPT. Calf Note #39 contains some important information regarding the use of refractometers.

One comment in Calf Note #39 was "*the relationship between total serum protein and IgG will change as the calf ages*". This is true. It is the objective of this Calf Note to clarify this observation.

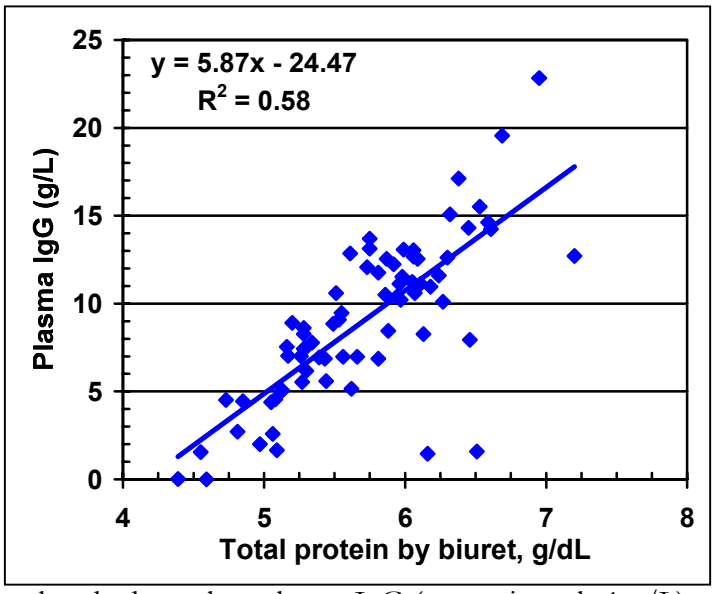


Experiment 1. A group of calves (n = 72) was purchased from local dairy farms and sale barns and transported to the APC Calf Research Unit in Ames, IA. The calves were 3 to 5 days of age on arrival at the facility, although actual birth date was not recorded. Calves were fed electrolytes on arrival and milk replacer from day 1 to day 3. On day 4, a sample of jugular blood was collected into evacuated containers containing EDTA, and the plasma was collected by centrifugation. The total protein was then determined using a clinical refractometer (Schuco Clinical Refractometer). The refractometer was calibrated for temperature prior to each sample. Remaining blood was centrifuged and plasma was stored (-30ºC) prior to analysis for IgG by turbidimetric immunoassay and total protein by biuret using an automated chemistry system (COBAS MIRA, Roche Diagnostic Systems, Somerville, NJ). We measured total protein by biuret to determine if the refractometer was effectively determining the total protein in our samples.

Our first measurement was to determine if the refractometer was providing an accurate measurement of the total protein in the plasma samples. To accomplish this, we compared the total protein measurements made with the refractometer with those made by the biuret chemistry

(laboratory) system. The correlation between these two measurements was 0.92. From the figure, it is clear that there is a high degree of relationship between the two methods of measuring total protein. In addition, the regression equation (0.91X + 0.39) indicates that as the total protein (estimated by biuret) increased, there was a corresponding increase in the total protein estimated by the refractometer. The rate of increase (0.91), or slope of the regression line, is quite close to 1.0, which corresponds to a one-unit increase in protein estimated by refractometer for a one-unit increase in total protein estimated by biuret. The intercept (0.39) is also close to zero, which means there is very little bias in the refractometer compared to the biuret method.

Answering the question of relationship between total protein and IgG was our next goal. In the second figure, the relationship between total protein, measured as biuret, and IgG is shown. The r^2 (a measure of the variation in plasma IgG that is explained by total protein is now 0.58. This means that about 58% of the variation in plasma IgG can be explained by the measurement of total protein. On the other hand, this also means that about 42% of the variation in plasma IgG in NOT accounted for by total protein. From the graph, it is clear that some calves with no measurable plasma IgG (plasma IgG = 0) still had protein in their blood stream – about 4.5 g/dL. Two other calves had very low plasma IgG (approximately 1 g/L) but > 6 g/dL of total protein.

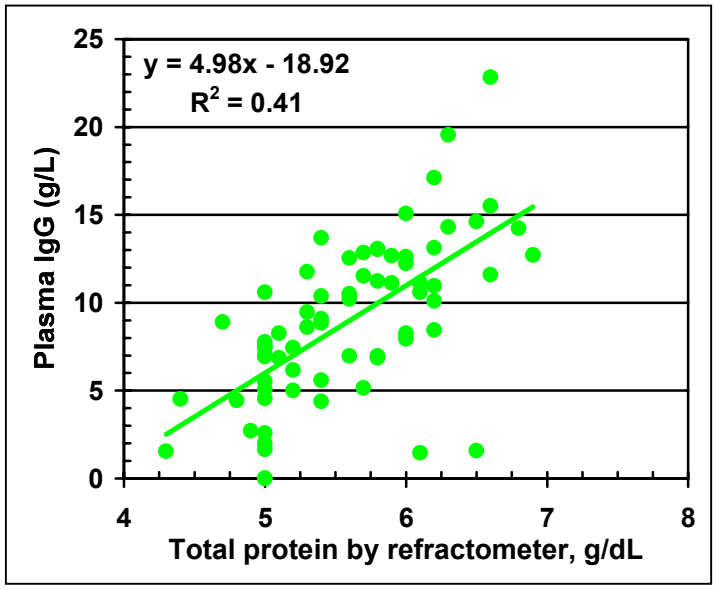


The final graph shows the relationship between plasma IgG and total protein when measured by the refractometer. The r^2 of this regression is only 0.41, which means that less than half of the variation in plasma IgG concentration can be explained by variation in total protein when estimated by the refractometer. Conversely, about 59% of the variation in plasma IgG is due to something OTHER than total protein when measured by the refractometer.

Why is the relationship between total protein (refractometer) and plasma IgG so low in this study? Well, we have a few theories for this observation:

- the refractometer is inherently less accurate than the biuret method. Using an automated method such as biuret (and the equipment we used to measured protein) is more accurate because there is greater control in measurement of the assay. That is why the biuret method gave a better prediction (r^2 = 0.58) than refractometer (r^2 = 0.41).

- the relationship between IgG and protein changed. The calves in this study were 4 to 9 days of age. However, because calves were purchased from sale barns, we had no ways to determine the actual age of each calf. As calves get older, the relationship between plasma IgG and total protein in the blood appears to decline. Therefore, in older calves, using a refractometer is less useful than in very young calves. The change in relationship between IgG and total protein can be caused by several factors:



  - a decline in the concentration of plasma IgG due to loss of IgG. The loss of IgG (half-life) is due to normal consumption of IgG in response to age, exposure to pathogens in the environment, and other factors.
  - change in concentrations of protein. The concentration of protein in the blood depends on many factors, including the amount of colostrum fed and protein consumed. As calves get older, the amount of protein in the blood is less dependent on the amount of colostrum found and more on the amount of protein consumed during normal feeding.

Using the equation in the final graph, it is possible to ESTIMATE the plasma IgG concentration in calves on the basis of total protein when measured by refractometer. Here's how it works. Let's say you measure total protein in the calf's plasma using your trusty refractometer and find it's 6 g/dL. On the basis of this study, the estimated plasma IgG would be 6 * 4.98 – 18.92 = 10.96 g/L. You can estimate any number of refractometer measurements using this equation. Remember, though, that the relationship between the refractometer measurement and IgG is only 41%.

Many vets use a refractometer and then use cutoff of 5.5 g/dL to indicate FPT. That is, when calves have total protein > 5.5, they are adequately protected, but when the total protein is < 5.5, they are not sufficiently protected. In this study, a total protein of 5.5 g/dL translates to a plasma IgG concentration of 8.47 g/L, which is usually classified as too low. To reach an IgG concentration of 10 g/L (which is classified as successful passive transfer) the total protein measurement needs to be 5.8 g/dL.

Well, the results of this study indicate that measurement of total protein by refractometer is more complicated than simply measuring total protein in the blood and estimating IgG. The age of the animal is very important. These data would suggest that when a calf is more than about 5 days of age, the ability of the refractometer to estimate FPT is reduced. And as calves get older, the usefulness of a refractometer will continue to decline.

Estimates of total protein that indicate FPT must be carefully evaluated. In several research evaluations, the total protein (measured by refractometer) that correlates to 10 g of IgG/L of plasma is higher than 5.5 g/dL.

Calves require an adequate mass of colostrum as early as possible for a healthy, productive life. A refractometer is a good tool – when used properly – to estimate the quality of the overall colostrum feeding program. Knowing the limitations of a tool usually makes that tool more useful. In the case of the refractometer, measuring total protein in blood of calves less than 5 days of age will increase the chance to make the correct decision regarding colostrum management.