Technical note: Serum total protein and immunoglobulin G concentrations in neonatal dairy calves over the first 10 days of age

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ABSTRACT

Efficacy of passive transfer of immunity in young calves is commonly assessed using total serum protein (STP) or serum immunoglobulin G (IgG) concentration tested within the first few days of life. To our knowledge, no research has measured changes in these concentrations over this period to establish an appropriate age range for testing. The aim of this study was to monitor changes in STP and serum IgG concentrations from birth until 10 d of age to provide a basis for recommendations for when passive transfer of immunity in dairy calves can be measured. Concentrations of STP and IgG of 12 calves were measured at 11 time points: at approximately 30 min before colostrum feeding, at 24 h after colostrum feeding, and daily from d 2 to 10 of age. Mean ($\pm$ standard deviation) STP and IgG concentrations were 4.61 ± 0.3 g/dL and 0.6 ± 0.6 mg/mL at birth, 5.83 ± 0.73 g/dL and 22.2 ± 9.6 mg/mL at 24 h after colostrum feeding, and 5.78 ± 0.52 g/dL and 16.1 ± 7.3 mg/mL at d 10 of age, respectively. The IgG concentration declined over subsequent days relative to IgG measured at 24 h at a rate of approximately 0.69 mg/mL per day, declining by $27.6 \pm 6.2\%$ (mean $\pm$ SD) on d 10. The concentration of STP did not decrease over time. Concentrations of IgG at 24 h after colostrum feeding were highly correlated with each of the measures of IgG over the 10-d period ($r \geq 0.97$). These correlations were supported by the Bland-Altman plots of agreement between the 24-h sample and subsequent samples. Compared with the reference value at 24 h, STP concentrations were highly correlated on d 2 and 3 ($r \geq 0.98$), highly correlated but variable from d 4 to 9 ($r \geq 0.88$), and lower at d 10 ($r = 0.76$). These results indicate that calves may be reliably tested for passive transfer of immunity using IgG or STP concentrations up to 9 d of age.

Key words: failure of passive transfer, colostrum feeding, calf health

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Passive transfer of immunity from colostrum is important for calf health; failure of passive transfer of immunity (**FPT**) has been related to increased morbidity and mortality in calves (Donovan et al., 1998; Vogels et al., 2013; Boccardo et al., 2016), lower productivity, and increased risk of culling later in life (DeNise et al., 1989; Faber et al., 2005). Passive transfer of immunity can be assessed by several methods. Radial immunodiffusion is considered the gold standard for directly assessing serum IgG concentrations. Serum total protein (**STP**) measured with a refractometer provides an indirect measure that is highly correlated with blood IgG concentration and is more practical for use on-farm (Deelen et al., 2014; Elsohaby et al., 2015; Thornhill et al., 2015). The suggested cut-off values for FPT using a refractometer are <5.2 g/dL STP in healthy calves (equivalent to 1 g/dL serum IgG; Tyler et al., 1996; Windeyer et al., 2014), and <5.5 g/dL STP in clinically ill calves (Tyler et al., 1996; McGuirk and Collins, 2004).

Serum IgG concentrations in the calf will gradually increase after colostrum IgG has been absorbed by the intestinal tract, with peak concentrations observed at approximately 24 to 36 h after feeding (Stott et al., 1979; Hassig et al., 2007). The onset of active immunity production by the calf begins during the first days of life (Hassig et al., 2007) and endogenous production increases around 1 to 2 wk of age, generating a secondary increase in IgG concentrations (Husband et al., 1972). Accurate measures of passive transfer of immunity must occur before this onset of active immunity. Also, maternal colostrum IgG has a half-life of approximately 10 d (Erhard et al., 1999; Hassig et al., 2007). Thus, some researchers have suggested that IgG should be measured by 7 d of age (McGuirk and Collins, 2004; Godden, 2008), whereas others have sampled earlier (Villaruel et al., 2013) or as late as 21 d of age (Tyler et al., 1999). To our knowledge, no research has been

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done to support these time frames for IgG and STP concentration sampling.

The aim of this study was to monitor changes in IgG and STP concentrations from birth until 10 d of age to determine when reliable estimates of passive transfer of immunity could be acquired. We hypothesized that STP concentration measured up to 10 d of age would reflect STP and IgG concentrations measured at 24 h after colostrum feeding.

This study was conducted from October to December 2015 at the University of British Columbia's (UBC) Dairy Education and Research Centre (Agassiz, BC, Canada). All procedures were approved by the UBC Animal Ethics Committee as outlined in protocol #A14-0245. Holstein dairy calves (6 male and 6 female, $n = 12$) were enrolled in the study at birth. Power analysis (following Hintze, 2008) showed that this sample was adequate to detect a change of 0.5 g/dL of STP and 5 mg/mL of serum IgG concentration with 80% power and α of 0.05. Estimates of variation for STP were based on the reported values in Villarroel et al. (2013) and for serum IgG concentration from a previous study conducted on the same experimental farm as the current study ($SD = 7$ mg/mL of serum IgG).

None of the calves suckled from their dam (monitored using an overhead video camera in the maternity pen: WV-CW504SP, Panasonic, Osaka, Japan) before they were separated on average 3.8 ± 3.3 h after birth. Calves were weighed (mean $\pm$ SD; 42.7 ± 6.0 kg of BW), housed in individual sawdust-bedded pens (1.22×2.44 m), and fed 4 L of colostrum through a nipple bottle approximately 30 min following separation from the dam. If the full 4 L of colostrum was not consumed, the remainder was fed via esophageal tube. The colostrum fed to each calf contained at least 50 mg/mL IgG as measured using a colostrometer (Kruuse Colostrum Densimeter, Langeskov, Denmark). All colostrum was frozen and thawed before feeding without pasteurization. After the initial colostrum feeding, all calves were provided 8 L/d of pasteurized whole milk divided into 2 feedings delivered using a nipple bottle at 0830 and 1600 h. No calves were identified as ill during routine visual health monitoring in the research facility.

Blood samples were collected daily from the jugular vein into untreated evacuated tubes. The first sample was taken approximately 30 min before colostrum feeding, the second sample was taken at 24 h following colostrum feeding, and subsequent samples were taken between 1500 and 1600 h each day until 10 d of age. After the final blood sample, all calves were returned to the herd and managed according to standard operating procedures. All blood samples were immediately centrifuged at $3,000 \times g$ for 15 min at 21°C, and the

serum was analyzed for total protein using a digital refractometer (Reichert AR 200, Reichert Inc., Depew, NY). The refractometer was calibrated weekly according to the manufacturer's guidelines, and each sample was measured again using a handheld refractometer (Reichert Vet 360, Reichert Inc.) to ensure consistent measurements within a single sample. Serum was pipetted into 2-mL cryovials and frozen at -20°C for later IgG analysis. All blood samples were analyzed for IgG concentrations using radial immunodiffusion (Chelack et al., 1993) at the SCCL Ltd. (Saskatoon, SK, Canada) laboratory.

All statistical analyses were performed in SAS (version 9.4; SAS Institute Inc., Cary, NC). Descriptive analyses were performed using PROC UNIVARIATE and probability distribution plots. No outliers were detected (data points that were beyond 3 SD from the mean) and no transformations were deemed necessary. Concentrations of IgG and STP from d 2 to 10 were expressed as a total and percentage change from the concentration measured 24 h after colostrum feeding. Only 1 calf did not achieve passive transfer of immunity (IgG < 10 mg/mL) and was excluded; therefore, all analyses, results, and interpretations are based upon animals that achieved passive transfer ($n = 11$).

We performed 3 analyses to assess (1) the total change in IgG and STP concentrations over time, (2) how the day of sampling affected each of IgG and STP concentrations, and (3) the agreement between IgG and STP concentrations on each day of sampling relative to the 24-h gold standard. In the first analysis, the influence of day of sampling on IgG and STP concentrations from d 2 to 10 was analyzed using PROC MIXED. The fixed effects included in this model were day of testing, birth weight, and sex of the calf. Day was specified as a repeated measure and calf as subject, using an autoregressive covariance structure.

In the second analysis, the effect of day of testing on the total change of IgG and STP concentrations was analyzed using PROC MIXED, where the intercept was set to zero 24 h after colostrum feeding. Birth weight and sex of calf did not explain any variation in the change in concentration of IgG and STP; thus, day of testing was the only effect included in the final model. Day was specified as a repeated measure and calf as subject, using an autoregressive covariance structure.

In the third analysis, we used Pearson correlations to measure (1) the correlation between IgG concentration measured at 24 h after colostrum feeding and at each of d 2 to d 10, (2) the correlation between STP concentration measured at 24 h after colostrum feeding and at each of d 2 to d 10, and (3) the correlation between IgG concentration at 24 h after colostrum feeding and

Figure 1. Boxplot of blood serum IgG concentrations of 12 Holstein dairy calves sampled at birth, 24 h after colostrum feeding, and from d 2 to 10 of age. Mean values are indicated by $\times$ and median by the solid black line. Individual calf values of IgG at each time point are represented by the solid black circles overlaying the boxplot.

Figure 2. Boxplot of blood serum total protein (STP) concentrations of 12 Holstein dairy calves sampled at birth, 24 h after colostrum feeding, and from d 2 to 10 of age. Mean values are indicated by $\times$ and median by the solid black line. Individual calf values of STP at each time point are represented by the solid black circles overlaying the boxplot.

STP concentration measured at each of d 2 to d 10. Following Hinkle et al. (2003), correlations were categorized as negligible ($r < 0.30$), low ($r = 0.30$ to 0.49), moderate ($r = 0.50$ to 0.69), high ($r = 0.70$ to 0.89), or very high ($r \geq 0.90$). Agreement between the 24-h measure and each subsequent measure for STP and IgG concentration was assessed using plots of the difference in measures between the 2 time points against their mean, following Bland and Altman (1986).

As expected, calves showed low values of IgG and STP concentrations before colostrum feeding and an initial peak at 24 h after colostrum feeding. IgG concentrations then decreased over time ($F_{1,98} = 76.11$, $P < 0.001$; Figure 1). Relative to 24 h after colostrum feeding, IgG declined to (mean $\pm$ SD) $92.4 \pm 3.8\%$ by d 2, to $76.5 \pm 7.5\%$ by d 7, and to $72.4 \pm 6.2\%$ by d 10. Concentrations of STP did not change over time ($F_{1,98} = 1.37$, $P = 0.24$; Figure 2).

Furthermore, the effect of sampling day on IgG concentrations was described by an equation with a slope of -0.69 ± 0.05 mg/mL per day ($t_{1,98} = -13.43$, $P < 0.001$). The equation can be described as follows: $\text{IgG}_{24\text{-h equivalent}} = \text{IgG}_{\text{day } n} + (n - 1) \times 0.69$, where n = number of days after birth that the blood sample was acquired. For the decline in STP concentration over time, the slope from 24 h after colostrum feeding until d 10 was not significant ($t_{1,98} = -1.79$, $P = 0.08$).

Concentrations of IgG measured from d 2 to 10 were very highly correlated with the reference value recorded 24 h after colostrum feeding; this correlation declined only slightly from $r = 0.99$ on d 2 to $r =$

0.98 on d 10 (Figure 3). Correlations were also very high between the reference (24-h) value for STP and values recorded through d 3 of age. The strength of the relationship remained high from d 4 to 9 ($r \geq 0.88$) and then declined on d 10 ($r = 0.76$). Measures of IgG and STP concentrations were also highly correlated at each day of measurement ($r \geq 0.88$), although on d 10 this

Figure 3. Pearson correlation coefficients between reference values of either IgG (■) or serum total protein (STP, ♦) concentrations measured 24 h after colostrum feeding and measured in subsequent days, and the relationship between IgG concentrations taken 24 h after colostrum feeding and STP concentrations (STP $\times$ 24 h IgG, ▲) measured from d 2 to d 10 of age ($n = 11$ Holstein dairy calves).

relationship was lower ($r = 0.79$). Similarly, STP measurements were very highly correlated with the gold standard IgG measure at 24 h after colostrum feeding until d 4 ($r \geq 0.88$) and highly correlated from d 5 to 9 ($r \geq 0.80$). The correlation was somewhat lower (but still considered high) on d 10 ($r = 0.73$; Figure 3).

Bland-Altman plots of the agreement between samples taken at 24 h after colostrum feeding and each subsequent sampling day are shown for STP (Figure 4) and IgG (Figure 5) concentrations. As expected for STP concentration, the lowest mean difference between the 24-h sample and any subsequent sample (i.e., highest agreement) was close to 0 [e.g., from d 2 (-0.036 ± 0.14 g/dL STP) to d 10 (0.11 ± 0.43 g/dL STP)].

As IgG concentration was affected by day of sampling, the lowest mean difference occurred between the 24-h sample and d 2 (1.8 ± 1.3 mg/mL IgG), and the mean difference increased over sampling day to d 10 (6.6 ± 2.5 mg/mL IgG).

Our study is the first to evaluate passive transfer status in the same individual over the first 10 d of life. The data from this study confirm and expand upon other reports on the decline in concentrations of maternal antibodies in newborn calves after birth, indicating a marked decline occurring around 10 d of age (Erhard et al., 1999; Hassig et al., 2007).

As with serum IgG, no previous study has provided justification for how late the sampling of STP concen-

Figure 4. Bland-Altman plots comparing the difference in STP concentrations measured at 24 h after colostrum feeding and (A) d 2, (B) d 3, (C) d 4, (D) d 5, (E) d 6, (F) d 7, (G) d 8, (H) d 9, and (I) d 10 of age for 11 Holstein dairy calves. The solid line indicates the mean difference between the 2 measures (24 h – subsequent day) and the dotted lines represent 2 SD from the mean difference. The x-axis represents the mean of the 24 h and subsequent day values.

trations can occur relative to the 24-h reference point. Concentrations of STP at 24 h after colostrum feeding were very highly correlated with those taken up to d 3 of age, and this correlation remained very high up to 9 d of age. These correlations were supported by the Bland-Altman plots of agreement between the 24-h sample and subsequent samples. We conclude that sampling STP concentration up to at least d 9 can provide a reliable estimate of passive transfer.

Serum IgG declined over time in this study, but the concentration at 24 h after colostrum feeding was highly correlated with concentrations recorded up to d 10. However, the Bland-Altman plots suggest a decline in agreement between a sample taken at 24 h

after colostrum feeding and up to 10 d after colostrum feeding. This decrease over time in IgG concentration was similar to that described by Husband et al. (1972) and Hassig et al. (2007). Husband et al. (1972) found a steady decline up to 16 d of age when calves were sampled at 1, 8, 16, 32, and 64 d after birth, and Hassig et al. (2007) found that IgG concentration decreased until 22 d of age. More recently, Furman-Fratczak et al. (2011) showed that IgG concentrations at 21 to 28 d of age were greater than those at 30 to 60 h of age.

We found that serum IgG concentration decreased by 0.69 ± 0.05 mg/mL per day; this decline is similar to that reported by Villarroel et al. (2013; 0.74 mg/mL per day in the first week of life when sampling from dif-

Figure 5. Bland-Altman plots comparing the difference in IgG concentrations measured at 24 h after colostrum feeding and (A) d 2, (B) d 3, (C) d 4, (D) d 5, (E) d 6, (F) d 7, (G) d 8, (H) d 9, and (I) d 10 of age for 11 Holstein dairy calves. The solid line indicates the mean difference between the 2 measures (24 h – subsequent day) and the dotted lines represent 2 SD from the mean difference. The x-axis represents the mean of the 24 h and subsequent day values.

ferent calves at different time points). Tests for passive transfer of immunity have typically been based upon measures taken at 24 h of age (e.g., serum IgG >10 mg/mL; Weaver et al., 2000). Based on the current results, we suggest that IgG values collected from 2 to 10 d can be used, although these values should be converted to the 24-h equivalent (where $\text{IgG}_{24\text{h equivalent}} = \text{IgG}_{\text{day } n} + (n - 1) \times 0.69$, and n = number of days after birth the blood sample was acquired). We caution that the rate of decline of IgG may be different for calves with FPT, and hydration and health status of the calf should be considered when calculating estimates of IgG. Future studies should investigate how STP and IgG concentrations decline in calves with FPT.

We also found that STP concentrations were highly correlated with the gold standard measurement of IgG concentration at 24 h after colostrum feeding up to 9 d of age, indicating that STP measures can be taken beyond 24 h. Previous studies have also found that STP and IgG concentrations were highly correlated (e.g., Tyler et al., 1996; $r = 0.76$) or moderately correlated, even when calves were sampled from 1 to 30 d of age (Villarroel et al., 2013; $r = 0.67$). Other authors have discussed the importance of considering age of the animal when sampling STP concentration; for example, Villarroel et al. (2013) measured STP concentration from calves at different ages and concluded that STP concentration from calves up to 1 wk of age could be used to evaluate FPT. Although those authors sampled each calf only once, their conclusion is broadly in agreement with the results of the current study, showing high correlations until d 10. For this reason, we argue that our study offers a more compelling reference for the decline in and agreement between IgG and STP concentrations over the first 10 d of life.

In conclusion, serum IgG concentrations of dairy calves that achieved passive transfer of immunity declined at a rate of approximately 0.7 mg/mL per day from 24 h to 10 d of age, but values on all days were highly correlated with reference values taken 24 h after colostrum feeding. Concentrations of STP on d 2 to 3 were most highly correlated with the 24-h reference value, but correlations remained very high from d 4 to 9. Concentrations of STP on each of these days were highly correlated with the gold standard IgG measure at 24 h after colostrum feeding. However, we caution that our study included only calves that achieved passive transfer; concentrations of IgG and STP that are below the passive transfer threshold may differ in their decline over time and measurement agreement. We conclude that IgG or STP concentrations measured up to 9 d of age may provide reliable estimates of passive transfer of immunity at 24 h after colostrum feeding.

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