

Serum Retinol and Beta-Carotene Concentrations in US Dairy Cows

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Beta-carotene is a carotenoid present naturally in plant tissue. In un-supplemented herbivore diets it serves as the primary source of vitamin A activity. In most modern livestock diets, however, vitamin A is added as a supplement, usually in the form of retinyl acetate. This raises the question of the role of beta-carotene in dairy cow diets in which adequate vitamin A has been added as a supplement.

There is some evidence that beta-carotene may have nutritional roles in addition to being a precursor for vitamin A. Specifically, dietary beta-carotene may benefit animal health and performance by serving as an antioxidant, enhancing immune function, and by promoting steroidogenesis, the latter especially within the corpus luteum. The strength of evidence supporting the importance of these functions in cattle is variable (Weiss, 1998). Studies with dairy cattle have revealed either positive or no effects of supplemental beta-carotene on reproduction and mammary gland health (Weiss, 1998). From a recent study (Arechiga *et al.*, 1998) designed to test effects of beta-carotene in cattle under heat stress, a significant increase in milk production of cows supplemented with 400 mg/day beta-carotene was reported in each of three experiments, regardless of ambient temperature. Other recent information from *in vitro* studies suggests that beta-carotene may stimulate the production of progesterone from the corpus luteum (Arikan and Rodway, 2000), although the results appear variable (O'Shaughnessy and Wathes, 1988).

The variability in both *in vivo* and *in vitro* responses to supplemental beta-carotene in cattle may be associated with variation in the beta-carotene status of the animals, prior to supplementation or tissue collection. In order to better evaluate the potential benefit of beta-carotene supplements, more data is needed concerning the beta-carotene status of US dairy cattle. With that as a goal, the objective of this study was to determine descriptive statistics for serum beta-carotene concentrations in a random sampling of healthy US dairy cattle. In addition, the sampling design allowed us to examine for regional differences, within a specific time period. Furthermore, the relationship between serum beta-carotene and retinol concentrations was evaluated.

Materials and Methods

Samples analyzed were from the 1996 National Animal Health Monitoring System study of US dairy herds. For this study, herds were selected from four regions across the country, Northeast (NY, PA, VT), Midwest (MN, WI, MI, IA, IN, IL, OH, MO), West (CA, OR, WA, ID, NM, TX), and Southeast (FL, KY, TN). The selection of herds was designed to reflect the size of the dairy cow population in each area such that total sampling was representative of the US dairy cow population (NAHMS, 1996). On each

farm, cows from the entire population of healthy adults were randomly selected for blood sampling. Cows designated as sick, or that were scheduled for culling were not included in the samples analyzed in this study. The blood samples used in this analysis were collected in April or May of 1995. The samples were frozen at -80°C during storage. Herds were designated as being “pastured” if at sometime during the year dry or lactating cows received and estimated 90% or more of their forage from pasture. No record was made of whether or not the cows from which blood samples were taken were currently at pasture. Herds were further classified by size; 1 – 1 to 49, 2 – 50 to 99, 3 – 100 to 299, and 4 – 300+.

The data analyzed for this report were not part of the original study design, so we were using the sample amount that remained after the designed analyses were completed. Therefore, in some cases there was not sufficient sample volume for beta-carotene and retinol analysis.

Beta-carotene and retinol were extracted from the samples with an equal volume of ethanol and twice the sample volume of hexane. Quantification was by a single, modified-reverse-phase HPLC procedure employing a C18 column and a mobile phase of acetonitrile, methylene chloride, and methanol (70:20:10). Detection was by absorbance at 225 nm (retinol) and 450 nm (beta-carotene). A commercial kit (Sigma) was used to determine cholesterol concentrations in serum.

Statistical evaluation was by a step-down procedure using a mixed-model ANOVA. (Proc Mixed, SAS). Initial fixed independent variables were region, pasture, and herd size. Herd was analyzed as a random variable. Serum cholesterol was included in the model as an independent covariate. A single interaction effect, pasture by region, was also included in the initial model. Variables were eliminated from the model if the significance (P) value for their effect in the aggregate model was greater than 0.2.

Results

Descriptive statistics

Samples with suitable volume for analysis were obtained from 358 animals distributed among 35 herds. There were 11 herds in the Midwest, 10 herds in the Northeast, 7 herds in the Southeast, and 7 herds in the West. The modal number of animals tested per herd was 10, with a range of 7 to 18. The herds were distributed approximately equally between pastured (17) and non-pastured (18). Herds were also distributed approximately equally among size classifications: 1 – 8, 2 – 10, 3 – 9, and 4 – 8. However, there was not even distribution of pastured herds among herd size classifications, as illustrated in figure 1. Thus, herd-size and pasture effects were confounded.

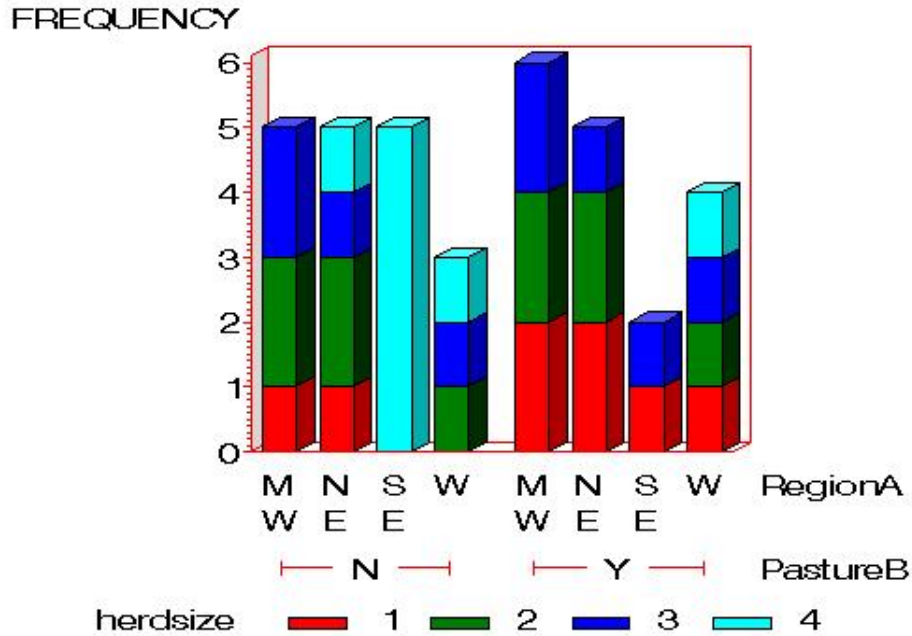


Figure 1. Bars are stacked by herd size, as indicated by shade of gray. The grouping of regions is by pasture (Y – yes, N – no). Note that all but one of the large herds were not pastured, while the large majority of the small herds were pastured, confounding the effects of herd size and pasture.

The distribution of serum beta-carotene concentrations is in figure 2. Note that the distribution is strongly skewed to the right, and is not normal. This distribution is characteristic of many biological variables in which there is a lower limit (zero) but no rigidly controlled upper limit. Log transformation results in an approximate normalization of the distribution, as illustrated in figure 3.

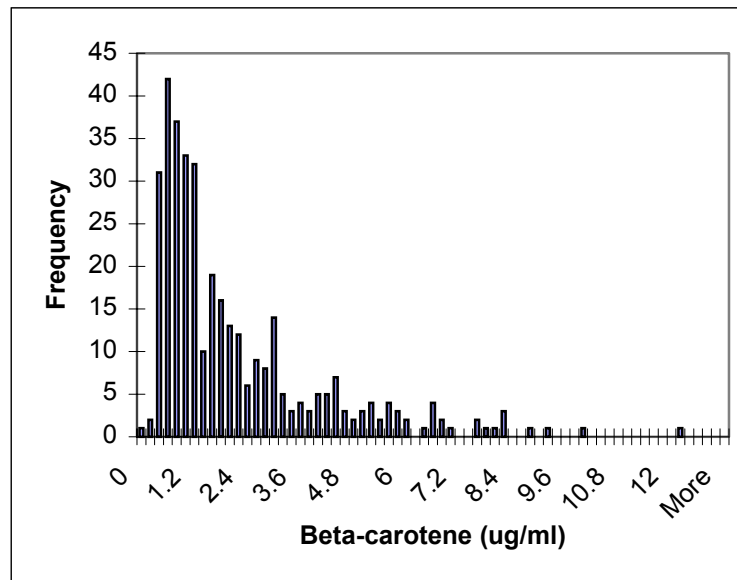


Figure 2. Frequency distribution of untransformed beta-carotene values.

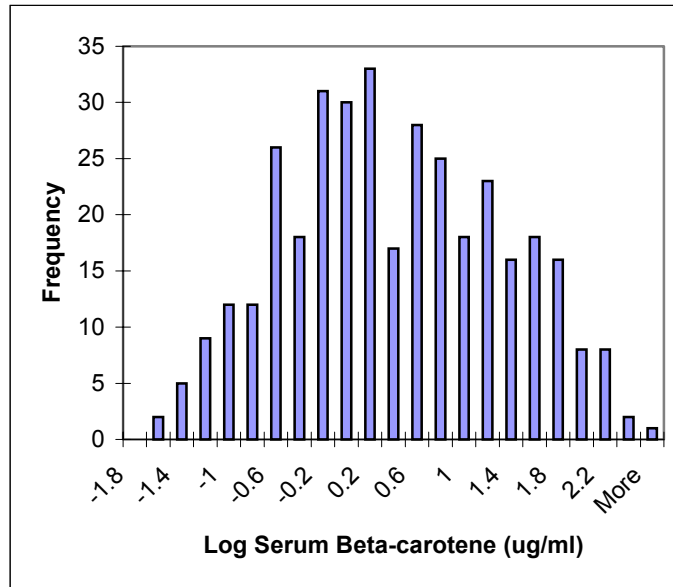


Figure 3. Distribution of log-transformed serum beta-carotene concentrations. This distribution deviates significantly ($p < 0.05$) from normal, but is far closer to normal than the untransformed data.

The distribution of serum retinol concentrations is in figure 4. This distribution is normal, which is characteristic of many biological variables that are under homeostatic control.

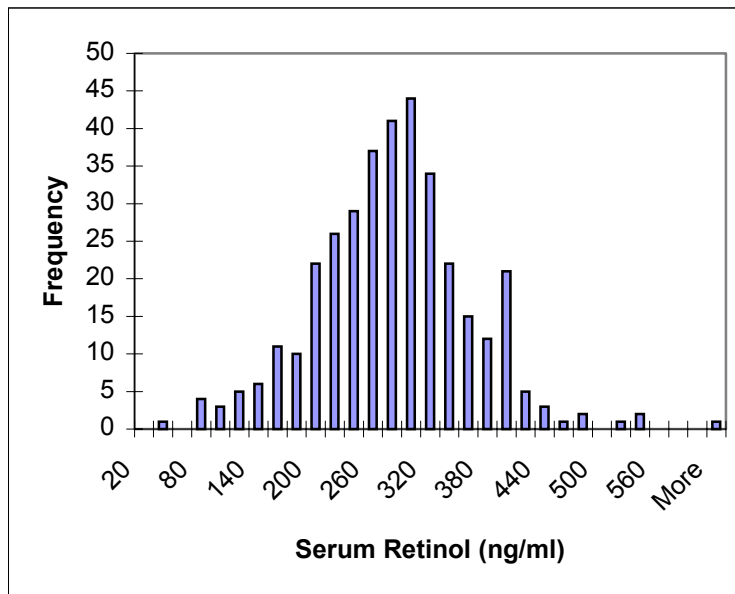
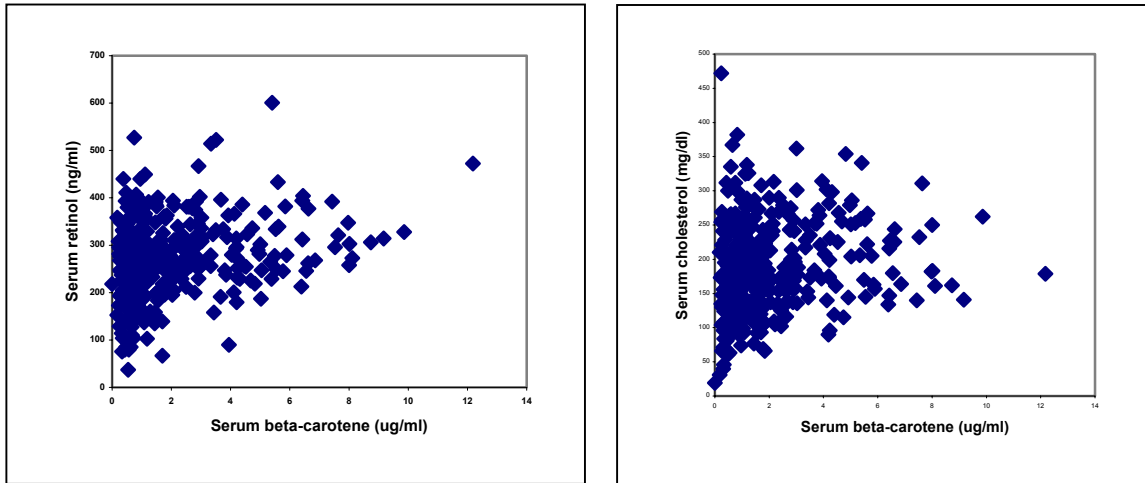


Figure 4. Distribution of serum retinol concentration. This distribution approximates normal.

Serum beta-carotene concentrations were correlated significantly ($p < 0.02$) with serum retinol and cholesterol, but the R^2 values were low, 0.06 and .025 respectively.

Scattergrams illustrating the association between serum beta-carotene, serum cholesterol, and serum retinol are in figures 5 and 6.



Figures 5 and 6. The association between serum beta-carotene and serum retinol and cholesterol concentrations. The associations between these variables are not strong, but note that while low values of beta-carotene are associated with both high and low values of retinol or cholesterol, no low values of these two variables are associated with higher values of beta-carotene.

Unadjusted beta-carotene means by region and pasture are in tables 1 and 2.

	Midwest	Northeast	Southeast	West
Mean ($\mu\text{g/ml}$)	1.24	2.14	2.15	2.67
Standard deviation	0.99	1.35	2.31	2.27

Table 1. Means and standard deviations of serum beta-carotene concentrations related to region.

	Pastured	Not pastured
Mean ($\mu\text{g/ml}$)	2.41	1.68
Standard deviation	2.0	1.64

Tables 2. Means and standard deviations of serum beta-carotene concentrations related to use of pasture in the herd. It is unknown whether or not the animals from which the measures were made were receiving pasture at the time of sampling.

In univariate analysis of log-transformed values, the Midwest mean is lower ($P < 0.05$) than each of the other three regions. None of the other regions are significantly different from one another. In univariate analysis of log-transformed values, pastured herds have higher ($P < 0.05$) mean serum beta-carotene than non-pastured herds.

Model building

Normal distribution is an important assumption for analysis of variance. Therefore, the log transformed beta-carotene values were used for model building. Independent variables remaining in the final model were herd ($P < 0.001$), cholesterol ($P < 0.001$), and

pasture ($P=0.08$), although pasture was marginally significant. Herd accounted for the major portion (68%) of the variation. Variables dropped from the model included region, herd size, and region by pasture interaction.

Discussion

Beta-carotene is the dietary source of vitamin A in the natural diets of ruminants. In this study, however, the correlation between serum retinol and serum beta-carotene was weak. Animals with low serum beta-carotene had a broad range of serum retinol concentrations. The lack of association between retinol and beta-carotene at the low end of the beta-carotene distribution probably reflects the addition of retinyl acetate to the diets of many of the animals sampled, thus making beta-carotene unnecessary as a dietary source of vitamin A. At the higher end of the serum beta-carotene concentration spectrum, there were no animals with low serum retinol. This perhaps indicates that when beta-carotene is available the animals will have adequate vitamin A status.

Beta-carotene, like alpha-tocopherol is carried nearly exclusively in the lipoprotein fraction of blood serum (Chew *et al.*, 1993). Therefore, the concentration of serum lipoproteins could be expected to influence serum beta-carotene concentration. Serum cholesterol also is exclusively a component of the lipoprotein fraction. Therefore, cholesterol can be used as an estimator of serum lipoprotein concentration. Figure 6 illustrates the relationship between serum cholesterol and beta-carotene. As would be expected, animals with low serum cholesterol did not have high beta-carotene concentrations, because there was little lipid fraction into which it could distribute. In contrast, animals with high serum lipoprotein concentrations, as indicated by high serum cholesterol, had a broad range of serum beta-carotene concentrations, probably related to dietary beta-carotene intake.

Beta-carotene is present in large concentration in most fresh forages, but diminishes with storage. Therefore, it might be expected that animals at pasture would have higher serum beta-carotene concentrations than those receiving stored forage. However, in this study it was not known whether or not the animals sampled were receiving pasture at the time of blood collection, only that some animals on the farm received pasture as their major forage source at some time of the year. The samples were collected in April and May, making it unlikely that pasture had been fed for very long in the Midwest or Northeast. The effect of pasture may have been both from current consumption of pasture, and from body stores of beta-carotene that had accumulated from previous pasture feeding.

Perhaps the most striking observation in this study was the large variance component due to herd. This means that environmental factors, likely related to nutrition, have a large influence on serum beta-carotene concentration. This implies that serum beta-carotene is readily amenable to manipulation by dietary management. In addition, the general distribution of serum beta-carotene values was well below the 3 $\mu\text{g}/\text{ml}$ concentration suggested as minimal by some authors (Frye *et al.*, 1991). Therefore, there may be an opportunity to manage serum beta-carotene in dairy cattle by dietary supplements. Further epidemiological research is necessary to better elucidate the relationship between serum beta-carotene concentrations and health and performance variables.

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