



Selective and time-dependent transfer of herb-derived terpenes into cow milk without sensory impacts

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ABSTRACT

Herb supplementation is increasingly researched in dairy nutrition, yet the availability of comparative data on the short-term transfer kinetics of herb-derived terpenes into milk and their sensory relevance remains limited. The major objective of the study was to reveal the short-term effects of feed supplementation (i.e., oregano, basil, thyme, rosemary, and yarrow) on milk's volatile compound composition, in accordance with the former findings establishing rapid transfer of terpenes from essential oils to dairy cow milk. Thirty-six Holstein–Friesian cows were assigned to 1 control and 5 herb-supplemented groups. Milk samples were collected at 0, 8, 16, and 24 h following bolus administration and analyzed on raw milk samples by GC-MS. Customer test was performed by 30 untrained panelists on pasteurized milk samples. Transfer of herb-derived terpenes was found to be compound- and plant-specific. Apolar monoterpenes, particularly p-cymene and α -pinene, were most consistently detected, with peak concentrations occurring at 16 to 24 h post-administration. Maximum concentrations ranged from trace levels to 1.28 ng/g (α -pinene, rosemary group). Polar phenolic terpenes (thymol, carvacrol, eugenol) showed restricted or delayed transfer despite high abundance in herbs. No significant differences were

observed in pasteurized milk color, aroma, flavor, or texture across all the treatments. Herb-derived terpenes can be transferred into milk within hours; however, concentrations remain below the sensory thresholds. The study demonstrates the feasibility of herb supplementation without compromising pasteurized milk sensory quality and highlights physicochemical constraints governing feed-to-milk transfer.

Key words: feed–milk transfer, monoterpenes, volatile compounds, dairy cow nutrition, sensory thresholds

INTRODUCTION

Herbs and their essential oils are frequently investigated as plausible dietary supplements in ruminant nutrition due to their diverse bioactive constituents, particularly terpenoids, such as monoterpenes and sesquiterpenes (Froehlich et al., 2017; Dorantes-Iturbide et al., 2022). These compounds are naturally present in many forage plants and aromatic herbs, and they can enter the animal organism through feed intake. Several studies have demonstrated that terpenes originating from pasture plants, conserved forages, or supplemented herbs can be transferred from the feed into the bovine milk (Linehan et al., 2024; El Hawat et al., 2026).

Previous researches indicate that the terpene transfer into milk is strongly influenced by compound-specific physicochemical properties, including molecular size, polarity, and lipophilicity, as well as metabolic transformation within the rumen and peripheral tissues (Viallonista et al., 2000; Lejonklev et al., 2016; Debong

Received March 22, 2026.

Accepted May 10, 2026.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

Table 1. Chemical composition of milk in the control and treatment groups (mean $\pm$ SE)

Day	Parameter	Control	Oregano	Basil	Thyme	Rosemary	Yarrow
1	Fat (%)	3.69 $\pm$ 0.16	3.69 $\pm$ 0.16	3.42 $\pm$ 0.09	3.35 $\pm$ 0.16	3.51 $\pm$ 0.14	3.71 $\pm$ 0.15
14	Fat (%)	3.29 $\pm$ 0.18	3.45 $\pm$ 0.11	3.48 $\pm$ 0.23	3.71 $\pm$ 0.09	3.61 $\pm$ 0.19	3.47 $\pm$ 0.27
1	Protein (%)	3.33 $\pm$ 0.11	3.24 $\pm$ 0.14	3.12 $\pm$ 0.15 ^a	3.13 $\pm$ 0.18	3.26 $\pm$ 0.16	3.13 $\pm$ 0.09
14	Protein (%)	3.49 $\pm$ 0.09	3.53 $\pm$ 0.06	3.39 $\pm$ 0.14 ^b	3.22 $\pm$ 0.25	3.62 $\pm$ 0.12	3.25 $\pm$ 0.07
1	Lactose (%)	4.51 $\pm$ 0.15	4.44 $\pm$ 0.11	4.84 $\pm$ 0.16	4.55 $\pm$ 0.15	4.59 $\pm$ 0.09	4.52 $\pm$ 0.15
14	Lactose (%)	4.36 $\pm$ 0.10	4.56 $\pm$ 0.11	4.67 $\pm$ 0.37	4.51 $\pm$ 0.08	4.74 $\pm$ 0.17	4.63 $\pm$ 0.13

^{a,b}Different superscripts indicate a significant difference ($P < 0.05$).

et al., 2021; Agatonovic-Kustrin et al., 2022). Apolar monoterpenes, such as α -pinene and p-cymene tend to be transferred more readily and persist longer in milk fat, whereas more polar compounds (e.g., thymol, carvacrol, linalool) are often detected at lower concentrations and their durations are found to be shorter in the same milk fraction. The appearance of terpenes in milk has been reported at as early as 8 h after ingestion, with concentrations declining after withdrawal of the relevant dietary source (Lejonklev et al., 2016; Masyita et al., 2022).

In parallel, the flavor and aroma of the milk are known to be sensitive to feed-derived volatile compounds (Cadwallader and Singh, 2009; Kilcawley et al., 2018; Masyita et al., 2022). Terpenes may contribute to the occurrence of characteristic herbal, minty, or thyme-like notes when being present at sufficiently high concentrations (Tornambé et al., 2008). However, several studies suggest that sensory effects occur only above certain concentration thresholds, and are therefore highly dependent on the supplementation level and the composition of the feed (Lejonklev et al., 2016; Masyita et al., 2022).

Despite the growing body of literature on terpene transfer, most studies have focused on individual herbs or essential oils—most commonly oregano—or on single compounds, often using different experimental designs, diets, dosages, and sampling schedules (Lejonklev et al., 2016; Koczura et al., 2021). Consequently, comparative assessment of the transfer kinetics of terpenes from multiple herb species under identical feeding, housing, and analytical conditions remains limited.

Furthermore, although the presence of feed-derived terpenes in milk has been documented, just a few studies have linked quantitatively the milk terpene concentrations with sensory outcomes, being assessed under controlled conditions. In particular, the concentration ranges at which herb-derived terpenes remain sensorially neutral in milk are not well defined, especially when multiple herbs with differing chemical profiles are applied simultaneously (Faulkner et al., 2018). This finding limits the ability to distinguish between analytically detectable transfer and practically relevant sensory impact.

Understanding the transfer kinetics of herb-derived terpenes into milk is relevant for several reasons. From a

product quality and authenticity perspective, milk aroma and flavor stability are regarded as the key factors in tailoring consumers' acceptance. Moreover, unintended feed-related flavor alterations may compromise the product consistency (Cadwallader and Singh, 2009; Kilcawley et al., 2018; Faccia et al., 2022; Wójtowski et al., 2023; El Hawat et al., 2026). At the same time, terpenes are increasingly used as markers of the feeding regimen and the assessment of the authenticity of geographical origin, making the knowledge of their transfer patterns important for milk traceability (Pouloupoulou et al., 2012; Ponnampalam et al., 2025).

In addition, there is growing interest in the controlled modulation of milk composition through dietary strategies, including the possibility of enriching milk with plant-derived bioactive compounds without altering its sensory profile (El-Sayed and Youssef, 2019). Establishing the upper limits of terpene transfer without any sensory perception is therefore essential for defining practical supplementation boundaries.

Based on these considerations, we hypothesized that terpene transfer into milk is compound-specific and time-dependent, and that the concentrations achieved under practical supplementation levels remain below the sensory perception thresholds. Accordingly, the aim of this study was to investigate the transfer kinetics of selected terpene compounds from 5 commonly used herbs—oregano, basil, thyme, rosemary, and yarrow—into the milk of dairy cows under identical experimental conditions, and to evaluate whether the resulting terpene concentrations in the pasteurized milk affect its organoleptic characteristics.

MATERIALS AND METHODS

Ethical Approval and Study Location

The animal experiment was approved by the Ethical Committee for Animal Use of the University of Debrecen, Hungary (protocol no. 22/2023 DEMÁB), and all procedures complied with national and institutional regulations for animal experimentation. The study was conducted on an intensive Holstein–Friesian dairy farm

located in Southeast Hungary (Homokszentgyörgy; 46°06'21.3"N, 17°33'33.9"E).

Animals and Experimental Design

In total, 36 multiparous Holstein–Friesian pregnant cows (with weights approximately of 700 kg) were enrolled in the study, and they were randomly assigned to 6 experimental groups (1 control and 5 herb-supplemented groups), with 6 cows per group. Each group consisted of 3 cows in their second lactation and 3 cows in their third lactation. At the start of the experiment, cows had 149.0 ± 30.44 DIM and an average daily milk yield of 48.46 ± 10.68 kg (mean $\pm$ SD; Table 1). Cows were maintained under commercial farm conditions and remained within their original production groups; therefore, individual DMI was not recorded. Based on farm records, the experimental herd ($n = 61$) had an average TMR intake of approximately 26.61 kg DM/d, with an estimated 10% refusals. These values are consistent with high-producing Holstein–Friesian cows at mid lactation. The absence of individual intake data should be considered when interpreting compound intake and transfer efficiency.

Administration of the 5 herbs (i.e., dried oregano, basil, thyme, rosemary, or yarrow) was carried out over a 14-d period, which served as an adaptation phase for the cows before milk sampling. Cows in the control group received only the basal TMR throughout this period. No separate adaptation or washout periods were applied before the start of herb supplementation.

The experimental design combined 2 complementary components. First, a short-term kinetic approach was applied to characterize the acute appearance and temporal dynamics of herb-derived terpenes in milk within 24 h following bolus administration, consistent with previous studies demonstrating rapid feed-to-milk transfer of volatile compounds (Viallonista et al., 2000; Lejonklev et al., 2013). Second, the 14-d feeding period was included to provide a practical feeding context and to allow the collection of bulk milk samples for organoleptic evaluation, reflecting cumulative exposure rather than kinetic processes. Thus, the study does not aim to assess long-term steady-state transfer, but rather integrates acute kinetic measurements with end-point sensory assessment under short-term supplementation conditions.

Feeding Regimen and Herb Supplementation

All cows were fed the same basal TMR ad libitum throughout the experiment, provided once daily at 0600 h. The TMR composition and nutritive values were determined by near-infrared spectroscopy (Table 2).

For the treatment groups, air-dried and ground whole-plant material of oregano (*Origanum vulgare* L.), basil

Table 2. Physical, nutritive, and bioactive composition of TMR diet

Item ¹	Concentration
Ingredient (g/kg of diet DM)	
Corn silage	411.63
Molasses	22.54
Grass silage	47.00
CCM	72.25
Alfalfa silage	58.95
Bristlecone	22.54
Yeast	3.20
Soybean	156.15
Soypreme	69.51
Premix	59.84
Rapeseed meal	75.24
Nutritive composition (g/kg of diet DM)	
CP	172
aNDFom	303
Lignin	28
NFC	422
NEL (MJ/kg)	6.87
Bioactive compound (mg/g)	
α -Pinene	0.06
Eucalyptol	0.00592
γ -Terpinene	0.35
p-Cymene	0.01
Camphor	0.00504
Linalool	0.34

¹CCM = corn cob mix, on-farm mixed; aNDFom = amylase neutral detergent fiber organic matter. Soypreme, rumen-non degradable protein, xylose plus full fat soy (UBM AGRO ZRT., Pilisvörösvár, Budapest, Hungary).

(*Ocimum basilicum* L.), thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), or yarrow (*Achillea millefolium* L.) was encapsulated into rumen boluses. Herb dosages were standardized to deliver 2 g of essential oil per cow per day, based on predetermined essential oil content (Table 3). This dosage is consistent with previously reported supplementation levels of essential oils in dairy cow nutrition, which typically range between 0.5 and 3 g/d depending on the compound and delivery method (Chaves et al., 2008; Lejonklev et al., 2016). Such levels have been shown to avoid adverse effects on rumen fermentation while still enabling measurable systemic absorption and transfer of volatile compounds into milk (Tornambé et al., 2008; Lejonklev et al., 2013). Therefore, the selected dose represents a physiologically relevant compromise between practical applicability under farm conditions and the analytical requirement to detect feed-derived terpenes in milk.

On d 1, the full daily herbal dose of each herb (Table 3) was administered as a single bolus following the first milking at 0900 h (defined as the 0 h time point). From d 2 to 14, the daily dosage was administered in 2 equal portions following the morning (0900 h) and afternoon (1700 h) milkings to ensure a more even distribution of the herbal dose throughout the day. The daily amount of 100 g of TMR split into 2 boluses of identical quantities was administered to cows in the control group after milk-

Table 3. Essential oil content and orally administered quantities of herbs used in the supplements

Herb	Essential oil content (wt/wt %)	Administered quantity (g/cow per d)
Oregano	2.2	90.91
Basil	1.9	105.26
Thyme	2.8	71.43
Rosemary	3.0	66.67
Yarrow	2.7	74.07

ings for 14 d. Feed intake was not measured, which is addressed as a limitation of the study. The TMR was formulated based on the AMTS software (version 4.18 and model CNCPS 6.55; Agricultural Modeling and Training Systems LLC, Cortland, NY) to cover the needs of a 700-kg cow with an average of 44 kg/d milk yield of 3.8% fat, and 3.3% protein with 10% feed refusal. The daily DMI was calculated to be 26.61 kg per cow.

Given the absence of a prolonged adaptation period, the applied supplementation level was selected to reflect realistic short-term feeding practices and to minimize the risk of excessive ruminal disturbance, while allowing the detection of terpene transfer into milk.

Based on the terpene concentrations determined in the basal TMR (Table 2) and the estimated DMI, cows were exposed to background levels of terpenes originating from the diet. However, this contribution is expected to be substantially lower than the standardized supplementation dose of 2 g of essential oil per cow per day. Previous studies have demonstrated that, although basal diets may contribute to low-level background presence of volatile compounds in milk, direct supplementation represents the dominant source of terpene intake and transfer (Viallonista et al., 2000; Lejonklev et al., 2013). Consequently, the detected temporal increases in terpene concentrations are primarily attributed to the administered herbal boluses rather than the basal diet.

Milk Sampling and Analytical Schedule

Milk samples for terpene analysis were collected before the first bolus administration (0 h) and subsequently at 8, 16, and 24 h after the first supplementation. This sampling design was chosen to capture early transfer dynamics based on previous reports indicating rapid appearance of feed-derived terpenes in milk (Viallonista et al., 2000; Lejonklev et al., 2013). Bulk milk samples for organoleptic evaluation were collected on d 14 of the experimental period were blast chilled on ice and were pasteurized within 8 h and subsequently stored at 4°C for 2 d before sensory evaluation. Samples were pasteurized at 63°C for 15 min for food safety reasons and stored at 4°C until sensory analysis. Individual milk samples designated for quality and bioactive compound analyses

Table 4. Detection limit for each bioactive compound

Compound	LOD value (ng/g)
α -Pinene	0.0300
β -Pinene	0.00805
α -Terpinene	0.0304
Eucalyptol	0.00452
γ -Terpinene	0.00450
p-Cymene	0.00307
Camphor	0.0387
Linalool	0.0171
Borneol	0.0100
Eugenol	0.113
Thymol	0.00814
Carvacrol	0.0227

were frozen immediately after collection on dry ice and stored at -20°C until further analysis.

Chemical Analyses

The bioactive composition of TMR, herbs, and milk samples was determined using HPLC and GC-MS methods. Limits of detection (LOD) for each terpene compound are provided in Table 4. In cases where compound concentrations did not exceed the LOD, values were recorded as zero to allow statistical evaluation, acknowledging that this approach may underestimate low-level presence.

HPLC Analysis of the Bioactive Compounds

The ECOM Quaternary HPLC system ECS05 with photodiode array PDA detector (ECOM s.r.o., Czech Republic, Prague) and autosampler was applied for the detection of the desired compounds. An RP-C18 column (250 mm $\times$ 4.6 mm, 3 μ m, Phenomenex, USA) was used for the separation of compounds. The gradient elution was performed with 50% water and 50% acetonitrile (VWR International, Hungary) for 5 min, followed by a gradual increase in the acetonitrile ratio to 100% for 10 min. Then, the acetonitrile-water ratio gradually returned to 50:50 in 5 min with a flow rate of 0.8 mL/min. The injection volume was 5 μ L. The UV wavelengths were set at 217 and 274 nm. The column oven temperature was 25°C. Before the HPLC analysis, the samples were filtered through 0.45- μ m polytetrafluoroethylene (PTFE) filters (Labex Ltd., Hungary). The chromatograms were processed with Clarity software 9.1 version (DATAA-PEX s.r.o., Prague, Czech Republic). The compounds were identified by comparison with the relative retention time of the standards.

For quantitative analysis, external calibration was performed using commercially available analytical standards of the target terpene compounds (purity $\geq$ 98%; Sigma-Aldrich, St. Louis, MO). Calibration curves were constructed by analyzing a series of standard so-

lutions prepared in appropriate solvent matrices over a concentration range covering expected sample levels (typically 0.001–5.0 µg/mL). Each calibration level was analyzed in triplicate. Linearity was assessed by least squares regression, and the goodness-of-fit was evaluated using the coefficient of determination (R^2), with all compounds showing satisfactory linearity ($R^2 \geq 0.995$). Limits of detection and limits of quantification (LOQ) were determined based on signal-to-noise ratios of 3 and 10, respectively, in accordance with established analytical validation guidelines (ICH, 2005; Eurachem, 2014). In addition, LOD values were verified experimentally by analyzing low-concentration standards and confirming consistent detection under repeatability conditions. Quantification was performed only for signals exceeding the LOQ, whereas values between LOD and LOQ were considered detectable but not reliably quantifiable. Method performance characteristics, including repeatability and instrumental precision, were monitored through replicate injections and periodic analysis of quality control standards throughout the analytical sequence.

HPLC Analysis of the Bioactive Composition of Herb Essential Oils

Air-dried plant material was finely ground using a Retsch MM200 ball mill (Germany). An accurately weighed 0.2-g portion of the powdered sample (Analytical Balance, Xingyun FA2204T, China) was transferred to a 10-mL volumetric flask (VWR International, Hungary) and brought to volume with 96% ethanol. The mixture was vortexed for 1 min and subjected to ultrasound-assisted extraction for 30 min at 25°C using an ultrasonic bath (NEY Ultrasonic 3 QT HEAT, Dentsply NeyTech, Yucaipa, CA) operating at 50 Hz. After extraction, samples were filtered through 0.45-µm membrane filters (VWR International, Hungary) and analyzed by HPLC. Separation was performed on a C18 column using a gradient mobile phase consisting of water and acetonitrile (VWR International, Hungary). The gradient started at 50:50 (vol/vol) for 5 min, followed by a linear increase to 100% acetonitrile over 10 min, and then returned to the initial 50:50 ratio within 5 min. The flow rate was maintained at 0.8 mL/min. The essential oil content of each herb was determined 1 wk before analysis using the Soxhlet extraction method according to ISO 659:1998 (ISO, 1998) by the Institute of Agricultural Chemistry and Soil Science, University of Debrecen, Debrecen, Hungary.

GC-MS Analysis of the Bioactive Composition of Herb Essential Oils

Volatile compound profiles were analyzed using GC-MS on an Agilent 8890 GC system coupled with an Agi-

lent 7000D triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). The mass spectrometer was tuned with perfluorotributylamine at m/z 69.0, 264.0, and 502.0. Automated sample preparation and injection were performed using Gerstel MAESTRO software, and data acquisition was carried out with Agilent Mass Hunter Acquisition software (version 10.0.384.1; https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software?utm_source). Compound identification was based on comparison with the NIST 17 Mass Spectral Library (https://www.nist.gov/programs-projects/tandem-mass-spectral-library?utm_source).

Headspace (HS) analysis was conducted using a Gerstel MPS Headspace system (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) equipped with a 2,500-µL, 65-mm syringe. For HS-GC-MS analysis, 0.5 g of air-dried, ground plant material was placed in 20-mL headspace vials sealed with PTFE-lined septa and aluminum crimp caps. Samples were incubated at 90°C for 20 min with agitation at 250 rpm. Following a 60 s flush, 2,000 µL of headspace was injected at 200 µL/s using a split ratio of 20:1, resulting in an effective injection volume of 100 µL. The inlet temperature was set to 250°C.

Chromatographic separation was achieved on an Agilent HP-5MS UI column (30 m × 250 µm × 0.25 µm). The oven temperature program started at 40°C (held for 1 min), increased to 180°C at 5°C/min without a final hold, and included a total run time of 29 min with a 3 min post-run. Helium (99.999%) was used as the carrier gas at a constant flow rate of 0.475 mL/min. The MS transfer line temperature was maintained at 220°C. Mass spectrometry was performed in electron ionization mode at 70 eV, with a source temperature of 230°C, a solvent delay of 1 min, and scan acquisition over an m/z range of 40 to 500.

Oregano exhibited a high concentration of carvacrol, whereas p-cymene, eugenol, and thymol were each present at levels exceeding 1%. In contrast, eucalyptol, γ-terpinene, and borneol were detected only in trace amounts (Table 5). Basil contained predominantly trace levels of most essential oil components, including eucalyptol, camphor, thymol, and carvacrol; however, p-cymene and eugenol were present at moderate concentrations (1%–3%).

Thyme showed the greatest chemical diversity among the analyzed herbs, with thymol as the dominant compound, followed by p-cymene. Additionally, α-pinene, γ-terpinene, eugenol, and carvacrol were detected at concentrations between 1% and 3%, whereas eucalyptol, camphor, and linalool occurred only in trace amounts. In rosemary, α-pinene and γ-terpinene were the predominant constituents, each exceeding 7%. Moderate levels (1%–3%) of p-cymene, eugenol, thymol, and carvacrol were observed, whereas camphor and borneol were present at concentrations below 1%.

Yarrow exhibited the lowest chemical diversity, with all detected compounds—including p-cymene, camphor, borneol, eugenol, thymol, and carvacrol—present at levels below 3%, with p-cymene being the most abundant.

Organoleptic Evaluation

Pasteurized milk samples intended for organoleptic analysis were subjected to pasteurization immediately after collection and subsequently stored at 4°C for 2 d before sensory evaluation. A total of 30 untrained panelists (10 females and 20 males) participated in the consumer test. Organoleptic evaluation was conducted in a single session before noon over a 2-h period, during which participants attended in sequential groups. Pasteurized milk samples were kept under refrigerated conditions throughout the assessment period. At each session, the required volume of milk was removed immediately before evaluation and served to participants, whereas the remaining samples were promptly returned to refrigeration. The assessment took place under natural lighting conditions, and sufficient space was provided to ensure that each participant was seated individually at a separate table. Each panelist received an evaluation sheet, 6 milk samples, and a cup of water for palate cleansing. Before the evaluation, participants completed a demographic questionnaire. Most panelists were between 18 and 45 yr of age; 3 were aged 46 to 55 yr, and 2 were older than 55 yr. One participant reported being a smoker. Regarding milk consumption habits, 13 participants consumed milk daily, 10 consumed it 2 to 3 times per week, 3 once per week, and 4 on a monthly basis. Each panelist received 50 mL of pasteurized milk at 13°C to 18°C from all 6 sample groups, served in plastic cups labeled with letters to facilitate identification (A—control, B—oregano, C—basil, D—thyme, E—rosemary, and F—yarrow). The sensory questionnaire was developed based on internationally recognized sensory evaluation methodologies (Jeon, 1996; Mendieta-Araica et al., 2011; Lejonklev et al., 2016; Faulkner et al., 2018). It should be noted that the sensory evaluation was conducted using a hedonic approach with untrained panelists, which is primarily suited for assessing general acceptability rather than detecting subtle differences between samples. More sensitive discriminative methods, such as triangle tests, are commonly recommended for identifying small sensory differences, particularly when expected effects are minor (Lawless and Heymann, 2010). Therefore, the applied methodology may have limited sensitivity to detect slight variations in aroma or flavor among treatments. Initially, samples were subjected to a general assessment, during which 4 key quality attributes—color, aroma, flavor, and texture—were rated using a 5-point hedonic scale. Subsequently, these attributes were evaluated in greater

Table 5. The content of bioactive compounds in herb essential oils

Compound (%)	Oregano	Basil	Thyme	Rosemary	Yarrow
α -Pinene	ND ¹	ND	1.75	7.63	ND
Eucalyptol	0.48	0.54	0.16	ND	ND
γ -Terpinene	0.08	ND	3.00	7.13	ND
p-Cymene	1.59	2.21	5.36	2.27	2.48
Camphor	ND	0.02	0.003	0.33	0.003
Linalool	ND	ND	0.08	ND	ND
Borneol	0.02	ND	ND	0.10	0.11
Eugenol	4.49	1.74	1.46	1.17	0.81
Thymol	3.49	0.89	12.25	2.86	1.07
Carvacrol	9.09	0.78	2.43	1.27	0.55

¹ND = not detected.

detail. The specific evaluation criteria and corresponding scales are presented in Table 6.

Statistical Analysis

Milk composition and terpene concentration data were analyzed using univariate ANOVA (GLM) and paired-sample *t*-tests, depending on data structure and detectability. Repeated measurements (0, 8, 16, and 24 h) were obtained from the same cows within each treatment group, resulting in within-subject dependency. To account for this, paired-sample *t*-tests were applied by comparing each post-supplementation time point to the baseline (0 h) within the same animals. This approach enabled identification of the time point at which terpene concentrations significantly exceeded initial values. Linear mixed-effects models (LMM), incorporating cow as a random effect, are generally recommended for repeated measures data as they explicitly account for within-subject correlation and improve statistical inference (Pinheiro and Bates, 2000). However, given the limited sample size ($n = 6$ cows per group) and the exploratory focus on predefined pairwise temporal comparisons, the use of paired *t*-tests was considered appropriate to avoid model overparameterization and instability. Only compounds detected at a minimum of 2 time points in at least 2 cows were included in statistical comparisons. For variables analyzed using the GLM, results are reported as LSM (estimated marginal means) together with their associated SE. Organoleptic data were analyzed using the Kruskal–Wallis nonparametric test. Statistical significance was defined at $P < 0.05$. All analyses were performed using SPSS version 23.0 (IBM Corp.). Given the limited sample size and short experimental duration, statistical analyses were intended to identify detectable trends and time-dependent patterns rather than definitive treatment effects. A methodological limitation of the present study relates to the statistical treatment of repeated measurements. Although paired comparisons were used to account for within-subject dependency, more advanced approaches such as LMM could provide

Table 6. Summary of the attributes and scales applied in the organoleptic evaluation

General evaluation		Detailed evaluation	
Attribute	Evaluation scale	Attribute	Evaluation scale
Color	1 (extremely unpleasant), 2 (unpleasant), 3 (average), 4 (pleasant), 5 (extremely pleasant)	Color	1 (white), 2 (yellowish white), 3 (yellow)
Aroma	1 (extremely unpleasant), 2 (unpleasant), 3 (average), 4 (pleasant), 5 (extremely pleasant)	Aroma	1 (not observed), 2 (mild), 3 (average), 4 (high), 5 (very extreme)
Flavor	1 (extremely unpleasant), 2 (unpleasant), 3 (average), 4 (pleasant), 5 (extremely pleasant)	Sweet	1 (not observed), 2 (mild), 3 (average), 4 (high), 5 (very extreme)
		Oxidized	
Texture	1 (extremely unpleasant), 2 (unpleasant), 3 (average), 4 (pleasant), 5 (extremely pleasant)	Barnyard	1 (not observed), 2 (mild), 3 (average), 4 (high), 5 (very extreme)
		Flavor	
		Sweet	
		Bitter	
		Creamy	
		Oxidized	
		Fruity	
		Barnyard	
		Astringent	
		Off-flavor	
		Garlic or onion	
		Salty	
		Texture	1 (not observed), 2 (mild), 3 (average), 4 (high), 5 (very extreme)
		Smooth	
		Thick	

improved handling of individual variability and temporal correlation by incorporating cow as a random factor. Future studies with larger sample sizes should adopt such modeling frameworks to enhance statistical robustness and generalizability of the findings.

RESULTS

Time-Dependent Transfer of Herb-Derived Bioactive Compounds into Milk

The transfer of herb-derived bioactive compounds into milk showed clear time-dependent and herb-specific patterns, as reflected by the number of cows in which the concentrations of individual compounds exceeded the LOD. In the oregano-supplemented group, only sporadic occurrence of the relevant compounds was observed at 8 h post-administration, whereas by 16 h several compounds, including γ -terpinene, p-cymene, eugenol, thymol, and carvacrol, were detected in cases of increasing number of cows. Further expansion was experienced by 24 h, when carvacrol and thymol were present above the LOD in cases of all or nearly all of the animals (Table 7).

Basil supplementation displayed a generally weaker transfer profile, with p-cymene being the most consistently detected compound across the sampling times, whereas other compounds appeared only intermittently and in cases of fewer cows, indicating limited transfer efficiency. In contrast, thyme supplementation led to the most pronounced and rapid transfer, as α -pinene and p-cymene were already detectable for all the cows even at the initial sampling time, and by 16 and 24 h, a broad

spectrum of compounds—including camphor, eugenol, thymol, and carvacrol—was detected in cases of most of the animals, highlighting that thyme is regarded as the most effective source of transferable bioactives.

Rosemary supplementation resulted in a consistent detection of α -pinene throughout the entire experimental period, with a progressive increase in the number of cows, whose milk exhibited the occurrence of p-cymene, camphor, linalool, and borneol at 16 and 24 h, whereas thymol and carvacrol remained undetectable. Yarrow exhibited the weakest overall transfer, with delayed and limited detection of p-cymene and camphor and only occasional appearance of thymol and carvacrol at the later time points.

Temporal Kinetics of Essential Oil Bioactive Compound Transfer from Diet to Milk

Oregano supplementation resulted in a clear time-dependent appearance of several essential oil-derived bioactive compounds in milk (Figure 1A). Among the detected monoterpenoids, p-cymene showed the highest transfer efficiency, with a mean concentration of 0.32 ng/g, and a pronounced increase over time, peaking at 24 h post-administration (0.99 ng/g).

Eucalyptol, γ -terpinene, borneol, thymol, and carvacrol were not detectable at early sampling points, but became quantifiable from 16 h onward, reaching concentrations of 0.50, 0.61, 0.06, 0.15, and 0.12 ng/g at 24 h, respectively. Apart from p-cymene and borneol, whose concentrations continued to be increased between 16 and 24 h, the remaining compounds' concentrations were stabilized after the first detection. α -Pinene, cam-

Table 7. The number of cows with concentrations over the level of detection

Hours after administration/ bioactive compound	1 ¹	2	3	4	5	6	7	8	9	10
Oregano										
0	—	1	0	2	0	0	0	0	0	0
8	—	0	0	2	0	0	0	0	0	0
16	—	3	4	6	0	1	3	1	5	4
24	—	6	4	6	1	2	5	0	6	6
Basil										
0	—	1	0	5	0	—	0	0	0	0
8	—	1	0	6	0	—	0	0	0	0
16	—	4	1	6	0	—	0	4	0	0
24	—	2	4	5	0	—	0	1	0	0
Thyme										
0	6	0	0	4	0	0	—	0	0	0
8	6	0	0	4	0	0	—	0	0	0
16	6	0	2	6	5	1	—	1	6	6
24	6	0	4	6	6	3	—	2	6	6
Rosemary										
0	6	—	0	3	0	0	0	0	0	0
8	6	—	0	3	0	0	0	0	0	0
16	6	—	0	5	5	1	3	3	0	0
24	6	—	0	6	5	1	5	3	0	0
Yarrow										
0	—	—	—	5	0	0	0	0	0	0
8	—	—	—	4	1	0	0	0	0	0
16	—	—	—	5	5	0	0	1	0	5
24	—	—	—	6	6	2	0	2	0	6

¹Row numbers of bioactive compounds: 1: α -pinene, 2: eucalyptol, 3: γ -terpinene, 4: p-cymene, 5: camphor, 6: linalool, 7: borneol, 8: eugenol, 9: thymol, and 10: carvacrol. — indicates compound not analyzed.

phor, and linalool were detected only in case of using the basal TMR and were absent from both oregano and milk samples. Notably, eugenol, despite its high abundance in oregano essential oil, was not detected in milk.

Basil supplementation led to the detection of a limited number of essential oil-derived compounds in the milk (Figure 1B), reflecting selective uptake and transfer. Eucalyptol and p-cymene were the only compounds being consistently detected at quantifiable levels. Eucalyptol appeared in milk at 16 h post-administration, and its concentration remained stable at 24 h. In contrast, p-cymene was already present in milk at 0 h and its concentration was increased progressively until 16 h, with no further change thereafter. Its early presence likely reflects background contributions from the basal TMR. Other basil constituents, including camphor, eugenol, thymol, and carvacrol, could not be detected in milk at any time points.

Because of supplementation with thyme, several bioactive compounds were detectable in milk, although generally at low concentrations (Figure 1C). α -Pinene and p-cymene exhibited the highest mean concentrations (0.24–0.44 ng/g), whereas α -pinene displayed more pronounced increase at 16 and 24 h compared with earlier sampling points. In contrast, γ -terpinene, camphor, thymol, and carvacrol were below the detection limits during the initial sampling times and could only be sporadically detected thereafter. Despite thymol being the predominant constituent of thyme essential oil, its transfer into

milk proved to be minimal, indicating restricted uptake, extensive metabolic transformation, or both. The detectability of α -pinene and p-cymene before supplementation suggests a background contribution from the basal diet. It should be noted that the detection of these compounds at time 0 was not consistent across all animals or treatment groups, despite their presence in the basal TMR. This variability is likely attributable to low background concentrations near the analytical detection limits, combined with interindividual differences in ruminal metabolism, absorption, and secretion into milk. Previous studies have demonstrated that feed-derived terpenes may appear sporadically in milk under such conditions, even when animals receive identical diets (Pouloupoulou et al., 2012; Lejonklev et al., 2013; Viallonista et al., 2000). In addition, strict handling and administration protocols were applied during the experiment, minimizing the likelihood of cross-contamination between treatment groups. Therefore, the occasional presence of α -pinene and p-cymene before supplementation is more plausibly explained by background dietary exposure and biological variability rather than by cross-contamination.

Supplementation with rosemary resulted in the uptake and translocation of several components of the essential oil into the milk (Figure 1D). α -Pinene, p-cymene, camphor, borneol, and eugenol were observed at varying concentrations, with α -pinene showing the highest mean level (1.28 ng/g) and a significant increase at 16 and 24 h post-admin-

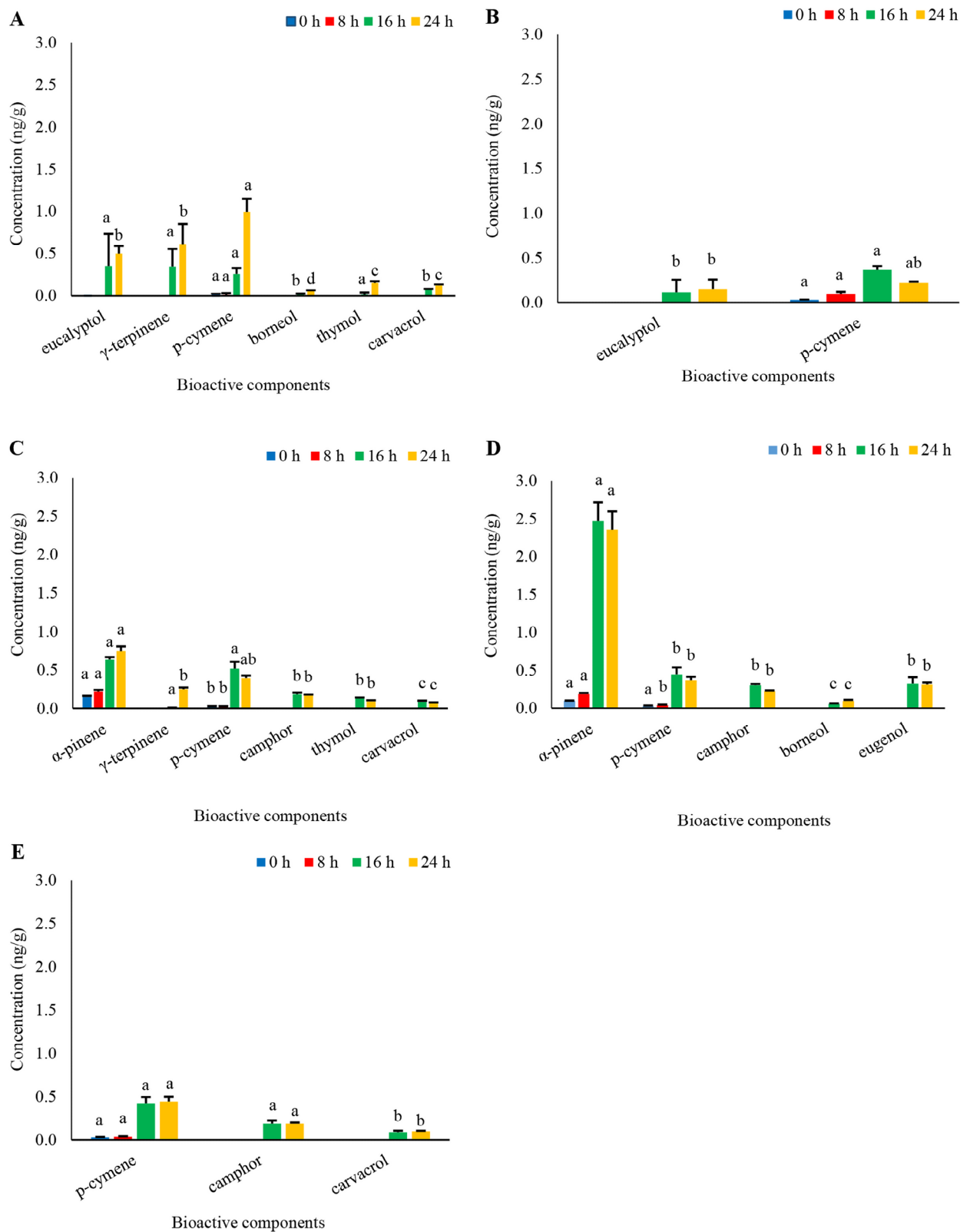


Figure 1. Temporal changes in milk concentrations of bioactive compounds derived from (A) oregano, (B) basil, (C) thyme, (D) rosemary, and (E) yarrow following dietary supplementation. Milk samples were collected at 0, 8, 16, and 24 h after the first administration. Data are presented as mean $\pm$ SD ($n = 6$ cows per treatment group). Different lowercase letters indicate significant differences ($P \leq 0.05$) between sampling times within the same treatment group and compound, based on pairwise comparisons.

Table 8. General evaluation of the main milk characteristics in the control and supplemented groups (LSM $\pm$ SE)

Attribute ¹ or group	Control	Oregano	Basil	Thyme	Rosemary	Yarrow	<i>P</i> -value
Color (n = 30)	3.65 $\pm$ 0.15	3.81 $\pm$ 0.15	3.61 $\pm$ 0.15	3.65 $\pm$ 0.15	3.52 $\pm$ 0.17	3.68 $\pm$ 0.14	0.85
Aroma (n = 30)	3.45 $\pm$ 0.17	3.65 $\pm$ 0.17	3.48 $\pm$ 0.15	3.42 $\pm$ 0.16	3.39 $\pm$ 0.16	3.42 $\pm$ 0.15	0.91
Flavor (n = 30)	3.26 $\pm$ 0.17	3.55 $\pm$ 0.18	3.52 $\pm$ 0.16	3.35 $\pm$ 0.17	3.32 $\pm$ 0.20	3.32 $\pm$ 0.21	0.88
Texture (n = 30)	3.87 $\pm$ 0.13	3.77 $\pm$ 0.15	3.94 $\pm$ 0.14	3.97 $\pm$ 0.18	3.87 $\pm$ 0.15	3.68 $\pm$ 0.17	0.21

¹The attribute was evaluated on a 5-point scale.

istration. Borneol followed a similar temporal pattern, as its concentrations were increased at later sampling points. In contrast, the concentrations of camphor and eugenol were found to be below the detection limits at 0 and 8 h and appeared only after supplementation. The presence of α -pinene and p-cymene before administration suggests a basal dietary contribution, whereas the time-dependent increase of other compounds' concentrations supports their absorption and transfer following rosemary intake.

Yarrow supplementation resulted in the occurrence of a narrow range of bioactive compounds in the milk (Figure 1E). The presence of p-cymene, camphor, and carvacrol could be observed exclusively in the supplemented group. Among these, p-cymene proved to be the predominant compound, with a mean concentration of approximately 0.23 ng/g, and displayed no significant temporal variations across the sampling points. In contrast, camphor and carvacrol were detected only at 16 and 24 h post-administration. The presence of p-cymene at 0 h likely reflects its background occurrence in the basal diet rather than treatment-specific uptake.

Across the examined 5 plant species, dietary supplementation with them resulted in the appearance of selected essential oil-derived bioactive compounds in milk, confirming their uptake and translocation, albeit with marked plant- and compound-specific differences (Figure 1). Oregano and rosemary showed the broadest spectrum and highest concentrations of the transferred compounds, whereas basil and yarrow exhibited more limited transfer profiles. p-Cymene was the most consistently detected compound across all the treatments, frequently influenced by background dietary contributions, whereas compounds such as thymol, carvacrol, and eugenol displayed restricted or delayed transfer despite their abundance in the respective essential oils. Temporal patterns generally indicated increased concentrations at 16 and 24 h post-administration, underscoring the time-dependent kinetics of absorption and secretion into the milk.

Organoleptic Evaluation of Pasteurized Milk Samples

As shown in Table 8, dietary supplementation with oregano, basil, thyme, rosemary, or yarrow did not significantly affect the general sensory characteristics of pasteurized milk compared with that of the control group

($P > 0.05$). The scores obtained for the color of milk ranged from 3.52 $\pm$ 0.17 in the rosemary group to 3.81 $\pm$ 0.15 in the oregano group, in comparison with 3.65 $\pm$ 0.15 in the control. Aroma scores were similarly consistent across all the treatments, varying between 3.39 $\pm$ 0.16 (rosemary) and 3.65 $\pm$ 0.17 (oregano), whereas the control averaged 3.45 $\pm$ 0.17. Flavor scores showed only minor variations, ranging from 3.26 $\pm$ 0.17 (control) to 3.55 $\pm$ 0.18 and 3.52 $\pm$ 0.16 for the milk samples stemming from the oregano- and basil-supplemented groups, respectively. Scores for the texture yielded data being comparable among all the studied groups, with values between 3.68 $\pm$ 0.17 (yarrow) and 3.97 $\pm$ 0.18 (thyme), relative to 3.87 $\pm$ 0.13 for the control.

The supplementation of the feed with different essential oils did not markedly affect the sensory characteristics of the pasteurized milk compared with that of the control group, as no significant differences were observed among treatments for any evaluated attributes ($P > 0.05$; Table 9). Color scores ranged from 1.23 $\pm$ 0.07 in the oregano group to 1.58 $\pm$ 0.11 in the thyme group, whereas sweet aroma varied between 2.42 $\pm$ 0.24 (yarrow) and 2.87 $\pm$ 0.20 (control). Sweet flavor scores were comparable across all the groups, with values from 2.90 $\pm$ 0.19 (yarrow) to 3.32 $\pm$ 0.20 (oregano). Characteristics for creamy flavor tended to be numerically higher in milk from the rosemary group (3.16 $\pm$ 0.15) in comparison with the control (2.39 $\pm$ 0.19), although this difference was not significant ($P = 0.13$). Texture attributes were also similar among the treatments, with smooth texture scores ranging narrowly from 3.39 $\pm$ 0.17 to 3.55 $\pm$ 0.16, and thick texture from 2.10 $\pm$ 0.22 to 2.81 $\pm$ 0.23. Overall, the inclusion of essential oils did not induce off-flavors or undesirable sensory changes in pasteurized milk.

DISCUSSION

The transfer of plant-derived volatile compounds from feed into milk has received increasing attention, primarily as a tool for tracing feeding systems and understanding diet-product interactions, rather than as a means of conferring added biological value to the milk. In this context, the present study aimed to characterize the appearance, temporal dynamics, and sensory relevance of herb-derived terpenoids in cow milk following

Table 9. Effect of dietary essential oil supplementation on the sensory attributes of milk (LSM $\pm$ SE)

Attribute ¹ or group	Control	Oregano	Basil	Thyme	Rosemary	Yarrow	P-value
Color (n = 30)	1.35 $\pm$ 0.09	1.23 $\pm$ 0.07	1.39 $\pm$ 0.08	1.58 $\pm$ 0.11	1.48 $\pm$ 0.11	1.48 $\pm$ 0.12	0.24
Sweet aroma (n = 30)	2.87 $\pm$ 0.20	2.77 $\pm$ 0.22	2.84 $\pm$ 0.21	2.68 $\pm$ 0.24	2.58 $\pm$ 0.20	2.42 $\pm$ 0.24	0.58
Oxidized aroma (n = 30)	1.52 $\pm$ 0.15	1.55 $\pm$ 0.15	1.32 $\pm$ 0.13	1.52 $\pm$ 0.16	1.42 $\pm$ 0.15	1.61 $\pm$ 0.20	0.82
Barnyard aroma (n = 30)	1.58 $\pm$ 0.21	1.42 $\pm$ 0.14	1.45 $\pm$ 0.15	1.42 $\pm$ 0.15	1.58 $\pm$ 0.19	1.52 $\pm$ 0.16	0.99
Sweet flavor (n = 30)	3.16 $\pm$ 0.19	3.32 $\pm$ 0.20	3.26 $\pm$ 0.18	3.00 $\pm$ 0.22	3.26 $\pm$ 0.20	2.90 $\pm$ 0.19	0.71
Bitter flavor (n = 30)	1.29 $\pm$ 0.09	1.32 $\pm$ 0.13	1.23 $\pm$ 0.07	1.32 $\pm$ 0.11	1.45 $\pm$ 0.16	1.42 $\pm$ 0.14	0.97
Creamy flavor (n = 30)	2.39 $\pm$ 0.19	2.48 $\pm$ 0.20	2.61 $\pm$ 0.20	2.81 $\pm$ 0.23	3.16 $\pm$ 0.15	2.48 $\pm$ 0.21	0.13
Oxidized flavor (n = 30)	1.26 $\pm$ 0.15	1.29 $\pm$ 0.14	1.23 $\pm$ 0.12	1.29 $\pm$ 0.14	1.23 $\pm$ 0.12	1.39 $\pm$ 0.15	0.83
Fruity flavor (n = 30)	1.61 $\pm$ 0.16	1.55 $\pm$ 0.17	1.52 $\pm$ 0.17	1.61 $\pm$ 0.17	1.52 $\pm$ 0.19	1.32 $\pm$ 0.12	0.81
Barnyard flavor (n = 30)	1.61 $\pm$ 0.19	1.68 $\pm$ 0.18	1.58 $\pm$ 0.18	1.65 $\pm$ 0.18	1.77 $\pm$ 0.21	1.35 $\pm$ 0.14	0.66
Astringent flavor (n = 30)	1.45 $\pm$ 0.16	1.48 $\pm$ 0.18	1.23 $\pm$ 0.08	1.42 $\pm$ 0.16	1.48 $\pm$ 0.18	1.65 $\pm$ 0.23	0.98
Off-flavor (n = 30)	1.90 $\pm$ 0.22	1.97 $\pm$ 0.22	1.65 $\pm$ 0.20	2.06 $\pm$ 0.24	1.84 $\pm$ 0.22	1.97 $\pm$ 0.25	0.89
Garlic or onion flavor (n = 30)	1.23 $\pm$ 0.11	1.13 $\pm$ 0.09	1.13 $\pm$ 0.10	1.16 $\pm$ 0.09	1.29 $\pm$ 0.15	1.00 $\pm$ 0.01	0.42
Salty flavor (n = 30)	1.35 $\pm$ 0.12	1.42 $\pm$ 0.12	1.29 $\pm$ 0.11	1.84 $\pm$ 0.22	1.65 $\pm$ 0.19	1.23 $\pm$ 0.10	0.19
Smooth texture (n = 30)	3.55 $\pm$ 0.16	3.42 $\pm$ 0.15	3.48 $\pm$ 0.17	3.39 $\pm$ 0.17	3.39 $\pm$ 0.17	3.39 $\pm$ 0.17	0.99
Thick texture (n = 30)	2.10 $\pm$ 0.22	2.19 $\pm$ 0.18	2.32 $\pm$ 0.22	2.52 $\pm$ 0.21	2.81 $\pm$ 0.23	2.23 $\pm$ 0.19	0.21

¹The attribute was evaluated on a 3-point scale.

bolus supplementation, while also assessing whether such supplementation induces detectable changes in milk organoleptic properties. As milk sampling was accomplished 8 to 24 h after administration of the herbal bolus twice a day, the major objective of the study was to reveal the short-term effects of feed supplementation on milk's volatile compound composition, in accordance with the relevant former findings of the literature establishing rapid transfer of terpenes from essential oils to dairy cow milk.

Our findings confirm that selected volatile compounds originating from herbs and the basal diet can be detected in milk; however, their occurrence is limited, compound- and plant-specific, and strongly influenced by the physicochemical properties, rather than by the biological activity typically attributed to essential oils. The limited occurrence of the studied volatile compounds has not resulted in sensory effects or organoleptic alterations in the milk samples; however, it was investigated for pasteurized milk, whose volatile constituents should be eliminated thanks to the heat treatment.

Transfer Kinetics and Compound-Specific Appearance in Milk

The appearance of terpenoid compounds in milk as a consequence of herb supplementation occurred within a relatively short timeframe, with several compounds detected between 8 and 24 h subsequent to the administration. This rapid appearance is consistent with the findings of earlier reports demonstrating that feed-derived volatiles may reach milk within hours after ingestion (Lejonklev et al., 2013; Faccia et al., 2022). Nevertheless, the extent of the transfer observed in the present study was modest, and only a subset of compounds present in the herbs or TMR could be detected in the milk, too.

Across all the treatments, apolar monoterpenes—most notably p-cymene and α -pinene—proved to be the most frequently detected compounds. In contrast, more polar phenolic terpenoids, such as thymol and carvacrol were largely absent or present only at trace levels, even when they were abundant in the administered herbs. This selective transfer highlights the central role of the polarity of the compound, the molecular structure, and the fat solubility in determining the extent of compound's occurrence in the milk. Milk fat acts as a preferential reservoir for lipophilic compounds, favoring the partitioning of apolar terpenes over polar molecules (Lejonklev et al., 2016; Poulopoulou and Hadjigeorgiou, 2021; Agatonovic-Kustrin et al., 2022; El Hawat et al., 2026). Temporal patterns provide further confirmation of the validity of this interpretation. In cases of several treatment groups, p-cymene and α -pinene were already detectable in milk samples collected before herb administration, indicating a background contribution from the basal TMR. Similar findings demonstrated that terpenoids present in control milk often reflect the volatile profile of the diet rather than supplementation alone (Lejonklev et al., 2016). This observation underscores the importance of accounting for the composition of the basal diet when evaluating feed-to-milk transfer, to avoid plausible overestimation of the impact of the supplementation. Although some compounds showed numerical increases at later sampling points, concentrations remained in the low nanograms per gram range throughout the experimental period. Within the 24-h observation period, no consistent increase in concentrations beyond the initial rise was observed; however, the experimental design does not allow conclusions regarding compound accumulation or steady-state dynamics. Previous studies indicate that longer-term sampling is required to evaluate accumulation processes and equi-

librium conditions in feed-to-milk transfer (Vialloninsta et al., 2000; Lejonklev et al., 2013).

Interpretation Limits Regarding Metabolism and Biotransformation

Several former studies have proposed plausible metabolic pathways influencing the fate of terpenoids in ruminants, including degradation, hydroxylation, interconversion, and elimination via hepatic or urinary routes (Tornambé et al., 2008; Malecky et al., 2009; Lejonklev et al., 2013; El Hawat et al., 2026; Priyashantha et al., 2026). The observed differences in terpene transfer among compounds can be primarily attributed to their physicochemical properties, particularly polarity and lipophilicity, which influence absorption, distribution, and partitioning into milk fat. Compounds with higher lipophilicity are generally more readily transferred into milk, consistent with the patterns observed in the present study. While metabolic processes such as ruminal transformation and hepatic metabolism may also influence the fate of terpenes, these mechanisms were not directly investigated in the present experiment. Therefore, their role in explaining the observed transfer patterns should be considered hypothetical and interpreted with caution. Previous studies have reported that plant secondary metabolites can undergo microbial and enzymatic transformations in ruminants, potentially affecting their bioavailability and transfer into milk (Lejonklev et al., 2013). However, further targeted studies are required to confirm these processes under the specific conditions applied here. The observed patterns in milk composition can sufficiently be explained by the differences in molecular polarity, volatility, and lipid affinity. Apolar compounds such as p-cymene and α -pinene are known to diffuse readily into lipid-rich compartments and to exhibit slower elimination rates compared with more polar compounds (Vialloninsta et al., 2000; Poulopoulou and Hadjigeorgiou, 2021). This single physicochemical behavior may account for their higher detection frequency and persistence in the milk relative to the polar phenolic terpenoids. Claims that terpenoids undergo extensive biotransformation before milk secretion (Sapra et al., 2008) cannot be confirmed or refuted based on the present data. Similarly, the possibility that certain compounds were preferentially used or retained by other tissues, rather than being secreted into the milk remains hypothetical. Overall, the interpretation of terpene transfer in this study is based primarily on experimentally observed concentration patterns, whereas mechanistic explanations related to metabolic transformation remain tentative and require further investigation.

Magnitude of the Detected Concentrations and Biological Relevance

A critical point in terms of interpretation of the present findings is the magnitude of compound concentrations analyzed in the milk. Across all the treatments and time points, the established terpenoid concentrations were in the nanograms per gram range. The determined concentrations were found to be below the reported bioactive intake levels by orders of magnitude. This observation has important implications for the interpretation of the biological relevance. Numerous former studies have documented antioxidant, antimicrobial, anti-inflammatory, or other biological effects of the examined essential oils and their components under given experimental conditions (Ghasemzadeh et al., 2016; Leyva-López et al., 2017; Hammoudi Halat et al., 2022; Kamelnia et al., 2023). However, such effects are typically observed at intake levels far exceeding those applied by us resulting in transferred concentrations measured in the present study. Therefore, while the presence of herb-derived compounds in milk can be analytically confirmed, they signify negligible contribution to the physiological condition of the consumers. Accordingly, the results do not underscore a functional food narrative or possible claims on enhanced health-promoting properties of milk derived from herb-supplemented cows. Instead, the detected compounds should be interpreted as markers of dietary origin and transfer dynamics rather than as nutritionally meaningful components.

Sensory Thresholds and Organoleptic Implications

Although volatile compounds could analytically be detected in the milk, no significant differences in the sensory attributes of pasteurized milk were observed as a result of the herb supplementation during feeding. This observation is consistent with the fact that pasteurization may largely contribute to the elimination of the volatiles, as well as the well-established understanding that volatile compounds affect milk aroma and flavor only when being present at or above specific sensory threshold concentrations (Tornambé et al., 2008; Wójtowski et al., 2023). Given the very low concentrations measured, the lack of perceptible sensory changes is therefore not unexpected. Similar conclusions have been reported previously, with no adverse sensory effects observed as a result of oregano supplementation (Lejonklev et al., 2016), and no differences were experienced in aroma or flavor across a range of oregano dosages (Lacerda et al., 2014). In addition, no detectable sensory differences were identified using duo-trio testing after oregano leaf supplementation (Tekippe et al., 2011). Even in cases when volatile compounds appear rapidly in milk, their

sensory impact remains limited unless concentrations approach or exceed the threshold levels (Honkanen et al., 1964). The international literature underscores that pasteurization can significantly influence the concentration and composition of volatile compounds in milk, resulting in either reductions or increases depending on processing conditions and compound stability (Faulkner et al., 2018). Therefore, the lack of detectable sensory effects in our samples may be attributed to these heat-induced changes, and the findings should be interpreted as applicable exclusively to pasteurized milk. The present results provide further evidence to support the notion that analytical detectability does not necessarily signify sensory relevance. In addition, the sensory scores should be interpreted with caution, as the applied hedonic test with untrained panelists may not be sufficiently sensitive to detect subtle differences among samples. Discriminative sensory approaches, such as triangle tests, are generally more appropriate for identifying small variations in flavor or aroma profiles (Lawless and Heymann, 2010). Consequently, the present findings indicate no detectable sensory differences under the applied experimental conditions rather than definitive sensory equivalence among treatments. From a practical perspective, this is advantageous for dairy production, as it suggests that herb supplementation at well-tailored levels does not compromise milk sensory quality. At the same time, these findings indicate that well-considered regulation of the supplementation levels is considered sufficient to preserve sensory neutrality. It is important to be aware of the fact that these supplementation levels are unlikely to lead to biologically meaningful concentrations of the given volatile compounds in the milk, and thus we might not expect plausible health promotion of consumers.

Implications for Feed–Milk Transfer Research

The results of this study contribute to a growing body of evidence demonstrating that feed-derived volatile compounds can be transferred into the milk in a compound-specific and time-dependent manner. However, the extent of this transfer is limited and governed primarily by the physicochemical properties, rather than by the biological activity of the compounds themselves. Composition of the background diet plays a critical role, and its plausible negligence leads to misinterpretation of the supplementation effects. From a methodological perspective, the study highlights the merits of the multi-time point sampling in terms of the comprehension of the transfer kinetics, as well as the importance of integrating chemical analysis with sensory evaluation. Without consumer testing, the assessment of volatile compounds and their presence in milk could be misinterpreted. Future research should prioritize integrated approaches combin-

ing rumen, plasma, and milk analyses to improve comprehension of transfer pathways, plausible physiological impacts, and secretion kinetics. Additionally, studies aiming at defining compound-specific sensory thresholds in milk matrices would further clarify the relationship between the transfer routes and consumer perception.

Overall Interpretation

In summary, herb supplementation of cow feed resulted in limited transfer of the selected terpenoids into the milk, in compliance with the relevant previous findings, as well as the applied methodological setup and the nature of the short-term sampling. The apolar compounds displayed greater propensity for secretion than the polar ones. The transfer occurred rapidly, but the concentrations detected remained low under the applied experimental conditions, being strongly influenced by the polarity of the compounds, fat solubility, and background diet. It should be noted, however, that the 24-h sampling period was designed to capture short-term transfer kinetics and may not have been sufficient to fully characterize longer-term transfer dynamics or potential steady-state levels, as suggested by previous studies on feed-to-milk transfer of volatile compounds (Viallon et al., 2000; Lejonklev et al., 2013). No sensory alterations were observed in the pasteurized milk samples yielded by the treatments, consistently with the impact of heat treatment and the fact that the bioactive concentrations remained below the sensory thresholds. Importantly, the detected levels were orders of magnitude lower than the reported bioactive intake levels, precluding possible functional or health-related implications. These findings reinforce the concept that herb-derived volatiles in milk primarily serve as indicators of establishment of nutrients' dietary origin and transfer dynamics rather than as contributors to nutritional or physiological functionality.

CONCLUSIONS

This study comparatively evaluated the short-term transfer kinetics of terpene compounds from 5 commonly used herbs into cow milk under controlled conditions. Herb-derived terpenes were detected in milk within 8 to 24 h after supplementation, with transfer largely influenced by compound polarity, lipid affinity, and background diet. Apolar monoterpenes showed the greatest transfer, whereas polar phenolic compounds exhibited limited or delayed appearance despite their abundance in the herbs. All detected concentrations were in the nanogram-per-gram range and produced no detectable sensory changes in pasteurized milk. These levels are far below those associated with terpene bioactivity and therefore do not support classifying milk as a terpene-re-

lated functional food. Study limitations include the short experimental period, a single supplementation dose, and the lack of consumer sensory evaluation. Future studies should investigate longer feeding periods, dose–response relationships, and integrated plasma–rumen–milk transfer mechanisms.

NOTES

The publication was supported by the GINOP-PLUSZ-2.1.1-21-2022-00157 and 2023-1.1.1-PIACI-FÓKUSZ-2024-00039 projects, both financed by the National Research, Development and Innovation Office, Budapest, Hungary. We also express our gratitude to the farm for making this project possible. This work is also supported by the University of Debrecen Program for Scientific Publication. Data availability statement: Data are contained within the article. The animal experiment was approved by the Ethical Committee for Animal Use of the University of Debrecen, Hungary (protocol no. 22/2023 DEMÁB), and all procedures complied with national and institutional regulations for animal experimentation. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: HS = headspace; LMM = linear mixed-effects models; LOD = limits of detection; LOQ = limits of quantification; ND = not detected; PTFE = polytetrafluoroethylene.

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