Agroforest Syst (2025) 99:106
https://doi.org/10.1007/s10457-025-01195-8

Silvopastoral systems with wild sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray) and lipid supplementation: a strategy to improve the fatty acid profile of milk in dairy livestock systems

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Received: 21 October 2024 / Accepted: 7 April 2025 / Published online: 19 May 2025

Abstract This study evaluated the effect of *T. diversifolia* on the fatty acid (FA) profile of milk, with and without lipid supplementation, through two trials. Trial 1 compared a monoculture system of Kikuyu grass—*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone (MONO)—with a silvopastoral system (ISS) incorporating Kikuyu grass and wild sunflower (5% of forage dry matter, FDM). The objective was to assess the effect of shrubs as grazing forage on the FA profile of dairy Holstein cows, using a Latin rectangle design with two periods and 12 cows (experimental units) in a crossover arrangement. Trial 2 evaluated the effect of increasing wild sunflower in the forage diet (17.5% of FDM) and supplementing concentrates with different lipid sources (LS1: Concentrate with commercial saturated rumen bypass fat; LS2: Concentrate with 1% soybean oil, 0.5% fish oil, and 1.5% rumen bypass omega-3 fat; LS3: Concentrate with 2.5% soybean oil and 0.5% fish oil). A completely randomized block design with a 2×3 factorial arrangement (two production systems×three lipid sources in the concentrate) was used. The milk FA profile was positively influenced by wild sunflower intake (5% FDM in trial 1, 17.5% in trial 2) and soybean/fish oil inclusion (trial 2). In trial 1, trans-vaccenic acid (TVA) increased by 10% compared to MONO. In trial 2, TVA increased by 41%. Overall, incorporating wild sunflower and lipid supplements into the ISS diet improved milk nutritional quality.

Keywords Fatty acids · Fish oil · Ruminal biohydrogenation · Soybean oil · Sustainable system

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Introduction

Bovine milk has long been considered an essential food during the early stages of mammalian growth and throughout the later stages of human life, primarily due to its content of protein, fat, minerals, and vitamins (Dror and Allen 2014; Lin et al. 2021; Guo et al. 2024). However, concerns have been raised regarding the relationship between milk consumption and the potential risk of cardiovascular diseases associated with saturated fat intake (Soedamah-Muthu et al. 2011; Jakobsen et al. 2021), as well as the environmental impact of bovine production systems in terms of greenhouse gas emissions. These concerns have fueled campaigns discouraging milk consumption and its products (Willett et al. 2019; Neethirajan 2024).

Nevertheless, modifying the fatty acid (FA) profile of milk and its derivatives to increase the proportion of monounsaturated and polyunsaturated FAs has been shown to positively influence of consumers (Markey et al. 2017; Calvo et al. 2023). This modification can be achieved by altering the cow's diet, supplementing it with sources rich in polyunsaturated FAs, such as vegetable oils or seeds like sunflower, flaxseed, and canola, which contain high levels of linoleic (C18:2 cis 9,12) and linolenic (C18:3 cis 9,12,15) acids (Bettero et al. 2017; Gallardo and Teixeira 2023). Additionally, marine-derived sources such as fish oil or seaweed, which are rich in eicosapentaenoic acid (EPA C20:5 cis 5,8,11,14,17) and docosahexaenoic acid (DHA C22:6 cis 4,7,10,13,16,19), can further enhance the FA profile (Angulo et al. 2012; Toral et al. 2017).

The main objective of these dietary modifications is to alter ruminal biohydrogenation (RBH) to increase the concentration of dietary polyunsaturated FAs in milk (Doreau et al. 2011; Polidori et al. 2018). This is achieved by employing strategies that protect FAs from ruminal degradation, allowing a significant proportion to bypass the rumen and reach the small intestine for absorption and subsequent transfer to the mammary gland (Prieto-Manrique et al. 2018).

The mechanisms involved in modifying the biohydrogenation process focus on the cytotoxicity effects of FAs like DHA and EPA on ruminal bacteria. These effects include alterations in plasma membrane fluidity (Maia et al. 2010; Tedeschi et al. 2021) and the saturation of the RBH process, which slows down the rate of biohydrogenation, particularly in its final stages (Vargas et al. 2019).

Over the last decade, one of the most significant advances in efforts to increase trans-vaccenic FA and conjugated linoleic acid (CLA) levels has been the combination of vegetable oils with fish oil or algae (Angulo et al. 2012; Toral et al. 2017). Likewise, the use of forage shrubs containing secondary metabolites (such as tannins, saponins, and flavonoids) has been explored. These compounds are associated with disruptions in plasma membrane functionality (primarily in bacterial and protozoan membranes) within the RBH process. Tannins, in particular, have been linked to substrate sequestration and competition, which inhibit the growth of these microorganisms (Kholif and Olafadehan 2021; Sun et al. 2022).

Based on this background, this research aims to address the following question: Can the inclusion of fats, vegetable oils, and the shrub *T. diversifolia* in dairy cows diet modify the fatty acid profile of milk? The finding of this study may contribute to expanding the scientific knowledge on sustainable dairy production in the tropical highlands silvopastoral systems, helping to bridge the existing information gap. Furthermore, these insights could assist in generating added value by offering differentiated milk products to both the industry and the consumers.

Materials and methods

Description of the production systems evaluated

This research was conducted at La Montaña Farm, University of Antioquia, in San Pedro de Los Milagros, Colombia. It is classified as a low-mountain humid forest with an average temperature of 15 °C and an altitude ranging between 2350 and 2500 m above sea level (Holdridge 1966). The evaluated systems were:

Intensive silvopastoral system (ISS): The system occupied an area of 1.4 ha and was composed of Kikuyu grass (*C. clandestinus*) and wild sunflower (*T. diversifolia,* 1110 shrubs/ha in Trial 1; > 2500 shrubs/ha in Trial 2). Shrubs for grazing were distributed in linear furrows every 8 m, and the alder trees (*Alnus acuminata* Kunth) were scattered in the paddock (40 trees in total). The electric tape was used to manage daily grazing strips of 380 m^2 with a one-day occupation period, 35 days of grass rest, and 70 days for wild sunflower rest. The shrub rest period was as follows: cows grazed along the same strip every two grass grazing cycles, ensuring both the dry matter (DM) supply and the agronomic recovery of the shrubs. This system did not receive fertilization during the evaluation period (Mejía-Díaz et al. 2017b).

Monoculture system (MONO): This system included kikuyu grass (*C. clandestinus*) covering a total surface area of 1.4 ha. Grazing was managed in daily strips of 380 m^2 (using electrical tape), with a one-day occupation period and 35 days of recovery. MONO was fertilized with synthetic nitrogen during each rotation cycle (42–0–5) at concentrations of 52.73 kg/ha and organic fertilizer (cow manure)

according to the farm management guidelines (Mejía-Díaz et al. 2017b).

Experiments and experimental design

This work derives from two independent trials conducted at different times and with different animals. It should be noted that this manuscript focuses exclusively on the fatty acid profile of milk. However, for general context, the most relevant results regarding production, milk chemistry quality, and dry matter intake, reported by other authors, are referenced below:

In trial 1, using methods to estimate dry matter intake, the research reported a total intake (kg/cow/day) of 20.2 for the ISS and 20.6 for the MONO. Voluntary forage intake evaluated in the pasture yielded a Kikuyu: wild sunflower ratio of 95.5:5 in the ISS. Milk production (L/cow/day) was 25.5 L for the ISS and 25.6 L for the MONO. For fat-corrected milk (FCM), values were reported for the ISS and the MONO as 23.1 and 22.9, respectively. Regarding fat and protein (% and kg), the ISS and the MONO values were 3.4% and 3.3%—0.9 kg and 0.8, respectively, and 2.8% and 2.8%—0.7 kg and 0.7, respectively (Mejía et al. 2017b, c).

In trial 2, differences ($p < 0.05$) were reported in total consumption (kg/cow/day) of 19.26 in the ISS and 17.26 in the MONO, showing a Kikuyu-to-wild sunflower ratio of 84.5:17.5 for the ISS. In milk production (L/cow/day), values with differences ($p < 0.05$) were reported in the ISS of 28.24 L and the MONO of 24.89 L. Likewise, FCM values of 24.6 and 21.9 were reported for the ISS and the MONO, respectively. In terms of fat and protein (% and kg), the values for ISS and MONO were 3.19% and 3.18%—0.89 kg and 0.79, respectively; and 3.00% and 2.89–0.82 kg and 0.72, respectively (Cardona et al. 2019).

Finally, in these and other studies, secondary metabolites have been characterized in *T. diversifolia* (used as a shrub in ISS) (Gallego-Castro et al. 2016; Mejía-Díaz et al. 2017a, b), in concentrations ranging from 1.04 to 7.63 g/kg for tannins (Santacoloma Varón and Granados 2012; Gallego-Castro et al. 2016; Cardona-Iglesias et al. 2017a; Mejía-Díaz et al. 2017b) and 4.34 g/kg for saponins (Cardona Iglesias et al. 2017b).

With the above context, the tests that were the basis of analysis for the response variables in the present work are described below:

Trial 1: The objective was to compare two production systems (MONO and ISS), evaluating the effect of *T. diversifolia* as grazing forage on the FA profile of the dairy Holstein cows (12 cows in total, 6 per system, with an average weight of 542.5 kg), with a milking period of 117 days and average daily production of 25.9 L/day). Cows in the MONO system grazed Kikuyu and were supplemented with concentrate (8 kg animal/day). In contrast, cows in the ISS system grazed Kikuyu and wild sunflower (accounting for 5% forage DM/day) and were supplemented with concentrate (8 kg animal/day). This trial was conducted during a 35-day rotation cycle in which treatments were switched. The proportion of Kikuyu and wild sunflower in the forage diet (95%: 5%) was estimated through dry matter (DM) intake using the indicator, agronomic, and grazing behavior method described by Mejía et al. (2017b).

At the time of the research, the ISS was in its third grazing cycle after establishment (a young system) and had limited wild sunflower shrub development. Research on DM intake found that the intake of cows in a production presented a ratio of 95% *C. clandestinus* and 5% *T. diversifolia* (Mejía et al. 2017b). The systems were compared using a Latin rectangle design with two periods (Periods I and II) and 12 cows (experimental units) in a change arrangement. Table 1 shows the fodder composition and concentrate used.

Trial 2: MONO and ISS were compared after increasing the percentage of wild sunflower in the diet (17.5% of DM) by including a more significant number of shrubs for browsing in the pasture and adding concentrates with different lipid sources (LS) (Table 2) in a completely randomized block design with a two x three factorial arrangement (two production systems, MONO and ISS, and three types of lipid sources in the concentrate). Twelve Holstein cows (with an average weight of 500 in the MONO and 538 kg in the ISS), with 113.5 and 105 milking days, respectively) and an average daily production of 24 L/day were used.

In the ISS, more shrubs were planted as replacements compared to Trial 1, and shrubs had higher growth and forage supply. Research on dry matter

Table 1 Nutritional content of forages and feed concentrate provided in the production systems during Trial 1 (Mejía-Díaz et al. 2017b)

System	GE cal/g	DM %	CP	NDF	ADF	Ca	P
Kikuyu	4085.3	14.1	21.6	55.1	24.7	0.4	0.4
Wild sunflower	3886.7	16.0	21.5	42.0	33.9	2.7	0.40
Concentrate	4015.0	88.0	14.1	18.8	6.8	1.78	0.34

GE: Gross energy; DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; Ca: Calcium; P: Phosphorus

Table 2 Description of treatments and the number of cows in Trial 2

System	Treatment	No. of cows
MONO	Kikuyu grass + concentrate with LS1: Commercial saturated rumen bypass fat	2
	Kikuyu grass + concentrate with LS2: 1% soybean oil, 0.5% fish oil and 1.5% rumen bypass fat omega-3	2
	Kikuyu grass + concentrate with LS3: 2.5% soybean oil and 0.5% fish oil	2
ISS	Kikuyu grass and wild sunflower (17.5% DM) + concentrate with LS1: Commercial saturated rumen bypass fat	2
	Kikuyu grass and wild sunflower (17.5% DM) + concentrate with LS2: 1% soybean oil, 0.5% fish oil and 1.5% rumen bypass fat omega-3	2
	Kikuyu grass and wild sunflower (17.5% DM) + concentrate with LS3: 2.5% soybean oil and 0.5% fish oil	2

MONO: Monoculture system with kikuyu grass; ISS: Intensive silvopastoral system with kikuyu, wild sunflower and alder; LS: type of lipid source in the concentrate

intake in dairy cows found a ratio of 82.5% of *C. clandestinus* and 17.5% of *T. diversifolia* (Cardona et al. 2019). Treatments are described in Table 2. Table 3 shows the bromatological composition of fodder and concentrates.

Field sampling and laboratory analysis

In both trials, cows were mechanically milked twice daily (morning and afternoon) and supplied feed concentrate. Milk samples were collected during each cow's morning and afternoon milking on the measurement days of each evaluation period. Finally, samples taken during morning milking were mixed for each test, and the same procedure was repeated for the samples taken during afternoon milking. Thus, sample pools were obtained for each period, and each sample was placed in a 50 ml bottle. Samples of all fodders and feed concentrates were also taken. They were sent to the Laboratory of Food and Human Nutrition (certified under ISO/IEC 17025:2005, with accreditation certificate 15-LAB-015) of the University of Antioquia, located at the University Research Headquarters,

Table 3 Nutritional content of fodder diets and feed concentrate provided in the production systems during Trial 2 (Adapted from Cardona et al. 2017b)

Chemical fraction, % DM	Kikuyu	Wild sunflower	LS1	LS2	LS3
DM, %	14.4	18	86.9	86.9	86.9
CP, %	22.1	18.3	15.6	15.6	15.6
NDF, %	57.4	44.3	11.7	11.7	11.7
ADF, %	25.2	33.3	4.5	4.5	4.5
EE, %	3.0	3.0	6.0	6.0	6.0
GE, cal/g	4367	4183	4547.2	4589	4521.4
ASH, %	12	15	7.5	7.5	7.5
Ca, %	0.7	0.9	1.5	1.5	1.5
P, %	0.4	0.4	0.4	0.4	0.4
NFE, %	5.6	19.4	59.2	59.2	59.2

DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; EE: Ethereal extract; ASH: Ashes: Ca: Calcium; P: Phosphorus; NFE: Nitrogen-free extract; LS1: Concentrate including commercial saturated rumen bypass fat; LS2: Concentrate including 1% soy oil, 0.5% fish oil and 1.5% rumen bypass fat omega-3; LS3: Concentrate including 2.5% soy oil and 0.5% fish oil

for evaluating the FA profile of milk using gas chromatography.

Fodder, concentrate feed, and milk samples were prepared using fat extraction and methylation according to the methods reported by Bligh and Dyer (1959) and AOAC (2002), respectively. After the fat extraction and methylation process, 100 µL of the organic phase of the sample was dissolved with 900 µL of hexane in a vial. The analysis was performed on an Agilent 7890B gas chromatograph with FID detector with autosampler 7963A, capillary column TR-CN100 60 m×250 µm×0.20 µm TEKNOKROMA, carrier gas: Helium with flow rate 1 mL/min, pressure 30.9 psi, injection volume of 1 µL, Split of 50:1, injector temperature with 220 °C, temperature program with 170 °C for 5 min then 5 °C/min up to 240 °C for 5 min, detector temperature of 250 °C, H2 flow with 30 mL/min, air flow with 350 mL/min and auxiliary gas (N2) flow with 25 mL/min. Signals were collected and manipulated using OpenLab CDS ChemStation software.

Using a TR-CN100 capillary column (60 m×250 µm×0.20 µm) reduces peak overlap compared to shorter columns (15 m and 30 m). It is suitable for observing independent peaks related to *trans* fatty acids. Likewise, flow conditions, pressure, injection volume, injector temperature, and detector temperature were standardized to use this column adequately. The method described above enabled the adequate identification and differentiation of the *trans* fatty acids present in the samples, thanks to the relationship between the efficiency, resolution, and column length.

The method of analysis and quantification for the determination of trans fatty acids is by the official method AOAC 996.06 (AOAC 2001), where a comparison is made with the retention times of each of the 11 fatty acids present (isomers C18:1, C18:2, and C18:3) in a mixture of a standard solution (*trans* standard). The fatty acids (FA) concentration was expressed in g/100 g of sample and are reported as g/100 of FA, standardized (Angulo et al. 2011) using the following formula:

$$\frac{g}{100gFA}(\%) = \frac{\frac{g}{100gsample}}{Tf} \times 100$$

where: ***Tf*** = Total fat.

Evaluated variables

FAs were quantified according to the official AOAC 996.06 method (AOAC 2002) and were identified by comparing sample retention times with those of a reference standard (FAME Mix OD INDUSTRY with 37 components: C4–C24). The atherogenicity index (AI) of FAs was estimated considering lauric (C12:0), myristic (C14:0), and palmitic (C16:0) FAs about the sum of monounsaturated FAs and polyunsaturated FAs (Ulbricht and Southgate 1991):

$$AI = \frac{[C12:0 + (4 * C14:0) + C16:0]}{\left[\left(\sum MUFA\right) + \left(\sum PUFA\right)\right]}$$

where, AI = Atherogenicity index; MUFA = Monounsaturated FA; PUFA = Polyunsaturated FA.

Tables 4 and 5 show the characterization of FAs from grasses, shrubs, and balanced foods used in Trials 1 and 2.

Statistical analysis

In Trial 1, data obtained from the fatty acid profile of milk in MONO and ISS were analyzed using a Latin rectangle design comprising two periods (Periods I and II) and 12 cows (experimental units) with a

Table 4 Fatty acid profile of foods used in Trial 1 (5% wild sunflower in the diet)

FA (g/100 g FA)	Trial 1		
	Kikuyu	Wild sunflower	Concentrate
C12:0 (lauric acid)	0.95	0.64	–
C14:0 (myristic acid)	0.83	0.88	0.43
C16:0 (palmitic acid)	31.65	33.63	16.91
C18:0 (stearic acid)	4.55	5.39	2.82
C18:1n9c (oleic acid)	13.41	16.05	25.25
C18:2n6c (linoleic acid)	13.70	17.21	51.29
C18:3n3 (α-linolenic acid)	24.16	18.37	1.93
∑SFA	44.54	44.51	20.83
∑MUFA	16.09	18.53	25.95
∑PUFA	39.37	36.95	53.22

∑SFA: Sum of saturated fatty acids; ∑MUFA: Sum of monounsaturated fatty acids; ∑PUFA: Sum of polyunsaturated fatty acids

Table 5 Fatty acid profile of foods used in Trial 2 (17.5% wild sunflower in the diet)

FA (g/100 g FA)	Kikuyu	Wild sunflower	LS1	LS2	LS3	SO	FO	SF
C12:0 (lauric acid)	1.59	1.01	–	–	–	–	0.08	1.25
C14:0 (myristic acid)	1.03	0.99	0.76	1.43	0.53	0.07	3.03	1.35
C16:0 (palmitic acid)	29.46	28.19	30.80	19.49	14.62	7.66	27.49	49.72
C18:0 (stearic acid)	4.84	5.77	4.47	4.68	4.43	3.62	7.89	5.14
C18:1n9c (oleic acid)	17.68	12.27	30.38	24.47	24.24	34.08	30.91	33.25
C18:2n6c (linoleic acid)	20.09	21.07	29.77	40.94	47.84	50.68	12.04	8.06
C18:3n3 (α-linolenic acid)	18.73	22.07	2.38	3.44	4.97	2.40	0.92	0.29
C20:5n3 (EPA)	–	–	–	0.71	0.36	–	1.36	–
C22:6n3 (DHA)	–	–	–	0.99	0.68	–	5.12	–
∑SFA	41.01	40.35	36.70	27.01	20.68	12.55	40.01	58.09
∑MUFA	19.58	14.60	30.79	26.68	25.28	34.37	37.91	33.55
∑PUFA	39.42	45.05	32.51	46.31	54.04	53.08	22.07	8.36

MONO: Monoculture; ISS: Intensive silvopastoral system; LS1: Concentrate including commercial saturated rumen bypass fat; LS2: Concentrate including 1% soy oil, 0.5% fish oil and 1.5% rumen bypass fat omega-3; LS3: Concentrate including 2.5% soy oil and 0.5% fish oil; SF: Saturated fat; FO: Fish oil; SO: Soybean oil; ∑SFA: Sum of saturated fatty acids; ∑MUFA: Sum of monounsaturated fatty acids; ∑PUFA: Sum of polyunsaturated fatty acids

change arrangement according to the following statistical model:

$$Y_{ijk} = \mu + V_i + P_j + S_k + \Sigma_{ijk}$$

where: Y_{ijk}= response variable; μ= general mean; V_i= i-th row effect (i=cows) distributed across N(0, σ_V^2); P_j= j-th column effect (j=Period I and Period II) distributed across N(0, σ_P^2); S_k= k-th effect of the system (k=MONO and ISS); Σ_{ijk}= identically distributed experimental error N(0, σ^2).

Trial 2 was based on a completely randomized block design with a two-x-three factorial arrangement (two production systems -MONO and ISS—and three types of lipid sources in the concentrate), using the following statistical model:

$$Y_{ijk} = \mu + B_i + S_j + L_k + S * L_{jk} + \delta_{ijk} + C_1 + C_2 + \Sigma_{ijk}$$

where: Y_{ijk}= response variable; μ= general mean; B_i= i-th block effect or rotation (i= 1.2); S_j= j-th system effect (j= MONO and ISS); L_k= k-th effect of the type of FA source on the feed concentrate (k= LS1, LS2, and LS3); $S * L_{jk}$= jk-th effect of the interaction between the system (j= MONO and ISS) and the type of fatty acid source on the feed concentrate (k= LS1, LS2, and LS3); δ_{ijk}= random error (0, σ2) associated with the repeated measurement (two observations) in each animal in i-th block (i= 1.2), j-th system (j= MONO and ISS), and k-th type of lipid source in the feed concentrate (k = LS1, LS2, and LS3); C_1, C_2= covariates (number of births, milking days); Σ_{ijk}= identically distributed experimental error N(0, σ^2).

The R Project software version 4.4.1 (R Core Team 2024) was used to analyze information from both trials, using the lmer function of statistical package lme4, using ANOVA and determining normality assumptions (Shapiro–Wilk test) and homoscedasticity (Bartlett's test). In Trial 1, the systems evaluated (MONO and ISS) were considered fixed effects, and the cows and evaluation period (Periods I and II) were considered random effects. In Trial 2, the system (MONO or ISS) and type of FA source in the concentrate (LS1, LS2, or LS3) were considered fixed effects, and the cows and block were considered random. The significance level was set at < 0.05.

Results

The results of the two trials are presented below:

Trial 1: A significant effect in milk fat of the production system on milk fat was observed for the TVA (p-value 0.04774), linoleic FAs (p-value 0.0023), and the sum of omega-6 (p-value 0.0017**), with higher content in ISS. Pentadecanoic and behenic FAs were higher in cow's milk from MONO than in ISS (Table 6).

Trial 2: The production system had a significant effect in favor of ISS. Including wild sunflower at 17.5% of DM available for consumption in ISS increased the TVA and CLA contents in the cows' milk in this system compared with MONO. The contents of pentadecanoic, stearic, and arachidic acid were higher in MONO than in ISS. The type of lipid source in the concentrate also significantly affected the concentration of several FAs in milk. Higher contents of lauric, myristic, and palmitic FA were found in the milk of cows that consumed concentrate with a high level of saturated fat (LS1).

LS2 concentrates with lipid sources rich in polyunsaturated fatty acids (PUFAs) increased the TVA compared to LS1 and LS3 and increased CLA compared to LS3. LS3 reduced lauric and palmitic FAs, increased stearic acid, and decreased AI compared with LS1 and LS2. There was a significant S*L interaction for TVA and CLA (Table 7).

Discussion

In Trial 1 (5% wild sunflower), the TVA content of milk from cows in ISS was significantly higher (10% increase) than MONO. Wild sunflower, Kikuyu grass, and concentrate were the sources of unsaturated FAs, and cows in ISS and MONO consumed the same feed concentrate and grass of very similar nutritional compositions (Mejía-Díaz et al. 2017b). The only difference in the diet was the consumption of *T. diversifolia* in ISS.

Ruminal biohydrogenation (RBH) is a detoxification process of unsaturated fatty acids by ruminal

Table 6 Fatty acid profile of the milk of cows in MONO and ISS (with 5% ingestion of DM of *T. diversifolia*) in high Colombian tropical regions

FA (g/100 g FA)	System		SEM	*p-value*
	MONO	ISS		
C4:0 (butyric acid)	1.14	1.12	0.0598	0.7355
C6:0 (caproic acid)	1.08	1.09	0.0491	0.6983
C8:0 (caprylic acid)	0.82	0.81	0.0589	0.5750
C10:0 (capric acid)	5.80	5.48	0.8836	0.4664
C12:0 (lauric acid)	2.62	2.62	0.2340	0.9804
C14:0 (myristic acid)	11.25	11.36	0.4850	0.6436
C15:0 (pentadecanoic acid)	1.14[a]	1.09[b]	0.0268	0.0184
C16:0 (palmitic acid)	29.30	30.05	0.6900	0.2035
C17:0 (heptadecanoic acid)	0.69	0.69	0.0281	0.9673
C18:0 (stearic acid)	15.71	15.07	1.0200	0.3792
C20:0 (arachidic acid)	0.19	0.18	0.0143	0.5589
C22:0 (behenic acid)	0.09[a]	0.07[b]	0.0045	0.0273
C14:1 (myristoleic acid)	0.83	0.81	0.0791	0.4170
C16:1 (palmitoleic acid)	1.08	0.91	0.1531	0.1298
C18:1n9*c* (oleic acid)	24.65	24.59	0.7430	0.9008
C18:1 *t*11 (TVA: *trans*-vaccenic acid)	0.96[b]	1.06[a]	0.0496	0.04774
C18:2 *c*9 *t*11 (CLA: conjugated linoleic acid)	0.36	0.37	0.0161	0.3086
C18:2n6*c* (linoleic acid)	1.55[b]	1.85[a]	0.1434	0.0023
C18:3n3 (α-linoleic acid)	0.40	0.39	0.0292	0.4840
C20:3n6 (eicosatrienoic acid)	0.08	0.08	0.0058	0.1337
C20:4n6 (arachidonic acid)	0.12	0.13	0.0067	0.1310
∑omega-3	0.39	0.38	0.0249	0.5349
∑omega-6	1.65[b]	1.95[a]	0.1440	0.0017**
∑SFA	67.75	68.58	0.9720	0.2537
∑MUFA	25.23	24.76	0.6490	0.2942
∑PUFA	2.03	2.21	0.1750	0.2367
AI	2.72	2.80	0.1450	0.3602

FA: Fatty acid; MONO: Monoculture system with the grass *C. clandestinus;* ISS: Intensive silvopastoral system with *C. clandestinus, T. diversifolia* and *A. acuminate* trees*;* SEM: Standard error of the mean; ∑omega-3: Sum of omega-3 fatty acids; ∑omega-6: Sum of omega-6 fatty acids; ∑SFA: Sum of saturated fatty acids; ∑MUFA: Sum of monounsaturated fatty acids; ∑PUFA: Sum of polyunsaturated fatty acids; AI: Atherogenicity index

Different letters in the same row indicate a significant difference between systems ($p < 0.05$)

Table 7 Composition of the fatty acid profile of milk from cows in MONO and ISS (17.5% DM intake of *T. diversifolia*) and the addition of different fats in the feed concentrate in high Colombian tropical regions

FA (g/100 g FA)	Type of lipid source in the concentrate (LS)			System (S)		SEM	*p-value*		
	LS1	LS2	LS3	MONO	ISS		S	L	S*L
C4:0 (butyric acid)	1.45	1.39	1.30	1.37	1.39	0.0835	0.8558	0.3667	0.8242
C6:0 (caproic acid)	0.93	0.89	0.75	0.85	0.86	0.0569	0.9021	0.1725	0.4914
C8:0 (caprylic acid)	0.55^{a}	0.53^{ab}	0.42^{b}	0.49	0.51	0.0108	0.6802	0.0336	0.1763
C10:0 (capric acid)	3.17	4.21	3.43	3.14	4.06	1.6185	0.3035	0.1563	0.4946
C12:0 (lauric acid)	1.77^{a}	1.74^{a}	1.40^{b}	1.65	1.62	0.0648	0.7398	0.0491	0.3934
C14:0 (myristic acid)	7.95^{ab}	8.05^{a}	6.57^{b}	7.62	7.43	1.8492	0.6108	0.0204	0.2995
C15:0 (pentadecanoic acid)	0.85^{b}	1.04^{a}	0.96^{a}	0.99^{a}	0.92^{b}	0.0016	0.0415	0.0004	0.4341
C16:0 (palmitic acid)	30.02^{a}	26.98^{b}	24.66^{c}	27.33	27.11	0.0003	0.6915	< 0.0001	0.4546
C17:0 (heptadecanoic acid)	0.55^{b}	0.74^{a}	0.67^{a}	0.67	0.63	0.0004	0.1451	< 0.0001	0.3400
C18:0 (stearic acid)	12.87^{b}	13.86^{b}	16.99^{a}	15.21^{a}	13.94^{b}	0.0005	0.0295	0.0015	0.3238
C20:0 (arachidic acid)	0.16^{b}	0.20^{a}	0.22^{a}	0.20^{a}	0.18^{b}	0.0003	0.0137	< 0.0001	0.7888
C22:0 (behenic acid)	0.05^{b}	0.08^{a}	0.07^{a}	0.07	0.07	0.0004	0.8443	< 0.0001	0.1023
C14:1 (myristoleic acid)	0.65^{a}	0.55^{ab}	0.42^{b}	0.54	0.56	0.0022	0.8109	0.0177	0.3158
C16:1 (palmitoleic acid)	1.17	1.08	1.06	1.11	1.10	0.0020	0.9622	0.6885	0.5685
C18:1n9*c* (oleic acid)	26.07^{ab}	22.69^{b}	29.36^{a}	26.96	25.13	0.0005	0.1029	0.0007	0.9520
C18:1 *t*11 (TVA: *trans*-vaccenic acid)	6.02^{b}	9.17^{a}	6.28^{b}	5.94^{b}	8.38^{a}	0.5561	0.0026	0.0039	0.0166
C18:2 *c*9 *t*11 (CLA: conjugated linoleic acid)	2.65^{ab}	3.15^{a}	2.26^{b}	2.43^{b}	2.95^{a}	0.0061	0.0076	0.0473	0.0143
C18:2n6*c* (linoleic acid)	2.28	2.12	2.01	2.04	2.24	0.0015	0.2622	0.4305	0.1553
C18:3n3 (α-linoleic acid)	0.38	0.38	0.36	0.36	0.39	0.0002	0.1666	0.5874	0.2938
C20:3n6 (eicosatrienoic acid)	0.06	0.07	0.06	0.06	0.06	0.0001	0.7974	0.6006	0.4476
C20:4n6 (arachidonic acid)	0.09^{ab}	0.10^{a}	0.08^{b}	0.09	0.09	0.0008	0.7995	0.0070	0.0477
C20:5n3 (EPA: eicosapentaenoic acid)	0.11	0.08	0.05	0.09	0.07	0.0004	0.4568	0.8123	0.1877
C22:6n3 (DHA: docosahexaenoic acid)	0.04	0.05	0.04	0.04	0.04	0.0006	0.6346	0.1343	0.263
∑omega-3	0.52	0.44	0.39	0.47	0.43	0.0291	0.6544	0.2390	0.4895
∑omega-6	0.52	0.44	0.39	0.47	0.43	0.0360	0.2297	0.4523	0.1987
∑SFA	60.46	59.82	56.77	59.80	58.24	0.8415	0.2459	0.1052	0.2418
∑MUFA	28.03^{ab}	25.27^{b}	30.81^{a}	29.20	26.87	0.7279	0.0676	0.0051	0.8638
∑PUFA	2.95	3.03	2.59	2.73	2.99	0.2360	0.1749	0.1793	0.1602
AI	2.07^{a}	2.10^{a}	1.51^{b}	1.80	1.99	0.0995	0.1005	0,0006	0.8613

FA: Fatty acid; LS1: Concentrate with commercial saturated rumen bypass fat; LS2: Concentrate with lipid mixture 1 (1% soybean oil, 0.5% fish oil and 1.5% rumen bypass fat omega-3); LS3: Concentrate with lipid mixture 2 (2.5% soybean oil and 0.5% fish oil); MONO: Monoculture system with grass *C. clandestinus;* ISS: Intensive silvopastoral system with *C. clandestinus, T. diversifolia* and *A. acuminate* trees*;* SEM: Standard error of the mean; S*L: System interaction by type of lipid source in the concentrate; ∑omega-3: Sum of omega-3 fatty acids; ∑omega-6: Sum of omega-6 fatty acids; ∑SFA: Sum of saturated fatty acids; ∑MUFA: Sum of monounsaturated fatty acids; ∑PUFA: Sum of polyunsaturated fatty acids; AI: Atherogenicity index

Different letters in the same row indicate a significant difference between diets and systems ($p < 0.05$)

microorganisms (Maia et al. 2010; Yang et al. 2019). TVA formation occurs in the last step of the RBH of PUFAs, a process mediated by the bacteria *Propionibacterium acnes* and *Butyrivibrio proteoclasticus* (Buccioni et al. 2012; Vasta et al. 2019). Therefore, factors that affect the population of these bacteria are directly involved in the alteration of RBH.

Tannins and saponins have been associated with the interruption of RBH and the consequent appearance of TVA in products such as meat and milk (Jayanegara et al. 2011; Vasta et al. 2019; Makmur

et al. 2022). Although secondary metabolites were not evaluated in this research, the results could indicate that a low intake of wild sunflower (5%) was sufficient to affect the biohydrogenation pathways of C18 GAs, possibly due to the tannin and saponin content in this plant (Cardona-Iglesias et al. 2017a; b; Mejía-Díaz et al. 2017b), which may have caused an interruption in the final biohydrogenation step for TVA saturation.

Another study with fistulated sheep showed that adding 100 mg/kg of condensed tannins decreased the population of *Butyrivibrio proteoclasticus*, among other bacteria, leading to a reduction in the RBH of C18 FAs (Costa et al. 2018). Similarly, Oso et al. (2018) determined that the methanol extract of *T. diversifolia* (concentration, 100 mg/ml) inhibited the growth of *P. acnes* under in vitro conditions in sensitivity tests. Ribeiro et al. (2016) fed Holstein × Cebu cows with an inclusion (DM) of 6.5% and 15.4% of *T. diversifolia* as a replacement for the sugar cane, corn, and place-based control diet, found an increase in the TVA content when comparing control treatment and the 6.5% inclusion with the 15.4% inclusion of *T. diversifolia*, both in the morning and afternoon milkings.

In Trial 2, high TVA content was again found in the ISS. The higher amount of wild sunflower in the diet (17.5% DM) and the lipid supplementation generated a 41% increase in this fatty acid compared to MONO. Additionally, conjugated linoleic acid (CLA, C18:2 *cis*9, *trans*11) or rumenic acid also increased in ISS milk.

Rumenic acid in ruminant milk and meat comes from two sources: One is an intermediate product of the RBH of linoleic acid, and the other is through the synthesis in mammary gland tissue and muscle tissue from TVA generated from the RBH of unsaturated FAs such as linoleic acid and linolenic acids. Therefore, the presence of rumenic acid in the milk and meat of ruminants is considered to relate to the RBH of unsaturated FAs directly or indirectly (Conte et al. 2022).

The higher proportion of substrate for biohydrogenation and the presence of secondary metabolites of the wild sunflower could have led to a high TVA content because of an incomplete RBH, which could follow both routes, producing rumenic acid. Some authors have shown that in addition to the secondary compounds in plants, such as tannins and saponins, RBH can also be influenced by long-chain unsaturated FAs (Maia et al. 2010; Huang et al. 2020).

RBH depends on the type and amount of fat entering the rumen (Huyen et al. 2020). Long-chain unsaturated FAs inhibit metabolism by ruminal microorganisms, and the higher the degree of unsaturation, the higher the inhibitory activity; therefore, RBH is modified through differential toxicity of different polyunsaturated FAs, including FAs from fish oil, EPA, and DHA, against ruminal bacteria (Maia et al. 2010). LS2 which contained 1% soybean oil, 0.5% fish oil, and 1.5% n3 bypass fat may have provided a greater substrate for RBH; possibly, the protection of n3 bypass fat was not as effective as expected.

Similar to the present study, Prieto-Manrique et al. (2018) demonstrated the synergistic effect of using ISS with *L. leucocephala* along with fat supplementation, specifically from sunflower oil. These authors reported a linear increase in the TVA content in milk as the addition of sunflower oil increased with 13.3 and 16.7 g/100 g of FA for diets with 20 and 40 g/kg of sunflower oil, respectively.

No investigations associate the combination of fats and this shrub in the specific case of a wild sunflower. However, Ribeiro et al. (2016) reported that it is possible to replace sugar cane with wild sunflower by up to 15% in the feed of milk-producing cows without affecting the productive and metabolic parameters of cows but with an essential impact on the FA profile of milk. In a study evaluating the combined effects of fish oil, soybean oil, and the inclusion of the aromatic plant *Satureja khuzistanica* in the diet of dairy cows, it was found that the secondary metabolites of the plant and fish oil significantly increased CLA and omega-3 (n-3) content (Golbotteh et al. 2024).

Research has identified lipid derivatives, in addition to sesquiterpene lactones and flavonoids, present in *T. diversifolia* leaves (Gallon et al. 2019). These may also contribute to the increase in polyunsaturated fatty acids. When alfalfa silage was included in the diet of dairy cows, omega-3 was significantly increased due to the action of lipid derivatives (Liu et al. 2024). This helps explain the significant interaction in the present work on the type of fat and the production system.

Currently, there are many strategies to improve the content of polyunsaturated fatty acids in bovine milk

through lipid supplementation (oilseeds, fish oil and fishmeal, vegetable oils, microalgae, feeding grasses, and forage shrubs) or genetic factors (Gebereyowhans 2024). Thus, this study confirms that wild sunflower and the addition of rumen-active fat can improve the fatty acid profile of milk, particularly TVA and CLA. It also opens the door for further research to evaluate the effect of secondary metabolites on the lipid profile of milk and other key metabolic processes in livestock systems with sustainable production models.

Conclusions

Incorporating 5% of wild sunflower (trial 1) into the silvopastoral system increased the content of beneficial milk fatty acids, such as trans-vaccenic acid, linoleic acid, and the sum of Omega-6 fatty acids. All of which are associated with positive effects on human health. In trial 2, increasing wild sunflower to 17.5% in the silvopastoral system and incorporating a mixture of lipid sources rich in polyunsaturated fatty acids (soybean oil, fish oil, and rumen bypass omega-3 fat), further increased trans vaccenic acid in milk, along with conjugated linoleic acid.

Silvopastoral systems with *T. diversifolia*, with or without polyunsaturated lipid supplementation, contribute to the production of milk with improved nutritional properties and potentially beneficial compounds for human health. Additionally, they may enhance consumer acceptance and consumption. The results demonstrate that silvopastoral systems, along with strategic fat and oil supplementation, contribute to the competitiveness of dairy farming as a model of sustainable livestock production in the tropical highlands of Colombia and similar regions.

Acknowledgements The authors thank the CODI-910-2014 project the University of Antioquia executed for allowing this research that has contributed to human capital formation. The results will be of importance to society in general.

Author contributions All listed authors have contributed substantially to funding, research design, analysis or interpretation of data, and manuscript drafting. All authors have approved the submitted version.

Funding Open Access funding provided by Colombia Consortium. This research is part of the doctoral thesis "Productive evaluation of intensive silvopastoral systems (ISPS) in high tropical dairy, with supplementation of creole fodder resources and lipid sources." Financed by the GRICA Group of the University of Antioquia (through sustainability resources) and the Sapiencia Fund—Medellín Higher Education Agency (Mayor's Office of Medellín, Antioquia, Colombia).

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The research protocol was based on the Colombian Code of Ethics for the Professional Practice of Veterinary Medicine, Veterinary Medicine and Zootechnics, Law 576, 2000.

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