Studies on some antioxidant vitamin concentrations in lambs with stiff-lamb disease

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SUMMARY

A lamb was examined first both clinically and pathologically which was brought from Altındere State farm to the Animal Hospital of the Faculty of Veterinary Science, University of Yüzüncü Yıl. In the histopathological examination, hyaline degeneration and necrosis were seen in muscle fibres. Then, blood samples obtained from twenty two lambs which were from the same farm were used for some antioxidant vitamins concentrations (vitamins E, C and A). Seven lambs (Group 1) showing clinical signs of stiff-lamb disease had significantly ($p<0.05$) lower vitamin E levels compared to the animals in Group 2 (suspected of stiff-lamb disease) and Group 3 (control animals). β-carotene concentration in the lambs showing clinical signs of the disease were also significantly ($p<0.05$) lower compared to the values obtained from control animals. However, vitamin C and retinol concentrations were not significantly different..

Key words: *Stiff-lamb disease, β-caroten, retinol, ascorbic acid, α-tocopherol, lamb.*

Beyaz kas hastalıklı kuzularda bazı antioksidan vitamin düzeyleri üzerine çalışmalar

ÖZET

Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, Hayvan Hastahanesine TİGEM Altındere çiftliğinden daha önce getirilen kuzulardan bir tanesi hem klinik hemde patolojik olarak incelendi. Histopatolojide, kas liflerinde hiyalin dejenerasyonu ve nekroz saptandı. Daha sonra aynı çiftlikteki 22 kuzudan alınan kan örnekleri ise bazı antioksidan vitaminler (vitamin E, C, ve A) yönünden analizleri yapıldı. Beyaz kas hastalığı ile ilgili klinik belirti gösteren 7 kuzuda (Grup 1) serum vitamin E konsantrasyonlarının bayazkas hastalığından şüpheli (Grup 2) ve kontrol (Grup 3) grubu kuzulara kıyasla istatistiki olarak önemli derecede ($p<0.05$) düşük olduğu gözlendi. β- karoten konsantrasyonlarının da, aynı şekilde beyaz kas hastalıklı kuzularda kontrol grubuna göre istatistiki olarak önemli derecede ($p<0.05$) düşük olduğu saptandı. Bununla beraber, bu kuzularda vitamin C ve retinol konsantrasyonlarının istatistiki olarak önemli derecede değişmediği tespit edildi.

Anahtar kelimeler: *Beyaz kas hastalığı, kuzu, β-karoten, retinol, ascorbik asid, α-tokoferol*

INTRODUCTION

Muscular dystrophy is one of the most important problem in growing lambs, kids calves and foals. This disease is most commonly seen in lambs and called as stiff-lamb disease (16). The disease has been reported from all over the world (5,16) and also from Turkey especially from Central, East and South East Anatolia (3).

Several studies have been published on clinical, histopathological and biochemical findings of this disease (3,5,6,7,8, 9,15,16). It is well known that the disease develops as a result of vitamin E and selenium (Se) deficiency. The role of amino-acids containing sulphur, polyansaturated fatty acid, some trace elements and other antioxidants in the development of the disease has also been suggested (3).

Vitamins A, C, and E are all antioxidant vitamins (1,10, 13). These vitamins have also some effects to each others. For instance, vitamin E protects the dissociation of vitamin A which is sensitive to oxygen in the organism (17). So, it can be suggested that vitamin E deficiency may indirectly effect vitamin A status too. Lipid peroxydation damages the microvascular endothelial cells, thereby increasing capillary Permeability. The lipid peroxide in the cell membrane can only be scavenged by vitamin E (2,11,13) producing a vitamin E free-radical complex (4,9). In the extracellular fluid vitamin C acts on this complex and removes the free-radical moiety, regenerating vitamin E (2,13,19).

All these gives an idea that in lambs with stiff-lamb disease which is caused by vitamin E and selenium deficiency, other antioxidant vitamins may also be affected. There were not so many report on this aspect. Thus, in the present study, the status of vitamin C, vitamin A and vitamin E in lambs with stiff-lamb disease were aimed to investigate.

MATERIALS AND METHODS

Materials

Blood samples from 22 lambs which were aged between 1 and 3 months were used as material. Seven lambs out of 22 were unable to stand, trembling of the limbs, moving stiffly with an arched back and frequently became recumbent, weak and had increased heart and respiration rate (Group 1), Seven lambs showing only weakness, stiffness and trembling (Group 2) and eight healthy lambs showing no clinical signs (Control) from Altındere State farm were the materials of the present study. Blood sera were collected and stored at –20°C until the analysis of the antioxidant vitamin concentrations.

Methods

Concentration of vitamin E as α-tocopherol was determined according to the method described by Martinek (12). Vitamin C concentration as ascorbic acid was determined by the method described by Omaye (14) and Vitamin A concentration as retinol and ß-carotene were determined according to the method described by Suzuki and Katoh (18). Statistical analysis was made by Duncan's multiple range test.

RESULTS

Clinical findings

A lamb brought to the Animal Hospital of the University of Yüzüncü Yıl from Altındere State farm was unable to

stand, moving stiffly with an arched back and frequently became recumbent. Trembling of the limbs, weakness, dullness, increased heart and respiration rate were also observed. The lamb was killed for histopathological examination.

Macroscopic and microscopic findings

Macroscopic lesions were only seen in heart and skeletal muscles. The lesions localised in the musculus papillaris and endocardium, which left ventricules had severer damage than the right. The affected muscles were speckle or diffuse pale-grey or white coloured and they were in mild rough constituency. Skeletal muscles of femur and humerus were also bilateral symetrically affected. These areas were in right constituency, lined, ribbon-like and were in cooked meat appearance. Microscopically; similar changes were seen both in skeletal and heart muscles (Figure 1). Hyaline degeneration and necrosis were seen in muscle fibres. These muscle fibres were swallen, dark basophylic or pale coloured and longitudinal and transversal lines were disappeared. Sometimes, multi nucleated giant cells and syncytia developed from degenerated muscle cells were seen. Calsification in the necrotic muscle fibres and mononuclear cell infiltration in the interstitium were also observed.

Then the Altındere State farm was visited. Blood samples from 22 lambs (Groups 1,2 and 3) were taken. Serum samples obtained from these bloods were analysed for the status of some antioxidant vitamins.

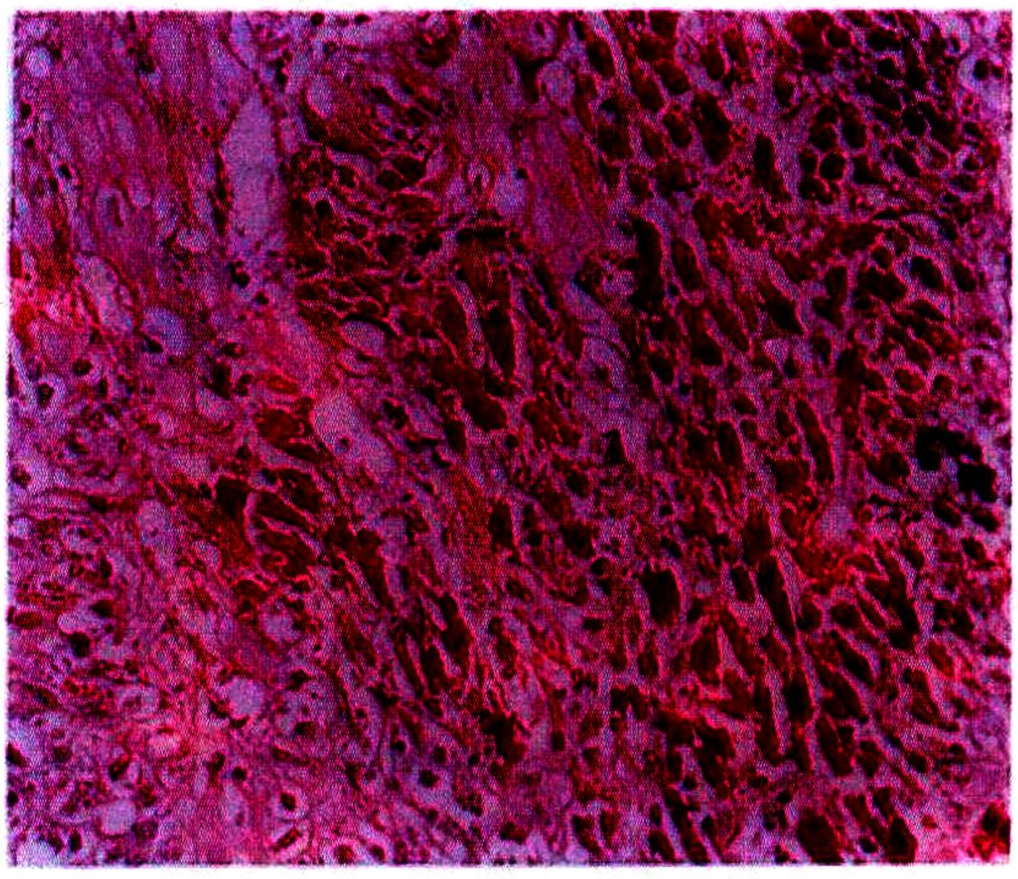

Figure 1: Hyaline degeneration and necrosis in the heart muscle.

Biochemical findings

The results of some antioxidant vitamin concentrations were given in Table 1. When β-carotene levels compared, the values obtained from Group 1 were significantly lower ($p< 0.05$) compared to the values obtained from Group 3. However, the differences were not significant when the values obtained from Group 1 and 2 compared. Retinol and ascorbic acid concentrations were not also significantly different in all groups. When α-tocopherol concentrations compared, the values obtained from Group 1 were significantly lower ($p<0.05$) compared to the values obtained from Group 2 and 3. Differences between Group 2 and 3 were also significant ($p>0.05$).

Table 1. β-carotene, retinol, ascorbic-acid and α-tocopherol c concentrations (mean± se) in lambs with stiff-lamb disease (Group 1), suspected animals (Group 2) and in controls (Group 3).

Vitamins	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=8)
β-carotene (μg/dl)	34.27 ± 7.76^a	43.80 ± 3.15^a	52.55 ± 6.69^b
Retinol (μg/dl)	150.96 ± 10.93	140.27 ± 9.33	142.96 ± 9.03
Ascorbic acid (mg/d)	1.78 ± 0.24	1.43 ± 0.26	1.52 ± 0.58
α-tocopherol (mg/dl)	0.09 ± 0.02^a	0.18 ± 0.01^b	0.32 ± 0.03^c

DISCUSSIONS

Stiff-lamb disease occurs in lambs, borne from dams which have been fed for long periods, usually during the winter months, on diets low in selenium and/or vitamin (16). The lambs used in the present study were also from dams which had been fed indoors during long winter which is seen in the region of Van. Although, selenium status could not be determined in the present study, vitamin E levels were significantly lower in the lambs showing clinical signs of stiff-lamb disease (Group 1) and animals suspected from stiff-lamb disease (Group 2) compared to healthy animals (Group 3). Pathological findings given in the present study also support the presence of stiff-lamb disease in this farm and similar pathological findings have been reported by several workers (3,5, 16).

Vitamin E is important for cell membrane integrity and many functions that occur at the cell membrane level, including drug metabolism, heme biosynthesis, mitocondrial electron transport and neuro-muscular functions (1). It functions in the body as a powerful antioxidant and is the primary defence against potentially harmful oxidations. Vitamin E prevents the oxidation of the unsaturated fatty acids by trapping free radicals. This defence system is aided by two other essential nutrients, selenium and ascorbic acid (13,16,19). There is evidence for ascorbate's involvement in the hydroxylation reactions and protection against lipid peroxidation. It enhances gut absorption of iron and may be involved in heme synthesis. The metabolism of folic acid, amino acid, cyclic nucleotides and glucose involves ascorbic acid. A role in the immune function is also indicated (10). Major dietary source of vitamin C for animals is grass as vitamin E. Because vitamin C is an antioxidant vitamin and it has also some sort of interaction with vitamin E, it's concentration on the blood of the animals with vitamin E deficiency may be affected. However, in the present study, vitamin C status in the lambs with stiff-lamb disease appeared to be unchanged.

Vitamin A serves to maintain the integrity of all epithelial tissues including germinal epithelium which provide protection against infectious organisms, as well as its well known role in vision (5,10,13). Adequate concentration of plasma retinol usually indicates dietary and tissue adequacy, but low concentrations do not always indicate dietary deficiency. Enzymes of small intestinal mucosa convert dietary β-carotene and retinal to the predominant form of vitamin A, retinol (10,13). There is also some indication that some of this process may take place in the liver and kidney (20).

Herbivorous animals obtain their vitamin A supplies from carotene which is present in the green plants. β-carotene is the most important source, with a higher provitamin A activity than the other forms of carotene. Although young, leafy pastures abundant quantities of carotene; old steamy dry grazing contains insufficient to supply the animals requirements. There are further losses of carotene when fodder are processed or preserved. Variation in the digestibility of carotene in plants due to year of harvest, species of the plant, dry matter content, and form of forage have been reported. Apart from the β-carotene supply level, various other factors affect the rate of absorption of carotene and its conversion to vitamin A. Thyroxine has a direct influence on the rate of conversion of carotene to vitamin A. If there is a shortage of iodine in the diet, thyroxine production is reduced and carotene conversion is impaired. An excess or deficiency of phosphorus in the diet can reduce the efficiency of carotene conversion. Nitrates or nitrites in the feed or in the drinking water can seriously effect the conversion of carotene to vitamin A. Chlorinated naphthalenes can prevent the conversion of carotene to vitamin A. The thyroid hormone; thyroxine and adrenal cortex hormone; cortisone have also been reported to play important role for the conversion of β-carotene into vitamin A and regulating the blood vitamin A level respectively. Vitamin A is also necessary for the synthesis of adrenocortical hormones (glucocorticoids, progesterone) (1,9,10,16,20). The reasons for the low level of β-carotene observed in the animals with stiff-lamb disease could be several. One or more reasons given above for the low levels of β-carotene may had taken place in the present study. However, other animals (Group 2 and 3) were living in the same environment, and fed with the same food. If it was so, β-carotene levels should have been low in the other groups too. Furthermore, suggesting a direct effect of vitamin E deficiency on the low levels of β-carotene is also controversial. However, some workers suggest that vitamin E protects the dissociation of vitamin A (17). In the absence of sufficient vitamin E, β-carotene could well be dissociated by oxidation in the lambs with stiff-lamb disease. It is also believed that apart from deficient oxidative vitamins, stress developed in the diseased animals may also have some sort of effect on the level of this vitamin.

In the present study, the status of selenium and glutathione peroxidase activities were not determined. The reason was that because their role in stiff-lamb disease is well known and several workers have been examined it well (7,8,9,15). Another thing is that in the present study, the feed and soil were not analysed in terms of these vitamins and elements. Future studies should include feed and soil samples together with blood samples to get a better picture.

In conclusion, animals showing the signs of stiff-lamb disease, apart from vitamin E and selenium deficiencies, it should be bear in mind that other vitamins especially β-carotene levels may also be affected. Therapy should include these vitamin as well.

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