NUTRITION, FEEDING, AND CALVES

Estimation of Plasma Volume in Holstein and Jersey Calves

J. D. QUIGLEY, III,[1] J. J. DREWRY, and K. R. MARTIN
Tennessee Agricultural Experiment Station,
Department of Animal Science,
The University of Tennessee, Knoxville 37901-1071

ABSTRACT

The concentration of immunoglobulin (Ig) G in the blood of neonatal calves shortly after birth is a widely used criterion to determine the degree of acquisition of passive immunity. Another method used to determine the biological mechanisms of IgG absorption is calculation of the apparent efficiency of IgG absorption. Estimation of the efficiency of IgG absorption requires the estimation of plasma volume in neonatal calves. Previous estimates of plasma volume in a few calves of varying breeds have been made; the estimates ranged from 7 to 14.5% of body weight (BW). Holstein (n = 97 from four farms) and Jersey (n = 49 from one farm) calves were fed fresh maternal colostrum or colostrum that had been previously frozen. Calves were fed 2 L of colostrum at 4.1 h (SE = 0.2; range = 0.3 to 11.0 h) and 12 h later. Plasma volume was measured by determining the concentration of Evans' blue dye in a jugular blood sample collected 10 min after injection of approximately 1.5 ml of 1.5% Evans' blue dye. Factors that affected plasma volume (milliliters) were BW, breed, and age at sampling; r^2 of the regression was 0.60. Factors that affected plasma volume (percentage of BW) were BW, breed, and age at sampling; r^2 of the regression was 0.08. Mean plasma volume for all calves was 3162 ml (SE = 79) and was 9.86% of birth BW (SE = 0.15%). Mean plasma volume was 2250 ml (9.71% of BW) and 3623 ml (9.94% of BW) for Jersey and Holstein calves, respectively. Body weight was the best predictor of plasma volume.
(**Key words**: calves, plasma volume, Holstein, Jersey)

Abbreviation key: **AEA** = apparent efficiency of absorption, **EBD** = Evans' blue dye, **PV** = plasma volume.

INTRODUCTION

The degree of acquisition of passive immunity in neonatal calves is usually determined by measuring circulating concentrations of Ig or IgG at 24 to 48 h after birth. Although this procedure can provide a measure of the degree of passive immunity, it does not indicate the efficiency with which Ig are absorbed prior to closure. Apparent efficiency of absorption (**AEA**) is calculated as [plasma IgG (grams per liter) × plasma volume (**PV**; liters) ÷ IgG intake (grams)] × 100.

Determination of PV or blood volume is necessary to estimate AEA. Previous estimates of PV vary. Möllenberg et al. (15) reported that PV increased from 5.3% of BW at birth to 6.5% of BW at 1 d of age. Calves in this study were fed colostrum (15% of BW in four feedings) after the initial PV measurement. McEwan et al. (10) reported an increase in PV from 6.6 to 9.3% of BW after calves were fed colostrum during the 1st d after birth. Most published studies used only a limited number of calves (usually <10) of various breeds, which might have influenced estimates of PV. Other factors that may influence colostral protein absorption, and possibly PV, include colostral management, sex of the calf, and age at which calves were fed and sampled. The freezing and storage of colostrum may influence absorption of colostral components, particularly colostral leukocytes (K. R. Martin and J. D. Quigley, III, 1997, unpublished data). Whether the freezing and thawing of colostrum influences absorption of colostral components and, possibly, PV is unknown. Objectives of this study were to determine whether the method of colostrum management (frozen and thawed colostrum vs. fresh maternal colostrum) influenced PV at 24 h after birth and to determine relationships among PV, breed, BW, sex, age at feeding, and intake of colostrum by calves.

MATERIALS AND METHODS

Calves from five dairy farms at the Tennessee Agricultural Experiment Station (Knoxville) were used in the study. Holstein calves (n = 97) from four farms and Jersey calves (n = 49) from one farm were used. Calves were not allowed to nurse the dam. Any calf that was not observed during calving and calves that were standing prior to removal from the dam

Received July 11, 1997.
Accepted December 9, 1997.
[1]Current address: American Protein Corporation, 2325 North Loop Dr., Ames, IA 50010.

were not used. Calves were removed from the dam as soon as possible after birth, weighed, and placed in individual hutches or pens in a calf barn.

Calves were offered fresh colostrum from the dam or previously frozen and thawed colostrum from a cow other than the dam. Frozen colostrum was obtained from donor cows that calved prior to use in this study. The colostrum was placed in 2-L plastic storage bags and stored at –20°C. Frozen colostrum was placed in warm tap water (temperature was not determined) and allowed to thaw during parturition. Colostrum was fed in two 2-L feedings approximately 12 h apart. Times of birth, colostrum feeding, and sampling were recorded.

An initial blood sample was collected by jugular puncture at approximately 24 h of age. Blood was collected into an evacuated tube containing sodium heparin. Immediately after the initial blood sample was collected, approximately 1.5 ml of 1.5% Evans' blue dye (**EBD**) were injected into the jugular vein using a 3-ml syringe. Syringes were weighed to the nearest milligram before and after injection to measure the amount of dye injected. Following a 10-min equilibration, a second jugular blood sample was collected. Single-point estimates of marker concentration have been used to estimate PV previously (2, 4, 9, 10, 11). Both blood samples were centrifuged, and plasma was collected and stored at –20°C prior to transport to the laboratory.

Plasma was analyzed for EBD by spectrophotometry (620 nm). Initial plasma samples from each calf were used to set absorbance of the spectrophotometer to 0. Then, 50 μl of 1.5% EBD solution (wt/vol) were added to 1.0 ml of initial plasma sample, and absorbance was determined and used as the standard for the calf. Absorbance of the postinjection plasma sample was determined, and PV was calculated (21).

Regression procedures were used to determine relationships between PV at 24 h of age and birth BW, sex of calf, farm of origin, ages at feeding and sampling, amounts of colostrum fed, method of colostrum management, and breed of calf (18). Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Initially, 110 samples from Holstein calves and 60 samples from Jersey calves were obtained. However, because of hemolysis or errors in dye injection or sample collection, only 97 and 49 samples, respectively, were used for data analysis. Descriptive data are presented in Table 1. Hemolysis may cause significant errors in EBD analysis (4, 7); therefore, hemolyzed samples were excluded from the study. Additional objectives of this study were to determine plasma IgG concentrations and estimates of AEA of IgG in calves; however, because of a freezer failure, all samples thawed for an undetermined period, precluding IgG analyses.

TABLE 1. Colostrum intake, ages at feeding and sampling, and estimates of BW and plasma volume (PV) in Holstein and Jersey calves.

Item	$\bar{X}$	SE	Minimum	Maximum
Colostrum intake, L	3.76	0.04	1.50	4.00
Age at first feeding, h	3.96	0.18	0.33	11.00
Age at sampling, h	24.3	0.07	22.75	28.75
BW, kg	32.2	0.7	17.3	48.6
PV, ml	3162	79	1694	5804
PV, % of BW	9.86	0.15	6.39	14.56
Holstein calves (n = 97)				
BW, kg	36.7	0.6	17.3	48.6
PV, ml	3622	82	1745	5804
PV, % of BW	9.94	0.20	6.39	14.56
Jersey calves (n = 49)				
BW, kg	23.4	0.4	18.5	31.5
PV, ml	2250	53	1694	3458
PV, % of BW	9.71	0.21	6.52	13.47

Evans blue dye binds to albumin and has been extensively used as a marker of PV (2, 4, 5, 8, 12, 15, 16, 22, 23). Reports generally indicate close correlation of estimates of PV with other methods (24), although van Waversveld and van Bruchem (23) indicated that EBD estimated total blood volume and not PV.

Multiple regressions of PV indicated that factors affecting PV (milliliters) were birth BW ($P < 0.0001$), breed ($P < 0.03$), and age at sampling ($P < 0.04$); r^2 of the regression was 0.60. The regression equation was PV (milliliters) = –2393.1 + 68.09 × BW (kilograms) + 404.1 × breed (0 = Jersey; 1 = Holstein) + 127.3 × age at sampling (hours).

Factors affecting PV (percentage of BW) were birth BW ($P < 0.005$), breed ($P < 0.02$), and age at sampling ($P < 0.06$); r^2 of the regression was 0.08. The regression equation was PV (percentage of BW) = 3.66 – 0.089 × BW (kilograms) + 1.27 × breed (0 = Jersey; 1 = Holstein) + 0.34 × age at sampling (hours).

Mean PV for all calves was 3162 ml (SE = 79) and was 9.86% of birth BW. Mean PV was 2250 ml (9.71% of BW) and 3623 ml (9.94% of BW) for Jersey and Holstein calves, respectively. Birth BW was the best predictor of PV (Figure 1) based on the amount of variation accounted for by each indepen-

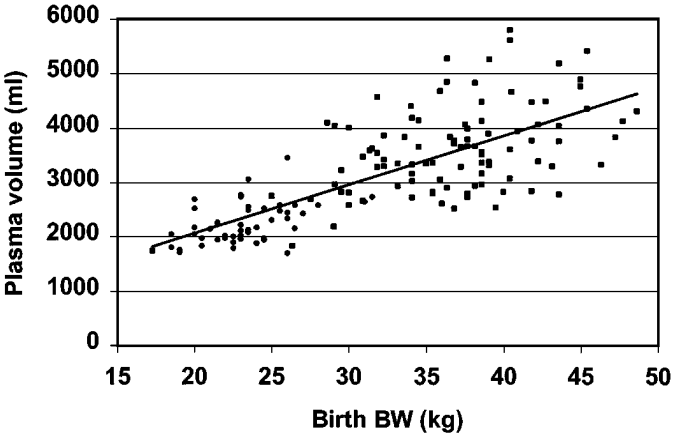

Figure 1. Relationship between plasma volume (milliliters) and birth BW of Jersey (●) and Holstein (■) calves.

dent variable. However, the relationship between BW at birth and PV was not linear, because birth BW was significant in the equation to predict PV as a percentage of BW. The negative coefficient indicated that as BW increased, PV as a percentage of BW decreased. The natural logarithm of BW improved the r^2 of the estimate of PV (milliliters) to 0.65. The relationship between PV and BW is well defined (4, 15, 19), and correlations usually exceed 0.8. Haxton et al. (7) reported that PV was more closely associated with total body surface area than with BW. In our study, however, metabolic BW ($BW^{0.75}$), total body surface area (7), or curvilinear terms of birth BW did not improve prediction. The greater variation in the present study may be related to the relatively small variation in calf BW and the effect of other factors (e.g., absorption of liquid from the gut, degree of physical activity) on PV. Differences in PV at a given BW make estimation of PV as a proportion of BW in neonatal calves difficult.

Estimates of PV were quite variable. Confidence intervals around predictions of PV as a percentage of BW (Figure 2) indicated considerable variation around PV prediction. For example, at 39.0 kg of BW, confidence intervals ranged from 6.0% of BW to 13.1% of BW around a mean of 9.58% of BW.

Breed of calf influenced estimates of PV at 24 h of age. Holstein calves had 404 ml more plasma than did Jersey calves at a similar BW and age at sampling. However, all Jersey calves were at one location; therefore, breed might have been confounded with location. Within Holstein calves, farm location (n = 4) had no effect on PV at 24 h, suggesting that location might not have been a significant factor that affected PV.

Estimates of PV increased as age at sampling increased. Others (13, 14) also reported that PV increased as age increased to approximately 24 h because of the absorption of water and protein from the gut. Conversely, Matte et al. (9) reported a decline in PV in calves fed colostrum at later ages. Estimates of PV declined from 14.5% of BW in calves that were initially fed colostrum at 6 h of age to 7.9% of BW in calves that were initially fed colostrum at 36 h of age. In the study by Matte et al. (9), all calves were sampled 6 h after colostrum was fed, indicating that advancing age may affect absorption of colostrum and estimates of PV. Changes in PV are dynamic and result from water redistribution between intracellular and extracellular fluid compartments or from the influx of water from the gut (13). Age at first feeding, age at second feeding, amount of colostrum consumed, and sex of the calf did not significantly affect estimates of PV in our study.

Colostrum management (fresh vs. frozen and thawed) had no effect on PV in calves at any location. Mean PV in calves fed fresh or frozen and thawed colostrum were 3230 ml (SE = 116) and 3097 ml (SE = 107), respectively. A lack of treatment effect indicated that colostrum management did not affect PV, although the freezing and thawing of colostrum kills viable leukocytes found in fresh colostrum and may affect absorption of colostral leukocytes (K. R. Martin and J. D. Quigley, III, 1997, unpublished data).

The loss of EBD from vascular circulation during the 10-min equilibration period might have influenced estimates of PV (8, 15, 16). Payne et al. (16) reported that losses of EBD from the vascular circulation in cattle differ from losses in humans and dogs; those researchers hypothesized that differences in vascular permeability among species might be

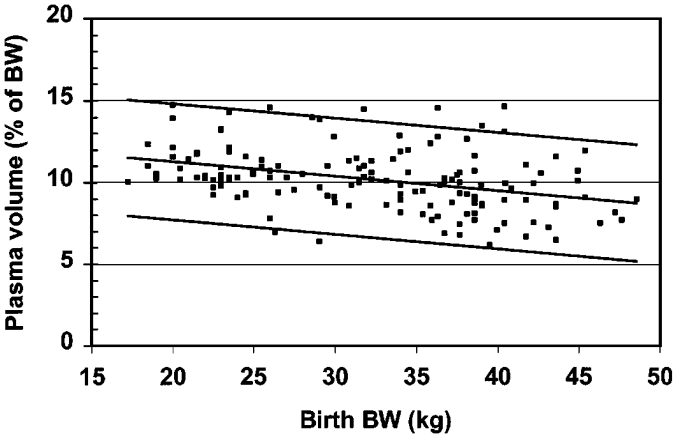

Figure 2. Regression and 95% confidence intervals of plasma volume (percentage of BW) and birth BW of Holstein calves. Data are corrected to 24 h of age at blood sampling.

TABLE 2. Estimates of plasma volume (PV) in Holstein and Jersey calves.[1]

	Age of Holsteins					Age of Jerseys				
BW	22 h	23 h	24 h	25 h	26 h	22 h	23 h	24 h	25 h	26 h
(kg)	(ml)									
20	...	...	...	...	...	1604	1719	1835	1950	2065
25	2279	2394	2509	2625	2740	1912	2028	2143	2258	2374
30	2587	2702	2818	2933	3048	2221	2336	2452	2567	2682
35	2895	3011	3126	3242	3357	2529	2645	2760	2875	2991
40	3204	3319	3435	3550	3665	...	...	...	...	...
45	3512	3628	3743	3859	3974	...	...	...	...	...
	(% of BW)									
20	...	...	...	...	...	8.03	8.94	9.84	10.75	11.65
25	9.11	9.58	10.04	10.50	10.96	7.58	8.31	9.03	9.75	10.48
30	8.62	9.01	9.39	9.78	10.16	7.28	7.88	8.49	9.09	9.69
35	8.27	8.60	8.93	9.26	9.59	7.07	7.58	8.10	8.62	9.13
40	8.01	8.30	8.59	8.88	9.16	...	...	...	...	...
45	7.81	8.06	8.32	8.57	8.83	...	...	...	...	...

[1]Corrected for 10-min sampling by multiplying estimated PV by 0.906.

responsible. Payne et al. (16) suggested that the loss of EBD from plasma was due to escape of albumin and dye into extravascular space, turnover of plasma albumin and metabolism of dye, and removal of unbound dye. Möllenberg et al. (15) suggested that PV estimated using EBD in blood samples taken 10 min after injections of EBD overestimated PV by 6.2% compared with serial sampling and extrapolation to time 0. Mackie (8) also reported that PV was overestimated by 12.6% when a 10-min sampling schedule was used compared with a continuous sampling schedule in mature sheep. Conversely, Greenleaf et al. (6) reported little difference between estimates of PV in humans injected with EBD using a 10-min sampling schedule and infusion and repeated sampling with ^{131}I. Age of the animal may also influence the rate of loss of EBD from circulation; younger animals lose EBD from circulation more rapidly than do older animals (22). Persson and Ullberg (17) reported that the overestimation caused by single-point sampling was highest in younger animals and declined as the animals aged. However, Persson and Ullberg (17) concluded that the mean overestimation in foals (approximately 5%) caused by single-point sampling was negligible, provided that albumin turnover was normal and no edematous state prevailed. Möllerberg et al. (15) reported that the rate of elimination of labeled albumin did not change in calves from birth to 90 d. If our data are corrected for the overestimated PV caused by our sampling technique, actual PV in our calves was 9.86% of BW × 0.906 or 8.93% of BW. The correction factor (0.906) is the mean of the overestimations reported by Mackie (8) and Möllenberg et al. (15).

Some researchers (1, 3, 20) have assumed that PV was a fixed percentage of BW (usually 7%). Our data suggest a more appropriate estimate is 8.93% of BW in calves fed 1.5 to 4 L of colostrum and sampled at approximately 24 h of age. Estimates of PV at various BW and ages at sampling are presented in Table 2. Our results suggest that estimates of PV based on BW are subject to considerable variation.

CONCLUSIONS

Plasma volume in neonatal calves was unaffected by method of colostrum management (fresh vs. frozen and thawed) and age at first feeding. However, PV was influenced by BW, age at sampling, and breed. Mean PV was 9.86% of BW, but, when adjusted for loss of EBD from plasma caused by single-point sampling, the mean PV was 8.9% of BW.

ACKNOWLEDGMENTS

The authors thank the superintendents and staff of the Tennessee Experiment Stations who collected samples in this study.

REFERENCES

1 Besser, T. E., A. E. Garmedia, T. C. McGuire, and C. C. Gay. 1985. Effect of colostral immunoglobulin G, and immunoglobulin M concentrations on immunoglobulin absorption in calves. J. Dairy Sci. 68:2033–2037.

2 Besser, T. E., and D. Osborn. 1993. Effect of bovine serum albumin on passive transfer of immunoglobulin G1 to newborn calves. Vet. Immunol. Immunopathol. 37:321–327.

3 Cruywagen, C. W. 1990. Effect of curd forming of colostrum on absorption of immunoglobulin G in newborn calves. J. Dairy Sci. 73:3287–3290.

4 Dalton, R. G., and E. W. Fisher. 1961. Plasma and blood volumes in Ayrshire cattle. Br. Vet. J. 117:115–119.

5 English, P. B. 1966. A study of water and electrolyte metabolism in sheep. II. The volumes of distribution of anti-

pyrine, thiosulfate, and T1824 (Evans blue) and values for certain extracellular fluid constituents. Res. Vet. Sci. 7:258–275.
6 Greenleaf, J. E., V. A. Convertino, and G. R. Mangseth. 1979. Plasma volume during stress in man: osmolality and red cell volume. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 47:1031–1038.
7 Haxton, J. A., M. D. Schneider, and M. P. Kaye. 1974. Blood volume of the male Holstein-Friesian calf. Am. J. Vet. Res. 35: 835–837.
8 Mackie, W. S. 1976. Plasma volume measurements in sheep using Evans' blue and continuous blood sampling. Res. Vet Sci. 21:108–109.
9 Matte, J. J., G. L. Girard, J. R. Seoane, and G. J. Brisson. 1982. Absorption of colostral immunoglobulin G in the newborn dairy calf. J. Dairy Sci. 65:1765–1770.
10 McEwan, A. D., E. W. Fisher, and I. E. Selman. 1968. The effect of colostrum on the volume and composition of the plasma of calves. Res. Vet. Sci. 9:284–286.
11 McEwan, A. D., E. W. Fisher, and I. E. Selman. 1970. An estimation of the efficiency of the absorption of immune globulins from colostrum by newborn calves. Res. Vet. Sci. 11: 239–243.
12 McKeever, K. H., W. A. Schurg, and V. A. Convertino. 1988. A modified Evans blue dye method for determining plasma volume in the horse. Equine Vet. Sci. 8:208–212.
13 McMurray, C. H., E. F. Logan, P. J. McParland, F. J. McRoy, and D. G. O'Neill. 1978. Sequential changes in some blood components in the normal neonatal calf. Br. Vet. J. 134: 590–597.
14 Molla, A. 1978. Immunoglobulin levels in calves fed colostrum by stomach tube. Vet. Rec. 103:377–380.
15 Möllerberg, L., L. Ekman, and S. Jacobsson. 1975. Plasma and blood volume in the calf from birth till 90 days of age. Acta Vet. Scand. 16:178–185.
16 Payne, E., J. W. Ryley, and R.J.W. Gartner. 1967. Plasma, blood, and extracellular fluid volume in grazing Hereford cattle. Res. Vet. Sci. 8:20–26.
17 Persson, S.G.B., and L.-E. Ullberg. 1979. Blood volume determination with Evans blue dye in foals. Acta Vet. Scand. 20:10–15.
18 SAS® User's Guide: Statistics, Version 6 Edition. 1988. SAS Inst., Inc., Cary, NC.
19 Spensley, M. S., G. P. Carlson, and D. Harrold. 1987. Plasma, red blood cell, total blood, and extracellular fluid volumes in healthy horse foals during growth. Am. J. Vet. Res. 48: 1703–1707.
20 Stott, G. H., and B. E. Menefee. 1978. Selective absorption of immunoglobulin IgM in the newborn calf. J. Dairy Sci. 61: 461–466.
21 Swenson, M. J. 1984. Blood circulation and the cardiovascular system. Pages 15–40 *in* Dukes' Physiology of Domestic Animals. M. J. Swenson, ed. Cornell Univ. Press, Ithaca, NY.
22 Thornton, J. R., and P. B. English. 1978. Body water of calves: change in distribution with diarrhoea. Br. Vet. J. 134:445–453.
23 van Waversveld, J., and J. van Bruchem. 1985. Estimation of blood (plasma) volume in sheep with Evans blue and bromsulphalein. Neth. J. Agric. Sci. 33:325–327.
24 Wagstaff, A. J., I. Maclean, A. R. Mitchell, and P. H. Holmes. 1992. Plasma and extracellular volume in calves: comparison between isotopic and 'cold' techniques. Res. Vet. Sci. 53: 271–273.