

## Article

# Comparative Analysis of Fatty Acid Profile and Fat-Soluble Vitamin Content in Sheep and Goat Milk of Organic and Conventional Origin

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**Abstract:** Consumers tend to perceive organic foods as more nutritious and safer in comparison to conventional, due to the perception that organic food is eco- and animal-friendly. Since fatty acids and fat-soluble vitamins in dairy produce are important nutrients, this study aims to assess possible differences in the amount of these components included in organic and conventional ewes and goat raw milk in Greece, while identifying the potential determining factors. A total of 48 samples were collected from organic and conventional sheep and goat farms in Greece (Thessaly) during the lactation period. A standardized questionnaire, including information about the farming system, and a sampling protocol were completed. Samples were studied for the fat-soluble vitamins (all-trans-retinol and  $\alpha$ -tocopherol) and the fatty acids profile, while the outcomes were statistically analyzed to explore any associations with the questionnaire data for potential factors affecting the results. According to the obtained results there are slight differences in milk fatty acid content, which indicates that organic and conventional sheep and goat milk in Greece do not have any substantial differences in terms of feeding strategies. Regarding the content of fat-soluble vitamins, the organic milk is generally found to contain a higher amount than the conventional one.

**Keywords:** organic; conventional; sheep; goat; milk; fatty acids content; fat-soluble vitamins

## 1. Introduction

Over the past few years, the demand by the consumers for organically produced food has been increasing. This growth can be attributed to two major factors: health aspects for the consumers and environmental concerns [1,2]. In addition, there are ethical issues considering that organic farming, based on the physical welfare of animals, using only organic feed, minimizes possible use of chemically synthesized allopathic medicinal products, promotes the biodiversity, protects the environment, is contrary to the genetic modification, and is distinguished for its high quality and the safety of products [3,4].

Recently, after relevant research, the scientific community has become interested in milk fat, as it has been proven to contain ingredients such as conjugated linoleic acid (CLA), unsaturated fatty acids, and polyunsaturated fatty acids (PUFA), especially those of the n-3 group, which have beneficial effects on human health, on the cardiovascular system and in prevention of cancer [5]. Linoleic acid (LA) and  $\alpha$ -linolenic acid (ALA) are essential fatty acids, as they cannot be synthesized by the human body [6], and they are the major n-6 and

n-3 fatty acids in milk. Moreover, numerous biological effects have been reported for CLAs, and several studies indicate that ALA has cardioprotective effects [7].

Furthermore, fat milk is a good source of fat-soluble nutritional vitamins, especially A (all-trans-retinol) and E ( $\alpha$ -tocopherol), which are known for their antioxidant activity [8–12]. The levels of the fat-soluble vitamins in milk are highly dependent on the type and the amount consumed in the feed. Higher concentrations of these vitamins are found in grass, legumes, and other green plants [13,14]. Vitamin A and E concentrations of milk follow a seasonal trend with higher values to be achieved during the outdoor grazing period [15–17].

The species and animal breed are some of the main factors affecting the production of milk fatty acids [18–21]. Furthermore, the levels of small- and medium-chain fatty acids are significantly higher in sheep and goat milk in relation to the cow [22]. Nutrition is one of the most important factors affecting the concentration of fatty acids in milk, and primarily that of the CLA, both in organic and conventional farms. Toledo and his associates [23] argued that composition of the milk and thereby its beneficial properties are largely determined by the food figure followed. Therefore, the effect of nutrition on milk components has been a subject of study for many researchers in various countries around the world [24–27]. In Denmark for example, conventional farms, compared to organic ones, use different pasture [28], while in Sweden the same diet is followed by both farming systems [23]. Considered that nutrition largely affects the concentration and the profile of fatty acids in milk, the direct comparison of conventional and organic systems is not easy. So far, studies in Greece [29] report that even though a statistically significant difference is noted in the fat content of goat milk, with organic milk to show increased levels, this cannot be evaluated, as when confounders are tested, no differences are observed. The latter is attributed to the fact that organic and conventional goat farming in Greece do not have any substantial difference in terms of feeding strategies. The feeding system in Greece is based on seasonal natural grazing and on supplementary feeding of home-grown roughages and concentrates. Differences that have been previously identified between organic and conventional sheep and goat farms in Greece are related mostly to the use of antibiotics that are associated consequently with different microbiological profiles of the milk produced but not with the feeding strategies [30].

With reference to CLA, which has beneficial effects in various disease conditions such as diabetes, obesity, cancer, and atherosclerosis, studies are showing that the stage of the lactation period affects, by a small percentage, the production of CLA in milk fat ewes [18]. This was also observed in Signorelli et al. [31], who argued that the CLA had maximum values at the end of the lactation period of sheep. Fatty acids, according to Urso et al. [32], were affected by grazing and the seasonality. The research in sheep, goats, and cows have concluded that fresh grazing increases CLA, oleic acid, and monounsaturated fatty acids (MUFA), while reducing the saturated ones in the fat of milk [33,34]. The grazing at early spring (young vegetative stage) contains linolenic acid, more than 40% of the total fatty acids, while at the end of spring (mature vegetative stage), the linolenic acid decreases. Sheep that consumed by grazing in young vegetative stage showed higher CLA concentrations in milk fat from when eating in pasture, in mature vegetative stage [33,35,36]. The importance of pasture was also pointed out in a study, where they observed the lowest concentration of CLA in fat cows from Nordic countries, in relation to European countries [37].

Most research so far on this topic is focused on cow's milk, yet sheep and goat milk are of special interest for Mediterranean countries such as Greece. Sheep and goat farming has been the main pastoral activity in Greece since ancient times and has survived until today as it is associated with products having deep cultural roots and characterized as designated origin. The main objective of this study was to compare the fatty acid profile and the fat-soluble vitamin (A and E) content of milk produced from organic and conventional dairy sheep and goat farms in Greece, taking under consideration the geographical vicinity, seasonality, feeding scheme, and other farming practices.

## 2. Materials and Methods

### 2.1. Area Description—Study Population

The study was carried out in the region of Thessaly, Central Greece in a total of 16 sheep and goat farms. The specific study area was selected as it is a predominantly rural area with substantial animal population, shows diversity in farming systems (conventional/organic), produces significant quantities of dairy produce, and also comprises various landscapes and climatic conditions. For comparison purposes, eight organic (four sheep and four goat) and eight conventional (four sheep and four goat) farms were selected, to be comparable in terms of number of animals kept, breeds, feeding system, productivity, milking method, and frequency. During the onsite visit, a valid certificate was required to assure the organic farm status.

### 2.2. Questionnaire

A standardized questionnaire was initially completed for each participating farm, during the first visit, to collect data with reference to the farming system, which would be analyzed as potential associated factors. Questions were categorized in the following sections: farm descriptive characteristics (number of animals, species, breeds), nutrition profile (type of feed, feed supply, feed storage), farming system (organic/conventional), premises profile, and milk production characteristics.

### 2.3. Sample Collection

All 16 farms were visited in three regular intervals (winter, spring, and summer) during lactation period, and 500 mL of bulk tank milk were collected in sterile glass bottles. A total of 48 samples were collected. A standardized sampling protocol was completed for all samples collected, to assure sample traceability and suitability: pH and temperature were recorded.

### 2.4. Sample Analysis

#### 2.4.1. Fatty Acid (FA) Profile Determination

The total fat content was determined using a milk analyzer based on Fourier transformed infrared (FTIR) technology (MILCOSCAN-FT1-FOSS, Hillerød, Denmark). The FA profile was determined in all milk samples according to Tsiplakou et al. [38] through the preparation of fatty acid methyl esters. Briefly, 8.5 mL of milk and 15 mL isopropanol were shaken into a separating funnel and then 11.25 mL hexane (three times) was added, and the mixture was shaken again. The hexane layer (20 mL) was separated after centrifugation ( $2520 \times g$  for 5 min at 5 °C) and it was evaporated after the addition of 7.5 mL 0.47 M aqueous  $\text{Na}_2\text{SO}_4$ . To 100 mg lipid were added 5 mL hexane. The mixture was vortexed and 0.2 mL of a mixture of 11.2 g KOH in 100 mL of methanol was added, then it was vortexed again and allowed to react for 5 min. Then, 0.5 g  $\text{NaHSO}_4 \cdot \text{H}_2\text{O}$  was added and the mixture was centrifuged for 5 min ( $5000 \times g$  at 25 °C). The supernatant layer was analyzed by gas chromatography using an Agilent 5975 C GC gas chromatograph (Agilent Technologies, Centerville Road, Wilmington, DE, USA) equipped with a Supelco SP Omegawax 320 capillary column (30 m  $\times$  0.32 mm i.d. with 0.25  $\mu\text{m}$  film thickness, and a flame ionization detector where helium was used as a carrier gas and injector temperature was at 240 °C. The sample split mode was 1/5. The initial column temperature was 70 °C for 5 min and then increased at a rate of 20 °C/min to a maximum temperature of 160 °C, where it remained for 3 min. Then, the temperature increased at a rate of 5 °C/min to a maximum temperature of 240 °C. The identification of the compounds was conducted using Sigma FAME standards with known concentrations. All samples were analyzed in triplicate.

#### 2.4.2. Fat Soluble Vitamins Determination

The fat-soluble vitamins were determined according to Kondyli et al. [39]. Briefly, 2 mL of milk with 5 mL absolute ethanol containing 0.1% (*w/v*) ascorbic acid and 2 mL of

50% (*w/v*) KOH were shaken and allowed to react at 80 °C for 20 min. Then the sample was allowed to cool, and it was mixed with 20 mL of petroleum ether: diethylether mixture (1:1), containing 0.01% butylated hydroxy toluene (BHT). The mixture was vortexed, 15 mL of water (2 °C) was added, and it was centrifuged for 15 min (2000× *g*). The organic layer was separated and evaporated.

The analysis for the vitamin E [40] was carried out on a Shimadzu CBM-20A liquid chromatograph equipped with an SIL-20AC auto-sampler and a CTO-20AC column oven. Detection was carried out using a Shimadzu RF-10AXL fluorescence detector set at 278 nm (excitation) and 339 nm (emission). The column used was a Waters I-Porasil, (125 Å, 10 µm, 3.9 × 300 mm; Waters Corp., Waltham, MA, USA). A total of 1 g of lipid was dissolved in 5 mL n-hexane before injection into the HPLC, then 2-propanol:n-hexane:absolute ethanol (2:97.5:0.5) at 1 mL/min was used as mobile phase.

The analysis for the vitamin A was determined with RP-HPLC-UV-VIS according to Mendoza et al. [41]. The analysis was carried out on a Shimadzu CBM-20A liquid chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) equipped with a Shimadzu SIL-20AC auto sampler and a Shimadzu CTO-20AC column oven (set at 300 °C). The detection was carried out using a Shimadzu SPDM20A diode array detector set at 325 nm. The column used was a Luna C18, 250 × 4.6 mm, 5 µm (Phenomenex, Torrance, CA, USA). The mobile phase was 100% methanol at a flow rate of 1 mL/min.

All samples were analyzed in triplicate.

## 2.5. Statistical Analysis

A database for the information collected from the sampling protocols and the questionnaires was created and analyzed using the statistical package SPSS v.20. Quantitative variables are presented as mean values with standard deviation. Qualitative variables are given as absolute frequencies with percentages. In univariate analysis, Chi-square test or Fischer's exact test was used to investigate associations between variables. A *p*-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Farms and Samples Descriptive Characteristics

Samples were collected from eight goat and eight sheep farms located in Thessaly. The farms' descriptive data, retrieved from the questionnaires completed, are summarized in Table 1. In total, 48 samples (24 goat's milk and 24 ewe's milk) were collected in three regular intervals (winter, spring, and summer) during lactation period.

**Table 1.** Farms' descriptive characteristics.

Characteristic		Frequency	%
Type	Organic	8	50.00
	Conventional	8	50.00
Prefecture	Larisa	9	56.25
	Magnessia	7	43.75
Farm area	Lowland	3	18.75
	Semimountainous	11	68.75
	Highland	2	12.50
Species	Goats	8	50.00
	Sheep	8	50.00
Breeds	Native	14	87.50
	mixed	2	12.50
Feed	Pasture + Compact Feed	1	6.25
	Compact + Hay	3	18.75
	Pasture + Compact Feed + Hay	12	75.00

**Table 1.** *Cont.*

Characteristic		Frequency	%
Pasture	Low	8	50.00
	Bushy	8	50.00
Milking	Manual	10	62.50
	Automatic	6	37.50

### 3.2. Fatty Acid Profile

The gross fat composition of sheep milk samples was 6.27% ( $\pm 1.74$ ) and of goat milk samples 6.00% ( $\pm 1.48$ ). The FA composition of the analyzed sheep and goat milk from organic and conventional farms is presented in Tables 2 and 3.

**Table 2.** Fatty acid profile in sheep milk (g/100 g total FAs).

	Organic (n = 12)				Conventional (n = 12)				<i>p</i> -Value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
C6:0	2.533	0.168	2.400	2.807	2.485	0.150	2.381	2.718	0.009
C8:0	1.524	0.101	1.444	1.689	1.495	0.090	1.433	1.635	0.000
C10:0	3.532	0.235	3.346	3.913	3.464	0.209	3.320	3.789	0.000
C12:0	4.090	0.272	3.874	4.531	4.012	0.242	3.844	4.387	0.000
C13:0	0.129	0.009	0.122	0.143	0.126	0.008	0.121	0.138	0.000
C14:0	12.560	0.835	11.897	13.915	12.319	0.742	11.806	13.473	0.000
C15:0	1.202	0.080	1.139	1.332	1.179	0.071	1.130	1.290	0.000
C16:0	34.030	2.262	32.235	37.702	33.377	2.010	31.987	36.504	0.000
C18:0	7.963	5.907	0.000	13.677	11.683	0.032	11.604	11.694	0.000
C20:0	0.172	0.011	0.163	0.190	0.168	0.010	0.161	0.184	0.023
C22:0	0.076	0.005	0.071	0.083	0.074	0.004	0.071	0.081	1.000
C10:1	0.354	0.024	0.336	0.392	0.347	0.021	0.333	0.380	0.000
C14:1	0.966	0.064	0.915	1.070	0.948	0.057	0.908	1.036	0.000
C16:1	1.782	0.118	1.688	1.974	1.748	0.105	1.675	1.912	0.000
C17:1	0.301	0.020	0.285	0.333	0.295	0.018	0.283	0.322	0.052
C18:1 cis/trans	24.567	3.929	13.006	27.752	25.375	1.528	24.318	27.752	0.233
C20:1	0.215	0.014	0.203	0.238	0.211	0.013	0.202	0.230	0.000
C18:2 cis/trans	2.923	0.253	2.333	3.224	2.948	0.178	2.825	3.224	0.381
C18:3n3/3n6cis	0.307	0.079	0.064	0.357	0.326	0.020	0.313	0.357	0.195
C18:4n3	0.787	0.838	0.519	3.444	0.537	0.032	0.515	0.587	0.002
SFA	67.80	4.47	62.35	79.98	67.20	1.93	64.20	68.39	0.081
MUFA	28.18	3.90	17.01	31.63	28.92	1.74	27.72	31.63	0.252
PUFA	4.02	0.97	3.00	6.92	3.81	0.23	3.65	4.17	0.014

**Table 3.** Fatty acid profile in goat milk (g/100 g total FAs).

	Organic (n = 12)				Conventional (n = 12)				<i>p</i> -Value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
C6:0	2.400	0.002	2.400	2.405	2.400	0.000	2.400	2.400	0.871
C8:0	1.444	0.001	1.444	1.447	1.444	0.000	1.444	1.444	0.945
C10:0	3.346	0.002	3.346	3.353	3.346	0.000	3.346	3.346	0.913
C12:0	3.875	0.003	3.874	3.883	3.874	0.000	3.874	3.874	0.913
C13:0	0.122	0.000	0.122	0.122	0.122	0.000	0.122	0.122	0.945

Table 3. Cont.

	Organic (n = 12)				Conventional (n = 12)				<i>p</i> -Value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
C14:0	11.900	0.008	11.897	11.924	11.897	0.000	11.897	11.897	0.825
C15:0	1.139	0.001	1.139	1.141	1.139	0.000	1.139	1.139	0.945
C16:0	32.241	0.021	32.235	32.308	32.235	0.000	32.235	32.235	0.994
C18:0	11.696	0.008	11.694	11.721	11.694	0.000	11.694	11.694	0.823
C20:0	0.163	0.000	0.163	0.163	0.163	0.000	0.163	0.163	0.945
C22:0	0.071	0.000	0.071	0.171	0.071	0.000	0.071	0.071	0.945
C10:1	0.336	0.000	0.336	0.336	0.336	0.000	0.336	0.336	0.945
C14:1	0.915	0.001	0.915	0.917	0.915	0.000	0.915	0.915	0.945
C16:1	1.688	0.001	1.688	1.692	1.688	0.000	1.688	1.688	0.945
C17:1	0.285	0.000	0.285	0.285	0.285	0.000	0.285	0.285	0.945
C18:1 cis/trans	24.511	0.016	24.507	24.562	24.507	0.000	24.507	24.507	0.803
C20:1	0.309	0.155	0.203	0.520	0.203	0.000	0.203	0.203	0.000
C18:2 cis/trans	2.848	0.002	2.847	2.854	2.847	0.000	2.847	2.847	0.899
C18:3n3/3n6cis	0.278	0.055	0.203	0.315	0.315	0.000	0.315	0.315	0.000
C18:4n3	0.432	0.141	0.090	0.519	0.519	0.000	0.519	0.519	0.004
SFA	68.40	0.040	68.39	68.54	68.39	0.000	68.39	68.39	0.720
MUFA	28.04	0.160	27.93	28.31	27.93	0.000	27.93	27.93	0.000
PUFA	3.56	0.190	3.15	3.68	3.68	0.000	3.68	3.68	0.003

### 3.2.1. Fatty Acid Profile in Organic and Conventional Sheep Milk

Sheep milk was found to contain a large number of FAs, some of which are very important for human health, including PUFA, vaccenic acid (VA), and CLA. Palmitic (C16:0), oleic (9-cis C18:1), stearic (C18:0), and myristic (C14:0) acids contributed most to total FAs. Their mean concentrations in the organic farms were 34.03, 24.56, 7.96, and 12.56 g/100 g total FAs, respectively, while in the conventional farms were 33.37, 25.37, 11.68, and 12.32 g/100 g total FAs, respectively.

The total concentration of short-chain saturated fatty acids (SCFA) in the organic farms (Caproic C6:0, Caprylic C8:0, and Capric C10:0) was 7.59 g/100 g total FAs, while in the conventional farms was 7.44 g/100 g total FAs.

Medium-chain saturated fatty acids (MCFA) consist of lauric (C12:0), tridecanoic (C13:0), myristic (C14:0), pentadecanoic acid (C15:0), and palmitic (C16:0). The total concentration in the organic farms of MCFA was 52.01 g/100 g total FAs, while in the conventional farms was 51.01 g/100 g total FAs.

Long-chain saturated fatty acids (LCFA) consist of stearic acid (C18:0), arachidic acid (C20:0), and behenic acid (C22:0). In the organic farms, the total concentration of LCFA was 8.21 g/100 g total FAs, while in the conventional farms it was 11.92 g/100 g total FAs.

The total concentration of saturated fatty acid (SFA) in organic milk (SFA = SCFA + MCFA + LCFA) was 67.80 g/100 g total FAs, and in conventional milk was 67.20 g/100 g total FAs.

Monounsaturated fatty acids (MUFA) consist of myristoleic acid (C14:1), palmitoleic acid (C16:1), heptadecenoic acid (C17:1), oleic (C18:1), and gondoic acid (C20:1). The total concentration of MUFA in organic farms was 28.18 g/100 g total FAs, and in conventional farms, 28.92 g/100 g total FAs. PUFA consist of linoleic (C18:2), cis/trans,  $\alpha$ -linolenic (C18:3), and stearidonic acid (C18:4). The total concentration of PUFA in organic farms was 4.02 g/100 g total FAs, while in conventional farms, 3.81 g/100 g total FAs.

Statistically significant differences between organic and conventional sheep milk were found in all saturated short-chain (C6:0, C8:0, C10:0), medium-chain (C12:0, C13:0, C14:0, C15:0, C16:0), and large-chain (C18:0, C20:0) fatty acids. In addition, significant differences



were also found in the proportion of some MUFA (C10:1, C14:1, C16:1, C20:1) and the PUFA stearidonic acid (C18:4n-3) between organic and conventional sheep milk.

### 3.2.2. Fatty Acid Profile in Organic and Conventional Goat Milk

Goat milk was found to contain a high proportion of MCFA which partly contributes to the specific “goaty” flavor of goat milk [42]. Palmitic (C16:0), oleic (9-cis C18:1), stearic (C18:0), and myristic (C14:0) acid contributed most to total FAs. Their mean concentrations in the organic farms were 32.24, 24.51, 11.69, and 11.90 g/100 g total FAs, respectively, and in the conventional farms were similar. In the organic farms, the total concentration of SCFA, MCFA, and LCFA was 7.19 g, 49.27 g, and 11.93 g/100 g total FAs, respectively, as well as in the conventional farms. The total concentration of SFA in organic milk was 68.40 g/100 g total FAs, and the same in conventional milk (68.39 g/100 g total FAs). The total concentration of MUFA in organic milk was 28.04 g/100 g total FAs and a little bit lower in the conventional 27.93 g/100 g total FAs. Finally, the total concentration of PUFA in organic farms was 3.56 g/100 g total FAs, while in conventional farms was 3.68 g/100 g total FAs.

A similar trend of significant effects was observed between organic and conventional goat milk with regard to the total content of gondoic acid (C20:1), as well as ALA (C18:3n-3) and stearidonic acid (C18:4n-3).

### 3.2.3. Associated Factors for Statistically Significant Differences in FA Profile

From the data analysis on the specific nutrient investigated, it became apparent that there are certain factors with significant association such as season, feeding scheme, and the type of farm (Tables 4 and 5).

In the comparative analysis of associated factors with the fatty acid profile of sheep milk, significant differences were observed with respect to compound feed, the organic farming system, and the moving farm that were positively affecting all saturated SCFA, MCFA, and LCFA profiles. In addition, significant differences of the above factors were also found in the proportion of MUFA and PUFA. No significant differences were observed with respect to season (winter vs. spring) or pasture type (natural or artificial).

In the comparative analysis of associated factors with the fatty acid profile of goat milk, significant differences were observed with respect to the farming system (organic vs. conventional) and the season (winter vs. spring; winter vs. summer) on the proportion of the MUFA gondoic acid (C20:1) as well as the PUFA ALA (C18:3n-3) and stearidonic acid (C18:4n-3). No significant differences were observed with respect to compound feed or pasture type (natural or artificial). Finally, no significant differences were observed in SCFA or MCFA profile.

### 3.3. Fat Soluble Vitamin Content

Vitamin A was found to be higher in organic sheep milk in comparison to conventional (Table 6), though without any statistically significant difference, while vitamin E was almost in the same amount. In organic goat milk, both vitamins A and E were found to be higher in comparison to conventional (Table 7). In the comparative analysis of associated factors (namely, compound feed, farming system, moving farm, season, and pasture type) to the vitamin content of sheep and goat milk, no statistically significant differences were noted ( $p$ -value > 0.05).

**Table 4.** Comparative analysis of associated factors with the fatty acid profile of sheep milk.

	C6:0	C8:0	C10:0	C12:0	C13:0	C14:0	C15:0	C16:0	C18:0	C20:0	C22:0	C10:1	C14:1	C16:1	C17:1	C18:1 cis/trans	C20:1	C18:2 cis/trans	C18:3n3/3n6cis	C18:4n3	SFA	MUFA	PUFA
<b>Farming system</b>																							
Organic vs. conventional	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	1.000	0.000	0.000	0.010	0.052	0.233	0.000	0.381	0.195	0.002	0.081	0.252	0.014
<b>Season</b>																							
Winter vs. spring	0.601	0.578	0.578	0.583	0.579	0.579	0.578	0.579	0.815	0.616	0.925	0.579	0.579	0.598	0.583	0.596	0.579	0.596	0.596	0.365	0.839	0.596	0.539
Winter vs. summer	0.271	0.232	0.231	0.241	0.233	0.233	0.232	0.231	0.118	0.295	0.332	0.233	0.234	0.265	0.235	0.090	0.233	0.019	0.164	0.341	0.048	0.068	0.118
<b>Compound feed</b>																							
Yes vs. no	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.023	1.000	0.000	0.000	0.010	0.052	0.494	0.000	0.301	0.619	0.002	0.814	0.449	0.035
<b>Moving farm</b>																							
Yes vs. no	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.021	1.000	0.000	0.000	0.009	0.049	0.154	0.000	0.301	0.123	0.002	0.497	0.170	0.040
<b>Pasture type</b>																							
Natural vs. artificial	0.494	0.286	0.355	0.413	0.409	0.300	0.320	0.397	1.000	0.537	1.000	0.413	0.415	0.506	0.563	0.133	0.399	0.084	0.159	0.290	0.116	0.124	0.180

**Table 5.** Comparative analysis of associated factors with the fatty acid profile of goat milk.

	C6:0	C8:0	C10:0	C12:0	C13:0	C14:0	C15:0	C16:0	C18:0	C20:0	C22:0	C10:1	C14:1	C16:1	C17:1	C18:1 cis/trans	C20:1	C18:2 cis/trans	C18:3n3/3n6cis	C18:4n3	SFA	MUFA	PUFA
<b>Farming system</b>																							
Organic vs. conventional	0.871	0.945	0.913	0.913	0.945	0.825	0.945	0.994	0.823	0.945	0.945	0.945	0.945	0.945	0.945	0.803	0.000	0.899	0.000	0.004	0.720	0.000	0.003
<b>Season</b>																							
Winter vs. spring	0.349	0.341	0.350	0.350	0.341	0.343	0.341	0.351	0.343	0.341	0.341	0.341	0.341	0.341	0.341	0.350	0.000	0.347	0.000	0.012	0.352	0.000	0.005
Winter vs. summer	0.629	0.644	0.574	0.574	0.644	0.646	0.644	0.977	0.644	0.644	0.644	0.644	0.644	0.644	0.644	0.663	0.041	0.664	0.039	0.094	0.760	0.011	0.033
<b>Compound feed</b>																							
Yes vs. no	0.960	0.847	0.727	0.727	0.847	0.704	0.847	0.983	0.699	0.847	0.847	0.847	0.847	0.847	0.847	0.849	0.887	0.825	0.521	0.390	0.604	0.950	0.991
<b>Pasture type</b>																							
Natural vs. artificial	0.961	0.851	0.734	0.734	0.851	0.710	0.851	0.983	0.706	0.851	0.851	0.851	0.851	0.851	0.851	0.853	0.887	0.829	0.528	0.390	0.610	0.950	0.991



**Table 6.** Fat-soluble vitamins content in organic and conventional sheep milk.

	Organic (n = 12)				Conventional (n = 12)				<i>p</i> -Value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Vit E (µg/mL)	2.51	1.22	1.88	4.09	2.26	1.12	0.64	4.68	0.233
Vit A (µg/mL)	0.90	0.21	0.56	1.06	0.93	0.25	0.55	1.28	0.275

**Table 7.** Fat-soluble vitamins content in organic and conventional in goat milk.

	Organic (n = 12)				Conventional (n = 12)				<i>p</i> -Value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Vit E (µg/mL)	3.26	2.35	0.76	8.29	2.53	1.86	0.35	6.04	0.242
Vit A (µg/mL)	0.75	0.27	0.51	1.38	0.57	0.15	0.35	0.77	0.388

#### 4. Discussion

Milk fat contains a wide variety of fatty acids, on average 65–70% saturated fatty acids and 30–35% unsaturated fatty acids. Amongst saturated fatty acids in milk fat, there are around 10–12% short- and medium-chain and 50–55% long-chain. These acids consist of stearic acid (8–10%), myristic acid (10–12%), and palmitic acid (25–27%). Amongst unsaturated fatty acids in milk fat, there are 25–30% monounsaturated, where oleic acid is the second most abundant fatty acid in milk fat.

The composition of milk fat varies slightly depending on the breed of animal, stage of lactation, season, geographical location, and feed composition. The stage of lactation and the feed are two important factors which may change the vitamin content in sheep and goat milk. The results obtained in this study indicate that organic sheep and goat milk was found to contain a higher amount of the fat-soluble vitamins in comparison to conventional. Although no significant differences were observed with respect to factors, such as stage of lactation, season, and feed composition, values of vitamins A and E varied slightly in conventional and organic farms.

Some authors have reported that ewe's milk contains higher amounts of vitamins [22,43] than the goat milk. In addition, a study showed higher contents of these vitamins in cow's and sheep's milks than in goat's milk [44], while other authors [45] have reported the opposite. In our study, the goat milk seems to be richer regarding the vitamin E while the sheep milk has more vitamin A in both organic and conventional milk compared to goat ones.

The fat content present in sheep and goat milk is a rich source of MCFA. SCFA and MCFA are de novo synthesized in the mammary gland, from rumen fermentation of acetic acid and  $\beta$ -hydroxybutyric acid, both influenced by the energy balance of diet and the specificity of rumen micro flora [21]. Significant differences in SCFA were not found between organic and conventional farms. Lower concentrations of SCFA and MCFA in conventional farms compared to organic farms may be attributed to the feeding system and to reduced synthesis of fatty acid in the mammary gland. The sum content of MUFA in goat milk was slightly higher in organic farms than in conventional ones because of the increase in oleic acid and in gondoic acid. Oleic acid is considered to be favorable for health, because it lowers LDL cholesterol and triacylglycerol contents and reduces the risk for artery disease. PUFA concentrations were higher in the sheep milk of organic farms compared to these of conventional farms whereas MUFA concentrations were lower in sheep organic farms than in conventional farms. On the contrary, PUFA concentrations were lower in the milk from goat organic farms compared to those of conventional farms, whereas MUFA concentrations were higher in the goat milk of organic farms than that of the conventional farms. These differences are mainly attributed to different feeding practices used by the two production systems, as it has been also previously reported in literature [46,47].

Tzamaloukas et al. [46] report that under organic farming practices, milk contained increased values of total MUFA and PUFA, and specific FA, such as oleic, conjugated linoleic, and linolenic acids, while total SFA levels were particularly decreased in organic samples. Differences were attributed to the differences in the feeding strategies applied in the farms. Relatively high levels of MUFA and PUFA in sheep and goat milk are probably due to a combination of increased dietary supply of PUFA, reduced rumen biohydrogenation, and possibly enhanced desaturase activity in the mammary gland, which have been shown to occur with increasing intakes of fresh forage in dairy diets [48–50].

Higher values of CLA were found in the sheep milk and in conventional farms, particularly, compared to organic farms. The CLA that was found in goat and sheep milk fat is mainly attributed to ruminal biohydrogenation of LA (C18:2) that leads first to vaccenic acid (C18:1) and finally to stearic acid (C18:0). Secondly, CLA is synthesized by the animal's tissues from vaccenic acid, another intermediate in the rumen biohydrogenation of unsaturated fatty acids [51,52]. Different feeding strategies have different impact on the chemical composition of goat milk [23]. Different nutrients, such as pasture, silage, and concentrates, along with various feeding strategies, have great impact on the content of FA in milk [25,53]. Manzi and Durazzo [54] report also that animal feed mainly affects the fatty acid profile of milk irrespective of the conventional or organic status, a statement that is also supported by Mollica et al. [55]. The fact there were no significant differences observed in our study indicates the possibility that organic and conventional sheep and goat milk in Greece do not have any substantial differences in terms of feeding strategies. The feeding system in Greece is based on seasonal natural grazing and on supplementary feeding which consists of home-grown roughages and concentrates. This system has great impact on the content of fatty acids in milk.

Limitations to be noted regarding this study are the restricted geographical area and the relatively low number of samples collected. Thessaly, as a predominantly rural area that hosts a significant proportion of lactating animal livestock, producing almost the 25% of the dairy products in Greece and having the majority of organic livestock farms, could be used as a snapshot of the aimed comparison, to achieve and present some indicative and preliminary data. Overall, there were some differences in milk composition and fatty acid content between the two farm types (organic and conventional) in sheep and goat milk. Thus, the results seem to show slight alterations in the concentration of MUFA and PUFA in organic and conventional milk. In future studies, milk fatty acid composition should be further controlled in order to reduce the content of saturated fatty acids and also to increase the content of unsaturated fatty acids in milk. The fact that no statistical differences were found between organic and conventional farms in fatty acid profile both in sheep and goat milk proves that the conventional farming system in Greece has common farming practices and management systems with the organic one.

## 5. Conclusions

Greek conventional milk from sheep and goat, in terms of fatty acids content, is almost the same as the organic one, as no significant differences were generally observed. Regarding the fat-soluble vitamins (all-trans-retinol and  $\alpha$ -tocopherol), the goat milk seems to be richer regarding the vitamin E, while the sheep milk is richer in vitamin A in both organic and conventional milk.

Due to the fact that the conventional livestock production system adopted in dairy goat and sheep breeding in Greece, particularly in small mountain farms, is considered semi-intensive, and fat composition can be attributed to the management system, season, and sampling period, there is a need for further research on the estimation of milk fat components and strategies that will impact the FA profile and lipophilic vitamins content.

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