

Cholesterol and fatty acid composition of lamb serum and offal as affected by alfalfa and concentrate

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ABSTRACT

This study was conducted to investigate the effects of fresh, ensiled and dried alfalfa on the fatty acid composition and cholesterol level of the serum, liver, kidney and spleen samples in yearling sheep. Forty Akkaraman lambs, 4 months of age, with an average body weight of 21 kg were used in the study. All diets were formulated to be isonitrogenous and isoenergetic. The lambs were divided into four groups according to feed: wheat straw as roughage (Control group, C); fresh alfalfa as roughage (AF group); ensiled alfalfa (AS group); and dried alfalfa (AD group). The alfalfa group had lower saturated fatty acid (SFA) and higher polyunsaturated fatty acid (PUFA) contents than the wheat straw group. In parallel, SFA concentrations in the serum and offal (liver, kidney and spleen) were significantly higher in the controls, whereas the highest PUFA amounts were recorded in lambs fed with fresh alfalfa. Serum and offal proportions of ω 3 and ω 6 fatty acids significantly increased except in serum ω 3 content of lambs fed with alfalfa. However, the cholesterol level decreased in all groups given alfalfa. In conclusion, the results of this study suggest that alfalfa consumption causes a significant decrease in cholesterol levels and a significant increase in PUFA levels in lambs. Additionally, it may be said that the risk of coronary heart diseases may decrease in humans who consume the offal of lambs fed with alfalfa.

Key words: alfalfa, fatty acid, cholesterol, lamb, serum, offal

Introduction

Liver is recognized as a valuable source of nutrients in human nutrition but there are few reports about the fatty acid composition of ruminant total liver lipids (KINSELLA and BUTLER, 1970; FLANZY et al., 1976; LANZA et al., 1980; HIDIROGLOU et al., 1987). In

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general, lipids in ruminant livers appear to contain more 18:0 (stearic acid) and less 16:0 (palmitic acid) than muscle, and more longer-chain polyunsaturated fatty acids (PUFA) although the results for 18:2 (linoleic acid) are inconsistent.

Numerous studies (BONANOME and GRUNDY, 1988; KANNEL et al., 1971) have confirmed that there is a strong relationship between the lipids consumed in human diet and total plasma cholesterol. Low intake of saturated fat and an increased ratio of polyunsaturated to saturated fatty acid are associated with a low risk of human coronary heart disease (HU et al., 1997; HU et al., 1999). Not only saturated and unsaturated fats are important, but also individual fatty acids (ARAUJO DE VÍZCARRONDO et al., 1998). It has been reported that high concentrations of cholesterol in the human diet lead to raised serum cholesterol level and, in turn, this exposes the consumer to the risk of arteriosclerosis and coronary heart diseases (GRUNDY et al., 1988).

Alfalfa is one of the most important legumes used in agriculture. Sometimes it is called as the “Queen of Forage” and it is grown for hay, pasture, silage and dehydrated meal. Alfalfa can be used for diets in all domestic animal species (CHEEKE, 2005) and it includes high levels of bioactive antinutritive factors such as saponins (SEN et al., 1998). Saponins have been reported to have hypocholesterolemic, anticarcinogenic, anti-inflammatory and antioxidant properties (RAO and GURFINKEL, 2000).

The aim of this study was to investigate the effects of fresh, ensiled and dried alfalfa on fatty acid composition and cholesterol levels in the serum, livers, kidneys and spleens of yearling sheep.

Materials and methods

Experimental design and diet regimens. In this study forty, 4-month-old Akkaraman lambs were used following approval from the local ethics committee. Trivalent foot and mouth disease vaccine [inactivated FMD virus strain A, O and Asia 1 produced by the FMD institute, Ankara-TURKEY, 1 mL] and biovalan enterotoxemia vaccine, including type C and D (Entovac-P, 1 mL) were subcutaneously injected into all animals. Prior to the experiment, a subcutaneous injection of Ivermectin (Ivomec-F) at a dose of 1 mL/50 kg was administered against internal and external parasites. The lambs were then divided into 4 equal groups with 10 animals in each, according to the diet regimen, and the initial body weight was homogeneous between the groups. The control group was fed with a wheat straw diet whereas the 3 other groups received alfalfa in fresh form (group AF), as silage (group AS) or dried (group AD). Rations were constituted of wheat straw or alfalfa and 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, Turkey. The experiment consisted of a 10 day pre-experimental period and 98 day sampling period. Feedstuffs and water were offered

ad libitum throughout the study. The animals were fed twice a day, at 08.00 am and 18.00 pm hours.

Table 1. Diet ingredients and chemical composition (in % of dry matter). Alfalfa was distributed in fresh form (group AF), as silage (group AS) or in dried form (group AD).

Diet regimens				
Ingredients	Control group	AF group	AS group	AD group
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

¹per kg including vitamin A 1 200 000 U, vitamin D₃ 200 000 U, vitamin E 5 000 mg, vitamin K₃ 100 mg, vitamin B₁ 100 mg, vitamin B₂ 50 mg, vitamin B₆ 10 mg, Niacin 500 mg Niacin, Calcium D-Pentotenate 300 mg and vitamin C 100 mg; ²per 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; ³Determined by calculation.

At the end of the study, six animals in each group were slaughtered. Blood samples were collected from the jugular vein before slaughtering and centrifuged for 5 min at 2260 × g to separate the sera. The serum samples were stored at -20 °C until analyzed. Following slaughtering, liver, spleen and kidney tissue was taken from carcass and stored at -20 °C until analysis.

Chemical analysis. The chemical composition of feed ingredients (crude protein) were analyzed according to ANONYMOUS (2000).

Lipid analysis. Lipid extraction from tissue specimens was carried out using the HARA and RADIN (1978) method in which a 3:2 (v/v) hexane isopropanol mixture was used. For this, 1 g tissue specimen was homogenized in 3:2 (v/v) 10 mL hexane-isopropanol mixture for 30 seconds. The tissue homogenate was centrifuged in 2 260 g for 10 minutes and the supernatant fraction was used for the tissue analysis.

Thereafter, 2% methanolate sulphuric acid (5 mL) was added to the lipid extracts in the hexane/isopropanol phase (5 mL). After incubation at 50 °C for 15 hours, which it is necessary for methylation, the mixtures were cooled at room temperature and the reaction was stopped by the addition of 5% sodium chloride (5 mL). After extraction with hexane (5 mL), the hexane phases, containing the produced fatty acid methyl esters, were removed using a pipette and treated with 2% KHCO₃ (5 mL). After evaporation at 45 °C under nitrogen flow, the mixtures were solved with 1 mL hexane, put into 2 mL closed auto sampler vials and analyzed by gas chromatography (CHRISTIE, 1992) (Shimadzu GC 17) using a 25 m long Machery-Nagel (Germany) capillary column with an internal diameter of 0.25 µm and a thickness of Permabond 25 micron film.

During the analysis, the column injection and detector heats were kept at 120-220 °C, 240 °C and 280 °C, respectively. The column heat program was regulated from 120 °C to 220 °C, heat increase was set to 5 °C/min until 200 °C and to 4 °C/min from 200 °C to 220 °C and kept at 220 °C for 8 min. The nitrogen gas was used as a carrier gas, and FID (Flame Ionization Detector) as detector. Before the analysis of the fatty acid methyl esters in samples, the mixtures were injected into standard fatty acid methyl esters and the residence times of each fatty acid were determined.

Analysis of cholesterol level with HPLC device. Cholesterol level was measured using the method described by KATSANIDIS and ADDIS (1999). One section of lipid extraction phase, 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 85 °C for 15 minutes. The tubes were cooled at room temperature, 5 mL pure water was added and the fluid was vortexed. After phase separation, the upper hexane phase was taken and its solvent was evaporated. Then it was solved with nitrogen flow in the acetonitril/methanol mixture (50% + 50%, v/v) put into autosampler vials, and prepared for analysis. The acetonitril/methanol (60% + 40%, v/v) mixture was used for the mobile phase. The mobile phase flow speed was 1 mL/min. A UV detector for analysis at 202 nm wave length Supelcosil LC 18 (15 × 4.6 cm, 5 µm; Sigma, USA) column was used for the column.

Statistical analysis. Data were subjected to analysis of variance, and significant differences were further subjected to Duncan's multiple range test from the SPSS 11.5 program for Windows. The results were considered as significant when p values were less than 0.05, 0.01 and 0.001.

Results

The fatty acid composition of four different diets is presented in Table 2. The highest proportion of saturated fatty acids was found in the wheat straw as forage or concentrates whereas the lowest proportions were observed in fresh alfalfa. However, monounsaturated fatty acids were more abundant in fresh alfalfa and less in the alfalfa hay. All forms of alfalfa (fresh, silage or hay) had larger amounts of polyunsaturated acids when compared to the wheat straw. Although the ω 3 fatty acids (linolenic acid) were mainly concentrated in silage or in hay, the ω 6 fatty acids (linoleic acid) were mainly found in fresh alfalfa and wheat straw.

The fatty acid compositions and cholesterol levels in serum and offal (liver, kidney and spleen) samples are given in Table 3. The SFA proportion in the livers ($P < 0.01$) and kidneys ($P < 0.001$) were found to be higher in lambs fed with wheat straw (controls) than lambs fed with alfalfa. However the lowest serum SFA concentrations were observed in the serum of lambs fed with fresh alfalfa. The serum and offal proportions of ω 3 and ω 6 fatty acids significantly increased in lambs fed with any form of alfalfa, except serum ω 3 content. However, cholesterol levels decreased in all groups given alfalfa.

Table 2. Determination of the fatty acid composition (in forages and concentrates) of the diet regimens expressed in % of DM (Dry Matter). Alfalfa was distributed in a fresh form (group AF), as silage (group AS) or in a dried form (group AD).

Fatty acids	Group C		Group AF		Group AS		Group AD	
	F	C	F	C	F	C	F	C
SFA	40.00	18.75	30.25	18.48	34.40	18.19	36.56	19.46
MUFA	29.70	27.07	30.85	27.21	24.80	27.96	23.50	28.17
PUFA	30.30	54.18	38.90	54.31	40.80	53.82	39.94	52.37
ω 3 (C18:3, n3)	10.15	2.02	15.57	1.42	23.49	1.32	21.18	1.53
ω 6 (C18:2, n6)	20.15	52.16	23.33	52.89	17.31	50.00	18.76	50.48

F: Forage (Wheat straw in controls, fresh alfalfa in the group AF, alfalfa silage in the group AS and alfalfa hay in the AD group); C: concentrates; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Table 3. Fatty acid composition (% of DM (Dry Matter)) and cholesterol levels in serum and offal [liver, kidney and spleen] from lambs fed with wheat straw (controls) or with alfalfa in fresh form (group AF), as silage (group AS) or in dried form (group AD). Results are expressed as means \pm standard deviations.

Fatty acids (%)	Group C	Group AF	Group AS	Group AD	P value
SFA (total)					
Serum	37.00 \pm 0.98 ^a	34.71 \pm 1.05 ^b	37.41 \pm 1.36 ^a	37.57 \pm 1.09 ^a	<0.05
Liver	36.74 \pm 1.00 ^a	32.40 \pm 0.88 ^b	32.67 \pm 0.70 ^b	31.24 \pm 1.04 ^b	<0.01
Kidney	32.50 \pm 1.04 ^a	24.92 \pm 0.36 ^b	24.45 \pm 0.92 ^b	24.85 \pm 0.33 ^b	<0.001
Spleen	33.74 \pm 1.42 ^a	29.76 \pm 0.45 ^b	29.16 \pm 0.86 ^b	32.35 \pm 1.11 ^a	<0.05
MUFA (total)					
Serum	37.67 \pm 1.44	37.81 \pm 1.73	37.61 \pm 2.37	36.77 \pm 2.00	NS
Liver	30.67 \pm 1.20	29.79 \pm 1.98	29.27 \pm 0.76	28.34 \pm 1.38	NS
Kidney	18.75 \pm 2.23 ^{ab}	20.19 \pm 0.23 ^a	17.84 \pm 0.78 ^{ab}	15.91 \pm 0.70 ^b	<0.05
Spleen	32.53 \pm 2.64	32.64 \pm 1.76	30.98 \pm 1.04	32.21 \pm 2.73	NS
PUFA (total)					
Serum	25.33 \pm 0.90 ^b	27.48 \pm 1.67 ^a	24.98 \pm 5.87 ^b	25.66 \pm 3.99 ^b	<0.05
Liver	32.59 \pm 0.58 ^b	37.81 \pm 2.49 ^a	38.06 \pm 0.55 ^a	40.42 \pm 0.99 ^a	<0.01
Kidney	48.75 \pm 2.46 ^c	54.89 \pm 1.21 ^a	57.71 \pm 2.08 ^{ab}	59.24 \pm 1.54 ^a	<0.001
Spleen	33.73 \pm 3.93 ^b	37.60 \pm 1.90 ^a	39.86 \pm 1.83 ^a	35.44 \pm 3.54 ^{ab}	<0.05
ω 3 (total)					
Serum	5.12 \pm 1.23	5.09 \pm 1.12	4.54 \pm 0.93	4.84 \pm 0.87	NS
Liver	6.12 \pm 0.35 ^b	8.09 \pm 1.32 ^b	11.54 \pm 0.50 ^a	12.34 \pm 0.64 ^a	<0.001
Kidney	1.52 \pm 0.11 ^c	5.05 \pm 0.26 ^b	6.21 \pm 0.38 ^a	6.27 \pm 0.34 ^a	<0.01
Spleen	2.95 \pm 0.42 ^c	5.82 \pm 0.13 ^b	5.96 \pm 0.12 ^b	7.16 \pm 0.39 ^a	<0.001
ω 6 (total)					
Serum	19.44 \pm 0.94 ^b	22.34 \pm 1.61 ^a	19.98 \pm 1.16 ^b	20.66 \pm 1.00 ^{ab}	<0.05
Liver	26.07 \pm 0.53 ^b	29.06 \pm 1.08 ^a	26.17 \pm 2.11 ^b	27.61 \pm 0.65 ^{ab}	<0.05
Kidney	46.55 \pm 1.31 ^c	49.77 \pm 0.80 ^{bc}	51.41 \pm 1.83 ^{ab}	52.80 \pm 0.58 ^a	<0.001
Spleen	27.71 \pm 0.53 ^b	30.02 \pm 1.08 ^{ab}	31.37 \pm 2.11 ^a	27.77 \pm 0.65 ^b	<0.05
Cholesterol (total)					
Serum, mmol/L	3.06 \pm 0.17 ^a	2.08 \pm 0.16 ^b	2.71 \pm 0.13 ^a	2.66 \pm 0.14 ^a	<0.01
Liver, mg/100g	386.27 \pm 6.75 ^a	348.80 \pm 7.83 ^b	356.33 \pm 8.84 ^b	358.21 \pm 9.28 ^b	<0.05
Kidney, mg/100g	398.58 \pm 1.16 ^a	349.72 \pm 12.47 ^b	376.71 \pm 10.57 ^{ab}	380.21 \pm 13.27 ^{ab}	<0.05
Spleen, mg/100g	411.27 \pm 7.53 ^a	363.12 \pm 8.47 ^b	373.82 \pm 6.82 ^{ab}	376.51 \pm 7.54 ^{ab}	<0.05

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

Discussion

In the present study, it was observed that feeding lambs with any form of alfalfa (fresh, silage or hay) significantly affected the serum and offal fatty acid composition by decreasing the saturated acid proportion and by increasing the polyunsaturated acids.

SFA level decreased and PUFA level increased in all alfalfa groups (AF, AS and AD) compared with the control group in this study. This might be a result of the higher SFA level and lower PUFA level in straw (fed to the control group) compared with alfalfa (Table 2). In a study reported by ENSER et al. (1998) it was determined that the level of PUFA increased in the liver tissue of ruminants fed with fresh grass. HIDIROGLOU et al. (1987) reported that the use of increased proportions of concentrate in animal feed led to increased SFA levels in cattle livers. The results of the present study are in agreement with these reports. On the other hand, ENSER et al. (1998) found that liver fatty acid content did not differ significantly between grass and concentrate-fed animals, despite the former having significantly higher muscle fatty acid content and fatter carcasses. At the same time, MILLER and RICE (1967) reported that liver and serum fatty acid contents did not differ significantly between roughage and concentrate-fed animals.

Serum and offal cholesterol levels were found to be lower in the groups given alfalfa than in the control group in this study. This may be due to the hypocholesterolemic effect of the saponin present in the alfalfa. Saponin has the effect of lowering serum cholesterol levels in rats (RAO and KENDALL, 1986; SIDHU and OAKENFULL, 1986), rabbits (MALINOW et al., 1981), chickens (MORGAN et al., 1972) and donkeys (MOREHOUSE et al., 1999). Saponins form 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 faecal defecation (JENKINS and ATWAL, 1994; MALINOW et al., 1981; MILGATE and ROBERTS, 1995; OAKENFULL and SIDHU, 1990). In addition, saponins may affect the enterohepatic circulation of bile acids by forming mixed micelles, which directly affect the reabsorption of bile acids from the terminal ileum (OAKENFULL and SIDHU, 1990). According to the information reported above, it may clearly be seen that feeding with plants containing saponin precisely affects the body's lipid metabolism. Our results were supported by the findings of these previous reports.

Conclusions

In conclusion, the results of this study clearly suggest that feeding yearling lambs with alfalfa causes a significant reduction in cholesterol levels and a significant increase in PUFA levels in the serum and offal. In the light of these findings, it may be said that the risk of coronary heart diseases may decrease in humans who consume the offal of lambs fed with alfalfa.

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SAŽETAK

U radu su istraženi učinci svježe, silirane i sušene lucerne na sastav masnih kiselina i razinu kolesterola u serumu te u uzorcima jetre, bubrega i slezene jednogodišnjih ovaca. U istraživanju je korišteno 40 janjadi akaraman pasmine, u dobi od 4 mjeseca, prosječne tjelesne mase od 21 kg. Obroci su bili ujednačeni s obzirom na sadržaj dušika i energije. S obzirom na upotrijebljenu krmu janjad je bila podijeljena u četiri skupine: kontrolna skupina je dobivala pšeničnu slamu, skupina AF je dobivala svježu lucernu, skupina AS siliranu lucernu te skupina AD sušenu lucernu. Lucerna je sadržavala manje zasićenih masnih kiselina (SFA) i više polinezasićenih masnih kiselina (PUFA) u odnosu na pšeničnu slamu. Usporedno, koncentracije SFA u serumu i iznutricama (jetrima, bubregu i slezeni) bile su značajno više u kontrolnoj skupini, dok su najviše koncentracije PUFA zabilježene u janjadi hranjene svježom lucernom. Omjer $\omega 3$ i $\omega 6$ masnih kiselina bio je značajno povišen, osim koncentracije $\omega 3$ u serumu janjadi hranjene lucernom. Međutim, razina kolesterola bila je snižena u svim skupinama hranjenim lucernom. Zaključno, rezultati ovoga istraživanja upućuju da hranidba lucernom dovodi do značajnoga sniženja razine kolesterola i značajnoga povišenja razine PUFA u janjadi. Dodatno bi se moglo reći da je u osoba koje konzumiraju iznutrice janjadi hranjene lucernom smanjen rizik od bolesti srca i krvnih žila.

Ključne riječi: lucerna, masne kiseline, kolesterol, janje, serum, iznutrice
