Animal Industry Report

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Two Methods to Determine IgG Concentration in Calf Serum

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Summary and Implications

The objective of this study was to develop a rapid, calf-side test to determine serum IgG concentration using caprylic acid **(CA)** fractionation followed by refractometry of the IgG-rich supernatant and compare the accuracy of this method with results obtained using refractometry of whole serum. Serum samples (n = 200) were obtained from 1 d old calves, frozen and shipped to the laboratory. Samples were allowed to sit at room temperature to thaw. Fractionation with CA was conducted by adding 1 ml of serum to a tube containing CA and 0.5, 1 or 1.5 mL 0.06 M acetic acid. The tube was shaken and allowed to react for 1 min and centrifuged for 0, 10 or 20 min. Refractive index of the fractionated supernatant **(nDf)** was determined using a digital refractometer. Whole, non-fractionated, serum was analyzed for IgG by radial immunodiffusion **(RID)** and refractive index **(nDw)**. The nDf and nDw were compared to serum IgG concentration. Mean serum IgG concentration was 19.0 mg/ml (SD = 9.7) with a range of 3.5 to 47.0 mg/ml. Serum nDw was positively correlated with IgG concentration (r = 0.86, n = 185). Fractionated samples treated with 1 ml 0.6 M AcO and 60 µl CA and not centrifuged prior to analysis resulted in a strong relationship between nDf and IgG (r = 0.80, n = 45). These results suggest that refractometry of whole calf serum provides a strong estimate of IgG concentration that can be used to determine if adequate passive transfer has occurred in 1 d old calves.

Introduction

Newborn calves are born aggamaglobulinemic, without any measurable circulating IgG or IgM. The newborn calf derives passive immunity by absorbing immunoglobulins **(IgG)** from colostrum **(MC)** provided within the first hours of life. In the calf, passively acquired immunity is of importance to the health of the calf for the extended period of time until they are capable of making their own antibodies.

Rapid postnatal growth of the intestine results in the replacement of fetal-type enterocytes by adult-type enterocytes leading to gut closure or cessation of macromolecule absorption from gut to blood. Neonates that obtain transfer of antibodies post-partum have short closure times, with cessation of macromolecule transport increasing after 12 h with a mean closure time of 24 h after birth.

Passive transfer of IgG can be determined by taking a blood sample from the calf at 24 h of age. Neonatal calves are classified as having failure of passive transfer **(FPT)** if their serum IgG concentration is less than 10 mg/ml at 24 h of age. Radial immunodiffusion **(RID)** is a direct measurement of IgG concentration and considered to be the gold standard to determine IgG concentration in bovine serum. Unfortunately RID assays require a relatively long incubation time (~24 h) that prevents the identification of FPT calves prior to gut closure. Refractometers, digital or optical, have been utilized to measure the total protein content in colostrum and calf serum. Protein solutions refract light, and refractometers use this property to measure the total protein in a solution. In the neonatal calf, IgG constitutes a large proportion of the protein in serum. This allows the measurement of TP to provide an estimation of serum IgG concentration.

Short chain fatty acids have long been recognized to be powerful plasma protein precipitants. Caprylic acid **(CA)** has been utilized to precipitate non-IgG proteins from a solution, leaving an IgG-rich supernatant. This technique has been adapted to determine the IgG content of mammary secretions from non-lactating dairy cattle and for the purification of serum for therapeutic uses in humans and horses.

The objectives of this study were to develop a rapid, calf-side test to determine serum IgG concentration using CA fractionation followed by refractometry of the IgG-rich supernatant and compare the accuracy of this method with results obtained using refractometry of whole serum.

Materials and Methods

Serum samples (5 ml) were obtained from 1 d old Holstein calves (n = 200) on a California calf ranch. Samples were frozen, packed on ice and shipped to Iowa State University. Samples were allowed to thaw at room temperature prior to analysis. One ml of bovine calf serum was added to a tube containing one of three acid treatments (Table 1) and mixed for 10 sec and allowed to incubate for 60 sec. Samples were then centrifuged for 0, 10 or 20 min prior to analysis of the supernatant. A digital refractometer (SPER Scientific, SCIENTIFIC model 300034; Scottsdale, AZ) was used to determine the nD of the fractionated serum and whole serum. IgG concentration was determined using radial immunodiffusion kits (Triple J Farms, Bellingham, WA).

The PROC CORR procedure of SAS was utilized to determine the relationship between IgG concentration and nD value for each treatment.

Table 1. Caprylic acid and acetic acid concentrations evaluated to determine the relationship between nD and RID obtained IgG concentration.

	TRT A	TRT B	TRT C
Serum (ml)	1.0	1.0	1.0
0.6 M acetic acid (ml)	0.5	1.0	1.5
Caprylic acid (µl)	45	60	75

Results

Two hundred serum samples were analyzed; 15 samples had IgG concentrations less than 3.43 mg/ml and were not included in the analysis of the relationship between nD and RID determined IgG concentration. The mean IgG concentration of the remaining 185 serum samples was 19.0 mg/ml (SD = 9.7) with a range of 3.5 to 47.0 mg/ml. A total of 150 (65%) of the samples had IgG concentrations greater than 10 mg/ml indicating adequate passive transfer had occurred, while 50 samples (25%) had IgG concentrations less than 10 mg/ml indicating FPT.

Whole serum nD was positively correlated with IgG (r = 0.86, $P \leq 0.00$; Figure 1). Correlation coefficients for the CA test treatment refractometry values compared to IgG were positive, but had weaker relationships compared to those observed between whole serum nD and IgG (Table 2). The correlation between TRT B samples that were not centrifuged and IgG (r = 0.80, $P \leq 0.001$) provided the strongest relationship for the CA test variables.

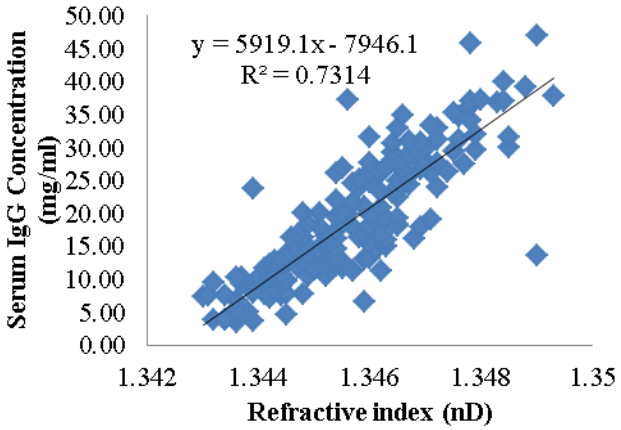

Figure 1. Serum IgG concentration as determined by RID compared to the refractive index of whole calf serum (n = 185).

Discussion

The percentage of calves utilized in this study that had FPT is slightly greater than the 19.2% estimated by utilizing data in the 2007 NAHMS report and identical to that reported in Ontario dairy calves. Failure of passive transfer introduces the risk of increased mortality and morbidity due to an increased susceptibility to pathogens and subsequent disease.

Table 2. Correlation coefficients between RID determined serum IgG concentration and brix or nD values.

		RID*nD	
	n	r	p - value
Treatment[1]			
Whole serum	185	0.86	<0.0001
TRT A - NC	41	0.61	<0.0001
TRT A -10	40	0.72	<0.0001
TRT A - 20	39	0.77	<0.0001
TRT B - NC	45	0.8	<0.0001
TRT B - 10	41	0.77	<0.0001
TRT B - 20	40	0.52	<0.0001
TRT C - NC	53	0.59	<0.0001
TRT C - 10	51	0.69	<0.0001
TRT C - 20	35	0.75	<0.0001

[1] Treatment number followed by the number of minutes allowed to sit prior to centrifugation, where NC = no centrifuge, TRT A = 0.5 ml acetic acid & 45 µ CA, TRT B = 1 ml acetic acid & 60 µl CA, TRT C = 1.5 ml acetic acid & 75 µl CA.

Research conducted at the University of Guelph utilized a refractometer to evaluate total protein in bovine calf serum at 24 to 36 h of age and again at 11 to 14 days of age. They observed a strong relationship between total protein and IgG concentration (R^2 = 0.7349), that was nearly identical to the value as we observed between nD of whole serum and IgG in this study.

There are many advantages of refractometry of whole serum to determine IgG over currently available methods. Radial immunodiffusion has a long incubation time (18 to 24 h) preventing identification of FPT calves prior to gut closure. Refractometry takes only the time necessary to obtain a blood sample, allowing it to incubate for the serum to separate and less than 15 sec for the refractometer to produce a digital reading. Refractometry does not require internal standards that may introduce error in determining the IgG concentration of serum. Digital refractometers are calibrated with distilled water that is readily available. A second strength of refractometers they often contain a temperature compensating device, thus reducing the confounding factor of temperature that can impact the accuracy of other methods to determine IgG concentration in serum. The simplicity of the refractometer to determine the IgG concentration of serum as compared to RID and ELISA, is a great advantage and will easily allow for the on-farm adaptation.

The primary disadvantage of refractometry to determine FPT in calves is that it requires the use of serum. Very few dairy farms own a centrifuge, however, data out of Canada reports that serum collected from blood tubes allowed to sit and clot had a total protein content (as determined by

refractometer) that was highly correlated (R^2 = 0.95, n = 234) to the total protein content of a duplicate sample that was centrifuged prior to serum collection. This suggests that producers could take a blood sample from calves, let it sit and use a transfer pipette to remove a small amount of the serum for analysis by refractometer.

Currently only 2.1% of U.S. dairy operations routinely measure passive transfer status of calves. The goal of any calf monitoring program is not to predict the health fate of each calf, but to monitor the success of passive transfer on an individual farm and provide additional support to FPT calves. If producers have access to simple tools that rapidly and accurately estimate IgG concentration, FPT calves and calves at risk of FPT could be identified and provided additional MC, or preventative care.

Conclusion

The objectives of this study were to determine if the CA could be adapted to serum and compare this method to refractometry of whole serum as a method to evaluate IgG concentration of neonatal calves. Adding 1 ml of serum to a tube containing 1 ml 0.06 M acetic acid, 60 µl caprylic acid, shaking the sample for 10 sec, allowing the sample to incubate for 1 min prior to analysis of supernatant resulted in a strong correlation between nD and RID. However, a stronger relationship exists between IgG and nD of whole calf serum. This study concludes that the refractive index of whole serum provides a rapid and accurate estimate of serum IgG concentration.

Acknowledgements

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