

Article

The Effects of Flax and Mustard Seed Inclusion in Dairy Goats' Diet on Milk Nutritional Quality

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Abstract: Our study evaluated the impact of incorporating flax seeds or a flax and mustard seeds' mixture into goats' diets to enhance milk polyunsaturated fatty acids (PUFAs). The incorporation of mustard seeds also aimed to slow the lipid oxidation process. A three-week feeding trial was conducted on 18 lactating goats, randomly distributed in three groups: control (C), FS (replacing 12% of the oil source with flax seeds), and FMS (replacing a quarter of flax seeds with mustard seeds). Flax seed inclusion improved the quality of milk fat by reducing saturated fatty acid concentration ($p = 0.004$) and increasing PUFA levels ($p = 0.001$). Both experimental groups significantly lowered the omega 6/omega 3 fatty acid ratio ($p < 0.001$). The FMS group showed a significantly higher total vitamin E concentration ($p = 0.007$). The fat oxidation parameters revealed that after 24 h of storage at room temperature, the p-anisidine value increased for the FS group compared to the C group, while the FSM group showed no significant difference, suggesting that the combined inclusion of flax and mustard seeds may prolong milk storage time by mitigating secondary oxidation products. This highlights the potential benefits of incorporating the studied seeds into goats' diets for improving milk quality and extending its shelf life.

Keywords: flax seeds; mustard seeds; milk antioxidant potential; milk quality; milk fatty acids



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1. Introduction

Lipids are essential macromolecules crucial for the proper functioning of all living organisms, particularly the human body. They can form membrane vesicles that transport bioactive lipo-soluble compounds such as proteins, hormones, or vitamins (A, D, E, and K) throughout the organism. Due to the lack of a specific enzyme involved in the synthesis of essential fatty acids (δ -12 desaturase), human metabolism is not capable of producing conjugated linoleic acid (CLA) and α -linolenic acid [1]. These fatty acids represent precursors for long-chain polyunsaturated fatty acids, which are essential for synthesising compounds involved in the complex inflammation process. Since humans cannot produce all essential lipids, and those provided externally are metabolised into various bioactive molecules, the quality of the diet is crucial for maintaining lipid homeostasis [2]. One of the most important strategies to influence the fatty acid content in animal-derived food products is animal nutrition. It is well known that feed ingredients can improve the fatty acid profile of milk, especially through supplementation with lipid sources [3,4].

Besides its content of protein and lipids, flax seeds represent an important source of α -linolenic acid; its inclusion in ruminants' diets leads to an increase in the concentration of omega-3 fatty acids in milk and meat [5,6]. Furthermore, it has been observed that fatty acids from flax seeds can significantly reduce methane production in ruminants [7,8]. Mitigating methane production is a major concern in reducing the greenhouse effect caused by gas emissions. However, special attention must be paid to the milk lipid oxidation

process, which occurs when the concentration of unsaturated fatty acids increases, giving rise to volatile compounds that influence its organoleptic properties [9]. Besides the production of off-flavour compounds, the lipid oxidation process can lead to the loss of nutrients and bioactive components, accompanied by the production of certain harmful substances to the human organism. This process can lead to specific conditions such as cancer, inflammation, or atherosclerosis [10].

Mustard seeds represent an alternative to conventional protein sources for ruminants, with potential uses in animal feeding and the vegetal oil industry due to their important nutritional characteristics and much higher content of phenolic compounds, compared to other oilseeds [11]. Moreover, they have a rich content of flavonoids, tocopherols, and ascorbic acid, which are particularly important in preventing or slowing down lipid oxidation [12].

Hence, the objective of this study was to analyse the nutritional profile of goat's milk after the inclusion of flax and mustard seeds in their diets. This investigation focused on evaluating how flax seeds contribute to enhancing milk polyunsaturated fatty acid levels and how their inclusion with mustard seeds may influence the milk's overall antioxidant status, considering the antioxidant properties of mustard seeds.

2. Materials and Methods

2.1. Experimental Design and Sample Collection

The feeding experiment was assessed according to the legislation in this field (Directive 2010/63/EU) [13], and the procedures were approved by the Ethical Commission of the National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Romania. Flax and mustard seeds were obtained from local varieties and provided by a producer from Southern Romania.

The feeding trial was performed using 18 multiparous goats, Carpathian breed, with an average live weight of 34.23 ± 6.08 kg. The goats were distributed under a completely randomised design in three groups, kept in individual boxes as follows: control group (C), fed a compound feed based on sunflower meal and sunflower oil; FS group, fed a diet where sunflower meal and sunflower oil were replaced with flax seeds, at an inclusion level of 12%; and FMS group, fed a diet where one-quarter of the flax seeds in ration FS were replaced with mustard seeds. During the experiment, dairy goats had continuous access to fresh water. The experimental period started on 1 September and lasted three weeks, after two weeks of adaptation. The milk samples were collected in the last week of the feeding trial. The proximate chemical composition of sunflower meal, flax, and mustard seeds used in this experiment is presented in Table 1.

Table 1. Chemical composition of sunflower meal, flax, and mustard seeds.

Parameters (%)	Sunflower Meal	Flax Seeds	Mustard Seeds
Dry matter	89.5	93.45	92.85
Crude protein	40.67	21.14	33.93
Crude fat	1.24	28.41	14.71
Crude fibre	21.4	24.84	20.67
Ash	7.86	3.89	5.23

The three diets (C, FS, and FMS) were designed to be isoenergetic and isonitrogenous, using the MFU and IDP as main parameters [14]. The compound feed was administered in limited quantities (900 g/kg/day), while the hay was fed ad libitum. The hay consumption was as follows: 0.848 ± 0.075 kg/day (C); 0.859 ± 0.074 kg/day (FS); 0.849 ± 0.083 kg/day (FMS). Table 2 presents the structure of the experimental diets.

Table 2. The structure of the experimental diets.

Ingredients in Concentrate (as Fed, %)	C ¹	FS ²	FMS ³
Maize	80.00	77.0	77.0
Sunflower meal	11.11	0.0	0.0
Sunflower oil	5.89	0	0
Flax seeds	0.0	20.0	15.0
Mustard seeds	0.0	0.0	5.0
Calcium carbonate	1.00	1.00	1.00
Sodium chloride	1.00	1.00	1.00
Mineral–vitamin supplement	1.00	1.00	1.00
Nutrients in total diet (g/day)			
DM ⁴ , as fed	1503.07	1514.60	1507.83
MFU ⁵	1.37	1.40	1.40
IDPE ⁶	118.91	119.99	118.84
IDPN ⁷	108.64	112.16	110.62
CP ⁸	143.70	148.68	147.11
EE ⁹	81.45	84.59	88.75
CF ¹⁰	254.49	249.32	248.87

¹ Control group, ² Inclusion of 12% flax seed, ³ Inclusion of 9% flax seeds and 3% mustard seeds, ⁴ Dry matter, ⁵ Milk feed units, ⁶ Intestinally digestible protein allowed by rumen-available energy, ⁷ Intestinally digestible protein allowed by rumen-available nitrogen, ⁸ Crude protein of the diets, ⁹ Fat content of the diets, ¹⁰ Total fibre content of the diets.

The fat supply was similar among groups (6% in total diet, approximatively), the only difference being the fatty acid profiles.

The milk samples were individually collected from each animal included in the experiment during the last week of the feeding trial using an automatic milking machine. The samples were stored at -4°C until the analysis was completed.

2.2. Chemical Analysis

2.2.1. Proximate Chemical Composition of the Milk Samples and Dietary Ingredients

The proximate chemical composition for the milk samples (crude protein, crude fat, total caseins, lactose, density, and pH) was assessed using Fourier-transform infrared spectroscopy (FTIR), with a CombiScope FTIR 200 system (PerkinElmer, Waltham, MA, USA), according to standard methods in this field (ISO 9622:2013) [13].

The crude protein analysis for the dietary ingredients was performed following the Kjeldahl method using a Kjeltac auto 1030 Tecator Instruments (Höganäs, Sweden), while the crude fat content was obtained using the Soxhlet determination, with Soxtec 2055 Foss Tecator equipment (Höganäs, Sweden). The content of the crude fibre was obtained using intermediary filtration with the Fibertec 2010 system Foss Tecator System (Höganäs, Sweden). The concentration of ash was determined using a Nabertherm Labotherm L15/11/P320 Comfort (Bremen, Germany) equipment.

2.2.2. Mineral Composition of the Milk Sample and Dietary Ingredients

The trace mineral composition (copper, iron, manganese, zinc, and magnesium) was obtained using flame atomic absorption/emission spectrometry (FAAS) with the Thermo Electron SOLAAR M6 Dual Zeeman Comfort system (Cambridge, UK), following the method described by [15]. The calcium concentration was determined using a complexometric approach that used ethylenediaminetetraacetic acid (EDTA) as a titration agent in the presence of murexide. To analyse phosphorus content, a colourimetric method with molybdovanadate and a Jasco V530 UV/VIS spectrophotometer (Japan Servo Co., Ltd., Tokyo, Japan) was used, as reported by [15].

2.2.3. Hydro-Soluble Components of the Milk Sample and Dietary Ingredients

The total polyphenol (TP) determination for the milk samples and dietary ingredients was assessed using the Folin–Ciocâlteu method as described by [16,17], and the content was expressed as mg gallic acid equivalents/gram of the dried sample.

The polyphenol profile for the dietary ingredients (flax and mustard seeds) was obtained following the method described by [18] using a high-performance liquid chromatography (HPLC) method with a Vanquish Core HPLC System (Thermo Fisher Scientific, Waltham, MA, USA) and a BDS HyperSil C18 column (250 mm × 4 mm, 5 µm particle dimension), with three solvents for the mobile phase (A-1% acetic acid; B-methanol; C-acetonitrile). The elution of the mobile phase was in a gradient as follows: 0–15 min: 5% B, 5% C; 15–20 min: 4% B, 15% C; 20–25 min: 3% B, 25% C; 25–40 min: 2% B, 38% C; 40–50 min: 5% B, 5% C, and a flow rate of 0.5 mL/min.

2.2.4. Lipo-Soluble Components of the Milk Sample and Dietary Ingredients

The fatty acid profile for the ratio of ingredients and milk samples was assessed using gas chromatographic techniques with a Perkin Elmer Clarus 500 system (Waltham, MA, USA), following the method described by [19].

The fat extraction from milk, flax, and mustard seed samples to determine isomers of vitamins E, A, D₃, and xanthophyll was performed using a method described by [20].

Isomers of vitamin E (alpha, gamma, delta) for milk, flax, and mustard seed samples were assessed using the HPLC method with the Vanquish Core HPLC system (Thermo Fisher Scientific, Bremen, Germany), a C18 column (Thermo Fisher Scientific, Waltham, MA, USA), and a mobile phase consisting of 4% water and 96% methanol, as previously described by [20].

The determination of vitamin A concentration for the milk samples was performed using the HPLC method, with a Vanquish Core HPLC system (Thermo Fisher Scientific, Waltham, MA, USA), as described by [20,21].

Vitamin D₃ content from the milk samples was determined using the HPLC method, using the Vanquish Core HPLC system (Thermo Fisher Scientific, Waltham, MA, USA), involving a mobile phase consisting of 85% methanol and 15% acetonitrile and an Accucore XL C18 column (150 × 4.6 mm, 4 µ particles size), (Thermo Fisher Scientific, Waltham, MA, USA).

Assessing the xanthophyll content (lutein, canthaxanthin, β-carotene) for flax and mustard seeds was performed using the HPLC method with a Finnigan Surveyor Plus chromatograph (Thermo-Electron Corporation, Waltham, MA, USA) using a mobile phase consisting of 75% acetone, 15% methanol, and 10% water, as described by [20].

2.2.5. Antioxidant Potential of the Milk Sample and Dietary Ingredients

The determination of the antioxidant capacity (AC) for the milk, flax, and mustard seeds using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) method was performed following the protocol described by [16] with a Jasco V-530 spectrophotometer (Japan Servo Co., Ltd., Tokyo, Japan), and the results are expressed as mM Trolox equivalents/kg of samples.

To determine the degradation state of the milk samples, we analysed primary and secondary degradation indices of lipid oxidation. The examination of the indicators that characterise the first stage of lipid oxidation (conjugated dienes (CDs) and conjugated trienes (CTs)) was conducted using a spectrophotometric technique described by [16]. The peroxidation value (PV) was measured using the ferric thiocyanate method, and the results are expressed as milliequivalents of oxygen per kilogram of lipids (meq. O₂/kg) [22]. The p-anisidine value was determined as an indicator for the secondary stage of lipid oxidation, using a method based on the reaction between p-anisidine and the aldehydic compounds under acidic conditions [22].

2.3. Statistical Analysis

The statistical analysis of the results was conducted using a bifactorial analysis of variance (ANOVA) with version 16 of Minitab® Statistical Software. Differences between mean values were considered significant at $p < 0.05$. Graphs were generated using Prism-GraphPad software, version 9.1.2 (San Diego, CA, USA). The Principal Component Analysis (PCA), which simultaneously represents the variables and observations in the PCA space (biplot), was used to determine the correlation pattern between the experimental groups and the analytical results. The PCA analysis was obtained using the XLStat software, version 19.01 by Addinsoft (New York, NY, USA).

3. Results

3.1. Fine Chemical Composition of Flax and Mustard Seeds

Besides the fact that both flax and mustard seeds presented important amounts of total protein and fat (Table 1), the studied seeds also represent an important source of trace elements, especially in the case of iron and zinc, as presented in Table 3.

Table 3. Trace elements and fatty acids profile of flax and mustard seeds and sunflower meal and oil.

Parameters	Flax Seeds	Mustard Seeds	Sunflower Meal	Sunflower Oil	SEM	<i>p</i>
Trace elements (mg/kg)						
Cooper	10.05 ^b	N.d. ¹	35.50 ^c	N.d. ¹	0.469	<0.0001
Iron	82.22 ^a	98.00 ^b	201.25 ^c	N.d. ¹	0.587	<0.0001
Manganese	26.30 ^b	20.85 ^a	59.18 ^c	N.d. ¹	0.581	<0.0001
Zinc	68.11 ^a	66.91 ^a	111.35 ^b	N.d. ¹	0.570	<0.0001
Fatty acids profile (g/100 g)						
Capric acid (C10:0)	N.d. ¹	0.120	N.d. ¹	N.d. ¹	-	-
Lauric acid (C12:0)	N.d. ¹	N.d. ¹	0.259	N.d. ¹	-	-
Myristic acid (C14:0)	0.065 ^a	0.140 ^a	0.373 ^b	0.101 ^a	0.034	0.001
Palmitic acid (C16:0)	5.395 ^a	5.680 ^a	11.48 ^b	6.460 ^a	0.286	<0.0001
Palmitoleic (C16:1)	0.105	0.325	0.352	0.133	0.091	0.171
Stearic acid (C18:0)	3.060 ^b	1.145 ^a	4.251 ^b	3.569 ^b	0.311	0.001
Oleic acid (C18:1n9)	18.15 ^a	21.04 ^a	29.94 ^a	35.24 ^b	1.097	<0.0001
Linoleic acid (C18:2n6)	15.99 ^b	13.12 ^a	52.14 ^c	52.99 ^c	0.424	<0.0001
Linolenic acid(C18:3n3)	56.33 ^c	10.20 ^b	0.748 ^a	0.521 ^a	0.118	<0.0001
Octadecatetraenoic acid (C18:4n3)	N.d. ¹	N.d. ¹	N.d. ¹	0.074	-	-
Eicosadienoic acid (C20:2n6)	N.d. ¹	0.09	0.07	0.038	-	0.283
Erucic acid (C22:1n9)	N.d. ¹	9.98	N.d. ¹	N.d. ¹	-	-
Arachidonic acid (C20: 4n6)	N.d. ¹	N.d. ¹	0.300	N.d. ¹	-	-
Eicosatrienoic acid (C20:3n6)	0.055 ^b	0.095 ^a	N.d. ¹	N.d. ¹	0.001	0.001
Nervonic acid (C24:1n9)	N.d. ¹	37.23	N.d. ¹	N.d. ¹	-	-
Other fatty acids	0.06	N.d. ¹	N.d. ¹	N.d. ¹	-	-

¹ Not determined, ^{a, b, c} Means within a row with no common superscript differs ($p < 0.05$).

As is shown in Table 3, flax seeds exhibited a remarkable amount of essential fatty acids, including α -linolenic and conjugated linoleic (CLA) acids, while mustard seeds presented a higher concentration of oleic acid than flax seeds. Notable amounts of nervonic (C24:1n9) and erucic (C22:1n9) acids were detected in the composition of mustard seeds. Also, both types of seeds presented a significantly higher content of α -linolenic acid than sunflower meal and oil.

Considering the high amounts of unsaturated fatty acids in the composition of the studied seeds was essential to determine their antioxidant potential. Table 4 presents the antioxidant composition and total antioxidant capacity determined for flax, mustard seeds, and sunflower meal

Table 4. Antioxidant composition and antioxidant capacity of flax, mustard seeds, and sunflower meal.

Parameters	Flax Seeds	Mustard Seeds	Sunflower Meal	SEM	<i>p</i>
α -tocopherol (mg/kg)	N.d. ¹	63.51 ^a	40.11 ^b	0.762	<0.0001
γ -tocopherol (mg/kg)	131.35 ^a	175.44 ^c	11.5 ^a	2.653	<0.0001
δ -tocopherol (mg/kg)	N.d. ¹	5.140 ^b	12.20 ^a	0.316	<0.0001
Total vitamin E (mg/kg)	131.3 ^c	243.8 ^b	63.81 ^a	3.333	<0.0001
Lutein (mg/kg)	5.507 ^b	7.163 ^b	3.60 ^a	0.393	0.002
Canthaxanthin (mg/kg)	0.560 ^b	1.020 ^a	N.d. ¹	0.094	0.026
β -carotene (mg/kg)	39.42 ^b	52.28 ^a	N.d. ¹	1.002	0.001
TP ² (mg/g GAE)	2.22 ^b	28.47 ^a	2.53 ^a	0.978	<0.0001
TAC ³ (mM eq. Trolox)	7.20 ^b	33.78 ^c	1.04 ^a	0.341	<0.0001

¹ Not determined, ² Total polyphenols content, ³ Total antioxidant capacity, ^{a, b, c} Means within a row with no common superscript differs ($p < 0.05$).

The mustard seeds exhibited a high content of lipo-soluble antioxidant compounds, mainly vitamin E. Furthermore, the mustard seeds also showed a higher concentration of total polyphenols than flax seeds and sunflower meal. The antioxidant activity of the flax and mustard seeds was also examined, as reported in Table 4. Mustard seeds had a higher total antioxidant capacity compared to flax seeds, which can be attributed to their remarkable composition of antioxidant compounds. Sunflower meal exhibited the least antioxidant capacity and had a reduced amount of antioxidant compounds, such as xanthophyll and vitamin E.

In terms of water-soluble antioxidants, the polyphenol profiles for the studied seeds were investigated and are presented in Table 5.

Table 5. Polyphenol profiles for flax and mustard seeds.

Polyphenols (mg/g)	Flax Seeds	Mustard Seeds	SEM	<i>p</i>
Phenolic acids				
Galic acid	0.121 ^b	0.612 ^a	0.009	<0.0001
Syringic acid	0.006 ^b	0.025 ^a	0.003	<0.0001
Protocatechuic acid	0.004	N.d. ¹	-	-
Chlorogenic acid	N.d. ¹	0.060	-	-
4-methoxy cinnamic acid	0.039 ^b	0.131 ^a	0.002	<0.0001
Vanillic acid	0.151 ^a	0.048 ^b	0.003	<0.0001
Caffeic acid	0.003 ^b	0.013 ^a	0.005	<0.0001
Ferulic acid	0.022 ^b	8.589 ^a	0.020	<0.0001
3-Hydroxybenzoic acid	N.d. ¹	1.663	-	-
p-coumaric acid	N.d. ¹	0.051	-	-
Ellagic acid	0.004	N.d. ¹	-	-
Flavonoids				
Epicatechin	N.d. ¹	0.027	-	-
Stilbene				
Resveratrol	0.002	N.d. ¹	-	-
Total	0.351 ^b	11.23 ^a		<0.0001

¹ Not determined, ^{a, b} Means within a row with no common superscript differs ($p < 0.05$).

Regarding the polyphenol composition, mustard seeds presented a greater content of the examined compounds, with ferulic acid being the predominant compound. In contrast, flax seeds presented a lower concentration of the studied polyphenols.

3.2. The Influence of the Flax and Mustard Seeds on the Milk Yield and Composition

The total content of protein ($p = 0.001$) and casein (0.001), as well as the density of milk ($p = 0.016$), were significantly higher in the FMS group, compared with the C and FS groups, as reported in Table 6.

Table 6. The proximate composition of milk.

Parameters	C ¹	FS ²	FMS ³	SEM	<i>p</i>
Proximate chemical composition					
Milk yield (mL)	256.9	266.6	273.4	30.03	0.927
Protein (%)	3.94 ^b	3.73 ^b	4.62 ^a	0.156	0.001
Fat (%)	4.87 ^a	3.56 ^b	4.77 ^a	0.141	<0.001
Casein (g/L)	38.78 ^b	37.12 ^b	45.63 ^a	1.514	0.001
Lactose (%)	4.48	4.36	4.51	0.072	0.362
Density (g/L)	1030 ^b	1030 ^b	1031 ^a	0.334	0.016
pH	6.58	6.58	6.64	0.018	0.031
Minerals composition					
Zinc (mg/kg)	31.44	28.69	24.34	2.638	0.192
Magnesium (%)	0.187	0.172	0.169	0.011	0.484
Calcium (%)	1.104	1.117	1.105	0.005	0.151
Phosphorus (%)	1.085	1.082	1.138	0.038	0.520

¹ Control group, ² Inclusion of 12% flax seed, ³ Inclusion of 9% flax seeds and 3% mustard seeds, ^{a, b} Means within a row with no common superscript differs ($p < 0.05$).

In the case of milk total fat content, the FS group led to a significantly ($p < 0.001$) lower concentration, compared with the C and FMS groups. The milk production, lactose, pH, and mineral content of the milk were not influenced by the experimental diets.

However, the inclusion of the studied oilseeds led to notable influences on the fatty acid composition of the milk, as presented in Table 7.

Table 7. The fatty acid profile of milk.

Fatty Acids (g/100 g)		C ¹	FS ²	FMS ³	SEM	<i>p</i>
Butyric acid	C 4:0	0.037	0.035	0.038	0.004	0.882
Caproic acid	C 6:0	1.153	1.118	1.083	0.050	0.602
Caprylic acid	C 8:0	2.544	2.518	2.436	0.088	0.654
Nonanoic acid	C 9:0	0.127	0.216	0.242	0.055	0.296
Capric acid	C 10:0	10.83	10.50	10.98	0.208	0.288
Undecanoic acid	C 11:0	0.476 ^b	0.552 ^{a, b}	0.582 ^a	0.027	0.023
Lauric acid	C 12:0	6.794 ^b	6.848 ^b	8.954 ^a	0.474	0.003
Tridecanoic acid	C 13:0	0.212	0.284	0.244	0.023	0.107
Myristic acid	C 14:0	12.74 ^b	12.91 ^b	14.87 ^a	0.509	0.008
Myristoleic acid	C 14:1	1.139 ^b	1.225 ^{a, b}	1.694 ^a	0.155	0.031
Pentadecanoic acid	C 15:0	0.299	0.322	0.278	0.061	0.325
Pentadecenoic acid	C 15:1	1.143 ^b	1.508 ^a	1.265 ^b	0.098	0.001
Palmitic acid	C 16:0	25.79	24.14	25.68	0.730	0.240
Palmitoleic acid	C 16:1	2.679	3.007	3.205	0.216	0.216
Heptadecanoic acid	C 17:0	0.295	0.327	0.279	0.022	0.315
Heptadecenoic acid	C 17:1	0.385 ^{a, b}	0.449 ^a	0.342 ^b	0.025	0.019
Stearic acid	C 18:0	4.980 ^a	4.151 ^{a, b}	3.349 ^b	0.397	0.019
Cis-oleic acid	C 18:1n9c	21.61 ^a	20.37 ^a	16.83 ^b	0.858	0.001
Trans-linoleic acid	C 18:2n6t	0.959 ^b	2.343 ^a	1.405 ^b	0.214	0.000
Cis-linoleic acid	C 18:2n6c	2.867 ^a	2.858 ^a	2.407 ^a	0.189	0.153
Arachidic acid	C20:0	0.014 ^b	0.168 ^a	0.057 ^b	0.018	<0.0001

Table 7. Cont.

Fatty Acids (g/100 g)		C ¹	FS ²	FMS ³	SEM	p
γ-linolenic acid	C 18:3n6	0.037 ^b	0.163 ^a	0.083 ^{a, b}	0.024	0.003
α-linolenic acid	C 18:3n3	0.302 ^b	0.991 ^a	0.896 ^a	0.092	<0.0001
Conjugated linoleic acid	CLA (c9, t11)	1.248	1.334	1.427	0.140	0.653
Eicosadienoic acid	C 20:2n6	0.081	0.081	0.072	0.060	0.895
Eicosatrienoic acid	C 20:3n6	0.094 ^b	0.161 ^a	0.096 ^b	0.016	0.011
Arachidonic acid	C 20:4n6	0.042 ^b	0.164 ^a	0.084 ^b	0.022	0.002
Other fatty acids		1.003	1.053	1.013	0.051	0.776
ΣSFA ⁴ (%)		66.3 ^{a, b}	64.10 ^b	69.06 ^a	0.957	0.004
ΣMUFA ⁵ (%)		26.96 ^a	26.56 ^a	23.33 ^b	0.623	<0.0001
ΣPUFA ⁶ (%)		5.738 ^b	8.291 ^a	6.591 ^b	0.441	0.001
Omega 3 fatty acids (%)		0.409 ^b	1.186 ^a	1.016 ^a	0.998	<0.0001
Omega 6 fatty acids (%)		5.329 ^b	7.104 ^a	5.574 ^b	0.360	0.004
Omega 6/ omega 3 ratio		15.01 ^a	6.31 ^b	5.93 ^b	0.992	<0.0001

¹ Control group, ² Inclusion of 12% flax seed, ³ Inclusion of 9% flax seeds and 3% mustard seeds, ⁴ Sum of the saturated fatty acids, ⁵ Sum of the mono-unsaturated fatty acids, ⁶ Sum of the poly-unsaturated fatty acids, ^{a, b} Means within a row with no common superscript differs ($p < 0.05$).

The FS experimental group led to an improvement in the quality of milk fat, by significantly ($p = 0.004$) reducing the concentration of saturated fatty acids (SFAs) and increasing ($p = 0.001$) that of poly-unsaturated fatty acids (PUFAs). The content of the omega-3 fatty acids, specifically α-linolenic acid, was also improved in the case of the FS and FMS groups. Moreover, the ratio between omega 6/omega 3 fatty acids was significantly lowered by both experimental groups ($p < 0.001$).

Considering the increase in the concentration of unsaturated fatty acids in the experimental groups, it was necessary to quantify the lipo-soluble compounds with antioxidant effects in the milk matrix, as presented in Table 8.

Table 8. The lipo-soluble antioxidants of milk.

Parameters	C ¹	FS ²	FMS ³	SEM	p
α-tocopherol (mg/kg)	13.40 ^b	12.70 ^b	19.37 ^a	1.257	0.001
γ-tocopherol (mg/kg)	3.86 ^b	4.36 ^{a, b}	4.89 ^a	0.227	0.009
δ-tocopherol (mg/kg)	8.610	9.177	9.440	0.521	0.511
Total vitamin E (mg/kg)	24.12 ^b	23.62 ^b	32.92 ^a	2.186	0.007
Vitamin A (mg/kg)	6.617	5.644	6.606	0.362	0.134
Vitamin D ₃ (μg/kg)	38.43	40.58	42.29	2.949	0.614

¹ Control group, ² Inclusion of 12% flax seed, ³ Inclusion of 9% flax seeds and 3% mustard seeds, ^{a, b} Means within a row with no common superscript differs ($p < 0.05$).

The FMS group led to a significantly higher concentration of the total vitamin E ($p = 0.007$), mainly due to the rise in gamma- and alpha-tocopherol contents. The concentration of the vitamins A and D₃ were not influenced by the experimental diets.

Regarding the composition of total polyphenols, as well as the antioxidant capacity of milk, they were not influenced by the experimental diets (Figure 1).

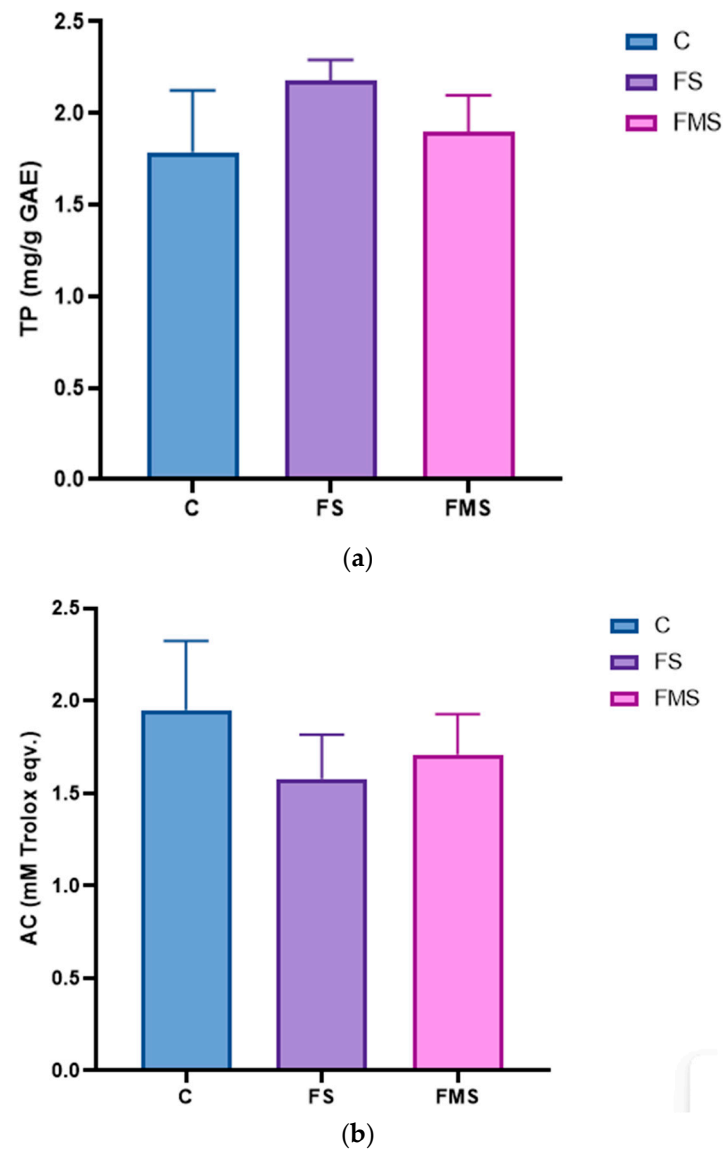


Figure 1. Total polyphenols content (a) and antioxidant capacity (b) of milk, for C—control group, FS—inclusion of 12% flax seed, FMS—inclusion of 9% flax seeds and 3% mustard seeds.

3.3. The Influence of the Flax and Mustard Seeds on the Milk Degradation Parameters

Considering the increase in the concentration of unsaturated fatty acids in the FS, as well as the elevated concentration of antioxidant compounds from mustard seeds, the susceptibility to oxidation of the fatty acids in milk was also tested.

The primary indicators of fatty acid oxidation were the concentrations of conjugated dienes, trienes, and peroxidation values, examined in fresh samples (t0) and after 24 h of storage at room temperature (t1) (Table 9).

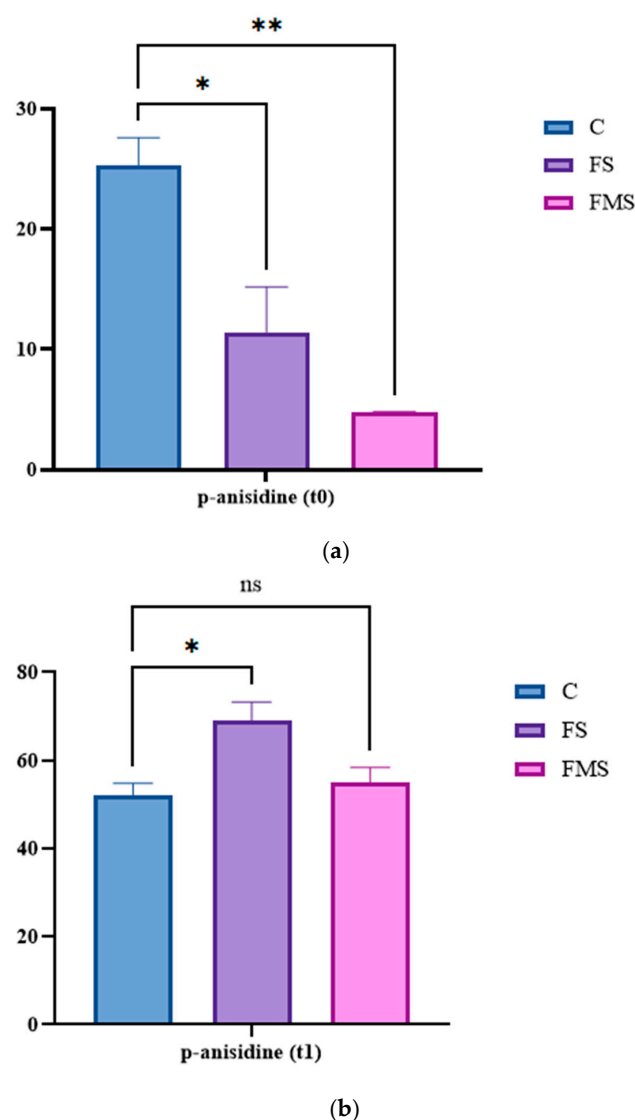
The concentration of conjugated dienes and the peroxidation value were not influenced by the experimental diets at both t0 and t1. However, the concentration of conjugated trienes significantly increased following the administration of both experimental diets at the initial time point (t0).

The results of the secondary oxidation indices assessment for the milk samples are presented in Figure 2.

Table 9. The concentrations of the conjugated dienes, trienes, and peroxidation value, for the fresh samples (t0) and after 24 h of storage at room temperature (t1).

Parameters	C ¹	FS ²	FMS ³	SEM	<i>p</i>
t0					
CD ⁴ (μmol/g)	54.16	66.95	73.16	5.880	0.104
CT ⁵ (μmol/g)	0.537 ^b	1.811 ^a	1.969 ^a	0.244	0.001
PV ⁶ (meq. O ₂ /kg)	0.134	0.169	0.163	0.035	0.350
t1					
CD ⁴ (μmol/g)	59.02	72.27	73.52	5.957	0.175
CT ⁵ (μmol/g)	2.227	2.772	2.421	0.179	0.140
PV ⁶ (meq. O ₂ /kg)	0.087	0.132	0.105	0.028	0.551

¹ Control group, ² Inclusion of 12% flax seed, ³ Inclusion of 9% flax seeds and 3% mustard seeds, ⁴ Conjugated dienes content, ⁵ Conjugated trienes content, ⁶ peroxidation value, ^{a,b} Means within a row with no common superscript differs ($p < 0.05$).

**Figure 2.** The concentration of the p-anisidine (a) at the initial time (t0), (b) after depositing of milk for 24 h at room temperature (t1), for C—control group, FS—inclusion of 12% flax seed, FMS—inclusion of 9% flax seeds and 3% mustard seeds. The results are presented as means \pm SEM; **, * represents significant differences between means (** $p \leq 0.01$; * $p < 0.05$), and “ns” represents no significant differences between means.

The concentration of p-anisidine significantly decreased in the experimental groups at the initial time, indicating a possible influence of flax and mustard seeds on the secondary oxidation processes of fatty acids. Additionally, after 24 h of storage at room temperature, a significant increase in its concentration was observed in the FS group compared to the C group. In the case of the FMS group, there were no differences observed in p-anisidine concentration compared to the C group.

Figure 3 presents the Principal Component Analysis (PCA), which simultaneously represents the variables and observations in the PCA space (biplot), used to determine the correlation pattern between groups and the analytical data. The PCA representation indicates that the experimental observations for FS and FMS groups were distributed in different quadrants at the bottom of the diagram, while the experimental observations for C were distributed in the upper quadrants of the PCA representation. The left bottom quadrant showed a strong association between the concentration of omega 3, omega 6, and PUFA with the inclusion of flax seeds in the goats' diet. Additionally, the FS group was strongly associated with the p-anisidine value, which is consistent with the data presented in our study. The bottom-right quadrant expressed higher associations between the lipo-soluble vitamins (specifically alpha-, gamma-tocopherol, and total vitamin E) and the inclusion of the mixture of flax and mustard seeds in goats' diets, confirming the observation presented in this study. The control group was strongly associated with the concentration of manganese and zinc and antioxidant capacity. The ratio between omega 6 and omega 3 fatty acids in the upper quadrants was associated with the C group, due to the higher value obtained in the study, the result indicating a negative meaning.

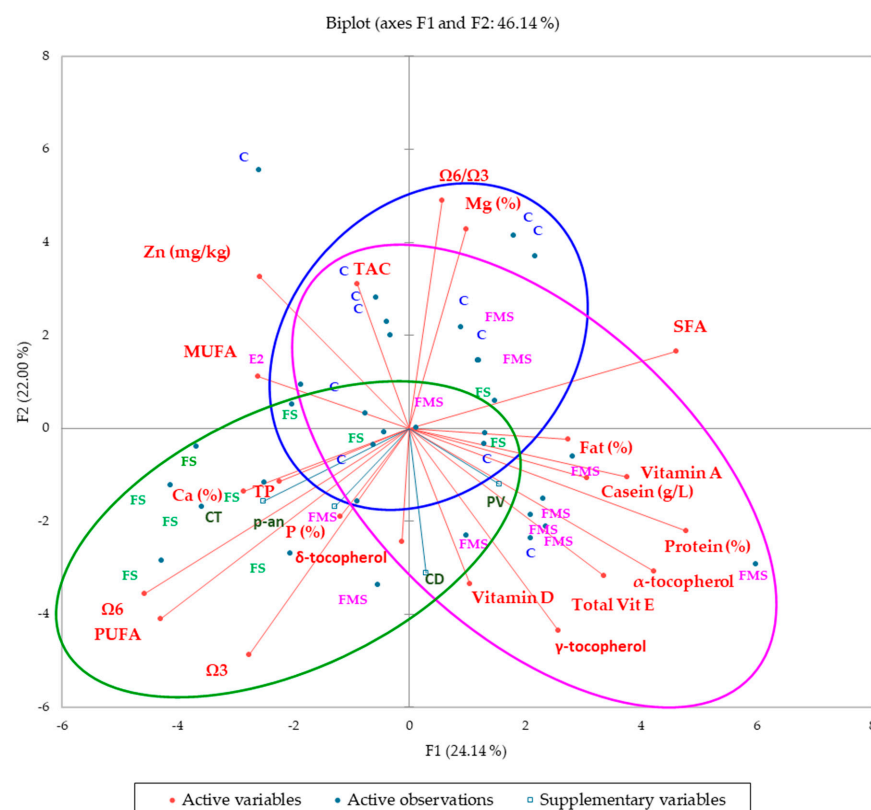


Figure 3. The Principal Component Analysis (PCA), representing the correlation between the experimental groups (C—control group, FS—12% inclusion of flax seed, FMS—inclusion of 9% flax seeds and 3% mustard seeds) and the observations (SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids, $\Omega 6$ -omega 6 fatty acids, $\Omega 3$ -omega 3 fatty acids, TAC-total antioxidant capacity, TP-total polyphenols, -omega 6 fatty acids TAC-total antioxidant capacity, CD-conjugated dienes, CT-conjugated trienes, p-an-p-anisidine, PV-peroxidation value).

4. Discussion

Regarding the chemical composition of the flax and mustard seeds, the literature presented contradictory data on the trace mineral composition [23–25], but it must be considered that the soil has a strong impact on plant mineral concentrations [26]. In the case of the fatty acid profile, both studied seeds, obtained from local varieties, presented remarkable amounts of the essential fatty acids, α -linolenic and CLA. These compounds are important because they play a crucial role in forming omega-3 and omega-6 fatty acids, which are vital for the optimal functioning of the human body [27]. In the case of mustard seeds, a higher content of oleic acid (compared with flax seeds) was observed, a fact that is supported by the literature [28].

The presence of nervonic acid (C24:1n9) in the composition of mustard seeds can be explained by the fact that nervonic acid is a product of the oleic acid elongation process, the main fatty acid found in mustard seeds. Furthermore, erucic acid (C22:1n9) was also present in mustard seeds, and a possible explanation could be that it represents the main synthesis precursor of nervonic acid. The presence of nervonic acid could have potential practical applications, considering its beneficial effects on the development of neurological functions [29]. The assessment of the antioxidant compounds for the studied seeds showed that mustard seeds were a significant source of vitamin E and total polyphenols compared to flax seeds, which aligns with the existing literature [30]. Additionally, the polyphenol profiles revealed a greater abundance of the studied polyphenols in mustard seeds compared to flax seeds. According to the literature, mustard is reported as a rich source of polyphenolic compounds. Furthermore, it can be observed that the predominant polyphenol detected in mustard seeds was ferulic acid, which is consistent with data reported in other studies [31]. Flax seeds exhibited small quantities of individual polyphenols, aligning with the literature, as flax seeds are not described as a rich source of polyphenolic compounds [32]. Mustard seeds exhibited remarkable antioxidant capacity compared to flax seeds, supported by their rich composition in antioxidant compounds [30].

The lack of significant influence of the studied seeds on milk yield was in line with the diet, which was formulated to be isoenergetic and isonitrogenous. However, a numerical increase in the FMS group was observed, which may be related to the results obtained by [33], who reported a significant increase in milk production in goats following the inclusion of mustard seeds (10 g mustard seeds/goat/day) in their diet.

The significant increase in the milk total protein composition for the FSM group may be due to the high protein content of mustard, as well as the presence of certain bioactive compounds that can enhance protein absorption at the ruminal level. A tendency in milk protein content, but not statistically significant, was also recorded in other studies that used mustard oil in the diet of ruminants [34]. Consistent with higher protein content, higher casein concentration was observed in FMS, which is particularly important for the cheese manufacturing industry, as these are the most important components aiding the milk coagulation process [35].

Milk density increased significantly in the FMS group, but for all three groups, the values were within the range presented in the literature for goat milk [36].

The total content of fat was decreased in the FS group, and a possible explanation can be the fact that increasing the intake of C18:2 and C18:3 fatty acids, provided by the diet, has the potential to inhibit the synthesis of fatty acids in milk. Additionally, the literature suggests that CLA may be the primary isomer responsible for reducing *de novo* synthesis of fatty acids and the expression of genes involved in fatty acid uptake, transport, and synthesis in the mammary gland [37].

In the case of the fatty acid profile of milk, the FS group led to a significant decrease in the content of SFAs, which are known for their negative implications for human health [38]. Moreover, the FS group led to a significant increase in PUFA content. The decrease in SFA and the increase in PUFA concentrations following the inclusion of flax seeds in the ruminants' diet have also been reported in the literature [39,40]. Incorporating flax seeds into the diet of ruminants can be an effective strategy to reduce the ruminal bio-

hydrogenation of unsaturated fatty acids while ensuring their high availability in the small intestine. The literature suggests that supplementing the diet of ruminants with raw or extruded flaxseed leads to an increase in the content of PUFA in the small intestine [41].

A possible explanation for these influences could be the increased intake of dietary PUFA from the FS diet. The inclusion of flax seeds leads to the inhibition of SFA synthesis with short (4:0–10:0) and medium (12:0–16:0) chains, resulting in a reduction in the total concentration of SFA in milk. Another possible mechanism is the inhibitory effect of trans-18 isomers of fatty acids, produced during their biohydrogenation in the rumen, on de novo synthesis of SFA with short and medium chains [39].

Also, the ratio of omega 6/omega 3 fatty acids decreased significantly in both experimental groups, a result also reported in the literature [42]. Additionally, in both experimental groups, a significantly higher concentration of α -linolenic acid can be observed, known for its beneficial effects on the human body. As for the effects of mustard seed inclusion into ruminants' diet, there are not many studies available, but it has been reported that an inclusion level of 10 g/goat/day may lead to a numerical decrease in the ratio of omega 6/omega 3 fatty acids, not statistically significant [33].

The experimental diets led also to influences in terms of antioxidant compounds in milk. Mustard seeds positively influenced the content of vitamin E in milk, especially by increasing the content of gamma and alpha tocopherols. The literature regarding the effects of mustard seeds on milk fat-soluble vitamins is scarce. However, it is known that the concentration of vitamin E in milk is influenced by the dietary fat content; as the fat intake, particularly PUFA, increases, there is a linear increase in the concentration of vitamin E, as observed in our study [43]. Another possible explanation could be that the intake of vitamin E from the diet may influence its concentration in milk. Mustard seeds provide an important amount of vitamin E in the diet, which is consistent with the increased composition of vitamin E in the milk from the FMS group [44]. Additionally, the literature details the negative effect of incorporating flax seeds into the ruminants' diet on the composition of milk tocopherols, as observed in our study (a numerically lower concentration of vitamin E was determined, not statistically significant) [45]. Increasing the concentration of vitamin E, especially of the alpha and gamma isomers, can have beneficial effects on milk quality, as they are known to be important antioxidant agents that can prevent the oxidation of unsaturated fatty acids [46].

The total polyphenol content and antioxidant capacity were not affected by the experimental diets. This can be influenced by various factors, including milk pH. The main transporters of polyphenols in milk are proteins. It is described in the literature that polyphenols such as chlorogenic acid or ferulic acid have a greater availability to transport proteins when the pH is lower (around 3) [47]. This could affect the concentration of polyphenols in milk; in our study, the milk had a pH in the range of 6.58–6.64.

The study of the fatty acids' degradation processes revealed that the concentration of conjugated dienes and the peroxidation value at t_0 and t_1 were not influenced by the experimental diets. However, the concentration of the conjugated trienes significantly increased following the administration of both experimental diets at the initial time point, t_0 , a fact also reported in the literature for milk enriched in unsaturated fatty acids, which showed high values of primary oxidation indices [48]. In terms of the parameters describing the secondary phase of the fatty acids' oxidation, the concentration of p-anisidine significantly decreased in the experimental groups at the initial time, t_0 , indicating a possible influence of flax and mustard seeds on the secondary oxidation processes of fatty acids.

Additionally, after 24 h of storage at room temperature, a significant increase in its concentration was observed in the FS group compared to the C group; however, in the case of the FMS group, there were no significant increases observed, compared with the C group. These results may suggest the beneficial effect of including flax seeds in combination with mustard seeds in goats' diets on the storage time of milk, potentially prolonging its duration by acting on secondary oxidation products. This fact could be attributed to the higher dietary supply of vitamin E in the FMS group, which is a powerful antioxidant.

Vitamin E can exert its antioxidant activity by chelating transitional metals and forming a complex that will prevent metal-catalysed pro-oxidative activity. Another mechanism of action can be through physicochemical reactions aimed to remove reactive oxygen species. In the case of lipid oxidation, tocopherols can react with peroxide radicals (LOO^-) and later with alkoxide radicals (LO^-), which appear during the propagation of the lipid oxidation reaction, leading to the slowing down or even stopping of the oxidation process [49].

5. Conclusions

The results obtained in this study have highlighted that supplementing goats' diets with flax seeds yielded a nutritionally enriched product, by positively influencing the concentration of PUFAs and omega 3 fatty acids and by reducing the ratio of omega 6/omega 3 fatty acids. Regarding compounds with antioxidant activity, supplementing goats' diets with a mixture of flax and mustard seeds not only helped to reduce the ratio of omega 6/omega 3 fatty acids but also to improve the concentration of tocopherols in milk. Additionally, following the supplementation of goats' diets with a mixture of flax and mustard seeds, a positive influence on milk secondary lipid oxidation indices was observed, as suggested by the value of p-anisidine, which did not significantly increase during storage.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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