

Variability in the Vitamin D₃ Content of 2% Milk from a Nationwide United States Department of Agriculture (USDA) Sampling

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Abstract

Objective: The objective of the study was to assess the vitamin D₃ content of 2% retail milk in the United States and its variability. Milk contains little natural vitamin D but is usually supplemented with vitamin D₃ for retail sale in the US. The fortification target is 400 IU (10 mcg) per quart (25% DV per 8 oz serving or 42 IU/100g). **Materials and Methods:** In 2001, the USDA sampled and analyzed vitamin D₃ in milk from 12 statistically selected supermarkets in the United States. In 2007, sampling was repeated, obtaining milk from 24 different supermarkets selected statistically to represent the nation. Analyses were done on the 2007 2% milk samples and repeated for the 2001 2% milk samples by HPLC with UV detection using an improved method specifically validated for vitamin D₃ in milk. Vitamin D₂ was also monitored to ensure that any samples fortified with D₂ instead of D₃ were detected. **Results:** None of the samples contained vitamin D₂. Of the 12 samples obtained in 2001, 7 were >10% below label value, 4 within 100-150% of label, and 1 above 150% of label. Three of the 24 samples from 2007 were below label value with 1 of the 3, despite the label claim, having no detectable vitamin D₃; 20 had values within 100-150% of label, and 1 above 150% of label. Analyses are ongoing of other types of fluid milk samples also obtained. **Significance:** Regulations from the US FDA require that the amount of vitamin D₃ in milk be at least equal to the nutrient value declared on the label. The acceptable range within limits of good manufacturing practices is 100–150% of label claims. The amount of vitamin D₃ on the label for 2% milk was met by less than half of the 2001 samples but about 83% of those from 2007. Due to the high levels of consumption, milk is an important source of vitamin D₃ for many North Americans, and current and accurate data are important to the assessment of intake, which could be significantly more or less than the label value, depending on the particular milk consumed.

Background

- A review of North Carolina dairies published in 1996 found that vitamin D₃ was added at 9 different points in the processing of milk at various dairies¹, including at the raw milk tanker or the homogenizer.
- When vitamin D₃ is added in a lipid soluble form prior to separation of the cream, low-fat and skim milks may be under-fortified and higher fat products over-fortified.
- In 2004, the FDA provided guidance² on the fortification of milk suggesting that a metering system be used when adding Vitamin D₃ after cream separation and before homogenization to allow for adequate mixing.

Acknowledgement. This work was supported by the USDA Agricultural Research Service. Partial support was received from the National Institutes of Health, agreement #Y1CN5010, and from The Beverage Institute for Health & Wellness, a division of The Coca-Cola Company.

Methodology

- Milk samples obtained from 12 locations in 2001 and 24 different locations in 2007 (Fig. 1) *
- 5.0 mL of sample spiked with an internal standard (D₂), saponified in methanolic KOH for 20 minutes at 60°C, and extracted with hexane.
- Hexane extract washed with dilute methanol and dried.
- Sample re-suspended in hexane/methylene chloride, applied to silica SPE cartridge, eluted with methylene chloride/2-propanol (99.8/0.2) and dried.
- Sample re-suspended in hexane/methylene chloride/alcohol (85/15/0.2), applied to HPLC ZORBAX SIL column (5µ), vitamin D fraction collected, and dried.
- Sample re-suspended in hexane/2-propanol (99.5/0.5), applied to a HPLC ZORBAX SIL column, vitamin D fraction collected, and dried.
- Sample is applied to a Vydac ODS column in acetonitrile/methylene chloride (75/25).
- Vitamin D₃ quantified by comparison of the UV peak areas with standards and corrected for recovery. Vitamin D₂ and vitamin D₃ are baseline resolved.

*In some cases, the milk purchased in one state was actually produced in another state.

Figure 1. Sampling Locations

Milk samples were obtained at statistically selected supermarkets in 2001 and again in 2007



Quality Control

- Methodology for the milk analysis was validated, including spike recovery studies, and a Skim Milk quality control (QC) material was developed for this study, with its vitamin D₃ content characterized by three laboratories³.
- The QC material was included in each batch of samples from both 2001 and 2007 (Figure 2): 2 of the 24 samples from 2007 were run in duplicate.
- The blind duplicates agreed within 5.5%, and all QC results fell within the previously established tolerance limits (+/-2SD of the 3-laboratory mean)
- The uncertainty in assayed values, based on the results for the QC material analyzed with the milk samples, was <3%.

Results

- Only 33% of the vitamin D₃ in 2% milk samples from 2001 fell within 100 to 150% of label value (400 IU [16 µg] per qt) (Figure 3a).
- 83% of the vitamin D₃ in 2% milk samples from 2007 fell within 100 to 150% of label value (Figure 3b).
- In 2007, the 2% milk sample from one site had no detectable vitamin D₃. This result was confirmed with a repeat analysis.
- Omitting the sample without vitamin D₃, the mean value for 2007 was 495 IU/qt (122 IU [3.06 µg] per 8 oz) with a 15% RSD, while for the 2001 sampling, the mean value was lower, 424 IU/qt (105 IU [2.64 µg] per 8 oz), with a 30% RSD.
- Vitamin D₃ in the 2% milk from the recent (2007) sampling ranged from <20 to 622 IU/qt, and averaged about 18% above label value.

Figure 3a. Vitamin D₃ in 2% Milk - (2001)

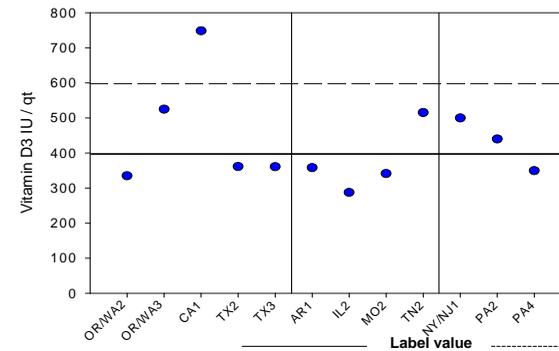


Figure 3b. Vitamin D₃ in 2% Milk - (2007)

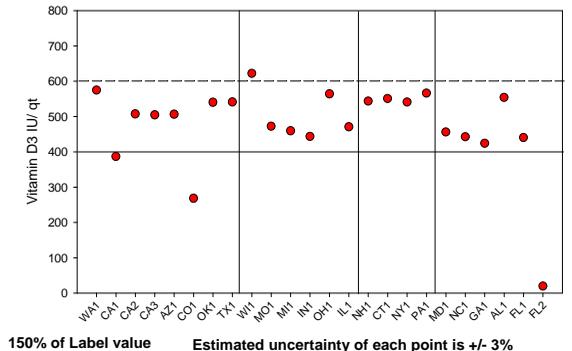
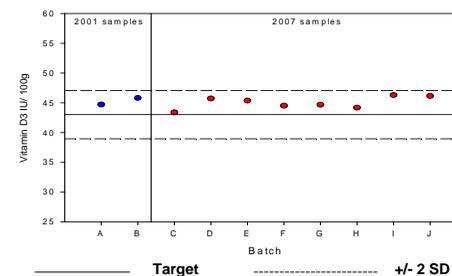


Figure 2. Quality Control Chart



Conclusions

- More than twice as many of the 2% milk samples from 2007 met the label levels for vitamin D₃ than the 2% milk samples collected in 2001.
- It is possible that the guidance from the FDA in 2004 may have had some impact on how the vitamin is added to the milk.
- Some dairies still have quality control problems with vitamin D₃ fortification of milk as evidenced by 12.5% of samples below the expected range.
- The accuracy and precision of the analyses, using the validated methodology, gives confidence in these estimated values for vitamin D₃ levels in milk.

Additional Planned Research

- Another sampling of milk from four of the original dairies is planned for 2008 – CA3, CO1, MD1 and FL2 (see Fig. 3b). This additional sampling may give some indication as to whether the level of fortification with vitamin D₃ by these dairies is consistent.
- Analysis of skim, 1%, whole, and 1% chocolate milk sampled along with the 2% milk is ongoing.

References

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