Analysis of published and clinical sample data to develop new ranges of liver and blood concentrations of vitamin A for diagnosis of vitamin A deficiency in cattle

***David Villar,**[1] DVM, PhD, DABVT; **David J. Schaeffer**[2] MS, PhD

[1]Grupo CIBAC, Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia
[2]College of Veterinary Medicine, University of Illinois, Champaign-Urbana, IL 61802
***Corresponding author:** Dr. David Villar **david.villar@udea.edu.co**

Abstract

In cattle, liver stores of vitamin A (VA) maintain blood VA concentrations until liver supplies are nearly exhausted, providing VA to tissues during insufficient dietary intake. Therefore, normal blood levels do not indicate adequate VA, but low blood levels indicate a deficiency. We hypothesized that previous diagnostic criteria for VA deficiency needed updating and that blood levels of VA could be used as indicators of liver VA deficiency. We performed multiple regressions of paired blood and liver sample data from published experimental and field case reports of VA deficiency in cattle to demonstrate this. We fitted various models using data from unpaired clinical samples submitted to the Iowa State University Diagnostic Laboratory by comparing fractiles of liver and plasma/serum samples. Using these relationships, we propose new ranges for severely deficient, deficient, marginal and adequate, and we also propose that blood samples alone can be used to diagnose deficiency in the presence of clinical signs of VA deficiency. Veterinary diagnostic laboratories can use this information to better estimate serum and liver VA concentrations for unpaired data and recommend to cattle practitioners the status of VA in their herds.

Key words: vitamin A deficiency, cattle, guidelines, regression models

Introduction

Vitamin A (VA) is required in cattle rations and is essential for regenerating the visual purple pigment rhodopsin necessary for dim-light vision, normal bone growth, and maintenance of normal epithelial tissues.[1,2] The numerous pathophysiological effects of deficiency in cattle include poor night vision and blindness,[3-8] poor bone growth,[9-12] atrophy of epithelial cells in tissues that have a secretory and covering role,[13] embryological development,[14,15] immune mechanisms,[9,16,17] weight gain,[9-11] and loss of reproductive functions.[15,18] Many reports about the clinical signs of hypovitaminosis A are from experimental studies.[11,13,19-21] Reports of naturally occurring hypovitaminosis A also provide potential associations of clinical signs or histopathological or other diagnostic findings with VA blood or liver concentrations.[3-8,14,22,23.] These studies support using VA liver or blood concentrations to diagnose deficiency.

The objectives of this study were to:

1. Find an appropriate statistical model to describe the relationship between liver and blood levels of VA based on individual animal-paired sample data in published reports.
2. Determine the relationship between liver and blood levels of VA using unpaired data from a diagnostic laboratory's clinical submissions.
3. Determine if the current diagnostic criteria (Puls')[24] for deficiency are adequate and propose revisions.
4. Evaluate the clinical relevance of differences in the distribution of deficiency using the published criteria compared to the proposed criteria.

Materials and methods

Acquisition and modeling of published data on concentrations of VA in paired blood and liver samples in cattle

A literature review harvested paired and unpaired serum/plasma and liver VA data for individual beef or dairy cattle, or herd data, from studies[13,26-29] and one thesis[30] published from 1920 to the present. Searches for vitamin A in cattle were carried out numerous times by both authors between December 2019 and December 2022. Searches used Google, Google Scholar, Pubmed, Chemical Abstracts, Dogpile.com, Proquest Digital Dissertations, Academic Search Ultimate Plus, Scopus, Web of Science, WorldCat Discovery, CrossRef and citations in retrieved publications. All languages were eligible but articles were excluded if there was no English, Spanish, French or Portuguese abstract or if they were not translatable by Google or Microsoft Translate. Articles without statistical data were excluded. Articles with strongly asymmetrical summary data (e.g., mean and median were statistically unequal, the median was greater than the mean, the skew was significantly different from zero, or the mean minus twice the standard deviation was negative) or that were missing data necessary for evaluating relationships (e.g., correlations between tissues within animals, dam and neonate, or feed and tissue concentrations) were also excluded.

Vitamin A articles were reviewed if they included the following initial terms:

1. Vitamin A AND Puls' guidelines
2. Vitamin A AND Puls AND cattle
3. Vitamin A AND deficiency AND (cattle OR bovine OR calf OR calves OR beef OR dairy)
4. 1 to 3 AND (liver OR hepatic) AND (serum OR blood OR plasma)
5. Subsequently, "ruminant" was added to the OR list.
6. Alternative terms in an article were searched for first, and if there were multiple hits, they were added to 1 to 6.

Initial terms for statistical analysis were:

1. 1 to 6 AND correlation OR regression
2. Subsequently, vii plus OR discriminant analysis OR (mean OR median) AND (standard deviation OR standard error OR confidence interval.)

The terms "distribution", "normal distribution", "Gaussian distribution" and "lognormal" were not reliably associated with the studies themselves but most often with a citation. Statistical terms were removed, replaced or added such as reference range, skew and kurtosis. Most individual animal data was in 1 thesis published before 1970.[30] Dry-weight (dw) was converted to wet-weight (ww) as ww = dw/4, as recommended in Puls' guidelines.[24]

Most articles that reported only descriptive statistics for liver and blood fraction failed to report their correlation or Pearson's correlation for data that were not Gaussian (normal distribution). We judged nonnormality by one or more of the following criteria: mean – 2 SD < 0, mean + 3 SD > maximum, mean < median, histogram, probability plot or quantile plot. Summary statistics were entered directly into Microsoft Excel[a]. Data from individual animals were extracted from PDF-formatted tables into Excel using Cogniview PDF2XL Enterprise[b] or copied into Excel. Data reported only in figures were extracted using manual digitization (CurveExpert Pro[c], Engauge Digitizer[d] or WebPlot Digitizer[e]); automatic digitization was usually impossible due to low resolution, an overlap of symbols, or extraneous marks on available copies. Excel tables of extracted data were verified by visual agreement of the extracted data and reported values and agreement of the descriptive statistics. Tables were merged and aligned by the animal for tissues or calf-dam pairs.

We fitted all the published data sets to 67 nonlinear regression models for consistent statistical analysis. The models with the most significant diagnostic scores of regression fit and those with the smallest AICs were retained for each dataset. We ordered the models for each dataset by mathematical simplicity, e.g., linear > quadratic > log (either serum or liver) > log-log (serum and liver) > other. Retained models were mathematically compared to the "best" model using a built-in option. Models with discontinuities, wide confidence intervals, sigmoidal (S-shaped), or multiple peaks and valleys, were excluded.

Animal care

No animals were involved in this research, only published data and the ISU-VDL database.

Statistical analysis of liver and serum concentrations from unrelated samples submitted to ISU-VDL data

Vitamin A serum and liver concentrations in samples from cattle presented to the ISU-VDL between 2001 and 2021 were provided by Dr. Kent J. Schwartz. Variables extracted from the dataset included accession ID, date, breed, state, farm, stage, Dx code and serum and liver VA concentrations. The ISU-VDL considered the accession ID a case regardless of the number of cattle included. When multiple cattle shared an accession ID, the database included the count, mean, median, low, high and standard deviation, but not the individual animal's values.

Outliers identified from histograms and outlier statistics from fits of continuous univariate distributions[b], such as normal, lognormal and logistic, were deleted.

Data with similar properties can be grouped and defined so that the areas under the probability density curve between points and zero are equal to specified fractions (fractiles) or include equal numbers of points (quantile). Order statistics include fractions, quantiles, ranks, minimum, maximum and median.

For each published study, the same fractiles and quantiles of the paired serum and liver concentrations were estimated using linear interpolation (Systat 13.1[f]). Spearman's rank correlation was calculated for each dataset. Quantiles for a single variable plotted against the fraction of the distribution is a Q plot. Quantiles from two variables plotted against each other is a quantile-quantile or Q-Q plot. The two variables in a Q-Q plot do not have to be paired or have the same distribution. It has been known since the 1930s that vitamin A concentrations in calves and their dams are significantly correlated.[26,27,30-32] We compared published equations from the paired dam and calf liver and serum VA concentrations[26,27,30-32] to our equations from unrelated adults and calves in the ISU-VDL data. A "herd" relationship is inherent in Q-Q plots, random sampling, statistical procedures, and distributions (*z*, *t*, F, and chi-square). Ordinary least squares (OLS) linear regression was depicted by a trend line through the means of a normal distribution of *y* values with the same shape at each *X*.

We grouped liver and serum VA concentrations into fractiles of their distribution; for example, 4 groups of about equal size are the quartiles[33-35] displayed in a box plot. The quartiles are equivalent to composite samples, for example, of bulk feeds. Vitamin A concentrations in sera and livers were separately grouped into 15 fractiles[b], and we fitted the values to the 67 nonlinear regression models[a], for y = liver and *x* = serum/plasma and x = serum/plasma and y = liver. If x and y are independently correlated with variable z (here, z = fractiles), they are correlated with each other.[33-35] Grouping changes tissue correlations from paired values within an animal to unpaired values from the group.

Results

Experimental and field studies establish bovine hypovitaminosis based on serum and liver VA concentrations

Puls' serum criteria (Table 1)[24] were the initial concentrations we used to establish a new range (Table 2) for deficient and corresponding changes for marginal and adequate. The idea of these new ranges was to close gaps in Puls' guidelines with an empty classification, which is hard to interpret by diagnosticians. The new ranges are based on experimental studies with large groups of calves on the clinical consequences of VA deficiency (squamous metaplasia of the parotid gland, ocular papilledema, increased cisternal cerebrospinal fluid, and poor vaccination response).[12,13,16,19,21] The studies reported a continuum of adequate versus deficient concentrations for paired serum and liver concentrations. Based on these studies, the following categories are proposed. At serum concentrations causing clinical deficiency (< 0.2 ppm), most liver concentrations were ≤ 7.5 ppm ww. At marginal serum levels (0.2 – < 0.3 ppm), most liver concentrations were 7.5 – 25 ppm ww. Serum ≥ 0.3 ppm or liver ≥ 25 ppm ww were adequate.

Table 1: Adaptation of Puls' interpretation of VA concentrations in bovines > 6 months of age.[24]

Diet	IU/kg diet (dry matter)	Serum (ppm, mg/L)	Liver (ppm, dry weight)
Deficient	< 1400	0.002 - 0.05	< 15 or < 30*
Marginal	1400	0.1 - 0.2	30 - 100
Adequate	2200 - 3900†	0.3 - 0.8	300 - 700
Toxic	> 3900‡	> 2	

* Based on the severity of the signs, Puls classified deficient in the liver into these subsets. The published ranges have gaps.

† This range is from Puls' sub-table "cattle"; the intake for adequate in his sub-table "cattle" is > 2500 IU/kg diet (dry matter).[24]

‡ Puls[24] did not give a dietary value for toxic; we used exceedance of the maximum of his adequate range†.

Table 2: Proposed classification of vitamin A concentrations in bovines > 45 days old based on 11 papers representative of reported clinical and pathological findings of hypovitaminosis A in cattle.[3-5,7,8,13,14,16,18,22,23] Numbers of liver and serum vitamin A samples in the Iowa State Veterinary Diagnostic Laboratory (ISU-VDL) database (2001 through 2021) for the proposed tissue concentration range are indicated.

Diet	IU/kg	Serum		Liver		
	Dry matter	mg/L	Number and percent of ISU-VDL samples	ppm dw	ppm ww*	Number and percent of ISU-VDL samples
Severely deficient	< 1400	0.002 - < 0.1	34 (8%)	< 15	< 3.75	338 (47%)
Deficient	< 1400	0.1 - < 0.2	76 (18%)	< 30	3.8 - <7.5	92 (13%)
Marginal	≥ 1400	0.2 - < 0.3	38 (9%)	30 to < 100	7.5 - < 25	159 (22%)
Adequate	> 2500	0.3 - 0.8	273 (68%)	100 to < 300	25 - 75	83 (12%)
Toxic	N/A	> 2	0	300–700	75 - 175	42 (6%)

* Wet weight (ww) = Dry weight (dw)/4.

These ranges fill gaps between VA categories in Puls' guidelines[24] and are consistent with reports of clinical or pathological effects correlated with the degree of hypovitaminosis A.

Eleven papers representative of reported clinical and pathological findings of hypovitaminosis A in cattle were selected to fill gaps in Puls' ranges[24] based on associations between clinical consequences and degree of VA deficiency in liver and serum samples.[3-5,7,8,13,14,16,18,22,23] All included data for VA concentrations in liver and sera from affected animals, and field case investigations ruled out other potential causes.

Puls did not assign the serum range 0.2 to 0.3 ppm to a category (Table 1),[24] creating a gap in which his marginal concentrations might become deficient. To establish preliminary serum VA ranges for severely deficient, deficient, marginal and adequate (Table 2), we compared our analyses to Puls' guidelines (Table 1).[24] Based on an uncertainty factor for situations where vitamin A may naturally drop, such as near parturition[36] and infections,[16,17] our marginal concentration (0.2 - < 0.3 ppm) for serum VA is between Puls' deficient and adequate.[24]

Correlation of serum or liver VA from adults and calves with different accession IDs

The best fit of the fractiles of VA concentrations in calf sera to adult sera in Table 3 was a linear relationship $R^2 = 0.977$. Liver concentrations were also fitted by a linear relationship: calf liver (ppm ww) = 0.5076 ± 1.488 + 0.334 ± 0.0082 adult liver (ppm ww), $R^2 = 0.985$. Linear models with a random intercept were significantly better than intercept-at-the-origin models.

For fractiles of unpaired liver and serum VA concentrations from Table 3, defining a and b as unknown regression coefficients, the power model (serum/plasma = a(total liver)^b and the modified power model (serum/plasma = $ab^{(\text{total liver})}$) had similar fit scores (879 and 833) and visual fits. Taking logarithms on both sides of each equation, the linearized power model (log-log) was simpler. A test of the difference for Akaike's Information Criterion (AICC) favored the power model (AICC = 196.0) over the modified power model (AICC = 209.5; $P = 0.0011$).

We retained the linear (all terms arithmetic) and the linearized power model (a and x logarithmic), both of which can be written as $y = a + bx$ (Table 5). The linearity of the Q-Q plot of the logarithms of the unrelated hepatic and serum VA concentrations was parallel to those for paired samples and produced similar equations.

Criteria derived from liver and serum samples from unrelated animals

Statistical criteria and visual inspection of plots indicated the paired fractiles were fitted by a power law, $y = ax^b$, which is linearized by taking logarithms on both sides, $\log(y) = a' + b \log(x)$, where $a' = \log(a)$ (Figure 1). Deficient liver

Table 3: Fractions (fractiles)* of serum and liver vitamin A distributions in adult bovines and calves in the Iowa State Veterinary Diagnostic Laboratory (ISU-VDL) database from 2001 through 2021.

Summary statistics and fractions (fractiles) of the distribution	Adult sera (ppm)	Calf sera (ppm)	Adult livers (ppm ww)	Calf livers (ppm ww)
N (Number of samples)	686	179	84	62
Minimum	0.02	0.02	1.00	1.00
Maximum	1.00	0.74	601.00	198.00
Arithmetic mean	0.25	0.25	86.13	34.27
Standard deviation	0.15	0.12	109.94	37.83
Fractiles				
1	0.03	0.04	1.00	1.00
5	0.08	0.08	1.00	1.00
10	0.11	0.11	1.9	2.00
20	0.14	0.14	13.3	10.50
25	0.15	0.16	18.00	11.00
30	0.16	0.17	20.40	12.00
40	0.19	0.21	38.10	16.30
50	0.21	0.23	54.00	27.00
60	0.24	0.26	80.00	37.10
70	0.30	0.28	96.00	39.90
75	0.33	0..30	106.00	42.00
80	0.36	0.32	122.40	49.20
Summary statistics and fractions (fractiles) of the distribution	**Adulta sera (ppm)**	**Calf sera (ppm)**	**Adult livers (ppm ww)**	**Calf livers (ppm ww)**
90	0.45	0.42	161.70	64.60
95	0.57	0.52	302.90	94.80
99	0.85	0.64	572.10	198.00

* Fractiles were calculated by Cleveland's algorithm in Systat 13.1[f].

concentrations (≤ 7.35 ppm ww) corresponded to ≤ 0.2 ppm in serum, and marginal liver concentrations of 7.35 - 24.23 ppm ww corresponded to 0.2 - < 0.3 ppm in serum (Table 2).

Based on these correlations, new ranges for liver VA concentrations are proposed in Table 2. The classification of bovine vitamin A concentrations in sera and liver from the ISU-VDL using Puls[24] (Table 1) and our (Table 2) schemes are reported in Table 4. At liver concentrations > 25 ppm ww, most serum concentrations would be within the normal range for serum 0.3 - 0.8 ppm. Most serum concentrations would be marginal (0.2 - < 0.3 ppm) at 7.5 to 25 ppm ww in the liver. For the liver, 430/714 (60.2%) samples were severely deficient or deficient. The larger category was extremely deficient (338/714, 47.3%). According to the new classification in Table 2, serum VA concentrations were severely deficient or deficient in 110 of 421 samples (26%). The majority (273, 68.4%) were adequate. The distributions of the revised concentration range for the ISU-VDL data, with most animals being beef cattle, are in Table 4.

Discussion

Most published regression analyses of VA and associated analytes (retinoids, β-carotene) correlate concentrations in the same tissue samples or multiple tissues from the same animals. Regression can also be carried out on grouped data weighted by the group size, e.g., histogram. This discussion focuses on correlations between tissues from unpaired samples, which we term "herd-based" (or composite-sample) correlation. Size-weighted regression on grouped data replicates the coefficients obtained from paired values,[37,38] but the standard errors are underestimated.

It is unclear what serum and liver concentrations are adequate for neonatal calves, but they are lower than for adults.[9,32,39,40-44] Adequate VA status in neonatal calves depends mainly on VA intake during the first days of life, and delaying colostrum intake for more than 12 hours after birth affects plasma and liver VA concentrations during the first month of life.[42] Despite supplementation in the first month of

Table 4: Classification of bovine vitamin A concentrations in sera and liver from the ISU-VDL using Puls[24] (Table 1) and our (Table 2) schemes are reported as counts (%). Concentrations are ppm (mg/L for serum and dry weight for liver). Puls' deficient includes our severely deficient and deficient, and the lower end of moderate. The majority of these animals are beef animals.

	New scheme (this paper)					
Puls' [24] scheme	**Severely deficient**	**Deficient**	**Moderate**	**Adequate**	**Toxic**	**Row total (This paper)**
Serum						
Deficient	74 (100)	0 (0)	0 (0)	0 (0)	0 (0)	74
Moderate	0 (0)	341 (55)	280 (45)	0 (0)	0 (0)	621
Adequate	0 (0)	0 (0)	0 (0)	253 (100)	0 (0)	253
Toxic	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)	5
Column total (Puls[24])	74	341	280	253	5	953
Liver						
Deficient < 30	58 (100)	43 (100)	0 (0)	0 (0)	0	101
Moderate	0 (0)	0 (0)	75 (100)	0 (0)	0	75
Adequate	0 (0)	0 (0)	0 (0)	21 (100)	0	21
Toxic	0	0	0	0	0	0
Column total (Puls[24])	58	43	75	21	0	197

* Neonates and suckling calves were omitted because adequate vitamin A concentrations in serum and liver were not established. Cattle of unreported age were excluded.

life, serum and liver concentrations could be at levels considered deficient in adults.[41-43] Retinol measured only in plasma does not accurately indicate VA status in calves from birth through 6 weeks of age. At 1 month of age, the only way VA could be adequately assessed was by measuring liver VA or doing a Relative Dose Response (RDR) assay.[44]

Statistical analysis of scattergrams digitized from published data[26-28] and one thesis[30] and for the ISU-VDL data (Table 3) showed that the liver stores of vitamin A maintain serum concentrations until supplies are nearly exhausted. These implied that animals use liver supplies in times of dietary scarcity and that clinical signs of VA deficiency do not develop until the liver supplies are nearly exhausted.

Correlations of VA from related samples

We used published or digitized data for individual animals to evaluate the correlation of hepatic VA or serum VA from related calves and adult females. This analysis helps to determine if postulated equations apply to non-related samples. Also, liver and serum VA correlations were developed for "within calves" and "within adult females." It is important to note that the methods of analysis of VA values were inconsistent across studies as the manuscripts reviewed spanned from the early 1900s to the present. However, differences in analytical methods could not be assessed in our analysis from published data.

A VA status level implies that all combinations of liver and serum VA concentrations within that bivariate range are possible. To summarize, the power-law equation should be used to regress VA concentrations:

$y = ax^b$ or in linearized form, $\log(y) = a' + b \log(x)$, where $a' = \log(a)$.

Correlation of serum or hepatic VA from unrelated adults and calves

Little VA is transferred across the placenta, and newborn calves obtain most of their required 500,000 IU of VA by ingestion of colostrum.[39] As they mature, calves obtain VA from milk, feed, and supplemental vitamins; therefore, linear relationships such as those we found are reasonable. We tested the hypothesis that the ordered vitamin A concentrations in the ISU-VDL serum or liver samples from a group of adults would be typical of ordering concentrations from calves under 6 months of age.[30,39] As this hypothesis proposed, Spearman rank correlations between adults and younger age groups were significant ($P > 0.9$) for each tissue. If a practitioner has tissue VA data for the group of adults in the herd, our equations estimate the tissue concentration distribution in the group of calves in the herd or between the dam and calf. Using fractiles to group concentrations for correlation analysis in a herd is analogous to Spearman's method: both are a correlation of ordered data. While analytical methods used by the ISU-VDL for VA would have changed with improved and new instrumental methods between 2010-2020, the direct effects would be on precision (repeatability) and sensitivity (detection and quantification limits).

Table 5: Regression models relating liver and plasma/serum vitamin A concentrations reported in the cited literature. All the models in this table used the data from Baker et al.[26] (1954; Tables 21 and 22) and DC Church[30] (1956; Tables 26-29). Neither author reported correlations or regression equations. Baker reported the vitamin A concentrations in the plasma and livers of 4 cows fed low-carotene rations (µg/100 ml) from November 1950 to July 1953. Church reported the plasma and vitamin A concentrations in varying numbers of steers from 1953 to 1955.

Models*		**Y = a (± se_a†) + b (± se_b†) × ± RMSE‡.**						
Y	**X**	**a (intercept)**	**se_a**	**b (slope)**	**se_b**	**RMSE**	**R^2**	**Pr**
Baker *et al.*								
plasma[a]	liver	21.95	6.05	0.02	0.03	30.53	0.01	0.453
liver[a]	plasma	129.39	28.10	0.54	0.72	151.00	0.01	0.453
log(plasma)[b]	liver	2.68	0.15	0.0018	0.00074	0.77	0.11	0.023
plasma[b]	log(liver)	8.43	9.95	4.28	2.31	29.65	0.07	0.079
liver[b]	log(plasma)	-39.05	78.99	61.33	26.04	144.01	0.11	0.023
log(liver)[b]	plasma	3.50	0.34	0.02	0.01	1.81	0.07	0.079
log(liver)[c]	log(plasma)	-0.07	0.84	1.34	0.28	1.52	0.34	< 0.001
log(plasma)[c]	log(liver)	1.95	0.22	0.25	0.05	0.66	0.34	< 0.001
DC Church								
plasma[a]	liver	1.88	1.43	0.11	0.06	23.19	0.06	0.05
liver[a]	plasma	21.08	1.64	0.49	0.25	101.76	0.06	0.05
log(plasma)[b]	liver	0.77	0.26	0.01	0.01	0.73	0.03	0.19
plasma[b]	log(liver)	-13.20	5.23	6.06	1.73	34.45	0.15	0.00
liver[b]	log(plasma)	21.24	2.03	1.95	1.48	108.03	0.03	0.19
log(liver)[b]	plasma	2.88	0.06	0.02	0.01	0.14	0.15	0.00
log(liver)[c]	log(plasma)	2.71	0.06	0.26	0.04	0.11	0.33	< .0001
log(plasma)[c]	log(liver)	-2.75	0.66	1.29	0.22	0.55	0.33	< .0001

* Models: [a]Linear; [b]semilog; [c]log-log

† se_a and se_b are the standard error of the respective regression coefficient

‡ RMSE (or ε) = root mean square error

Correlation of vitamin A in unrelated liver and serum samples

This study developed a mathematical relationship between unpaired vitamin A concentrations in bovine sera and livers submitted to the ISU-VDL. Current definitions[24] (Table 1) were used to establish if vitamin A concentrations were adequate, marginal or deficient. For the ISU-VDL data, 26.1% of sera and 68.4% of liver samples were deficient. The high percentages of animals with low VA concentrations are not surprising because these are diagnostic lab samples; this sample population may or may not represent the accurate underlying population distribution of VA levels in U.S. beef and dairy cattle. The results are consistent with liver stores of vitamin A maintaining serum concentrations until supplies are nearly exhausted when the diet contains insufficient VA. A normal blood level cannot be interpreted to ensure that current intakes are adequate, but a low level would indicate a deficiency. A possible explanation is the limited availability of vitamin A due to losses by ruminal destruction when ruminants are fed a high-concentrate diet.[45] Unfortunately, the VSU-VDL did not include diet; thus, we cannot link the diagnostic lab data to the dietary levels of VA.

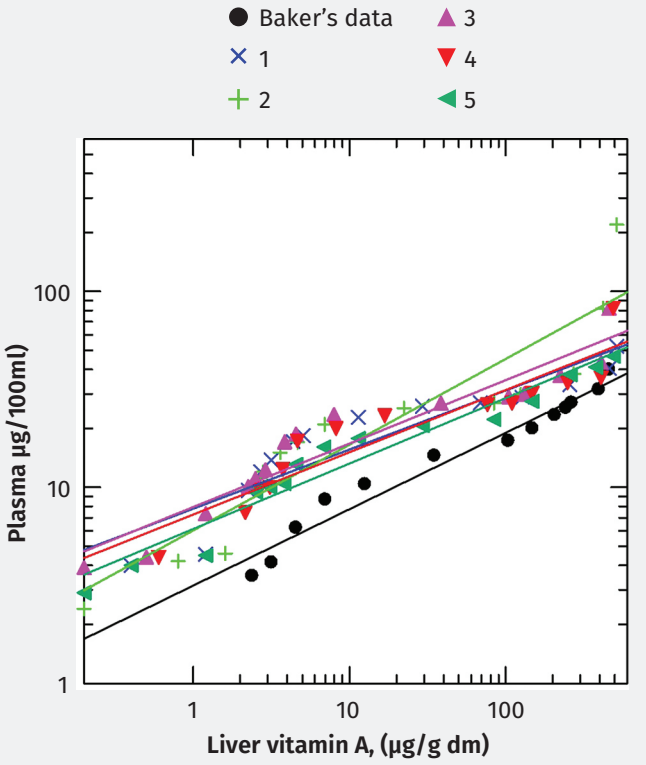
A literature review and data analyses indicated that the continuum between deficient and adequate vitamin A status should be revised because deficiencies occur at higher serum and liver concentrations than the 1994 guidelines.[24] Unlike previous proposals, our serum and liver VA ranges are bivariate criteria to be met jointly.

Conclusion

Most published regressions relating VA concentrations in bovine liver and serum/plasma were unified by the log-log relationship log(serum) = *a*'+ *b* log(liver). The relationship provides a missing connection between Puls' criteria for VA in these tissues. Ranges for classifying deficient VA status based on clinical and experimental studies occur at higher serum and liver concentrations than Puls' guidelines.

Our data analyses for reported relationships between serum and liver VA from the same animal showed that regressions of the fractiles of the serum and liver concentrations from non-paired animals attained a similar relationship. Using these relationships, we proposed new ranges for serum and liver

Figure 1: Log-log plots of vitamin A (VA) quantiles for plasma and liver for 97 plasma-liver pairs from Baker's[26] tables 21-22. Five random samples of 50 pairs each were created using SAS PROC SURVEYSELECT[g]. SYSTAT[f] was used to independently estimate the quantiles (1, 5, 10, 20, 25, 30, 40, 50, 60, 70, 75, 80, 90, 95, 99) for each replicate and tissue to create the figure. The 1% and 99% quartiles for the original data were omitted due to outliers in the original data. The correlations' similarity of random subsets and the original data supports our "herd" regression concept.



(Table 2) and emphasized that these bivariate criteria must be met jointly. Veterinary diagnostic laboratories can use this information to better estimate serum and liver VA concentrations for unpaired data from the herd and recommend to cattle producers the status of VA in their herds.

Conflict of interest

The authors declared no potential conflict of interest concerning this article's research, authorship and publication.

Funding

The authors received no financial support for this study.

Acknowledgments

We thank Dr. Kent Schwartz from Iowa State University for sharing the data while David Villar was invited in June 2021. We thank the anonymous reviewers for their significant comments. We are indebted to two associate editors, Dr. Capik and Dr. Fajt, for substantial edits that focused on veterinary practitioners for this research.

Author contributions

DV conceived of the study, obtained the data during a sabbatical at the Iowa State Veterinary Diagnostic Laboratory in (2021) and wrote the initial draft. He compiled and reviewed publications on vitamin A deficiency, developed the proposed changes in Puls' criteria and prepared the related tables. He edited and approved the final version to be published.

DJS conducted literature reviews for published statistical relationships for VA in bovine (and other species) tissues, carried out all the statistical analyses in the manuscript, prepared the figures, and developed supplemental data tables. He edited and approved the final version to be published.

Endnotes

[a]Excel version 2105. Microsoft Corporation. (2021) Available at: **https://office.microsoft.com/excel**.

[b]Cogniview PDF2XL Enterprise version 5.2.2.304. Cogniview-The PDF to Excel Company, Roswell, GA.

[c]CurveExpert Pro 2.7.3. Hyams Development. Madison, Alabama. **https://curveexpert.net/**.

[d]Engauge Digitizer. Mark Mitchell, Baurzhan Muftakhidinov and Tobias Winchen et al. **http://markummitchell.github.io/engauge-digitizer**. DOI: **10.5281/zenodo.3941227**.

[e]WebPlotDigitizer. Ankit Rohatgi, author. Pacifica, CA. **https://automeris.io/WebPlotDigitizer**

[f]Systat 13.1 Inpixon, Palo Alto, CA.

[g]SAS Institute Inc. 2016. SAS® 9.4 Language Reference: Concepts, Sixth Edition. Cary, NC.

Abbreviations

VA	Vitamin A
log or $\log_e$	Natural logarithm
ww	Wet weight
dw	Dry weight
ppm	Parts per million
ISU-VDL	Iowa State University Veterinary Diagnostic Laboratory

References

1. McDowell LR. Vitamin A. In: McDowell LR, ed. *Vitamins in Animal and Human Nutrition*. 2nd ed. Academic Press; 2000:48-53.

2. Frye TM, Williams SN, Graham TW. Vitamin deficiencies in cattle. *Vet Clin North Am Food Anim Pract* 1991; 7(1):217-228. doi:**10.1016/S0749-0720(15)30817-3**

3. Divers TJ, Blackmon DM, Martin CL, Worrell DE. Blindness and convulsions associated with vitamin A deficiency in feedlot steers. *J Am Vet Med Assoc* 1986; 189(2):1579-1582. PMID:3793597

4. Van der Lugt JJ, Prozesky L. The pathology of blindness in newborn calves caused by hypovitaminosis A. *Onderstepoort J Vet Res* 1989; 56(2):99-109. PMID:2748139

5. Van Donkersgoed J, Clark EG. Blindness caused by hypovitaminosis A in feedlot cattle. *Can Vet J* 1988; 29:925-927. PMID:17423169

6. Millemann Y, Benoit-Valiergue H, Bonnin JP. et al. Ocular and cardiac malformations associated with maternal hypovitaminosis A in cattle. *Vet Rec* 2007; 160:441-443. doi:**10.1136/vr.160.13.441**

7. Paulsen ME, Johnson L, Young S, et al. Blindness and sexual dimorphism associated with vitamin A deficiency in feedlot cattle. *J Am Vet Med Assoc* 1989; 1947:933-937. PMID:2703427

8. Read DH. Congenital blindness in dairy calves in California due to maternal vitamin A deficiency. Proceedings of the 39th Conference of the New Zealand Society for Veterinary Pathology, *N Z Vet J*. 2014;62:5, 305. doi:**10.1080/00480169.2014.907736**

9. Franklin ST, Sorenson CE, Hammell DC. Influence of vitamin A supplementation in milk on growth, health, concentrations of vitamins in plasma, and immune parameters of calves. *J Dairy Sci* 1998; 81(10):2623-2632. doi:**10.3168/jds.S0022-0302(98)75820-5**

10. Kumagai H, Chaipan Y, Mitani K. Effects of periparturient vitamin A supplementation on vitamin A concentrations in colostrum and milk from dairy cows, and plasma retinol concentrations, feed intake and growth of their calves. *Anim Sci J* 2001; 72:126-133. doi:**10.2508/chikusan.72.126**

11. Gallina AM, Helmboldt CF, Frier HI, et al. Bone growth in the hypovitaminotic A calf. *J Nutr* 1970; 100(1):129-141. doi:**10.1093/jn/100.1.129**

12. Cousins RJ, Eaton HD, Rousseau JE, et al. Biochemical constituents of the dura mater in vitamin A deficiency. *J Nutr* 1969; 97(3): 409-418. doi:**10.1093/jn/97.3.409**

13. Eaton HD, Lucas JJ, Nielsen SW, et al. Association of plasma or liver vitamin A concentrations with the occurrence of parotid duct metaplasia or of ocular papilledema in Holstein male calves. *J Dairy Sci* 1970; 53(12):1775-1779. doi:**10.3168/jds.S0022-0302(70)86477-3**

14. Hill BD, Holroyd RG, Sullivan M. Clinical and pathological findings associated with congenital hypovitaminosis A in extensively grazed beef cattle. *Aust Vet J* 2009; 87(3):94-98. doi:**10.1111/j.1751-0813.2009.00398.x**

15. Clagett-Dame M, Knutson D. Vitamin A in reproduction and development. *Nutrients* 2011; 3(4):385-428. doi:**10.3390/nu3040385**

16. McGill JL, Kelly SM, Guerra-Maupome M, et al. Vitamin A deficiency impairs the immune response to intranasal vaccination and RSV infection in neonatal calves. *Sci Rep* 2019; 9:15157. doi:**10.1038/s41598-019-51684-x**

17. Chew BP, Hollen LL, Hillers JK, et al. Relationship between vitamin A and β-carotene in blood plasma and milk and mastitis in Holsteins. *J Dairy Sci* 1982; 65:2111-2118. doi:**10.3168/jds.S0022-0302(82)82469-7**

18. Davis RE, Madsen LL. Carotene and vitamin A in cattle blood plasma with observations on reproductive performance at restricted levels of carotene intake. *J Nutr* 1941; 21(2):135-146. doi:**10.1093/jn/21.2.135**

19. Eaton HD. Chronic bovine hypo- and hypervitaminosis A and cerebrospinal fluid pressure. *Am J Clin Nutr* 1969; 22(8):1070-1080. doi:**10.1093/ajcn/22.8.1070**

20. Eaton HD, Rousseau JE, Hall RC, et al. Reevaluation of the minimum vitamin A requirement of Holstein male calves based upon elevated cerebrospinal fluid pressure. *J Dairy Sci* 1972; 55(2):232-237. doi:**10.3168/jds.S0022-0302(72)85465-1**

21. Mikkilineni SR, Rousseau JE, Hall RC, et al. Sodium and potassium concentration of brain and dura matter in chronic hypovitaminosis A of calves. *J Dairy Sci* 1973; 56(3):395-398. doi:**10.3168/jds.S0022-0302(73)85184-7**

22. Booth A, Reid M, Clark T. Hypovitaminosis A in feedlot cattle. *J Am Vet Med Assoc* 1987; 190(10):1305-1307. PMID:3583885

23. Parker EM, Gardiner CP, Kessell AE, et al. Hypovitaminosis A in extensively grazed beef cattle. *Aust Vet J* 2017; 95(3):80-84. doi:**10.1111/avj.12560**

24. Puls R. *Vitamin Levels in Animal Health: Diagnostic Data and Bibliographies*. 1994; Sherpa International. Clearbook B.C., Canada.

25. Eaton HD, Rousseau JE, Dicks MW, et al. Association between plasma and liver concentrations of vitamin A or of tocopherol in Holstein male calves fed fixed intakes of these vitamins. *J Dairy Sci* 1956; 41(10):1456-1459. doi: **10.3168/jds.S0022-0302(58)91113-5**

26. Baker FH, Pope LS, MacVicar R. The effect of vitamin A stores and carotene intake of beef cows on the vitamin A content of the liver and plasma of their calves. *J Anim Sci* 1954; 13(4);802-807. doi:**10.2527/jas1954.134802x**

27. Parrish DB, Wise GH, Hughes JS. Effect of vitamin A supplements upon the state of vitamin A in blood serum of the dairy cow and in blood serum and liver of its neonatal calf. *J Biol Chem* 1948; 172(2):355-365. doi:**10.1016/S0021-9258(19)52720-X**

28. Wright KK, Hall RC. Association between plasma and liver vitamin A levels in the calf; weanling pig, rabbit and rat; and adult goat fed fixed intakes of vitamin A. *J Nutr* 1979; 109:1063-1072. doi:**10.1093/jn/109.6.1063**

29. Rousseau JE, Dembiczak CM, Doge KL, et al. Relationship between plasma vitamin A and liver vitamin A in calves fed a vitamin A depletion ration and calves fed minimum levels of vitamin A or carotene. *J Dairy Sci* 1954; 37(11):1376-1382. doi:**10.3168/jds.S0022-0302(54)91416-2**

30. Church DC. *Vitamin A studies with beef cattle*. PhD thesis. Oklahoma Agricultural and Mechanical College; 1956. **https://core.ac.uk/download/pdf/215185183.pdf**

31. Wheeler RR, Heswig PH, Brannon WF, et al. The carotene and vitamin A content of plasma and their calves in the Northern Great Basin. *J Anim Sci* 1957; 16(2):525-536. doi:**10.2527/jas1957.162525x**

32. Wise GH, Caldwell MT, Hughes JS. The effect of the prepartum diet of the cow on the vitamin A reserves of her newborn offspring. *Science* 1946; 103(2681): 616-618. doi:**10.1126/science.103.2681.616**

33. Bera AK, Ghosh A, Xia Z. 2011. Fractiles on quantile regression with applications. **Fractilesonquantileregression.pdf (mysmu.edu). http://www.mysmu.edu/faculty/aurobindo/papers/fractilesonquantileregression.pdf**

34. Phoon K-K, Quek S-T, Huang H. Simulation of non-Gaussian processes using fractile correlation. *Probabilistic Eng Mech* 2004; 19(4):287-292. doi:**10.1016/j.probengmech.2003.09.001**

35. Rodriguez RN, Yao Y. Five things you should know about quantile regression. *Proceedings of the SAS Global Forum 2017 Conference*. AS Institute Inc. SAS525-2017. **https://support.sas.com/resources/papers/proceedings17/SAS0525-2017.pdf**

36. Goodwin TW, and Wilson AA. Studies in vitamin A. 15. The variations in the serum β-carotene and vitamin A levels of cows near parturition. *Biochem J* 1951; 49(4):499-503. doi:**10.1042/bj0490499**

37. Freund RJ. Some observations on regression with grouped data. *Am Stat* 1971; 25(3):29-30. doi:**10.2307/2683321**

38. Schwiebert J. Computing regression statistics from grouped data. *J Econ Soc Meas* 2014; 39(4):283-303. doi:**10.3233/JEM-150416**

39. Puvogel G, Baumrucker C, Blum JW. Plasma vitamin A status in calves fed colostrum from cows that were fed vitamin A during late pregnancy. *J Anim Physiol Anim Nutr* 2008; 92:614-620. doi:**10.1111/j.1439-0396.2007.00757.x**

40. Waldner CL, Uehlinger FD. Factors associated with serum vitamin A and vitamin E concentrations in beef calves from Alberta and Saskatchewan and the relationship between vitamin concentrations and calf health outcomes. *Can J Anim Sci* 2017; 97(1):65-82. doi:**10.1139/cjas-2016-0055**

41. Nezvesky L, Eaton HD, Johnson RE, et al. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the neonatal calf. *J Dairy Sci* 1950; 33(5):315-323. doi:**10.3168/jds.S0022-0302(50)91906-0**

42. Zanker IA, Hammon HM, Blum JW. Beta-carotene, retinol and alpha-tocopherol status in calves fed the first colostrum at 0-2, 6-7, 12-13 or 24-25 hours after birth. *Int J Vit Nutr Res* 2000; 70(6):305-310. doi:**10.1024/0300-9831.70.6.305**

43. Nonnecke BJ, Horst RL, Hammell DC, et al. Effects of supplemental vitamin A on retinoic acid concentrations in the plasma of preruminant calves. *Int J Vitam Nutr Res* 2000; 70(6):278-286. doi:**10.1024/0300-9831.70.6.278**

44. Hammell DC, Franklin ST, Nonnecke BJ. Use of the relative dose response (RDR) assay to determine vitamin A status of calves at birth and four weeks of age. *J Dairy Sci* 2000; 83(6):1256-1262. doi:**10.3168/jds.S0022-0302(00)74992-7**

45. Rode LM, McAllister T, Cheng KJ. Microbial degradation of vitamin A in rumen fluid of steers fed concentrate, hay, or straw diets. *Can J Anim Sci* 1990; 70:227-233. doi:**cdnsciencepub.com/doi/10.4141/cjas90-026**