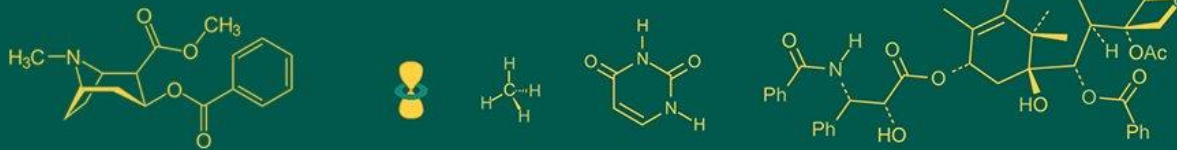


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
 ISSN Online: 2617-4707  
 IJABR 2024; SP-8(5): 406-408  
[www.biochemjournal.com](http://www.biochemjournal.com)  
 Received: 16-02-2024  
 Accepted: 23-04-2024

**Anisha J Perumbilly**  
 Assistant Professor,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Tirunelveli, TANUVAS, Tamil  
 Nadu, India

**M Balagangatharathilagar**  
 Professor and Head,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Tirunelveli, TANUVAS,  
 Tamil Nadu, India

**M Kalaivanan**  
 Assistant Professor,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Theni, TANUVAS, Tamil  
 Nadu, India

**Vishnurahav RB**  
 Assistant Professor,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Tirunelveli, TANUVAS, Tamil  
 Nadu, India

**M Madeena Begum**  
 Assistant Professor,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Tirunelveli, TANUVAS, Tamil  
 Nadu, India

**Corresponding Author:**  
**Anisha J Perumbilly**  
 Assistant Professor,  
 Department of Veterinary  
 Clinical Complex, VCRI,  
 Tirunelveli, TANUVAS, Tamil  
 Nadu, India

## Quantitative determination of 25-Hydroxyvitamin D in adult captive Asian elephants (*Elephas maximus*) from Southern tropical regions of Tamil Nadu

Anisha J Perumbilly, M Balagangatharathilagar, M Kalaivanan, Vishnurahav RB and M Madeena Begum

DOI: <https://doi.org/10.33545/26174693.2024.v8.i5Sf.1232>

### Abstract

Vitamin D, one of the fat-soluble vitamins, has a crucial role in mineral metabolism, immunomodulation and as an antioxidant. Clinical conditions of osteoarthritis, gastrointestinal diseases, and skin and ocular pathologies are the commonly encountered problems in captive Asian elephants (*Elephas maximus*). Most of these conditions are suspected to arise from deficiency of vitamin D. Still its metabolism in elephants is not clearly understood and a reference interval for it has not yet been established, limiting the prompt diagnosis and treatment of diseases in these animals. An immunoassay-based quantitative determination of total 25-hydroxyvitamin D (25(OH)D) was carried out in blood samples received from apparently healthy elephants at Centralised Clinical Laboratory, VCC, VCRI, Tirunelveli. The samples submitted as a part of the general health checkup of animals from a period of September 2022 to August 2023 were selected for the study. The mean value of 25(OH)D estimated in ten adult female Asian elephants in captivity is  $11.15 \pm 1.98$  ng/mL. This information obtained from this preliminary study expands the knowledge about this analyte and helps in assessing and establishing the reference interval for vitamin D status in captive elephants.

**Keywords:** Asian elephant, *Elephas maximus*, Vitamin D, 25-hydroxyvitamin D

### Introduction

Vitamin D is one of the fat-soluble vitamins and has a vital role in bone mineralization by maintaining calcium and phosphorous homeostasis under the influence of parathyroid hormone [1]. Its role in cell proliferation, maintenance of epithelial integrity, and immunomodulant and antioxidant function has also thrived recently [2, 3]. Also, low concentrations of this vitamin in serum are reported to be related to a greater risk of active tuberculosis [4]. Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol) are the two forms of vitamin D in the blood, both of which are structurally different at their side chain. The source of Vitamin D2 is a diet of plant origin while D3 is produced on the skin photochemically by ultraviolet radiation from 7-dehydrocholesterol [5]. Irrespective of the source both forms of the vitamin are hydroxylated at C25 cytochrome P450 vitamin D 25 hydroxylases in the hepatic tissue forming 25-hydroxyvitamin D2/D3. It is the major metabolite that could be used to evaluate the general status of vitamin D in the blood [6]. Further hydroxylation at the Cα1 position by the renal tissue converts it to the biologically active form of 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) by the enzyme of 25-hydroxylase [7, 8]. Although structurally different each hydroxylated form has comparable functions.

Asian elephants are tropical herbivores and, like other mammals they might be relying mainly on the cutaneous synthesis of vitamin D. Since the synthesis of this vitamin is inversely associated with skin pigmentation [9], elephants might be more prone to vitamin D deficiency with improper housing and management conditions. Osteoarthritis, gastrointestinal problems, eye, nail, skin pathologies, and wound lesions are commonly encountered in captive elephants [10]. Reproductive failure is also a frequently encountered problem in elephants under captive management [11]. Though the deficiency of vitamin D is found to be connected with diseases of bones and different non-skeletal diseases in pet animals and reproductive disorders in different livestock species [12], the interaction of these clinical conditions with vitamin D deficiency in elephants is still mysterious.

Various studies on this vitamin and its connection with the metabolism of calcium and associated diseases have been carried out in different domestic animals and its administration is already an adopted treatment regime in these animals. But assessing vitamin D homeostasis is still a major concern in wildlife management due to the lack of established reference intervals for these parameters [13]. This prevents proper diagnosis and treatment of diseases and the adoption of better management practices for them. To bridge this knowledge gap and to have a better knowledge of the metabolism of vitamin D in elephants, as a preliminary step the current brief investigation was conducted measuring the serum level of vitamin D in elephants maintained under captive management.

### Materials and Methods

The estimation of 25(OH)D was carried out in blood samples of elephants submitted at the Centralised Clinical Laboratory, Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli as a part of the routine general health checkups. The samples were collected from ten female Asian elephants in captivity aged between 18 and 65 years. All samples were collected in serum vials without any anticoagulant and received at the laboratory in sealed thermocol ice boxes from August 2022 to March 2023. All animals were reported to be clinically healthy at the time of collection of blood samples, had adequate exposure to sunlight and access to *ad libitum* feed and water, and were maintained under captivity in the Tirunelveli, Madurai, and Trichendur districts of Tamil Nadu.

Serum was separated by centrifuging the sample at 3000 rpm for 10-12 minutes. Quantitative determination of 25(OH)D was carried out in ichromα II using ichromα™ vitamin D estimation kits manufactured by Boditech Med Incorporated, Republic of Korea by strictly following the manufacturer's instructions. The test uses a sandwich immunodetection method and detects both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> as the total 25(OH)D level. Briefly, antigen-antibody complexes are formed by binding detector antibodies in the buffer with antigens in the sample. Biotinylated capture antibody complex in the buffer also binds to the antigen-antibody complexes. These new complexes formed migrate on the nitrocellulose matrix and are captured by the immobilized streptavidin on the test strip. The instrument detects and processes the fluorescence signal by the detector antibodies.

### Results and Discussion

The study estimated the serum concentration of 25(OH)D to evaluate the level of vitamin D in ten apparently healthy female elephants. Quantitative vitamin D estimation was carried out as a preliminary study to establish a reference interval of vitamin D in captive Asian elephants. The 25(OH)D in ten serum samples ranged from 8.53 ng/mL to 14.26 ng/mL and the mean value was  $11.15 \pm 1.98$  ng/mL. A mean value of  $15.86 \pm 7.5$  ng/ml for 25(OH)D was reported by Miller *et al.* [16] in 72 serum samples collected from 14 African elephants. An average serum 25(OH)D<sub>2</sub> of  $7.02 \pm 0.85$  ng/ml was reported by Childs-Sanford *et al.* [17] in Asian elephants maintained under a northern temperate climate. The estimated range of 25(OH)D<sub>3</sub> in domestic equine species is 2-7 ng/mL [18]. The variation in the concentration obtained in Vitamin D concentration by

various researchers could be due to the change in management conditions of the animals.

**Table 1:** Concentration of 25(OH)D (ng/mL) in captive Asian Elephants

Sl. No.	Name of the Elephant	Age	25(OH)D (ng/mL)
1	Sundaravalli	16	13.61
2	Parvathy	26	8.53
3	Gurungundivalli	28	9.13
4	Gomathi	29	9.5
5	Beegam	49	12.3
6	Kandhimathi	53	11.42
7	Jeny	53	9.25
8	Lalitha	57	14.26
9	Ponni	61	11.28
10	Sundari	65	12.19
	Mean		$11.15 \pm 1.98$

Given the complication of the homeostasis mechanism of vitamin D and its role in the health and diseases in animals [14, 15], it is the need of the hour to establish a complete consistent method for veterinary clinical practice. Vitamin D concentration in blood is affected by several extrinsic (geographical location, exposure to sunlight, feed) as well as intrinsic (age, sex, breed, physiological status and disease conditions) factors [19, 20]. The effect of these factors on the result obtained is uncertain. Though the results obtained is comparable with the values reported by various authors in their studies, a comprehensive study in different heterogenous groups with large sample size needs to be conducted to find out the influence of these factors on the metabolism of vitamin D and to establish the reference interval for vitamin D in elephants. This may further help in implementing a better diagnostic approach of clinical conditions and improving management practices in elephants. As far as the author's knowledge no similar study was conducted in Asian elephants. This brief investigation forms a base for establishing reference intervals for Vitamin D and understanding its metabolism in Asian elephants.

### Convulsion

This study investigated serum vitamin D levels in captive Asian elephants, revealing a mean concentration of  $11.15 \pm 1.98$  ng/mL, with individual values ranging from 8.53 ng/mL to 14.26 ng/mL. These findings, while consistent with some previous studies, highlight the need for standardized veterinary practices due to the multifactorial influence on vitamin D levels, including geographical factors and animal demographics. Establishing robust reference intervals through comprehensive research will enhance diagnostic accuracy and inform better management practices, ultimately contributing to the welfare of Asian elephants. This study provides a foundational step toward understanding vitamin D metabolism in these animals.

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