Animal Nutrition 19 (2024) 261–271

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Variations in the milk lipidomic profile of lactating dairy cows fed the diets containing alfalfa hay versus alfalfa silage

Kaizhen Liu [a, b, c, †], Meiqing Chen [a, c, †], Guoxin Huang [a, c], Chuanyou Su [a, b, c], Wenhao Tang [a, c], Ning Li [a, c], Jiyong Yang [a, c], Xufang Wu [a, c], Boxue Si [a, c], Shengguo Zhao [a, c], Nan Zheng [a, c], Yangdong Zhang [a, c, *], Jiaqi Wang [a, c, *]

[a] Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[b] Henan International Joint Laboratory of Nutrition Regulation and Ecological Raising of Domestic Animal, College of Animal Science and Technology, Henan Agricultural University, Zhengzhou 450046, China
[c] State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

ARTICLE INFO

Article history:
Received 20 October 2023
Received in revised form
12 June 2024
Accepted 30 June 2024
Available online 30 September 2024

Keywords:
Dairy cow
Alfalfa silage
Alfalfa hay
Milk fat
Lipidomic profile
Fatty acid

ABSTRACT

Alfalfa is primarily stored as silage or hay in livestock production. Previous research has shown that the storage method of grass significantly influences milk composition. This study aimed to investigate milk production performance and lipid composition in dairy cows fed diets consisting of alfalfa hay or alfalfa silage as roughage. Forty-two mid-lactation Holstein dairy cows were selected and randomly divided into three groups, each receiving a total mixed ration consisting of alfalfa hay (AH), 50% alfalfa silage + 50% alfalfa hay (AHAS), or alfalfa silage (AS). The results showed that milk fat content ($P = 0.049$) and milk fat yield ($P < 0.001$) were significantly higher in the AH and AHAS groups compared to the AH group. With increased supplementation of alfalfa silage in the diet, ω-3 polyunsaturated fatty acid content increased significantly ($P < 0.001$), while ω-6 polyunsaturated fatty acid content ($P = 0.007$) and the ratio of ω-6 to ω-3 polyunsaturated fatty acids decreased ($P < 0.001$). The contents of sphingomyelins, phosphatidylserines, phosphatidylethanolamines, and phosphatidylglycerols in the AHAS and AS samples were higher than in the AH samples, although the differences were not statistically significant. Additionally, the content of phosphatidylcholines was significantly higher in the AS group compared to the AH group ($P = 0.032$). In conclusion, feeding dairy cows a diet consisting of alfalfa silage can increase the major phospholipid content and polyunsaturated fatty acid composition in raw milk, which is more conducive to human health. These findings provide valuable insights into the benefits of alfalfa silage for dairy cows.

1. Introduction

With the enhancement of human health awareness, milk is extensively favored by consumers. Beyond its nutritional value, milk plays a key role in regulating the immune system, supporting gut microbiota, and preventing cardiovascular and cerebrovascular diseases (Ren et al., 2021). Lipids, which account for 3% to 5% of milk, are important nutrients with significant impacts on human health (Liu et al., 2018). Previous studies have highlighted the essential roles of phospholipids (Anto et al., 2020; Contarini and Povolo, 2013), ω-3 polyunsaturated fatty acids (ω-3 PUFA), and the ratio of ω-6 to ω-3 PUFA in promoting human health (Brick et al., 2016). Phospholipids and ω-3 PUFA have been shown to positively regulate cardiovascular health and promote infant growth and development (Abedi and Sahari, 2014).

Currently, researchers aim to regulate the lipid profile of raw milk and dairy products through dietary modifications, environmental management, and optimization of processing technology (Dabija et al., 2018; Murphy et al., 2016; Shingfield et al., 2005). The

* Corresponding author.
E-mail addresses: zhangyangdong@caas.cn (Y. Zhang), jiaqiwang@vip.163.com (J. Wang).
† Both authors contributed equally to this work.
Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.06.009

contents and compositions of milk lipids are susceptible to variations induced by dairy breed, season, forage, and feeding practices (Barca et al., 2018; Chilliard et al., 2016; Garcia et al., 2012; Liu et al., 2017). Previous research indicated higher levels of medium-chain triglycerides, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in goat milk, while cow milk contained higher levels of ceramides (Cer), triacylglycerol (TAG), and diacylglycerols (DAG) (Li et al., 2017). Diet composition, including roughage, protein, and energy, is a major factor affecting raw milk quality. For example, dairy cows fed a diet supplemented with flaxseed produced milk with increased levels of ω-3 PUFA (Huang et al., 2021). Additionally, the species of roughage, processing, and storage methods can alter milk composition, especially the content and yield of milk fat (Broderick et al., 2002; Elgersma, 2015; Liu et al., 2016, 2020).

Roughage, accounting for 40% to 60% of the total mixed ration (TMR), is essential feed for ruminants. As a legume forage, alfalfa is regarded as superior roughage for dairy cows due to its high protein content and palatability. Fresh alfalfa is mainly conserved as alfalfa hay or alfalfa silage for long-term storage. Other legume forages, such as sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*), have shown that preservation methods affect fatty acid content, with total FA and C18:3 concentration decreasing and C16:0 concentration increasing more in hay than in silage (Rufino-Moya et al., 2022). However, studies on the variations in the milk lipidomic profile of dairy cows induced by alfalfa hay and alfalfa silage are limited.

The term “lipidomics” was first proposed by Han and Gross (Han and Gross, 2003). As an efficient method for analyzing the properties of all lipid molecules in an organism, lipidomics has been widely used in research on meat quality and food lipid oxidation (Li et al., 2020; Tu et al., 2022). Recently, the milk lipid profiles of humans, dairy cows, goats, donkeys, camels, and even soy milk have been investigated using lipidomic analysis (Li et al., 2017; Wang et al., 2020; Zhang et al., 2021). Lipidomics is mostly conducted using nuclear magnetic resonance spectroscopy, liquid chromatography-mass spectrometry (LC-MS), or gas chromatography-mass spectrometry (GC–MS), with LC-MS providing abundant molecule identification and simultaneous quantitation (Want et al., 2010; Dunn et al., 2011; Zhao et al., 2022). Additionally, milk fatty acid profiles of cows, humans, goats, camels, and donkeys are usually identified and quantified by GC–MS (Barca et al., 2018; Chen et al., 2021).

In the current study, we hypothesized that the milk production performance and lipid profile of dairy cows differ when fed a diet consisting of alfalfa silage or alfalfa hay.

2. Materials and methods

2.1. Aimal ethics statement

The experimental protocols were approved by the Animal Care Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China) in accordance with the guidelines for animal experimental welfare and ethical inspection in China (approval no. IAS 2021–222).

2.2. Experimental animal and feeding management

This trial was conducted at AUSTASIA Farming in Chifeng, Inner Mongolia, China. Forty-two healthy mid-lactation Holstein cows, averaging 124.9 ± 2.5 days in milk and 37.5 ± 1.04 kg average milk yield, were selected for the study. The cows were randomly divided into three groups of 14 cows each and housed in the same stall with free and continuous access to fresh water. Three types of total mixed rations (TMR) were prepared: one with alfalfa hay (AH), one with 50% alfalfa silage and 50% alfalfa hay (AHAS), and one with alfalfa silage (AS) (Table 1). The nutrient compositions of alfalfa hay, alfalfa silage and other feed are detailed in Table S1. The percentages of dry matter (DM), ash, calcium (Ca) and phosphorus (P) in the feeds were determined by the methods 930.15, 942.05, 935.13 and 946.06, respectively, according to AOAC (2016). Ash concentrations were measured by igniting samples at 550 °C for 8 h with the aid of a chamber muffle furnace (Thermo Fisher Scientific, China). Crude protein (CP) concentrations were analyzed using the Kjeldahl method with the Kjeltec 8400 (Foss, Denmark) (McKenzie and Wallace, 1954). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were measured according to the protocol of the Ankom 220 fiber analyzer (ANKOM Technology, USA) (Van Soest et al., 1991).

Three TMRs were formulated to be isoenergetic and isonitrogenous, with 46% roughage and 54% concentrate. The lactating cows were fed at 10:00, 16:30, and 22:00, with each cow receiving an amount designed to leave 5% residual feed each day. The trial consisted of a three-week adaptation period followed by a six-week experimental period. The daily matter intake (DMI) of each cow was recorded throughout the entire experimental period.

2.3. Milk yield record and sample collection

Each animal was milked four times daily at 09:30, 15:30, 21:30, and 03:30. Milk yield from each milking was recorded using a

Table 1
The ingredients and nutrient compositions of the diets (% DM basis).

Item	Dietary treatment[1]		
	AH	AHAS	AS
Ingredients			
Corn silage	19.05	19.05	19.05
Alfalfa hay	25.84	12.93	0.00
Alfalfa silage	0.00	12.93	25.85
Oaten hay	0.78	0.78	0.78
Corn meal	10.67	10.67	10.67
Steam-flaked corn	14.83	14.83	14.83
Soybean meal	10.35	8.01	5.61
Extruded full-fat soybean	5.16	3.99	2.54
Cotton seed	0.76	2.36	2.96
Corn fiber	0.96	2.83	6.08
Cane Molasses	1.87	1.87	1.87
Rumen-bypass soybean meal	2.65	2.65	2.65
Premix[2]	4.97	4.97	4.97
Fatty acid calcium	0.41	0.41	0.41
Palm fat powder	1.26	1.26	1.26
Methionine hydroxy analogue	0.08	0.08	0.08
Rumen-bypass lysine	0.16	0.16	0.16
Urea	0.21	021	0.21
Total	100.00	100.00	100.00
Nutrient level[3]			
Dry matter	55.00	55.00	55.00
Crude protein	18.44	18.44	18.44
NE_L, Mcal/kg	1.40	1.40	1.40
Neutral detergent fiber	21.49	21.91	22.30
Acid detergent fiber	13.34	14.10	15.19
Ether extract	3.92	4.18	4.22
Ash	8.60	8.43	8.26
Ca	1.04	1.04	1.04
P	0.37	0.38	0.39
C/F	54:46	54:46	54:46

DM = dry matter; C/F = concentration to roughage ratio; NE_L = net energy for lactatioin.

[1] AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage.

[2] Each kilogram of premix dry matter contains vitamin A 134.57 kIU, vitamin D 36.77 kIU, vitamin E 825.85 IU, Fe 985.97 mg, Cu 183.77 mg, Zn 919.99 mg, Mn 915.14 mg, Se 7.36 mg, and Co 13.80 mg.

[3] NE_L was calculated according to NRC (2001) and others were measured values.

DeLaval automatic monitoring system (DeLaval Co., Ltd, Sweden). Raw milk samples were collected on the last day of each week. The milk samples from the four milking sessions were combined in a ratio of 3:3:2:2, resulting in a 200-mL composite sample for each day, which was then aliquoted into four 50 mL EP tubes. One milk sample was stored at 4 °C for milk composition analysis, while the other three samples were stored at −80 °C for later analysis.

On the last day of the trial, blood was collected from the tail vein before morning feeding and immediately centrifuged to prepare plasma. Additionally, rumen fluid samples (approximately 200 mL each) were collected from each cow using an oral stomach tube. The pH value was measured immediately using a portable acidity meter (Tianqi Mdt InfoTech Ltd., Shanghai, China). The samples were then filtered through a 4-layer cheesecloth and aliquoted into six sterilized 5 mL tubes. Samples of plasma and rumen fluid were stored at −80 °C until further analysis.

2.4. Plasma biochemistry analysis

Plasma samples of each dairy cow were thawed on ice. Subsequently, plasma biochemical parameters including total protein (TP), albumin (ALB), globulin (GLOB), glucose (GLU), urea, total cholesterol (TC), triglyceride (TAG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by an automatic biochemistry analyzer (BS200, Mindray). The albumin/globulin (A/G) ratio was calculated.

2.5. Production performance and milk composition analysis

The DMI was measured using a method from our previous study (Liu et al., 2022). Milk composition was analyzed using an Ultramilker (UL40AC), and somatic cell count (SCC) was measured using a DeLaval somatic cell analyzer. The 4% fat-corrected milk (FCM) was calculated based on milk yield and milk fat (Gaines, 1928). Energy corrected milk (ECM) for each cow was calculated using milk yield, milk fat, and milk protein (Tyrrell and Reid, 1965). The feed efficiency was calculated by dividing ECM by DMI.

2.6. Rumen fermentation parameter analysis

A tube of rumen liquid sample was thawed on ice, then centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was collected to measure ammonia nitrogen (NH_3—N) and microbial crude protein (MCP) concentrations as described in Liu et al. (2022). The volatile fatty acid (VFA) concentrations in all samples were measured using an Agilent 7890A gas chromatographer (Agilent 7890A-7000B, Agilent, Beijing, China).

2.7. Fatty acid analysis

The milk fatty acid (FA) profiles in all samples were analyzed using a method previously developed in our laboratory with slight modifications, following the procedure described by Wang et al. (2021) and Chen et al. (2023). Briefly, 2 mL of milk was mixed with 25 μL of internal standard C19:0 FA methyl ester and 4 mL of n-hexane/isopropanol mixed solution (3:2, vol:vol). After vortexing and centrifugation, the n-hexane phase was collected. Then, 1.3 mL of n-hexane was added, centrifuged, and the upper n-hexane phase was collected, repeating the process twice. Next, 2 mL of methanolic NaOH solution (20 g/L) was added to the extracted n-hexane to undergo saponification and alkali-catalyzed methyl esterification. This was followed by acid-catalyzed methyl esterification by adding 2 mL of acetyl chloride-methanol solution (100 mL/L). After cooling the n-hexane phase to room temperature, 5 mL of ultrapure water was added and diluted to 10 mL. The n-hexane samples extracted from milk were diluted 25-fold and subsequently analyzed by GC—MS.

2.8. Lipidomic analysis

Lipidomic analysis was performed using a method described by Hu et al. (2021). Briefly, the lipid was extracted from milk by the following steps: a 100-μL milk sample was placed into a glass tube with a Teflon lined cap, then 0.75 mL of methanol was added to the tube and vortexed. Lipidomics were performed using an ultrahigh-performance liquid chromatography-tandem Q-Exactive Orbitrap mass spectrometer (UPLC-Q-Exactive Orbitrap/MS, Thermo Fisher Scientific, Waltham, MA). The protocol referenced previous research (Yuan et al., 2012).

2.9. Statistical analysis

The comparisons among three groups were performed in variance analysis with the PROC MIXED model in SAS. Data were presented as mean and SEM. Statistical significance was defined as $P \leq 0.05$ and highly significant differences as $P < 0.01$. The graphs were generated by GraphPad Prism 8.0 software or R software.

The statistical model of milk yield, milk component, SCC, and milk component yield including treatment, experimental week, sampling data, covariate, the interactions between treatment and experimental week, and experimental week as the repeated measurement, with initial data for these indexes as covariate, and cows as a random effect, was represented as follows:

$$Y_{ijt} = \mu + T_i + p_{ji} + W_t + TW_{it}\,(B + \Phi_j)\,X_{ij} + e_{ijt}$$

where Y_{ijt} denotes the milk yield, milk component, SCC, or milk component yield of *j* cow in the *i* treatment and week t; μ denotes the overall mean; T_i means the effect of treatment; p_{ji} means random effect of cows *j* in treatment *i*; W_t represents the effect of week *t*; TW_{it} is the treatment and week interaction; *B* stands for the regression coefficient of the covariate; Φ_j represents the skew deviation of *i* treatment; X_{ij} means the milk yield or milk component of cow *j* in treatment *i*; e_{ijt} denotes the residual error.

The statistical model for DMI and feed efficiency included treatment, experimental week, sampling date, the interaction between treatment and experimental week, and experimental week as a repeated measurement, with cows as a random effect, as follows:

$$Y_{ijt} = \mu + T_i + p_{ji} + W_t + TW_{it} + e_{ijt}$$

where Y_{ijt} is the DMI or feed efficiency of cow *j* in treatment *i* in week *t*; μ is the overall mean; p_{ji} means random effect of cows *j* in treatment *i*; T_i denotes the effect of treatment *i*; W_t represents the effect of week *t*; TW_{it} means the treatment and week interaction; e_{ijt} stands for the residual error.

3. Results

3.1. Plasma biochemistry

Some plasma biochemical indexes of dairy cows showed divergence among the three groups (Table 2). Plasma TAG concentration in AHAS cows was higher than that in AH cows, and AS cows tended to have higher levels than AH cows ($P = 0.087$). Total cholesterol and LDL levels in AHAS group were significantly lower than in the AH and AS groups ($P = 0.001$, $P < 0.001$), while levels of AST and urea were higher in the AHAS group ($P = 0.017$, $P < 0.001$).

Table 2
The concentrations of plasma biochemical indexes in dairy cows fed alfalfa silage or alfalfa hay.

Item	Dietary treatment[1]			SEM	*P* value
	AH	AHAS	AS		
GLU, mmol/L	4.99	5.35	5.23	0.150	0.606
ALT, U/L	23.49^{b}	29.13^{a}	30.18^{a}	0.955	0.005
AST, U/L	25.61^{b}	32.19^{a}	27.32^{b}	1.007	0.017
TAG, mmol/L	0.94	1.22	1.17	0.056	0.087
Urea, mmol/L	4.79^{b}	6.67^{a}	5.00^{b}	0.230	< 0.001
TC, mmol/L	4.58^{a}	4.13^{b}	4.97^{a}	0.096	0.001
HDL, mmol/L	1.58	1.67	1.84	0.075	0.359
LDL, mmol/L	3.01^{a}	2.46^{b}	3.13^{a}	0.058	< 0.001
TP, g/L	77.68	73.33	73.84	1.572	0.470
ALB, g/L	36.62^{ab}	40.38^{a}	33.40^{b}	0.930	0.007
GLOB, g/L	28.71	30.08	27.98	0.652	0.434
A/G	1.27^{b}	1.35^{a}	1.20^{c}	0.017	< 0.001

ALB = albumin; A/G = the albumin to globulin ratio; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GLOB = globulin; GLU = glucose; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; TAG = triglyceride; TC = total cholesterol; TP = total protein; SEM = standard error of the means.
[a-c] Within a row, means without a common superscript differ at $P \leq 0.05$.
[1] AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage.

Alanine aminotransferase levels were significantly higher in the AHAS and AS groups compared to the AH group ($P = 0.005$). Albumin levels in the AHAS group were significantly higher than those in the AS group ($P = 0.007$). The A/G was significantly different among the three groups (AHAS = 1.35, AH = 1.27, AS = 1.20, $P < 0.001$). Glucose, HDL, TP, and GLOB concentrations were similar among the three groups ($P > 0.05$).

3.2. Production performance and milk composition

The milk yield and milk composition varied among dairy cows fed AH, AHAS, and AS diets (Table 3). Total milk solids content, milk protein content, milk fat content, and milk fat yield were significantly higher in AHAS and AS cows than in AH cows ($P < 0.001$, $P = 0.050$, $P < 0.001$, $P = 0.040$), whereas milk yield was significantly higher in AH and AHAS cows than that in AS cows ($P = 0.011$). Dry matter intake, 4% FCM, ECM, feed efficiency, SCC, milk protein yield, lactose, lactose yield, and total milk solids yield showed no significant difference among the three groups ($P > 0.05$).

3.3. Rumen fermentation parameters

The ruminal fermentation parameters differed among the three groups (Table 4). The pH value of the rumen fluid in AH cows were significantly higher than that in AS cows ($P = 0.016$). The NH_3–N concentration in AH cows had the great tendency increase than that in AS cows ($P = 0.088$). Regarding VFA, the molar concentration of isobutyric acid and the A/P ratio in AS cows was greatly higher in AS cows compared to AH and AHAS cows ($P = 0.052$, $P = 0.067$). However, valeric acid was higher in AH cows than that in AHAS and AS cows ($P = 0.119$). There was no significant difference in total VFA and other individual VFA among the three groups.

3.4. Milk fatty acid composition

A total of 70 FA in milk samples were detected using GC–MS (Table S2), with 36 FA differing significantly between the AS and AH groups (Fig. 1A, B and C, $P < 0.05$). The FA content in alfalfa hay, silage, and the TMRs is shown in Table S3. The contents of C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C10:1 c3, C10:1 c9, C11:0, C12:0, C13:0, C14:0, C15:0, C15:1 t10, C17:0, C17:1 c10, C18:0, C18:3 c9c12c15, C19:1 c10, C20:0, C22:1 c13, C22:2 c13c16, C22:3 c13,c16c19, and C22:5 c7c10c13c16c19 in milk were significantly higher in AS cows than in AH cows ($P < 0.05$). Whereas the contents of C4:0, C16:0, C16:1 t9, C17:0 iso, C18:1 t6, C18:1 t9, C18:1 t11, C18:2 c9c12, C18:2 c9t11, and C18:2 t9t11 were significantly lower in AS cows than in AH cows ($P < 0.05$). The results showed a significant decrease in PUFA, ω-6 PUFA content, and the ω-6/ω-3 PUFA ratio with silage in the diet ($P = 0.004$, $P = 0.007$, $P < 0.001$, Table S2). In contrast, the ω-3 PUFA content significantly increased with alfalfa silage in the diet ($P < 0.001$, Table S2).

Table 3
The milk performance of cows and milk components.

Item	Dietary treatment[1]			SEM	*P*-value		
	AH	AHAS	AS		Treatment	Week	Treatment × week
DMI, kg/d	21.87	22.88	22.60	0.131	0.238	0.001	0.795
Feed efficiency[2]	1.64	1.60	1.67	0.023	0.626	< 0.001	< 0.001
Milk composition, %							
Fat	3.82^{b}	4.33^{a}	4.41^{a}	0.050	< 0.001	< 0.001	0.049
Protein	3.26^{b}	3.29^{a}	3.30^{a}	0.006	0.050	0.800	0.218
Lactose	4.74	4.76	4.77	0.010	0.236	0.288	0.755
Total milk solid	12.44^{b}	13.03^{a}	13.13^{a}	0.053	< 0.001	< 0.001	< 0.001
SCC, × 10^3 cells/mL	14.30	12.27	13.98	1.440	0.334	0.450	0.444
Yield, kg/d							
Milk	36.14^{a}	35.78^{a}	33.70^{b}	0.309	0.011	0.020	0.489
4% FCM[3]	33.02	35.21	35.20	0.491	0.501	< 0.001	< 0.001
ECM[4]	35.83	37.68	37.70	0.500	0.700	< 0.001	< 0.001
Fat	1.30^{b}	1.46^{a}	1.46^{a}	0.090	0.040	< 0.001	< 0.001
Protein	1.10	1.10	1.10	0.130	0.471	< 0.001	< 0.001
Lactose	1.60	1.59	1.59	0.020	0.408	< 0.001	< 0.001
Total milk solid	4.22	4.36	4.36	0.055	0.910	< 0.001	< 0.001

AH = the diet containing alfalfa hay; AHAS = the diet containing 50% alfalfa hay and 50% alfalfa silage; AS = the diet containing alfalfa silage; DMI = dry matter intake; ECM = energy corrected milk; FCM = fat corrected milk; SCC = somatic cell count; SEM = standard error of the means.
[a,b] Within a row, means without a common superscript differ at $P \leq 0.05$.
[1] AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage.
[2] Feed efficiency = ECM/DMI.
[3] 4% FCM = [0.40 × milk yield (kg/d)] + [15 × milk fat yield (kg/d)].
[4] ECM = [12.95 × milk fat yield (kg/d)] + [7.20 × milk protein yield (kg/d)] + [0.327 × milk yield (kg/d)].

Table 4
Rumen fermentation indexes of dairy cows.

Item	Dietary treatment[1]			SEM	*P*-value
	AH	AHAS	AS		
Acetic acid, mmol/L	55.66	55.04	57.26	0.991	0.667
Propionic acid, mmol/L	19.23	17.98	17.05	0.595	0.334
Isobutyric acid, mmol/L	0.82	0.83	0.93	0.020	0.052
Butyric acid, mmol/L	10.62	10.48	10.13	0.265	0.752
Isovaleric acid, mmol/L	1.28	1.22	1.30	0.035	0.667
Valeric acid, mmol/L	1.63	1.56	1.35	0.057	0.119
Total VFA, mmol/L	89.24	87.11	88.02	1.728	0.141
A/P ratio	3.01	3.09	3.38	0.068	0.067
MCP, mg/L	333.46	326.94	318.32	21.732	0.508
NH_3–N, mg/L	10.16	9.22	7.81	0.436	0.088
pH	6.80[a]	6.64[b]	6.69[b]	0.026	0.016

A/P ratio = acetate to propionate ratio; MCP = microbial crude protein; VFA = volatile fatty acids; SEM = standard error of the mean.
[a,b] Within a row, means without a common superscript differ at $P \leq 0.05$.
[1] AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage.

3.5. Milk lipidomic profile

From the results of milk production performance, both milk fat content and yield increased with the amount of alfalfa silage fed to cows. To further analyze the milk lipid variation among the three groups, lipidomics was used to investigate the lipid profiles. A total of 709 lipid molecules were detected in positive (516) and negative (193) ion modes by the mass spectrometer (Table S4 and S5), including triacylglycerol (TAG), diacylglycerols (DAG), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidic acids (PA), phosphatidylserines (PS), phosphatidylinositols (PI), phosphatidylglycerols (PG), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), lysophosphatidylserine (LPS), lysophosphatidylglycerols (LPG), sphingomyelins (SM), ceramides (Cer), monohexosylceramide (Hex1Cer), dihexosylceramide (Hex2Cer), and acylcarnitine (AcCa) (Fig. 2A). Among the 18 lipid species in Fig. 2B, TAG (16.74%), PE (14.75%), and Hex1Cer (13.19%) were the most abundant lipids, followed by DAG (11.77%), PC (11.63%), SM (10.50%), and PS (7.38%).

Multivariate statistical analysis using unsupervised principal component analysis (PCA) modeling was performed to analyze lipid composition divergence among the three groups. The PCA plot visualized the discrimination between the AH and AS groups, but less discrimination between the AH and AHAS groups, and AHAS and AS groups (Fig. 2C). Moreover, the cluster heatmap plot showed that lipid composition between the AH and AS groups differed greatly, followed by the comparison of the AH and AHAS groups, and AHAS and AS groups (Fig. 2D). Subsequently, comparative analysis of lipid subclasses and molecules in the AHAS and AS groups compared with the AH group was performed. Results showed that TAG, SM, PS, PG, PE, and PC in the AS and AHAS groups all tended to be higher, with PC showing extremely higher levels in

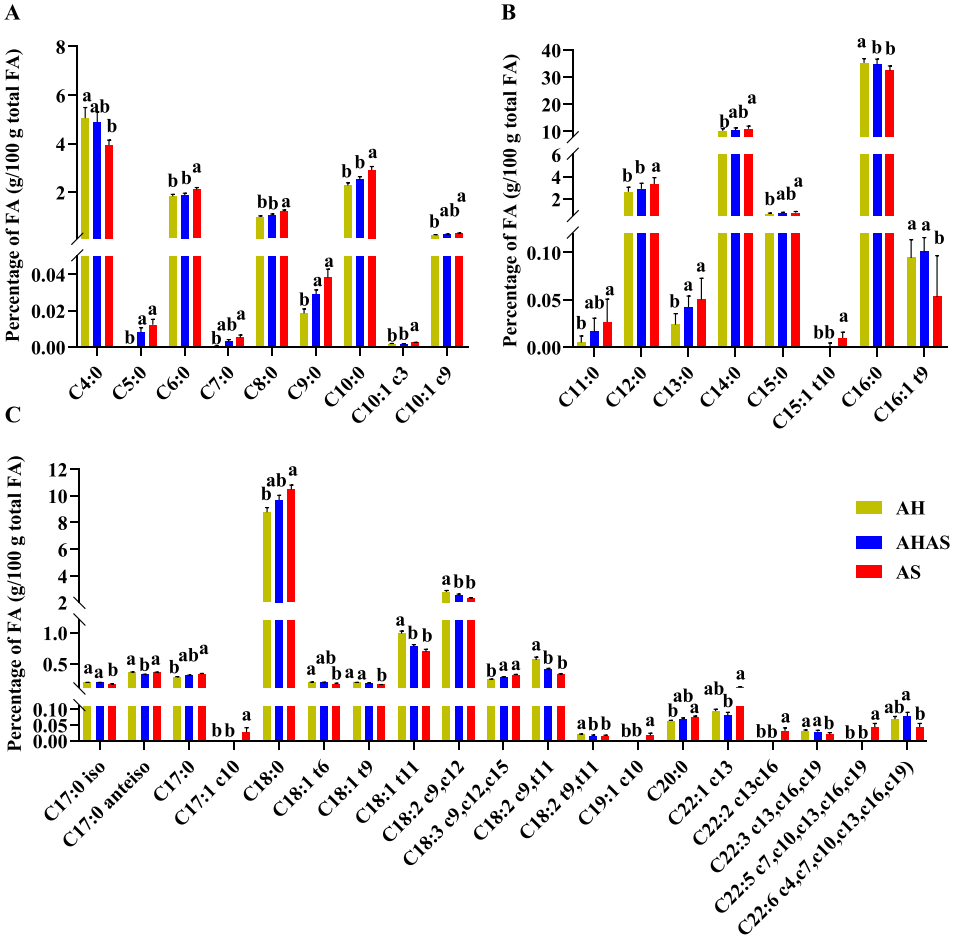

Fig. 1. The fatty acid (FA) concentrations among three groups. AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage. (A, B and C) Percentages of FA < C10, C11 to C16, and > C17, respectively. The different letters on the bars indicate significant differences between the three groups ($P < 0.05$).

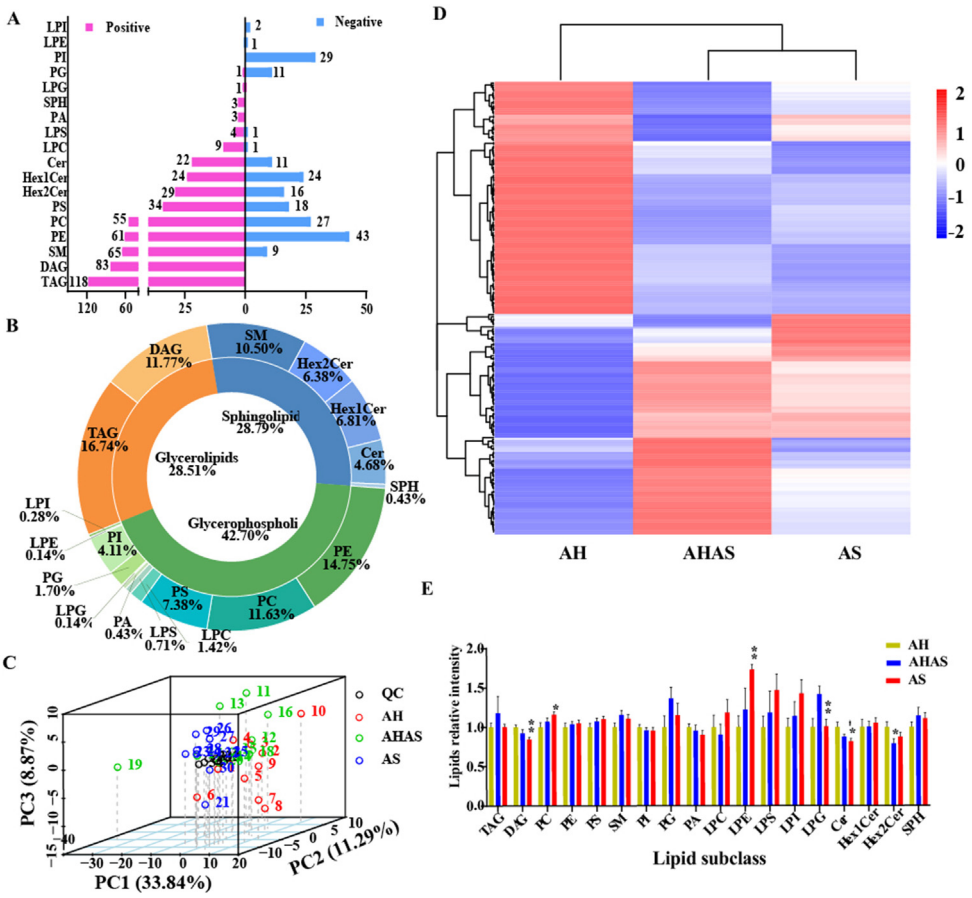

Fig. 2. Lipid subclasses and molecules in the milk of dairy cows. AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage. (A) The number of lipids in each subclass. (B) The percentage distribution map of lipid subclasses. (C) The principal component analysis (PCA) plot of individual lipid molecules among the three treatments. (D) The heatmap of differential lipid molecules. (E) The differences of each lipid subclass of AHAS and AS compared to AH group. *, $P < 0.05$; **, $P < 0.01$. Cer = ceramides; DAG = diacylglycerols; Hex1Cer = monohexosylceramide; Hex2Cer = dihexosylceramide; LPC = lysophosphatidylcholine; LPE = lysophosphatidylethanolamine; LPG = Lysophosphatidylglycerols; LPI = lysophosphatidylinositol; LPS = lysophosphatidylserine; PA = phosphatidic acids; PC = phosphatidylcholines; PE = phosphatidylethanolamines; PG = phosphatidylglycerols; PI = phosphatidylinositols; PS = phosphatidylserines; SM = sphingomyelins; SPH = sphingosine; TAG = triacylglycerol.

the AS group than the AH group. Conversely, PI, PA, DAG, and Cer all tended to be lower, with DAG and Cer significantly lower in the AS group compared to the AH group (Fig. 2E).

Under the conditions of VIP >1, *P*-value <0.05, and fold change (FC) ≥ 2 or FC ≤ 0.5, a total of 220 lipid molecules were identified as different in the three comparisons. Among these, 115 exhibited significant differences between the AH and AHAS groups, 169 between the AH and AS groups, and 38 between the AHAS and AS groups (Fig. 3A–C). These differential lipid molecules mainly belonged to the Hex2Cer (30), DAG (28), PC (26), PE (25), TAG (22), and Hex1Cer (19) subclasses (Fig. 3D). In glycerides, 7 molecules in TAG and 1 in DAG in the milk of AS cows were significantly higher than in AH cows ($P < 0.05$). However, 12 molecules in TAG and 20 in DAG were significantly lower in AS cows than in AH cows (Fig. 4A and B). Compared with the AH group, the relative intensity of 25 lipid molecules in PC varied significantly in dairy cows fed alfalfa silage. Figure 4C shows that PC (38:3), PC (16:1e_18:1), PC (15:0_14:0), PC (16:2e_17:0), PC (18:0_18:1), PC (18:0_16:0), PC (38:2), PC (16:1e_16:0), PC (12:1e_18:1), PC (14:1e_16:0), PC (12:0e_18:1), PC (40:5), PC (18:3e_18:0), PC (40:4), PC (27:0), PC (18:0_15:0), PC (40:1), PC (20:0_20:3), PC (38:0), PC (39:1), PC (37:0), and PC (42:1) in the milk of AS cows were significantly higher than in AH cows ($P < 0.05$). In Fig. 4D, PE (14:0p_17:1), PE (14:1e_18:2), PE (16:0_18:2), PE (16:0_20:4), PE (16:0p_18:1), PE (16:1e_18:1), PE (16:1e_18:3), PE (18:0_18:0), PE (18:0_18:1), PE (18:0_22:5), PE (18:0e), PE (18:0p_18:1), PE (18:1_20:4), PE (18:1_21:0), PE (18:1e_18:1), PE (18:1e_20:3), PE (18:1p_18:1), PE (35:2e), and PE (35:3e) in AS cows were significantly higher than in AH cows ($P < 0.05$). Eighteen milk lipid molecules in Hex1Cer and 30 in Hex2Cer were significantly reduced in dairy cows fed alfalfa silage compared to cows fed alfalfa hay (Fig. 4E and F). Fig. 4G and H showed that the AS group had significantly higher PS (18:0_18:1), PS (18:0_22:5), PS (18:1_21:0), PS (18:1_20:2), PS (18:1_24:0), PS (18:0_20:2), PS (36:4e), PS (40:5), PI (16:0_16:0), PI (19:1_18:0), PI (18:0_21:1), PG (16:0_18:1), PG (18:0_18:1), PG (16:0_14:0), PG (16:0_16:0), SM (d18:1_23:0), SM (d34:1), SM (d42:1), SM (d43:5), SM (d35:0), SM (d43:1), and SM (d28:1) ($P < 0.05$). Additionally, Fig. 4H shows that 14 lipid molecules of Cer in AS cows were significantly reduced compared to those in AH cows ($P < 0.05$). Furthermore, 4 lipid molecules of LPC, 3 of LPE, 6 of LPS, and 1 of LPI were significantly higher in the milk of AS cows than in AH cows, but 1 PA was significantly reduced in the milk of AS cows compared to AH cows (Fig. 4I).

4. Discussion

This research found that feeding dairy cows alfalfa silage increased milk fat content by 15.45% and milk fat yield by 12.31% compared to those fed alfalfa hay. Previous studies have shown that milk fat content increased by 3% to 10% in cows fed alfalfa silage

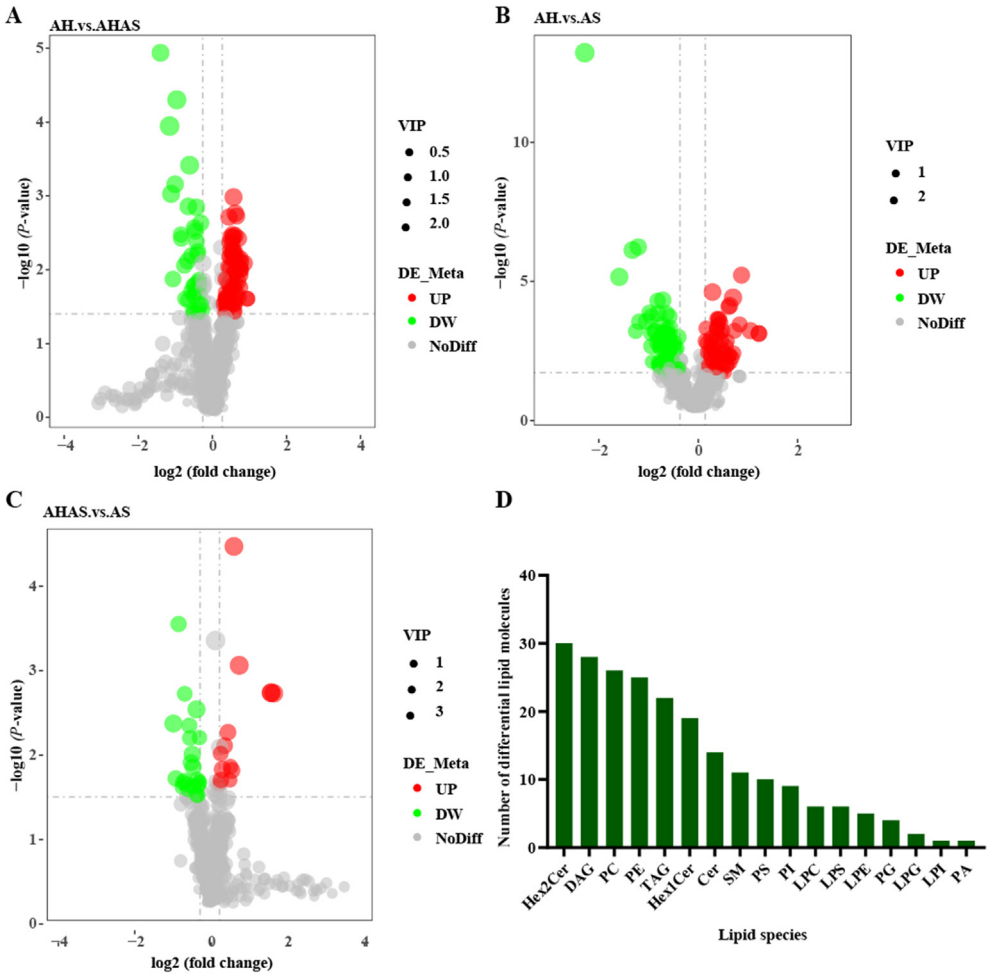

Fig. 3. Pair-wise comparisons of lipid molecules among three dietary treatments. (A, B, and C) The differential lipids between AH and AHAS, AH and AS, and AHAS and AS, respectively. (D) The number of differential lipids in each subclass. AH mean the diet containing alfalfa hay; AHAS means the diet containing 50% alfalfa hay and 50% alfalfa silage; AS means the diet containing alfalfa silage. DE_Meta means the differential metabolites; DW means down; NoDiff means the undeferential metabolites. Cer = ceramides; DAG = diacylglycerols; Hex1Cer = monohexosylceramide; Hex2Cer = dihexosylceramide; LPC = lysophosphatidylcholine; LPE = lysophosphatidylethanolamine; LPG = lysophosphatidylglycerols; LPI = lysophosphatidylinositol; LPS = lysophosphatidylserine; PA = phosphatidic acids; PC = phosphatidylcholines; PE = phosphatidylethanolamines; PG = phosphatidylglycerols; PI = phosphatidylinositols; PS = phosphatidylserines; SM = sphingomyelins; SPH = sphingosine; TAG = triacylglycerol.

versus alfalfa hay (Broderick, 1995; Calberry et al., 2003). The digestible energy value of silage (per kg DM) is about 1.24 times that of hay (Thomas et al., 1969). The higher milk fat content and yield in the AHAS and AS groups in the present study is partly due to the higher digestible energy in alfalfa silage compared to alfalfa hay. Our previous studies also found that milk fat was significantly higher in cows with higher total milk solids (Liu et al., 2022), which is consistent with the present study. Thus, we preliminarily conclude that the main reason for the high total milk solids content is due to the high milk fat content, although this has not been reported in previous research.

Moreover, there was no significant difference in milk protein production between cows fed alfalfa silage and those fed alfalfa hay, consistent with findings from previous studies (Broderick, 1995; Calberry et al., 2003; Plaizier, 2004). The current research also exhibited no significant differences in milk protein, possibly due to similar rumen MCP concentrations among the three groups. The study also showed minimal differences in lactose content, as lactose is a relatively stable component in milk and is usually less affected by diet, season, and other factors (Nichols et al., 2018).

Plasma biochemistry reflected both the health condition and the nutrient transportation of cows (Wu et al., 2018). Nutrients digested in the gastrointestinal tract first enter the bloodstream, and subsequently reach the mammary gland to produce milk components in dairy animals. The present study showed that the plasma biochemistry indexes varied among the three groups. Triacylglycerol and urea concentrations in plasma were higher in the two groups fed alfalfa silage compared to those fed alfalfa hay, likely due to the higher lipid and protein metabolism induced by the higher DMI.

The liver was the major organ for lipid and protein metabolism. For example, the concentrations of TAG, CHOL, GLU, ALT, AST, ALB, and urea are all associated with lipid metabolism in the host. ALB has a role in transporting steroids and FAs in the blood (Tairon et al., 2016). Therefore, we speculate that milk lipid compositions might differ due to the variations in milk fat content and plasma biochemistry indexes related to lipid metabolism among the three groups.

In the current study, results indicated that the contents of ω-3 PUFA, ω-6 PUFA, the ω-6/ω-3 PUFA ratio, and a total of 37 FAs in the milk of cows fed alfalfa silage and hay were altered. Previous research

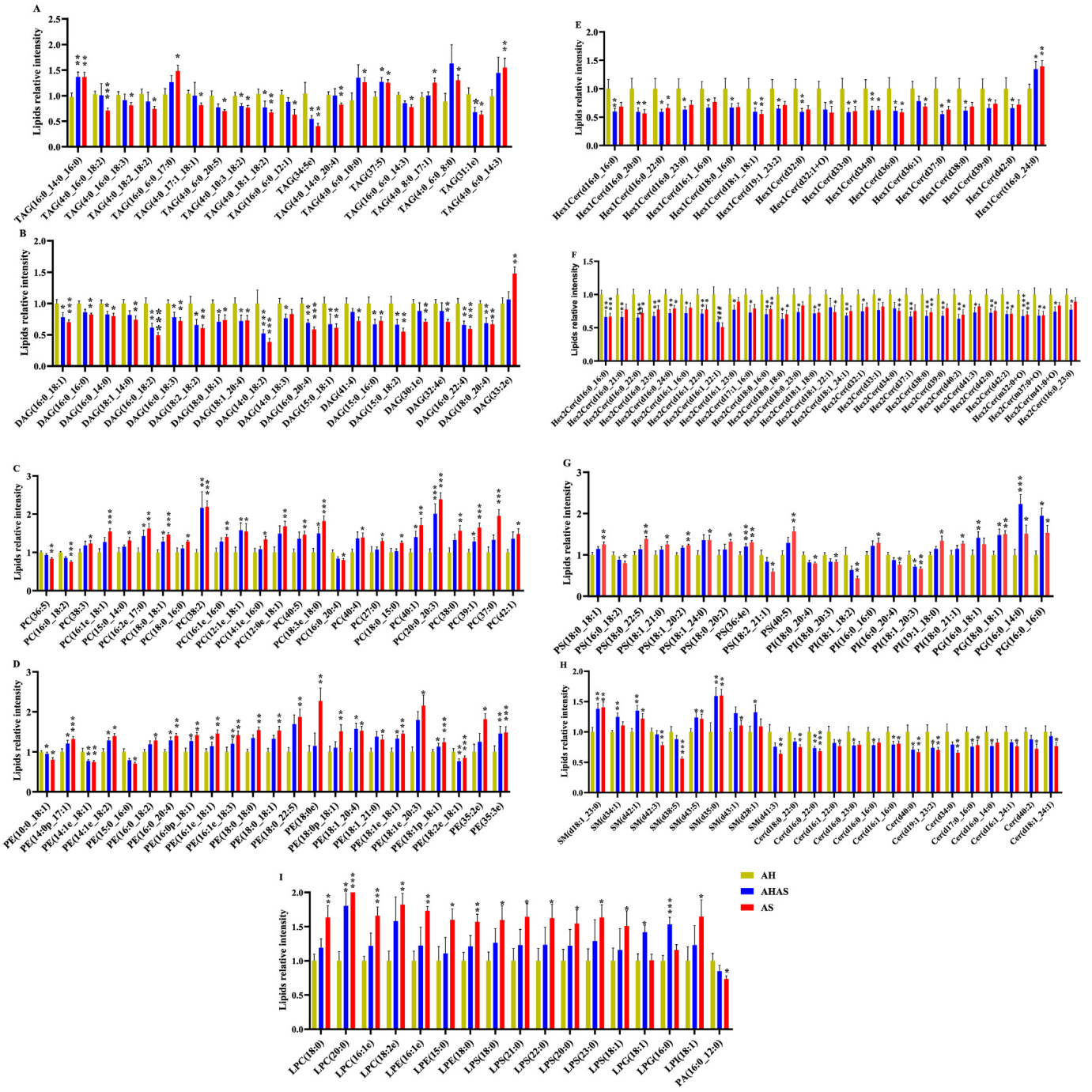

Fig. 4. Differences in milk lipid molecules among the three dietary treatments. AH, the diet containing alfalfa hay; AHAS, the diet containing 50% alfalfa hay and 50% alfalfa silage; AS, the diet containing alfalfa silage. (A, B, C, D, E, and F) The differential lipids in TAG, DAG, PC, PE, Hex1Cer, Hex2Cer, respectively. (G) The differential lipids in PS, PI and PG. (H) The differential lipids in SM and Cer. (I) The differential lipids in LPC, LPE, LPS, LPG, LPI, AcCa, and PA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Cer = ceramides; DAG = diacylglycerols; Hex1Cer = monohexosylceramide; Hex2Cer = dihexosylceramide; LPC = lysophosphatidylcholine; LPE = lysophosphatidylethanolamine; LPG = lysophosphatidylglycerols; LPI = lysophosphatidylinositol; LPS = lysophosphatidylserine; PA = phosphatidic acids; PC = phosphatidylcholines; PE = phosphatidylethanolamines; PG = phosphatidylglycerols; PI = phosphatidylinositols; PS = phosphatidylserines; SM = sphingomyelins; SPH = sphingosine; TAG = triacylglycerol.

has reported that forage species and conservation methods are primary factors influencing the milk FA profile (Shingfield et al., 2005; Glasser et al., 2013; Halmemies-Beauchet-Filleau et al., 2013; Jaakamo et al., 2019). We found that major ω-3 PUFA, such as ALA (C18:3 c9, c12, c15) and DPA (C22:5 c7, c10, c13, c16, c19) were significantly increased in the milk of AS cows compared to AH cows. ALA was the highest FA (about 33.06 g/100 g FA. Table S3) in the milk of both AH and AS cows. It is notable that an average of 4.84 g ALA per 100 g FA was detected in TMR, but only an average of 0.27 g ALA per 100 g FA was measured in milk samples (Table S2 and S3). This might be due to hydrogenation of unsaturated FAs during the conservation and mixing of forages. Additionally, a significant portion of ALA might be converted to other PUFAs, such as DHA, EPA, and docosapentaenoic acid (DPA). Moreover, a substantial amount of ALA is hydrogenated in the rumen by microorganisms, such as Ruminococcaceae_NK4A214_group, Christensenellaceae_R-7_group, and *Eubacterium coprostanoligenes*

(Huang et al., 2021; Gulati et al., 2022). Fermented forages, as roughage, are beneficial for producing milk fat with higher ω-3 PUFA content compared to hay (Elgersma, 2015; Rufino-Moya et al., 2022). In addition, researchers have found that feeding dairy cows with high quality forages results in milk richer in beneficial PUFA (Dewhurst et al., 2006; Liu et al., 2020). Thus, milk from cows fed on alfalfa silage was richer in ω-3 PUFA than milk from cows fed alfalfa hay.

The sum of all ω-6 PUFA concentrations in AH cows was significantly higher than in AS cows, likely due to the higher LA concentration in AH cows. The World Health Organization suggests that the ω-6/ω-3 PUFA in food should range from 5 to 10, with a lower ω-6/ω-3 PUFA ratio being beneficial for human health, especially in reducing the risk of cardiovascular diseases and cancers (Xiang et al., 2015; Candela et al., 2011). Moreover, decreasing the ω-6/ω-3 PUFA ratio in food can alleviate inflammatory responses because ω-6 PUFAs have pro-inflammatory activity, while ω-3 PUFAs have the anti-inflammatory activity (Warner et al., 2019). This study exhibited that the ω-6/ω-3 PUFA ratio in the milk was significantly reduced when alfalfa silage was increased in the TMR. Previous studies have found that the ω-6/ω-3 PUFA ratio in milk was three times greater in dairy cows fed TMR diets compared to those grazing, as well as when dairy cows were fed TMR with a lower forage-to-concentrate ratio (Barca et al., 2018; Cavaliere et al., 2018).

This study further investigated the divergence of lipid profiles in the milk of dairy cows fed alfalfa hay and alfalfa silage using lipidomics. Phospholipids and sphingolipids are two types of polar lipids (Fahy et al., 2005). The major phospholipids include PC, PE, PS, and PI, followed by PG, PA, LPC, LPE, and LPS (Song et al., 2022). Sphingolipids consist of SM, cerebrosides, ceramides, and gangliosides. SM is also considered a phospholipid because it shares the same head group as phospholipids (Liu et al., 2018; Song et al., 2022). The current research showed a higher intensity of PC, PE, and SM lipid molecules in the milk of AS and AHAS cows compared to AH cows. Liu et al. (2017) reported that most polar lipids exhibit a positive correlation with milk fat content. Additionally, other studies have noted a positive correlation between PC and PE content and milk fat content (Argov-Argaman et al., 2020; Zhao et al., 2022). These findings are consistent with the present study, which found significantly higher levels of lipid molecules in PC, PE, PS, PI, PG, and SM in the cows with the higher fat content. Previous research has indicated that phospholipids are critical components of the milk fat globule membrane and play numerous physiological roles in developing and maintaining brain health (Contarini et al., 2013; Anto et al., 2020). The major phospholipids, such as PC, PE, PS, and SM, have been reported to reduce the risk of cardiovascular diseases and aid in cholesterol absorption (Liu et al., 2017; Anto et al., 2020). Thus, this study suggests that raw milk from dairy cows fed alfalfa silage may have potential health benefits.

To date, few studies have compared the lipid variations in raw milk from dairy cows fed diets consisting of alfalfa silage versus hay. However, it has been shown that milk phospholipids and SM are higher in dairy cows fed fresh pasture-based diets in spring compared to those fed corn silage-based diets in winter (Christelle Lopez, 2014). We speculate that feeding diets based on alfalfa silage would improve the major phospholipid content compared to alfalfa hay. In summary, milk may be more beneficial for human health when dairy cows are fed a diet consisting of alfalfa silage rather than alfalfa hay. More research is required to support this view.

5. Conclusion

Dairy cows fed a diet based on alfalfa silage exhibited significantly higher milk fat content and yield compared to those fed alfalfa hay. Further investigation into the variation in milk lipidomic profiles revealed that numerous lipid molecules in PC, PE, PS, PI, PG, and SM were significantly increased in dairy cows fed alfalfa silage compared to those fed alfalfa hay. Moreover, ω-3 PUFA was significantly increased, while the ω-6/ω-3 PUFA ratio was decreased in cows fed alfalfa silage compared to those fed alfalfa hay. In conclusion, feeding TMR based on alfalfa silage to dairy cows could regulate the lipid and PUFA composition of raw milk toward meeting human dietary demands. These findings provide critical insights for producers regarding the application of alfalfa in dairy cow feeding practices.

Credit Author Statement

Kaizhen Liu: Writing - Original draft and Formal analysis. **Meiqinq chen**: Writing - Review & Editing and Conceptualization. **Guoxin Huang:** Writing - Review & Editing, and Formal analysis. **Chuanyou Su**: Methodology and Validation. **Wenhao Tang**: Writing - Review & Editing and Validation. **Ning Li:** Formal analysis. **Jiyong Yang**: Methodology and Validation. **Xufang wu:** Validation and Methodology. **Boxue Si**: Formal analysis and Validation. **Shengguo Zhao**: Conceptualization and Project administration. **Nan Zheng:** Investigation and Resources. **Yangdong Zhang**: Supervision and Resources. **Jiaqi Wang**: Writing - Review & Editing, Supervision, and Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This study was supported by National Key R&D Program of China (2022YFD1301000) , The Agricultural Science and Technology Innovation Program (ASTIP-IAS12) and The earmarked fund for CARS (CARS-36). The authors would like to express their sincere gratitude to all the farm workers at AUSTASIA Farming of Inner Mongolia (Chifeng, Inner Mongolia, China) for their invaluable support. Special thanks also go to the students of our laboratory who were involved in the animal trial.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.06.009.

References

Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr 2014;2(5):443–63. https://doi.org/10.1002/fsn3.121.

Anto L, Warykas SW, Torres-Gonzalez M, Blesso CN. Milk polar lipids: underappreciated lipids with emerging health benefits. Nutrients 2020;12(4). https://doi.org/10.3390/nu12041001.

AOAC International. Official methods of analysis. 20th ed. AOAC International; 2016.

Argov-Argaman N, Cohen-Zinder M, Leibovich H, Yishay M, Eitam H, Agmon R, Hadaya O, Mesilati-Stahy R, Miron J, Shabtay A. Dietary pomegranate peel improves milk quality of lactating ewes: emphasis on milk fat globule membrane properties and antioxidative traits. Food Chem 2020;313. https://doi.org/10.1016/j.foodchem.2019.125822.

Barca J, Carriquiry M, Olazabal L, Fajardo M, Chilibroste P, Meikle A. Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation. J Anim Physiol Anim Nutr 2018;102(3):620–9. https://doi.org/10.1111/jpn.12826.

Brick T, Schober Y, Bocking C, Pekkanen J, Genuneit J, Loss G, Dalphin JC, Riedler J, Lauener R, Nockher WA, Renz H. Omega-3 fatty acids contribute to the asthma-

protective effect of unprocessed cow's milk. J Allergy Clin Immunol 2016;137(6):1699–1706 e1613. https://doi.org/10.1016/j.jaci.2015.10.042.
Broderick GA. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J Dairy Sci 1995;78(2):320–9. https://doi.org/10.3168/JDS.S0022-0302(95)76640-1.
Broderick GA, Koegel RG, Walgenbach RP, Kraus TJ. Ryegrass or alfalfa silage as the dietary forage for lactating dairy cows. J Dairy Sci 2002;85(7):1894–901. https://doi.org/10.3168/jds.S0022-0302(02)74264-1.
Calberry JM, Plaizier JC, Einarson MS, McBride BW. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on production and rumen conditions of lactating dairy cows. J Dairy Sci 2003;86(11):3611–9. https://doi.org/10.3168/jds.S0022-0302(03)73967-8.
Candela CG, Gómez López LM, Loria VL. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Nutr Hosp 2011;26(2):323–9. https://doi10.1590/S0212-16112011000200013.
Cavaliere G, Trinchese G, Musco N, Infascelli F, De Filippo C, Mastellone V, Morittu VM, Lombardi P, Tudisco R, Grossi M, Monda V. Milk from cows fed a diet with a high forage: concentrate ratio improves inflammatory state, oxidative stress, and mitochondrial function in rats. J Dairy Sci 2018;101:1843–51.
Chen M, Wang F, Wu X, Si B, Pan J, Zheng N, Zhang Y, Wang J. Updating the fatty acid profiles of retail bovine milk in China based on an improved GC-MS method: implications for nutrition. Front Nutr 2023;25(10):1204005. https://doi.org/10.3389/fnut.2023.1204005.
Chilliard Y, Ferlay A, Loor J, Rouel J, Martin B. Trans and conjugated fatty acids in milk from cows and goats consuming pasture or receiving vegetable oils or seeds. Ital J Anim Sci 2016;1(4):243–54. https://doi.org/10.4081/ijas.2002.243.
Christelle Lopez VBB, Olivia Ménard. Polar lipids, sphingomyelin and long-chain unsaturated fatty acids from the milk fat globule membrane are increased in milks produced by cows fed fresh pasture based diet during spring. Food Res Int 2014;58:59–68. https://doi.org/10.1016/j.foodres.2014.01.049.
Contarini G, Povolo M. Phospholipids in milk fat: composition, biological and technological significance, and analytical strategies. Int J Mol Sci 2013;14(2):2808–31. https://doi.org/10.3390/ijms14022808.
Dabija AI, Mironeasa S, Oroian M, Sion I. Study concerning milk quality – raw material for dairy industry. ETP Inte J Food Eng 2018:14–21. https://doi.org/10.18178/ijfe.4.1.14-21.
Dewhurst RJ, Shingfield KJ, Lee M, Scollan ND. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim Feed Sci Technol 2006;131(3–4):168–206. https://doi.org/10.1016/j.anifeedsci.2006.04.016.
Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, Brown M, Knowles JD, Halsall A, Haselden JN, Nicholls AW. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc 2011;6(7):1060–83. https://doi.org/10.1038/nprot.2011.335.
Elgersma A. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives. Eur J Lipid Sci Technol 2015;117(9):1345–69. https://doi.org/10.1002/ejlt.201400469.
Fahy E, Subramaniam S, Brown HA, Glass CK. Dennis. A comprehensive classification system for lipids. J Lipid Res 2005;46(5):839–61. https://doi.org/10.1194/jlr.E400004-JLR200.
Gaines WL. The Energy basis of measuring milk yield in dairy cows. Illinois Agr. Expt. Sta., Bull. 1928;308.
Garcia C, Lutz NW, Confort-Gouny S, Cozzone PJ, Armand M, Bernard M. Phospholipid fingerprints of milk from different mammalians determined by 31P NMR: towards specific interest in human health. Food Chem 2012;135(3):1777–83. https://doi.org/10.1016/j.foodchem.2012.05.111.
Glasser F, Doreau M, Maxin G, Baumont R. Fat and fatty acid content and composition of forages: a meta-analysis. Anim Feed Sci Technol 2013;185(1–2):19–34. https://doi.org/10.1016/j.anifeedsci.2013.06.010.
Gulati SK, Thomson P, Ha WK, Lee WJ, Lee JH, Choi JH, Ko KH, Park SI, Cox G, Scott TW. Transfer rates of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids into cow's milk in pasture based and feed-lot management systems. Eur J Lipid Sci Technol 2022;124(6):1–8. https://doi.org/10.1002/ejlt.202100106.
Halmemies-Beauchet-Filleau A, Kairenius P, Ahvenjarvi S, Toivonen V, Huhtanen P, Vanhatalo A, Givens DI, Shingfield KJ. Effect of forage conservation method on plasma lipids, mammary lipogenesis, and milk fatty acid composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J Dairy Sci 2013;96(8):5267–89. https://doi.org/10.3168/jds.2013-6571.
Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. J Lipid Res 2003;44(6):1071–9. https://doi.org/10.1194/jlr.R300004-JLR200.
Huang G, Guo L, Chang X, Liu K, Tang W, Zheng N, Zhao S, Zhang Y, Wang J. Effect of whole or ground flaxseed supplementation on fatty acid profile, fermentation, and bacterial composition in rumen of dairy cows. Front Microbiol 2021;12:760528. https://doi.org/10.3389/fmicb.2021.760528.
Hu X, Zhang W, Chi X, Wang H, Liu Z, Wang Y, Ma L, Xu B. Non-targeted lipidomics and transcriptomics analysis reveal the molecular underpinnings of mandibular gland development in apis mellifera ligustica. Dev Biol 2021;479:23–36. https://doi:10.1016/j.ydbio.2021.07.016.
Jaakamo MJ, Luukkonen TJ, Kairenius PK, Bayat AR, Ahvenjarvi SA, Tupasela TM, Vilkki JH, Shingfield KJ, Leskinen HM. The effect of dietary forage to concentrate ratio and forage type on milk fatty acid composition and milk fat globule size of lactating cows. J Dairy Sci 2019;102(10):8825–38. https://doi.org/10.3168/jds.2018-15833.
Li J, Tang C, Zhao Q, Yang Y, Li F, Qin Y, Liu X, Yue X, Zhang J. Integrated lipidomics and targeted metabolomics analyses reveal changes in flavor precursors in psoas major muscle of castrated lambs. Food Chem 2020;333:127451. https://doi.org/10.1016/j.foodchem.2020.127451.
Li Q, Zhao Y, Zhu D, Pang X, Liu Y, Frew R, Chen G. Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-Exactive Orbitrap Mass Spectrometry. Food Chem 2017;224:302–9. https://doi.org/10.1016/j.foodchem.2016.12.083.
Liu K, Zhang Y, Huang G, Zheng N, Zhao S, Wang J. Ruminal bacterial community is associated with the variations of total milk solid content in Holstein lactating cows. Anim Nut 2022;9:175–83. https://doi.org/10.1016/j.aninu.2021.12.005 (2022).
Liu N, Pustjens AM, Erasmus SW, Yang Y, Hettinga K, van Ruth SM. Dairy farming system markers: the correlation of forage and milk fatty acid profiles from organic, pasture and conventional systems in The Netherlands. Food Chem 2020;314:126153. https://doi.org/10.1016/j.foodchem.2019.126153.
Liu S, Zhang R, Kang R, Meng J, Ao C. Milk fatty acids profiles and milk production from dairy cows fed different forage quality diets. Anim Nutr 2016;2(4):329–33. https://doi.org/10.1016/j.aninu.2016.08.008.
Liu Z, Logan A, Cocks BG, Rochfort S. Seasonal variation of polar lipid content in bovine milk. Food Chem 2017;237:865–9. https://doi.org/10.1016/j.foodchem.2017.06.038.
Liu Z, Rochfort S, Cocks B. Milk lipidomics: what we know and what we don't. Prog Lipid Res 2018;71:70–85. https://doi.org/10.1016/j.plipres.2018.06.002.
Mckenzie HA, Wallace HSA. The Kjeldahl determination of nitrogen: a critical study of digestion conditions of temperature, catalyst, and oxidizing agent. Aust J Chem 1954;7:55–70. https://doi.org/10.1071/CH9540055.
Murphy SC, Martin NH, Barbano DM, Wiedmann M. Influence of raw milk quality on processed dairy products: how do raw milk quality test results relate to product quality and yield? J Dairy Sci 2016;99(12):10128–49. https://doi.org/10.3168/jds.2016-11172.
Nichols K, Bannink A, Pacheco S, van Valenberg HJ, Dijkstra J, van Laar H. Feed and nitrogen efficiency are affected differently but milk lactose production is stimulated equally when isoenergetic protein and fat is supplemented in lactating dairy cow diets. J Dairy Sci 2018;101(9):7857–70. https://doi.org/10.3168/jds.2017-14276.
NRC (National Research Council). In: Nutrient requirements of dairy cattle. 7th rev. Washington (DC): The National Academy Press; 2001.
Plaizier JC. Replacing chopped alfalfa hay with alfalfa silage in barley grain and alfalfa-based total mixed rations for lactating dairy cows. J Dairy Sci 2004;87(8):2495–505. https://doi.org/10.3168/jds.S0022-0302(04)73374-3.
Ren G, Cheng G, Wang J. Understanding the role of milk in regulating human homeostasis in the context of the COVID-19 global pandemic. Trends Food Sci Technol 2021;107:157–60. https://doi.org/10.1016/j.tifs.2020.09.027.
Rufino-Moya PJ, Bertolin JR, Blanco M, Lobon S, Joy M. Fatty acid profile, secondary compounds and antioxidant activities in the fresh forage, hay and silage of sainfoin (Onobrychis viciifolia) and sulla (Hedysarum coronarium). J Sci Food Agric 2022;102:4736–43. https://doi.org/10.1002/jsfa.11834.
Shingfield KJ, Salo-Vaananen P, Pahkala E, Toivonen V, Jaakkola S, Piironen V, Huhtanen P. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. J Dairy Res 2005;72(3):349–61. https://doi.org/10.1017/S0022029905000919.
Song Y, Cai C, Song Y, Sun X, Liu B, Xue P, Zhu M, Chai W, Wang Y, Wang C, Li M. A comprehensive review of lipidomics and its application to assess food obtained from farm animals. Food Sci Anim Resour 2022;42(1):1–17. https://doi.org/10.5851/kosfa.2021.e59.
Tairon PD, Silva Carlo AT. Marques. Intake, digestibility, milk yield and indicators of the metabolic status of native ewes fed supplemented diet under grazing system. Ital J Anim Sci 2016;14(2):272–9. https://www.tandfonline.com/loi/tjas20.
Thomas J, Brown L, Emery R, Benne E, Huber J. Comparisons between alfalfa silage and hay. J Dairy Sci 1969;52(2):195–204. https://doi.org/10.3168/jds.S0022-0302(69)86529-X.
Tu CH, Qi XE, Shui SS, Lin HM, Benjakul S, Zhang B. Investigation of the changes in lipid profiles induced by hydroxyl radicals in whiteleg shrimp (*Litopenaeus vannamei*) muscle using LC/MS-based lipidomics analysis. Food Chem 2022;369:130925. https://doi.org/10.1016/j.foodchem.2021.130925.
Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Wang F, Chen M, Luo R, Huang G, Wu X, Zheng N, Zhang Y, Wang J. Fatty acid profiles of milk from Holstein cows, Jersey cows, buffalos, yaks, humans, goats, camels, and donkeys based on gas chromatography-mass spectrometry. J Dairy Sci 2021;105(2):1687–700. https://doi.org/10.3168/jds.2021-20750.
Wang L, Li X, Liu L, Zhang DH, Zhang Y, Chang YH, Zhu QP. Comparative lipidomics analysis of human, bovine and caprine milk by UHPLC-Q-TOF-MS. Food Chem 2020;310:125865. https://doi.org/10.1016/j.foodchem.2019.125865.
Want EJ, Wilson ID, Gika H, Theodoridis G, Plumb RS, Shockcor J, Holmes E, Nicolson JK. Global metabolic profiling procedures for urine using UPLC-MS. Nat Protoc 2010;5(6):1005–18. https://doi.org/10.1038/nprot.2010.50.
Warner DR, Warner JB, Hardesty JE, Song YL, King TN, Kang JX, Chen CY, Xie S, Yuan F, Prodhan MA, Ma X. Decreased ω-6: ω-3 PUFA ratio attenuates ethanol-induced alterations in intestinal homeostasis, microbiota, and liver injury. J Lipid Res 2019;60(12):2034–49. https://doi.org/10.1194/jlr.RA119000200.

Wu X, Sun H, Xue M, Wang D, Guan LL, Liu J. Serum metabolome profiling revealed potential biomarkers for milk protein yield in dairy cows. J Proteonomics 2018;184:54–61. https://doi.org/10.1016/j.jprot.2018.06.005.

Xiang M, Harbige LS, Zetterstrom R. Long-chain polyunsaturated fatty acids in Chinese and Swedish mothers: diet, breast milk and infant growth. Acta Paediatr 2015;94(11):1543–9. https://doi.org/10.1080/08035250500251601.

Yuan M, Breitkopf SB, Yang X, Asara JM. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. Nat Protoc 2012;7(5):872–81. https://doi.org/10.1038/nprot.2012.024.

Zhang X, Li H, Yang L, Jiang G, Ji C, Zhang Q, Zhao F. Corrigendum to comparative lipidomics profiling of donkey milk with cow and human milk by UHPLC-Q-Exactive Orbitrap Mass Spectrometry. Food Compos Anal. 2021;101:103988. https://doi.org/10.1016/j.jfca.2021.104242. J Food Comp Analy 2022;106.

Zhao Y, Yu S, Zhao H, Li L, Li Y, Tu Y, Jinag L, Zhao G. Lipidomic profiling using GC and LC-MS/MS revealed the improved milk quality and lipid composition in dairy cows supplemented with citrus peel extract. Food Res Int 2022;161:111767. https://doi.org/10.1016/j.foodres.2022.111767.