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Research paper

Conserved grassland forage – is the composition of cow's milk influenced by feeding silage compared to hay?

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ABSTRACT

This study aimed to investigate the impact of feeding either silage or hay (harvested from the same parent plant material) on cow's milk composition. Numerous milk constituents (e.g., protein fractions, free amino acids, amines, fatty acid profile, B-vitamins, vitamin E) were analyzed. Results showed that crude protein content was only numerically higher in hay milk compared to silage milk (3.11 and 2.92 %, respectively). Free amino acids showed hardly any differences with values for sum of free essential amino acids of 6.50 and 5.09 mg L^{-1} for hay milk and silage milk, respectively. For vitamin B_1, B_2 and B_6, no differences were found. Vitamin B_{12} and vitamin E were significantly higher in silage milk (3.83 μg L^{-1} and 0.39 mg L^{-1}, respectively) compared to hay milk (2.50 μg L^{-1} and 0.26 mg L^{-1}, respectively). Fatty acid profile was comparable for both types of milk, and sensory analysis showed only minor differences.

1. Introduction

Including forages of various types in ruminants' diets is not only a physiological necessity but also of economic and ecological importance, potentially benefitting net human food supply (Ertl et al., 2015). Feeding high-forage diets (>70 % of feed dry matter) has been linked to increased rumen microbial diversity (Wang et al., 2020) and a decreased requirement for concentrates (Patel, 2012), which is particularly relevant in regions with a large percentage of permanent grassland. While ensiling grassland forages is still the predominant conservation method due to its reduced dependence on weather conditions, silages also carry the risk of causing late blowing defects in cheese; these are more likely to occur if poor quality silages are fed that are potentially contaminated with clostridial endospores (Bernardes et al., 2018). Nevertheless, when ensiled properly, silage is superior in preserving nutrients including vitamins and tocopherols, which can carry over to the milk (Kalač, 2011). If fresh grass or forage conserved as hay is fed as the sole forage source, with no ensiled ration components, the milk can be marketed as a dairy specialty known as "hay milk." This food quality certification, along with cultural traditions and consumers' willingness to purchase specialty products, has increased the market share of hay milk in Central Europe. To date, about 15 % of Austria's milk production is declared as hay milk (BML, 2024). Hay milk is recognized as a "traditional specialty guaranteed" (TSG) product (European Commission, 2016). Traditional hay milk farming in the Austrian Alpine Arc was awarded the status of a Globally Important Agricultural Heritage System (GIAHS) by the FAO (FAO, 2023).

In a recent study, we demonstrated that feeding cows hay instead of silage from the same parent forage source improved feed intake and consequently energy and nutrient intake of dairy cows, resulting in higher milk energy output (Bauer et al., 2025). A higher abundance of water-soluble carbohydrates in hay may have improved palatability. It is known that forage type and plant species impact milk composition, but research on the effects of forage conservation method on the chemical composition of milk is scarce. Data is often confounded through different parent plant material (Shingfield et al., 2005), harvest dates (Coulon et al., 1997), or the effects of grazing (Scherzer et al., 2019). Van den Oever et al. (2021) studied the composition of milk from cows

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that were kept under similar conditions and fed either silage or hay. They found significantly increased contents of essential fatty acids (FAs) such as linoleic acid and α-linolenic acid, as well as the amine putrescine, and the amino acid lysine in the milk of cows fed hay. Although silage milk was found to have higher levels of vitamin B_{12} and spermine, these effects were leveled out with regard to daily milk secretion (Van den Oever et al., 2021). Vitamin B_{12} is exclusively synthesized by bacteria and archaea found predominantly in the rumen and ultimately excreted in milk, and is considered as essential in human nutrition (Matte et al., 2011). Silage milk has been shown to have higher levels of vitamin E (Van den Oever et al., 2021), which can be explained by silage's lower exposure to oxygen and light during storage, resulting in an increased abundance of provitamin A, carotenoids, and tocopherol (Beeckman et al., 2010; Kalač, 2011). Lopez et al. (2024) studied the FA composition of retail milk in Italy and found that TSG hay milk presented a more favorable milk profile with regard to polyunsaturated fatty acids (PUFAs), branched-chain fatty acid (BCFAs), and linoleic acid, when compared to milk labeled as a "mountain product," high-quality fresh milk, and extended shelf-life milk. Consumer surveys in Italy have shown that interest in hay milk is high, as it is often associated with local supply chains, sustainability, and traceability (Peira et al., 2020). Renes et al. (2020) studied several effects such as forage conserved as either silage or hay on quality aspects of sheep milk cheese and did not find a difference regarding conservation method. A study by Velik et al. (2015) investigated the impact of different grass conservation methods on cow's milk FA profile for three consecutive years. Each year, forages were obtained from the same cut and field. While no differences in milk composition were observed in the cows fed differently dried hay, silage-based diets led to lower contents of ω-3 FAs as well as ω-6 FAs.

Given the inconclusive findings, and based on the previous study by Van den Oever et al. (2021), the present study aimed to investigate the effects of feeding grassland forages harvested from the same parent plant material, on the same harvest date, and conserved either as hay or silage, on a wide range of milk constituents. Unlike in the abovementioned study by Van den Oever et al. (2021), where haylage with a DM content of 56 % was compared to hay, the wilted grassland forage for ensiling or barn-drying in the present study had a dry matter (DM) content of 38 % when harvested, i.e., 18 percentage points lower, which is closer to the conditions prevalent in practical farming. Consequently, the present study aimed to investigate the effects of feeding grass conserved as grass silage or hay on the composition of cow's milk.

2. Animals, material, and methods

2.1. Animals and milk sampling

The data for this study were collected as part of a larger-scale feeding experiment examining the effects of feeding grass silage versus hay on dairy cow's feed intake, performance, and fecal microbiota, as well as on the apparent total tract nutrient digestibility (Bauer et al., 2025). The experiment was approved by the Ethics Committee of BOKU University, Vienna, Austria, in accordance with good scientific practice guidelines and national legislation (protocol no. BOKU-2022/007). The feeding trial was carried out at the organic dairy farm of the Secondary School for Agriculture and Environmental and Resource Management HBLA Ursprung, Elixhausen, Salzburg, Austria, from January to February 2023. Pursuant to the European regulations on organic farming (EU, 2018/848; European Parliament and Council of the European Union, 2018), cows were kept in a loose-housing system with cubicles. In order to guarantee individual feeding, the feed bunk was equipped with transponder-regulated CALAN gates (American Calan Inc. Northwood, NH, USA). Five primiparous and thirteen multiparous (second to ninth lactation) Holstein cows were allocated into two homogenous groups, based on days in milk (DIM), milk yield (MY), body weight (BW), and parity. The groups received either grass silage or hay, harvested from the first cut on May 16, 2022, and obtained from one 4 ha field consisting of approximately 65 % grass (*Lolium multiflorum* and *L. perenne*) and 35 % legumes (mainly composed of *Trifolium pratense* and *T. repens*) as well as from 9 ha of permanent grassland composed of roughly 75 % grass (mainly *Dactylis glomerata*, *L. perenne*, *L. multiflorum*, *Trisetum flavescens*, *Alopecurus pratensis*, *Anthoxanthum odoratum*), 20 % legumes (mainly *T. repens*, *T. pratense*), and 5 % herbs (mainly *Taraxacum officinale* and *Plantago lanceolata*). All forages were treated equally before ensiling or barn-drying, i.e., forages were cut using a front-and-rear disc mower combination (Alpha Motion, Pöttinger, Grieskirchen, Austria; Disco 3200s, Class, Harsewinkel, Germany), with an integrated impeller conditioner. Forages were tedded (HIT 8.9 T, Pöttinger, Grieskirchen, Austria) twice on the day of mowing (1:00 p.m. and 7:30 p.m.) to ensure optimal wilting conditions and windrowed after 24 h (Center-swath rake, Top 702C, Pöttinger, Austria). To ensure similar plant material in the silo and the barn-drying box, swaths for either ensiling or barn-drying were picked up in an alternating order using a loading wagon (PRIMO 401 132, Pöttinger, Austria) (Bauer et al., 2025).

Barn-drying of the wilted forage was performed using a dehumidifier placed in front of the ventilator (Heutrocknung SR GmbH, Lengau, Austria) at 29 °C until a DM content of 90 % was reached. The remaining wilted forages for ensiling were inoculated with Bonsilage Plus (Wilhelm Schaumann GmbH, An der Mühlenau, Germany; *Pediococcus pentosaceus*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*) at a rate of approximately 3×108 cfu kg^{-1} of fresh material, compacted with a 14-t wheel loader in a horizontal bunker silo (basal area 80 m^2, 1 m filling height after compaction), and immediately sealed with 2 layers of plastic sheets (Bauer et al., 2025). In total, 10 silage samples were analyzed for preservation quality receiving an average grade of 1.7 (where 1 = very good and 5 = very poor; DLG, 2006). Relative proportions of lactic, acetic, propionic, and butyric acid were 44.7, 45.1, 1.9 and 8.7 %, respectively. The total amount of fermentation acids was 51.9 g kg^{-1} DM (Bauer et al., 2025).

Additionally, every animal received 3.6 kg DM of pelleted concentrate per day, consisting of soybean cake, sugar beet pulp, wheat bran, molasses, minerals, and a vitamin premix at a rate of 510, 227, 180, 30, 43, and 10 g kg^{-1} DM, respectively, in equal shares before milking (5:00 a.m. and 3:00 p.m.). Prior to receiving silage and hay, all animals received a total mixed ration (TMR) for five weeks, followed by a 14-day adaptation period to the experimental feeds (silage or hay), which they were offered *ad libitum*. Data were collected for 21 days following the 14-day adaptation period. The forage DM intake was 13.9 and 16.3 kg, and total DM intake was 17.5 and 19.9 kg per day for the silage and the hay group, respectively. The animals' daily ingested ration, including both forages and the concentrate, contained 2.97 and 2.95 kg crude protein (CP), 8.17 and 9.36 kg neutral detergent fiber (NDF), and 104 and 117 MJ net energy lactation (NE_L) for the silage and the hay group, respectively. Cows entered the 2x3 herringbone milking parlor twice a day, at 6:00 a.m. and 4:30 p.m. Automatic recording of individual milk yields and the collection of individual milk samples were guaranteed through the use of an ICAR-approved milk meter (Metatron MB/Metatron P21, GEA Farm Technologies, Bönen, Germany). The average milk yield was 25.9 and 27.4 kg per day for the silage and the hay groups during the data collection period, respectively ($p = 0.07$). Milk samples were collected from each cow on d 6, 12, and 20 of the 21-day data collection period. For each sampling day, two subsamples (morning and evening; approx. 125 mL each) were collected and pooled. Subsequently, the samples from the three milking days were pooled prior to chemical analysis.

2.2. Chemical analysis

All chemical analysis were performed in quadruplicate.

2.2.1. Protein fractions

Total nitrogen (TN), non-casein N (NCN), and non-protein N (NPN) were determined by the *Kjeldahl* method according to the corresponding IDF/ISO standard (ISO/IDF, 2001a; ISO/IDF, 2001b; ISO/IDF, 2004). Nitrogen from whey protein was calculated from the difference between NCN and NPN, and casein N from TN and NCN, respectively. Protein equivalents were calculated from nitrogen data determined using the factor 6.38.

2.2.2. Free amino acids, polyamines, and biogenic amines

Sample preparation was performed as described by Van den Oever et al. (2021), and ultra-high performance liquid chromatography (UHPLC) analysis was conducted according to Van den Oever & Mayer (2022a). The applied UHPLC method was used to separate a total of 23 free amino acids (fAAs) as well as 13 polyamines (PAs) and biogenic amines (BAs) simultaneously. The following free amino acids were detected: aspartic acid (Asp), serine (Ser), asparagine (Asn), glycine (Gly), glutamic acid (Glu), glutamine (Gln), histidine (His), threonine (Thr), arginine (Arg), alanine (Ala), proline (Pro), γ-aminobutyric acid (GABA), cystine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), norvaline (Nor), ornithine (Orn), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and tryptophan (Trp), as well as the following amines: colamine (Col), agmatine (Agm), histamine (Him), ethylamine (Ea), octopamine (Oct), tyramine (Tym), putrescine (Put), cadaverine (Cad), tryptamine (Try), 2-phenylethylamine (Pea), 3-methylbutylamine (Mba), spermidine (Spd), and spermine (Spn).

2.2.3. Vitamin B_1

Sample preparation was performed as described by Van den Oever et al. (2021). UHPLC analysis was conducted according to Schmidt et al. (2017a) in order to separate the following native forms (vitamers) of vitamin B_1: thiamine, thiamine monophosphate, and thiamine diphosphate.

2.2.4. Vitamin B_2 and B_6

Sample preparation was performed as described by Van den Oever et al. (2021), and UHPLC analysis was conducted according to Schmidt et al. (2017b). The following native forms of vitamin B_2 were separated: riboflavin and flavin mononucleotide, as well as the B_6-vitamers: pyridoxine, pyridoxamine, pyridoxal, pyridoxamine phosphate, pyridoxal phosphate, and pyridoxic acid.

2.2.5. Vitamin B_{12}

Sample preparation and UHPLC analysis were performed according to Van den Oever & Mayer (2022b).

2.2.6. Vitamin E

Sample preparation and HPLC analysis were performed as described by Van den Oever et al. (2021). In whole milk, the main form of vitamin E is α-tocopherol (>85 %), while γ-tocopherol or α-tocotrienol are only minor components (Haug et al., 2007). Since only α-tocopherol was found in the analyzed raw milk samples, it is described as vitamin E throughout this study.

2.2.7. Fatty acids

Sample preparation and gas chromatography analysis were performed as described by Van den Oever et al. (2021). Peaks were identified using SUPELCO 37 component standard as reference (Supelco Co., Ltd).

2.3. Sensory analysis

A sensory panel consisting of ten panelists from the Lebensmitteltechnologisches Zentrum (LMTZ) in Wieselburg (Austria), who have been trained in the sensory description of milk samples, performed a paired comparison with pooled hay milk and silage milk samples, respectively, as well as a quantitative descriptive analysis. Results were evaluated by a Student's *t*-test, and differences between hay milk and silage milk were considered to be significant when $p \leq 0.05$. Further details were described by Van den Oever et al. (2021).

2.4. Statistical analysis

Statistical analysis was performed with SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA) using the generalized linear models (proc glm) procedure. The statistical model for the concentration of milk constituents (Tables 1–4) included the fixed effect of treatment (silage, hay) and the milk yield of cows as a covariable. Milk yield was significantly affected by treatment (Bauer et al., 2025). As differences in milk production between individual cows may affect milk composition, milk yield was included as a covariate to show the results for an average milk yield (26.6 kg d^{-1}). Interactions between treatment and milk yield were only considered at $p \leq 0.10$. The statistical model for the fatty acid composition (Table 5) considered only the treatment as a fixed effect. The statistical model for the daily secretion of milk constituents included the fixed effect of treatment, and, as a covariable, the days in milk (DIM).

Models were chosen showing the smallest root mean square error. Results are displayed as least squares means (LS means) and the standard error of the mean (SEM). Significance was declared at $p \leq 0.05$ and tendencies at $p \leq 0.10$.

3. Results & discussion

Effects of feeding conserved forages as silage or hay on the concentration of milk constituents are shown in Tables 1–5, and Fig. 1. The effect on the total daily secretion of the respective milk constituents can be found as supplementary material in the corresponding tables in Appendix A (Supplementary Material Tables S1–S4). Daily quantities of secretion constituents are reported as well, as these provide insights into the daily production output. Furthermore, the effect of the treatment (silage *versus* hay feeding) on the chemical composition of cow's milk in response to their daily milk yield is shown for certain milk components (isoleucine, serine, sum of free essential amino acids, vitamin $B_{12,}$ and vitamin E) in Fig. S1 and S2. Because cows were randomly assigned to the feeding groups, it was assumed that groups were similar before the onset of the feeding trial. Effects on DM intake and performance can be found in the companion paper (Bauer et al., 2025).

3.1. Protein fractions

The effects of feeding conserved forages as either silage or hay on

Table 1
Effects of the forage conservation method (silage vs. hay) on cow's milk protein fractions.[a]

Item	Treatment (T) (LS means)			*p*-value		
	Silage (*n* = 9)	Hay (*n* = 9)	SEM	T	Milk yield	T x MY
CP	2.92	3.11	0.064	0.151	0.002	
NPN (mg L^{-1})	281.8	243.1	7.128	<0.001	0.021	
NPN in CP equivalents	0.18	0.16	0.005	0.002	0.021	
True protein	2.79	2.96	0.065	0.100	0.003	
Casein	2.32	2.45	0.064	0.184	0.030	
Whey protein	0.47	0.51	0.023	0.294	0.006	
% of casein of true protein	83.12	82.91	0.789	0.856	0.149	

[a] Abbreviations and calculations are: LS means, least squares means; CP, total Nitrogen x 6.38; NPN in CP equivalents, NPN (mg L^{-1}) x 6.38; True protein = (total nitrogen – NPN) x 6.38; casein = (total nitrogen – non-casein nitrogen) x 6.38; whey protein = (non-casein nitrogen – NPN) x 6.38. Values are in % (w/w), unless stated otherwise. Interactions were only considered if $p < 0.10$; least squares means were then calculated for the overall mean of milk yield (MY) (26.6 kg d^{-1}; 21 d).

Table 2
Effects of forage conservation (silage vs. hay) on the concentration of free amino acids (fAA; mg L^{-1}) in cow's milk.[a]

Item	Treatment (T) (LS means)			*p*-value		
	Silage (*n* = 9)	Hay (*n* = 9)	SEM	T	Milk yield	T x MY
fEAA						
Arg	4.61	4.52	0.290	0.825	0.298	
His	0.76	0.90	0.079	0.208	0.763	
Ile	1.24	1.41	0.085	0.162	0.036	0.102
Leu	0.81	1.09	0.066	0.009	0.207	
Lys	2.14	2.70	0.356	0.287	0.207	
Met	0.26	0.27	0.019	0.683	0.085	
Phe	0.48	0.48	0.039	0.904	0.208	
Thr	2.10	1.80	0.122	0.106	0.986	
Trp	0.59	0.56	0.024	0.385	0.149	
Val	1.47	2.02	0.142	0.016	0.370	
fNEAA						
Ala	41.15	40.11	0.920	0.435	0.344	
Asn	0.86	0.75	0.057	0.199	0.609	
Asp	3.72	3.53	0.705	0.851	0.049	
Cys	0.54	0.57	0.058	0.667	0.231	
Gln	11.0	9.34	0.992	0.263	0.546	
Glu	70.66	60.02	7.656	0.342	0.064	
Gly	7.67	5.25	0.707	0.029	0.817	
Orn	0.67	0.64	0.045	0.602	0.737	
Pro	3.88	3.21	0.405	0.262	0.625	
Ser	1.92	1.47	0.141	0.153	0.354	0.072
Tyr	1.13	0.99	0.038	0.028	0.041	
GABA[b]	0.001	0.006	0.004	0.385	0.268	
$\sum$ fEAA	14.47	15.75	0.797	0.275	0.532	
$\sum$ fNEAA	143.2	125.8	9.186	0.204	0.066	
$\sum$ fAA	157.0	141.1	9.303	0.244	0.076	
% of NPN						
$\sum$ fEAA	5.09	6.50	0.261	0.139	0.249	0.037
$\sum$ fNEAA	51.02	52.59	3.379	0.747	0.011	
$\sum$ fAA	55.94	58.91	3.428	0.550	0.010	

[a] Abbreviations and calculations are: LS means, least squares means; fEAA, free essential AA; fNEAA, free non-essential AA; Interactions were only considered if $p < 0.10$; least squares means were then calculated for the overall mean of milk yield (MY) (26.6 kg d^{-1}; 21 d).
[b] GABA: γ-aminobutyric acid.

Table 3
Effects of the forage conservation method (silage vs. hay) on the concentration of polyamines and biogenic amines (mg L^{-1}) in cow's milk.[a]

Item	Treatment (T) (LS means)			*p*-value		
	Silage (*n* = 9)	Hay (*n* = 9)	SEM	T	Milk yield	T x MY
Agm	1.95	1.56	0.229	0.250	0.385	
Col	8.80	8.04	0.799	0.515	0.136	
Ea	0.97	0.70	0.114	0.107	0.544	
Mba	7.4×10^{-2}	6.3×10^{-2}	1.8×10^{-2}	0.775	0.553	
Put	5.4×10^{-2}	5.1×10^{-2}	0.7×10^{-2}	0.791	0.575	
Spd	6.7×10^{-2}	6.4×10^{-2}	1.8×10^{-2}	0.903	0.682	
Spn	0.36	0.37	0.044	0.833	0.724	
Try	0.03	0.12	0.044	0.161	0.785	
Tym	0.11	0.08	0.026	0.522	0.357	
$\sum$ Biogenic amines	12.41	11.05	1.017	0.361	0.164	
$\sum$ Polyamines	2.43	2.05	0.263	0.322	0.487	
$\sum$ Biogenic amines: free AA	0.24	0.20	0.026	0.297	0.019	
$\sum$ Polyamines: free AA	4.7×10^{-2}	3.6×10^{-2}	6.0×10^{-3}	0.223	0.098	
Spn: Spd	0.17	0.16	0.033	0.937	0.834	
Spn: Put	6.75	7.27	0.656	0.583	0.346	
Spn: ORN	0.54	0.60	0.071	0.561	0.897	
Spn: ARG	0.08	0.09	0.011	0.738	0.527	
Put: ORN	8.6×10^{-2}	8.5×10^{-2}	1.4×10^{-2}	0.950	0.624	
Put: ARG	1.2×10^{-2}	1.2×10^{-2}	2.0×10^{-3}	0.918	0.832	
Put: Agm	2.9×10^{-2}	$3.5 \, 10^{-2}$	4.0×10^{-3}	0.300	0.837	
Spn: Agm	0.19	0.26	0.034	0.193	0.526	
Spn: MET	1.45	1.37	0.168	0.716	0.146	
Spd: MET	0.288	0.288	0.075	0.579	0.423	

[a] Abbreviations are: LS means, least squares means; $\sum$ Polyamines = putrescine + agmatine + spermidine + spermine; ND, not detectable; NC, not calculated. Interactions were only considered if $p < 0.10$; least squares means were then calculated for the overall mean of the milk yield (MY) (26.6 kg d^{-1}; 21 d).

protein fractions, i.e., crude protein (CP), true protein, casein, whey protein and non-protein nitrogen (NPN) were minor (Table 1). True protein tended to be higher in hay milk compared to silage milk (+6.1 %). For NPN, the content was higher in silage milk (+15.9 %). A similar trend was shown for NPN in CP equivalents, which was likewise found to be higher (+12.5 %) in the milk of cows fed silage. Ensiling forages results in higher NPN contents compared to (barn) drying due to anaerobic fermentation processes (Givens & Rulquin, 2004). Since grass silages contain lower amounts of rapidly available carbohydrates (e. g., sugars) NPN cannot be efficiently used for microbial protein synthesis in the rumen. No interactions between treatment and milk yield were found for any of the protein fractions.

The content of crude protein in hay milk was comparable to the previous study by Van den Oever et al. (2021), while silage milk showed a numerically lower content in the present study. The content of true protein as well as casein and whey protein concentrations were lower in both silage and hay milk compared to Van den Oever et al. (2021). Results obtained can be compared to literature (e.g., Coulon et al., 1997; Manzocchi, Martin, et al., 2020), even though the crude protein content was lower in the present study. Manzocchi, Hengartner, et al. (2020) reported no significant differences in physico-chemical properties of milk from a hay-based diet. Coulon et al. (1995) stated that cows fed grass silage yielded more milk compared to hay-fed cows, but with lower contents of protein and casein. Protein synthesis is a highly complex physiological process that occurs in the mammary gland and requires vast amounts of energy. The content of milk protein can be influenced by a number of factors, including breed, genetics, age, health status, and nutrition. Differences in gross milk composition and protein profile of three Scandinavian breeds (i.e., Swedish Red, Danish Holstein, and Danish Jersey) were studied by Gustavsson et al. (2014). A reduced milk protein percentage in cow's milk is usually a result of diet, indicating that not enough energy was provided or that essential nutrients were lacking in the ration. Other factors include diet quality, dry matter intake, and the digestibility of forages like grass silage. Farmers producing hay milk often face the problem of low protein contents. As labeling regulations require the protein content in the milk to reflect the amount stated on the packaging (e.g., 3.3 %), this can pose a major problem in the production of commercial retail milk. More true milk protein could be produced by providing diets that are low in rumen-degradable protein or that are balanced for optimal microbial protein synthesis, as both diets generally increase intestinal supplies of amino acids available to the mammary gland for protein synthesis (Alothman et al., 2019; Leiber et al., 2004; Linn, 1988; Murphy & O'Mara, 1993).

3.2. Free amino acids, polyamines and biogenic amines

No treatment effect was observed for the total concentration of free amino acids ($\sum$ fAAs) (Table 2). However, with regard to free essential amino acids ($\sum$ fEAA), there was an increased concentration of Leu (+34.6 %) and Val (+37.4 %) in the milk of cows that were fed hay. The treatment effect for Ile interacted with the milk yield of cows, particularly when cows were fed hay (Supplementary Material Table S2; Fig. S1). An interaction between treatment and milk yield was also

Table 4
Effects of the conservation method (silage vs. hay) on the concentration of vitamins B_1, B_2, B_6, B_{12}, E, and the vitamers of B_1, B_2 and B_6 in cow's milk.[a]

Item	Treatment (T) (LS means)			*p*-value		
	Silage ($n = 9$)	Hay ($n = 9$)	SEM	T	Milk yield	T x MY
Vitamin B_1 (mg L^{-1})	0.14	0.14	0.008	0.577	0.119	
Vitamin B_2 (mg L^{-1})	1.08	1.05	0.054	0.645	0.096	
Vitamin B_6 (mg L^{-1})	0.85	0.83	0.056	0.756	0.556	
Vitamin B_{12} (μg L^{-1})	3.83	2.50	0.188	0.001	0.212	0.007
Vitamin E (mg L^{-1})	0.39	0.26	0.012	0.001	0.001	0.022
Vitamers (μmol L^{-1})						
Thiamine	0.38	0.39	0.028	0.769	0.767	
Thiamine monophosphate	0.16	0.12	0.032	0.415	0.088	
Thiamine diphosphate	ND	ND	NC	NC	NC	
Riboflavin	2.72	2.64	0.145	0.684	0.147	
Flavin mononucleotide	0.15	0.14	0.019	0.689	0.086	
Pyridoxamine	0.39	0.40	0.006	0.322	0.316	
Pyridoxamine phosphate	0.08	0.06	0.018	0.366	0.668	
Pyridoxal	0.50	0.52	0.032	0.685	0.942	
Pyridoxal phosphate	4.05	3.90	0.032	0.749	0.552	
Pyridoxic acid	0.34	0.31	0.065	0.051	0.234	0.042

[a] Abbreviation: LS means, least squares means; Interactions were only considered if $p < 0.10$; least squares means were then calculated for the overall mean of the milk yield (MY) (26.6 kg d^{-1}; 21 d).

observed for Ser (Supplementary Material Table S2; Fig. S1). Ser was slightly increased in the silage group when milk yield increased, whereas it steadily decreased in the hay group. The sum of fEAA was only numerically higher in hay milk, while the sum of fNEAA was numerically higher in silage milk. Of the free non-essential amino acids ($\sum$ fNEAAs), the concentrations of Gly (+46.1 %) and Tyr (+14.1 %) were significantly elevated in silage milk.

Although CP content was lower in hay compared to silage (117 vs. 136 g kg DM^{-1}), the higher DM intake in the hay group resulted in an equal daily CP intake in both groups (2.97 vs. 2.95 kg CP). Since the daily intake of non-fiber carbohydrates (NFC) was significantly higher in hay-fed cows (4.02 vs. 5.17 kg NFC; Bauer et al., 2025), it can be assumed that their microbial protein synthesis was greater, which could potentially have altered the milk's amino acid profile. As one of the branched-chain amino acids (BCAAs), valine is an essential amino acid and cannot, or not sufficiently, be synthesized by animal cells (NASEM, 2021). Valine has been linked to milk protein content, meaning that its higher abundance aligns with the numerically higher milk protein content in hay-fed cows (Table 1). Free amino acids in raw milk can come from the bloodstream or be due to proteolysis after milking (Kunji et al., 1996). Compared to the previous study by Van den Oever et al. (2021), Leu and Val were also elevated in hay milk in the present study. The sum of fAA (as well as sum of fEAA and fNEAA) was numerically higher in the present study. Higher concentrations of essential amino acids are not only nutritionally relevant in human diets, but can also affect the production of fermented dairy products. Milk protein has a high biological value, as it is a good source of essential amino acids (Haug et al., 2007). The main source of essential amino acids is intact protein (also depending on the respective casein and whey protein content of the milk), and not the low level of free amino acids in milk (a substantial part of the NPN fraction). However, this intact protein in milk must first be broken down by various proteinases and peptidases in order to be absorbed and utilized, whereas free amino acids are directly available. Especially in the production of fermented dairy products and also during the early stages of cheese manufacturing, a higher content of free amino acids can have a positive effect on starter cultures. It is well known that many lactic acid bacteria (LAB) isolated from dairy products

Table 5
Least squares means for the effects of the forage conservation method (silage vs. hay) on the fatty acid composition of cow's milk fat.[a]

Item (g 100 g^{-1} milk fat)	Silage ($n = 9$)	Hay ($n = 9$)	SEM	*p*-value
C4:0	4.86	4.94	0.13	0.658
C6:0	3.00	3.08	0.07	0.425
C8:0	1.54	1.62	0.04	0.155
C10:0	3.05	3.26	0.13	0.246
C11:0	0.38	0.38	0.02	0.992
C12:0	3.10	3.33	0.14	0.249
C13:0	0.17	0.15	0.01	0.158
C14i	0.11	0.13	0.01	0.025
C14:0	11.16	11.2	0.31	0.911
C15i	0.28	0.29	0.01	0.420
C15ai	0.49	0.52	0.02	0.345
C14:1	0.91	0.89	0.07	0.807
C15:0	1.41	1.08	0.04	<0.001
C16i	0.22	0.24	0.01	0.046
C16:0	29.11	28.01	0.69	0.274
C17i	0.38	0.52	0.01	0.034
C17ai	0.34	0.38	0.01	0.007
C16:1n9	0.11	0.14	0.01	0.008
C16:1n7	1.23	1.35	0.07	0.236
C17:0	0.69	0.66	0.32	0.034
C18:0	10.38	10.65	0.32	0.554
C18:1$\sum$t_isomers	3.60	3.05	0.20	0.073
C18:1t11	2.21	1.91	0.11	0.076
C18:1n9c	16.95	18.03	0.46	0.114
C18:2n6c	1.71	1.80	0.07	0.344
C20:0	0.13	0.14	0.01	0.443
C18:3n3	0.68	0.76	0.04	0.144
C18:2c9t11	0.81	0.67	0.06	0.122
C22:0	0.04	0.06	0.01	0.371
C20:3n6	0.01	0.03	0.01	0.096
C20:4n6	0.01	0.01	0.01	0.948
C22:2	0.01	0.02	0.01	0.452
C24:0	0.00	0.01	0.01	0.639
C20:5n3	0.05	0.07	0.01	0.300
C22:5n3	0.06	0.05	0.01	0.478
$\sum$ *de novo* fatty acids	26.70	2.74	0.623	0.411
$\sum$ SFAs	66.37	66.31	0.622	0.949
$\sum$ MUFAs	25.02	25.37	0.579	0.670
$\sum$PUFAs	3.28	3.36	0.111	0.630
$\sum$OBCFAs	4.47	4.25	0.099	0.130
ω6-fatty acids	1.72	1.84	0.06	0.211
ω3- fatty acids	0.799	0.88	0.03	0.106
Fatty acid ratios				
ω6: ω3	2.17	2.10	0.074	0.515
C18:2c9t11: C18:1t11	0.36	0.36	0.018	0.810

[a] Abbreviations are: SFA, saturated fatty acids, $\sum$ = C4:0 + C6:0 + C8 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA, monounsaturated fatty acids, $\sum$ = C14:1 + C16:1n9 + C16:1n7 + C18:1 $\sum$t_isomers + C18:1t11 + C18:1n9c; PUFA, polyunsaturated fatty acids, $\sum$ = C18:2n6c + C18:3n3 + C18:2c9t11 +C20:3n6 + C20:4n6 + C22:2 + C20:5n3 + C22:5n3; OBCFA, odd- and branched-chain fatty acids, $\sum$ = C11:0 + C13:0 + C14i + C15i + C15ai + C15:0 + C16i + C17i + C17ai + C17:0; $\sum$*de novo* fatty acids = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0 (C16:0 has been excluded from this summary, since it is partially derived from dietary sources and fat depot as well).

are auxotrophic for several amino acids (the requirement is strain dependent and can range from 4 up to 14 different amino acids). In milk, the amounts of free amino acids and peptides are very low. Therefore, LAB depend on an effective proteolytic system that allows the degradation of milk proteins (caseins) for their growth in milk (Kunji et al., 1996). However, some LAB strains lack sufficient proteolytic activity for protein cleavage and must rely on the availability of free amino acids or on other synergistic strains to provide amino acids for growth and metabolism during fermentation. One well-described example is the associative growth between *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in yoghurt, as each organism provides compounds that benefit the other. The *Streptococcus* benefits from the stronger proteolytic activity of the *Lactobacillus* (providing free amino acids and small peptides), and in return, it supplies carbon dioxide and

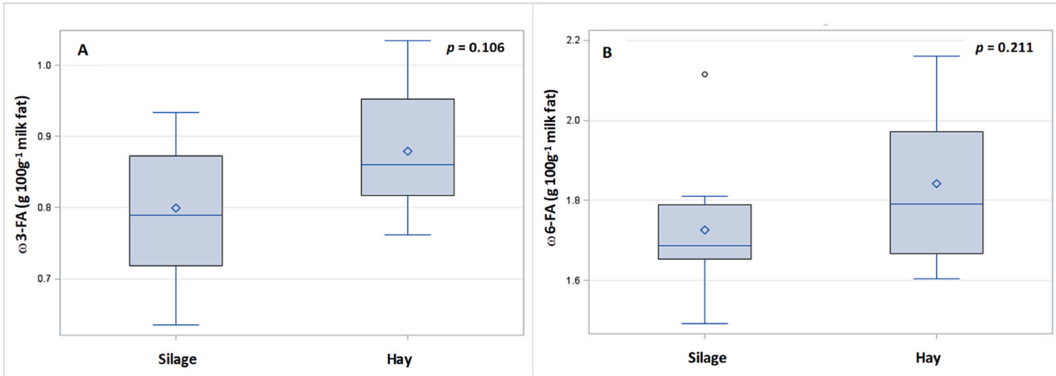

Fig. 1. Comparison of the concentration of ω3-fatty acids (A) and ω6-fatty acids (B) in milk of cows either fed with silage or hay (n = 9; for each treatment). Box-whisker plots show median (–), lower and upper quartiles, and means (◊).

formate, which stimulate the *Lactobacillus*. Thus, this synergistic effect of a mixed starter culture has a positive impact on growth rate, cell density, coagulation, acidification, aroma, and other characteristics (Marshall & Tamine, 1997).

With regard to biogenic amines and polyamines, there was neither a treatment effect nor an interaction between treatment and milk yield found for any of the amines analyzed (Table 3). There also was no effect on the daily secretion of polyamines (Supplementary Material Table S3). The content of biogenic amines in raw milk is generally very low. Results obtained showed that Col had the highest concentration in both silage milk and hay milk (being also more than two times higher compared to the findings of Van den Oever et al., 2021). Col appears in cellular membranes as part of phospholipids, hence, there was no significant difference between the two types of milk. In general, biogenic amines (BAs) can be formed and degraded as a consequence of normal metabolism in plants, animals, and microorganisms, and are mainly produced via decarboxylation of amino acids and transamination of aldehydes and ketones. In eukaryotic cells, biosynthesis of BAs is essential since these compounds act as precursors for the synthesis of hormones, alkaloids, nucleic acids, and proteins. Some of these BAs play an important role in metabolism. They have relevant functions in the human nervous system as neurotransmitters (e.g., noradrenaline, adrenaline, dopamine, serotonin, histamine), or they have a significant effect on the cardiovascular system (increasing or decreasing blood pressure), or they play a significant role in the regulation of metabolism and digestion acting as a precursor of hormones (e.g., catecholamine), as mediators (e.g., histamine, serotonin, tyramine), or as components of co-enzymes (e.g., cysteamine), respectively. Histamine and serotonin influence gastric and intestinal ion secretion and intestinal motility, whereas histamine is also a mediator of acute anaphylaxis and vascular permeability and strongly affects immune responses. Naturally occurring polyamines (e.g., agmatine, putrescine, spermidine, spermine) appear ubiquitously in all mammalian cells, and have specific physiological and regulatory functions that are indispensable for cell growth and protein biosynthesis, but anti-inflammatory properties and antioxidant activity have also been reported. While the abundance of several biogenic amines is considered to be a natural antinutritional factor associated with food poisoning, hypersensitivity and toxicity, polyamines are considered essential compounds of all living cells (Bardócz et al., 1995; Loeser, 2000; Shalaby, 1996). Therefore, accurate information about the actual levels of biogenic amines and polyamines in the milk of cows fed either silage or hay is crucial, as these components play an important biological role in metabolism and have significant functions in human physiology.

3.3. B-vitamins

No treatment effect was observed with regard to vitamins B_1, B_2, and B_6 (Table 4). Ruminally digested NDF has been associated with changes in vitamin B_2 and B_6 contents in milk (Castagnino et al., 2016). Although intake of NDF was significantly higher in the hay-fed group (Bauer et al., 2025), it did not affect the concentrations of vitamins B_2 and B_6 in the two types of milk. Shingfield et al. (2005) reported slightly higher concentrations of vitamin B_1 and B_2, and a lower concentration of vitamin B_6 in cows fed hay. B-vitamins are synthesized by certain microorganisms in the cow's rumen and excreted into the milk. The content of B-vitamins produced in the rumen may influence the concentrations of these vitamins in cow's milk, although this has not been extensively studied (McDowell, 2000; Spreer, 2018). Effects regarding the ratio of forage-to-concentrate on the ruminal synthesis of B-vitamins were published by Seck et al. (2017). Apart from diet, genetics may also influence the ruminal synthesis of B-vitamins, as reported by Poulsen et al. (2014), where significantly higher levels of vitamin B_2 were found in milk from Danish Jersey cows compared to milk from Danish Holstein cows.

Vitamin B_{12} is synthesized exclusively by bacteria, making ruminant products, especially dairy products, an excellent source of this vitamin (Martens et al., 2002). The high level of microbial activity in the rumen causes a constant production of the vitamin, which is consequently excreted into milk (Duplessis et al., 2019). In the present study, vitamin B_{12} content was positively affected when cows were fed silage as the sole forage source (+53.2 %), likely due to microbial fermentation processes during ensiling, which is in line with the findings of Van den Oever et al. (2021). However, the concentration of vitamin B_{12} decreased in this group when milk yield was increased, potentially due to the presence of butyric acid as well as an uncommon lactic to acetic acid ratio in the silage that might have adversely affected rumen microbial activity with increasing dry matter intake (Bauer et al., 2025). On the other hand, the concentration of B_{12} in the milk increased slightly in the hay group when milk yield was elevated (Supplementary Material Table S4; Fig. S2). An increased dry matter intake of hay likely fostered ruminal conditions more conducive to vitamin B_{12} synthesis. The concentration of vitamin B_{12} in silage milk is in accordance with results by Duplessis et al. (2019), who reported that vitamin B_{12} concentration in milk was positively related to percentage of fiber in the feed. Given the superior bioavailability of vitamin B_{12} of animal origin when compared to the synthetic form of the vitamin (used for fortification of food or as dietary supplement) (Matte et al., 2011), it is crucial to determine the effects of feeding on its abundance in cow's milk. Raw milk samples analyzed contained only "true" vitamin B_{12}, even though ruminal microorganisms have the ability to synthesize the biologically non-active analogue (i.e., pseudo-cobalamin) as well. The concentration of vitamin B_{12} is presented as sum of the vitamers of vitamin B_{12} (i.e., methylcobalamin, 5′-deoxyadenosylcobalamin, and hydroxocobalamin; all converted into cyanocobalamin). One glass of cow's milk (250 mL) per day already covers approximately 36 % and 34 % of an adult's daily requirement of vitamin B_2 and B_{12}, respectively, which highlights the nutritional value of milk. Regarding vitamin B_1 and B_6, almost 10 % of the recommended daily intake are covered by one glass of milk (Belitz et al., 2004; Combs & McClung, 2017). In cow's milk, all B-vitamins are present in relevant concentrations (Graulet & Girard, 2017).

3.4. Vitamin E

The content of vitamin E was higher (+50 %) in silage milk compared to hay milk (Table 4), which had also been observed by Van den Oever et al. (2021). There was also an interaction for treatment and milk yield, resulting in a steadily decreasing vitamin E content with increased milk yields in both groups (Supplementary Material Table S4; Fig. S2). Conservation of forages as silage enables a better retention of α-tocopherol when compared to hay production (due to certain losses during drying caused by the exposure to environmental oxygen). Hence, silage is a richer source of tocopherols than hay due to higher losses of these compounds during grass drying and hay storage (Ballet et al., 2000). While vitamin E contents have been reported to be higher in silage as compared to hay (alfalfa), it requires fat to be effectively absorbed in the body (Weiss & Wyatt, 2003), meaning that a greater concentration of the vitamin in feed does not necessarily lead to a higher vitamin E concentration in milk.

The concentration of α-tocopherol was lower compared to findings by Shingfield et al. (2005). Niero et al. (2018) reported a comparable content of vitamin E in milk of silage-fed cows. The concentration of vitamin E in milk is affected by nutrition, season, stage of lactation, and to a far lesser extent by breed and genetics. Pastured cows typically show higher levels of vitamin E in their milk than those fed a diet of conserved feed (Schingoethe et al., 1978). Ensiled grasses seem to contain higher levels of available tocopherols (Kalač, 2011). As the pelleted concentrate offered each cow provided the same amount of vitamins, silage may have offered a greater amount of vitamin E than hay.

3.5. Fatty acids

The vast majority of FAs in hay and silage milk did not differ between groups, a finding attributable to the comparable forage-to-concentrate ratios in the diets and the only marginal effects of forage type on fecal microbial alpha diversity in cows (Bauer et al., 2025). However, certain FAs showed some significant differences between silage milk and hay milk, e.g., pentadecanoic acid (C15:0), which was significantly higher in milk from silage-fed cows. As one of the most common odd-chain FA, pentadecanoic acid is primarily found in dairy fat. Furthermore, while concentrations of unbranched, odd-chain FAs (C11:0 and C13:0) showed no difference between the two treatments, the content of branched-chain FAs (C14i, C16i, C17i, C17ai) was significantly higher in the milk fat of the hay-fed cows. A possible explanation for this discrepancy is the difference in microbial activity in the rumen compared to microbial activity during ensiling, as both branched-chain FAs and odd-chain FAs are considered indicators for bacterial metabolism. Branched-chain fatty acids (BCFAs) are rumen-derived FAs comprising approx. 2 % of bovine milk FAs. Certain benefits for human health have been reported for BCFAs, as these FAs demonstrate anti-inflammatory properties (Picklo et al., 2022; Shingfield et al., 2005). Contents of FAs were not different with regard to PUFAs. The two main PUFAs found in milk fat, linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3), also remained unaffected by the treatment, with 1.71 and 1.80 g 100 g^{-1} milk fat, and 0.68 and 0.76 g 100 g^{-1} milk fat in the silage and hay group, respectively. A slight trend (but not statistically significant) towards a higher concentration of ω3-FAs was observed in the hay milk (Fig. 1), while ω6-FAs showed only a numerical difference in favor of hay milk. This is only partially consistent with some literature sources, as Benbrook et al. (2018) found that the amount of ω3-FAs increased (by approx. 50 %), while the amount of ω6-FAs decreased (by approx. 50 %) when cows were fed a nearly 100 % forage-based diet. However, the results of the current study are comparable to values reported by Scherzer et al. (2019), especially with regard to proportions of SFA, MUFAs, and PUFAs in milk from grazing cows and silage-fed cows. Shingfield et al. (2005) also observed similar values of FAs in milk for different forage conservation methods (hay and differently treated silage). Compared to the previous study by Van den Oever et al. (2021), linoleic acid and α-linolenic were not significantly higher in hay milk in the present study. Both linoleic acid and α-linolenic acid are essential for animals, and provide important information about the diet of ruminants, as they indicate the part of dietary FAs which pass the rumen unharmed. MUFAs and PUFAs could also be affected by genetics and cattle breed, as reported by Manuelian et al. (2019). That study found that Holstein Friesian cows produced more milk with greater content of saturated FAs, and short- and medium-chain FAs compared to dual-purpose cows (Simmental and Alpine Grey), while the latter group produced milk with higher concentrations of monounsaturated FAs and long-chain FAs. These characteristics of the FA profile of dual-purpose cows could be seen as more relevant to human nutrition.

3.6. Sensory properties

A paired comparison was performed with 10 samples each of hay milk and silage milk. The trained panelists had difficulties discriminating between these two types of milk since mean values of 5.3, 5.5, and 6.2 (out of the highest possible score of 10) resulted in a correct identification of silage milk in three independent sessions. Similar findings were reported by Schaeren et al. (2005). Regarding quantitative descriptive analysis, some marginal differences between hay milk and silage milk were recognized regarding certain characteristics. In silage milk, a yellowish color was observed, while hay milk was evaluated to be more viscid. Moreover, the attribute odor intensity was perceived higher with respect to silage milk. The final session is shown in Fig. 2.

Comparable findings were reported by Shingfield et al. (2005), who stated that forage conservation method had no effect on the organoleptic properties of milk. Furthermore, Lopez et al. (2024) investigated sensory properties of retail milk samples (e.g., hay milk and extended shelf-life milk), but no significant difference could be observed for various attributes. Furthermore, Phillips et al. (1995) found out that the perceived color of milk also correlates to physical measurement using the CIELAB color space. Manzocchi, Martin, et al. (2020) also confirmed a positive linear increase in perceived color intensity and instrumentally measured L*a*b*-indexes in milk and cheese.

4. Conclusions

This study focused on possible differences in milk composition from cows that were fed either silage or hay. Both silage and hay were

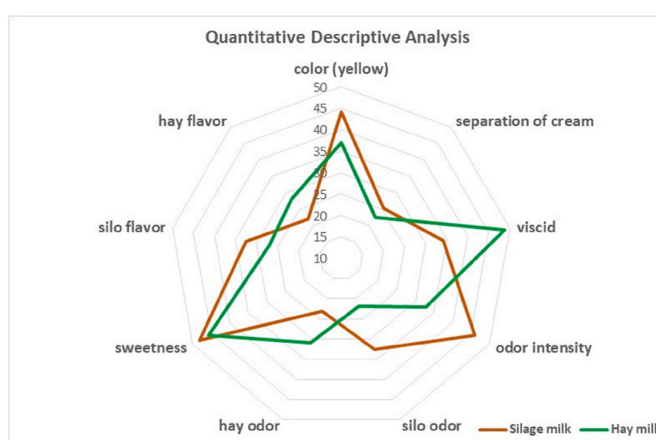

Fig. 2. Sensory evaluation using quantitative descriptive analysis for the differentiation between silage milk and hay milk.

harvested from the same parent plant material, making the two types of milk directly comparable. Milk composition was investigated with regard to protein fractions, free amino acids, polyamines, FAs, and the vitamins B_1, B_2, B_6, B_{12}, and E. Interestingly, hay milk showed a tendentially higher protein content compared to silage milk. According to literature, farmers producing hay milk often face the problem of low protein contents, barely meeting the required content for the production of commercial retail milk. Milk composition showed no significant differences regarding numerous chemical components (e.g., most free amino acids, polyamines and biogenic amines, vitamin B_1, B_2, and B_6), while significantly higher concentrations of vitamin B_{12} were found in silage milk, likely due to enhanced microbial fermentation processes during ensiling. Furthermore, vitamin E was also significantly higher in the milk of silage-fed cows due to better retention of tocopherols when forages are preserved as silage rather than hay. Essential FAs linoleic acid and α-linolenic acid, which typically represent the fatty acid profile of hay milk, were not significantly elevated in the present study. Sensory analysis showed only minor differences, such as a higher viscosity in hay milk, or a more yellowish color and a stronger odor intensity in silage milk.

Limitations of the present study (e.g., seasonal changes, longer storage periods of differently conserved forages, different cattle breeds) might require further investigation. However, future studies will benefit from the data provided due to the extensive analyses on numerous nutritionally important milk constituents. Furthermore, this study shows that there are hardly any differences in milk composition when feeding hay or silage, provided that the silage was produced under practical conditions.

CRediT authorship contribution statement

Sabrina P. Van den Oever: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Katrin Bauer:** Writing – original draft, Visualization, Software, Resources, Investigation, Data curation. **Andreas Haselmann:** Software, Data curation. **Matthias Schreiner:** Validation, Investigation. **Birgit Fuerst-Waltl:** Software, Data curation. **Werner Zollitsch:** Software, Data curation. **Qendrim Zebeli:** Writing – review & editing, Methodology, Conceptualization. **Helmut K. Mayer:** Writing – review & editing, Supervision. **Wilhelm Knaus:** Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2025.106475.

Data availability

Data will be made available on request.

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