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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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2 **based colostrum replacer using Evans blue dye and hematocrit values at various time**

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ABSTRACT

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25 Twenty-eight Holstein calves were blocked by birth date and randomly assigned to one of two

26 treatments to investigate the effect of colostrum replacer (**CR**) feeding regimen on plasma

27 volume (**PV**). Treatments were: 1) one feeding of CR (C_1; 3L of reconstituted CR 675 g of

28 powder providing 184.5 g of IgG at birth) or 2) two feedings of CR (C_2; 2L of reconstituted CR

29 at birth and 1 L of reconstituted CR at six h). By 6 h of age, all calves had received 3L of CR

30 providing 184.5 g of IgG. Plasma volume was estimated at six, 12, 18, and 24 h after birth using

31 Evans blue dye (**EBD**). No treatment effects were noted at any time points ($P > 0.05$). Mean PV

32 for all calves regardless of treatment at six, 12, 18, and 24 h were 78.6, 89.2, 83.9, and 90.7 mL

33 kg^{-1} of BW, respectively. Plasma volume was correlated with hematocrit (**HCT**), initial HCT,

34 and treatment. Hematocrit was correlated with PV, initial HCT, and body weight. Hematocrit for

35 six, 12, 18 and 24 h after birth can be predicted with an initial precolostral HCT determination.

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RÉSUMÉ

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38 Vingt-huit veaux de race Holstein ont été classés par leurs dates de naissance et soumis

39 aléatoirement à l'un de deux traitements afin d'examiner l'effet d'un régime alimentaire de

40 remplacement de colostrum (**RC**) sur le volume plasmatique (**VP**). Les traitements étaient : 1)

41 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

42 dose totale à la naissance, 1/3 de la dose totale 6 heures plus tard). Le volume plasmatique a été

43 estimé six, douze, dix-huit et vingt-quatre heures après la naissance. Le colorant bleu d'Evans

44 (**CBE**) a été utilisé pour estimer le VP; l'effet du traitement n'a été observé à aucun moment sur

45 le VP ($P > 0,05$). Indépendamment du traitement, les moyennes de VP pour tous les veaux à six,

46 douze, dix-huit et vingt-quatre heures étaient 78,6; 89,2; 83,9 et 90,7 mL kg^{-1} de poids corporel

47 (**PC**), respectivement. Le VP a été corrélé à l'hématocrite (**HtC**), l'HtC initial et au traitement.

48 L'hématocrite a été corrélé au VP, à l'HtC initial et au poids. L'hématocrite à six, douze, dix-

49 huit et vingt-quatre heures après la naissance peut-être prédit par une détermination initiale de

50 l'HtC avant l'ingestion de colostrum.

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

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54 **Abbreviations: AEA,** apparent efficiency of absorption; **CR**, colostrum replacer; **EBD** Evans

55 Blue Dye; **HCT** hematocrit; **I** μg of dye injected; **IgG** immunoglobulin G; **PV** plasma volume; **S**

56 absorbance of standard; **U** absorbance of unknown

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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

92   basis using HCT values at each time point. The last objective of this study was to determine if

93   HCT could be predicted using precolostral HCT values.

94   <div align="center">**MATERIALS AND METHODS**</div>

95   **Experimental Design and Treatment Diets**

96   This experiment was reviewed and approved by the University of New Hampshire

97   Institutional Animal Care and Use Committee (# 111205 and 130105).

98   Twenty-eight Holstein calves were born from multiparous and primiparous cows and

99   blocked by birth date. Calves were removed from dams within 30 min of birth, weighed and had

100   their navels dipped with a 7 % tincture of iodine. Calves were individually housed for the

101   duration of the experiment. All calves received 3 sachets of Calf's Choice Total [™]Gold CR

102   containing a total IgG mass of 184.5 g reconstituted, according to manufacturer's instructiona to

103   a final volume of 3L (The Saskatoon Colostrum Co., Saskatoon, SK). One sachet of product

104   contains 225 g of powder. Therefore, all calves received 675 g of powder. Assuming 950 g kg^{-1}

105   (DM) each calf received 641 g of solids with a final volume of 3 L. Total solids equaled 210

106   g/L. Calves were assigned to one of two treatments: 1) one feeding of CR (C_1) or 2) two feedings

107   of CR (C_2). Calves fed a single feeding of CR received the total dose at 0 h (3L CR, 184.5 g

108   IgG). Calves assigned to two feedings received 2/3 of the total CR dose at 0 h (2L CR, 123 g

109   IgG) and the remaining 1/3 of CR at six h (1L CR, 61.5 g IgG). All calves had consumed 3L of

110   CR by six h. Calves were fed via nipple bottle with the first feeding of CR given at 0 h (within

111   30 min of birth). If the calf did not consume the entire volume of CR, it was administered via

112   esophageal tube to ensure the total dose was received.

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Evans Blue Dye Procedure

Blood samples for PV analysis were collected at six, 12, 18, and 24 h of age. The stock

solution of EBD was prepared by weighing 2 g of 750 g kg^{-1} EBD into a volumetric flask mixed

with 100 mL of 9 g L^{-1} sterile NaCl and stored at 3ºC. At all sampling times, 10 mL of blood was

collected into Vacutainer tubes containing freeze-dried Na-heparin (Kendall, Mansfield, MA,

USA) via jugular venipuncture using a 22-gauge needle. After the pre-injection blood sample

was collected, approximately 1.5 mL of stock solution was injected into the jugular vein. The

syringe was weighed prior to and after injection so exact amount of dye administered was

known. After injection, the dye was allowed to equilibrate for 10 min before a post-injection

blood sample was obtained into 10 mL Na-heparinized Vacutainer tubes. Blood samples were

centrifuged (CentraMP4R; International Equipment Company; Needham HTS, MA, USA) at

1,310 × g at - 4°C for 20 min (within 15 min of collection). The plasma was harvested and stored

at -20°C until PV analysis via spectrophotometry. Absorbance of the spectrophotometer

(Beckman DU 520; Brea, CA, USA) was set to 620 nm. Upon analysis, plasma samples were

thawed and 1 mL of pre-injection plasma was transferred into a cuvette and the

spectrophotometer was blanked against the sample. Fifty µl of standard dye solution (prepared

by adding 1 mL of stock solution to a 100-mL volumetric flask which was diluted with 9 g L^{-1}

NaCl solution) was added to the plasma blank, vortexed and absorbance read. Finally,

absorbance of the post-injection sample was obtained. The PV was calculated using the equation:

[1 / ((U ÷ S) x 7.142857)] × I, where U = absorbance of unknown (post-injection sample); S =

absorbance of standard (pre-injection sample + 50 µl of standard dye solution); and I = µg of dye

injected (mL of stock injected × 15 mg dye mL^{-1} stock × 1000), 7.142857 is equal to the amount

138   of dye in the standard plasma sample (50 µL of dye containing 0.15 mg dye mL^{-1} of standard

139   dye solution added to 1 mL of plasma = 7.5 µg ÷1.05mL).

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141   **Blood Collection for Hematocrit**

142   A subsample of blood collected for PV analysis was obtained for evaluation of packed

143   cell volume. An initial sample was obtained at birth before CR feeding for HCT analysis as well.

144   Capillary tubes were centrifuged (Haematokrit 210; Andreas Hettich GmBH & Co; Germany) at

145   16,060 × g at 25°C for 5 min.

146   **Statistical Analysis**

147   Plasma volume and HCT values at six, 12, 18, and 24 h were analyzed using the mixed

148   procedure of SAS® (Version 9.3; SAS Institute, Inc. Cary, NC, USA) as a randomized complete

149   block design according to the following model:

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

151   Where:
152   Y = the dependent variable;
153   μ = the overall mean;
154   B_i = the random effect of block i (i = 1,…14);
155   C_j = the fixed effect of the jth CR feeding regimen (k = 1, 2);
156   H_k = the fixed effect of the kth hour (k = 6, 12, 18, and 24);
157   E_{ij} = the residual error $\sim N(O_1\sigma^2 e)$.
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159   Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure

160   of SAS® (Version 9.3; SAS Institute, Inc. Cary, NC, USA). Least square means were

161   determined for each treatment. Significant treatment effects were noted at $P \leq 0.05$ and trends

162   were noted at $0.05 < P \leq 0.10$. Regression analysis was conducted using the regression procedure

163   of SAS using PV (g kg^{-1} BW) and HCT. Variables used in the regression analysis for PV were:

164   sex, HCT, initial HCT, BW and treatment. Variables used in the regression analysis for HCT

165   were: sex, initial HCT, BW, treatment, hour (for overall value), and PV (mL). Correlation

166   coefficients were determined using the correlation procedure of SAS (Version 9.3; SAS Institute,

167   Inc., Cary NC, USA),

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169   **Results**

170   Mean BW was 46 ± 4.61 kg, mean initial HCT was 39.4 ± 6.82 %, mean HCT was $35.2 \pm$

171   5.83 %, and mean PV was $3,866 \pm 2022$ mL (Tables 1 and 2). Three calves on the C_1 and two

172   calves on C_2 treatment groups were fed using an esophageal tube feeder. The calves on treatment

173   C_1 required all of the CR to be fed in this manner. Whereas, one calf on C_2 required all of the CR

174   fed in this manner while the other calf consumed its first feeding via nipple bottle, but was fed

175   the total six h dose with the esophageal feeder. Initially, 28 samples per time point were

176   obtained; however, due to sampling error or hemolysis, 4 calves were removed from analysis (2

177   per treatment). Analyses of PV (mL, and mL kg^{-1} of BW) along with HCT data are presented in

178   Table 1. Mean PV for all calves regardless of treatment at six, 12, 18, and 24 h were 78.2, 89.2,

179   83.9, and 90.7 mL kg^{-1} of BW, respectively. Treatment did not affect PV expressed as either mL

180   or mL kg^{-1} of BW at any time point (Table 1). Hematocrit did not vary at any time point (Table

181   1). Mean, minimum and maximum values for PV, BW, initial HCT and HCT are in Table 2.

182   Correlation coefficients (Table 3) for PV showed strong relationships between PV, HCT, initial

183   HCT and treatment. Prediction equations for PV as a percent of BW had coefficients of multiple

184   determinations (r^2) values that ranged from 0.19 for the 12 h prediction equation to 0. 32 for the

185   6 h equation suggesting a weak relationship (Table 4).

186   Prediction equations for HCT had r^2 values that ranged from 0.69 for the 6 h equation to

187   0.84 for the 24 h equation (Table 5. Body weight and initial HCT can be used to predict HCT at

188   12 and 24 h. Whereas, these variables plus sex of the calf can predict HCT at six and 18 h

189   (Figure 1).

190   **Discussion**

191   Evans blue dye has been used as a marker of PV in cattle (Dalton and Fisher, 1961;;

192   Payne et al., 1967; Möllenberg et al., 1975; Thornton and English, 1978; Besser and Osborn,

193   1993), sheep (English, 1966; Mackie, 1976; van Waversveld and van Bruchem, 1985), horses

194   (McKeever et al., 1988), and pigs (McCance and Widdowson, 1959). Estimates of PV using

195   EBD and other methods typically indicate close correlation; however, van Waversveld and van

196   Brushem (1985) reported that EBD estimated total blood volume but not PV. However a strong

197   correlation was found between PV and hematocrit ($P = 0.006$). Quigley et al. (1998), with 146

198   calves sampled, also reported large variability in PV among calves even with the same BW (39

199   kg) with PV as mL kg^{-1} of BW ranging from 60 – 131. This is similar to calves used on the

200   current study with a BW of 40 kg having PV ranging from 67 – 131.

201   Research in human physiology has indicated that PV can be estimated by using a

202   patient's height, weight, and HCT; however, equations are only reliable at normal HCT.

203   Sprenger et al. (1987) were able to modify equations and allow for accurate calculation of PV

204   even with the use of pathological HCT but PV was measured using 51CR-method and not EBD.

205   Quigley et al. (1998) reported that birth BW was the best predictor of PV, though the relationship

206   was not linear; however, multiple regression analysis using HCT and BW yielded no correlation

207   with PV. Our results may have been affected by the use of EBD and perhaps a more accurate

208   method of PV estimation should be used in the future.

209   There was no difference in PV between treatments across the 4 sampling times.

210   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.

234   Results from this experiment provide researchers with values to allow determination of

235   AEA when CR is fed. Also, determination of AEA at different time points over the first 24 h of

236   life which will allow for a more accurate determination of when IgG absorption is maximized.

237   Hematocrit was not different between treatments. Hematocrit is a measure of packed cell

238   volume, which is an indicator of hydration status. Though the total volume was identical,

239   spreading the feeding between 2 time periods may have affected hydration status. Overall, HCT

240   decreased from birth to 24 h indicating an increase in hydration status. Hematocrit, sex,

241   treatment and initial HCT were correlated with PV in this study. Initial BW, sex and precolostral

242   HCT can be used to predict HCT at six,12, 18 and 24 h after birth in Holstein calves receiving

243   CR.

Conclusion

245   Feeding CR in either one or split into two feedings did not affect PV, or HCT

246   measurements.   Plasma volume was highly correlated to sex, HCT, initial HCT and feeding

247   regimen. Prediction equations to determine PV utilizing BW, sex, feeding regimen, initial HCT

248   and HCT had correlation coefficients that ranged from 0.25 to 0.40 indicating a weak

249   relationship. However, a better relationship was found for predicting HCT with r^2 ranging from

250   0.69 to 0.84. An initial precolostral blood sample along with BW can accurately predict HCT at

251   12, and 24 h. Those values along with an adjustment for sex of the calf can accurately predict

252   hematocrit at six and 18 h after birth. Researchers evaluating CR can utilize these data to

253   accurately determine AEA and pinpoint when IgG absorption is maximized..

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314   Table 1. Calves, body weight, plasma volume and hematocrit for calves fed colostrum replacer

315   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

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317   [1]Treatment C_1 = all colostrum replacer is fed at time 0 h, C_2 = colostrum replacer fed 2/3 at time

318   0 h and 1/3 at 6h.

319   [2]Standard error.

320   [3]Repeated measures by hour for plasma volume (mL) was not different (P< 0.13).

321   [4]Repeated measures by hour for plasma volume (% of body weight) was not different (P = 0.54).

322   [5]Repeated measures by hour for hematocrit was not different (P= 0.99).

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345   Table 2. Mean, minimum and maximum values and standard deviations for data used in

346   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

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

363 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

364

365 [1]Plasma volume in mL.

366 [2]Body weight.

367 [3]Hematocrit.

368 [4] Initial Hematocrit.

369 [5]Treatment- feeding regimen (one feeding of CR or 2 feedings of CR).

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372   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

373   [1]Variables with blank spaces indicate that P >/= 0.25.

374   [2]Hour corresponds to time after birth.

375   [3]Sex ,1 = bull, 2 = heifer.

376   [4]Body weight.

377   [5]Initial Hematocrit.

378   [6]Hematocrit.

379   [7]Treatment 1 = fed at 0h, 2 = fed in 2 feedings.

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389   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

390

391   [1]Variables with blank spaces indicate that P >/= 0.25.

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393   [2] Sex ,1 = bull, 2 = heifer.

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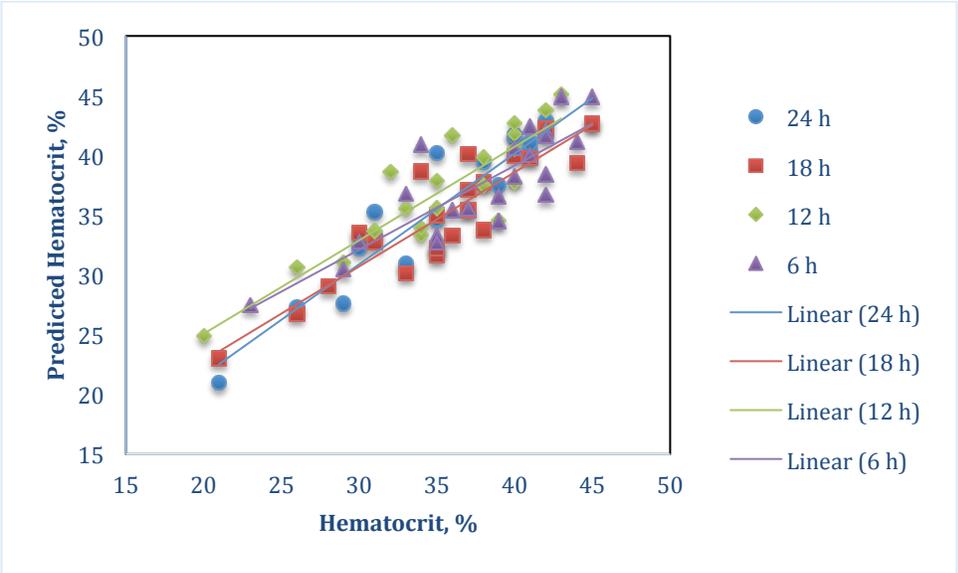395   [3]Body weight.

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Figure 1. Predicted hematocrit versus measured hematocrit.

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