



نسبة فيتامين (د) عند النساء في مدينة السليمانية , إقليم كردستان واستخدام مقياس الطيف الكتلي لقياس نسبة هذا الفيتامين في صفار البيض البلدي والتجاري

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الخلاصة

يعتبر فيتامين (د) من الفيتامينات الضرورية لدائمة صحة الانسان. ويعتبر التعرض لأشعة الشمس المصدر الرئيسي للحصول على هذا الفيتامين. إن عدم تعرض النساء في هذه المنطقة لأشعة الشمس يعد سببا رئيسيا لنقص فيتامين (د). هناك القليل من المواد الغذائية الحاوية على فيتامين (د) وهذه الاغذية (الماكولات) قد لا تشكل جزء من النظام الغذائي لهن. في الجزء الاول من هذا البحث تم تقييم نسبة فيتامين (د) لدى ١١٢٨ من النساء من النتائج المتوفرة في احدى مختبرات المدينة. ووجد بأن اكثر من ٧٩% من هذه النتائج هي اقل من الحد الطبيعي. في الجزء الثاني من الدراسة باستخدام طريقة الفصل الكروماتوغرافي عالي الكفاءة للسوائل تم ايجاد نسبة فيتامين (د) في صفار البيض البلدي والبيض التجاري المتوفر في الاسواق المحلية وجدنا بان صفار البيض البلدي يحتوي على نسبة من فيتامين (د) يضاهي النسبة الموجودة في البيض التجاري بمعدل ٧,٦٦ من المرات.





يجب اخذ مسألة الاهتمام بالحفاظ على نسبة فيتامين (د) لدى النساء ضمن الحدود الصحية الطبيعية بنظر الاعتبار, و توجيه النساء لاهتمام بهذا الموضوع. الكلمات الدالة : ضوء الشمس الطبيعي, فيتامين د, بيض, مقياس الطيف الكتلي

## Vitamin D Status Among Women Living in Sulaimani, Kurdistan Region and HPLC-MS Analysis for Measuring Vitamin D in Organic and Non-organic Eggs

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### ABSTRACT

*Vitamin D is a superstar of our health. Sun exposure is the major source of vitamin D for most humans, and the primary cause of vitamin D deficiency in women living in Kurdistan is the lack of appreciation of the sun. Very few foods naturally known to contain vitamin D, and these foods are not a part of these women daily meals. In the first section of this study, the measured serum 25(OH), D levels for 1128 women and girls were considered. Over 79% of these results were below the standard range value.*





*In the second part of this study, we use analysis with the Hight Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) method to elucidate the ratio of Vitamin D between commercially produced eggs (non-organic eggs) and Vitamin D in eggs produced by hens raised on pasture (organic eggs). Data showed vitamin D content in organic eggs 7.66 fold higher than non-organic eggs. It is clear that attention should pay to the vitamin D status of women in Kurdistan.*

**Key words:** Natural sunlight, Vitamin D, Eggs, Mass Spectrometry

## 1. INTRODUCTION

Vitamin D is a superstar for our health; over 800 references are highly cited on Vitamin D and Cancer [1]. Vitamin D deficiency has recently been considered a public health problem of epidemic proportions[2]. Vitamin D deficiency among women, in particular among those of reproductive age, is of particular concern because it can have adverse consequences for the mother, fetus, infant and child [3]. It also has a greater risk for preeclampsia during pregnancy, breast cancer, and premenstrual syndrome[4]. The best and most natural way to get vitamin D is by sunbathing, or, when that is not possible, by taking supplements. However, most people will do neither and are thus severely vitamin D deficient [5-7].

Researchers out of Sweden conducted a study on Northern European women to determine the effects of sun avoidance on health. They looked at data from 29,518 women aged 25 to 64 years old who participated in the Melanoma in Southern Sweden (MISS) study. They found that





women who avoided the sun were twice as likely to die compared to women who got higher amounts of sun exposure. There was a dose-dependent inverse relationship between sun exposure and all-cause mortality. It means that the rate of all-cause mortality decreased with every increase in ratings of sun exposure [8]. Vitamin D-producing UVB rays cannot pass through glass. So you will get absolutely no boost to your vitamin D levels if the sunlight passes through a window before hitting your skin [9].

Sulaimania is one of the cities with sun exposure approximately all year round. The average seasonal Sunshine duration hours is not less than 7.6 hours per day as shown in Figure 1. Vitamin D deficiency is a major health problem in all age groups among girls and women. Women residing in these areas tend to spend small time in the sunlight due to cultural and social reasons. In addition to prominent religion (Islam) that require wearing special kind of cloths which reduces the skin exposure to light. Furthermore, many Kurdish women avoid sun exposure to maintain a lighter skin color tone, as a dark skin color is often considered undesirable.

Vitamin D deficiency is associated with bone loss and bone fractures women. This preventable disorder, is a common and significant public health problem for older physically challenged women living in the community [8-10].





The study is only about women because we believe that women in the orient society are at greater risk of being Vitamin D deficient. It is because of physiological characteristics of being a female such as pregnancy, menstruation [11] and low availability of vitamin D-rich foods [10].

Besides getting vitamin D through sunlight, we can also get it through certain food like egg yolk which is the most available kind of foods in Kurdistan that contain vitamin D, and one of the top 10 foods highest in vitamin D [13].

In the second part of the study, the difference between the vitamin D level in organic and commercially produced chicken eggs is considered using High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS). Mass spectrometry (MS) has been described as the smallest scale in the world, not because of its size; it weighs a molecule [14]. Unique features of MS include its capacity for direct determination of the nominal mass of an analyze, and to produce and detect fragments of the molecule that correspond to discrete groups of atoms of different elements that reveal structure features [14].

## **1. MATERIALS AND METHODS**

The first part of the study was conducted to assess the Vitamin D status among 1128 results of Circulating 25(OH) D levels taken from the clinics in Sulaimania for women and girls of different ages that are living in Sulaimania.





However, information regarding the vitamin D status in the region is limited. Measuring the serum concentration of 25(OH)D<sub>3</sub> is the most reliable method for determining vitamin D status because it provides a measurement of the amount of vitamin D available to tissues [11, 12]. Circulating 25(OH)<sub>2</sub>D levels reflect the amount of sunlight carefully to which the epidermis is exposed and the dietary [12], [13].

In the second part of the study we analysis with the High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) method used to obtain the difference in the levels of vitamin D in egg yolks of commercially produced chickens or chickens raised in cages and eggs produced by hens raised on pasture. (HPLC-MS) has been used because of its superior selectivity and sensitivity.

We used 10 gm. of egg yolk from each organic and commerce eggs for the analysis. Sample preparation for studying vitamin D in egg yolks and we performed it as below:

The commercially produced chicken egg was extracted twice by mixing two times with 100 ml acetonitrile (Gradient grade for liquid chromatography). After agitation for 30 minutes, the mixture was centrifuged at 15000 rpm for 10 minutes. The extracts were combined, centrifuged and then the supernatant was added to a separating funnel. 200 ml hexane was added to the supernatant for defatting and mixed well for 10 minutes. The aqueous layer was separated into a Quick fit round-bottom flask and vacuum dried using rotary evaporator (BUCHI) at 40°C.





Two ml acetonitrile was added to the precipitate and solicted for 2 minutes until dissolved. The mixture was centrifuged at 14000 rpm for 5 minutes and then filtered by passing through a Millipore micro filter with 0.2 micrometer pores. Separation was carried out with an HPLC-MS (Waters 2545-USA) Quaternary Gradient Module, and it equipped with system fluidics organizer (Waters-SFO).It coupled with SQ detector and operated in positive ionization mode at range from  $m/z = 200-900$  with two scans/min, combined with Photodiodes Array detector (Waters 2998) with sampling rate 2 points/sec (Lambda range 190-800 nm). A C18/4.5 x 155 mm RP column (XBridge) was used for separation with a solvent system consisting of A: Deionized water, B: Acetonitrile each contains 0.1% formic acid. The following gradient was applied: 0-2 minutes 30% A, and 70% B, 2-10 minutes a linear from 25 to 5%, B linear from 75% to 95%. Ten microliters of the extract were injected using auto-sampler (Waters 2767), and run with flow rate 1 ml/min using auto-sampler (Waters 2767). Vitamin D was identified in the samples by comparison to the retention time and MS data and retention time of official standard.

## **2. RESULTS AND DISCUSSION**

The usual range of Serum concentrations of 25-hydroxy Vitamin D is between (30 and50) ng/ml, Level between (10-20) ng/ml is deficient, and range between (5 and10) ng/ml is sever deficiency.



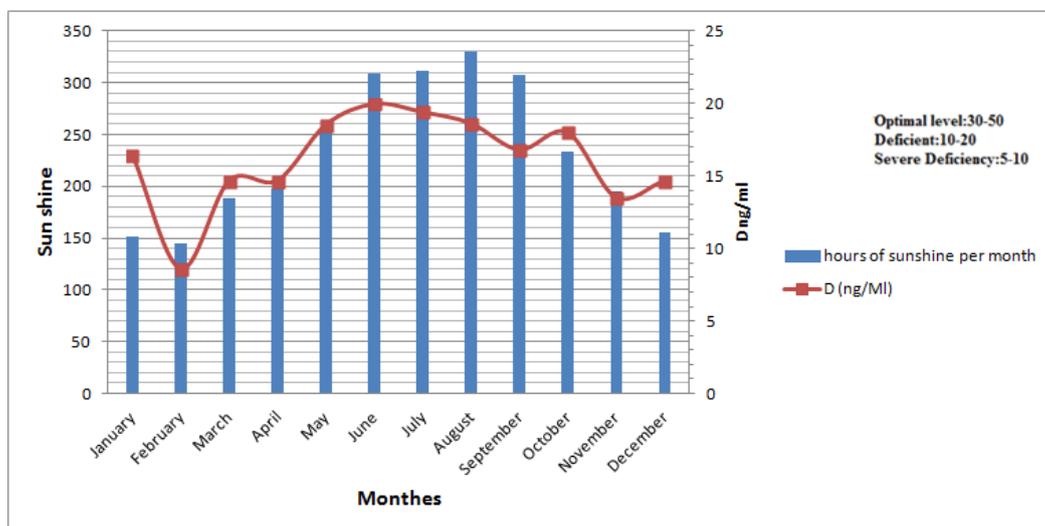


**Table 1:** Vitamin D levels

25 –Hydroxy Vitamin D	Rang (ng/ml)	Total patient 1128
Sever Deficiency	5-10	44.12%
Deficiency	10-20	34.16%
Normal	30-50	5.50%

From Table (1) over79% of the results were below the normal range value, 34.516% of them were deficient, defining sever deficiency status 44.12% of the results were below sever deficient range.





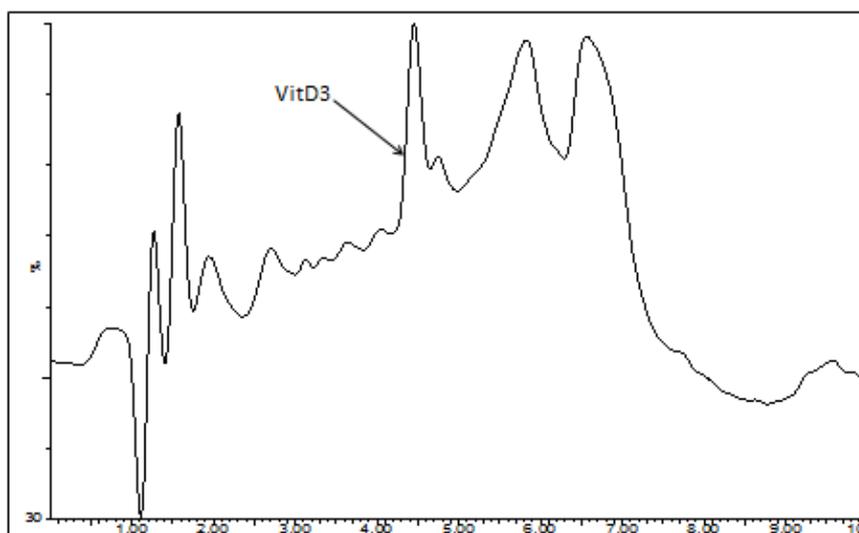
**Figure1:** Monthly variation in the mean values of primary circulating form of Vitamin D and the monthly sunshine hours in Sulaimani

Fig (1) shows the Monthly variation of serum 25-hydroxyvitamin D concentrations in the 1128 results; monthly Sunshine duration in Sulaimani is also shown. Most of the Vitamin D level values are below the optimal level. Despite the fact that July and August are the sunniest months of the year, the results show that Vitamin D levels have not increased during that period. The reason for that is because most women in our society are completely covered from head to toe. The sun might not even hit their faces because of a lack of outdoor activities. Vitamin D is produced when the sun hits the bare skin, but tanning is not part of Kurdish culture. Vitamin D supplements can provide the required dose, but unfortunately, the society is not well educated in this problem. Foods containing

Vitamin D include salt water fish, cow liver, and fortified milk. These are not a part of a typical Kurdish meal. Egg yolks are part of a daily breakfast meal. However, it is not sufficient. Without enough Vitamin D, one cannot form enough of the hormone Calcitriol (VitaminD<sub>3</sub>). This, in turn, leads to insufficient calcium absorption from the diet. In this case, the body must take Calcium from its stores in the skeleton which weakens existing bones. High fractured rates are frequently reported around this area, which at times leads to the death of old women.

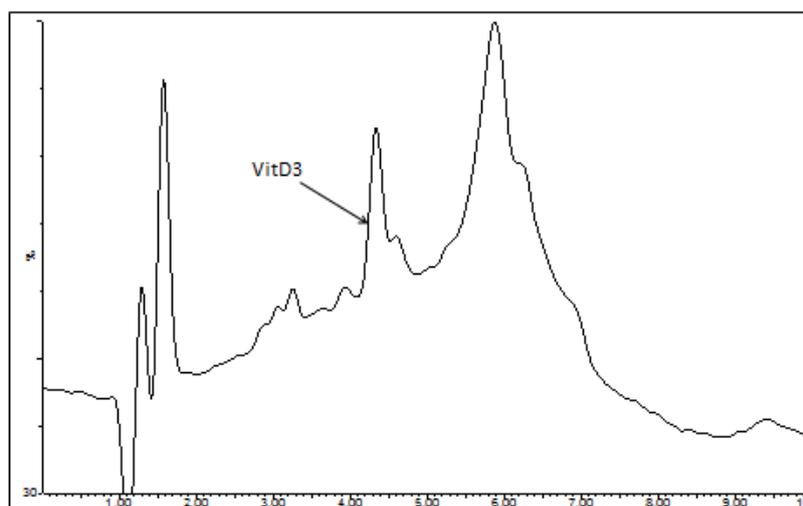
#### 4. ESTIMATION OF VITAMIN D<sub>3</sub>

To test whether free-range farming of hens provides eggs with more vitamin D in comparison to eggs produced commercially, we performed HPLC/MS method [16]. Vitamin D was detected in egg yolk after extraction by acetonitrile a peak of 384.22 {M+H} was detected at retention time 4.4 minutes (Figures 2, 3 and 4).

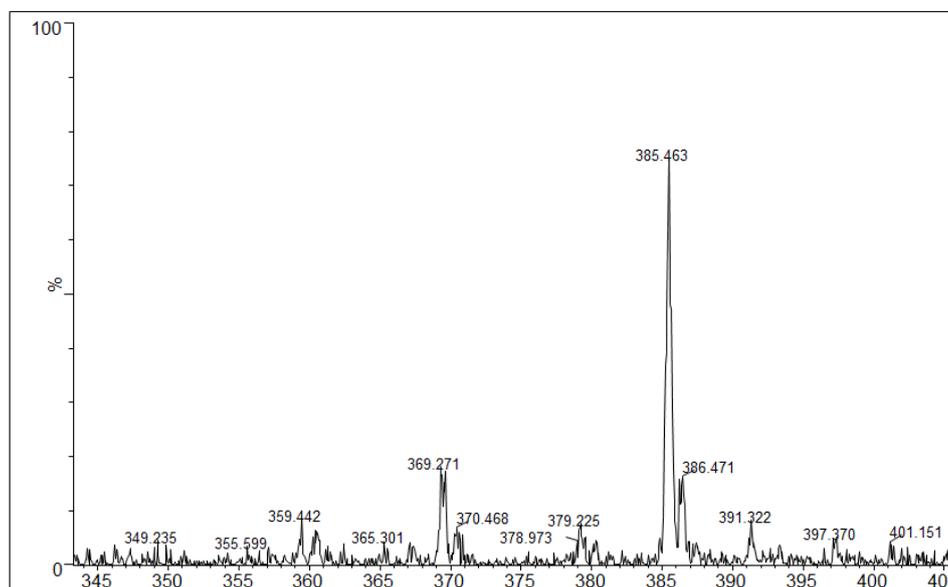


**Figure2:** Represents HPLC-MS TIC Chromatogram of organic =egg acetonitrile extract.

The arrow shows mass peak of Vitamin D3 ( $MW\{M+H^+\}=385.22$ ),  
rt=4.4min



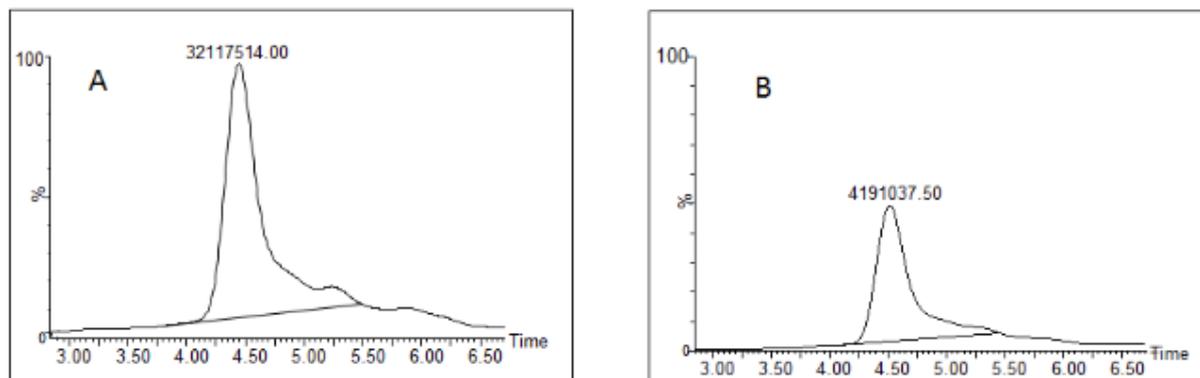
**Figure 3:** Represents HPLC-MS TIC Chromatogram of non-organic =egg acetonitrile extract. The arrow shows mass peak of Vitamin D3( $MW\{M+H^+\}=385.22$ ), rt=4.4min



**Figure 4:** Shows mass peak ( $MW\{M+H^+\}=385.46$ ) of VitD3 peak (in organic egg) at retention time=4.4min,incomparision to the mass of the MW of the authentic compound

Relative quantification of vitamin D was calculated based on peak area of the complex which showed significant differences of vitamin D in each egg. Figure 5A and 5B shows more than seven fold increase in vitamin D content in the organic eggs in comparison to non-organic eggs.

Similar data was reported about vitamin D content in eggs produced by hens that exposed to sunlight compared to that produced indoors in factory farms. It has been shown that the vitamin D3 content of egg yolk was three- to fourfold higher in the free groups that were exposed to sunlight. It has been concluded that free-range farming provides an efficient alternative to fortify eggs with vitamin D [17].



**Figure 5:** Extracted Ion Chromatogram of VitD3 (MW{M+H<sup>+</sup>}=385.46).

A: Represents mass peak area (32117514.0) in organic eggs EIC, and B:

Represents VitD3 (EIC) mass peak area (4191037.50) in NON-organic

eggs The peak area ratio between VitD3 organic and non-organic =

1:7.66

## 5. CONCLUSIONS

A significant proportion of the kurdistan women involved in this study had suboptimal vitamin D status.

1. Cultural factors appear to affect negatively the vitamin D status of women in Sulaimani, such as avoiding the sunshine to maintain a light skin tone and wearing special clothes by most of the women.
2. Consumption of Eggs from hens raised on pasture is suggested for improving vitamin D status; it would be difficult to obtain the necessary 25µg of vitamin D through this source alone.
3. Considerable attention should be paid to the vitamin D status of women in Kurdistan. Exposure to sun, organic egg consumption and



Vitamin D supplementation may be viable options to improve the  
vitamin D status.

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