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Calf Note #277 - Plasma Volume: The Hidden Variable in AEA

Introduction

In our previous Calf Note, we explored why apparent efficiency of absorption (**AEA**) of immunoglobulin G (IgG) is never 100%, emphasizing the dynamic exchange between intra- and extravascular spaces. That discussion highlighted an important reality: AEA is not a fixed biological constant, but rather a calculated estimate influenced by several physiological and methodological factors.

Another key component of AEA is plasma volume (**PV**), which is used to calculate the total amount of IgG absorbed into the circulation. Plasma volume may be measured using one of several methods, or estimated using body weight (**BW**), because PV has been shown to be highly related to BW in many species of animals. However, the variation in PV is important to our understanding of AEA and – importantly – variation in calculating AEA.

Plasma Volume: Assumption vs. Reality

In most studies of passive transfer, plasma volume is assumed to be a fixed proportion of BW, typically ranging from 7% to 10% of BW. This assumption simplifies calculations of AEA, since PV is required to convert serum IgG concentration (g/L) into total circulating IgG mass. However, PV is not fixed. It is a biological variable that changes with hydration status, feeding, age, and physiological adaptation after birth. Treating PV as a constant introduces error into AEA calculations—error that may be substantial depending on conditions.

Measuring Plasma Volume: Methods and Limitations

Researchers have used several techniques to estimate plasma volume in neonatal calves, including dye dilution and isotopic methods.

Evans Blue Dye. The most common approach uses Evans Blue dye (**EBD**), which binds to albumin and is assumed to remain within the vascular space. After injection, blood samples are collected, and PV is calculated based on dilution. However, this method has limitations, including equilibration time. Typically, a 10-minute period is used before sampling. During this time, some dye may leave the vascular space or be metabolized or redistributed. Therefore, many researchers include a correction factor to account for this loss of dye in the equilibration period (e.g., Quigley et al., 1998a).

Other researchers have repeatedly measured EBD concentrations after injecting the initial dose into the animal. They can then plot a regression line and estimate the amount at time zero (i.e., intercept), which corrects for the equilibration error.

Other researchers have used radioactive isotopes such as ^{131}I-labeled proteins (e.g., Möllerberg et al., 1975) to estimate PV. Of course, these approaches are more difficult to manage and are no longer commonly implemented.

The relationship between BW and PV (milliliters) in young calves is shown in Figure 1, which is from Quigley et al. (1998a).

In this study, we reported that PV was related to birth BW, breed (Holstein or Jersey), and age at sampling. The regression equation was:

PV = -2393.1 + 68.09 × BW + 404.1 × breed + 127.3 × age

Where BW is calf BW, kg; breed is 0 = Jersey, 1 = Holstein; age = hours of age. The r^2 of the regression = 0.60.

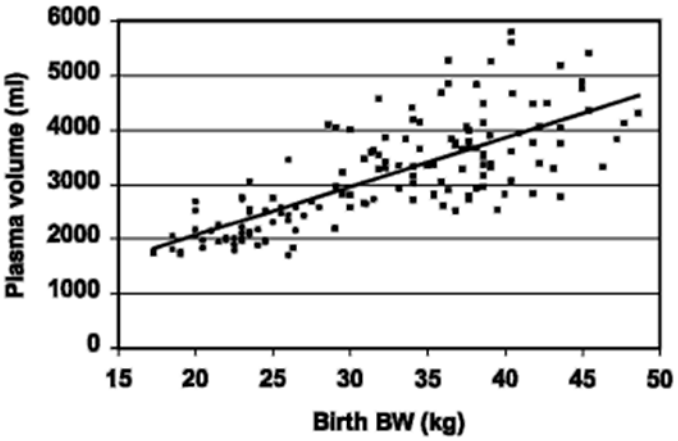

Figure 1. Relationship between plasma volume (milliliters) and birth BW of Jersey (●) and Holstein (■) calves.

Generally, the relationship between BW and PV is higher than 0.80; in this study, we worked with day-old calves that were experiencing rapid and variable PV expansion due to colostrum intake, absorption of large amounts of protein, fat, and lactose – all of which can influence body fluid dynamics and overall PV. Our data – unadjusted for experimental error – reported that PV was 9.9% of BW for Holstein calves and 9.71% of BW for Jersey calves. When corrected for loss of EBD during the equilibration period, the values were 9.0% and 8.8% of BW, respectively.

Range of Reported Plasma Volumes

A review of the calf colostrum literature shows that assumed or measured PV values typically range from ~7% of BW to >14% of BW. Interestingly, Matte et al. (1982) fed calves one feeding of colostrum at 6, 12, 24, 36, or 48 h of age. They measured PV at 6 h post-feeding and reported that PV, measured using EBD, declined from 14.5% of BW when measured at 12 h of age, to 9.2% at 54 h of age. McEwan et al. (1968) reported that PV in calves (n = 5) consuming colostrum increased from 6.6% to 9.3% of BW, and, in calves fed milk instead of colostrum, PV increased from 6.3% to 8.7% of BW. There was no difference between milk or colostrum feeding, but a large effect of liquid consumption. Plasma volume was measured at 72 h of age in this study. This range may seem modest, but its impact on AEA is significant.

Table 1 shows a sample of published studies and the ranges in reported PV. It is clear that there is significant variation in the published estimates – from 5.3% to 14.5% of BW as PV.

Author	No calves	Method	Breed	BW, kg	Age, h	PV
Matte et al. (1982)	40	EBD	Holstein		12	14.5
					18	13.9
					24	12.0
McEwan et al. (1968)	13	EBD			Prefeed	6.4
					Postfeed	9.0
Möllerberg et al. (1975)	9	^{131}I	Swedish	33.0	Birth	5.3
		EBD		34.0	1	6.5
		EBD		96.0	90	4.9
Dalton and Edwards, 1961	102	EBD	Ayrshire		1-3 wk	6.6
Cabral et al. (2015)	28	EBD	Holstein	46.6	6	7.9
		EBD			12	8.9
		EBD			18	8.4
		EBD			24	9.1
Quigley et al. (1998a)	49	EBD	Jersey	23.4	24	8.8
	97	EBD	Holstein	36.7	24	9.0
Thornton and English (1975)		EBD			~7 d	7.9
Quigley et al. (1998b)	20	EBD	Holstein	40.5	24	9.2
Drewry et al. (1999)	43	EBD	Holstein		25	8.5
Husband et al. (1973)		EBD	Holstein	40.0	24	6.3

PV = plasma volume, % of BW

The Impact of Feeding and Hydration on PV

Plasma volume is not static in the neonatal calf—it changes rapidly after birth, particularly in response to feeding.

Some factors that influence fluid dynamics in the calf,

and consequently PV, include feeding liquids (colostrum, milk, milk replacer), which increase fluid intake. Absorption of fluid expands PV and can occur within hours after feeding. As a result, a calf sampled at 24 hours may have a different PV than the same calf at 32 or 48 h. Additional feedings (e.g., milk replacer after colostrum) further influence PV. Thus, variation in the way calves are fed during a research trial introduces additional variability.

This means that PV at the time of blood sampling is a moving target.

Timing of Blood Sampling: Another Layer of Variation

The timing of serum IgG measurement is another critical factor. Common sampling times include 24, 28–32 h, and 48 h. However, these time points are not physiologically equivalent. Continued absorption and redistribution of IgG may occur, and PV may expand due to ongoing feeding. Absorbed IgG may move into extravascular compartments with later sampling times. Some IgG may be metabolized and filtered by the kidney for excretion in the urine.

If calves are not managed identically between birth and sampling (e.g., differences in milk replacer feeding after colostrum), then measured serum IgG concentration reflects both IgG mass and PV at that moment, and differences in PV can obscure true differences in IgG absorption.

Implications for AEA Calculations

AEA is typically calculated as:

AEA (%) = [Serum IgG (g/L) × Plasma Volume (L)] / IgG Intake (g) × 100

Each component carries uncertainty:

Serum IgG → influenced by timing and analytical method

IgG intake → often estimated, not directly measured

Plasma volume → assumed or imperfectly measured

Among these, PV is often treated as the most "certain"—when in fact it may be one of the largest sources of error. The decision to use a specific factor for PV (e.g., 7% vs. 10% of BW) has implications for the final values.

Table 2 shows the effect of calculating PV as 10% of BW to 5.5% of BW for a 40-kg calf fed 150 g of IgG and with a serum IgG concentration of 15 g/L. The calculated AEA declines from 40% to 22% simply based on the selected multiplication factor. This is an important consideration for any research reporting IgG absorption data.

PV	BW	Serum IgG	IgG Intake	AEA
10.0%	40	15	150	40%
9.5%	40	15	150	38%
9.0%	40	15	150	36%
8.5%	40	15	150	34%
8.0%	40	15	150	32%
7.5%	40	15	150	30%
7.0%	40	15	150	28%
6.5%	40	15	150	26%
6.0%	40	15	150	24%
5.5%	40	15	150	22%

Putting It Together

When we interpret AEA values, we must recognize that they are not precise measurements, but estimates derived from a dynamic biological system.

Variation in plasma volume arises from:

- Biological differences among calves
- Feeding and hydration status
- Timing of sampling
- Methodological differences in measurement (e.g., dye vs. isotope, correction vs. no correction)
- Assumptions used in calculations

These factors can meaningfully alter calculated AEA—even when the underlying biology of IgG absorption is unchanged.

Practical Take-Home Points

- Plasma volume is not a constant; it is a dynamic variable
- Assumed PV values (7–9.8% of BW) can significantly affect calculated AEA
- Methods used to measure PV (e.g., Evans Blue dye) introduce variability
- Feeding and hydration status before sampling influence PV
- Timing of blood sampling adds another layer of variation
- Differences in AEA among studies may reflect methodology as much as biology

Research Recommendations

Selection of a PV factor should consider breed and time of feeding and sampling. Data from Cabral et al. (2015) and Quigley et al. (1998a) suggest that a factor of approximately 9% of BW for Holstein calves sampled at 24 h of age is appropriate.

Feeding programs should be closely controlled and accurately reported. Additional feedings of colostrum or other liquids prior to blood sampling will introduce variability into the calculations.

Sampling at a fixed time point – preferably 24.00 h of age for colostrum feeding studies – will help reduce error in PV calculations.

If EBD is used, the 10-min correction factor of Quigley et al. (1998a) of 0.906 is recommended.

Summary

Plasma volume is a critical—but often underappreciated—component of AEA calculations. Because PV is dynamic and difficult to measure precisely, it introduces inherent variability into estimates of IgG absorption. Recognizing the limitations of PV assumptions and measurement techniques helps us better interpret AEA values—not as fixed truths, but as approximations within a complex and evolving physiological system.

Understanding and accounting for plasma volume will improve our ability to evaluate passive transfer and refine how we interpret serum IgG data in neonatal calves.

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