EFFECTS OF VITAMIN E SUPPLEMENTATION ON MYOFIBRILLAR PROTEINS OF FAT-TAILED AWASSI MALE LAMBS

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ABSTRACT

In this study, the effect of Vitamin E supplementation (for 75 days, 45 mg/day Vit E [DL-α tocopheryl acetate]) on myofibrillar proteins in the M. Longissimus dorsi *of Awassi male lambs was investigated. The lambs were divided into two groups consisting of a control (CG, n = 12) and an experimental (VG,* n = *12) treatment starting at the beginning of the fattening period. Both the control group and the Vitamin E group were fed with a concentrate and grass hay close to* ad libitum *by biweekly adjustment of the amount offered. In addition, the Vitamin E group received the same concentrate plus a supplement of 45 mg of Vitamin E (DL-α tocopheryl acetate) per lamb per day for 75 days. The effects of Vitamin E on myofibrillar proteins in the* M. Longissimus dorsi *were determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gels indicated that Vitamin E supplementation to diet had a significant effect on actin, myosin and tropomyosin. The density of these bands increased in samples from the Vitamin E-supplemented group. Also, the pH values of the* M. Longissimus dorsi *muscles were determined in the present study, and differences between the control and the Vitamin E-supplemented groups were found insignificant.*

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INTRODUCTION

The ability of oxidative compounds to alter muscle protein functionality lends to the logical speculation that antioxidants should be able to prevent protein functionality changes or to improve functionality. Protection of muscle proteins against functional deterioration by antioxidants had been demonstrated (Wan *et al.* 1993; Decker *et al.* 2000; Xiong 2000).

Vitamin E cannot be synthesized by monogastric and ruminant animals. The Vitamin E requirement of these animals must be met by diets that include green plants or commercial vitamin concentrates. It is reported that there is much interest in the use of high levels of Vitamin E for improving meat quality. In addition, animals fed with diets containing high Vitamin E during fattening period store increased quantities of the vitamin in the muscle tissue (Gökalp *et al.* 1996; Aksoy *et al.* 2000). Vitamin E has a positive effect on quality traits of meat and meat products (Bosi *et al.* 2000; Dufrasne *et al.* 2000; Gatellier *et al.* 2001; Macit *et al.* 2001, 2002; Yaprak *et al.* 2002). Macit *et al.* (2001) and Yaprak *et al.* (2002) reported that Vitamin E supplementation had significant effect on meat quality traits of Morkaraman and Awassi lambs. In addition, Aksu *et al.* (2004) reported that Vitamin E supplementation to diets of Awassi lambs had no significant effect on intramuscular or intermuscular fatty acid composition of lamb muscle.

The proportion of myofibrillar proteins in the muscle is higher than those of other proteins, according to findings of Lawrie (1990) and Pearson and Young (1989). In addition, Pearson and Young (1989) reported that these proteins consist of myosin (50%), actin (20%) and tropomyosin (3%). Insufficient Vitamin E increases the activity of dipeptidase enzyme and proteolytic degradation in muscle tissue (Lawrie 1990). Also, Lobley *et al.* (1971) determined structural changes due to Vitamin E insufficiency in the myosin protein. Aksu *et al.* (2003) reported that the effects of dietary Vitamin E on actin and myosin proteins were not significant in Morkaraman lambs fed with a diet that included a supplement of 45 mg Vitamin E per lamb in a day during a 75-day fattening period. No information is available on the effect of Vitamin E supplementation on myofibrillar proteins in Awassi lamb muscles. Thus, the present study was conducted to determine the effect of dietary Vitamin E myofibrillar proteins in the muscle tissue obtained from Awassi lambs fed with diets containing commercial Vitamin E.

MATERIALS AND METHODS

A total of 24 fat-tailed Awassi male lambs were subjected to intensive feeding at the Research and Application Farm of the Agricultural Faculty in

Erzurum, Turkey. The animals were randomly assigned to two treatment groups of 12 animals each, at 8 months of age. During the last 75 days before slaughter, the lambs received the same basic diets. The Vitamin E-supplemented group (VG, $n = 12$) received 45 mg Vitamin E (DL-α-tocopheryl acetate) per head, per day in the form of a premix (Evitse, Topkim, Turkey) added to the concentrate. The control group (CG, $n = 12$) received a similar diet without supplemental Vitamin E in the concentrate. At the end of the fattening period, lambs were randomly selected from each group for slaughter. The concentrate feed was composed of barley (42%), corn (24%), soybean meal (10%), wheat bran (4%), molasses (8%), sunflower meal (8%), premix (0.05%), salt (0.95%) and dicalcium phosphate (3%). It had 91% dry matter, 13.3% crude protein, 10.5% crude fiber, 3.2% ether extract, 55.9% nitrogen free extract and 2.5 MJ ME/kg. Grass hay was fed daily at a level of 340 g and 316 g per lamb during the fattening period for CG and VG groups, respectively.

After slaughter, carcasses were chilled at 4C for 24 h. The properties of myofibrilllar proteins and pH values were determined from chops cut from *M. longissimus dorsi* (LD) muscle.

Isolation of Myofibrillar Proteins in Muscle

Minced meat sample (2.5 g) was homogenized using an Ultra-Turrax (DI 18 Yellow line dispenser, IKA, Brazil Ltd, Taquara, RJ, Brasil) at 6.000–24.000/min, and 25 mL of buffer solution (3C, pH 7.6, 0.05 M sucrose, 0.05 M tris, 1 mM ethylene diamine tetraacetic acid [EDTA]). After centrifugation (1000 × g for 10 min), the supernatant was decanted. Myofibril proteins were resuspended in 25 mL of a buffer solution (pH 7.6, 3C, 0.05 M tris, 1 mM EDTA) and centrifuged at 1000 × g for 10 min. The supernatant was decanted, and the treatment was repeated with 25 mL of KCl solution (3C, 0.15 M KCl). After isolation, the samples were frozen (–40C) then lyophilized (Parrish *et al.* 1973; Claeys *et al.* 1995).

Sample Preparation for SDS-PAGE

Ten-milligram lyophilized myofibrillar protein was dissolved in 2 mL sample buffer (Tris, glycerin, SDS, pH 6.8) and 10 µL 2-mercaptoethanol at room temperature. Sample solutions were then filtered to remove connective tissue (Claeys *et al.* 1995).

Analysis of SDS-PAGE

Characterization of myofibrillar proteins was carried out using SDS-PAGE. A vertical slab gel system (SE 600 Series, Hoefer Scientific Instru-

ments [HSI], San Francisco, CA) was used to prepare a 1.5-mm-thick gel. A 5% stacking gel and 12.5% separating gel were used to separate the protein bands. Electrophoresis was conducted at 80 and 130 volt, respectively. The sample (40 µL) was injected in each sample slot (Laemmli 1971; Aksu *et al.* 2002). The SDS-PAGE profiles of myofibrils were determined visually on the gel and/or by photographing the gel.

pH Analysis

The pH value of the samples was determined with a pH meter (SCHOTT L 6880, Laboratory Star pH, Germany). The pH value was measured using a direct probe by thrusting the probe into the muscle (Ockerman 1985).

RESULTS AND DISCUSSION

Electrophoretic patterns of myofibrillar proteins of control and Vitamin E groups are shown in Fig. 1. The use of Vitamin E in Awassi lamb diets affected actin protein partially. As seen in Fig. 1, the density of the actin bands was lower in the control group (e, f, g and h) than in the Vitamin E group (a, b, c and d). Vitamin E supplementation had a significant effect on myosin bands. As seen from myofibrillar bands, myosin bands were weakened in the control group, as compared to the Vitamin E supplementation group (a, b and

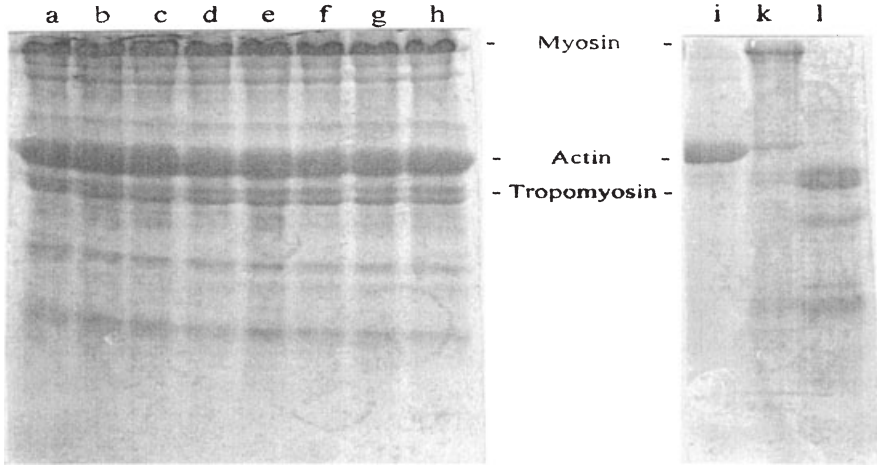


FIG. 1. ELECTROPHORETOGRAM (SDS-PAGE) OF MYOFIBRILLAR PROTEINS IN VITAMIN E AND CONTROL GROUPS
Groups (a), (b), (c) and (d), Vitamin E group; groups (e), (f), (g) and (h), control group; (i), standard actin; (k), standard myosin; (l), standard tropomyosin.

c) except for band d. In the same way, it was found that the density of band e of the control group was higher than those of the other bands in the control group (f, g and h) (Fig. 1). Myosin is the major myofibrillar protein in skeletal and cardiac muscles, making up more than one-third of the total protein in these two tissues. In addition, it has been reported that some changes in structure of myosin can occur because of insufficient Vitamin E in the tissue (Lawrie 1990). Aksu *et al.* (2003) determined that Vitamin E supplementation to the diets in Morkaraman male lambs (75 days, 45 mg/day Vitamin E [DL-α tocopheryl acetate]) had an effect on actin and myosin (SDS-PAGE). Some of the weak actin bands disappeared during the preparation of samples for SDS-PAGE (Fig. 1). It was reported that if myosin is purified and subjected under electrophoresis conditions that denature the protein, the individual polypeptide subunits can be dissociated from each other and visualized after staining. First, the heavy chains of myosin are situated near the top of the polyacrylamide gel because of their size; second, there are three myosin light chain bands near the bottom of the gel (Lowey and Hold 1972; Pearson and Young 1989).

The SDS-PAGE gels indicated that, for tropomyosin bands, significant differences occurred between the control and Vitamin E supplemented groups. The density of tropomyosin bands increased in the Vitamin E-supplemented group (a, b, c and d) compared to that of the control group (e, f, g and h) (Fig. 1). Aksu *et al.* (2003) found similar results in samples from the *M. Longissimus dorsi* muscle of Morkaraman male lambs fed with diets with and without supplemental Vitamin E.

The use of Vitamin E in the diet had no effect on pH value. The pH values of control and Vitamin E groups were 5.85 ± 0.06 (e, f, g and h: 5.92, 5.87, 5.78, 5.84) and 5.84 ± 0.03 (a, b, c and d: 5.82, 5.87, 5.82, 5.83), respectively. These pH values were nearly the same in the control and Vitamin E-supplemented groups, indicating an insignificant effect of pH on changes in proteins in the muscle tissue. Johansson *et al.* (1994) reported that proteolytic activity is blocked because of pH values' effects on structural changes of myofibrillar proteins moving close to isoelectric points. Loss of protein functionality is minimized by inhibition of lipid oxidation. The effectiveness of antioxidants is dependent on pH. A maximal gel strength is produced at pH 6.00. However, antioxidant-washed myofibrils form stronger gels than control myofibrils at $pH > 5.8$ but not at $pH < 5.7$ (Xiong *et al.* 1993; Xiong 2000).

CONCLUSION

In conclusion, the present study showed that Vitamin E supplementation to diets of Awassi lambs had a significant effect on actin protein. In addition,

the effect of dietary Vitamin E on myosin and tropomyosin proteins was relatively significant. But a more detailed larger-scale experiment should be conducted to confirm and clarify the possible role and positive influence that Vitamin E supplementation may have on myofibrillar proteins.

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