Vitamin D deficiency is common in many countries, including Saudi Arabia. Several studies suggest that vitamin D deficiency is linked with chronic and/or metabolic bone disease in adults and rickets in children. To minimize the extent of deficiency, various preventative measures have been taken at the population level, which includes fortification of vitamin D in milk and beverages. Recently, the American Academy of Pediatrics has issued guidelines to prevent rickets in infant and children. All infants should have a minimum intake of 200 IU of vitamin D per day. The Food and Drug Administration (FDA) in the United States recommends that milk should be fortified with vitamin D at a level 10-15 ng/mL (400-600 IU/L). Underfortification does not solve the purpose of fortification and continues the risk of deficiency whereas overfortification may cause intoxication and its serious consequences. Therefore, an optimum level 10 ng/mL (±20%) is considered an acceptable limit in many countries. However, the level may vary more widely due to several factors at the stages of production, transportation and/or storage, which necessitates surveillance programs.

The vitamin D content of fortified milk in different countries is highly variable. According to a US FDA survey only 26% of 669 milk samples collected from three states were within the range of fortification, whereas in another study, 15.8% of 158 fortified skim milk samples had vitamin D concentrations within 81-
120% of the claim. In a 1992 report on the vitamin D content of fortified milk and infant formula, Holick et al reported that 26 of 42 milk samples contained less than 80% of the claim. The US Department of Agriculture data reported that three types of fluid milk (skim, 1% fat, and 2% fat) collected from twelve locations sampled across the United States in 2001 showed that few of the values for the 36 samples were above the range of fortification, and one-third of the samples of all types fell below the minimum required level. In another study of 45 fortified milk samples from an Ontario retail market indicated that 20% of whole milk samples contained the recommended levels of vitamin D. The majority of the samples were over-fortified, while 27% were under fortified.

In 2009, data published from the United Arab Emirates indicated that 39% of samples of different brands of milk were within an acceptable range of fortification, whereas 31% were found to be under fortified and 30% over fortified. In a survey conducted in the eastern province of Saudi Arabia, the vitamin D content in fresh dairy products varied from 40-400 IU/L (1-10 ng/mL). The discrepancy between labeled and measured levels of vitamin D, particularly in skim milk, may be due to light degradation. The objective of current study was to measure the level of vitamin D in fortified low fat liquid milk and compare the results with the label claim. Further, we evaluated intra- and inter-batch variation, and inter-producer variation.

**MATERIALS AND METHODS**

We purchased 125 milk samples, (200 mL, fat content, 1-1.3%) of five selected brands [Almarai (A), Saudia (B), Nadec (C), Nada (D), and Alsafi (E)] in five replicates from five retailers at local markets of Riyadh, Saudi Arabia. Samples were stored at room temperature 23°C (3)°C until analyzed. However, after opening the seals, samples were refrigerated (4°C) until analyzed. Five samples of each batch from five different producers were analyzed to determine intra- and inter-batch production variations.

The vitamin D level was determined using a published liquid chromatography method from Acquity Ultra Performance System (UPLC) (Waters Associates Inc, Milford, MA, USA) composed of a quaternary pump, an autosampler, and a column thermostat, with the photodiode array detector set at 265 nm, reversed-phase and UPLC BEH C_{18} (bias -8.6%, and precision 4.9% at 7.5 ng/ml). The data were collected with a Pentium IV computer using Empower Chromatography Manager Software.

**RESULTS**

The 125 milk samples (fat content, 1-3%) were analyzed within first 40% of the period of validity. The mean measured level of vitamin D was 10.2 (1.6) ng/mL with a range of 7.1-13.9 ng/m. In twenty-five of 125 samples (20%), vitamin D levels were outside the ±20% of the claim. Thirteen samples (10.4%) were underfortified, and 12 samples (9.6%) were overfortified.

To measure the co-efficients of variation, we analyzed five samples of each batch (intra-batch, n=5), and inter-batch (n=25) (Table 1). The coefficients of variation were in the range of 1.6 - 20.8% for intra-batch variation, and 7.9 - 18.9% for inter-batch variation. Further, inter-producer variation (n=125) was 16.1%.

**DISCUSSION**

To prevent a deficiency of vitamin D at the population level, the chief consumable food (generally milk) is fortified with vitamin D in many nations. In the Saudi Arabia, milk is fortified with vitamin D at level 400 IU/L (10 ng/mL). However, the level may vary widely due to several factors, which include inaccurate addition of vitamin D at the production level and/or degradation of the vitamin D because of improper storage during transportation and/or at the retailer site. Underfortification increases the risk of rickets in children and of osteomalacia in adults, whereas overfortification can cause vitamin D intoxication and its serious consequences. Therefore, it is necessary to monitor the level of fortification to determine the accuracy of the whole process. For this purpose a survey study was conducted during 2013-2014 to determine the level of vitamin D fortification in commercially available milk in the market of Riyadh, Saudi Arabia.

Several studies in the past two decades have indicated a wide discrepancy between the contents and claims of vitamin D in commercial milk. However, in recent years the discrepancy has lessened substantially, which could be due to recent developments in technology and public health awareness. According to a survey report of US Department of Agriculture, a nationwide sampling program to update values in the National Nutrition Database for Standard Reference sample collected and analyzed in 2001 indicated that 5 (41.7%) of 12 low fat milk samples had vitamin D concentrations within 400-600 IU (10µg/quart), whereas samples collected from the same sites in 2007-2008 showed that 21 (87.5%) of 24 low fat milk samples had vitamin D concentrations within 400-600 IU (10µg/quart) in 2007-08.

In the present study, we analyzed 125 fortified low-fat milk samples which were purchased from major retailers in Riyadh, Saudi Arabia. The results of these analy-
sis revealed that 100 of 125 (80%) samples were within a range of 80% to 120% of recommended vitamin D concentrations, whereas 25 of 125 (20%) were outside the range (10.4% under-fortified, 9.6% over fortified). A limitation of the study is that only five major retailers in the Riyadh area were included and the study did not examine full-fat or non-fat milk samples. Further studies on other milk brands and on milk samples of different fat content are needed.

Table 1. Measured levels of vitamin D in 125 samples from five producers of fortified low-fat cow milk.

| Producer | <8 ng/mL | 8-12 ng/mL | >12 ng/mL |
|----------|----------|------------|-----------|
|          | Mean     | SD         | n (%)     | Mean     | SD         | n (%)     | Mean     | SD         | n (%)     |
| A        | NA       | NA         | 0         | 11.2     | 0.7        | 23 (92)   | 13.6     | 0.5        | 2 (8)     |
| B        | 7.6      | 0.3        | 6 (24)    | 10.0     | 0.9        | 15 (60)   | 13.0     | 0.5        | 4 (16)    |
| C        | 7.4      | 0.2        | 2 (8)     | 9.5      | 1          | 23 (92)   | NA       | NA         | 0         |
| D        | 7.7      | 0.2        | 3 (12)    | 9.4      | 1.2        | 22 (88)   | NA       | NA         | 0         |
| E        | 7.2      | 0.1        | 2 (8)     | 10.8     | 1.2        | 17 (68)   | 12.4     | 0.2        | 6 (24)    |

SD: standard deviation. NA: not available.

In conclusion, considering the precision and accuracy of the assay, we conclude that vitamin D content in the majority of fortified low fat cow milk samples on the Riyadh market matches label claims. Further, the observed differences from label claims in 15% of the samples are small and not likely to be clinically important.

Conflict of interest
The authors report no conflict of interest.

REFERENCES
1. Alsuwaida AO, Farag YM, Alsayyari AA, Mousa DH, Alherjaili FF, Al-Harbi AS, Houssawi AA, Mittal BV, Singh AK, Prevalence of vitamin D deficiency in Saudi adults. Saudi Med. J. 2013;34:814-8.
2. Holick MF, Chen TC, Vitamin D deficiency: a worldwide problem with health consequences. Am. J. Clin. Nutr. 2008;87:1080S-65.
3. Sills IN, Skuza KA, Horlick MN, Schwartz MS, Rapport R, Vitamin D Deficiency rickets. Reports of its demise are exaggerated. Clin Pediatr. (Phila). 1994;33:491-3.
4. Chen TC, Shao Q, Heath HIll, Holick MF, An update on the vitamin D content of fortified milk from The United State and Canada. N. Eng. J. Med., 1993;329:1507.
5. Vin T, Polyvixi K, Suzanne MR, Chen, Perez Alberto A, Holick MF, Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. Am. J. Clin. Nutr. 2003;77:1478-83.
6. Lawrence MG, Frank RG, Section on breastfeeding and Committee on Intake. American Academy of Science, Clinical Report on “Prevention of rickets and vitamin D Deficiency: New guidelines for vitamin D intake. Pediatrics 2003;111:908-10.
7. US Department of Health and Human Services. Food and Drug Administration, Code of Federal Regulations. Title 21, Food and Drugs 131: Milk and Cream. Washington. DC 2007.
8. Koufika P, Chen TC, Holick MF, Vitamin D intoxication associated with an over the counter supplements. N. Eng. J. Med., 2001;345:66-7.
9. US Food and Drug Administration, Centre for food Safety AND Applied Nutrition. Grade “A” pasteurized milk ordinance. Washington, DC: US Food and Drug Administration, 2003.
10. Tanner JT, Smith J, Delfbaugh P, Angyal G, Villalobos M, Bueno P, McCarrahan ET, Wher HM, Muniz JF, Hollis BW, Survey of vitamin content of fortified milk. J. Assoc. Off. Anal. Chem. 1988;71:607-10.
11. Holick MF, Shao Q, Liu WW, Chen TC, Muniz JF , Wehr CT, N. Eng. J. Med., 1992;326:1178-81.
12. Holden JM, Lemar LE, Exler J, Vitamin D in foods: development of the US department of agriculture database. Am. J. Nutr 2008; 87: 1092S-65.
13. Faulkner H, Hussein A, Foran M, Szejarto L, A survey of vitamin A and D content of fortified milk in Ontario. J. Dairy Sci. 2000;83:1210-6.
14. Louis CL, Abdulkadar AHW, Madduri VR, A study on vitamin D vitamin A and edible oils available in the United Arab Emirates, Int. J. Food Science and Nutrition, 2009; 60:1-9.
15. Ali MS, Abdulmohsen AE, Al Fashan M, Sadat NA, *Fortification with vitamin D: Comparative study in the Saudi Arabia and US markets, J Family Community Med. 2013; 20:49-52.
16. Renken SA, Warthesen JJ, Vitamin D stability in milk. J. Food Sci. 1993;58:502-5.
17. Alvi SN, Eltabache C, Hammami MM. Determination of vitamin D in fortified milk by Ultra-performance liquid chromatography World J Pharmacy and Pharm Sci. 2015; 4: 170-81.