

Factors Affecting Serum Total Protein and Immunoglobulin G Concentration in Replacement Dairy Calves

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Abstract

Measurement of serum Total Protein (TP) or Immunoglobulin G (IgG) is frequently used within the dairy industry to monitor failure of passive transfer (FPT) in calves. Through such monitoring, modifications to colostrum handling and feeding techniques can be incorporated into farm practices to improve calf management. Most studies establishing cutoff values of TP or IgG for determination of FPT have been based on data primarily obtained from Holstein calves. The purpose of this study was to determine factors associated with TP and IgG serum concentration in dairy replacement calves. Factors that were studied included, breed (Holstein and Jersey), weight, age, hydration status (packed cell volume) and rectal temperature. A total of 673 calves 1 to 30 days of age were included in the study; 411 Jersey and 262 Holstein calves. Jersey calves had consistently higher TP and IgG serum concentration than Holstein calves. TP concentration was approx. 0.5 g/dL higher, while IgG concentration was more than double that of Holstein calves. This indicates that the cutoff values established for TP (>5.0 mg/dL) and IgG (>1000.0 mg/dL) in Holstein calves are not appropriate to be used in Jersey calves, needing more research to establish adequate cutoff values for Jersey calves. Another factor strongly associated with both TP and IgG concentration was calf age. Maximum concentrations were achieved at 2-3 days of age. After that, concentrations decreased by approx. 0.07 g/dL of TP and 74 mg/dL of IgG per day. Our results indicate that there is a narrow window for optimal evaluation of colostrum management in dairy replacement calves, and that different breed of cattle need their own established reference values.

Keywords: Failure of passive transfer (FPT); Calve; Colostrums; Total protein (TP); Serum, Jersey; Breed; Age; Weight; Hydration

Abbreviations: FPT: Failure of Passive Transfer; IgG: Gamma Immunoglobulin; TP: Total Protein

Introduction

Evaluation of dairy replacement calf health is generally divided into the pre-weaning and post-weaning periods; with an average weaning age of 8.6 weeks [1]. In 2007, the NAHMS Dairy study found that the pre-weaning period accounts for about 81% of total dairy replacement calf mortality [1]. Accordingly, management strategies to optimize health during this period will have the greatest impact on overall dairy calf mortality. Cows do not confer immunity transplacentally, but rather calves acquire protection from pathogens through ingestion of colostrum in a process called passive transfer [2]. While morbidity and mortality risks are not exclusively correlated with passive transfer status, multiple studies have reported an increased risk for morbidity and mortality in calves with low levels of circulating immunoglobulins [3-5]. Colostrum has great amounts of immunoglobulins, with the majority being Immunoglobulin G (IgG), although IgA and IgM are also present and may be important [2,6-8]. Immunoglobulins are absorbed by enterocytes in the gut and aid in disease prevention until the calf's own immune systems develops [2]. In addition to immunoglobulins, colostrum contains other disease-fighting constituents, such as leukocytes, lactoferrin, lysozyme [2], and cytokines [2,9]. Colostrum also serves as an important source of nutrition for the neonate, offering richer fat and vitamin contents than whole milk [10]. Higher concentration of immunoglobulins have been associated with improved production within the herd, specifically improved average daily gain [11] and increased milk yield [12].

There are three primary immunoglobulins found in colostrum: IgA, IgG and IgM. IgG is found in the highest concentration, and represents up to 90% of the immunoglobulins found in colostrum [13], though both IgA and IgM remain valuable contributors to passive immunity

in the calf [7,8]. When adequate amounts of immunoglobulins are not absorbed to transfer immunity, the calf is determined to have failure of passive transfer (FPT), which has been convened to be defined in terms of serum IgG concentration <1000 mg/dL [14]. However, some researchers have used different cut off limits to define FPT such as 800 mg/dL [15], 750 mg/dL [16], and even as low as 350 mg/dL [17]. A strong correlation has been observed between IgG concentrations and TP in serum [18,19]. This relationship allows measuring serum TP concentration via refractometer under field conditions as a proxy for IgG concentration. Using the commonly accepted cutoff limit of 1,000 mg/dL of IgG to define FPT has resulted in a broad variation in cutoff values for serum TP in Holstein calves between 5.0 and 5.5 g/dL [5,20-24].

FPT has been associated through univariate analysis studies with up to a 90% increased risk rate in morbidity and up to 39% of calf mortality [5,25], and up to 180 days of age [11,20]. However, multivariate analysis studies differ in their results; some studies showed no association between IgG or TP in serum and morbidity or mortality [26,27], while others did find an association [17,20] although not for all diseases [20].

This variability in results, in combination with the absence of data for Jersey calves under current feeding conditions, calls for research

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to determine if factors such as calf breed, age, weight and temperature affect IgG and TP concentration in serum. Some differences known between Holstein and Jersey cattle include significant discrepancy in immunoglobulin content in colostrum [28] and decreased total volume of distribution due to the smaller size of Jersey calves [29]. Immunoglobulin concentration in Holstein cow colostrum has been reported to be on average 55.9 g/L compared to the much higher concentration of 90.4 g/L in Jersey cow colostrum [28,30]. The differences in colostrum properties could potentially alter immunoglobulin absorption and create significant differences in serum TP and IgG levels that have been previously reported between the two breeds [31,32]. A study from 1969 showed that Jersey calves that were allowed to suckle from their dams up to 3 days of life had significantly higher serum IgG concentration during the first 16 weeks of life compared to Holstein calves with the same management [31]. However, to our knowledge there are no studies published using current standards of calf rising away from the dam.

It is reasonable to assume that when two calves of different body weights are fed the same amount of colostrum, the final concentration of IgG and TP in the smaller calf would be higher than in the larger calf due to the smaller volume of distribution in the smaller calf. However, previous studies found no association between body weight and IgG concentration among Holstein calves [11,20]. In review, the current body of research supports evaluation of the passive transfer status of calves younger than one week of age, although a specific timeframe has not been scientifically proven.

The objectives of this study were (1) to determine if calf breed, age, weight, temperature and hydration status influence serum TP concentration and IgG in dairy calves, and (2) whether commonly accepted cutoff values for FPT in Holstein calves are applicable to Jersey calves.

Materials and Methods

Study Design

This was a cross-sectional study in which each calf was sampled only once.

Sampling Population

Heifer calves from three commercial dairy farms in Oregon were enrolled in the study between December 2008 and June 2010. Animals chosen for inclusion in the study were purebred Jersey or Holstein heifer calves between 1 and 30 days of age. Colostrum management protocols on all three farms specified delivery of at least 4 quarts of colostrum by esophageal tube prior to 12 hours of life. The exact number, timing, and volume of individual feedings varied between animals and farms.

Biological tests

Blood samples were obtained via jugular venipuncture and collected into serum separator tubes (BD VACUTAINER® SST, Becton Dickinson Diagnostics, Franklin Lakes, NJ) and EDTA tubes (BD VACUTAINER® EDTA, Becton Dickinson Diagnostics, Franklin Lakes, NJ). A unique sampling identification number was assigned to each calf to track individual animals throughout the study. Parameters recorded included animal ID, birth date, date and time of blood draw, rectal temperature (digital thermometer), weight via girth tape (Weight-By-Breed Dairy Management Tape, C06070N, Nasco, Fort Atkinson, WI)).

All samples were processed within four hours of collection. During

the second year only, blood from each EDTA tube was drawn into a capillary tube to determine PCV. The EDTA and serum separator tubes were centrifuged at 3,000 RPM for 10 minutes to allow removal of serum, respectively. Serum samples were transferred into 2 mL cryopreservation vials (ML5355, Market Lab, Inc., Detroit, MI) for storage. At this time, fresh sample TP was determined using a digital refractometer (Model 300027, Sper Scientific Ltd., Scottsdale, AZ). Serum samples were stored frozen until laboratory analyses were performed.

For laboratory analyses, samples were allowed to warm to room temperature via overnight exposure to ambient air, and then TP and IgG were measured in thawed samples in the same manner and with the same equipment as before freezing. Serum samples were tested for IgG concentration using a commercial kit for single radial immunodiffusion assay (SRID, Single Radial Immunodiffusion Kit – Bovine IgG, Catalog No. 240-60, VMRD, Inc. Pullman, WA). The SRIDkit included 4 standards with IgG concentrations of 400, 800, 1,600 and 3,200 mg/dL. Each well was inoculated with 3 μ L of sample. After incubating at room temperature for approximately 24 hours, the precipitation ring of each well was read using an immunoviewer (TRANSIDYNE General Corporation Calibrating Viewer, Ann Arbor, MI). IgG concentration in the test samples was determined according to manufacturer instructions using the curve generated by the standards in each kit.

Statistical analyses

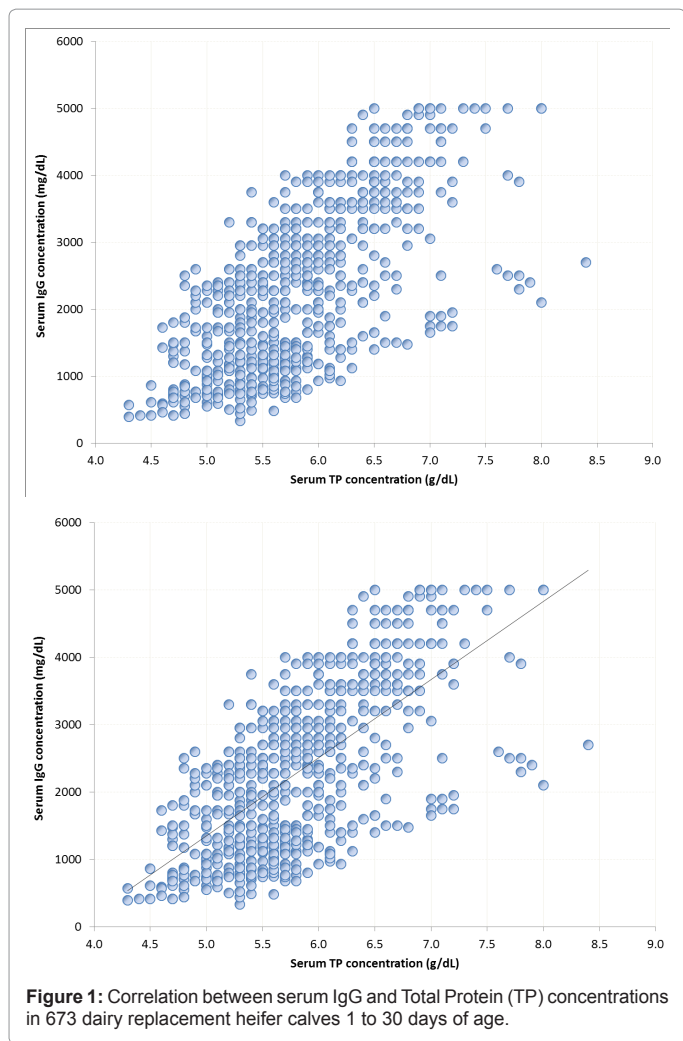
Stepwise multivariate linear regression analysis was used to determine the effect of breed, body weight and age on serum TP and IgG concentrations in dairy calves. Alpha to enter and leave the model was established at 15%. All analyses were performed using commercial statistical software (Minitab 15, Minitab Inc. State College, PA).

Results

In total, 673 calves were included in the study; 411 Jersey and 262 Holstein. Serum TP and IgG were moderately correlated ($r=0.670$, $P<0.001$, Figure 1). The resulting TP cutoff value corresponding to an IgG concentration of 1,000 mg/dL was 4.7 g/dL. In fact, it varied between Holstein calves (5.06 g/dL) and Jersey calves (4.3 g/dL).

Only calves sampled during the second year of the study ($N=434$) had hematocrit information, and their data was used for stepwise regression to analyze the effect of dehydration on TP and IgG concentration in serum. Univariate analysis on these animals showed that hematocrit was not a significantly associated with serum TP ($P=0.951$) or serum IgG concentration ($P=0.779$). Regression analysis of the other factors on all 673 calves showed that age, breed and weight were associated with IgG concentration (Table 1), while age, breed and temperature were associated with serum TP concentration (Table 2). None of the variables were highly correlated to each other. The highest correlation was between age and weight ($r=0.468$).

The effect of age on IgG (Figure 2) and TP concentration (Figure 3) was consistent in both breeds. However, this relationship was not linear, as can be observed in the graphs. The highest values for TP and IgG concentration were obtained at days 2 and 3 of age (Figures 2 and 3). The decrease was significant up to approximately 2 weeks of age ($P<0.001$), at which point TP remained similar in both breeds at approximately 5.5 g/dL, while IgG concentration remained constantly higher in Jersey calves. To eliminate a potential effect that older age may have had on the other factors in the regression analyses, they were repeated including only calves up to 7 or 14 days of age.



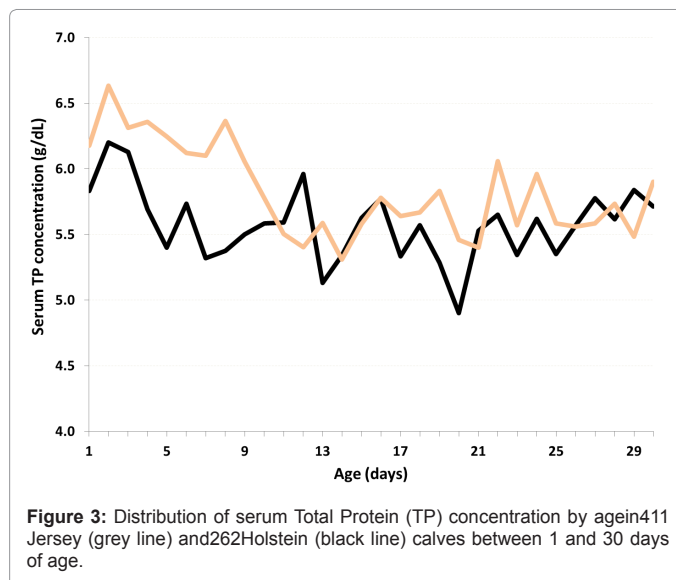
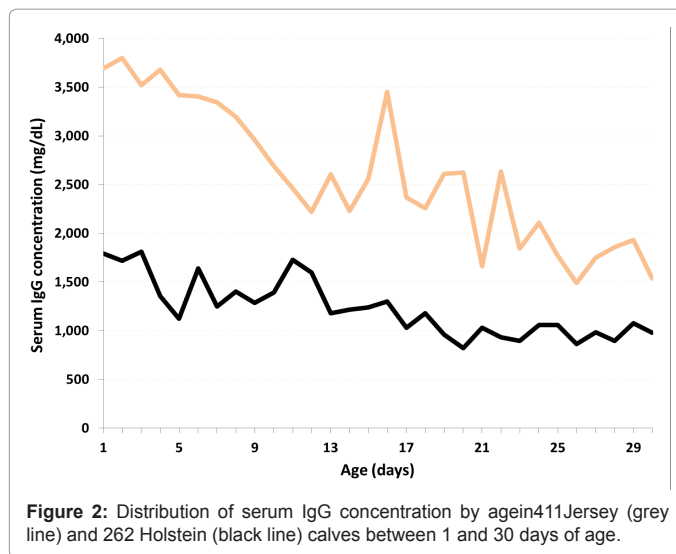
	1-30 days of age N=673	1-14 days of age N=483	1-7 days of age N=257
Constant	1278	978.6	1861
Jersey breed	1759	2080	1986
Age (days)	-66.2	-104.8	-74.0
Weight (lbs)	8.4	12.6	-

Table 1: Regression analysis results for factors associated with IgG concentration (mg/dL) in dairy replacements calves at different ages (alpha to enter was 15%).

Temperature was no longer associated with TP concentration (Table 2). Factors associated with IgG concentration remained the same, although the parameters changed (Table 2). These results emphasize the need for sampling at a young age, and to be consistent to be able to compare results over time.

All Jersey calves had IgG and TP concentrations above commonly accepted standard of 1000 mg/dL to determine presence of FPT (minimum was 1150 mg/dL in a 10 day old calf). However, 20/411 (4.9%) of the Jersey calves had TP values <5.0 g/dL; all but one were over 10 days old.

Morbidity and mortality information could only be obtained for 91 Jersey calves that were sampled at 2-3 days of age in this study. Of these, 33 developed scours, 15 developed pneumonia, and 20 were afflicted with both, leaving 23 healthy calves for comparison. Only one



calf died during the study, making statistical analyses for mortality risk impossible. Calves were diagnosed with pre-weaning scours at an average of 6.83 ± 1.81 days, and with pneumonia at an average of 36.71 ± 9.05 days (Figure 4). Mean \pm SD for IgG and serum TP concentrations calves are presented in Table 3. There was no significant difference in serum TP ($P=0.924$) or IgG ($P=0.348$) between healthy calves and those that developed scours, pneumonia, or both. Using the commonly accepted definition of FPT of 1,000 mg/dL of IgG, there was no difference in risk of scours, risk for pneumonia or risk for developing both diseases compared to healthy calves. There was also no difference in risk of morbidity using standard TP cutoff values of 5.0 g/dL or 5.5 g/dL. Survival analyses showed no difference in median time to develop morbidity between calves that were above or below 5.5 g/dL (Figure 5) or 6.0 g/dL (data not shown). Because there was only one calf that died, FPT could not be evaluated as a risk factor for mortality.

Discussion and Conclusions

Traditional research in Holstein calves has established cutoff values for FPT at IgG concentration at 1,000 mg/dL in serum [14], which has

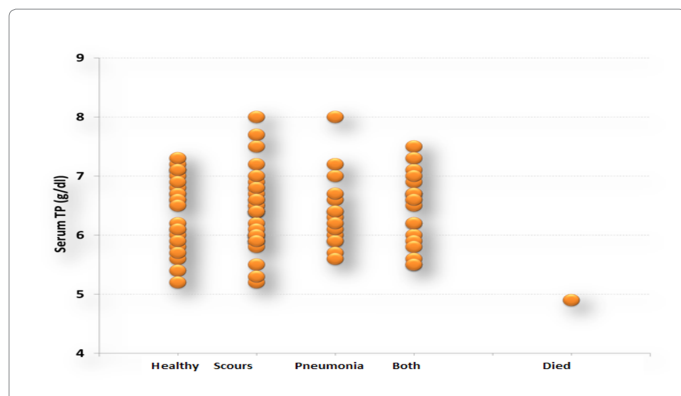


Figure 4: Comparison of serum TP concentration among 91 Jersey calves (1-3 days old at sampling) according to morbidity group.

	1-30 days of age N=673	1-14 days of age N=483	1-7 days of age N=257
Constant	10.67	6.19	6.06
Jersey breed	0.28	0.36	0.49
Age (days)	-0.024	-0.074	-0.067
Temperature (°F)	-0.047	-	-

Table 2: Regression analysis results for factors associated with total protein concentration (g/dL) in dairy replacement calves at different ages (alpha to enter was 15%).

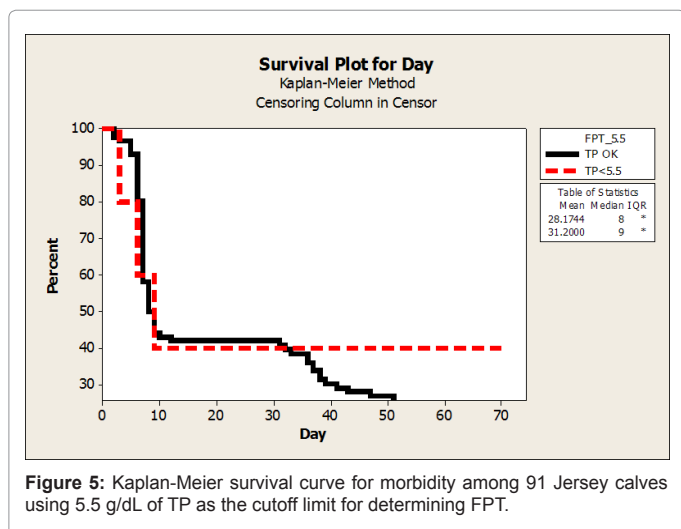


Figure 5: Kaplan-Meier survival curve for morbidity among 91 Jersey calves using 5.5 g/dL of TP as the cutoff limit for determining FPT.

corresponding values of serum TP concentration between 5.0 and 5.5 g/dL [5,20-24]. As in previous studies [19,21,22], we found that serum IgG and TP concentrations were correlated, but less so than in other studies. The correlation was similar to other studies for Holstein calves, but differed in Jersey calves. Cutoff values for TP concentration corresponding to 1,000 mg/dL of IgG varied greatly between breeds. In fact, this cutoff value had to be estimated in Jersey calves, as none had IgG concentration below 1,150 mg/dL. Another likely reason for this discrepancy was the inclusion of older calves in this study. The less than perfect correlation between IgG and TP concentration would have resulted in 4.9% of Jersey calves to have been classified as having FPT using the proxy of serum TP<5.0 g/dL as the cutoff limit, but 0% if IgG<1,000 mg/dL would have been used. This poses a practical problem for veterinarians working with Jersey calves.

Our study found that Jersey calf serum IgG and TP concentrations were significantly and consistently higher than those in Holstein calves. In fact, IgG concentrations were more than twice that of Holstein calves at most ages. TP concentrations were on average approximately 0.5 g/dL higher than in Holstein calves throughout the first week of age (Table 2). These findings indicate that applying Holstein cutoff values to manage Jersey calves could result in inadequate monitoring programs that can have detrimental consequences such as increased morbidity and mortality in these calves. Actual biological implications of these differences were not apparent as there was no difference in IgG or TP concentrations between healthy calves and those that developed diarrhea, pneumonia or both. Only one calf died, making evaluation of mortality impossible.

As seen in Figures 2 and 3, the differences in IgG and TP concentrations between the two breeds decreased over time. These results are similar to those obtained by Tennant et al. [31], which supports an inherent breed difference that may not be addressed by using Holstein cutoff values in Jersey calves. This is most obvious when comparing TP and IgG differences between breeds. TP concentrations seemed to equalize approximately at 2 weeks of age, while IgG concentrations remained constantly different.

The effect of age on both TP and IgG concentration was important (Figures 2 and 3). These results emphasize the need for sampling not only at a young age, but also to be consistent in the selection of calves within that age range to be able to compare results over time. TP and IgG concentration were highest at 2 and 3 days of age. This peak represents the systemic absorption and circulation of maternal antibodies, which occurs primarily in the first 24 to 48 hours, needing some lag time to show in serum. Sampling calves at 2-3 days of age will guarantee estimation of the highest TP and IgG concentrations achieved on a farm. Our findings indicate that calves up to one week old could be sampled to evaluate FPT, but cutoff values to establish inadequate TP concentrations should be adjusted as they decrease approximately by 0.07 g/dL each day within the first week of age. IgG concentration decreased by 74 mg/dL each day.

Our data also supports the hypothesis that weight influences IgG concentration, but not TP concentration. This result is further proof of the limited use of TP as a proxy for IgG concentration, and that interpretation of low TP concentration as an indicator of FPT in calves (especially Jersey calves) has to be done with caution. We agree that these measurements can be used to indicate whether there is a problem with the colostrum management program by determining the proportion of calves with low values. However, absolute values cannot be used to predict morbidity or mortality risk.

Limited amount of research in Jersey calf management has been published in the last 25 years. This has resulted in the necessity to assume that standards of care acceptable for Holstein calves are applicable to Jersey calves. While the farms in this study did not experience significant differences in morbidity and mortality between

Parameter	N	Total Protein	Range	Ig G	Range
Healthy calves	23	6.42 ± 0.62	5.2-7.3	3,904 ± 616	2,950 –5,000
Scours	33	6.33 ± 0.69	4.9 –8.0	3,561 ± 826	2,000 –5,000
Pneumonia	15	6.40 ± 0.63	5.6 –8.0	3,547 ± 802	2,100 –5,000
Both	20	6.45 ± 0.59	5.5 –7.5	3,770 ± 738	2,600 –5,000
All calves	91	6.39 ± 0.63	4.9 –8.0	3,688 ± 753	2,000 –5,000

Table 3: Comparison of serum total protein and IgG between calves with different morbidity.

calves categorized as having FPT or being healthy using Holstein cutoff values, it is a limited cohort and may not represent all types of dairy management practices across the US. However, our findings indicate that there are significant breed differences, demonstrating the need for future research to focus specifically on Jersey calves.

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