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Research Article

Fatty acid profile and health lipid indices in the milk of ewes feeding with soybean oil

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Abstract

The aim of this study is to increase conjugated linoleic acid (CLA) and the unsaturated fatty acid content in the total fatty acids in the milk obtained from Akkaraman sheep by adding soybean oil to the ration and to produce a more functional food. In the study, 75 Akkaraman (live weight=63.5±1.02 kg) were used in the early stage of lactation (41±0.5 days at the beginning of the experiment) in a private sheep farm. In this study, three groups were formed balanced and equal according to lactation day, live weight and lactation number. The first group consisted of the control group (C) and the other groups were given 34g/day (SO1) and 63g/day (SO2) soybean oil respectively addition to ration. The study lasted four weeks, two weeks adaptation time and two weeks study time. The CLA ratio was 146% higher in SO1 group than in the control group and 108% higher in the SO2 group than in the control group ($p<0.001$). PUFA ratio had the highest value in SO2 group ($p<0.001$). As the amount of SO in the ration increased, n-6 ratio increased and n-3 ratio decreased. The contribution of SO and time had no significant effect on atherogenic index (AI), thrombogenic index (TI) and h/H. As a result, it was concluded that adding soybean oil to the ration (34g of soybean oil/per ewe a day) increased the CLA ratio in total milk fatty acids. Also adding 34g of soybean oil to the ration was sufficient to increase the CLA rate and decrease the SFA: UFA ratio.

Keywords: Conjugated linoleic acid, Fatty acid, Milk fat, Sheep

Soya Fasulyesi Yağı ile Beslenen Koyunların Sütünde Yağ Asidi Profili ve Bazı Sağlık Lipit Endeksleri

Öz

Çalışmamızın amacı rasyona soya yağı ilave edilerek Akkaraman koyunlarının sütünde toplam yağ asitleri içerisinde CLA oranını ve sütteki doymamış yağ asidi oranını artırarak daha fonksiyonel bir gıda üretilmesini sağlamaktır. Çalışmada, laktasyonun erken döneminde (deneyin başında 41 ± 0.5 günde), 75 baş Akkaraman ırkı koyun (canlı ağırlık = 63,5±1,02 kg) kullanılmıştır. Birinci grup kontrol grubunu oluşturmuş diğer gruplara ise sırası ile rasyona ilave 34g/gün (SO1) ve 63g/gün soya yağı (SO2) verilmiştir. Çalışma iki hafta adaptasyon süresi ve iki hafta da çalışma süresi olmak üzere dört hafta sürmüştür. CLA oranı SO1 grubunda kontrol grubuna göre %146 ve SO2 grubunda kontrol grubuna nazaran %108 fazla orana sahiptir ($p<0,001$). MUFA oranı SO1 grubunda en yüksek değere sahip olmakla birlikte ($p<0,01$) SO2 grubunda kontrol grubu ortalamasının altında bulunmuştur. PUFA oranı ise SO2 grubunda en yüksek değere sahip olmuştur ($p<0,001$). Rasyondaki SO miktarı arttıkça n-6 oranı artarken n-3 oranı düşmüştür ($p<0,001$). Sonuç olarak çalışmamızda rasyona soya yağı katılmasının CLA oranını artırdığı, CLA oranını artırmak ve SFA:UFA oranını düşürmek için rasyona günlük 34g soya yağı katılmasının yeterli olduğu sonucuna varılmıştır.

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Anahtar Kelimeler: Konjuge linoleik asit, Yağ asidi, Süt yağı, Koyun

1. Introduction

Fat, which is one of the most important components of milk, contains many fatty acids. Because of its anticarcinogenic effect, conjugated linoleic acid (CLA), which is found only in ruminant, has come to the fore. In addition, studies in many animal species have shown that CLA reduces skin, stomach, colon, breast, liver cancers. It was stated that it prevents vascular stiffness in rabbits and hamsters. Also, when CLA added to the ration at a rate of 0.05%, it reduces body fat by 60% and has an anti-inflammatory effect (Kritchevsky et al., 2004).

CLA isomers are synthesized with two different ways in meat and milk of ruminants. It is either synthesized by biological hydrogenation of linoleic acid by bacteria or by the conversion of transvacenic acid (C18: 1, t-11) to CLA through Δ^9 desaturase enzyme in udder tissues (Köknaroğlu, 2007; Grinari et al., 2002).

In milk components, only milk fat is manipulated by the ration given to the animal. Energy balance, fat content and fatty acid composition in the ration directly affect the fat content and fatty acid composition in milk. The addition of vegetable oils to the ration affects the fatty acid composition of the milk and is also an energy source for the ration. Fat sources such as flaxseed oil, soybean, safflower and sunflower oil, which have a high content of unsaturated fatty acids, are used to increase the proportional amount of CLA and unsaturated fatty acids in milk. However, the effect of these oil sources varies according to the dose used in the ration.

In order to change the fatty acid composition of milk, the addition of vegetable oil to the ration has been investigated by many researchers. The researchers stated that the addition of sunflower, canola, flaxseed and sunflower oil to the ration increased the amount of CLA compared to the control groups (Zhang et al., 2006a; Zhang et al., 2006b; Maia et al., 2011). Bodas et al. (2010) and Gómez-Cortés et al. (2011) found that the addition of soybean oil to the ration increased the amount of CLA in milk.

Although sheep farming in Turkey is usually done as meat production rather than as milk production, milk sheep farming is very common in Erzincan for the production of Erzincan Tulum cheese from Akkaraman. Akkaraman sheep constitute approximately 45% of Turkey's sheep. The average lactation period is 140-150 days and lactation milk yield is between 60-65 kg. All milk produced from Akkaraman is used in the production of Erzincan Tulum Cheese.

The aim of our study is to increase the CLA and the unsaturated fatty acid content in the total fatty acids in the milk of Akkaraman sheep by adding soybean oil to the ration and to produce a more functional food.

2. Material and Method

In the study, 75 Akkaraman sheep (live weight=63.5±1.02 kg) were used in the early stage of lactation (41±0.5 days at the beginning of the experiment) in a private sheep farm. In this study, three groups were formed balanced and equal according to lactation day, live weight and lactation number. Each group received 1 kg of

concentrated ration and ad libitum wheat straw (particle size> 4 cm) and water daily. Feeding was performed daily at 09.00 and 19.00 hours. The first group consisted of the control group (C) and the other groups were given 34g/day (SO1) and 63g/day (SO2) soybean oil respectively addition to ration. The study was continued for a total of four weeks, with two weeks of adaptation period. Milk samples were collected on days 0, 7, 14, 21 and 28 of the study.

For the extraction of fat from milk, Jiang et al. (1998)'s method was modified as follows. 10 ml milk sample was placed in 50 ml plastic tube. 22.5 ml of isopropanol was added to the tube and shaken vigorously. Subsequently, 12.5 ml of hexane was added to the tube, and the mixture was stirred for an additional 3 minutes. The mixture was centrifuged at 4000 rpm for 5 minutes, the supernatant was transferred to a glass balloon and the subphase was washed twice with 12.5 ml of hexane solvent. Finally, the combined organic phases were dried with sodium sulfate (Na2SO4) and the solvent was removed in the evaporator at 30˚C. For methylation, 0.1 g of oil was taken and put into 15ml tube. 1ml 2N KOH solution (prepared in methanol) was added and vortexed for 2 minutes. Then 10ml hexane was added on it and mixed thoroughly and centrifuged for 10 minutes at 7000rpm. The upper phase is taken and 1 microliter is injected into GC.

Fatty acids were analyzed by Perkin Elmer Clarus 500 Chromatography; FID (flame ionization detector) and Restek (Rtx-2330) capillary column (30 m x 0.25 mm x 0.2 μm) were used. The operating condition of the GC was as follows: helium flow 1 ml/min; flame-ionization detector (FID) at 250 ˚C; split–splitless injector at 250 ˚C. The starting temperature of the oven was 120 ˚C and it was waited at this temperature for 2 minutes and increased by 2 ˚C per minute and reached 180 ˚C. It was increased by 4 ˚C per minute to 200 ˚C and then left to stand for 3 minutes at this temperature.

A mixture of methyl esters of 37 fatty acids was used as the standard in the identification of fatty acids (Food Industry FAME Mix-Restek). The standard of Cayman Chemical was used to determine the CLA. The fatty acid composition was expressed as a percent amount (%) of fatty acid per total fatty acids. Fatty acids could be determined from C8 to C22:6n3 (DHA).

The different groups of FA were defined as follows:

SFA: saturated fatty acids =C14:0+C15:0+C16:0+C18:0+C20:0+C22:0 +C23:0+C24:0; MUFA: monounsaturated fatty acids = C14:1+C15:1+C16:1+C17:1+C18:1;

PUFA: polyunsaturated fatty acids = C18:2n6c+C18:2n6t+C18:3n6+C18:3n3 +CLA+C20:2+C20:3n6+C20:4+C20:5;

UFA: unsaturated fatty acids = PUFA+MUFA;

h/H: hypocholesterolemic/hypercholesterolemic = (C18:1 + PUFA)/(C14:0 + C16:0);

AI: atherogenicity index (AI = (C12:0 + (C14:0 x 4) + C16:0)/UFA);

TI: thrombogenicity index = (12:0 + 16:0 + 18:0)/[(0.5×MUFA)+(0.5×n-6 PUFA)+(3×n-3 PUFA)+(n-3 PUFA/n-6 PUFA)], calculated according to Ulbricht and Southgate (1991) and Voblikova et al. (2020).

The data were analyzed with General Linear Model method in SPSS 17.0 and Duncan multiple comparison test was used for multiple comparisons (Duncan, 1955).

3. Results

The fatty acid composition of soybean oil (SO) and concentrated diet were given in Table 1. In both, the major fatty acid was linoleic acid (C18:2N6C). However, α-linolenic acid (C18:3N3) was about three times higher in soybean oil than concentrated diet. When soybean oil had more MUFA (6,2%) and PUFA (27,1%) than concentrated diet, it had less SFA (49,3%) than concentrated diet.

Table 1. Fatty acid composition of concentrated diet and soybean oil (g/100g fatty acids)

Fatty Acid	Soybean oil	Concentrated diet
C14	0,17	0,56
C16	10,04	21,64
C18	2,47	3,51
C18:1N9C	27,16	25,44
C18:2N6C	53,95	43,70
C18:3N3	3,93	1,41
C20:1	0,28	0,40
C22	0,12	0,30
C20:3n3	0,38	0,74
SFA	12,81	26,01
MUFA	27,45	25,84
PUFA	58,26	45,85

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

The milk fatty acid compositions belonging to the experimental groups were given in Table 2. C8, C10, C12, C18:2N6C ($p<0.001$) C11, C22:1N9 ($p<0.01$), C13, C18:3N6 ($p<0.05$) had the highest ratio (g/100g milk fatty acid) in the SO2 group, while C16:1, C17 , C17:1, C20, C21, C22 ($p<0.001$), C18:3N3 (ALA), C20:3n3, C23, C24:1 ($p<0.01$), C18:1N9C ($p<0.05$) had the lowest ratio (g/100g milk fatty acid). The ratio of C20:5N3 (EPA) and C18:3N3 (ALA) in milk were higher in the control group compared to the experimental groups ($p <0.01$). The addition of soybean oil to the ration had no effect on ratio of C16, C18:2N6T and C22:6n3 (DHA). C18:1N9C and C18:2N6C ratios were 10% and 127% higher in SO2 group than the control group, respectively. The CLA ratio was 146% higher in SO1 group than in the control group and 108% higher in the SO2 group than in the control group ($p<0.001$). The ratio of C18:3N6 was found to be more in SO1 (35%) and in SO2 (108%) than the control group.

Table 2. Fatty acid compositions in milk of the treatment groups (g/100g milk fatty acids)

	Treatment				p		
	C	SO1	SO2	SEM	D	T	DxT
C8	1,256[a]	1,229[a]	1,661[b]	0,198	***	ns	*
C10	3,719[a]	3,698[a]	5,196[b]	0,701	***	ns	*
C11	0,045[a]	0,061[a]	0,122[b]	0,033	**	ns	ns
C12	2,389[a]	2,262[a]	3,161[b]	0,397	***	*	ns
C13	0,077[a]	0,087[a]	0,127[b]	0,022	*	ns	ns
C14	8,309[b]	7,341[a]	8,713[b]	0,576	**	ns	ns
C14:1	0,531	0,483	0,440	0,037	ns	ns	**
C15	0,986	0,950	1,090	0,059	ns	ns	ns
C16	29,199	27,482	27,458	0,815	ns	ns	**
C16:1	1,421[b]	1,110[a]	1,048[a]	0,163	***	***	ns
C17	1,382[b]	1,005[a]	0,828[a]	0,231	***	**	ns
C17:1	0,584[c]	0,373[b]	0,289[a]	0,124	***	**	*
C18	13,344	15,076	12,802	0,970	ns	ns	*
C18:1N9C	31,561[b]	31,367[b]	28,334[a]	1,478	*	ns	**
C18:2N6T	0,242	0,212	0,256	0,018	ns	***	**
C18:2N6C	2,311[a]	3,936[b]	5,257[c]	1,205	***	ns	ns
C18:3N6	0,034[a]	0,046[b]	0,071[c]	0,015	*	ns	ns
C20	0,356[b]	0,308[b]	0,255[a]	0,041	***	ns	*
C18:3N3 (ALA)	0,591[b]	0,440[a]	0,421[a]	0,076	**	***	ns
CLA	0,723[a]	1,769[b]	1,509[b]	0,445	***	***	**
C21	0,050[b]	0,032[a]	0,026[a]	0,010	***	*	**
C20:2	0,127	0,098	0,121	0,013	ns	ns	ns
C20:3N6	0,062	0,065	0,092	0,013	ns	**	ns
C22	0,128[b]	0,096[a]	0,084[a]	0,018	***	*	ns
C22:1N9	0,235[a]	0,214[a]	0,282[b]	0,029	**	ns	ns
C20:3n3	0,111[b]	0,066[a]	0,064[a]	0,022	**	***	ns
C23	0,040[b]	0,027[a]	0,021[a]	0,008	**	ns	**
C20:5N3 (EPA)	0,081[b]	0,060[a]	0,066[a]	0,009	**	***	**
C24	0,016	0,022	0,020	0,002	ns	**	ns
C24:1	0,045[b]	0,032[a]	0,026[a]	0,008	**	ns	**
C22:6n3 (DHA)	0,030	0,035	0,022	0,006	ns	**	*

C: Control group, SO1: 34g/day soybean oil, SO2: 63g/day soybean oil, SEM: Standart error, D: Group of diet, T: Time

Although MUFA ratio had the highest value in SO1 group ($p<0.01$), it was found to be below the mean of SO2 group in the control group (Table 3.). PUFA ratio had the highest value in SO2 group ($p <0.001$). The addition of SO to the ration and diet time had no statistical effect on SFA and UFA ratios. As the amount of SO in the ration increased, n-6 ratio increased and n-3 ratio decreased ($p<0.001$). n-6 ratio increased by 114% in SO2 group compared to control group. n-6/n-3 ratio was found to be three times higher in SO2 group compared to the control group

($p<0.001$). The contribution of SO and time had no significant effect on AI, TI and h/H.

Table 3. Some total fatty acid compositions and health lipid indices in milk of the treatment groups

	Treatment				p		
	C	SO1	SO2	SEM	D	T	DxT
SFA	61,295	59,677	61,565	0,834	ns	ns	**
MUFA	35,100^{b}	35,349^{b}	31,929^{a}	1,557	**	ns	**
PUFA	3,589^{a}	4,958^{b}	6,370^{c}	1,135	***	ns	ns
UFA	38,689	40,308	38,298	0,870	ns	ns	**
n-6	2,650^{a}	4,259^{b}	5,676^{b}	1,236	***	ns	ns
n-3	0,812^{b}	0,601^{a}	0,573^{a}	0,107	***	**	ns
n-6/n-3	3,434^{a}	8,567^{b}	10,355^{c}	2,934	***	**	*
AI	1,790	1,503	1,754	0,127	ns	ns	**
SFA:UFA	1,678	1,508	1,645	0,074	ns	ns	**
TI	2,173	2,101	2,148	0,030	ns	ns	**
h/H	0,972	1,072	0,984	0,045	ns	ns	*

C: Control group, SO1: 34g/day soybean oil, SO2: 63g/day soybean oil, SEM: Standart error, D: Group of diet, T: Time, SFA: Saturated fatty acid, MUFA: Mono unsaturated fatty acid, PUFA: Polyunsaturated fatty acid, UFA: unsaturated fatty acid, Aİ: atherogenic index, TI: thrombogenic index

4. Discussion and Conclusion

The addition of fat to the ration increases the energy content of the ration and affects the fatty acid composition of the milk, especially when the sheep are away from fresh green plants. Nudda et al. (2014) reported a positive correlation (R^2=0.78) between the CLA ratio in milk and the richness of the ration in terms of linoleic and α-linolenic acid. In our study, higher ratio of CLA were detected in SO1 and SO2 groups compared to the control group, probably due to the high ratio of α-linolenic acid in soybean oil. This suggests that a large part of the CLA was synthesized not the result of biohydrogenation in rumen, but the result of delta-9-desaturase activity in udder tissue. This idea confirms results of Lock and Garnsworthy (2002) in cow and Gómez-Cortés et al. (2009) in sheep. Bodas et al. (2010) in their study with the addition of Palm, olive oil, linseed and soybean oil, they reported that soybean oil increased the CLA ratio in milk more than other oils. In addition, the result of SO contribution, the increase of C18:2N6C ratio in milk and the decrease of C18:3N3 (ALA) ratio in milk are compatible to the result of Gómez-Cortés et al. (2009).

If we look at the effect of milk dietary fat on human health as a result of the addition of SO, the increase in PUFA and CLA in milk fat is similar to that of Castro et al. (2009) and Gómez-Cortés et al. (2011) (except PUFA). High levels of unsaturated fatty acids in soybean oil are thought to have an effect on this result. Contrary to the results of this study, Cieslak et al. (2010) reported that rapeseed oil

reduces n-6 and flaxseed oil both n-3 and n-6. The World Health Organization (WHO) recommends that the n-6 / n-3 ratio be between 5: 1 and 10: 1. This ratio is recommended to be 4: 1 to prevent cardiovascular diseases. furthermore, as the ratio of n-6 / n-3 decreases; It is known that there are a decrease in intravenous inflammation and disease risk factors (Konukoglu 2008). In our study, it was determined that the addition of soybean oil increased ratio of n-6 / n-3. Mierlita (2016), on the other hand, reported that grazing sheep in pasture decreased n-6 / n-3 ratio compared to indoor feeding. It is considered to be proatrogenic as it constitutes a large part of milk fat, saturated fatty acids (mostly lauric, myristic and palmitic acid) (Valeille et al. 2005). It is considered to be proatrogenic as it constitutes a large part of milk fat, saturated fatty acids (mostly lauric, myristic and palmitic acid) (Valeille et al. 2005). While adding soybean oil to the ration is expected to decrease the atherogenic index, we did not find any effect like Castro et al. (2009). Contrary to these study results, it has been reported that soybean oil Gómez-Cortés et al. (2011) and flaxseed oil Cieslak et al. (2010) reduce the atherogenic index.

As a result, it was concluded that adding soybean oil to the ration (34g of soybean oil/per ewe a day) increased the CLA ratio in total milk fatty acids. Also adding 34g of soybean oil to the ration was enough to increase the CLA rate and decrease the SFA: UFA ratio. However, in our study, the fact that soybean oil was not effective on important health parameters such as AI and TI made it necessary to carry out new studies.

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