Efficacy of Oral Administration of a Reliable AD3E Treatment on Vitamin D3 Deficiency in Najdi Sheep

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Efficacy of Oral Administration of a Reliable AD3E Treatment on Vitamin D3 Deficiency in Najdi Sheep

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Abstract
This study was aimed to assess the efficacy of oral treatment of commercial product of vitamin D3 (VITOL-80 C ORAL®, Interchemie, Holland) in growing Najdi sheep suffering from musculoskeletal illness due to vitamin D3 deficiency in Basra province, Iraq. Using a Najdi sheep model bred in Iraq, here we focused on measuring the serum levels of total vitamin D3, calcium, phosphorus, parathyroid hormone (PTH), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) as well as complete blood count (CBC) and clinical examinations pre and post-treatment with VITOL-80 C ORAL®. No significant changes ($P > 0.05$) of the vitamin D status were recorded in Najdi sheep with vitamin D3 deficiency post treatment with (VITOL-80 C ORAL®). However, a sharp ($P < 0.0001$) decline of the total serum vitamin D3 concentration were observed in those Najdi sheep per-administration (21.95 ± 1.82 ng/ml) and post-administration (22.29 ± 1.34 ng/ml) of vitamins therapy contrast to control healthy Najdi sheep (89.75 ± 6.84 ng/ml). An interaction between vitamin D3 status and the serum concentrations of calcium/phosphorus, PTH, ALP and ALT was observed. With vitamin D3-deficient Najdi sheep; values of CBC, and calcium/phosphorus concentrations were lower while PTH, ALP and ALT were higher than the healthy control Najdi sheep; thus, no significant changes ($P > 0.05$) of these values were recorded post treatment of (VITOL-80 C ORAL®). In conclusion, vitamin D3 deficiency threats the health of local Najdi sheep and has a potential role through suppressing their immunity. Oral administration of the commercial product as a source of vitamin D3 is not effective suggesting involvement of vitamin D receptors (VDR) and/or dysfunction of liver and kidneys.

Keywords: *Vitamin D3 deficiency, Najdi sheep, musculoskeletal illness, calcium/phosphorus, PTH, oral therapy*

Introduction

Vitamin D3 (1,25-dihydroxyvitamin D3) or Cholecalciferol is the main member of the fat-soluble vitamins responsible for hemostasis of calcium and phosphorus that involved in bones formation and remodeling, and fetal development, and thought it has a crucial rule in immunity [1-3]. The main source of vitamin D3 in animals and human comes from direct exposure to UVB sunlight to the skin converting the 7-dehydroxycholesterol to Cholecalciferol or from diet, which enriched or supplemented with the analogous form of vitamin D3 [2,4].

Musculoskeletal tissues, intestine, liver, kidneys, and parathyroid glands are the main sites of action of vitamin D [5]. Two successive hydroxylation reactions are activating vitamin D to its hormonal form. The first one takes place in liver tissue, resulting in 25-dihydroxyvitamin D3 (the in active metabolite of vitamin D3). Then it is converted in kidney to 1,25dihydroxyvitamin D3 by the renal cytochrome p450 enzyme [6]. By the regulating action of the parathyroid hormone (PTH) on the circulatory status of vitamin D, plasma ionized calcium and phosphorus are kept within the tight physiological limit [2,7]. Moreover, vitamin D may have the ability to cure cancer with the possibility of treating cancer through cell growth suppression [8]. Vitamin D might also be involved in the prevention of cardiovascular disorders, high blood pressure, and diabetes mellitus as well as assists in the treatment of inflammatory bowel disease, psoriasis, multiple sclerosis, and rheumatoid arthritis [9]. However, vitamin D probably plays a role in the development of muscles based on the receptors of vitamin D located in muscles and muscle weakness occurs in vitamin D deficiency. Further tissues have vitamin D receptors such as colon, breast, prostate, immune cells and brain [10-11].

Vitamin D deficiency is commonly induced by insufficient sunlight exposure or decreases its concentration in diet. It is characterized by poor appetite, the lack of growth, reduce productivity, reduce reproductive efficiency, and in developed cases by osteodystrophy. The lack of vitamin

D complex in feed prompts an insufficiency of vitamin D in the tissue [12]. To our knowledge, very little studies were done evaluating the physiopathology status of vitamin D of local livestock in Iraq.

However, to date, no single study has been attempted to assess the vitamin D status and the efficacy of the treatment of ruminants in Basra, particularly in sheep including Najdi sheep that bred in Basra province. Najdi sheep is a domestic sheep breed native in the central part of Najd region of the Arabian Peninsula (Saudi Arabia) that are adapted to live under desert conditions [13]. However, broad ranges of problems are facing breeding Najdi sheep in Basra such as in-adaptation to the environmental conditions, decrease fertility, affecting with endemic and seasonal diseases and etc [14]. Therefore, we used the Najdi sheep that bred in Basra as an experimental model to explore the relationship between their vitamin D status and the disorders mentioned earlier as well as evaluating the efficacy of the oral administration of the reliable source of vitamin D3 (VITOL-80 C ORAL®) in treatment of vitamin D deficiency in growing Najdi sheep.

Materials and Methods

Animals and Area of the Study

This study was conducted in Basra governorate / Iraq during the period from November to December 2019. Forty Najdi sheep (a breed of domestic sheep native to "Najd" region of the Arabian Peninsula) aged 3 to 12 months were used in this study. The sheep were selected from two closed herds which were housed in one-side closed barns where the concentrated grain feed with hay and tap water were provided ad lib with no way for grazing. Twenty Najdi sheep were categorized as sheep with suspected vitamin D deficiency group based on signs of lameness, bending of large bones, low weight gain, decrease body condition score, rough hair condition score and etc, these sheep were only treated with recommended doses of vitamin D3. Whereas other 20 clinically healthy Najdi sheep at same age group, were categorized as control healthy sheep. The hematological, the serological, and the biochemical analysis were done in the Clinical Pathology and the Central Research Unite, College of Veterinary Medicine, University of Basrah, Iraq and in the Hematology and Biochemistry Laboratories of "Bayan Group

Laboratories", Basra, Iraq. This experiment was conducted according to the rules and the guidelines of the using and keeping of "experimental-animals" provided through the General Scientific Committee of College of Veterinary Medicine, University of Basrah, Iraq.

Clinical examination

Routine clinical examination were performed to all Najdi sheep included: the examination of skin, eyes, hair coat condition, body condition score, superficial lymph node and gait as well as the vital signs which included (rumen motility, body temperature, respiratory rate and heart rate).

Rumen motility was observed in growing Najdi sheep using manual palpation of the left paralumbar area and pushed by hand for three minutes feeling it as the rumen pushing back. Mean of rumen contraction per minute was applied in this study and the scoring system was depended on three scores of rumen motility as: I- Normal (about 1-2 powerful movement per minute, II- Weak (less than one weak movement per minute) and III- Cessation of rumination (no movement).

Body condition score is an easy useful method describing how thin or fat sheep are. The BCS was done using numerical score from 1 (very thin sheep) to 5 (very fat sheep) depending on the muscle/fat deposition around the loin region [15-17]. Sheep may be given half scoring such as 1.5 if it between BCS 1 and BCS 2.

Hair coat condition quality as animal main welfare indicator was adopted for all sheep in the current study. Two basic points were applied in the hair coat condition scoring: Normal (shiny, homogeneous and adheres to the body) and Rough/Poor (rough scarify hair coat, uneven, matted and frequently longer than normal) hair coat condition score [16,18].

Collection of Blood Sample

Samples of Blood were aseptically collected according to methods of [19]. Five milliliters of blood were collected aseptically from jugular vein, and divided into two tubes (4ml paced in a plain tube for biochemistry and 1 ml placed in EDTA tube for complete blood count analysis).The plain tube was left overnight for clotting or centrifuged immediately at 1500 rpm for 10 minutes, and serum was collected and

kept in deep freeze at – 20 °C till the day of estimation of Vitamin D3, PTH ALT, ALP, Calcium and Phosphorus concentrations.

Treatment Protocol

A commercial complex of vitamins A, D3, E and C was used in this study. VITOL-80 C ORAL® (Interchemie, Holland) complex of vitamin A "retinol palmitae" (50000 IU/ml), vitamin D3 "Cholicalciferol" (10000 IU/ml), vitamin E "α-tocopherol acetate" (20 mg/ml), and vitamin C "ascorbic acid" (100 mg/ml) was drench orally to the Najdi sheep had vitamin D3 deficiency at dose rate of 1 ml/40 kg body weight for 5 days. Treated Najdi sheep were monitoring daily post vitamins inoculation and the final clinical examination was performed at day seven post last oral dose. The blood samples were re-collected to evaluate the serum values of vitamin D3, PTH, ALT, and ALP as well as evaluation of CBC to the treated Najdi sheep.

Determination of Vitamin D3concentrations

Najdi sheep serum samples were tested by the enzyme linked immune sorbent assay ELISA kit to assess their vitamin D3 concentration using biotin-double antibodies sandwich technique. All the assay procedures were done following the manufacturer's instructions (Bioassay Technology/ China, www.bt-laboratory.com).

Determination of Parathyroid Hormone (PTH) concentrations

Parathyroid hormone (PTH) was estimated using Serum PTH kit following the manufacturer's instructions (abbot\Germany). The concentration of PTH in serum was analyzed automatically according to the instruction manual of ARCHITECT System operation (Germany). The ARCHITECT intact PTH assay is a two– step sandwich immunoassay for quantitative determination of intact PTH in serum and plasma using "CMIA" technique fixable test protocol (Chemiflix).

Determination of Alkaline Phosphatase (ALP) concentrations

Alkaline Phosphatase was estimated using Serum Alkaline phosphatase Kit following the manufacturer's instructions (abbot\Germany). The concentration of ALP in serum was analyzed automatically according to the instruction manual of ARCHITECT System operation (Germany).

This was done by using p-nitrophenyl phosphate (pNPP) as a substrate to measure the concentration of Alkaline Phosphatase in serum.

Determination of Alanine Aminotransferase (ALT) concentrations

Alanine Aminotransferase was estimated using Serum Alanine Aminotransferase Kit following the manufacturer's instructions (abbot\Germany). The concentration of Alanine Aminotransferase in serum was analyzed automatically according to the instruction manual of ARCHITECT System operation (Germany).

Determination of Calcium and phosphorus Concentrations

Calcium estimation was done by using serum calcium and phosphorus Kits following the manufacturer's instructions (abbot\Germany). The concentration of calcium in serum was analyzed automatically according to the instruction manual of ARCHITECT System operation (Germany). Serum calcium and phosphorus estimated by photometric color (Arsenazo-III method) test.

Determination of complete blood count (CBC)

The blood samples collected in EDTA tubes were analyzed for complete blood count (CBC) in the Hematology Laboratory of Bayan Group Laboratories / Basra, Iraq using automatic blood analyzer (SYSMIX®, JAPAN). The examined parameters were: RBCc, HGB, HCT, MCV, MCH, MCHC, total WBCc and differential WBCc.

Statistical Analysis

The data of the current Najdi sheep experiment were statistically analyzed either by using the software JMP 11, SAS Institute Inc. or by Microsoft Excel. Pair student t test were adopted to assess the differences between the results at ($P < 0.05$).

The Results

The results of the clinical examination

The examination of the vital signs of the growing Najdi sheep showed significant ($P < 0.05$) increases in respiratory rate and heart rate and non-significant ($P > 0.05$) increase in rectal temperature of Najdi sheep suffering from vitamin D3 deficiency when compared with the healthy

control (Table 1). Nevertheless, no significant ($P > 0.05$) differences were recorded between vitamin D3-deficient sheep pre and post-treatment with oral administration of vitamin D3 (Table 1).

However, many clinical manifestations were observed during the clinical examination of the Najdi sheep with vitamin D3 deficiency (Table 2); while no significant ($P > 0.05$) differences in those clinical signs were observed following recommended oral doses of "VITOL-80 C ORAL®". The main signs observed in vitamin D3-deficeint Najdi sheep were; decrease body condition score (Figures 1, 3-12), decrease ruminal motility (Figure 2), decrease weight gain (100% in both pre and post-treatment), signs of rickets (10% in both pre and post-treatment), lameness (100% in both pre and post-treatment) (Figures 3-15), loss of appetite (100% and 60% in both pre and post-treatment respectively), rough hair coat condition (100% in both pre and post-treatment) (Figures 3-15), joint enlargement (50% in both pre and post-treatment) (Figures 7 and 12), bending of large bone (70% in both pre and post-treatment) (Figures 3-6), recumbency (10% in both pre and post-treatment) (Figure 4.8), diarrhea (25% and 0% in both pre and post-treatment respectively) (Figures 8 and 11), pale mucous membrane (40% and 70% in both pre and post-treatment respectively) (Figure 13), congested mucous membrane (50% and 20 in both pre and post-treatment respectively) (Figures 14 and 15), nasal discharge (80% and 20% in both pre and post-treatment respectively) (Figures 8, 12, 14 and 15), lacrimation (60% and 20% in both pre and post-treatment respectively) (Figures 8, 12, 14 and 15), coughing (80% and 20% in both pre and post-treatment respectively) (Figures 12 and 15), and dyspnea (10% and 0% in both pre and post-treatment respectively) (Figure 12).

The results of serum vitamin D3 concentrations

Serum vitamin D3 concentrations were sharply ($P < 0.0001$) decreased (21.95±1.82 ng/ml) in Najdi sheep with signs of musculoskeletal illness, poor growth, poor appetite, decrease body condition score, and poor hair coat condition score when compared to the control healthy Najdi sheep (89.75±6.84 ng/ml), as shown in (Figure 16). However, no considerable variation ($P > 0.05$) of vitamin D3 concentrations (22.29±1.34 ng/ml) in the Najdi sheep that had vitamin D3 deficiency was noticed post oral treatment with reliable vitamin D3 (Figure 16).

The results of serum calcium and phosphorus concentrations

Serum calcium (7.45±0.19 mg/dl) and phosphorus (3.49±0.31 mg/dl) concentrations were significantly ($P < 0.05$) lower in Najdi sheep with vitamin D3 deficiency when compared to the healthy control (9.42±0.25 mg/dl), (5.58±0.26 mg/dl) respectively. Whereas no significant changes were recorded in calcium (7.53±0.27 mg/dl) and phosphorus (3.56±0.21 mg/dl) concentrations post oral treatment with reliable vitamin D3 product (Figure 17).

The result of the serum parathyroid hormone (PTH)

Our main finding were that the lowest vitamin D3 concentrations Najdi sheep had significantly ($P < 0.05$) higher serum PTH concentrations. Moreover, estimation of the levels of serum PTH post oral treatment with vitamin D3 showed no significant increase ($P > 0.05$) of its concentrations in Najdi sheep had vitamin D3 deficiency (Figure 18).

The result of serum enzymes (ALT, ALP) concentrations

Similarly to PTH, Serum ALT and ALP concentrations were significantly ($P < 0.05$) higher in Najdi sheep with lower serum concentrations of vitamin D3 suggesting skeletomuscular, liver, and kidneys disorders. However, the assessment of serum enzymes (ALT and ALP) did not show marked differences ($P > 0.05$) post oral treatment with reliable vitamin D3 product (Figure 19).

The result of complete blood count (CBC)

The analysis of blood samples showed significant decreases ($P < 0.05$) of most RBC in Najdi sheep that had lowest vitamin D3 concentrations. While the estimation of platelets count showed a marked increase ($P < 0.05$) of its numbers in Najdi sheep with lowest vitamin D3 concentrations. However, no significant changes ($P > 0.05$) in all haemogram values were recorded post oral treatment with reliable product of vitamin D3 (Table 3). The assessment of leukocyte count revealed marked increases ($P < 0.05$) of all leukogram values (except Eosinophil) in Najdi sheep with lowest vitamin D3 concentrations. Nevertheless, no significant changes ($P > 0.05$) in all leukogram values

were recorded post oral treatment with reliable product of vitamin D3 product (Table 4).

The Discussion

In the current study, the healthy control Najdi sheep were found to have higher levels of serum vitamin D3 (89.75±6.84 ng/ml), this finding proved the previous related studies done by [20-22] who have reported that the lambs and calves with good health and without skeletomuscular illness had high levels of vitamin D. However, a sharp decline (21.95±1.82 ng/ml) of vitamin D3 concentration was recorded in the other group of Najdi sheep which had poor body and hair condition scores combined with other skeletomuscular illness. Despite the similarity of the environmental and the nutritional status of the Najdi sheep in the current study, noteworthy variations in circulating vitamin D3 concentrations were noticed. This could be attributed to the alteration of the nutritional behavior between individuals or due to malfunctions of the kidneys and liver that affecting the metabolism and the activation of vitamin D3 [2,6-7]. However, impairment of the VDR is not being excluded [2]. It is possible that the Najdi sheep with the lowest circulating levels of vitamin D3 were born from ewes had severe vitamin D deficiency, since there is a considerable correlation between maternal and neonatal plasma vitamins D2 and D3 [23]. Additionally, poor nutrition with declined vitamin D in pregnant may impact the fetal vitamin D status increasing the risk of chronic diseases in later life [24].

Serum calcium and phosphorus levels were found to be significantly down-regulated in the lowest vitamin D3 Najdi sheep, while their levels were unregulated in the highest vitamin D3 Najdi sheep. The reductions of circulating calcium [25] and phosphorus [26-27] could be attributed to vitamin D deficiency. Thus, severe deficiency of vitamin D might present signs of hypocalcemia, while mild deficiency exhibits only moderate decrease in circulating levels of calcium or phosphorus or both [28]. Moreover, vitamin D3 deficiencies will dominantly induce hypocalcemia as approved by our current study, which consequently stimulate the proliferation of PTH [3,29-30]. This could explain why the treatment with vitamin D3 product in Najdi sheep of the current study did not maintain normal circulatory levels of vitamin D3.

Despite we have strictly followed the protocol of treatment; the circulatory levels of vitamin D3, calcium, and phosphorus were kept down-regulated. This our finding could be attributed as a result of VDR involvement, which led mainly to suppress the activation of vitamin D3 [31]. Another reason of dropping the circulatory vitamin D3 concentration is the failed in the hydroxylation reactions that are activating vitamin D to its hormonal active form (1,25(OH)2D3) in liver and kidneys [6,32], liver and kidneys disorders of growing Najdi sheep could also induce this impairment. However, a single previous related study has stated that the total plasma levels of calcium and phosphorus in sheep were not significantly affected by intramuscular injection of vitamin D3 suggesting absence its [33]. Furthermore, other study conducted on sheep model has demonstrated that different protocols of intramuscular injection of vitamin D3 did not produce circulatory vitamin D3 concentrations greater than those in normal grazing sheep during summer [34]. Thus for more efficient uses of vitamin D3 therapy, repeated dosing is recommended.

In the present study, ALP was dramatically higher in the lowest vitamin D3 Najdi sheep, which appear consistent with the findings from previous studies of domestic animals [23,30]. It seems that the elevation of ALP was caused by osteodystrophy and/or osteomalacia as a result of vitamin D3 deficiency in Najdi sheep, since it is combined with higher serum PTH levels and marked a decline of serum phosphorus levels [30,35]. Moreover, Najdi sheep with the lowest circulating vitamin D3 was also had higher serum ALT levels. This our finding suggest that the deficiency of vitamin D3 could mainly induce myopathy which consequently raises the levels of ALT [36], and can reflect one or more disorders including: a skeletal muscle damage [37-39], acute and chronic liver diseases [40-41] and liver flock infestation [42-43]. There is evidence from our finding that the deficiency of vitamin D3 could be attributed to the liver damage as a result of liver diseases or infestation with liver flock that interfering with the treatment.

In the current study, considerable differences of CBC values between healthy and vitamin D3 deficient Najdi sheep were noticed. This finding

provides evidence that the alterations of CBC values in Najdi sheep were due to vitamin D3 deficiency. In addition, alteration in CBC values was probably led to decrease appetite and making Najdi sheep more susceptible to be infected since all the sheep with declined circulating vitamin D3 had respiratory signs during winter season. Previous studies suggest that there is a greater risk and prevalence of individual anemia with deficiency of vitamin D [44] and there is strong evidence that vitamin D has a protective role against anemia by supporting erythropoiesis [45]. Therefore in the current study, it is logical that the treatment with oral administration of vitamin D3 did not induce a noticeable change in CBC values since it did not sustain a normal circulatory level of vitamin D3.

Clinically, Najdi sheep with declined vitamin D3 concentration showed divers signs of skeleto-myopathy. Numerous studies have reported that the deficiency of vitamin D is associated with many musculoskeletal and non-musculoskeletal health problems [12,23,35,46]. However in the current study, the Najdi sheep with low levels of vitamin D3 had numerous clinical manifestation of illness. Our study suggests that the Najdi sheep with lower serum levels of vitamin D3 are more susceptible to be infected during winter season and not adapted to the environmental conditions. However, we found evidence that the low levels of vitamin D3 suppress the immunity, since vitamin D plays a significant immune-regulatory role in innate and adaptive immune system [47].

Conclusions

We conclude that vitamin D3 deficiency is closely involved in calcium and phosphorus down-regulation of Najdi sheep, which induced various manifestations of illness. Hypocalcemia due to deficiency of the active form of vitamin D3 has markedly up-regulate PTH production in Najdi sheep that could induce further physiological disorders. However, it also implicates that vitamin D3 deficiency in Najdi sheep cannot be treated successfully through oral vitamin D3 therapy and the VDR has a suspicious role in this process. In final consideration, the liver dysfunction due to liver flock infestation and/or liver diseases was probably the main cause of vitamin D3 deficiency in Najdi sheep.

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Conflict of Interest

No conflict of interests is declared by authors for the contents in this manuscript

Informed consent

None

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Table 1: The results of vital clinical signs of healthy control, vitamin D3 deficient and treated Najdi sheep

Najdi sheep Groups	No.	Temperature Cº	Respiratory rate Breaths/Minute	Heart rate Beats/Minute
Healthy Control	20	39.02±0.42 ab	27.69±1.72 c	83.31±1.87 c
Before Treatment with Vit D3	20	39.85±0.71 a	40.5±2.86 a	90.60±3.41 a
Post Treatment with Vitamin D3	20	39.81±1.43 a	32.6±1.90 bc	86.90±2.98 bc

a,b,c values in different letters within column are significantly different at P < 0.05.

Table 2: The percentage of clinical symptoms and sings of vitamin D3-deficient Najdi sheep pre and post-treatment oral administration of vitamin D3

Clinical signs and symptoms	Pre-Treatment		Post-Treatment	
	No. of sheep	Percentage	No. of sheep	Percentage
Decrease weight gain	20	100%	20	100%
Signs of rickets	2	10%	2	10%
Lameness	20	100%	20	100%
Loss of appetite	20	100%	12	60%
Hair coat condition	20	100%	20	100%
Joint enlargement	10	50%	10	50%
Bending of large bone	14	70%	14	70%

Recumbency	2	10%	2	10%
Diarrhea	5	25%	0	0%
Normal mucous membrane	2	10%	2	10%
Pale mucous membrane	8	40%	14	70%
Congested mucous membrane	10	50%	4	20%
Nasal discharge	16	80%	4	20%
Lacrimation	12	60%	4	20%
Coughing	16	80%	4	20%
Dyspnea	2	10%	0	0%
Total number of sheep			20	

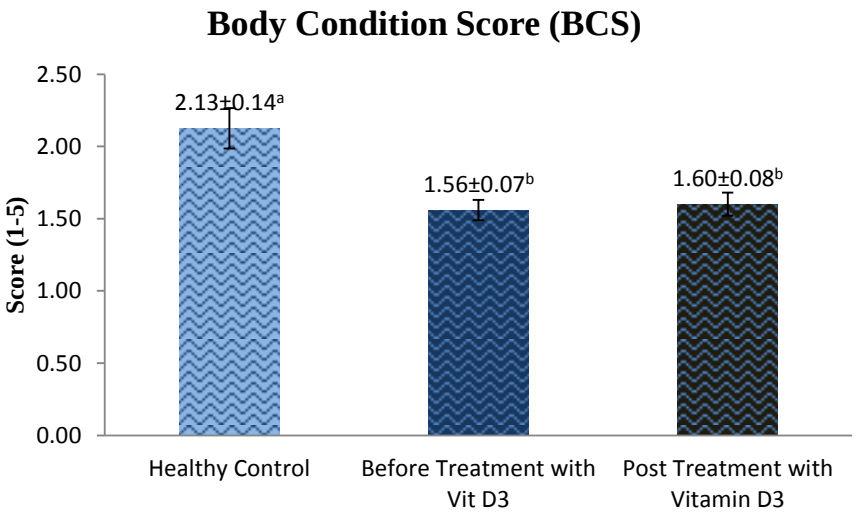

Figure 1: Means ± S.E. of Body Condition score in healthy control, vitamin D3 deficient, and treated Najdi Sheep.
[a,b] values in different letters are significantly different at P < 0.05.

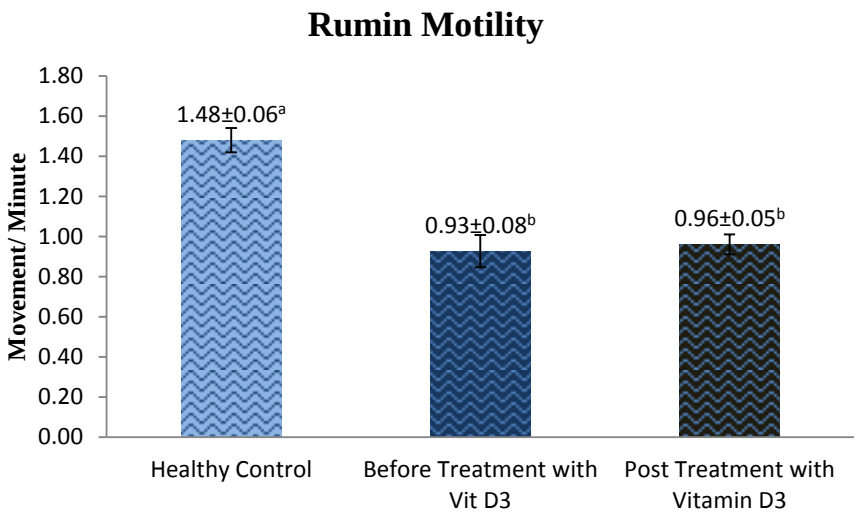

Figure 2: Rumen motility (Means ± S.E of movement per minute) in health control and vitamin D3 deficient Najdi Sheep.
[a,b] values in different letters are significantly different at P < 0.05.

Figure 3: Photo of 7 months old male vitamin D3 deficient lamed Najdi sheep showing rough hair coat condition and bending of the right and left metacarpal bones.

Figure 4: Photo of 6 months old male vitamin D3 deficient lamed Najdi sheep showing a marked bending of the right metatarsus bone combined with loss of condition.

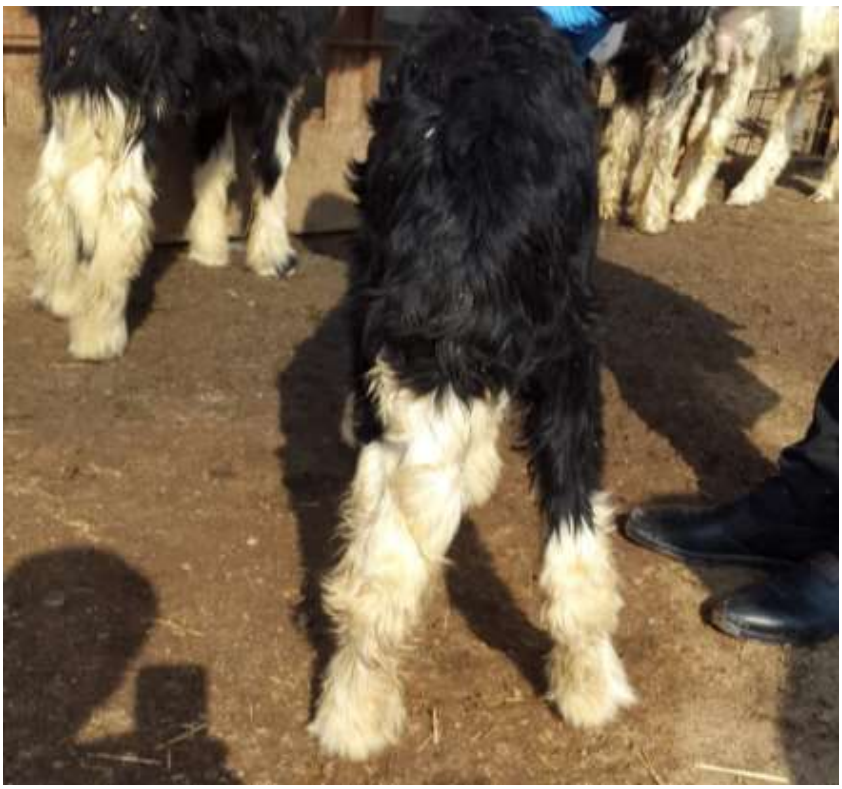

Figure 5: Photo of 6 months old male vitamin D3 deficient lamed Najdi sheep showing a marked bending of the left metatarsus bone combined with rough hair coat condition and poor body condition score.

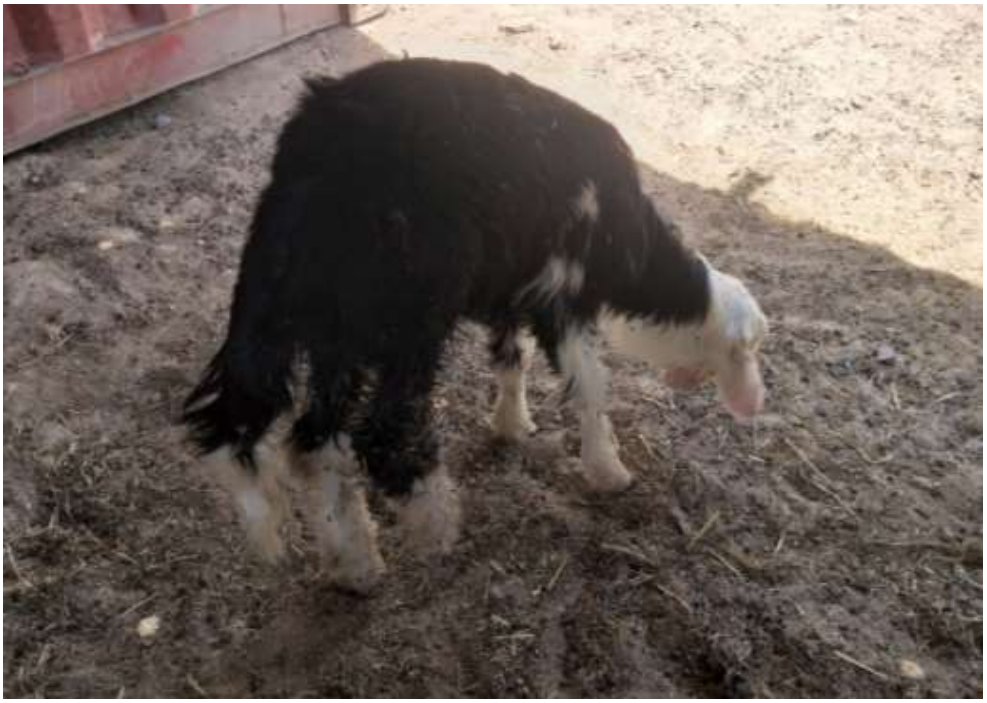

Figure 6: Photo of 5 months old female vitamin D3 deficient lamed Najdi sheep showing a bending of the right metatarsus bones combined with rough hair coat condition, loss of condition and rough hair coat condition.

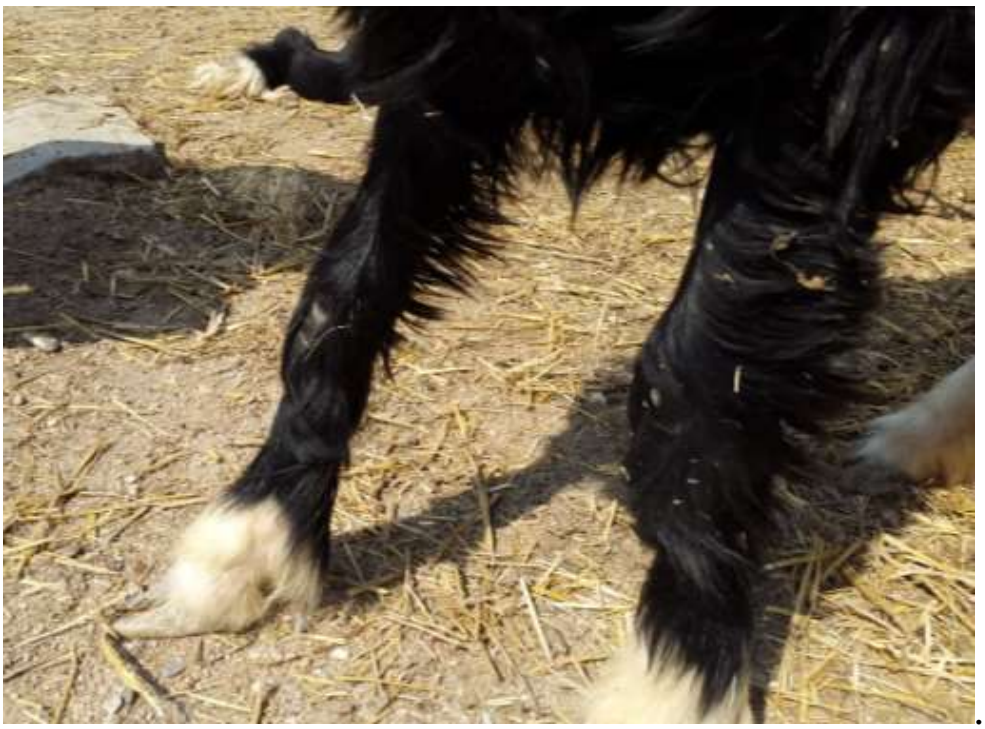

Figure 7: Photo of 4 months old male vitamin D3 deficient lamed Najdi sheep showing bending of the right metacarpal bone with enlargement of carpal joints.

Figure 8: Photo of 3 months old female vitamin D3 deficient Najdi sheep showing recumbency, loss of condition, rough hair coat condition, nasal discharge and lacrimation.

Figure 9: Photo of 6 months old female vitamin D3 deficient lamed Najdi sheep showing, loss of condition, rough hair coat condition and poor body condition score.

Figure 10: Photo of 3 months old male vitamin D3 deficient lamed Najdi sheep showing, loss of condition, rough hair coat condition and poor body condition score.

Figure 11: Photo of 3 months old female vitamin D3 deficient Najdi sheep showing diarrhea, rough hair coat condition and poor body condition score.

Figure 12: Photo of 3 months old male vitamin D3 deficient lamed Najdi sheep showing marked signs of dyspnea and frequent coughing, rough hair coat condition, poor body condition score and loss of condition.

Figure 13: Photo of 7 months old female vitamin D3 deficient Najdi sheep showing pale mucous membrane.

Figure 14: Photo of 8 months old female vitamin D3 deficient lamed Najdi sheep showing nasal and ocular discharges congested mucous membrane.

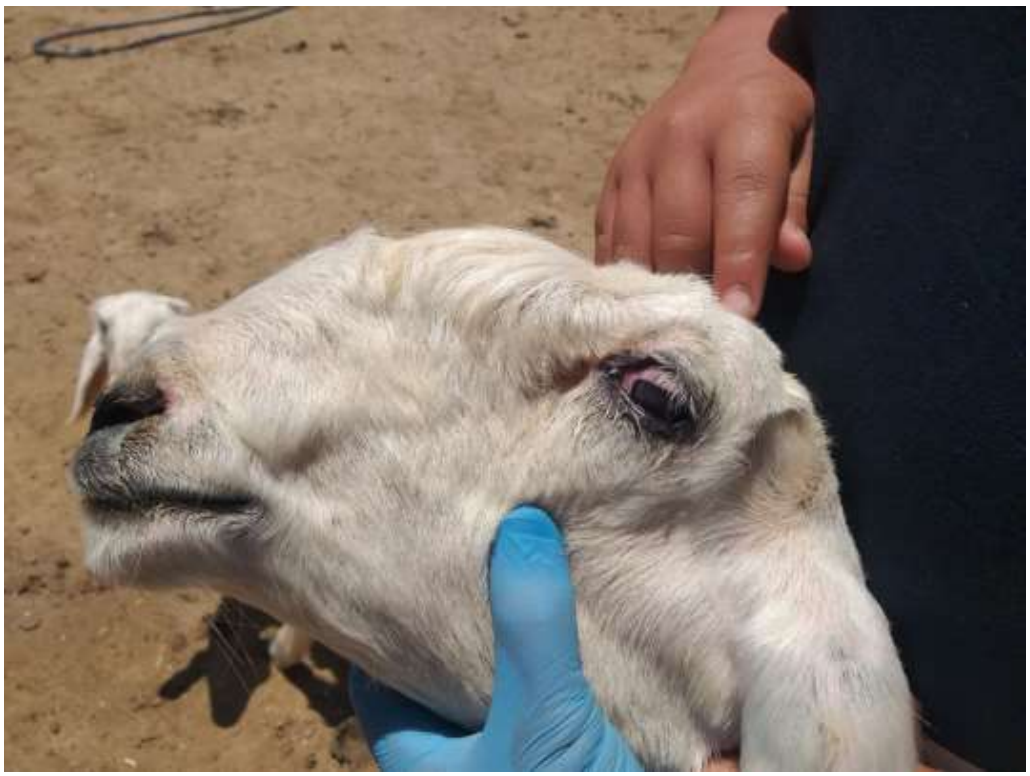

Figure 15: Photo of 6 months old female vitamin D3 deficient lamed Najdi sheep showing nasal and ocular discharges, nose redness, loss of condition and rough hair coat condition.

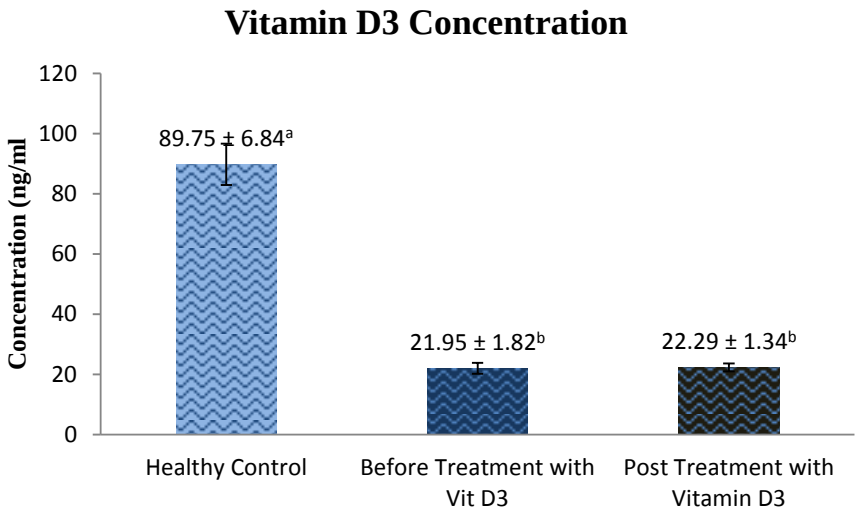

Figure 16: Means ± S.E. of serum vitamin D3 concentrations in healthy control, vitamin D3-deficient, and treated Najdi Sheep.
[a,b] values in different letters are significantly different at P < 0.05.

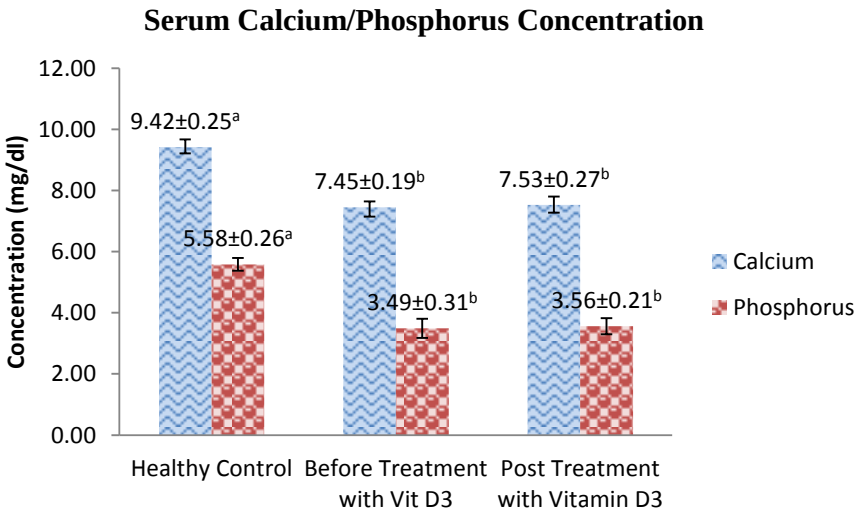

Figure 17: Means ± S.E. of serum calcium and phosphorus concentrations in healthy control, vitamin D3-deficient, and treated Najdi Sheep.
[a,b] values in different letters within similar columns are significantly different at P < 0.05.

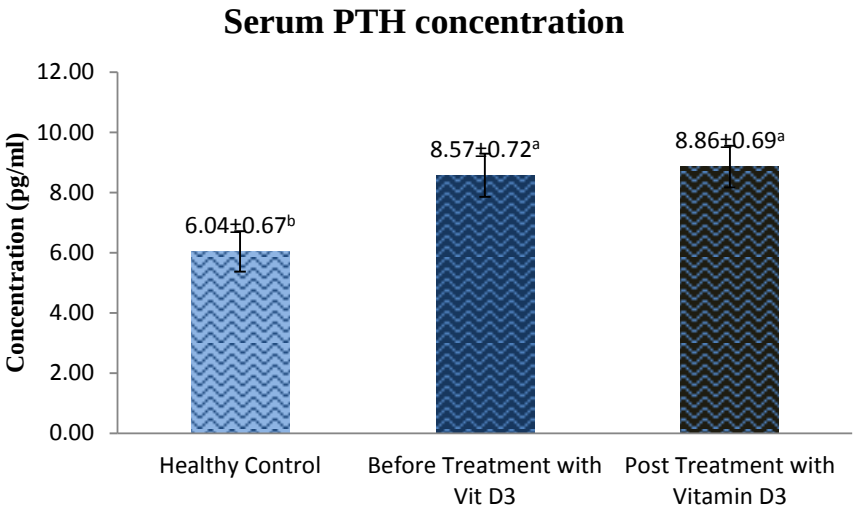

Figure 18: Means ± S.E. of serum PTH concentrations in healthy control, vitamin D3-deficient, and treated Najdi Sheep.
[a,b] values in different letters are significantly different at P < 0.05.

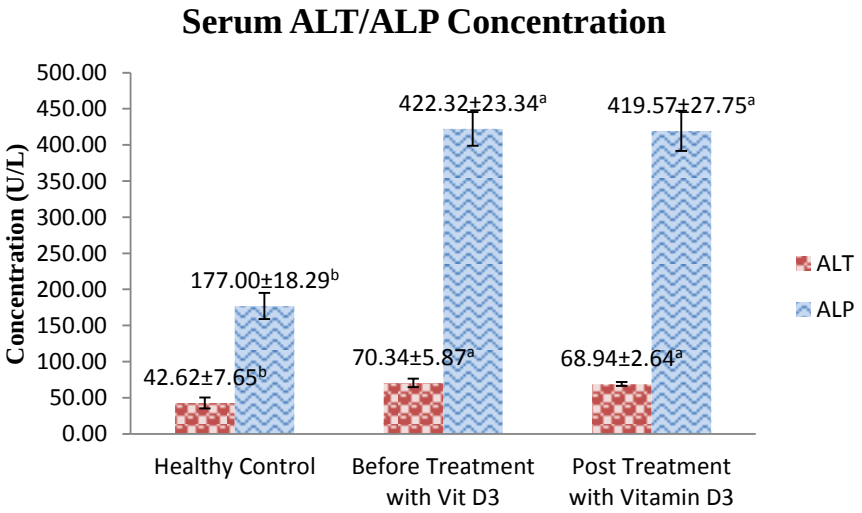

Figure 19: Means ± S.E. of serum ALT and ALP concentrations in healthy control, vitamin D3-deficient, and treated Najdi Sheep.
[a,b] values in different letters within similar columns are significantly different at P < 0.05.

Table 3: The values of Haemogram of healthy control, vitamin D3-deficient, and treated Najdi sheep (Mean±SE)

Groups	RBC $^{x}10^{6}$ /µL	HGB g/dl	HCT %	MCV Fl	MCH pg	MCHC g/dl	PLT $^{x}10^{3}$/µL
Healthy Control	5.69±0.47 a	10.32±0.26 a	23.47±0.45 a	41.41±0.45 a	19.86±1.51 a	47.75±3.4 a	334.69±38.57 b
Vit. D3 Defic.	4.63±0.18 b	8.34±0.21 b	20.01±1.65 b	39.52±0.46 b	18.01±0.83 b	48.01±3.34 a	461.26±34.19 a
Post Treat.	4.74±0.28 b	8.14±0.26 b	19.96±0.96 b	38.97±0.25 b	17.80±0.91 b	48.11±4.02 a	448.84±67.28 a

^{a,b} values in different letters within column are significantly different at P < 0.05.

(Healthy Control): Clinically healthy Najdi sheep, (Vit. D3 Defic.): Najdi sheep with Vitamin D3 Deficiency, (Post Treat.): Najdi sheep with Vitamin D3 Deficiency treated with oral doses of vitamin D3.

(RBC): red blood cells, (HGB): hemoglobin, (HCT): hematocrit, (MCV): mean corpuscular volume, (MCH): mean corpuscular hemoglobin, (MCHC): mean corpuscular hemoglobin concentration, and (PLT): platelets.

Table 4: The values of leukocytes count in control and vitamin D3 deficient Najdi sheep (Mean±SE)

Groups	WBC 10^{3}/µL	NEUT 10^{3}/µL	LYMPH 10^{3}/µL	MONO 10^{3}/µL	EO 10^{3}/µL	BASO 10^{3}/µL
Healthy Control	9.02±0.83 b	0.39±0.09 b	6.33±0.62 b	0.56±0.12 b	0.02±0.01 a	1.33±0.29 b
Vit. D3 Defic.	11.03±0.78 a	0.54±0.07 a	7.98±0.73 a	0.88±0.2 a	0.02±0.1 a	1.58±0.3 a
Post Treat.	10.92±0.65 a	0.52±0.05 a	7.82±0.85 a	0.91±0.09 a	0.02±0.1 a	1.53±0.2 a

^{a,b} values in different letters within column are significantly different at P < 0.05.

(Healthy Control): Clinically healthy Najdi sheep, (Vit. D3 Defic.): Najdi sheep with Vitamin D3 Deficiency, (Post Treat.): Najdi sheep with Vitamin D3 Deficiency treated with oral doses of vitamin D.

(WBC): white blood cell, (NEUT): neutrophils, (LYMPH): lymphocyte, monocyte (MONO), (EO): eosinophil, and (BASO): basophil.