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Estimating plasma volume in neonatal Holstein calves fed one or two feedings of a lacteal-based colostrum replacer using Evans blue dye and hematocrit values at various time points.

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ABSTRACT

Twenty-eight Holstein calves were blocked by birth date and randomly assigned to one of two treatments to investigate the effect of colostrum replacer (**CR**) feeding regimen on plasma volume (**PV**). Treatments were: 1) one feeding of CR (**C_1**; 3L of reconstituted CR 675 g of powder providing 184.5 g of IgG at birth) or 2) two feedings of CR (**C_2**; 2L of reconstituted CR at birth and 1 L of reconstituted CR at six h). By 6 h of age, all calves had received 3L of CR providing 184.5 g of IgG. Plasma volume was estimated at six, 12, 18, and 24 h after birth using Evans blue dye (**EBD**). No treatment effects were noted at any time points ($P > 0.05$). Mean PV for all calves regardless of treatment at six, 12, 18, and 24 h were 78.6, 89.2, 83.9, and 90.7 mL kg^{-1} of BW, respectively. Plasma volume was correlated with hematocrit (**HCT**), initial HCT, and treatment. Hematocrit was correlated with PV, initial HCT, and body weight. Hematocrit for six, 12, 18 and 24 h after birth can be predicted with an initial precolostral HCT determination.

RÉSUMÉ

Vingt-huit veaux de race Holstein ont été classés par leurs dates de naissance et soumis aléatoirement à l'un de deux traitements afin d'examiner l'effet d'un régime alimentaire de remplacement de colostrum (**RC**) sur le volume plasmatique (**VP**). Les traitements étaient : 1) Un dosage de RC (**C_1**; 184,5 g de IgG à la naissance) ou 2) deux dosages de RC (**C_2**; 2/3 de la dose totale à la naissance, 1/3 de la dose totale 6 heures plus tard). Le volume plasmatique a été estimé six, douze, dix-huit et vingt-quatre heures après la naissance. Le colorant bleu d'Evans (**CBE**) a été utilisé pour estimer le VP; l'effet du traitement n'a été observé à aucun moment sur le VP ($P > 0,05$). Indépendamment du traitement, les moyennes de VP pour tous les veaux à six, douze, dix-huit et vingt-quatre heures étaient 78,6; 89,2; 83,9 et 90,7 mL kg^{1} de poids corporel

(**PC**), respectivement. Le VP a été corrélé à l'hématocrite (**HtC**), l'HtC initial et au traitement. L'hématocrite a été corrélé au VP, à l'HtC initial et au poids. L'hématocrite à six, douze, dix-huit et vingt-quatre heures après la naissance peut-être prédit par une détermination initiale de l'HtC avant l'ingestion de colostrum.

Key words: calf, plasma volume, colostrum replacer, hematocrit

Abbreviations: AEA, apparent efficiency of absorption; **CR**, colostrum replacer; **EBD** Evans Blue Dye; **HCT** hematocrit; **I** μg of dye injected; **IgG** immunoglobulin G; **PV** plasma volume; **S** absorbance of standard; **U** absorbance of unknown

INTRODUCTION

Apparent efficiency of absorption (**AEA**) is commonly calculated in neonatal calf research as another means of viewing Immunoglobulin G (**IgG**) absorptive data. The AEA can be estimated using the equation: [plasma IgG (g/L) x BW (kg) x 0.09/IgG mass consumed (g)] x 100% (Quigley et al., 1998). Within the equation, the value 0.09 refers to PV, which has been measured in the calf at 24 and 48 h of age. Prior to the work of Quigley et al. (1998), Mollenberg et al. (1975) reported PV increasing from 53 mL (plasma)kg^{-1} of BW at birth to 65 ml kg^{-1} of BW at one day old after being fed 150 g of colostrum kg^{-1} of BW in four feedings and McEwan et al. (1968) indicated that PV increased from 66 ml kg^{-1} of BW at birth to 93 ml kg^{-1} of BW at the end of d one after calves were fed colostrum. Quigley et al. (1998) attributed the discrepancies in PV values in the aforementioned studies to the small number of calves used. These data are also limited by PV being measured only at birth and 24 h after birth. Researchers feeding CR utilize the equation of Quigley et al. (1998). However, that equation was derived from calves fed colostrum. Most researchers studying colostrum feed calves 4L while adequate passive transfer can occur in calves fed 3 L of CR. Therefore, we question the appropriateness of using the equation of Quigley et al. (1998) for calves fed CR. An objective of this study was to provide PV of calves fed CR for the determination of AEA at different time points such as six, 12, and 18 h after birth.. The second objective of this experiment was to determine if there were differences in PV due to feeding regimen of CR using EBD at six, 12, 18, and 24 h after birth in Holstein dairy calves. The third objective was to determine PV at different time points and to determine if there was any correlation between HCT values and PV determined by the EBD procedure. If HCT values are correlated with PV, then it is possible that PV could be calculated on a per calf

basis using HCT values at each time point. The last objective of this study was to determine if HCT could be predicted using precolostral HCT values.

MATERIALS AND METHODS

Experimental Design and Treatment Diets

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (# 111205 and 130105).

Twenty-eight Holstein calves were born from multiparous and primiparous cows and blocked by birth date. Calves were removed from dams within 30 min of birth, weighed and had their navels dipped with a 7 % tincture of iodine. Calves were individually housed for the duration of the experiment. All calves received 3 sachets of Calf's Choice Total ™Gold CR containing a total IgG mass of 184.5 g reconstituted, according to manufacturer's instructiona to a final volume of 3L (The Saskatoon Colostrum Co., Saskatoon, SK). One sachet of product contains 225 g of powder. Therefore, all calves received 675 g of powder. Assuming 950 g kg^{-1} (DM) each calf received 641 g of solids with a final volume of 3 L. Total solids equaled 210 g/L. Calves were assigned to one of two treatments: 1) one feeding of CR (C_1) or 2) two feedings of CR (C_2). Calves fed a single feeding of CR received the total dose at 0 h (3L CR, 184.5 g IgG). Calves assigned to two feedings received 2/3 of the total CR dose at 0 h (2L CR, 123 g IgG) and the remaining 1/3 of CR at six h (1L CR, 61.5 g IgG). All calves had consumed 3L of CR by six h. Calves were fed via nipple bottle with the first feeding of CR given at 0 h (within 30 min of birth). If the calf did not consume the entire volume of CR, it was administered via esophageal tube to ensure the total dose was received.

115

116 **Evans Blue Dye Procedure**

117 Blood samples for PV analysis were collected at six, 12, 18, and 24 h of age. The stock
118 solution of EBD was prepared by weighing 2 g of 750 g kg^{-1} EBD into a volumetric flask mixed
119 with 100 mL of 9 g L^{-1} sterile NaCl and stored at 3ºC. At all sampling times, 10 mL of blood was
120 collected into Vacutainer tubes containing freeze-dried Na-heparin (Kendall, Mansfield, MA,
121 USA) via jugular venipuncture using a 22-gauge needle. After the pre-injection blood sample
122 was collected, approximately 1.5 mL of stock solution was injected into the jugular vein. The
123 syringe was weighed prior to and after injection so exact amount of dye administered was
124 known. After injection, the dye was allowed to equilibrate for 10 min before a post-injection
125 blood sample was obtained into 10 mL Na-heparinized Vacutainer tubes. Blood samples were
126 centrifuged (CentraMP4R; International Equipment Company; Needham HTS, MA, USA) at
127 1,310 × *g* at - 4°C for 20 min (within 15 min of collection). The plasma was harvested and stored
128 at -20°C until PV analysis via spectrophotometry. Absorbance of the spectrophotometer
129 (Beckman DU 520; Brea, CA, USA) was set to 620 nm. Upon analysis, plasma samples were
130 thawed and 1 mL of pre-injection plasma was transferred into a cuvette and the
131 spectrophotometer was blanked against the sample. Fifty µl of standard dye solution (prepared
132 by adding 1 mL of stock solution to a 100-mL volumetric flask which was diluted with 9 g L^{-1}
133 NaCl solution) was added to the plasma blank, vortexed and absorbance read. Finally,
134 absorbance of the post-injection sample was obtained. The PV was calculated using the equation:
135 [1 / ((U÷ S) x 7.142857)] × I, where U = absorbance of unknown (post-injection sample); S =
136 absorbance of standard (pre-injection sample + 50 µl of standard dye solution); and I = µg of dye
137 injected (mL of stock injected × 15 mg dye mL^{-1} stock × 1000), 7.142857 is equal to the amount

of dye in the standard plasma sample (50 µL of dye containing 0.15 mg dye mL^{-1} of standard dye solution added to 1 mL of plasma = 7.5 µg ÷1.05mL).

Blood Collection for Hematocrit

A subsample of blood collected for PV analysis was obtained for evaluation of packed cell volume. An initial sample was obtained at birth before CR feeding for HCT analysis as well. Capillary tubes were centrifuged (Haematokrit 210; Andreas Hettich GmBH & Co; Germany) at 16,060 × g at 25°C for 5 min.

Statistical Analysis

Plasma volume and HCT values at six, 12, 18, and 24 h were analyzed using the mixed procedure of SAS® (Version 9.3; SAS Institute, Inc. Cary, NC, USA) as a randomized complete block design according to the following model:

$$Y_{ijk} = \mu + B_i + C_j + H_k + E_{ijk}$$

Where:
Y = the dependent variable;
µ = the overall mean;
B_i = the random effect of block i (i = 1,…14);
C_j = the fixed effect of the j^{th} CR feeding regimen (k = 1, 2);
H_k = the fixed effect of the k^{th} hour (k = 6, 12, 18, and 24);
E_{ij} = the residual error ~$N(O_1\sigma^2 e)$.

Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS® (Version 9.3; SAS Institute, Inc. Cary, NC, USA). Least square means were determined for each treatment. Significant treatment effects were noted at $P \leq 0.05$ and trends were noted at $0.05 < P \leq 0.10$. Regression analysis was conducted using the regression procedure of SAS using PV (g kg^{-1} BW) and HCT. Variables used in the regression analysis for PV were: sex, HCT, initial HCT, BW and treatment. Variables used in the regression analysis for HCT

were: sex, initial HCT, BW, treatment, hour (for overall value), and PV (mL). Correlation coefficients were determined using the correlation procedure of SAS (Version 9.3; SAS Institute, Inc., Cary NC, USA),

Results

Mean BW was 46 ± 4.61 kg, mean initial HCT was 39.4 ± 6.82 %, mean HCT was 35.2 ± 5.83 %, and mean PV was 3,866 ± 2022 mL (Tables 1 and 2). Three calves on the C_1 and two calves on C_2 treatment groups were fed using an esophageal tube feeder. The calves on treatment C_1 required all of the CR to be fed in this manner. Whereas, one calf on C_2 required all of the CR fed in this manner while the other calf consumed its first feeding via nipple bottle, but was fed the total six h dose with the esophageal feeder. Initially, 28 samples per time point were obtained; however, due to sampling error or hemolysis, 4 calves were removed from analysis (2 per treatment). Analyses of PV (mL, and mL kg^{-1} of BW) along with HCT data are presented in Table 1. Mean PV for all calves regardless of treatment at six, 12, 18, and 24 h were 78.2, 89.2, 83.9, and 90.7 mL kg^{-1} of BW, respectively. Treatment did not affect PV expressed as either mL or mL kg^{-1} of BW at any time point (Table 1). Hematocrit did not vary at any time point (Table 1). Mean, minimum and maximum values for PV, BW, initial HCT and HCT are in Table 2. Correlation coefficients (Table 3) for PV showed strong relationships between PV, HCT, initial HCT and treatment. Prediction equations for PV as a percent of BW had coefficients of multiple determinations (r^2) values that ranged from 0.19 for the 12 h prediction equation to 0. 32 for the 6 h equation suggesting a weak relationship (Table 4).

Prediction equations for HCT had r^2 values that ranged from 0.69 for the 6 h equation to 0.84 for the 24 h equation (Table 5. Body weight and initial HCT can be used to predict HCT at

12 and 24 h. Whereas, these variables plus sex of the calf can predict HCT at six and 18 h (Figure 1).

Discussion

Evans blue dye has been used as a marker of PV in cattle (Dalton and Fisher, 1961;; Payne et al., 1967; Möllenberg et al., 1975; Thornton and English, 1978; Besser and Osborn, 1993), sheep (English, 1966; Mackie, 1976; van Waversveld and van Bruchem, 1985), horses (McKeever et al., 1988), and pigs (McCance and Widdowson, 1959). Estimates of PV using EBD and other methods typically indicate close correlation; however, van Waversveld and van Brushem (1985) reported that EBD estimated total blood volume but not PV. However a strong correlation was found between PV and hematocrit ($P = 0.006$). Quigley et al. (1998), with 146 calves sampled, also reported large variability in PV among calves even with the same BW (39 kg) with PV as mL kg^{-1} of BW ranging from 60 – 131. This is similar to calves used on the current study with a BW of 40 kg having PV ranging from 67 – 131.

Research in human physiology has indicated that PV can be estimated by using a patient's height, weight, and HCT; however, equations are only reliable at normal HCT. Sprenger et al. (1987) were able to modify equations and allow for accurate calculation of PV even with the use of pathological HCT but PV was measured using 51CR-method and not EBD. Quigley et al. (1998) reported that birth BW was the best predictor of PV, though the relationship was not linear; however, multiple regression analysis using HCT and BW yielded no correlation with PV. Our results may have been affected by the use of EBD and perhaps a more accurate method of PV estimation should be used in the future.

There was no difference in PV between treatments across the 4 sampling times. Regardless of treatment, mean PV for all calves at six, 12, 18, and 24 h were 78.2, 89.2, 83.9

211 90.7 mL kg^{-1} of BW, respectively. Quigley et al. (1998) reported PV at 24 h at 98.6 mL $kg^{-1}$of
212 BW. The authors also adjusted for loss of EBD from plasma caused by single-point sampling by
213 multiplying PV as mL $kg^{-1}$of BW by 9.06 (correction factor determined as the mean
214 overestimations reported by Mackie (1976) and Möllenberg et al. (1975)), which resulted in a
215 new mean PV of 89 mL kg^{-1} of BW. Adjusting PV at 24 h by this same method reduces our
216 estimate of PV to 82.5 mL kg^{-1} of BW. Adjusting for EBD loss at six, 12 and 18 h results in
217 new PV values of : 71.1, 81.1, 76.3 ml kg^{-1} . Quigley et al. (1998) also indicate that age at
218 sampling averaged 24.3 h but ranged from 22.75 – 28.75 h. All calves used in the current study
219 had samples obtained within ±15 min from designated time point (six 12, 18, or 24 h). The
220 differences in sampling time may have impacted PV values. Colostrum intake type and volume
221 (3L of CR vs, 1.5-4L colostrum) were also inconsistent between studies. The current study
222 utilized CR with esophageal tube feeding used to ensure the total volume of CR was consumed.
223 Quigley et al. (1998) utilized colostrum with intake averaging 3.76 L but ranging from 1.50 –
224 4.00 L. Differences in volume of colostrum consumed may have affected hydration status and,
225 therefore, PV of their calves.
226 Calves on the current study were also sampled at four total time points, as compared to
227 one time point in the previously mentioned study. This may have affected the integrity of the
228 blood vessel leading to an increase in loss of EBD during the equilibration period. Samples
229 obtained at six, 12, 18 h and 24 h may have been slightly inaccurate because of the rate of loss of
230 EBD from circulation. Younger animals lose EBD from circulation more rapidly than older
231 animals (Thornton and English, 1978). By 24 h of age, calves were more apt to struggle during
232 administration of dye and blood sampling. This may have increased EBD loss from circulation
233 therefore adding to sample inaccuracy.

Results from this experiment provide researchers with values to allow determination of AEA when CR is fed. Also, determination of AEA at different time points over the first 24 h of life which will allow for a more accurate determination of when IgG absorption is maximized.

Hematocrit was not different between treatments. Hematocrit is a measure of packed cell volume, which is an indicator of hydration status. Though the total volume was identical, spreading the feeding between 2 time periods may have affected hydration status. Overall, HCT decreased from birth to 24 h indicating an increase in hydration status. Hematocrit, sex, treatment and initial HCT were correlated with PV in this study. Initial BW, sex and precolostral HCT can be used to predict HCT at six,12, 18 and 24 h after birth in Holstein calves receiving CR.

Conclusion

Feeding CR in either one or split into two feedings did not affect PV, or HCT measurements. Plasma volume was highly correlated to sex, HCT, initial HCT and feeding regimen. Prediction equations to determine PV utilizing BW, sex, feeding regimen, initial HCT and HCT had correlation coefficients that ranged from 0.25 to 0.40 indicating a weak relationship. However, a better relationship was found for predicting HCT with r^2 ranging from 0.69 to 0.84. An initial precolostral blood sample along with BW can accurately predict HCT at 12, and 24 h. Those values along with an adjustment for sex of the calf can accurately predict hematocrit at six and 18 h after birth. Researchers evaluating CR can utilize these data to accurately determine AEA and pinpoint when IgG absorption is maximized..

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Table 1. Calves, body weight, plasma volume and hematocrit for calves fed colostrum replacer either all at birth or split between two feedings.

	Treatement[1]		
Item	C_1	C_2	SE[2]
BW, kg (+/- SD)	44.90 +/- 4.0	46.70 +/- 5.1	
Initial HCT, %	38.25+/- 3.36	41.09 +/- 3.50	
PV[3], 6h, mL	3,522	3,590	289
PV, 12h, mL	3,612	3,967	289
PV, 18h, mL	3,221	4,376	289
PV, 24h, mL	3542	3,615	289
PV, 6h, mL kg^{-1} BW	78.5	78.7	8.2
PV, 12h, mL kg^{-1} BW	90.2	88.2	8.2
PV, 18h, mL kg^{-1} , BW	80.0	87.9	8.2
PV, 24h, mL kg^{-1} BW	81.4	100.0	8.2
HCT[5], 6h, %	36.8	38.0	1.3
HCT, 12h, %	34.3	35.1	1.3
HCT, 18h, %	33.8	35.4	1.3
HCT, 24h, %	34.2	35.0	1.3

[1]Treatment C_1 = all colostrum replacer is fed at time 0 h, C_2 = colostrum replacer fed 2/3 at time 0 h and 1/3 at 6h.

[2]Standard error.

[3]Repeated measures by hour for plasma volume (mL) was not different (P< 0.13).

[4]Repeated measures by hour for plasma volume (% of body weight) was not different (P = 0.54).

[5]Repeated measures by hour for hematocrit was not different (P= 0.99).

Table 2. Mean, minimum and maximum values and standard deviations for data used in development of prediction equations.

Variable	n	Mean	SD	Minimum	Maximum
PV, mL	95	3,430	1,333	1376	7176
BW, kg	97	45.87	4.68	40.0	56.0
Initial HCT, %	97	39.63	6.90	24.0	54.0
HCT, %	94	35.37	5.78	20.0	45.0

Table 3. Correlation coefficients for plasma volume, sex, body weight, hematocrit, initial hematocrit, and treatment.

Item	PV[1] (mL)	Sex	BW[2]	HCT[3]	Hi[4]	Trt[5]
PV (mL)	1.000	0.03	-0.12	-0.21	-0.26	0.13
		P = 0.74	P =0.28	P = 0.06	P = 0.02	P =0.24
Sex		1.000	-0.51	-0.01	0.07	-0.08
			P <0.0001	P= 0.92	P = 0.49	P = 0.39
BW			1.000	0.33	0.09	0.20
				P=0.0005	P = 0.38	P = 0.04
HCT				1.000	0.81	0. 08
					P <0.0001	P = 0.41
Hi					1.000	0.06
						P = 0.51
Trt						1.000

[1]Plasma volume in mL.

[2]Body weight.

[3]Hematocrit.

[4] Initial Hematocrit.

[5]Treatment- feeding regimen (one feeding of CR or 2 feedings of CR).

Table 4. Prediction equations for plasma volume (% of BW) at various time points[1].

Hour[2]	Intercept	Sex[3]	BW[4]	Initial HCT[5]	HCT[6]	Trt[7]	r^2
			kg	%			
6	21.80	-	-0.28	-0.11	-	1.66	0.32
12	19.93	-	-	-0.29	-		0.19
18	14.38	2.51	-	-	-0.28	-	0.22
24	11.58	2.80	-	-	-0.31	2.56	0.31

[1]Variables with blank spaces indicate that P >/= 0.25.

[2]Hour corresponds to time after birth.

[3]Sex ,1 = bull, 2 = heifer.

[4]Body weight.

[5]Initial Hematocrit.

[6]Hematocrit.

[7]Treatment 1 = fed at 0h, 2 = fed in 2 feedings.

Table 5. Prediction equations for hematocrit[1]

Hour	Intercept	Sex[2]	BW[3], kg	Initial HCT, %	r^2
6	-3.74	2.05	0.33	0.58	0.69
12	-2.59	-	0.26	0.71	0.74
18	-13.7	2.08	0.43	0.64	0.77
24	-12.14	-	0.39	0.73	0.84

[1]Variables with blank spaces indicate that P >/= 0.25.

[2] Sex ,1 = bull, 2 = heifer.

[3]Body weight.

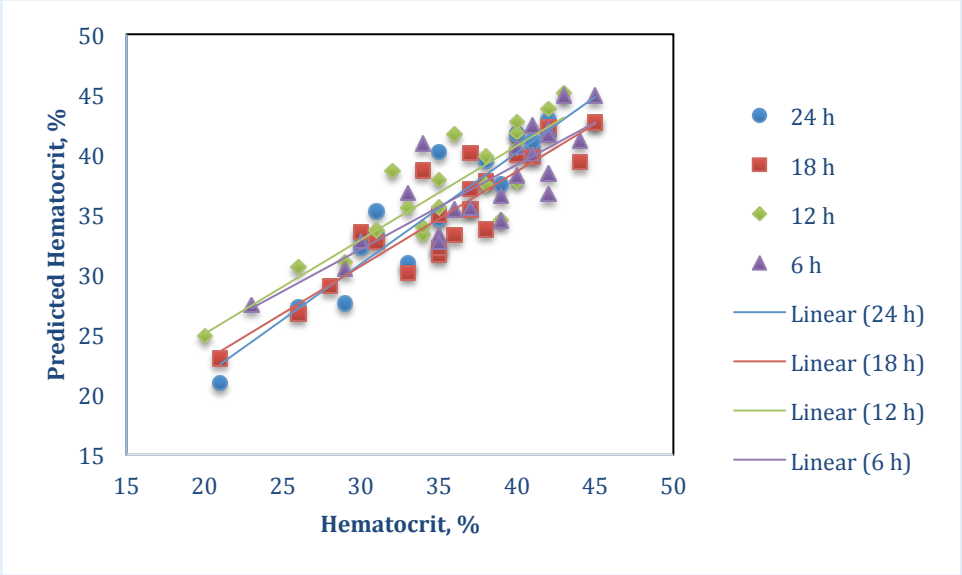

Figure 1. Predicted hematocrit versus measured hematocrit.