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# The Effect of Alfalfa on Retinol, α-Tocopherol and Cholesterol Levels in Muscle and Tail Fat Tissues in Yearling Sheep*

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In this study, the effects of fresh, ensiled or dried alfalfa on retinol, α-tocopherol and cholesterol levels in yearling sheep were investigated. In the study, 40 Akkaraman male yearling sheep, 4 months of age and an average body weight of 21 kg, were used. All diets used in the present study were prepared as isonitrogenous and isoenergetic. The alfalfa forms used in rations composed the experimental groups. With regard to groups were fed with wheat straw as roughage was Control group (C-Group) or fed with fresh alfalfa was AF group, ensiled alfalfa was AS group and dried alfalfa was AD group. Retinol levels in muscular and tail adipose tissue were found to be higher in alfalfa feeding groups compared to control group. The highest α-tocopherol level in *deltoideous muscle* tissue was detected in AF group. Cholesterol levels in muscular and tail adipose tissue were found to be lower in alfalfa feeding groups compared to control group.

As a conclusion it can be said that, decrasing cholesterol levels in yearling sheep by feeding with alfalfa, is extremely important for human beings that consume red meat, as it can reduce the risk of coronary heart diseases.

***Keywords*:** *Alfalfa, cholesterol, retinol, α-tocopherol, yearling sheep, meat.*

### Yoncanın Toklularda Kas ve Kuyruk Yağ Dokularındaki Retinol, A-Tokoferol ve Kolesterol Düzeyleri Üzerine Etkisi

Bu çalışmada, taze, silaj ve kuru yoncanın toklularda retinol, α-tokoferol ve kolesterol düzeyleri üzerine etkisi araştırılmıştır. Bu amaçla, çalışmada yaklaşık 21 kg canlı ağırlıkta, 4 aylık yaşta 40 adet Akkaraman erkek toklu kullanılmıştır. Araştırma rasyonları izonitrojenik ve izokalorik olarak hazırlanmıştır. Rasyonlarda yoncanın kullanılış şekli ise deneme gruplarını oluşturmuştur. Buna göre, rasyona kaba yem olarak buğday samanı ilave edilen grup Kontrol Grubunu (C-Grubu), taze yonca ilave edilen grup AF-Grubunu, yonca silajı ilave edilen grup AS-Grubunu ve kuru yonca ilave edilen grup ise AD-Grubunu oluşturmuştur. Kaslar ve kuyruk yağı dokusundaki retinol düzeyleri kontrol grubuna göre yonca tüketen gruplarda daha yüksek, düzeyde bulunmuştur. *M. Deltoideus* kas dokusundaki en yüksek α-tokoferol düzeyi AF grubunda tespit edilmiştir. Kas ve kuyruk yağı dokularında kolesterol seviyesi, kontrol grubuna göre yonca tüketen gruplarda daha düşük düzeyde tespit edilmiştir.

Sonuç olarak yonca tüketen gruplardaki kolesterol seviyesinin düşmesi bu tokluların etlerini tüketen insanlarda koroner kalp hastalıkları riskini azaltacağı için önemlidir.

***Anahtar sözcükler:*** *Yonca, kolesterol, retinol, α-tokoferol, toklu, et.*

## Introduction

Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. Meat contains 58–64% water, 24–31% protein, 6–14% fat, 1% carbohydrates and less than 0.1% vitamins and minerals. Meat is consumed carefully in terms of the amount of saturated fat and cholesterol (66 mg/100 g). Coronary heart disease (CHD) is the major cause of death in some parts of the world, and saturated fatty acids and cholesterol have been implicated as an important dietary risk factor for CHD (1).

Retinol, the form of vitamin A, is a fat soluble vitamin, which is important in vision and bone growth. Vitamin A is required in the production of rhodopsin (visual pigment used in low light levels) and glycoprotein synthesis. It is also essential for the correct functioning of epithelial cells (2).

Vitamin E is the primary lipid-soluble antioxidant in biological systems, with α-tocopherol being the most biologically active form. Dietary supplementation with vitamin E increases the amount of α-tocopherol deposited in muscle and fat tissues (3). Deposition

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of α-tocopherol in muscle prevents lipid and pigment oxidation since it acts directly on cell membranes (4).

Alfalfa, known as the "Queen of Forages", is the world's most important and widely grown forage legume. Alfalfa is rich in proteins, vitamins (such as vitamin A, $B_1$, $B_6$, C, E and K) and minerals, providing highly nutritious hay and pasture for animals (5, 6). In addition to its high fiber content, alfalfa contains high levels of bioactive antinutritive factors including 2-3% saponins (7), which are steroid or triterpenoid glycosides. Saponins have been shown to have hypocholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant activities (8). The hypocholesterolemic activity of saponins is well documented, with clearly defined molecular mechanisms (9).

The aim of this study was to investigate the effects of fresh, ensiled and dried alfalfa on retinol, α-tocopherol and cholesterol levels in muscle and tail fat tissues of yearling sheep.

## Materials and Methods

**Experimental Design and Diet:** In this study 40 Akkaraman male yearling sheep, 4-months-old, were used following approval of local ethics committee (University of Firat, 18.04.2007 date and 2007/15 decision). All animals used in the study were vaccinated (against foot and mouth disease, enterotoxaemia) and treated against internal and external parasites prior to the experiment. Yearling sheep were allotted in 4 equal groups (n = 10 in each group) according to the diet regimen, and their initial body weights were homogeneous between groups. The control group was fed with a diet of wheat straw whereas the 3 other groups received alfalfa in the fresh form (group AF), silage (group AS) or in a dried form (group AD). Rations were constituted by wheat straw or alfalfa, and their concentrates were designed to be isocaloric and isonitrogenous (Table 1). The experiment was carried out in individual cages using the facilities at the Veterinary Control and Research Institute in Elazig. The yearling sheep were adjusted to experimental feed for 10 days and following 98 days of sampling period. Feedstuffs and water were offered *ad libitum* throughout the study. The animals were fed twice a day, at 8.00 am and 6.00 pm.

At the end of the study, six animals in each group were slaughtered. Following the slaughtering, about 100 g of muscle samples from the *M. semimembranosus* (MSM), *M. gluteobiceps* (MG), *M. longissimus* dorsi (MLD), *M. deltoideus* (MD) muscles and 100 g of tail fat were taken from each animals, and stored -20$^{o}$C until analysis.

**Table 1.** The formulation of diets, (%).

| | Diet regimens | | | |
|---|---|---|---|---|
| Ingredients | Group C | Group AF | Group AS | Group AD |
| Wheat straw | 40.30 | - | - | - |
| Fresh alfalfa | - | 73.00 | - | - |
| Silage alfalfa | - | - | 73.00 | - |
| Dried alfalfa | - | - | - | 72.00 |
| Maize | 25.70 | 23.90 | 23.90 | 21.00 |
| Soybean meal | 21.00 | - | - | 2.00 |
| Wheat bran | 9.00 | - | - | 2.00 |
| Vegetable oil | 2.30 | 2.40 | 2.40 | 2.30 |
| Dicalcium Phosphate | 0.80 | - | - | - |
| Salt | 0.60 | 0.60 | 0.60 | 0.60 |
| Vitamin premix[1] | 0.20 | - | - | - |
| Mineral premix[2] | 0.10 | 0.10 | 0.10 | 0.10 |
| Chemical composition | | | | |
| ME (kcal/g)[3] | 2 460 | 2 500 | 2 500 | 2 500 |
| Crude protein (%) | 15.80 | 16.00 | 15.90 | 15.70 |

[1]per kg including vitamin A 1 200 000 U, vitamin $D_3$ 200 000 U, vitamin E 5 000 mg, vitamin $K_3$ 100 mg, vitamin $B_1$ 100 mg, vitamin $B_2$ 50 mg, vitamin $B_6$ 10 mg, Niacin 500 mg Niacin, Calcium D-Pentotenate 300 mg and vitamin C 100 mg; [2]per kg including Fe: 5 000 mg, Zn: 5 000 mg, Cu: 1 000 mg, I: 200 mg, Co: 50 mg, Se: 30 mg, P: 54 000 mg, Ca: 319 000 mg, NaCl: 100 000 mg, antioxidant: 15 000 mg; [3]Determined by calculation.

**Chemical Analysis:** Crude protein was analyzed according to AOAC (10).

**Analysis of Cholesterol and Vitamin (A, E) amount with HPLC Device:** Cholesterol level was measured by using the method described by Katsanidis and Addis (11). One section of lipid extraction phase which was divided into two sections was put into tubes with caps, and 5% KOH solution was added (KOH solution was prepared in 100% ethanol). After mixing thoroughly, it was kept at 85ºC for 15 minutes. Tubes were cooled at room temperature, 5 mL pure water was added and fluid was vortexed. After phase separation, upper hexane phase was taken and its solvent was evaporated. Then it was solved with nitrogen flow in acetonitryl/methanol mixture (50% + 50%, v/v) taken to autosampler vials, and prepared for analysis.

For the mobile phase, acetonitryl/methanol (60% + 40%, v/v) mixture was used. Mobile phase flow speed was 1 ml/min. A UV detector was used for the analysis at 202 nm wave length. Supelcosil LC 18 (15 x 4.6 cm, 5 µm; Sigma, USA) column was used.

Chromatographic analysis was performed using an analytical scale (15 cm× 0.45 cm I.D.) Supelcosil LC 18 DB column with a particle size 5 µm (Sigma, USA). HPLC conditions were as follows: mobile phase 75:25 (v/v/): acetonitrile: methanol; a flow rate of 1 ml/min; column temperature 30 °C. The detection was performed in UV dedector (Shimadzu, SPD,10$A_{VP}$), 326 nm for retinol, and 215 nm for α-tocopherol and cholesterol.

**Statistical Analysis:** Data were subjected to analysis of variance. Significant differences were further subjected to Duncan's multiple range test of SPSS 11.5 program for Windows (12). Results were considered as significant when p values were less than 0.001.

## Results

Retinol, α-tocopherol and cholesterol levels in muscular and tail fat in research groups were measured and presented in table 2.

**Table 2.** Retinol, alpha-tocopherol and total cholesterol level in muscles and tail fat in yearling sheep (mean±se;n = 10).

| | Group C | Group AF | Group AS | Group AD | P value |
|---|---|---|---|---|---|
| *Retinol (µg/100g)* | | | | | |
| *Semi-membranosus* | 2.50 ± 0.00$^{b}$ | 4.58 ± 0.42$^{a}$ | 2.91 ± 0.42$^{b}$ | 4.58 ± 0.42$^{a}$ | < 0.001 |
| *Longissimus dorsi* | 4.66 ± 0.73 | 5.91 ± 0.58 | 4.08 ± 0.58 | 4.33 ± 0.83 | NS |
| *Deltoideus* | 5.83 ± 0.83$^{b}$ | 11.66 ± 2.10$^{a}$ | 8.33 ± 1.05$^{ab}$ | 10.83 ± 0.83$^{a}$ | < 0.05 |
| *Gluteobiceps* | 2.13 ± 0.32$^{b}$ | 3.05 ± 0.22$^{a}$ | 3.33 ± 0.00$^{a}$ | 3.33 ± 0.00$^{a}$ | < 0.01 |
| *Tail fat* | 5.00 ± 0.00 | 5.00 ± 0.00 | 6.67 ± 1.05 | 5.83 ± 0.83 | NS |
| *Alpha-tocopherol (µg/g)* | | | | | |
| *Semi-membranosus* | 1.45 ± 0.13 | 1.88 ± 0.18 | 1.20 ± 0.28 | 1.23 ± 0.35 | NS |
| *Longissimus dorsi* | 2.91 ± 0.67 | 3.51 ± 0.88 | 2.66 ± 0.64 | 2.41 ± 0.61 | NS |
| *Deltoideus* | 1.32 ± 0.18$^{b}$ | 2.23 ± 0.34$^{a}$ | 1.60 ± 0.23$^{ab}$ | 1.38 ± 0.11$^{b}$ | < 0.05 |
| *Gluteobiceps* | 1.48 ± 0.20 | 2.42 ± 0.42 | 2.11 ± 0.51 | 2.19 ± 0.53 | NS |
| *Tail fat* | 1.22 ± 0.24 | 1.79 ± 0.49 | 1.31 ± 0.40 | 1.14 ± 0.30 | NS |
| *Cholesterol (total) (mg/100g)* | | | | | |
| *Semi-membranosus* | 34.03 ± 0.93$^{a}$ | 28.18 ± 1.72$^{b}$ | 31.01 ± 2.40$^{ab}$ | 28.60 ± 1.56$^{b}$ | < 0.05 |
| *Longissimus dorsi* | 50.17 ± 2.96$^{a}$ | 20.40 ± 3.19$^{b}$ | 27.52 ± 2.14$^{b}$ | 28.84 ± 4.03$^{b}$ | < 0.001 |
| *Deltoideus* | 58.28 ± 3.07$^{a}$ | 46.23 ± 3.95$^{b}$ | 51.76 ± 1.25$^{b}$ | 50.93 ± 2.27$^{b}$ | < 0.05 |
| *Gluteobiceps* | 52.51 ± 1.89$^{a}$ | 30.93 ± 2.87$^{b}$ | 42.63 ± 3.86$^{ab}$ | 46.00 ± 6.25$^{a}$ | < 0.05 |
| *Tail fat* | 69.93 ± 3.02$^{a}$ | 48.90 ± 3.70$^{b}$ | 63.95 ± 5.80$^{a}$ | 69.00 ± 9.38$^{a}$ | < 0.05 |

Different superscripts in the same row indicate significant differences between diet regimens.

## Discussion

Retinol level in *deltoideous* (P<0.05) and *semimembranosus* (P<0.001) muscles was the highest in AF and AD groups. Muscle tissue of gluteobicebs was higher in groups fed with alfalfa compared to control group fed with wheat straw (P<0.01), while *longissimus* muscle and tail fat tissues were no statistically different between the groups (P>0.05). Elevated level of retinol in muscles can be related to the higher contents of alfalfa for beta-carotene. Similarly, Smith (13) found an elevated vitamin A level in grazing animals. Our results also supported this report.

Alpha-tocopherol level in muscle tissue of *deltoideous* was the highest in AF group (P<0.05), while other muscles and fat of tail tissues were no statistically different between the groups (P>0.05). Turner et al., (14) evaluated α-tocopherol accumulation in muscle of lambs finished on pasture or concentrates. *Longissimus muscle* from lambs grazing alfalfa or ryegrass had similar (P>0.05) α-tocopherol concentrations and those

concentrations were similar to values obtained when the concentrate diet supplemented with 150 IU of vitamin E/kg was fed. Our results also supported these report.

Cholesterol levels in muscular and tail adipose tissues were lower in groups fed with alfalfa compared to control group fed with wheat straw. This may be due to the hypocholesterolemic effect of saponin present in the alfalfa. Saponin has lowering effect on the serum cholesterol level in rat (15, 16), rabbit (17), chicken (18) and donkeys (19). Saponins compose insoluble complexes with cholesterol in the digestive system. Therefore, they inhibit the intestinal absorption of endogenous and exogenous cholesterol and the raising of the bile acid and neutral sterols by fecal defecation (17, 20-22). In addition, saponins can affect enterohepatic circulation of bile acids by forming mixed micelles, which directly affect the reabsorption of bile acids from terminal ileum (22). According to the information reported above, it may clearly be seen that feeding with plants containing saponin affects exactly the lipid metabolism of body.

Solomon *et al.*, (23, 24) carried out two separated studies, and in the first study they used 77.23% alfalfa ration, and 45% in the second study for comparison of cholesterol level. As a result, the researchers found cholesterol levels of 64.18 mg/100 g and 74.7 mg/100 g for the first and the second study, respectively.

As a conclusion, the lowest cholesterol level was detected in particularly muscle tissue of groups fed with alfalfa, and the highest levels of α-tocopherol and retinol in some muscle in groups fed with alfalfa were evaluated as remarkable. This is crucial for human beings who consume red meat, since it may decrease the risk of coronary heart diseases.

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