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Calf Note #39 – Using a refractometer

Introduction

Measuring the degree of passive transfer in newborn calves can tell you a lot about the level of management in your calf raising enterprise. Studies have consistently shown that the incidence of death and disease is affected by the immunoglobulin (Ig) status of calves shortly after birth. Further, performance of calves (growth, intake, disease resistance) is profoundly affected by their immune status achieved in the first 24 hours.

One method used widely to estimate the degree of passive transfer in calves is the refractometer. This instrument is widely used by veterinarians to determine the overall health status of calves. This Calf Note will provide some insight into the use of a refractometer, and ways to interpret the results.

How does a refractometer work?

The refractometer works by shining a beam of light through a sample of liquid. The device measures the amount of light that is refracted (or bent) from the light path due to the constituents in the sample. In blood, proteins will cause light to bend. The greater the protein, the more light is bent from the light path. In the photo, this student is determining the total protein in a sample of plasma from calves that have just arrived from a sale barn.

What does it measure?

Instead of measuring serum IgG, the refractometer measures total serum protein. In newborn calves, there is usually a close correlation between total protein and IgG in the blood, since largest protein consumed in colostrum is IgG. The correlation between total serum protein and IgG in calves 24 hours of age is approximately 0.71. This means that about 50% of the variation in total protein in the blood of calves at 24 hours of age can be attributed to the IgG fraction (see figure below).

How much total protein is required in my newborn calves?

Most dairy professionals suggest the following guidelines for using total protein to estimate the level of passive transfer in calves?

- >5.5 g/dl: successful passive transfer
- 5.0 to 5.4 g/dl: moderately successful passive transfer

- <5.0 g/dl: failure of passive transfer

Note however, in the figure that serum protein of 5.0 g/dl is equivalent to 1,000 mg/dl (or 10 g/L), which many dairy professionals consider successful passive transfer. Using data in the figure, criteria would be:

- > 5 g/dl: successful passive transfer
- 4.75 to 5.0: moderately successful passive transfer
- < 4.75: failure of passive transfer

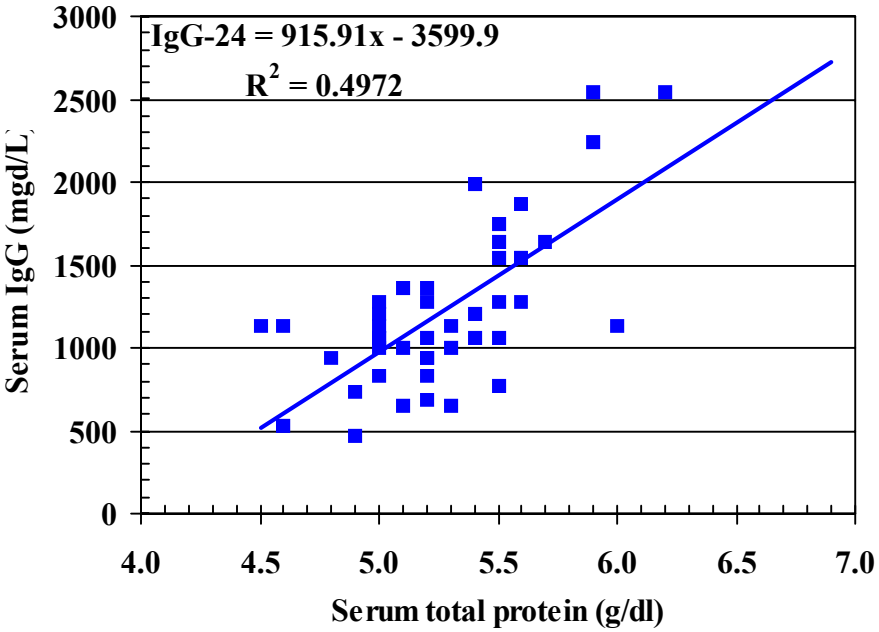

Figure 1. Relationship between serum total protein and IgG in calves at 24 hr of age. From: C. Adams, unpublished.

As you can see, the relationship between serum protein and serum IgG is not absolute, and more data is required to identify the reasons for variation among readings and colostral, animal, and environmental factors. Variation between two different groups of animals is shown in the differences between Figures 1 and 2.

How accurate is it?

The refractometer is quite accurate in measuring the index of refracted light, which is closely related to total protein in blood. However, there are several factors to consider in determining the validity of the refractometer measurement in estimating the degree of passive transfer in calves:

- *The quality of the instrument.* Low cost refractometers may be sufficiently accurate to measure the general categories (above),

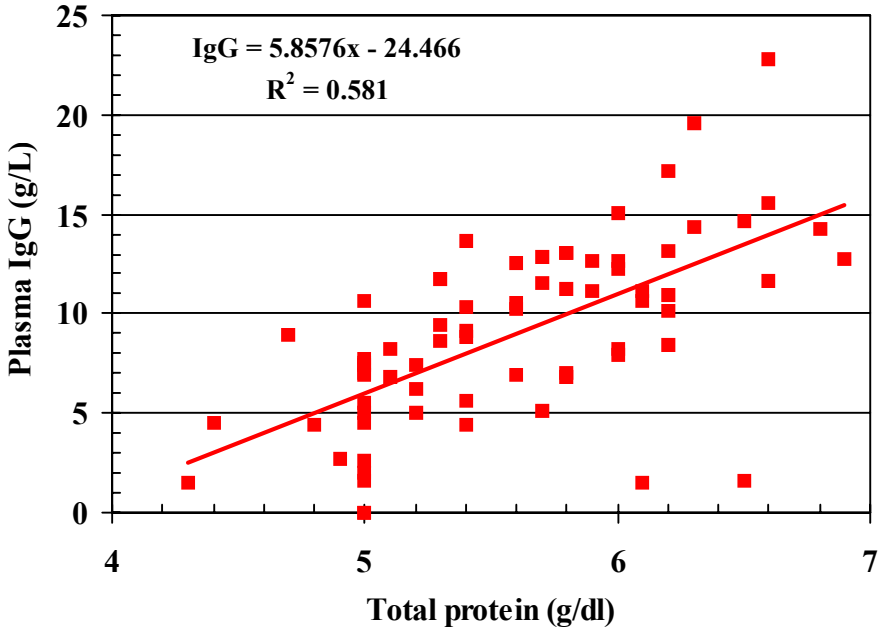

Figure 2. Correlation between plasma IgG and plasma total protein. From: Jaynes et al., 2000.

but may not be able to differentiate accurately between small increments of total protein, say between 5.1 and 5.2 g/dl. Check the accuracy and precision of your instrument before purchasing it. The more accurate instruments are generally quite expensive. Generally, on-farm refractometers should not be considered experimental instruments. Instead, they should be used as management tools for on-farm application. Highly sophisticated, expensive (>$1,000 U.S.) refractometers may be used in clinical or experimental conditions.

- *The age of the animal.* The relationship between total serum protein and IgG will change as the calf ages. Absorption of dietary proteins other than IgG and movement of IgG from the blood into other body pools can influence the accuracy of the measurement. Therefore, it's best to determine refractometer measurements in calves older than 1 day of age and less than 3 days of age. It's best to wait until the calf is at least 24 hours of age to ensure complete absorption of IgG from the intestine. After about 3 days of age, the relationship between IgG and total protein will change.
- *The types of proteins absorbed.* In normal colostrum, the relationship between IgG and other, non-Ig proteins is fairly constant. Thus, the relationship between refractometer measurements and passive transfer will be satisfactory. However, if the relationship is changed - for example, by using colostrum supplements, then the accuracy of the refractometer may be affected.

What if blood protein is low when measured with a refractometer?

There are several items to consider if your calves don't reach the target level:

- *The instrument.* Be sure the refractometer is working properly. Handle it carefully and take care of it when it is not in use. Regular maintenance, care and calibration will provide you an instrument with a long useful life. Reliance on an instrument that is inaccurate and malfunctioning will only harm the colostrum management program.
- *The temperature.* Refractometers are dependent on the temperature of the sample being measured. Differences in temperature can have a major impact on refractometer readings.
- *The quality of colostrum fed.* If calves are consuming an insufficient mass of IgG, total serum protein (and IgG) will be inadequate. For more information on measuring colostrum quality see *Calf Note #22* Using the colostrometer for measuring colostrum quality. Poor quality colostrum is a serious problem for calf growers. Poor quality colostrum may provide inadequate IgG at any realistic colostrum intake. If poor colostrum is a problem, consider using stored colostrum and/or colostrum supplements.
- *The intake of colostrum.* Are calves consuming all the colostrum offered? If not, you should consider using an esophageal feeder to force the calves to consume an adequate volume of colostrum. Of course, intake of large amounts of poor quality colostrum still may not provide adequate serum protein concentration.
- *The age of the calves.* The relationship between total protein and IgG changes as calves get older (see above). Be sure calves are in the appropriate age range for protein measurement when you take blood samples. In addition, the age at which calves are fed colostrum will influence the refractometer measurement. Calves fed colostrum at later ages (> 2-4 hours of age) will not absorb IgG in colostrum as efficiently as calves fed colostrum as soon as possible after birth.

- *The size of the calves*. Larger calves will have a lower serum protein calves compared to smaller calves fed the same mass of IgG. This is because the blood volume of larger calves is greater, and thus, dilutes out the protein (and IgG).

Some of my calves give me extremely high readings - what's going on?

There are a couple of possibilities. First, the calves might be dehydrated. An accurate protein measurement depends on the blood volume. In cases of dehydration, water leaves the circulation, concentrating the other blood constituents. This can lead to very high total protein readings (up to 8 g/dl). Alternatively, check the performance of your refractometer. It may be malfunctioning.

Adding a colostrum supplement to colostrum doesn't make a big difference in calves total protein readings. Why?

Again, there are several possibilities that you can't determine differences when adding a commercial colostrum supplement to colostrum:

- *The colostrum supplement isn't absorbed*. Absorption kinetics of some commercial supplements are relatively poor and don't contribute significantly to total protein in the calf.
- *There's not enough protein in the supplement*. Most colostral supplements contain large amounts of protein (>50%) and therefore should contribute to total protein in the calf. For example, absorption of a 454 gram dose that contains 60% protein will provide 454 x 0.6 = 272.4 grams. If these 272.4 grams are completely absorbed into the blood of a 40 kg calf (assuming 9% plasma volume), then there should be an increase of 75 grams/liter, or 7.5 grams/100 ml. Thus, if a calf has a birth total protein content of 4.0 g/dl, at 24 hours, there should be 11.5 g/dl. This protein concentration is much higher than levels seen in calves, so the total amount of protein in supplements should **not** contribute to the lack of refractometer increase. Proteins that do not contribute to immune response are typically used for protein synthesis, converted to energy, or are excreted in the urine. A typical condition, hyperproteinurea, occurs in calves for the first day or so as calves excrete excess protein from colostrum or colostrum supplements.
- *Calves are fed colostrum and supplement too late*. As calves get older, their ability to absorb IgG intact diminishes until the gut "closes" at about 24 hours of age. Feeding colostrum or supplement late (after 12 hours) won't contribute markedly to overall circulating IgG.
- *The refractometer is not accurate enough to determine differences*. Most hand-held refractometers are only accurate to + or - 0.2 g/dl. This means that it cannot effectively distinguish between 5.0 and 5.2 g/dl. Consider the following example. A producer feeds a colostrum supplement in addition to normal, fair quality colostrum (30 g of IgG/L). The producer adds a colostrum supplement that provides 45 grams of IgG. Assuming a typical efficiency of IgG absorption (25%) for both colostrum and supplement, the following will occur:
    - initial total protein content of the blood = 4.0 g/dl
    - BW of the calf = 40 kg x 0.09 plasma volume = 3.6 L of plasma
    - added protein from colostral IgG = 30 g/L x 4 L x 0.25 / 3.6 L of plasma = 0.83 g/dl increase
    - added protein from supplement = 45 grams x 0.25 / 3.6 = 0.31 g/dl increase
    - thus, calves fed colostrum will have 4.0 + 0.8 = 4.8 g/dl; calves fed colostrum + supplement = 4.0 + 0.8 + 0.3 = 5.1 g/dl. Depending on the quality of the

refractometer, variation in calf body weight, age at first feeding and many other factors, the difference (0.3 g/dl) may not be discernible using a hand-held refractometer. A more accurate clinical refractometer or other tests to directly measure IgG should be used in this case.

Summary.

Well, there you have it. The refractometer is a useful tool in managing the colostrum feeding program. Proper use and interpretation of the results can help you in better rearing healthy, productive calves.

Appreciation is expressed to Dr. Carol Adams for supplying serum data in the figure used in this "Calf Note".

Written by Dr. Jim Quigley (10 November 1998).

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