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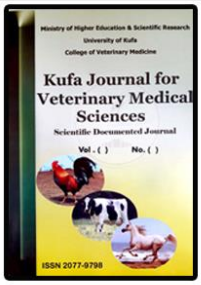

# The influence of vitamin C deficiency on dermatosis in neonatal dairy calves

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**Abstract**

Vitamin C is an important water-soluble antioxidant, which plays necessary roles in an animal's body, especially in neonatal dairy calves. Neonatal dairy calves receive vitamin C from their dam, and in unique situations, it can cause problems within the calf, specifically skin lesions, or dermatosis. This study aims to determine the effects of vit. C deficiency on the skin and coat integrity of the neonate calves. In this study, 10 neonatal dairy calves were used. Five calves were served as the control group, and the other 5 calves were tested for clinically affected with ascorbic acid deficiency. The clinical findings showed that severe scaling, alopecia in different areas of the body, pinna erythematous, purpuric, and pruritus. Samples of plasma were collected to estimate the value of vitamin C and some blood film parameters. The findings also revealed that significant decreases in the level of vitamin C, counting of erythrocytes, concentration of haemoglobin, and packed cell volume. Furthermore, there were significant changes in the neutrophils. Histopathological results revealed hyperkeratosis and congestion in the skin layers. After affected calves were treated with vitamin C, there was improvement and gradual disappearing of the skin gross changes. This study found a close relationship between the vitamin C deficiency in dairy calves and dermatosis (skin integrity).

***Key words:*** Vitamin C deficiency, ascorbic acid, neonatal dairy calves, dermatosis.

## دراسة تاثير نقص فيتامين ج في اصابات الجلد في العجول حديثة الولادة

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**الخلاصة:**

يعد فيتامين ج من الفيتامينات الذائبة في الماء ومن اهم مضادات الاكسدة ز اذ يلعب دورا اساسيا ومهما في اجسام الحيوانات ولا سيما بالعجول حديثة الولادة والتي تعتمد بشكل كلي على امهاتها بالحصول على فيتامين ج. وفي حالة حرمانها من الحصول عليه لاي سبب كان تبدأ عدة مشاكل صحية بالظهور على العجول حديثة الولادة مثل افات الجلد والتهاباته. تتناول هذه الدراسة التي اعتمدت 10 عجول حديثة الولادة قسمت الى مجموعتين من 5 عجول. اعتبرت المجموعة الاولى كمجموعة سيطرة للمقارنه فيما كانت المجموعة الاخرى مجموعة دراسة تاثير نقص فيتامين ج سريريا ومختبريا ونسجيا مرضياً.

العجول التي عانت من نقص فيتامين ج اظهرت نتائج دراستها من الناحية السريرية انها عانت من حالات تساقط الشعر خاصة في مناطق الفخذين والعنق وجذع الحيوان. كذلك علامات احمرار الجلد وتقشره بشكل حاد وايضا انتشار للبقع النزفية بينما اظهرت عينات الدم التي تم سحبها للفحوصات المختبرية نقصان ملحوظ في محتوى

المصل من فيتامين ج وايضا نقصان في كريات الدم الحمراء (RBC) وخلايا العدلات (Neutrophils) و الحجم الكريي المضغوط (PCV) وصبغ الدم(Hb) مقارنة بمجموعة السيطرة بنفس العمر والظروف.
كذلك بين القطع النسجي المرضي للجلد علامات فرط التقرن والاحتقان. ايضا اثبتت الدراسة تثاير فيتامين ج في زوال كل هذه العلامات خلال 5 اسابيع من العلاج للعجول المصابة. تستنتج هذه الدراسة التي اجريت على مدى عامين (2016-2018) في محافظة البصرة وتؤكد الدور المهم لنقص فيتامين ج في اصابت الجلد في العجول حديثة الولادة.

**Introduction**

Vitamin C (vit C), also known as ascorbic acid, is one of the most important water-soluble vitamins, playing a major role as an antioxidant factor for numerous cellular functions (1, 2). Vitamin C is found in most of the biological fluids of the body, including plasma, tissues, and leukocytes (2, 3, 4, 5). Other aspects of vit C refer to its role in synthesizing collagen and other connective tissues, which decreases the hydroxylation reaction, increase the integrity of the mucosal epithelium (capillary fragility), increase wound healing, bone growth (osteoblast vitality), the absorption of minerals and ions ($Na^+$, iron, etc.), maintains an immunologically important role through neutrophils, lymphocytes, macrophage interferon activity, reduces the plasma histamine, prevents neonatal calf diarrhea, remains a key factor in fertility, and establishes the differentiation of mesenchyme-derived cells (5,6,7,8,9, 10).

Most mammals, especially ruminants, synthesize ascorbic acid in the liver and therefore it is often not considered an essential nutrient (2, 11). Calves being synthesizing vit C between 2 to 3 weeks of age, however, the levels of Vit C is not as much as in adults until the calves reach 3-4 months of age. Therefore, a young calf relies on ascorbic acid that before found in milk, which is relatively low, making hypo-vitaminosis C a potential problem for dairy calves (2, 12, 13,14). Furthermore, vit C in milk is often exposed to air and light, which may destroy vit C before it can be consumed by the calf (2,8).The plasma vit C levels then rapidly decreased such that vit C concentrations in 6-week-old and 3-month old calves were significantly lower compared to that of their dams (9,12).

In fact, there are many reasons leading to ascorbic acid deficiency can be summarize as the fallowing: (15) refers to vit C degradation in rumens, and adult cattle are more susceptible to vit C deficiency, leading to calves having similar levels of deficiency. (6,9,16,17,18,19**).** However, several studies have conclusively documented decreasing vit C levels in the blood during stress and diseases in cattle and other ruminants (9,19,20,21). Genetic factors are reported as one of the causes of vit C deficiency (9). Whatever condition that decreases the availability of vit C precursors, such as glucose and galactose, may result in insufficient endogenous synthesis. For example, high milk-producing dairy cows have an elevated demand for glucose by the mammary gland in order to produce lactose; hence, they may synthesize less vit C (19, 22).

Ketosis also causes ascorbic acid deficiency (19). Furthermore, (7) reported three conditions in modern dairy calf rearing operations that suggest a need for ascorbic acid supplements: 1) rapidly growing animals with tissues needing ascorbic acid; 2) a diet that is low in ascorbic acid; and 3) many infection organisms require an adequate antibody response, where ascorbic acid is an essential nutrient for the immune system.

It was clear that the deficiency in growing calves between 2-10 weeks of age shows clinical signs of dermatosis, with moderate to severe scaling and crusting, alopecia, easy epilation of hair on the head or limbs with erythema and purpura, and lesions without pruritus or aching. (23,24,25,26, 27).

(27) Referred to dermatopathological analysis which revealed mild orthokeratotic hyperkeratosis, mild acanthosis, congestion of the dermis, and follicular keratosis and alopecic structures of the hair follicles. However, when treated with ascorbic acid, hair loss ceased, and new hairs regrew within 3 weeks. Based on the clinical findings in this case, the low plasma levels of ascorbic acid and the dermatopathological features were typical of vitamin C-responsive dermatosis; the former diagnosis was confirmed.

The pathogenesis of vitamin C-responsive dermatosis is not known. Ascorbic acid deficiency has been qualitatively related to mucosal barrier dysfunction, impaired resistance to infectious organisms, and the production of defective basement membrane collagen. Ascorbic acid is directly involved in the production of collagen. Various severe stressors, such as infectious diseases, metabolic diseases, and high fevers, result in temporary growth defects in hair shafts. Apoptosis of the hair matrix keratinocytes, with or without dysplastic hair shafts, are histological features of the anagen defluxion (24, 27). According to above, vit. C is very important to health and integrity of the skin and coat of the animals and the present study will report the influence of ascorbic acid in the neonate of local dairy calves.

**Materials And Methods**

In this study, 10 neonatal local dairy calves were divided into two groups, each one consisting of 5 animals for vitamin C deficiency. The first group (healthy) served as control group, while the second group included weather-stressed calves between 20-37 days old that were clinically affected with vit C deficiency.

The study was conducted during Oct. 2016 until Dec. 2018 in the Basrah government of southern Iraq. The clinical and physical examinations were made and clinical signs were reported. Blood samples were also collected (according for each case), taking 12 ml of blood from each calf. Each blood sample was then divided into 2 ml EDTA tubes for hematocrit (PCV) and centrifuged through the hematocrit centrifuge methods (nuve® NT 715). Hemoglobin concentrations (Hb) were also assessed using the Sahli's method (MARIENFELD®), Erythrocyte (RBC) levels were counted by manual routine methods (Neubauer improved, hemocytometer. MARIENFELD®), and neutrophils were performed using routinely prepared Giemsa-stained blood films (28, 29, 30).

For serum extraction, 10 ml of blood was collected by EDTA tubes and centrifuged at 1800 rounds for 10 min. for plasma vitamin C estimation using the colorimetric assay kit (Elabscience® by Spectrophotometer 536nm). The biopsy was taken to study the skin lesions, including alopecia, scaling, and crusting. A skin biopsy specimen was obtained after applying local infiltration anesthesia with a subcutaneous infiltration of 4 ml of lidocaine hydrochloride. The specimen was fixed in a 10% buffered formalin solution for histopathological examination. For the prevention of focal dermatitis secondary to skin biopsy, the calf was given a topical spray of tetracycline for 5 days.

STATISTICAL ANALYSIS: All statistical analyses were performed

using SPSS statistical software version 20 (IBM SPSS Statistics 20), using one-way ANOVA to compare the control group to the clinically and laboratory affected group.

**Results**

The definitive diagnosis of vit C deficiency is based on history, physical examination, plasma ascorbic acid concentration, and histopathology findings. The diagnosis was also dependent on the responses to treatment, where affected calves were given 1g/day when hair loss ceased and hairs had regrown within 5 weeks after treatment as shown by figure-4**.**

The physical examinations of affected calves showed that before moderate to severe scaling; alopecia in the thigh, neck, trunk, and pinna; occasional crusting, as seen in Figures-1 and figure-2 while extremities are usually erythematous and purpuric without pain or pruritus, as shown in Figure-1.

Figure 1: A depiction of widespread alopecia, erythema on the thigh with purpura of the skin as in arrows.

Figure 2 Remaining hair around the alopecic areas was plucked easily; crusting and scaling are seen without pruritus (ear pinna).

Table 1 shows the visible and highly significant decrease in plasma vit C levels in the clinically affected dairy calves compared to the control group, where $P < 0.05$. Hematological analysis revealed a significant decrease in RBC count, Hb, and neutrophil, as shown in Table-1, where P value is $P < 0.05$, except PCV, where the statistical analysis revealed $P > 0.05$. The histopathological changes of skin showed orthokeratotic hyperkeratosis, congestion, alopecic of the hair follicles, and mild acanthosis causing the dermatosis, as shown in Figure-3.

**Table 1. Values of plasma vit C levels and some hematology parameters.**

| Parameter | Control calves group | Clinically affected calves group | P value |
|---|---|---|---|
| Plasma vitamin C µg/ml | 4.6 ±0.7 | 0.89± 0.064 | P <0.01 |
| Erythrocyte $x10^6$ | 7.8±1.3 | 4.7± 0.9 | P <0.05 |
| Hemoglobin% | 12 ± 0.3 | 8.2± 0.8 | P <0.05 |
| Packed cell volume | 40± 1.7 | 36 ± 2.9 | P >0.05 |
| Neutrophils | 4200± 1.5 | 2000± 1.9 | P <0.05 |

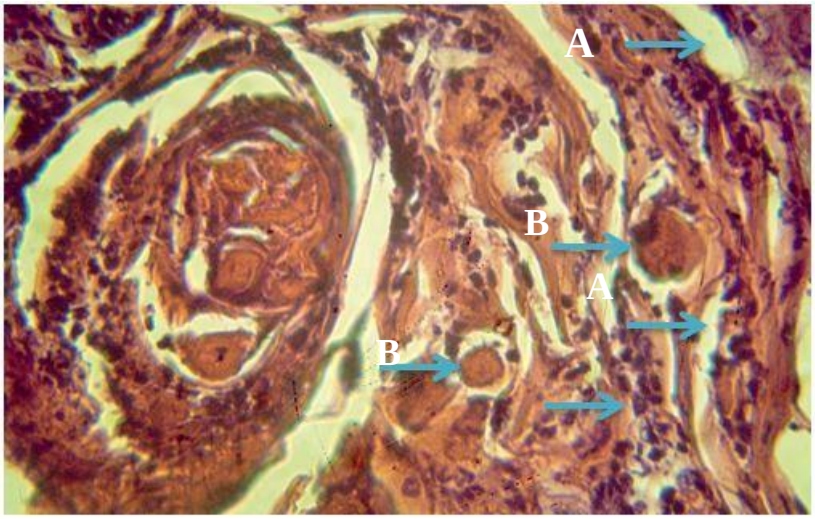


Figure 3. A: Clear hyperkeratosis and B: congestion of the skin of affected calves as explained in arrow.

**A** **B**

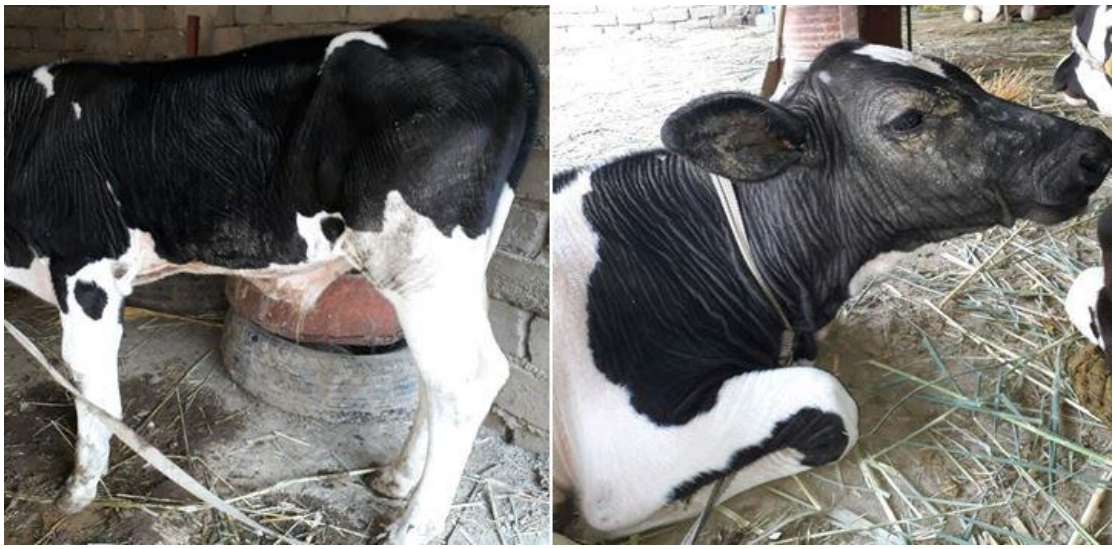

Figure 4: (A and B): Hair loss ceased and hairs had regrown within 5 weeks after treatment with vitamin C.

**Discussion**

The clinical findings in this study correspond to those described by (9, 23, 24, 27, 31). The purpura and skin petechial hemorrhage may be due to the capillary fragility and collagen deficiency as explained by (7, 10, 31, 32).

A hand full of studies reported that vit C has an important role to maintain the skin from collagen deficiency and protects it from the UV and the erythema. Moreover, the deficiency of vit C might lead to skin lesions and hemorrhages in some cases. This might be due to the lack of intracellular substances formation (31, 33, 34, 35). According to our knowledge, the pathogenicity of vit C on dermatosis is not yet known (24, 27).

Table-1 Show that highly significant decrease in plasma vit C levels in the clinically affected calves compared to the control group. This difference could be caused by thermal stress factors of the husbandry, low levels of vit C in dam's milk due to diseases (such as livers disorders) or weather stress, high milk yield, which consumes high levels of glucose (the precursor of ascorbic acid) or mastitis in the dam, as reported by (2,9,10,14,19). Because the neonatal calf chiefly depends on the exogenous source of vit C from the dam's milk during the first 4 months after birth, the low levels of vit C in the dam's milk create a deficiency of vit C in the plasma of neonatal dairy calves, causing many problems for them (2). Hematological analysis revealed significant decreases in RBC count, Hb, PCV, and Neutrophile, as shown in Table-1. These results are in agreement with results found in (31,32,35), who revealed that vit C deficiency impairs the absorption of iron and copper that necessary for erythrocytes and leukocytes formation. However, these results did not agree with those found in (14), who argued that vit C played no role in hematology. In contrast (8, 36, 37) argue that vit C plays an important role and reported that normal levels of vit C lead to well immune activity. While vit C deficiency reduces immunity and leukocyte (neutrophils) function.

The histopathological changes of skin showed that hyperkeratosis and congestion causing the dermatosis, as shown in Figure-3. This result agreed with (23, 24, 25, 27,39, 40) who reported similar results.

***Conclusions:*** Ultimately, this study was concluded that there was a major role between vit C deficiency and skin lesions and dermal changes (dermatosis) in neonatal dairy calves, and more study is needed to understand the connection between vit C and dermatosis. Additional studies are specifically needed in Iraq, especially since vit C deficiency may be caused by thermal weather stress or issues in milk production.

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