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Original

Nutritional muscular dystrophy in broiler thigh muscles: pathological analysis of a problem in the field

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ABSTRACT

Objective. This study aims to use histopathologic method to investigate nutritional muscular dystrophy in broilers due to vitamin E deficiency. **Materials and methods.** Tissue samples taken from 20 dead chicks and total 28 blood samples sent by taking from diseased also eight chicks were analyzed. The amount of vitamin E determined in feed analysis was found to be 5% less than the amount declared to be present in the feed ration. **Results.** The average amount of α-tocopherol in blood serums was measured as 0.285 μg/g. Plasma calcium and phosphorus levels were found to be high, whereas sodium, potassium, and magnesium levels were found to be normal levels. Histopathologically, nutritional muscular dystrophy was defined in 18 of 20 chicks (90%). In the histopathologic examination of muscular sections, varying degrees of hyaline degenerations, necrosis, mineralization, lipidosis, and mononuclear cell infiltrations were observed. **Conclusions.** It was determined that when the fat content of the ration was increased, vitamin and mineral levels, particularly vitamin E, changed within the ration content, and the health of the chicks deteriorated, resulting in histopathologic damages in different organ tissues. The study concludes that the poultry farming industry should attach importance to feed management systems for chick's the proper and healthy feeding.

Keywords: Chick; Histopathology; Muscular dystrophy; Vitamin E deficiency (Sources: MeSH, AIMS).

RESUMEN

Objetivo. Este estudio tiene como objetivo utilizar el método histopatológico para investigar la distrofia muscular nutricional en pollos debido a la carencia de vitamina E. **Materiales y métodos.** Se analizaron muestras de tejido tomadas de 20 polluelos muertos y un total de 28 muestras de sangre tomadas de ocho polluelos enfermos. La cantidad de vitamina E determinada en el análisis de los piensos resultó ser un 5% inferior a la cantidad declarada como presente en la ración. **Resultados.** La cantidad promedio de α-tocoferol en los sueros sanguíneos arrojó un resultado de 0.285 μg/g. Los niveles plasmáticos de calcio y de fósforo resultaron ser elevados mientras que los de sodio, potasio y magnesio, normales. A nivel histopatológico, se encontró una distrofia muscular nutricional en 18

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de 20 polluelos (90%). En el examen histopatológico de las secciones musculares se observaron diversos grados de degeneraciones hialinas, necrosis, mineralización, lipidosis e infiltraciones de células mononucleares. **Conclusiones.** Se determinó que a medida que aumentaba el contenido de grasa de la ración, los niveles de vitaminas y minerales, en particular de vitamina E, cambiaban dentro del alimento y la salud de los polluelos se deterioraba y provocaba daños histopatológicos en diferentes tejidos de los órganos. El estudio concluye que la industria avícola debe dar importancia a los sistemas de control de piensos para que los polluelos reciban una alimentación adecuada y saludable.

Palabras clave: Polluelos; histopatología; distrofia muscular; carencia en vitamina E (*Fuentes: MeSH, AIMS*).

INTRODUCTION

Poultry nutrition is ensured by a sufficient amount of quality, basic, and effective nutritional elements in rations, namely proteins, carbohydrates, fats, vitamins, and minerals. The lack or insufficient amounts of one or more of these nutritional elements in rations may result in the recess of growth, low yield, illness, and even mortality (1,2). Vitamin E or α-tocopherol, which is the active form of vitamin E that dissolves in fat, is required for the proper nutrition of chickens (3,4,5,6). The presence of antioxidants such as vitamin E in the ration may reduce the toxic effects of free radicals (7,8,9,10). Vitamin E eliminates harmful free radicals by protecting polyunsaturated fatty acids present in cellular and subcellular membrane phospholipids, which are the main target of the radicals against lipid peroxidation, ensuring the survival of the cell (11,12). Vitamin E deficiency causes exudative diathesis, muscular dystrophy, and encephalomalacia in chicks, ventricular muscle dystrophy and swollen knees in turkeys, and nutritional myopathy in ducks. Nutritional myopathy occurs as a result of vitamin E deficiency accompanied by sulfur-containing amino acids (methionine and cysteine), and it often concurs with exudative diathesis (13,14). Nutritional muscular dystrophy resulting from vitamin E and selenium deficiency in broilers causes changes that are similar to those caused by white muscle disease in lambs (15,16). Vitamin E plays several significant roles in poultry nutrition. The most effective antioxidant in nature, vitamin E, is essential not only for normal reproduction but also for preventing encephalomalacia (13,17). The cellular effects of vitamin E may be more significant than its antioxidant properties (18,19). Vitamin E changes the ratio and proliferation of T helper cells found in the thymus and spleen, increasing cellular and humoral immunity. In vitamin E deficiency, the phagocytic activities of macrophages decrease (20,21,22). Furthermore, the most significant effect of vitamin E and selenium on cells is tissue regeneration (7). The initial histopathological change as a result of their deficiency is the hyaline degeneration. Extravasation separates muscular fiber groups (13,23,24). Microthrombosis of arterioles and capillaries cause obstruction, leading to degeneration and necrosis of muscular fibers, which are essentially seen as pale lines in breast and thigh muscles (25,26).

Oils are routinely added to chick feed at varying degrees to increase energy concentration. However, the addition of fats (e.g., soybean oil) into the feed increases the absorption of fat-soluble vitamins with minerals such as calcium (27), their over-addition causes a decrease in cholesterol values, as they contain polyunsaturated fatty acids, as well as an increase in lipid peroxidation (12). Consequently, essential fatty acids are destroyed, aldehydes reacting with the free amino groups in proteins reduce the availability of amino acids, and active peroxides formed during oxidation may destroy activities of water-soluble vitamins such as biotin, as well as vitamins A, D, and E (13,28). The accumulation of lipid peroxidation by-products plays a role in toxic degenerations, functional anomalies, and pathologic changes in many tissues (28).

There is a high correlation not only between the amount of α-tocopherol intake through diet and plasma levels but also between α-tocopherol plasma and liver levels. Also, it is reported that α-tocopherol concentration in blood serum or plasma may reflect vitamin E intake in the last feeding (7).

Taking these as a starting point, the objective of this present study was to investigate the nutritional muscular dystrophy occurring due to vitamin and mineral imbalances in the feed as a result of the extra addition of fats to the ration in a poultry business by using the histopathological method. Histopathological findings were supported by biochemical parameters. The study also intends to reveal the distribution of lesions in organs and muscles and the relation mainly Vitamin E deficiency.

MATERIALS AND METHODS

Animals, Samples and Diets. The study was conducted on 20 dead (21-day-old) broiler chicks sent from a private poultry business for a necropsy to Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pathology. In the study, 28 blood samples taken from diseased chicks were also analyzed. Eight of 28 blood samples belonged to the other diseased chicks. Systematic necropsies of all the dead chicks were performed. All procedures in this study were approved by the Animal Ethical Committee of Hatay Mustafa Kemal University (Approval No. 2020/07-8).

In the anamnesis taken, clinical symptoms such as elasticity in the joints of the chicks, swollen knees, difficulty in walking, lethargy, and head leaning forward were mentioned. Regarding the ration, it was stated that extra soybean oil was added to the ration used in animal nutrition. The vitamin values of the feed sent by the poultry business were compared with those determined in analyses. Both values are presented in Table 1.

Table 1. Vitamin levels that should be present in feeds given to chicks subject to this study and the vitamin levels identified in laboratory analyses

Vitamins	RA	AR	%D
Folic acid, mg/Kg	1.000	569	-43.1
Niacin, mg/Kg	25.000	20.000	-18.4
Vitamin A, IU/Kg	5.500.000	7.440.000	35.3
Vitamin B1, mg/Kg	1.000	709	-29.1
Vitamin B12, mg/Kg	9	<0,050	-
Vitamin B2, mg/Kg	4.000	3.990	-0.25
Vitamin B6, mg/Kg	2.000	1.650	-17.5
Vitamin D3, IU/Kg	2.500.000	2.264.000	-9.4
Vitamin E, mg/Kg	30.000	28.500	-5
Vitamin K3, mg/Kg	1.500	1.453	-3.1

RA: Required amount* (per 1 Kg); AR: Analysis result; %D: % Difference
*****Amounts in feed registration

Minerals. To obtain serum, blood samples were centrifuged at 3000 rpm for 10 minutes. Blood serum potassium (K), sodium (Na), calcium (Ca), phosphorus (P), and magnesium (Mg) levels were measured at Hatay Mustafa Kemal University Hospital Central Laboratory using the biochemistry devices (Advia 1800, Siemens, Germany): sodium and potassium were measured using ion-selective electrode (ISE), magnesium using blue xylidine, phosphorus using phosphomolybdate/UV, and calcium using the Arsenazo III method.

HPLC Conditions. To obtain serum, blood samples were centrifuged at 2500 rpm for 10 minutes. Vitamin E (α-tocopherol) levels in blood serums were measured using the HPLC method at the Technology and Research Development Practice and Research Center (Shimadzu, Kyoto, Japan). Determination of α-tocopherol levels was made under the IUPAC standard method 2432 (29). The HPLC method was briefly equipped with the following system: LC-20 AD liquid chromatography, Which is a degasser (DGU-20A3), 30^{o}C column oven (CTO-20A), 20 µl injection loop, Gradient pump (LC- 20AD SP), SCL-10A system controller. Detection was achieved using a diode-array detector (RF-10AXL) at the 292 nm wavelength. α-Tocopherol was successfully separated on the C18 ODS-3 (4.6 mmx250mmx5µm) column (GL Science, Tokyo, Japan) at a flow-rate of 0.7 ml/min, with a mobile phase of Propan-2-ol/hekzan (1:99, v/v).

Histopathologic analysis. Tissue samples taken from internal organs (heart, liver, spleen, lung, kidney, and intestine), brains, and thigh muscles in particular (*m. flexor cruris medialis* and *m. adductor profundus*) were fixed in 10% buffered formaldehyde solution. Sections at a thickness of five microns taken from paraffin blocks and prepared using routine tissue monitoring procedures were stained with Hematoxylin-Eosin (HE) and reviewed under light microscope (Olympus BX50F, Tokyo, Japan).

RESULTS

Feed analysis and biochemical parameters. Vitamin analyses of the feed were performed by a private poultry business. Vitamin values required in the feed content and the actual values measured in the feed are given in Table 1. According to the analysis, the vitamin E content of the feed was 5% less than the required amount, and other vitamin values except for vitamin A were also less than the required amounts (e.g., folic acid -43.1%, vitamin B1 -29.1%). Moreover, according to the analysis results, the vitamin A content of the feed was +35.3% more than the amount required in the feed. In the light of anamnesis and macroscopic findings taken, despite according to deficiency in other vitamins, Vitamin E deficiency, which is of primary importance and causing deaths in chickens was taken into account. Also, macroscopic changes related to other vitamin deficiencies were not found.

The average amount of alpha-tocopherol levels in blood serums measured using the HPLC method was found to be 0.285ug/g. Alpha-tocopherol peak levels could not be measured in the blood serums of two broiler chicks. The alpha-tocopherol peak levels of these two chicks were evaluated at zero.

The average amounts of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), and potassium (K) were found to be 5.6 ± 4.6 mg/dL, 7.5 ± 1.4mg/dL, 1.6 ± 1.3mg/dL, 160.5 ± 25.7mmol/L, and 29.08 ± 13.04mmol/L, respectively. Blood serum mineral levels are given in Figure 1.

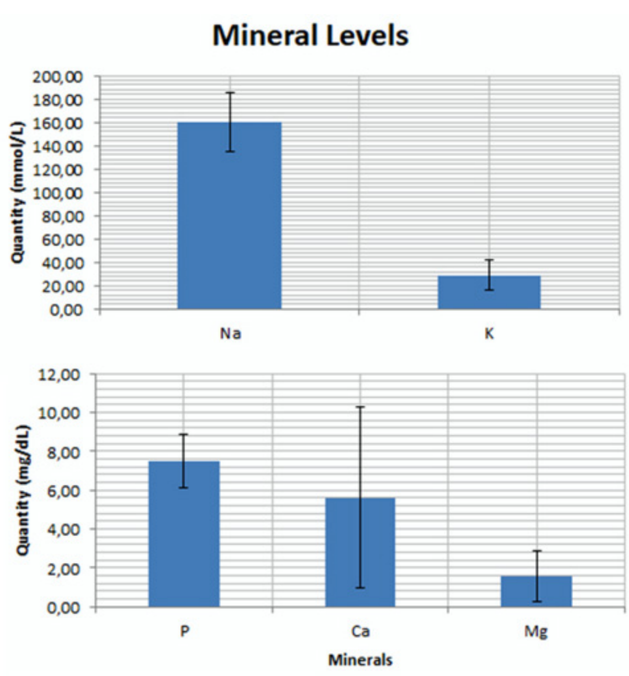

Figure 1. Average and standard errors for mineral levels in blood serums of the chicks in this study.

Pathologic findings. Macroscopic analyses of the chicks revealed acute muscular dystrophy, ventricular necrosis, and edemas which clinically resembled exudative diathesis. Macroscopically, pale yellowish-white areas were observed in the thigh ventral muscles of the chicks that underwent necropsy, particularly in *m. flexor cruris medialis* and *m. adductor profundus* (Figure 2A). Pale areas were seen in the pectoral muscles of a chick. Whitish grey pale areas were observed in livers, lungs were dark red in color, hyperemia was detected in the brain, bleeding of the pericardial sac was observed in five chicks, and the hematoma was observed in the neck region of two chicks. Extracellular fats were determined between muscular fibers.

Histopathologically, hyaline degeneration, swelling, and fragmentation of muscular fibers, striation loss, and hypereosinophilic muscular fibers were observed in ventral muscles of the thigh, particularly in *m. flexor cruris medialis* and *m. adductor profundus* (Figure 2B, C). Muscular fibers were seen to have shrunk and ruptured. Mineralization was identified in some muscular fibers. In addition to a small number of mononuclear cell infiltrations, prominently fat vacuoles were seen within muscular fibers (Figure 3A, B). Hyperemia, mild hyaline degeneration in the pectoral muscles in one of chicks, and pseudoeosinophilic granulocytes within muscular fibers were seen. In four chicks, hyperemia, bleeding, mononuclear cells, pseudoeosinophilic granulocyte infiltration in the heart, fat vacuoles within muscular fibers, and hyaline degeneration in the myocardium were observed (Figure 3C). In the brain, hyperemia, edema, neuronophagia, and focal bleeding areas were observed. In one chick, hyperemia, edema, neuronophagia, gliosis in pons and medulla oblongata, and multifocal bleeding areas were detected (Figure 3D, E). In the cerebellum, hyperemia, edema, mononuclear cell infiltrations, gliosis, and neuronophagia were observed. Focal hemorrhage areas in the substantia alba, hyperemia, and mononuclear cell infiltration in the meninges were observed in one of chicks (Figure 3F).

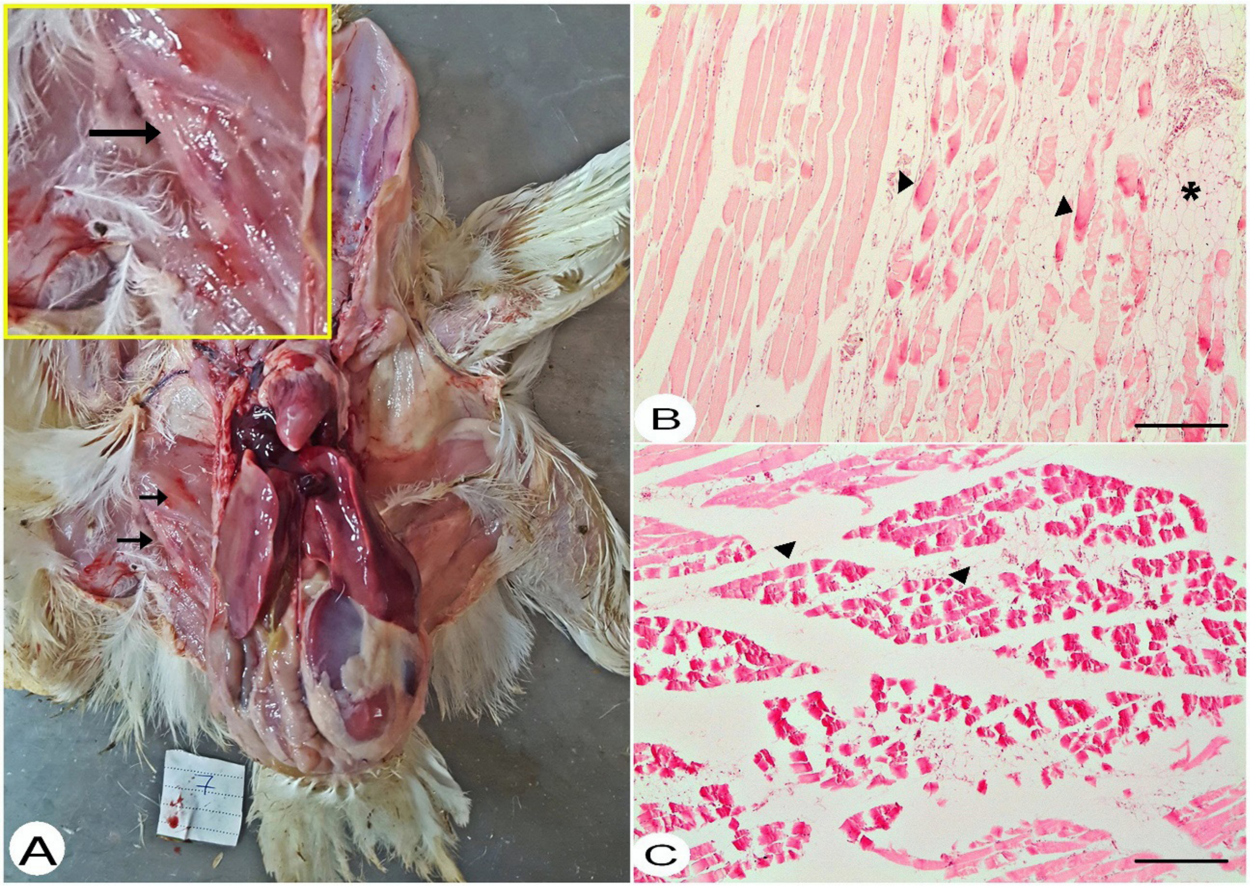

Figure 2. Ventral muscles of thigh **A.** Pale, yellowish white areas (Arrows), **B-C.** Degeneration (arrow heads) and lipidosis (asterisk), HE, bar= 200 µm (B-C).

Severe hyperemia in the lungs and lymphoid hyperplasia was observed in chicks, whereas mild mononuclear cell infiltration in the interstitial tissue, edema, hemorrhage, pseudoeosinophilic granulocytes, and thrombosis was observed in only one chick. Severe congestion in the liver, multifocal mononuclear cells, heterophil granulocyte infiltrations, thrombosis in the portal area, more significant heterophils in the portal area and around the vena centralis, degeneration in hepatocytes around the portal area and necrosis, degeneration and necrosis in bile ducts were observed. Hyperemia, bleeding, edema in kidneys, degeneration, and necrosis in tubules, mononuclear cells, few heterophil granulocytes in the interstitial tissue, mononuclear cell infiltrations in the pelvis, hyaline cylinders, and hyaline droplets were determined in one chick. Degeneration and desquamation in L. epithelialis of intestines, mononuclear cells including dense lymphocytes, macrophage, a small number of heterophil granulocyte infiltrations, and hyperplasia in crypts were observed in the propria. Also, hyperemia, bleeding in the spleen, necrosis in the lymphoid tissue, and heterophil granulocyte infiltrations were seen.

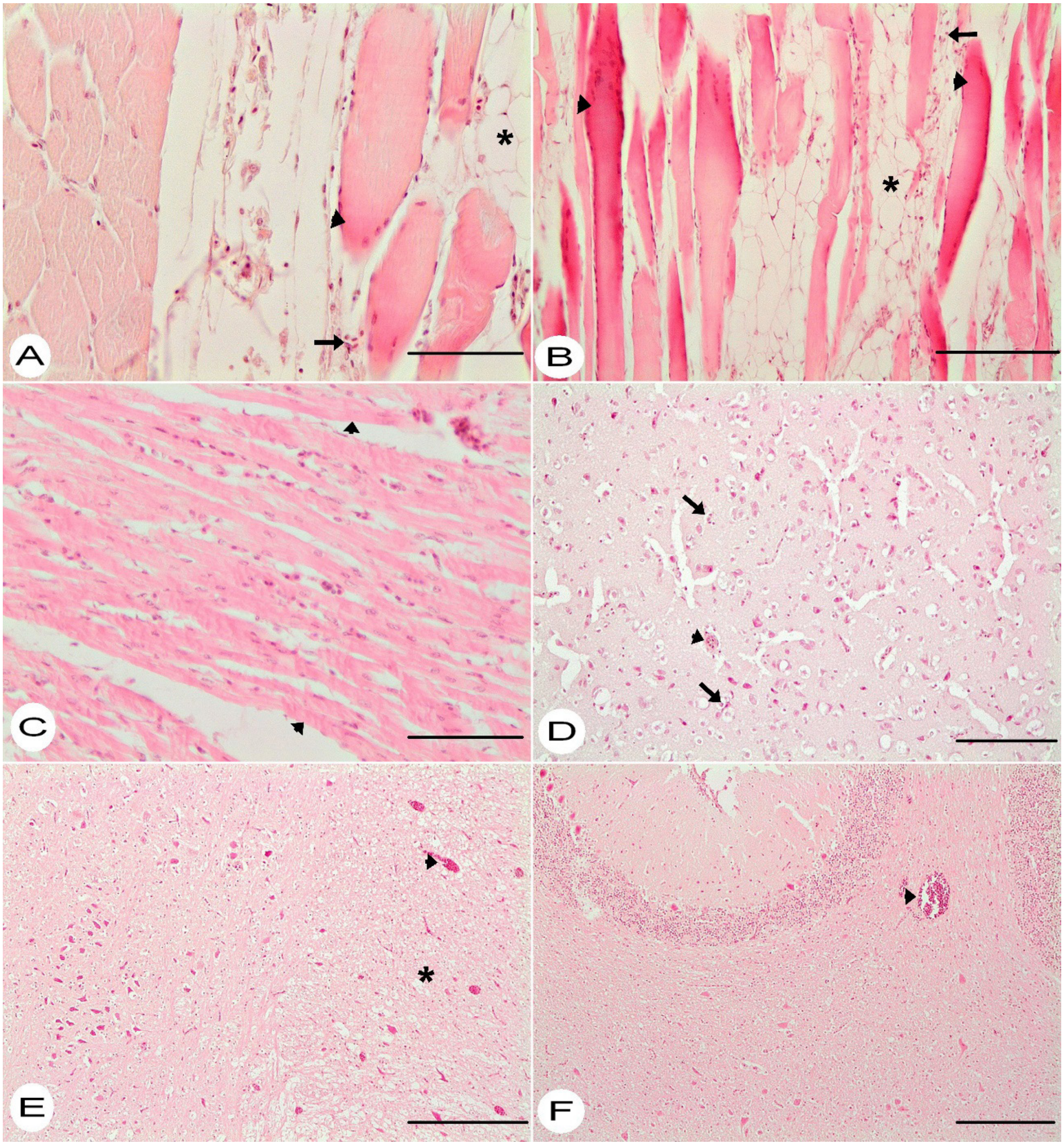

Figure 3. Mineralization in muscular fibers (arrow heads), mononuclear cell infiltrations (arrow) and fat vacuoles (asterisk) in fibers (**A, B**), Hyperemia and hyaline degeneration (arrow heads) in the heart (**C**), Hyperemia (arrow head), edema, neurophagia (arrow) in brain (**D**), Hyperemia (arrow head), edema (asterisk), and gliosis in pons and medulla oblongata (**E**), Hyperemia (arrow head), edema in the cerebellum (**F**). HE, bar= 50 µm (A, C), 100 µm (B, D), 200 µm (E, F).

DISCUSSION

Plant oils and animal fats are used in combination as natural components of poultry feed, but they are also added to increase the energy levels of the feed. In addition to this effect, oils and fats improve the consistency and taste of the feed. Plant-based oils such as soybean oil and rapeseed oil, and animal fats such as cow fat, bone meal, and poultry fat are added to chicken feed (30). It is reported that animal fats rich in saturated fatty acids are more difficult to digest for poultry animals than unsaturated plant-based oils (30). In addition to the physiological stress caused by higher live weights due to high-energy rations, increasing metabolic speed is considered another stress source that increases the need for vitamin E (3). Some researchers have reported that the vitamin E content of the feed should be a minimum of 50-80 mg/kg for healthy broiler chicks under normal circumstances (31). Vitamin E deficiency alone results in lipid peroxidation (11).

However, the over-addition of high-energy soybean oil that contains unsaturated fatty acids to the ration leads to vitamin and mineral imbalances, vitamin E deficiency due to lipid peroxidation, and eventually muscular dystrophy development caused by reduced amino acid levels in tissue due to vitamin E deficiency. Vitamin E and amino acids play an essential role in the protection against this illness. It was found that the poultry business from which the chicks were obtained fed the animals on rations with vitamin E amounts lower than the required amount in this study. Moreover, the addition of extra soybean oil into the ration caused a reduction in fat peroxidation, which, in turn, led to improper functioning among animals. Furthermore, deficiencies of folic acid, niacin, vitamins and minerals such as vitamin B1, B2, B6, D3, and K3, and vitamin E resulting from extra soybean oil addition led to changes in muscular tissues, as expected. Hence, feed analyses indicated that the vitamin E content was 5% lower than the amount reported by the company. The average amount of alphatocopherol in blood serum was identified as 0.285ug/g (Table 2). Considering the serum vitamin E levels in a previous study, plasma vitamin E concentration of 0.5-1 μg/ml was reported to be too low for many animal species, and vitamin E concentration below 0.5 μg/ml was reported as insufficient (7). Accordingly, the plasma vitamin E level identified in this study should be considered insufficient. Likewise, a previous study found a linear correlation between vitamin E levels in the ration and plasma alpha-tocopherol concentration (32). Moreover, although the vitamin E content of fresh grass is high (33), it may significantly decrease in the storage and preservation process (7). According to the feed analysis results in this research, it is believed that deficiencies in other vitamin levels, as well as vitamin E, occur possibly in the storage and preservation process as well as due to the addition of extra soybean oil.

Table 2. Average and standard errors for vitamins and mineral levels in blood serums of the chicks in this study.

Vitamin-Minerals	X ±SD
Na (mmol/L)	160.5 ± 25.7
K (mmol/L)	29.08 ± 13.04
P (mg/dL)	7.5 ± 1.4
Ca (mg/dL)	5.6 ± 4.6
Mg (mg/dL)	1.6 ± 1.3
α**-tokoferol amount (ug/g)**	0.285

Na: Sodium, K: Potassium, P: Phosphorus, Ca: Calcium, Mg: Magnesium

Vitamin E and niacin addition to the feed is reported to prevent swollen knees in the early stages (3). Researcher stated that the swollen knee problem might occur in turkeys despite vitamin E in their diets (34). In this study, niacin deficiencies and vitamin E deficiency in the diet used at the researched farm helped understand the causes of swollen joints detected in the clinically monitored chicks and reported in medical history. On the other hand, significant hypocalcemia and hyperphosphatemia development occur in chicks with vitamin D deficiency (35, 36). Vitamin D and its derivatives increase calcium (Ca) absorption, which may result in increased phosphorus (P) absorption (37, 38). However, despite vitamin D deficiency in the ration subject to this study, plasma Ca and P levels were found to be much higher than the values reported by Kurtoğlu et al (39). Bartholomew et al (40) reported that a plasma Na level of 150.86 mEg/L was sufficient and that correction of selenium deficiency increased plasma Na levels in chickens. In this study, no significant blood serum Na, K, and Mg deficiency was identified. One of the characteristics of vitamin E deficiency is the change in the fat color. The brownish color of fat is reported to be associated with possible ceroid accumulation in many animal species (3). However, in this study, no significant macroscopic color change was observed in the fats of chicks with particularly muscular dystrophy and vitamin E deficiency.

In this study, bleeding in the pericardial sac and hematomas in the neck area of chicks were observed on a macroscopic basis. Exudative diathesis occurred due to increased abnormal permeability of capillary vessel walls resulting from vitamin E deficiency. Ames (3) identified edema in subcutaneous fat tissues as exudative diathesis in respect to abnormal permeability of capillary vessel walls. In this regard, hematomas observed in the pericardial sac and ventral sections of the neck area are considered to be resulting from excessive abnormal permeability of vessel walls in this study.

In the case of vitamin E deficiency, about four-week-old chicks demonstrate symptoms of nutritional myopathy, particularly in breast muscles. Just like excessive myopathy occurring in gizzard and heart muscles of chicks suffering vitamin E and selenium deficiency (13), nutritional muscular dystrophy is identified in ventral muscles of the thigh, particularly in *m. flexor cruris medialis* and *m. adductor profundus* of three-week-old chicks, in the pectoral muscles of one chick, and in the myocardium of four chicks in addition to thigh muscles. Additionally, hyaline degeneration and necrosis in thigh ventral muscles, expansion and fragmentation of muscular fibers, loss of striation and hypereosinophilic, mineralization, a small number of mononuclear cell infiltrations, and significant intensive lipidosis were observed in interstitium within muscular fibers in this study. Similarly, de Brot et al (41) and Kuttappan et al (42) reported increased fibrosis, degenerative myopathic lesions, fat cells, microscopic vacuolar degeneration, moderate mineralization, occasional regeneration, interstitial inflammation, and multifocal edema formations in broiler breast muscles.

Researchers have reported that three-week-old broiler chicks do not have sufficient immunity, and defense against environmental pathogens mostly occurs via maternal antibodies and innate immunity (20,22). In another studies, as an antioxidant, vitamin E was reported to reduce inflammation or pathology occurring due to free radicals under normal metabolic circumstances (43, 44). El-Hak et al (45) reported impacts of vitamin E deficiency on various internal organ tissues (e.g., liver, kidneys) in laboratory and farm animals. In this present study, we believe that infections observed in internal organs (liver, spleen, kidneys, lungs, and intestines) could be due to environmental pathogens since the chicks lacked sufficient immunity, as reported in the study as mentioned earlier, and there was a decrease in their cellular defense activities resulting from vitamin E deficiency.

In conclusion, this study suggests that poultry businesses should have effective feed management systems that include the preparation of rations according to a dietary protocol, proper feed storage processes, and verification of vitamin and mineral levels in rations. Also, this study contributes to the relevant literature by revealing that increasing soybean oil amounts in the feed and the addition of extra amounts have led to a decrease of vitamin E levels in the blood, resulting in the development of pathologic findings. Moreover, the study found that the lack of a feed management system in the poultry business from which the chicks were obtained led to damages in the internal organs of the animals, even animal mortality, due to vitamin and mineral deficiencies in rations. Therefore, based on these findings, we believe more extensive and multidisciplinary experimental and field studies should be conducted to research new and effective feed management systems and their positive contributions to the farming industry.

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Conflict of interest

The authors declare no competing interests.

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