

## RESEARCH REGARDING THE EFFECTS OF REPLACING SUNFLOWER MEAL WITH FLAXSEED CAKE IN DAIRY COW DIETS ON THE PRODUCTION AND CHEMICAL COMPOSITION OF MILK

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### Abstract

*This study investigates the effects of substituting 30%, 60% and 100% sunflower meal concentrate mixture with flaxseed meal on milk production and chemical composition, with special emphasis on lipid profile and fatty acid content. The research was conducted over a 30-day period on a herd of 28 Romanian Black Spotted dairy cows, divided into four groups (one control group and three experimental groups). Milk samples were analyzed in an accredited laboratory to determine dry matter, protein, lipid and fatty acid content. The results showed that flaxseed meal supplementation did not negatively affect total lipid or saturated fatty acid content, but significantly increased omega-3 fatty acids and reduced trans and omega-6 fatty acids, thus improving the nutritional quality of the milk. These findings support existing research and highlight the potential of flaxseed cake as a functional ingredient that enhances milk quality for health-conscious consumers. The study opens important perspectives for integrating this nutritional strategy into modern dairy cow farming practices.*

**Key words:** dairy cows, flaxseed cake, fatty acids, milk, nutrition, sunflower meal.

### INTRODUCTION

Currently, at the national level, milk and dairy products hold an essential place in consumer preferences, being regarded as staple foods in the daily diet. This widespread appreciation is due to the remarkable nutritional composition of milk, which provides the body with all essential amino acids, five different types of proteins, fatty acids, four forms of lactose, as well as a wide range of essential minerals and enzymes (Enea et al., 2023).

Milk is not just a common food item, but a fundamental component of the human diet, with a significant contribution to maintaining health. Dairy products, in particular, are vital sources of nutrients that support the harmonious development of the body and can have a positive impact on overall well-being. Due to its rich content of proteins, lipids, carbohydrates, vitamins, and minerals, milk is recognized as a complex food, indispensable in a balanced and healthy diet (Defa et al., 2023).

An important factor that influences both the quality and nutritional value of milk is the diet

of the dairy cows. The composition of milk is directly shaped by the feed the animals consume, with significant effects on its fatty acid profile, vitamin content, and bioactive compounds. Cows nourished with a balanced, nutrient-dense diet, especially one that includes high-quality forages tend to produce milk of superior nutritional quality and improved sensory attributes. In this context, the use of oilseed cakes has gained considerable attention in recent years (Puppel et al., 2012; Puppel et al., 2013).

Oilseed cakes, which are residual by-products obtained after the extraction of oil from oleaginous seeds, represent a valuable resource in animal nutrition. These feed materials are typically rich in proteins, essential fatty acids, fiber, and various bioactive compounds, depending on the type of seed from which they originate (Abedini et al., 2022). When incorporated into the diet of dairy cows, oilseed cakes not only act as nutritionally dense and cost-effective alternatives to conventional feed but also have the potential to improve the nutritional profile of milk, particularly by

enhancing its lipid content with beneficial unsaturated fatty acids.

Moreover, the use of oilseed cakes in cattle feeding practices contributes to sustainable agricultural systems, by reintegrating agro-industrial by-products into the food chain and reducing feed-related waste. Their inclusion supports both environmental and economic sustainability, aligning with contemporary demands for eco-conscious livestock production and functional food development (Oliveira et al., 2016).

Flax has been known and valued for centuries as a source for extracting vegetable oil. One of the by-products of this process is flaxseed cake, traditionally used as animal feed, particularly for cattle. Pressed flaxseed cake retains a significant amount of valuable compounds such as residual lipids, high-quality proteins, soluble fibers, and lignans, which endow it with high potential for extended nutritional and functional applications (Coşkuner & Karababa, 2007; Gutiérrez et al., 2010).

The aim of this research is to investigate the possibility of replacing conventional protein sources in dairy cow feed, specifically sunflower meal, with residues derived from the extraction of extra virgin oils, namely flaxseed cake and to evaluate their impact on milk yield and quality.

## MATERIALS AND METHODS

The study was conducted over a 30-day period on a dairy farm located in the south-central region of Romania, involving 28 Romanian Black Spotted dairy cows (Bălta cu Negru Românească). The animals were divided into four groups, respectively one control group and three experimental groups. Each experimental group underwent a 7-day adaptation period to adjust to the new feed formulation, which included flaxseed cake.

All cows received the same basal diet, consisting of alfalfa hay, corn silage, and a concentrate mixture. The distinction among the groups was made through the composition of the concentrate. In the control group, the concentrate was composed of corn grain, barley, wheat bran, sunflower meal, monocalcium phosphate. In the experimental groups, sunflower meal was replaced with flaxseed cake

at inclusion rates of 30%, 60%, and 100%, respectively. All animals had free access to mineral blocks throughout the trial. The concentrate mixture was administered individually during milking.

The samples were taken from the feed components of the rations and concentrate mixtures and sent to the laboratory, where their chemical composition was determined (dry matter – DM, crude protein, calcium – Ca, phosphorus – P). The energy value of the feed (expressed in UFL – feed units for milk) and the protein value (expressed in PDIN – digestible protein in the intestine based on nitrogen, and PDIE – digestible protein in the intestine based on energy) were obtained by calculation.

The milk production of the cows was measured daily. Milking was performed automatically, and milk samples were collected in sterile containers, stored at 4°C, and transported under controlled conditions to the laboratory analyses. To assess the influence of flaxseed cakes on the quality of milk production, milk samples were collected at the end of the experimental period and sent to an accredited food product analysis laboratory in Romania. The following chemical parameters were determined: dry matter (DM); protein; lipids; fatty acids.

For the statistical analysis of the experimental data, Microsoft Excel was used to calculate the arithmetic mean and the standard error of the mean (SEM). The comparison of means between the control group and the experimental groups (30%, 60%, and 100% flaxseed cake) was performed using analysis of variance (ANOVA), followed by the Tukey post-hoc test to verify whether the differences between means were statistically significant.

The software provided the p-values, which were interpreted as follows:  $p > 0.05$  (NS – the difference between means is not significant),  $p < 0.05$  (\*) – the difference is significant),  $p < 0.01$  (\*\*) – the difference is distinctly significant),  $p < 0.001$  (\*\*\*) – the difference is highly significant). In this way, the real impact of flaxseed cake supplementation on the chemical and lipid parameters of cow's milk was determined. The Table 4 present the mean  $\pm$  SEM values for each parameter, together with the p-values comparing the control group to each experimental group (30%, 60%, and 100% flaxseed cake).

## RESULTS AND DISCUSSIONS

To provide a clearer understanding of the feeding structure applied during the experiment groups, regardless of the protein source included in the concentrate mixtures. Table 1 presents the composition and nutritional value of the rations used for both the control group and the three

experimental groups. The rations were formulated to ensure comparable levels of energy and digestible protein across all groups, regardless of the protein source included in the concentrate mixtures.

The detailed composition and nutritional values of the basal diet and the concentrate mixtures are shown in Table 1.

Table 1. Composition and nutritional value of the rations

Ingredients	kg	DM (Kg)	UFL	PDIN (g)	PDIE (g)	Ca (g)	P (g)
Basal ration							
Alfalfa hay	7.8	6.8	4.9	702	608.4	78	14.8
Ensiled corn	22	5.7	4.6	286	374	26.4	11
Concentrate mixture							
Control group	7.2	88.5	104.8	10302.2	10706.8	306.4	693.3
Experimental group 1	7.2	88.5	106.4	10285.1	10974.7	304.7	684.7
Experimental group 2	7.2	88.5	106.5	10448	11217.6	301	707.2
Experimental group 3	7.2	88.5	106.7	10676.6	11549.2	295.8	738.9
Total ration							
Control group	37	31.4	26.8	2717.6	2735.2	230.8	101.5
Experimental group 1	37	31.4	26.8	2717.6	2735.2	230.8	101.5
Experimental group 2	37	31.4	26.8	2724.8	2771.2	230.5	102.5
Experimental group 3	37	31.4	26.9	2739.2	27922.8	230	104.8

As shown in Table 1, replacing sunflower meal with flaxseed cake at varying inclusion levels did not significantly affect the energy content or digestible protein supply of the rations, thus providing a nutritionally balanced framework for evaluating the experimental effects. While

the Table 1 described the overall nutritional values of the rations, Table 2 provides a detailed breakdown of the concentrate mixtures used for each group, emphasizing how the inclusion of flaxseed cake replaced sunflower meal in the experimental diets.

Table 2. Composition of the concentrate feed mixtures in the study groups' rations

Group	Quantity (kg)					
	Corn	Barley	Wheat bran	Sunflower meal	Flaxseed cake	Monocalcium phosphate
Control group	56.5	14.3	9	19.2	0	1
Experimental group 1	56.5	14.3	9	13.5	5.7	1
Experimental group 2	56.5	9.3	14	7.8	11.4	1
Experimental group 3	56.5	2.3	21	0	19.2	1

The gradual replacement of sunflower meal with flaxseed cake in the experimental groups' rations allowed for the investigation of the effects of this change without significantly altering the nutritional balance of the diets. This strategy ensured the necessary consistency between groups and provided an optimal framework for analyzing the specific impact of flaxseed cake

inclusion on milk production and composition. To highlight the differences in the nutritional profile of the concentrate feed mixtures, depending on the level of sunflower meal replacement with flaxseed cake, the nutritional values expressed per kilogram of feed are presented in Table 3.

Table 3. Nutritional value of the concentrate feed mixtures used in the study groups' rations

Group	Nutritional value (expressed per kilogram of feed)					
	DM (Kg)	UFL	PDIN (g)	PDIE (g)	Ca (g)	P (g)
Control group	6.36	3.9	465	400	747.4	480.1
Experimental group 1	7.26	4.9	688	563	751.1	488.6
Experimental group 2	7.26	4.9	688	563	751.1	488.6
Experimental group 3	7.26	4.9	688	563	751.1	488.6

The values presented in Table 3 indicate a slight increase in energy and digestible protein content in the concentrate mixtures that included flaxseed cake, without significant changes in calcium, phosphorus, or carotene levels. This nutritional balance between groups is essential for an objective evaluation of the effects of flaxseed cake on milk production and composition.

Throughout the experimental period, milk production remained constant across all groups, including the control group and the experimental groups supplemented with 30%, 60%, and 100% flaxseed cake. The daily yields recorded over the entire experimental period were  $22 \pm 0.51$  l/cow/day (control group),  $21.2 \pm 0.52$  l/cow/day (experimental group 1, 30% flaxseed cake),  $21.6 \pm 0.48$  l/cow/day (experimental group 2, 60% flaxseed cake), and  $21.5 \pm 0.47$  l/cow/day (experimental group 3, 100% flaxseed cake), with no statistically significant differences

observed between the groups. These results indicate that the inclusion of flaxseed cake in the cows' diet did not negatively impact milk yield, confirming the stability of production performance while allowing for improvements in milk quality parameters.

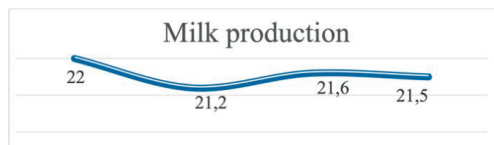


Figure 1. Daily milk production in control and experimental groups

The supplementation of dairy cow rations with linseed cake at levels of 30%, 60%, and 100% generated significant changes in the chemical composition of milk, especially regarding the lipid profile and fatty acid content, as shown in Table 4.

Table 4. Statistical interpretation of the chemical and lipid parameters of cow's milk following flaxseed cake supplementation

Parameter	Control group (mean $\pm$ SEM)	Experimental group 1 (mean $\pm$ SEM)	p Control vs 30%	Experimental group 2 (mean $\pm$ SEM)	p Control vs 60%	Experimental group 3 (mean $\pm$ SEM)	p Control vs 100%
Dry matter (%)	12.39 $\pm$ 0.31	12.75 $\pm$ 0.2 <sup>NS</sup>	0.8814	13.08 $\pm$ 0.31 <sup>NS</sup>	0.5044	13.52 $\pm$ 0.49 <sup>NS</sup>	0.1233
Lipids (g/100g)	4.20 $\pm$ 0.15	4.33 $\pm$ 0.08 <sup>NS</sup>	0.8799	4.44 $\pm$ 0.12 <sup>NS</sup>	0.5315	4.60 $\pm$ 0.14 <sup>NS</sup>	0.1316
Proteins (g/100g)	3.32 $\pm$ 0.10	3.49 $\pm$ 0.09 <sup>NS</sup>	0.6248	3.65 $\pm$ 0.10 <sup>NS</sup>	0.1136	3.86 $\pm$ 0.10 <sup>**</sup>	0.004
$\Sigma$ Trans fatty acids (%)	2.28 $\pm$ 0.04	2.16 $\pm$ 0.06 <sup>NS</sup>	0.261	2.05 $\pm$ 0.05 <sup>**</sup>	0.0071	1.90 $\pm$ 0.02 <sup>***</sup>	0
$\Sigma$ Saturated fatty acids (%)	68.04 $\pm$ 0.92	68.57 $\pm$ 2.43 <sup>NS</sup>	0.9987	69.05 $\pm$ 1.97 <sup>NS</sup>	0.9915	69.70 $\pm$ 3.75 <sup>NS</sup>	0.9645
$\Sigma$ Monounsaturated fatty acids (%)	23.15 $\pm$ 0.32	23.42 $\pm$ 0.23 <sup>NS</sup>	0.9268	23.66 $\pm$ 0.36 <sup>NS</sup>	0.6578	23.98 $\pm$ 0.31 <sup>NS</sup>	0.2605
$\Sigma$ Polyunsaturated fatty acids (%)	3.19 $\pm$ 0.06	3.23 $\pm$ 0.04 <sup>NS</sup>	0.9752	3.26 $\pm$ 0.10 <sup>NS</sup>	0.8847	3.30 $\pm$ 0.05 <sup>NS</sup>	0.6662
$\Sigma$ Omega-3 fatty acids (%)	0.48 $\pm$ 0.00	0.63 $\pm$ 0.01 <sup>***</sup>	0	0.77 $\pm$ 0.01 <sup>***</sup>	0	0.96 $\pm$ 0.02 <sup>***</sup>	0
$\Sigma$ Omega-6 fatty acids (%)	2.82 $\pm$ 0.04	2.67 $\pm$ 0.05 <sup>NS</sup>	0.2483	2.53 $\pm$ 0.05 <sup>**</sup>	0.0057	2.34 $\pm$ 0.07 <sup>***</sup>	0
C10:0 acid (%)	3.67 $\pm$ 0.10	3.71 $\pm$ 0.11 <sup>NS</sup>	0.9894	3.76 $\pm$ 0.06 <sup>NS</sup>	0.8967	3.81 $\pm$ 0.09 <sup>NS</sup>	0.7009
C12:0 acid (%)	4.18 $\pm$ 0.12	4.24 $\pm$ 0.16 <sup>NS</sup>	0.9878	4.30 $\pm$ 0.16 <sup>NS</sup>	0.9146	4.37 $\pm$ 0.07 <sup>NS</sup>	0.7344
C14:0 acid (%)	12.07 $\pm$ 0.24	12.09 $\pm$ 0.30 <sup>NS</sup>	0.9999	12.11 $\pm$ 0.19 <sup>NS</sup>	0.9992	12.14 $\pm$ 0.12 <sup>NS</sup>	0.996
C14:1 acid (%)	1.06 $\pm$ 0.02	1.08 $\pm$ 0.02 <sup>NS</sup>	0.9147	1.10 $\pm$ 0.02 <sup>NS</sup>	0.5725	1.12 $\pm$ 0.03 <sup>NS</sup>	0.2353

Parameter	Control group (mean $\pm$ SEM)	Experimental group 1 (mean $\pm$ SEM)	p Control vs 30%	Experimental group 2 (mean $\pm$ SEM)	p Control vs 60%	Experimental group 3 (mean $\pm$ SEM)	p Control vs 100%
C15:0 acid (%)	0.87 $\pm$ 0.03	0.90 $\pm$ 0.04 <sup>NS</sup>	0.894	0.92 $\pm$ 0.03 <sup>NS</sup>	0.6475	0.96 $\pm$ 0.02 <sup>NS</sup>	0.1766
C16:0 acid (%)	28.75 $\pm$ 0.54	28.60 $\pm$ 0.54 <sup>NS</sup>	0.9974	28.46 $\pm$ 0.32 <sup>NS</sup>	0.9821	28.27 $\pm$ 0.73 <sup>NS</sup>	0.9265
C16:1 acid (%)	1.37 $\pm$ 0.05	1.37 $\pm$ 0.05 <sup>NS</sup>	1	1.37 $\pm$ 0.03 <sup>NS</sup>	1	1.37 $\pm$ 0.02 <sup>NS</sup>	1
C17:0 acid (%)	0.43 $\pm$ 0.01	0.43 $\pm$ 0.01 <sup>NS</sup>	1	0.44 $\pm$ 0.01 <sup>NS</sup>	0.8363	0.44 $\pm$ 0.01 <sup>NS</sup>	0.8363
C18:0 acid (%)	9.56 $\pm$ 0.25	9.74 $\pm$ 0.31 <sup>NS</sup>	0.9644	9.90 $\pm$ 0.20 <sup>NS</sup>	0.8086	10.11 $\pm$ 0.29 <sup>NS</sup>	0.4854
C18:1 cis acid (%)	20.71 $\pm$ 0.60	20.96 $\pm$ 0.63 <sup>NS</sup>	0.9935	21.19 $\pm$ 0.97 <sup>NS</sup>	0.9571	21.49 $\pm$ 0.34 <sup>NS</sup>	0.8441
C18:1 trans- vaccenic acid (%)	1.72 $\pm$ 0.05	1.54 $\pm$ 0.03*	0.0495	1.38 $\pm$ 0.07***	0.0001	1.16 $\pm$ 0.03***	0
C18:2 cis acid (%)	2.46 $\pm$ 0.09	2.37 $\pm$ 0.05 <sup>NS</sup>	0.7226	2.30 $\pm$ 0.05 <sup>NS</sup>	0.2689	2.19 $\pm$ 0.04*	0.0211
C18:3 cis n3 acid (%)	0.48 $\pm$ 0.03	0.60 $\pm$ 0.01*	0.0191	0.70 $\pm$ 0.02***	0	0.84 $\pm$ 0.03***	0
C20:0 acid (%)	0.17 $\pm$ 0.00	0.15 $\pm$ 0.00***	0.0007	0.13 $\pm$ 0.00 <sup>NS</sup>	0	0.11 $\pm$ 0.00***	0
C20:4 n6 acid (%)	0.13 $\pm$ 0.01	0.13 $\pm$ 0.00 <sup>NS</sup>	1	0.14 $\pm$ 0.00 <sup>NS</sup>	0.3911	0.14 $\pm$ 0.01 <sup>NS</sup>	0.3911
C20:5 n3 acid (%)	0.12 $\pm$ 0.00	0.11 $\pm$ 0.00***	0.2716	0.11 $\pm$ 0.00***	0.2716	0.10 $\pm$ 0.00**	0.0055
C4:0 acid	2.02 $\pm$ 0.09	2.21 $\pm$ 0.03 <sup>NS</sup>	0.2804	2.37 $\pm$ 0.05*	0.0121	2.60 $\pm$ 0.10***	0
C6:0 acid	1.56 $\pm$ 0.04	1.73 $\pm$ 0.05 <sup>NS</sup>	0.0643	1.89 $\pm$ 0.03***	0.0002	2.10 $\pm$ 0.06***	0
C8:0 acid	1.63 $\pm$ 0.03	1.69 $\pm$ 0.02 <sup>NS</sup>	0.4749	1.75 $\pm$ 0.03*	0.0349	1.82 $\pm$ 0.03***	0.0006
$\Sigma$ Omega-9 fatty acids (%)	20.67 $\pm$ 0.31	20.78 $\pm$ 0.31 <sup>NS</sup>	0.9982	20.88 $\pm$ 0.54 <sup>NS</sup>	0.9881	21.02 $\pm$ 0.60 <sup>NS</sup>	0.9491

The mean values and standard deviations of the analyzed parameters are presented in Table 4. Statistical analysis showed that dry matter, lipids, and proteins did not undergo significant modifications at 30% and 60% flaxseed cake compared to the control ( $p > 0.05$ ); however, at 100% flaxseed cake, a significant increase in protein content was observed ( $p = 0.004$ ). This may be attributed to the additional protein contribution from the flaxseed cake, confirming the observations of Huang et al. (2022), who reported similar improvements in milk protein composition following supplementation with flaxseed.

Regarding the fatty acid profile, the data indicate important modifications. The omega-3 fatty acid content increased significantly ( $p < 0.05$ ) in all experimental groups, from 0.48% (control) up to 0.96% (100% flaxseed cake), demonstrating the high efficiency of including this ingredient in cow rations to improve milk quality. Similar results were reported by Caroprese et al. (2010), who showed a significant increase in omega-3 fatty acids in the milk of cows fed flaxseed or flaxseed cake, as well as by Puppel et al. (2023),

who reported a 132% increase in linolenic acid (C18:3 n3) and a 194% increase in CLA (C18:2 cis9 trans11) in the milk of organically fed cows supplemented with flaxseed cake.

On the other hand, the trans fatty acid content decreased significantly, from 2.28% to 1.90% ( $p < 0.05$ ). This result is relevant, as trans fatty acids are considered risk factors for human cardiovascular health. These observations are consistent with the research carried out by Oliveira et al. (2021), who showed that supplementation with omega-3-rich vegetable oils reduces the concentration of trans fatty acids in milk. Although Puppel et al. (2023) reported up to a 40% reduction in saturated fatty acids (SFA), especially palmitic acid (C16:0) and myristic acid (C14:0), in this study no significant differences were observed between groups for these parameters ( $p > 0.05$ ).

Although this study did not observe statistically significant increases in monounsaturated fatty acids (MUFA), including oleic acid (C18:1 cis9) ( $p > 0.05$ ), the trend aligns with increases of up to 10% reported by Puppel et al. (2023) after six weeks of flaxseed cake supplementation.

Additionally, the levels of vaccenic acid (C18:1 trans11), an important precursor for CLA synthesis in the mammary gland, increased significantly, confirming the trends reported by Puppel et al. (2023), where a 59% increase was recorded.

Comparisons with recent scientific literature, including studies by Puppel et al. (2023), Huang et al. (2022), Oliveira et al. (2021), and earlier research by Caroprese et al. (2010), validate the consistency of the significant results obtained in this research particularly for protein content, omega-3 fatty acids, and trans fatty acids, and support the hypothesis that flaxseed cake supplementation represents an effective nutritional strategy, applicable both in intensive and organic production systems.

## CONCLUSIONS

Overall, the findings of this study clearly demonstrate that flaxseed cake supplementation in the diets of dairy cows, at increasing inclusion levels of 30%, 60%, and 100%, positively influences milk composition, particularly by enhancing the lipid and fatty acid profiles.

The significant increase in omega-3 fatty acids, coupled with the reduction in trans and omega-6 fatty acids, highlights flaxseed cake as a valuable functional ingredient that improves the nutritional quality of milk without negatively impacting general parameters such as total lipids or saturated fatty acids.

This study confirms the potential of flaxseed cake use for producing milk with enhanced functional value, aligning with the growing demands of health-conscious consumers and opening important perspectives for integrating this approach into modern dairy farming practices.

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# REPRODUCTION, PHYSIOLOGY, ANATOMY



