

## Clinical and hematological profiles of vitamin D3 deficiency in Najdi lambs

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### Abstract

This study assessed the clinical and hematological impacts of vitamin-D3 deficiency in growing Najdi lambs raised in Basra, Iraq. A musculoskeletal illness experimental design was tested using 100 Najdi lambs; their ages range from 3-12 months. Lambs were examined for general clinical symptoms, serum was tested for vitamin D3 levels, and whole blood was tested for a complete blood count. Lambs with low serum vitamin D3  $17.77 \pm 1.07$  ng/ml displayed various symptoms such as; decreased body condition score, reduced weight gain 28.5%, signs of rickets 7.5%, lameness 42.5%, loss of appetite 53.7%, poor hair coat condition 52.5%, joint enlargement 27.5%, bending of large bone 33.7%, recumbency 12.5%, diarrhea 25%, pale mucous membrane 58.7%, congested mucous membrane 31.2%, nasal discharge 68.7%, lacrimation 40%, coughing 65%, dyspnea 26.2% and decrease ruminal motility. However, clinically healthy lambs exhibit high  $89.75 \pm 7.06$  ng/ml serum vitamin D3 levels. Lambs with low serum vitamin D3 levels showed significant increases in body temperature  $39.36 \pm 0.07$  °C, respiratory rate  $39.02 \pm 1.86$  breath/m, and heart rate  $89.22 \pm 1.74$  beat/m compared to lambs with high serum vitamin D3 levels. Furthermore, hematological parameters in lambs with low serum vitamin D3 levels differed markedly from those with high serum vitamin D3 levels based on complete blood count analysis. In conclusion, this study proves that musculoskeletal disorders and other clinical and hematological illnesses are associated with the down-regulation of vitamin D3 concentration in Najdi lambs.

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### Introductions

As a fat-soluble vitamin, vitamin D is primarily involved in intestinal absorption of calcium and phosphorus with a role in calcium/phosphorus hemostasis, bone formation, and immune modulation (1,2). More evidence, particularly in extraskelatal functions, suggests that vitamin D plays a role in iron homeostasis and erythropoiesis (2,3). It has been shown that vitamin D is associated with inflammatory cytokines and the antimicrobial peptide hepcidin in several observational studies of healthy and diseased populations. Other in vitro studies suggest that

vitamin D is involved in this association by acting on inflammatory cytokines (4-6). The broad functions of vitamin D are now better understood, and the discovery has provided the non-skeletal consequences of vitamin D deficiency that most tissues in the body contain vitamin D receptors (7). A vitamin D deficiency is caused by insufficient UVB exposure and inadequate vitamin D supplementation in feed, resulting in poor appetite, growth, osteomalacia, and osteodystrophy in advance (8). Vitamin D deficiency causes several non-skeletal disorders, including inflammatory reactions, neoplastic, cardiovascular disorders, autoimmune diseases, poor weight

gain, reduced reproductive efficiency, bending of large bones, and enlargement of joints, among others (9,10). Despite the critical physiological role of vitamin D3, little research has been done evaluating vitamin D status in Iraqi livestock, mainly sheep, since their production in Basra faces many problems, such as infections (11-15) or nutritional disorders (16,17), or adaptation and survival issues (18,19).

However, a previous related study was the first to assess vitamin D3 status in growing Najdi lambs bred in Basra province and evaluated the effectiveness of therapy for vitamin D3 deficiency. Therefore, the current study was designed to assess the effect of vitamin D3 deficiency on the clinical and hematological parameters in growing Najdi lambs bred in Basra, Iraq.

## **Materials and methods**

### **Ethical approval**

The Institutional Animal Care and Use Committee approved the experiment at the University of Basrah, Iraq, according to its rules and guidelines in 2019.

### **Experiment design**

In the current study, 100 Najdi lambs (a primary domesticated sheep breed native to the Najd region of Saudi Arabia) were used between November and December 2019, aged between three to twelve months old, from different farms located south of Basra province, Iraq. Various enclosed flocks of Najd sheep were used, housed in semi-enclosed pens where water and good fodder were provided as desired without grazing facilities. 80 lambs out of 100 examined were found to have suspected symptoms of vitamin D deficiency, including decreased body condition scores, reduced weight gain, considerable bone bending, and mainly lameness, among the other signs (Group A). Twenty clinically healthy sheep of the same age group were categorized as controls (Group B).

### **Clinical examination**

Clinical examinations of the hair coat, skin, mucous membranes, body temperature, and superficial lymph nodes and reviews of the musculoskeletal, digestive, respiratory, and cardiovascular systems were commonly performed on suspected Najdi lambs.

### **Rumen motility**

The rumen motility was measured in the study as Saleh *et al.* (17) described. This study used the mean rumen contraction per minute as a scoring system and three ruminal movement levels: 1. Normal motility, around one to two solid movements/minute; 2. Weak motility is around one or less than one weak movement/minute; and 3. No ruminal movement.

### **The body condition scoring (BCS)**

Applying BCS is the more accessible and beneficial score to describe the amount of muscle and fat in sheep, and it was determined using a numerical scale ranging from 1 [very thin] to 5 [very fat] based on the amount of muscle and fat deposition around the loin region (16,20,21). If the sheep scored between BCS 1 and BCS 2, they may receive a half score of 1.5.

### **Hair coat condition score (HCS)**

For all lambs in the present study, the quality of the wool was used as an indicator of health status (animal welfare). Two primary factors were taken into account when scoring the condition. When the hair is homogenous, addressed to the body, and shiny, it is considered Normal. When the hair is longer than the standard, matted, uneven, or scarified rough, it is regarded as Poor/Rough (16,22).

### **Blood sample collection**

Five millilitres of blood were collected aseptically from the jugular vein according to Jackson *et al.* (23) and then separated into two different tubes to conduct biochemistry and CBC testing. One millilitre of blood was put in a tube with anticoagulant (EDTA) for CBC analysis, while four millilitres were placed in a plain tube. Ten minutes were spent centrifuging the clotted blood at 1500 rounds per minute (rpm) or left overnight for clotting; serum was collected and placed at -20 centigrade until vitamin D3 evaluation.

### **Estimation of lamb serum vitamin D3 levels**

Serum vitamin D3 levels of Najdi lambs were assessed using the Biotin double antibody sandwich technique ELISA kit (Bioassay Technology/China). All the procedures were done according to the manufacturer's instructions.

### **Complete blood count (CBC)**

An automatic blood analyzer (SYSMIX®, JAPAN) was used to analyze EDTA blood samples for CBC. The parameters examined were red blood cell counts, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total and differential white blood cell counts.

### **Statistical analysis**

A pair student's t-test and Microsoft Excel software were applied to evaluate whether the results differed, and determination of the significant statistical differences was done at  $P < 0.05$  using JMP®11 NC: SAS Institute Inc. software Version 11 and Microsoft Excel.

**Results**

**Determination of serum Vitamin D3 concentration**

As shown in table 1, there was a statistically significant difference between lambs suspected of vitamin D3 deficiency (group A) and healthy lambs (group B).

Table 1: Values of serum vitamin D3 in suspected deficient and control Najdi lambs

Groups	No. of animal	Mean ± St. (ng/ml)
A	80	17.7±1.07
B	20	89.75±7.06

**Evaluation of vital signs**

Vital signs (body temperature, respiration rate, and heart rate) were higher in lambs with low serum vitamin D3

Table 2: Results of vital signs in both vitamin D3-deficient and normal lambs

Groups	No.	Body temperature (C°)	Respiration (breaths/minute)	Heartbeats (beats/minute)
Group A	80	39.36±0.07 a	39.02±1.86 a	89.22±1.74 a
Group B	20	39.02±0.09 b	27.69±1.72 b	83.31±1.87 b

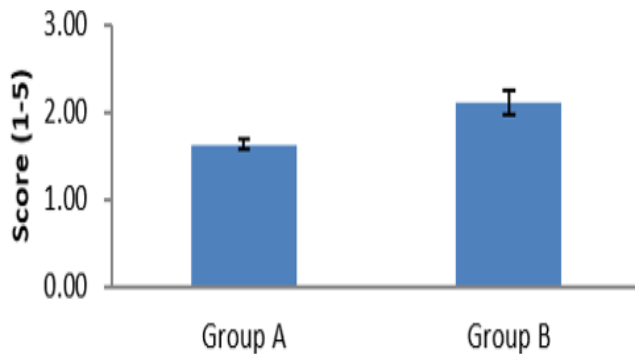


Figure 1: Body condition scores in both vitamin D3-deficient and normal lambs.

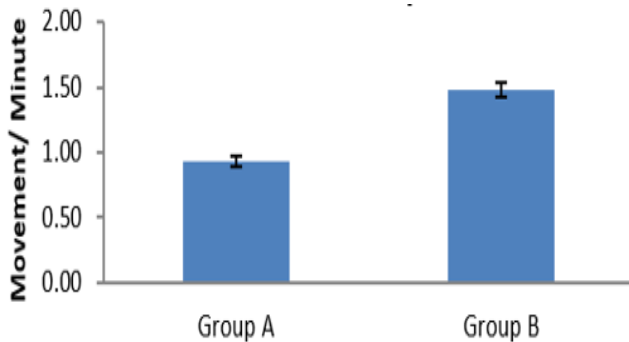


Figure 2: Ruminal motility (movement/min.) in vitamin D3-deficient and normal lambs.

concentrations than in lambs with high serum vitamin D3 concentrations (Table 2).

**Clinical signs and symptoms of vitamin D3-deficient Najdi lambs**

When examining lambs suspected of deficiencies in vitamin D3, several clinical manifestations were noted, including a decreased body condition score and ruminal motility (Figures 1 and 2), a decreased weight gain, signs of rickets, lameness, loss of appetite, poor hair coat condition, enlargement of joints, bending of large bones (Figures 3 and 4), recumbency (Figure 5), diarrhea, pale mucous membrane, congested mucous membrane, nasal discharge, lacrimation, coughing, dyspnea and decreased ruminal motility. The results of clinical signs and symptoms are listed in table 3 for animals suspected of vitamin D3 deficiency.



Figure 3: A ten-month-old male sheep of the Najdi breed had a deficiency of vitamin D3, showing marked enlargement of joints and bending of metacarpus bone (left) with poor hair and body conditions.



Figure 4: A vitamin D3 deficient eight-month-old male sheep of Najdi breed had marked enlargement of joints and metatarsus bone (right) bending with poor hair and body conditions.



Figure 5: A 3-month-old female lamb of the Najdi breed had a deficiency of vitamin D3, showing marked depression, poor body and hair condition, and recumbency.

Table 3: Signs/symptoms recorded in lambs with suspected vitamin D3 deficiency

Signs/symptoms	Animal number (%)
Weight gain	22 (27.5)
Signs of rickets	6 (7.5%)
Lameness	34 (42.5%)
Loss of appetite	43 (53.7%)
Hair coat condition	42 (52.5%)
Joint enlargement	22 (27.5%)
Bending of large bone	27 (33.7%)
Recumbency	10 (12.5%)
Diarrhea	20 (25%)
Normal mucous membrane	8 (10%)
Pale mucous membrane	47 (58.7%)
Congested mucous membrane	25 (31.2%)
Nasal discharge	55 (68.7%)
Lacrimation	32 (40%)
Coughing	52 (65%)
Dyspnea	21 (26.2%)

#### Evaluation of complete blood count

Interestingly, as shown in table 4, a significant decrease in most hemogram values was observed in lambs with lower serum concentrations of vitamin D3 compared to lambs with high serum levels of vitamin D3. Nevertheless, MCHC values were non-significantly decreased in lambs with lower serum levels of vitamin D3. The number of platelets in lambs with low vitamin D3 was significantly higher than in control lambs.

Additionally, detailed leukogram counts of the control and vitamin D3 deficient Najdi lambs are shown in table 5. All leukogram values except eosinophil were markedly higher in lambs with lower serum levels of vitamin D3 than other lambs with higher serum levels of vitamin D3.

Table 4: Hemogram values in vitamin D3-deficient and normal lambs

Groups	Mean $\pm$ St.						
	RBC $\times 10^6/\mu\text{L}$	Hb/g/dl	PCV/%	MCV/fl	MCH/pg	MCHC/g/dl	PLT $\times 10^3/\mu\text{L}$
A	4.70 $\pm$ 0.24 <sup>b</sup>	8.76 $\pm$ 0.16 <sup>b</sup>	19.91 $\pm$ 1.06 <sup>b</sup>	39.97 $\pm$ 0.35 <sup>b</sup>	18.38 $\pm$ 0.91 <sup>b</sup>	48.22 $\pm$ 2.45 <sup>a</sup>	453.57 $\pm$ 20.09 <sup>a</sup>
B	5.69 $\pm$ 0.47 <sup>a</sup>	10.32 $\pm$ 0.26 <sup>a</sup>	23.47 $\pm$ 0.45 <sup>a</sup>	41.41 $\pm$ 0.45 <sup>a</sup>	19.86 $\pm$ 1.51 <sup>a</sup>	47.75 $\pm$ 3.4 <sup>a</sup>	334.69 $\pm$ 38.57 <sup>b</sup>

Table 5: Leukogram values in vitamin D3-deficient and normal lambs

Groups	Mean ( $\times 10^3/\mu\text{L}$ ) $\pm$ St.					
	Total WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
A	10.64 $\pm$ 0.35 <sup>a</sup>	0.53 $\pm$ 0.06 <sup>a</sup>	7.77 $\pm$ 0.29 <sup>a</sup>	0.9 $\pm$ 0.10 <sup>a</sup>	0.02 $\pm$ 0.10 <sup>a</sup>	1.48 $\pm$ 0.10 <sup>a</sup>
B	9.02 $\pm$ 0.83 <sup>b</sup>	0.39 $\pm$ 0.09 <sup>b</sup>	6.33 $\pm$ 0.62 <sup>b</sup>	0.56 $\pm$ 0.12 <sup>b</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	1.33 $\pm$ 0.29 <sup>b</sup>

#### Discussion

The current study examined vitamin D3 deficiency and blood parameters of Najdi lambs from Basra province.

Twenty Najdi sheep with good body condition scores and typical vital signs were selected as the control group; these lambs normally had higher circulatory concentrations of vitamin D3. Previous studies reported that healthy calves

and lambs without musculoskeletal disorders have higher levels of vitamin D3 in their blood (24-26). The present study confirmed this finding. Nevertheless, in the current study, vitamin D3 concentrations were dramatically decreased in another group of lambs that showed various signs of illness, along with musculoskeletal disorders. When serum levels of vitamin D3 fall below 25 nmol/l (10 ng/ml), vitamin D deficiency occurs, affecting main vitamin D functions such as calcium/phosphorus metabolism and hemostasis (27,28). Since most lambs in this study had serum vitamin D3 levels above ten ng/ml, they did not show severe signs of rickets. However, this study presents the first evidence that serum vitamin D3 concentration decline is closely related to the severity of skeletomuscular illness, combined with poor body and hair condition scores recorded in Najdi lambs bred in Basra province, Iraq. There were remarkable variations in serum vitamin D3 concentrations among Najdi sheep, even though the nutritional and environmental conditions were similar. This may be due to varying nutritional behavior, liver or kidney dysfunction, or genetics. Najdi lambs with lower vitamin D3 concentrations may have been born from vitamin D-deficient ewes, as maternal and neonatal plasma vitamin D2 and vitamin D3 have a significant correlation (8), and fetal vitamin D status may be affected in pregnancy by poor nutrition and decreased vitamin D, leading to chronic diseases later in life (29).

Najdi sheep in the current study showed diverse clinical manifestations due to a sharp decline in serum vitamin D3 concentration, including loss of appetite, reduced ruminal movement, reduced productivity, reduced weight gain, lameness, joint enlargement, bending of large bones, and decreased osteomalacia and osteodystrophy. Several relevant previous studies (8,10,30-33) have confirmed that vitamin D3 deficiency is associated with a wide range of musculoskeletal and non-musculoskeletal health problems, including clinical rickets, osteomalacia, and osteodystrophy in adults, lameness, bending of large bones, reduced reproductive effectiveness, poor weight gain, diminished appetite, and reduced productivity. Although the study was conducted in the winter season, our results revealed that lambs with low circulatory vitamin D3 concentrations displayed elevated body temperatures, respiratory rates, and pulse rates, along with respiratory signs such as nasal discharge, congested mucous membranes, coughing, and dyspnea. Interestingly, this study found that Najdi lambs with lower blood levels of vitamin D3 were more susceptible to disease and may not be well adapted to their environment. Despite this, there is evidence that the lower circulatory concentration of vitamin D suppresses Najdi lambs' immunity since vitamin D regulates the innate and adaptive immune systems (34). According to a previous study, the body temperature, pulse rate, and respiratory rate of lambs with vitamin D deficiency were within normal ranges (31).

An accurate and rapid diagnosis of medical disorders begins with a complete blood count (CBC). A wide range of illnesses can be diagnosed or differentiated based on an accurate interpretation of the CBC supported by clinical examination and other diagnostic tests (35,36). As a result of the current study, significant differences were observed in hematological parameters among control and lambs that have declined vitamin D3 concentrations. Our findings suggest that vitamin D3 deficiency was responsible for the changes in hematological parameters in lambs, which affected feed consumption and made lambs more prone to infection, as most of the vitamin D3 deficient Najdi sheep showed respiratory symptoms. In addition, the elevation of the total white blood cells in lambs with low vitamin D3 levels may reflect vitamin D3's potential role in immunity. Sim *et al.* (4) report that vitamin D deficiency is associated with an increased risk and prevalence of anemia and vitamin D supports erythropoiesis, which protects against anemia (37). Moreover, serum vitamin D levels have been shown to modulate systemic cytokine production and white blood cell production (4,38).

## Conclusions

In addition to causing various musculoskeletal and other serious illnesses, vitamin D3 deficiency also causes immunosuppression in the lambs, making them susceptible to infection. Moreover, using the complete blood count to indicate health status can help determine how vitamin D3 deficiency impacts lambs and support accurate therapy.

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## Conflict of interest

There is no conflict of interest.

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أعراض مختلفة مثل؛ انخفاض درجة حرارة الجسم، انخفاض زيادة الوزن ٢٨,٥٪، علامات الكساح ٧,٥٪، العرج ٤٢,٥٪، فقدان الشهية ٥٣,٧٪، خشونة الشعر ٥٢,٥٪، تضخم المفاصل ٢٧,٥٪، التواء العظام الكبيرة ٣٣,٧٪، الرقود ١٢,٥٪، الإسهال ٢٥٪، شحوب الأغشية المخاطية ٥٨,٧٪، احتقان الأغشية المخاطية ٣١,٢٪، إفرازات الأنف ٦٨,٧٪، التدمع ٤٠٪ والسعال ٦٥٪ وضيق التنفس ٢٦,٢٪ وانخفاض حركة الكرش. أما الحملان السليمة سريريا فقد أظهرت مستويات طبيعية للعلامات الحيوية وفيتامين د ٣ في الدم ٧,٠٦±٨٩,٧٥ نانوغرام / مل. أظهرت الحملان ذات المستويات المنخفضة من فيتامين د ٣ في الدم زيادات كبيرة في درجة حرارة الجسم ٣٩,٣٦±٠,٠٧ درجة مئوية، ومعدل التنفس ٣٩,٠٢±١,٨٦ نفس / م، ومعدل ضربات القلب ١٧٤±٨٩,٢٢ نبضة / م مقارنة بالحملان التي تحتوي على نسبة عالية من فيتامين د ٣. علاوة على ذلك، اختلفت المعايير الدموية في الحملان ذات المستويات المنخفضة من فيتامين د ٣ في المصل بشكل ملحوظ عن الحملان التي تحتوي على مستويات عالية من فيتامين د ٣ في الدم بناء على تحليل تعداد الدم الكامل. في الختام، تقدم هذه الدراسة دليلاً على أن الاضطرابات العضلية الهيكلية، فضلاً عن الأمراض السريرية وأمراض الدم الأخرى، مرتبطة بانخفاض تركيز فيتامين د ٣ في الحملان النجدية.

## الملاحح السريرية والدموية لنقص فيتامين د ٣ في الحملان النجدية

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### الخلاصة

قيمت هذه الدراسة التأثيرات السريرية والدموية لنقص فيتامين د ٣ في تربية الحملان النجدية التي تربي في البصرة، العراق. تم اختبار تصميم تجريبي خاص باعتلال الجهاز العضلي الهيكلي باستخدام ١٠٠ حمل نجدية تتراوح أعمارهم بين ٣-١٢ شهرا. تم فحص الحملان بحثاً عن الأعراض السريرية العامة، وتم اختبار مستويات فيتامين د ٣ في المصل، وفحص الدم الشامل. ظهرت على الحملان التي تحتوي على مستوى منخفض من فيتامين د ٣ ١٧,٧٧±١,٠٧ نانوغرام / مل