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ABSTRACT

Compared with maize silage- and concentrate-based diets, herbage-based diets were repeatedly shown to favourably influence the milk fatty acid (**FA**) profile. However, it is unclear how the herbage feeding mode (grazing vs indoor green-feeding) and conservation (fresh herbage vs hay vs silage) modify the milk FA profile. Therefore, the aim of the present experiment was to investigate the effect of different herbage utilisation methods (including herbage feeding mode and herbage conservation method) on the ruminal biohydrogenation of dietary FA and the consequences on the milk FA composition in cows of two breeds (Holstein and Montbéliarde). Concomitant effects of botanical composition and phenological stage of the herbage on milk FA profile were controlled for by harvesting barn-dried hay and silage simultaneously as first cut from the same ryegrass-dominated grassland in a semi-mountainous region. Seven weeks later, the first regrowth of the same plot was used as fresh herbage, either grazed or fed indoor (indoor green-feeding). Twenty-four Montbéliarde and 24 Holstein cows were randomly allocated to four groups of 12 cows balanced by breed, parity, and milk yield. In a free-stall barn, three groups were given *ad libitum* access to hay, silage, or fresh herbage, respectively. The fourth group was strip-grazing. All cows were supplemented with 3 kg DM/day of the same energy-rich concentrate. After 2 weeks of adaptation to the forage, samples of forage, concentrate, milk, blood, and rumen fluid were collected. Fatty acid composition of forages, rumen fluid, and milk was analysed by gas chromatography. Haymaking reduced total FA content of the herbage, in particular that of linoleic acid (**LA**) and α-linolenic acid (**ALA**). Still, rumen fluid lipids of hay-fed cows had the highest proportion of rumenic acid, LA, ALA, and total polyunsaturated fatty acids (**PUFAs**). Milk fat from hay-fed cows had the highest proportion of LA, and the apparent transfer rates from feed to milk of LA and ALA were higher in hay-fed cows than in silage-fed cows. The proportion of PUFAs was highest in milk fat from grazing and indoor green-fed Montbéliarde cows and lowest in silage-fed cows of both breeds. In conclusion, the herbage utilisation method affects the ruminal biohydrogenation of LA and ALA, whereby herbage drying particularly increases their transfer from herbage to milk.

Implications

Effects on ruminal and milk fatty acid composition of herbage harvested from the same meadow but fed as hay, silage, or fresh herbage indoors or strip-grazed were investigated in Holstein and Montbéliarde cows. Feeding hay instead of silage and fresh herbage affected the ruminal biohydrogenation of linoleic and α-linolenic acids and their proportions in the rumen fluid lipids, but milk fatty acid profiles did not differ. When fresh herbage

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was fed either indoor or by strip-grazing, especially to Montbéliarde cows, the milk fat had a higher proportion of nutritionally relevant polyunsaturated fatty acids and conjugated linoleic acids than milk from Holstein cows.

Introduction

Grassland-based production systems are of great interest because of the positive perception of the consumers concerning the quality of milk and the resulting dairy products in nutritional and extrinsic respect (i.e. animal welfare, preservation of landscape and biodiversity, etc.). Establishing these systems is often encouraged through specific milk payment schemes. A characteristic feature of milk fat produced by cows fed grass-based diets is its higher proportion of polyunsaturated fatty acids (**PUFAs**), especially α-linolenic acid (C18:3n-3; **ALA**) and C18:2*c*9*t*11 (the most important isomer of conjugated linoleic acid; **CLA**), as well as of lipophilic vitamins (e.g. α-tocopherol) and provitamins (e.g. β-carotene) compared with milk from cows fed diets with high proportions of maize silage and concentrate (Ferlay et al., 2008; Butler et al., 2011). The higher ALA proportion in milk fat derived from grass-based diets results from the higher dietary intake of ALA, even though it is hydrogenated or completely saturated through ruminal biohydrogenation (**RBH**) before duodenal absorption (Khiaosa-Ard et al., 2015). However, different herbage utilisation methods may affect the proportion of PUFA in the herbage. Indeed, different herbage wilting and conservation practices such as haymaking or ensiling were also found to alter the proportions of ALA and linoleic acid (**LA**; 18:2*c*9*c*12) in the herbage lipids because of the differing lipolytic activity in the herbage and oxidation of herbage fatty acids (**FAs**) (Glasser et al., 2013). Despite the differences found in fresh, dried or ensiled herbage, only few differences have been reported so far concerning the milk FAs composition of cows fed differently conserved herbage (Shingfield et al., 2005; van den Oever et al., 2021). However, to our knowledge, no study directly linked the FAs profiles of rumen fluid and milk in dairy cows fed different herbage-based diets. Plant species diversity and the prevalence of different plant compounds (e.g. polyphenols), which is affected by botanical composition and phenological stage of the herbage, influence the RBH of dietary PUFAs (Khiaosa-Ard et al., 2009). Grazing compared with indoor green-feeding allows dietary selection by the cows and thus may modify their lipid and FAs intake or the intake of plant compounds, which in turn would affect the milk FAs profile. However, milk fat LA and ALA proportions did not differ between grazing and indoor green-fed cows with herbage from intensively managed grasslands, whereas both FAs were clearly more abundant in milk from cows grazing on Alpine pastures compared with cows kept indoor fed with green herbage from the same pastures (Leiber et al., 2005). Nutrient losses deriving from cutting, transport and plant respiration in the barn in indoor green-feeding instead of direct ingestion by grazing cows might also influence the herbage FAs content and proportions in the lipids, with consequences on the milk FAs profile. In addition, although dietary effects on milk FAs profile were reported to be of higher magnitude than breed effects, different cow breeds may still exhibit different responses to dietary factors that could influence the milk FAs profile (Lawless et al., 1999).

The aim of the present experiment was to investigate the effect of herbage utilisation method on milk FAs composition, through variations in RBH of dietary FAs in Holstein cows (**Ho**), that prioritise milk secretion, and Montbéliarde cows (**Mo**) that rather prioritise fat deposition (Pires et al., 2015). We hypothesised (1) that, in comparison to fresh herbage feeding (grazing and indoor green-feeding), any herbage conservation method, but especially haymaking, modifies the rumen fluid and milk FAs composition; (2) that grazing compared with indoor green-feeding modifies rumen fluid and milk FAs composition through dietary selection and intake behaviour; and (3) that the effect of the herbage utilisation method differs depending on the dairy cow breed. Findings on sensory properties of the milk and on cheese quality based on separate samplings of bulk milk obtained from the current experiment were reported in Manzocchi et al. (2021).

Material and methods

Experimental design and animals

The experiment took place in 2019 at the INRAE research facility Marcenat, where an extensive dairy production system with seasonal calving in autumn/winter is practised in a semi-mountainous area (45°15′N, 2°55′E, 1 100–1 200 m above sea level). Twenty-four late-lactating Ho and 24 Mo cows were randomly allocated to four groups of 12 cows each, balanced by breed, parity, and milk yield. After the pre-experimental period of 4 weeks, the cows were 251 ± 25 days in milk (mean ± SD), produced 14.3 ± 3.3 kg milk/day and had a BW of 651 ± 56 kg. Their average milk fat and protein percentages were 3.8 ± 0.4% and 3.2 ± 0.3%, respectively. During the pre-experimental period, all cows were kept in a free-stall barn and fed 1st cut hay (per kg on DM basis: 92 g CP, 619 g NDF, 357 g ADF) at *ad libitum* access plus 5 kg DM of 2nd cut hay (per kg on DM basis: 108 g CP, 530 g NDF, 332 g ADF) and up to 5 kg DM of a concentrate (per kg on DM basis: 175 g CP, 266 g NDF, 146 g ADF), allowing for adaptation to a herbage-based diet. The experiment consisted of 15 days of adaptation to the respective experimental herbage followed by a 2-week experimental period. After allocation to the groups, the first group was fed hay *ad libitum*. The second group was fed grass silage *ad libitum* and had free access to up to 2 kg/day DM of the same hay-fed to the hay-group, to ensure an adequate DM intake despite the high grass silage proportion in the diet. The third group was fed fresh herbage indoors (i.e. indoor green-feeding) in an amount that was adjusted to allow for 5% refusals. The three groups were kept in a free-stall barn. The fourth group was strip-grazing on the same portion of the plot where all other forages were originating from, with allocation of a new pasture strip every 3–4 days. All cows were supplemented with 3 kg/day DM of concentrate (per kg on DM basis: 172 g maize, 140 g wheat, 140 g barley, 140 g wheat bran, 140 g sunflower cake, 119 g rapeseed meal, 50 g cereal grains, 20 g molasses and 79 g mineral-vitamins premix) and had free access to mineral blocks (with 2 g P, 60 g Ca, 120 g Mg, 120 g Na/kg, SODI SoMag, Salins). Free access to water was always guaranteed to all cows. All cows were milked in a herringbone milking parlour at 0630 and 1600 h, and the milk yield was recorded automatically at each milking. Intake of hay and silage was recorded daily with automatic weighing troughs. Fresh herbage intake of the indoor green-fed group was measured daily by weighing the amount of fresh herbage offered and subtracting the amount of refusals. Individual feed intake of indoor green-fed cows was estimated by dividing the ingested amount of the group by 12. Herbage intake on pasture was estimated for each cow according to INRA's equation for the estimation of the intake capacity (Supplementary Material S1) based on age, week of lactation, week of gestation, maximal potential milk yield, BW, and body condition score (INRA, 2018). Owing to the associated uncertainties, feed and FAs intakes of the grazing and indoor green-fed groups were not included in the statistical analysis. The body condition score was graded on a scale from 0 to 5 (Bazin et al., 1984) by two independent, trained assessors at the beginning and at the

end of the experiment in the morning after milking and cows were concomitantly weighed on a scale (DeLaval, France).

Experimental forages

Methods on herbage production and detailed botanical composition of the herbage were previously reported in Manzocchi et al. (2021). Hay and silage were harvested simultaneously as first cut from the same intensively managed, ryegrass-dominated permanent (semi-natural) grassland (6 ha) on 15 May 2019. The phenological stage was determined on 100 randomly picked grasses (BBCH: 53, Hack et al., 1992). Herbage for silage was not wilted before ensiling, chopped, and stored in a plastic bag silo. Herbage for haymaking was dried for 72 h, raked, and baled (at 740 g/kg DM). Bales were ventilated with hot air to reach 880 g/kg DM during 2 days. As silage needs at least six weeks of fermentation before use, it was obviously not possible to simultaneously compare conserved and fresh forage in the same growing cycle. Therefore, the latter originated from the same plot but after seven weeks of regrowth, when the phenological stage of the herbage (BBCH: 47) was similar to that of the herbage at the first cut. Half of the plot was delimited with electric fences, divided into strips, and allocated to grazing. Twice per week, a new strip was opened. The stocking density was 4 cows/ha, and initial herbage biomass on the sward was 2 700 kg DM/ha (above 5 cm). Fresh herbage for indoor green-feeding was harvested daily with a cutting and loading truck on the other half of the plot.

Samplings and analysis

Feeds

Samples of hay, silage, and fresh herbage fed indoors were collected twice weekly for the determination of DM proportion, proximate composition, and FAs analysis. Pasture herbage samples were collected weekly on five 1 m × 10 cm strips across the plot and pooled. An aliquot was dried (60 °C, 72 h), and another aliquot was preserved at −20 °C until proximate and FAs analysis, respectively. Simulated bites of the grazing cows were sampled according to the method described by Coppa et al. (2015a) on day 17 of the experiment, pooled to one sample per cow and also preserved at −20 °C until analysis. Concentrate samples were collected once per week and stored at −20 °C until analysis. Fresh herbage and silage samples were dried (60 °C, 48 h). All herbage and concentrate samples were analysed for DM, ash, CP, NDF, and ADF according to Coppa et al. (2015a). Hemicellulose proportion was calculated as the difference between NDF and ADF. The digestibility of the organic matter was estimated with the pepsin-cellulase digestibility assay as described by Aufrère and Michalet-Doreau (1983). Fermentation quality of the silage was analysed according to VDLUFA (2018). Another aliquot of each hay, silage, and fresh herbage, simulated bite, and concentrate sample was frozen, lyophilised, and later subjected to FAs analysis as detailed by Ferlay et al. (2010).

Blood and rumen fluid

Blood samples were taken from the tail vein after the morning milking with ethylenediaminetetraacetic acid-containing tubes (Terumo) on day 18 of the experiment. Samples were centrifuged at 1 200*g* for 20 min at 4 °C, and the obtained plasma was immediately stored at −20 °C. After thawing, the plasma concentrations of non-esterified FAs (Kit NEFA-HR2, Fujifilm WAKO), glucose, urea, and β-hydroxybutyrate (Kit 981379, 981818, 984325, respectively, all ThermoScientific) were analysed on a chemistry analyser (Arena 20 XT Chemistry System, ThermoScientific). On the same day, 50 ml of rumen fluid was collected from each animal using an oro-ruminal probe, after at least 2 h of fasting, and discarding the first 200 ml drawn from the probe. The rumen fluid, probably originating from the central rumen (Shen et al., 2012), was immediately filtered through a 250-μm nylon pore cloth and frozen at −20 °C. Subsequent to lyophilisation, lipids in 100 mg of sample were methylated with 0.5 M NaOH in methanol and methanol-acetyl chloride (10:1 v/v) and the FAs profile was analysed as detailed by Zened et al. (2011). The following ratios between FAs and their precursors in the rumen (Enjalbert et al., 2017) were calculated: C18:2*t*11*c*15-to-ALA, rumenic acid (**RA**)-to-LA, vaccenic acid (**VA**)-to-(C18:2*t*11*c*15 + RA), and C18:0-to-VA.

Milk

Milk samples were collected on four consecutive milkings per week during the pre-experimental and experimental periods. They were preserved with bronopol at 4 °C and analysed for concentrations of fat, protein, casein, lactose, and urea with a spectrometric method (MilkoScan FT6000, Foss). Yield of energy-corrected milk was calculated as milk yield (kg/day) × (0.38 × milk fat (%) + 0.24 × milk protein (%) + 0.17 × milk lactose (%))/3.14. On the last day of the pre-experimental period and on day 18 of the experiment, individual samples from morning and evening milks were collected and preserved at −20 °C. Prior to FAs analysis, milk samples were lyophilised and pooled according to milk yields obtained during morning and evening milking. Milk lipids in lyophilised samples were methylated by adding 2 ml of 0.5 M sodium methoxide and 1 ml methanolic HCl (5% HCl v/v in methanol) at 50 °C for 5 min, and FAs composition was analysed according to Ferlay et al. (2010). Apparent recovery rates of ALA and LA (secreted, % of intake) were calculated assuming a FAs proportion in milk fat of 933 ± 2.0 g/kg (mean ± SD) as proposed by Glasser et al. (2007) and dividing daily milk FAs yield by daily intake of the respective FAs.

Statistical analysis

All data were analysed with generalised linear models in SAS (version 9.4, SAS Institute Inc.). The models used for the analysis of all cow-derived data included herbage utilisation method, breed, and their interaction as fixed effect. Individual cow was considered as the experimental unit. The pre-experimental data on yield and composition of the milk, milk FAs profile, blood plasma metabolites, and BW centred to the breeds' averages were included as covariates in the model. All data from one hay-fed cow were omitted from the statistical evaluation, as the animal suffered from severe claw problems. Comparisons among dietary treatments were performed with Fisher's protected LSD using the 'pdiff' and 'slice' option of the Least Square means statement of the generalised linear model procedure in SAS. Normality and homoscedasticity of data and residues were tested with the Shapiro-Wilk test and by visual inspection of skewness and kurtosis. All variables are reported as Least Square means and SEM for dietary treatments in the tables, except for data on rumen FAs proportions, rumen fluid FAs ratios, and plasma non-esterified FAs, which were log-transformed for statistical analysis and presented as arithmetic means and SE. Proportions of plant functional groups and composition of selected bite samples of Ho and Mo cows were compared by Student's *t*-test (Supplementary Tables S1). Differences were considered as significant when $P < 0.05$ and as a tendency for $0.05 \leq P < 0.10$.

Results

Herbage proximate and fatty acid composition

The NDF proportion did not greatly differ between forages (Table 1). The silage had a higher ADF proportion and a lower pro-

Table 1
Effect of herbage utilisation method (n = 3) on the proximate nutrient composition (in g/kg DM, if not else indicated) of the experimental herbages, and proximate nutrient composition of the concentrate fed to dairy cows.

			Fresh herbage					
Item	Hay	Silage[1]	Pasture (offered)	Simulated bites	Indoor	SEM	*P*-value	Concentrate
DM	882	196	290	287	287		<0.001	90.4
Organic matter	905[ab]	890[a]	906[ab]	908[a]	902[b]	2.4	<0.001	88.5
CP	131[b]	146[ab]	125[b]	161[a]	123[b]	7.8	<0.001	17.9
NDF	527	563	558	532	548	15.8	0.28	18.6
ADF	282[b]	349[a]	287[b]	265[c]	290[b]	6.9	<0.001	6.81
Hemicellulose	245[ab]	213[b]	270[a]	267[a]	257[a]	10.1	0.004	11.7
Digestibility[2](%)	67.0[b]	52.6[c]	68.3[b]	72.7[a]	67.8[b]	11.5	<0.001	80.4
Fatty acids								
Total fatty acids	8.99[b]	15.5[ab]	14.6[ab]	19.8[a]	15.7[ab]	2.50	0.01	14.6
C16:0	2.06[b]	2.47[ab]	2.42[ab]	3.12[a]	2.79[ab]	0.35	0.07	3.45
C18:0	0.15[b]	0.23[b]	0.23[b]	0.36[a]	0.31[ab]	0.05	0.01	0.43
C18:1*c*9	0.23[b]	0.59[a]	0.39[b]	0.42[a]	0.54[ab]	0.07	0.02	5.44
C18:2n-6	1.31[b]	3.03[a]	1.97[b]	3.01[a]	2.29[ab]	0.33	0.001	3.74
C18:3n-3	3.11[b]	6.27[ab]	6.24[ab]	9.02[a]	6.40[ab]	2.08	0.01	0.32
Other fatty acids	1.85[b]	2.85[ab]	3.46[ab]	3.90[a]	3.53[ab]	0.47	0.03	1.23

[a-c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Fermentation quality: pH = 4.9; per kg DM, lactic acid, 21.5 g; acetic acid, 79 g; propionic acid, 2.6 g; butyric acid, <1 g; NH_3-N, 53.6 mg.
[2] Digestibility of the organic matter estimated according to Aufrère and Michalet-Doreau (1983).

portion of hemicellulose compared with the other forages. The estimated digestibility of the organic matter was lower (−15.1%) in silage compared with hay and fresh herbage. The estimated digestibility of the organic matter of selected bites of the herbage was higher (+4.4%) than that of the fresh herbage offered either on pasture or indoors. Furthermore, the proportions of NDF and ADF of selected bites were numerically lower than that of the herbage offered indoors. Compared with silage, proportions of total FAs, C18:1*c*9, LA and ALA were numerically lower in hay by −6.5, −0.4, −1.7 and −3.2 g/kg DM, respectively. Proportions of total FAs and ALA were similar in silage and fresh herbage, but LA proportion was higher (+1 g/kg DM) in silage than in offered fresh herbage. The simulated bites were richer in total FAs (+4.7 g/kg DM), LA (+4.5 g/kg DM) and ALA (+2.7 g/kg DM) than the herbage offered indoors. DM proportion and proximate composition of the simulated bites as well as proportions of plant functional groups did not differ in the simulated bites of the two breeds (Supplementary Table S1). The silage had a relatively high concentration of acetic acid (79 g/kg DM) and underwent a relatively strong proteolytic process (53.6 mg NH_3-N/kg DM).

Intake, milk yield, composition, BW, and blood plasma metabolites

The DM intake was reduced by 3.6 kg/d in the silage-fed compared with the hay-fed group (Table 2). Still, the hay-fed cows ingested lower amounts of C16:0, C18:0, LA and ALA compared with the silage-fed group. The hay-fed cows had the highest milk yield of all groups, whereas silage-fed and grazing cows had an intermediate milk yield and indoor green-fed cows had the lowest milk yield. The milk of the hay-fed cows had higher protein (+0.26%) and casein (+0.20%) percentages than that of the silage-fed and indoor green-fed cows. Grazing cows had the highest, and the hay-fed cows had the lowest milk urea concentration. Montbéliarde had a lower milk yield (−1.6 kg/day) and higher concentrations of milk protein (+0.30%), casein (+0.26%) and urea (+27 mg/l) than Ho. Silage-fed cows tended to have lower corrected BW, at similar body condition score than hay-fed cows. Plasma glucose was higher in the hay-fed cows (+107 mg/l) compared with all other groups. Except for the higher plasma β-hydroxybutyrate level (+79 µmol/l) in grazing cows, we found no difference in other blood plasma metabolites between grazing and indoor green-feeding cows and between breeds.

Fatty acid profile of the rumen fluid

The hay-fed cows had the highest proportions of C18:1*c*9, C18:2*c*9*t*11, LA, ALA and total PUFAs (+1 g/100 g of FAs) in the rumen fluid lipids than all other groups (Table 3). The silage-fed cows had the lowest ruminal proportions of ALA and VA, and the highest proportion of saturated FAs of all groups. Both groups fed conserved herbage had a lower ruminal proportion of C18:2*t*11*c*15, whereas the highest C18:2*t*11*c*15 proportion was found in the rumen fluid of grazing cows. Both groups fed fresh herbage had lower proportions of RA than cows fed conserved herbage. Grazing Mo had the highest ruminal proportion of VA of all groups (Fig. 1). The ratio of C18:2*t*11*c*15-to-C18:3n-3 was the highest in rumen fluid of grazing Mo and lowest in the hay-fed cows of both breeds. Compared with the cows fed fresh herbage, the ratio of RA-to-LA was higher, and those of VA-to-(C18:2*t*11*c*15 + C18:2*c*9*t*11) were lower in rumen fluid from cows fed conserved herbage.

Fatty acid profile of the milk

Effect of herbage utilisation method

Proportions of C10:0, C12:0 and C14:0 were higher in milk fat from hay-fed cows than in all other groups (Table 4). The total proportion of saturated FAs was consequently higher (+2.65 g/100 g of FAs) in milk fat from hay- and silage-fed cows compared with that of the indoor green-fed cows (Table 5). Odd-chain FAs were more abundant in milk fat from hay-fed cows (+0.83 g/100 g of FAs) than in that from silage-fed cows, whereby milk fat from grazing and indoor green-fed cows had intermediate values (Table 5). The proportion of *iso* FAs (C13:0 *iso*, C14:0 *iso*, C15:0 *iso*, and C16:0 *iso*) was lower (−0.25 g/100 g of FAs) in milk fat from silage-fed cows than in milk fat from all other groups (Table 4). *Anteiso* FAs, in particular C15:0 *anteiso* and C17:0 *anteiso*, were less abundant in milk fat from silage-fed cows than in hay-fed cows, whereas milk fat from grazing and indoor green-fed cows had intermediate values. Milk fat from hay-fed cows contained more LA (+0.17 g/100 g FAs) than that from all other groups (Table 4). The proportions of some long-chain saturated FAs, such as C22:0, C23:0, and C24:0, as well as that of some long-chain PUFAs (C20:2n-6 and C20:3n-3) were higher in milk fat from hay-fed cows than in that from silage-fed cows. The n-6-to-n-3 FAs ratio was greater in milk fat from cows fed conserved herbage than in those fed fresh herbage (Table 5). We observed no effect of the herbage utilisation method on the

Table 2
Effect of herbage utilisation method (HUM: n = 12, except for hay, with n = 11) on intake (herbage conservation only), milk yield, milk composition, blood plasma metabolites, and BW of Holstein (Ho) and Montbéliarde (Mo) dairy cows (n = 24 and n = 23, respectively).

	Herbage utilisation method (HUM)									
			Fresh herbage		Breed (B)			*P*-values		
Item	Hay	Silage	Pasture	Indoor	Ho	Mo	SEM	HUM	B	HUM × B
DM intake (kg/day)	20.6	16.9	(20.7)[1]	(20.5)	18.9	18.7	0.539	<0.001	0.79	0.55
Fatty acids intake (g/day)										
Total fatty acids	204	246	(397)	(322)	227	223	6.0	<0.001	0.60	0.45
C16:0	46.9	44.3	(65.2)	(59.0)	45.9	45.4	1.18	0.13	0.71	0.51
C18:0	4.03	4.43	(7.58)	(6.62)	5.68	5.72	0.098	0.007	0.62	0.46
C18:1*c*9	21.4	25.4	(22.2)	(24.3)	23.6	23.2	0.302	<0.001	0.32	0.44
C18:2n-6	34.7	50.3	(63.8)	(50.8)	43.0	42.0	1.04	<0.001	0.49	0.40
C18:3n-3	55.2	80.2	(163)	(115)	68.5	67.0	2.26	<0.001	0.60	0.44
Milk yield (kg/day)	15.9[a]	14.5[b]	14.4[b]	12.3[c]	15.3	13.7	0.52	<0.001	0.008	0.93
Energy-corrected milk yield (kg/day)	15.3[a]	13.0[b]	13.7[b]	11.9[c]	14.0	13.2	0.50	<0.001	0.10	0.95
Milk constituents (%, if not else stated)										
Fat	3.68	3.51	3.72	3.70	3.54	3.74	0.089	0.41	0.09	0.35
Protein	3.36[a]	3.09[b]	3.23[ab]	3.10[b]	3.03	3.33	0.052	0.002	<0.001	0.28
Casein	3.02[a]	2.79[b]	2.92[ab]	2.80[b]	2.75	3.01	0.046	0.001	<0.001	0.30
Lactose	4.83	4.82	4.67	4.62	4.77	4.76	0.057	0.08	0.69	0.53
Urea (mg/l)	204[c]	270[b]	287[a]	222[c]	235	262	9.5	<0.001	0.05	0.63
Blood plasma metabolites										
NEFAs (μmol/l)	57.8[a]	42.9[b]	58.5[a]	62.0[a]	53.9	56.1	8.02	0.014	0.31	0.90
BHB (μmol/l)	366[b]	298[c]	469[a]	390[b]	389	372	23.4	<0.001	0.73	0.77
Glucose (mg/l)	644[a]	573[b]	507[b]	531[b]	567	560	25.7	0.01	0.99	0.15
Urea (mg/l)	97.9[b]	130.4[a]	146.6[a]	119.0[ab]	116	130	8.93	0.001	0.07	0.43
BW (kg)	644[ab]	628[b]	642[ab]	653[a]	620	664	5.81	0.04	<0.001	0.57
Corrected BW[2]	595	554	(541)	(563)	557	592	16.6	0.06	0.002	0.52
Body condition score (0–5)	1.9	1.6	1.7	1.7	1.5	2.0	0.17	0.63	<0.001	0.78

Abbreviations: NEFAs = non-esterified fatty acids; BHB = β-hydroxybutyrate.
[a-c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Values in brackets are arithmetic means and were not considered for statistical analysis, as they are based on estimated feed intake.
[2] Corrected BW (kg) = BW (kg) − 4 × DM intake (kg/d) (Chilliard et al., 1987).

desaturation indexes. The apparent transfer rates of LA (18%) and ALA (6%) were 1.8-fold and 2-fold higher, respectively, in hay-fed cows than in the silage-fed cows. Accordingly, hay-fed cows had higher LA yields (+1.83 g/day) than all other groups, and higher ALA yields (+0.83 g/day) than silage- and indoor green-fed cows. Minor FAs (<0.10 g/100g of total FAs) are reported in Supplementary Table S2.

Effect of breed

Milk fat from Ho had a higher proportion of saturated FAs (+2.8 g/100 g of total FAs; Table 5), especially of C16:0 (+2.5 g/100 g of total FAs; Table 4) compared with that from Mo. The proportion of MUFAs (−3.1 g/100 g of total FAs; Table 5), in particular of C18:1*c*9 (−1.6 g/100 g of total FAs; Table 4), was lower in milk fat from Ho than in that from Mo. Milk fat from Ho had a higher proportion of C14:1*c*9 (Table 4) and a higher C14:1*c*9-to-C14:0 ratio (Table 5) than that from Mo. The milk fat from Ho had a lower C18:1*c*9-to-C18:0 ratio than that from Mo, irrespective of the diet. There were no differences in apparent transfer rates of LA and ALA between breeds in the hay- and silage-fed groups.

Interactions between herbage utilisation method and breed

The proportion of VA was higher (+1.0 g/100 g of total FAs) in milk fat from cows fed fresh herbage than in that from hay- and silage-fed cows, except grazing Ho (Figure 2). In addition, the proportion of PUFAs was higher in milk fat from grazing and indoor green-fed Mo (5.46 and 5.30 g/100 g of total FAs, respectively), whereas lower proportions were found in milk fat from grazing Ho (4.17 g/100 g of total FAs) as well as in hay-fed Ho (3.85 g/100 g of total FAs) and Mo (4.22 g/100 g of total FAs). The proportions of some intermediates of the RBH of ALA such as C18:2*t*11*t*15 (0.05 vs 0.02 g/100 g of total FAs), C18:2*t*11*c*15 (0.46 vs 0.26 g/100 g of total FAs), and C18:2*t*11*c*13 (0.12 vs 0.07 g/100 g of total FAs) were higher in milk fat from Mo than Ho when fed fresh herbage. Consequently, the total CLA proportion was higher in the milk fat from grazing (2.00 g/100 g of total FAs) and indoor green-fed Mo (1.87 g/100 g of FAs) than in that from grazing and indoor green-fed Ho (1.07 and 1.25 g/100 g of total FAs, respectively), whereas with conserved herbage (i.e. hay and silage), total CLA proportion did not differ between breeds.

Discussion

Effect of herbage conservation method on herbage composition

As expected from literature, the hay had lower proportions of total FAs and ALA compared with unwilted silage and fresh herbage, mainly due to oxidative losses during wilting and drying as well as leaf losses during harvesting of the dry material (Boufaïed et al., 2003). It was not possible to use conserved and fresh herbage from the same growing cycle, but the phenological stage at the time of harvest was similar, as also supported by the similar proportions of total FAs and ALA found in the silage and the offered fresh herbage either by strip-grazing or indoor green-feeding (Renna et al., 2020). Furthermore, we suppose that the effect of the conservation *per se* was much greater than that of the phenological stage. This was shown for example under practical farming conditions (Coppa et al., 2015b). The silage had a lower digestibility of the organic matter compared with all other forages. This was probably the consequence of changes in carbohydrate composition (i.e. higher ADF proportion) during the process of ensiling, when water-soluble carbohydrates and part of the hemicellulose are degraded to organic acids that may have been lost via liquid effluent and volatilisation during silage feed out (Dewhar et al., 1963). The strong proteolytic processes that occurred during ensiling, as evidenced by the relatively high proportion of NH_3-N in

Table 3
Effect of the herbage utilisation method (HUM; n = 12, except for hay, with n = 11) on the fatty acid profile of the rumen fluid lipids of Holstein (Ho) and Montbéliarde (Mo) dairy cows (n = 24 and n = 23, respectively).

Fatty acids (FAs)[1]	Herbage utilisation method (HUM)				Breed (B)			*P*-values		
			Fresh herbage							
(g/100 g of total FAs)	Hay	Silage	Pasture	Indoor	Ho	Mo	SEM	HUM	B	HUM × B
Saturated FAs	64.8^{b}	67.4^{a}	62.2^{c}	60.0^{d}	64.2	63.0	0.460	<0.001	0.02	0.14
Odd- and branched-chain	9.19^{a}	7.45^{b}	6.78^{b}	8.64^{a}	7.90	8.09	0.356	<0.001	0.48	0.91
Odd-chain	3.57^{a}	3.36^{a}	2.48^{c}	2.96^{b}	3.06	3.11	0.113	<0.001	0.49	0.68
iso	2.23^{a}	1.67^{b}	1.90^{b}	2.47^{a}	2.04	2.09	0.092	<0.001	0.56	0.98
anteiso	3.38^{a}	2.43^{b}	2.40^{b}	3.20^{a}	2.79	2.89	0.160	<0.001	0.47	0.91
MUFAs	9.37^{bc}	8.43^{c}	12.58^{a}	10.0^{b}	9.61	10.6	0.504	<0.001	0.05	0.01
PUFAs	4.40^{a}	3.12^{b}	3.53^{b}	3.56^{b}	3.56	3.70	0.175	<0.001	0.31	0.44
Individual FAs										
C6:0	6.25^{b}	8.06^{a}	1.72^{c}	3.15^{c}	4.61	4.99	0.538	<0.001	0.47	0.74
C7:0	0.43^{b}	0.70^{a}	0.09^{c}	0.14^{c}	0.33	0.35	0.044	<0.001	0.56	0.96
C12:0	0.60^{b}	0.34^{c}	0.81^{a}	0.95^{a}	0.67	0.68	0.049	<0.001	0.91	0.47
C13:0	0.15	0.13	0.12	0.13	0.13	0.14	0.011	0.38	0.67	0.26
C13:0 *iso*	0.20^{c}	0.14^{b}	0.16^{ab}	0.23^{a}	0.19	0.17	0.012	0.03	0.58	0.78
C14:0	2.20^{a}	1.73^{b}	1.76^{b}	2.15^{a}	1.88	2.03	0.107	<0.001	0.15	0.49
C14:0 *iso*	0.60^{a}	0.38^{c}	0.48^{b}	0.63^{a}	0.50	0.55	0.028	<0.001	0.08	0.65
C15:0	2.09^{a}	1.82^{b}	1.48^{c}	1.81^{b}	1.77	1.83	0.073	<0.001	0.45	0.62
C15:0 *anteiso*	2.76^{a}	2.09^{b}	2.06^{b}	2.80^{a}	2.36	2.47	0.160	<0.001	0.42	0.84
C15:0 *iso*	0.96^{b}	0.84^{c}	0.94^{bc}	1.22^{a}	0.97	1.01	0.046	<0.001	0.52	0.80
C16:0	15.5^{a}	13.1^{b}	13.6^{b}	13.8^{b}	13.8	14.2	0.312	<0.001	0.25	0.92
C17:0	0.91^{a}	0.70^{c}	0.80^{b}	0.89^{a}	0.83	0.82	0.018	<0.001	0.64	0.05
C17:0 *anteiso*	0.62^{a}	0.34^{c}	0.34^{c}	0.40^{b}	0.43	0.41	0.026	<0.001	0.74	0.80
C17:0 *iso*	0.49^{a}	0.31^{c}	0.32^{c}	0.39^{b}	0.39	0.37	0.019	<0.001	0.41	0.63
C18:0	30.9^{b}	36.6^{a}	37.4^{a}	31.1^{b}	35.1	32.9	1.05	<0.001	0.04	0.89
C18:1*c*9	2.14^{a}	1.52^{b}	1.12^{c}	1.23^{c}	1.47	1.53	0.097	<0.001	0.52	0.33
C18:1*c*11	0.43^{a}	0.30^{b}	0.43^{a}	0.45^{a}	0.39	0.42	0.016	<0.001	0.03	0.21
C18:1*c*12	0.08	0.16	0.13	0.11	0.13	0.11	0.020	0.06	0.30	0.23
C18:1*t*6/7/8	0.27^{ab}	0.25^{ab}	0.30^{a}	0.22^{b}	0.25	0.28	0.021	0.05	0.13	0.04
C18:1*t*9	0.18^{a}	0.16^{a}	0.17^{a}	0.10^{b}	0.14	0.16	0.017	0.006	0.38	0.16
C18:1*t*10	0.38^{a}	0.28^{b}	0.35^{a}	0.26^{b}	0.31	0.32	0.018	0.001	0.68	0.006
C18:1*t*11	4.16^{b}	3.26^{c}	7.70^{a}	5.71^{a}	4.75	5.64	0.382	<0.001	0.02	<0.001
C18:1*t*12	0.31^{b}	0.38^{a}	0.29^{b}	0.22^{b}	0.30	0.30	0.022	<0.001	0.80	0.90
C18:1*t*13/14	0.68^{b}	0.95^{a}	0.95^{a}	0.76^{b}	0.86	0.82	0.041	<0.001	0.28	0.24
C18:1*t*15	0.28^{c}	0.44^{a}	0.36^{a}	0.29^{b}	0.33	0.35	0.019	<0.001	0.77	0.90
C18:1*t*16	0.41^{c}	0.64^{a}	0.67^{a}	0.52^{b}	0.56	0.54	0.027	<0.001	0.31	0.16
C18:2n-6	2.01^{a}	1.35^{b}	1.40^{b}	1.47^{b}	1.51	1.59	0.036	<0.001	0.08	0.14
C18:2*c*9*t*11	0.54^{a}	0.37^{b}	0.15^{c}	0.20^{c}	0.30	0.33	0.042	<0.001	0.29	0.10
C18:2*t*11*c*15	0.33^{c}	0.44^{c}	0.87^{a}	0.64^{b}	0.53	0.62	0.060	<0.001	0.22	0.13
C18:3n-3	1.50^{a}	0.95^{c}	1.10^{bc}	1.24^{b}	1.22	1.16	0.070	<0.001	0.45	0.47
Ratios										
C18:2*t*11*c*15/C18:3n-3	0.24^{c}	0.46^{b}	0.81^{a}	0.53^{b}	0.47	0.57	0.041	<0.001	0.07	0.001
RA/LA	0.27^{a}	0.27^{a}	0.11^{b}	0.15^{b}	0.19	0.20	0.022	<0.001	0.85	0.33
C18:1*t*11/(C18:2*t*11*c*15 + RA)	5.12^{b}	4.20^{b}	7.50^{a}	7.11^{a}	6.00	6.04	0.538	<0.001	0.86	0.21
C18:0/C18:1*t*11	7.74^{b}	11.96^{a}	5.5^{c}	5.88^{bc}	8.28	7.29	0.703	<0.001	0.007	0.03

Abbreviations: MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; RA = rumenic acid; LA = linoleic acid
[a–c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Not presented, when < 0.12 g/100g of total FAs.

the present experiment, probably also influenced the digestibility of the organic matter of the silage. This suggests that ensiling promoted the activity of heterofermentative lactic acid bacteria, which would explain the high concentration of acetic acid. However, the relatively high concentration of acetic acid guaranteed a high aerobic stability of the silage during feed out (Danner et al., 2003).

Effect of the herbage utilisation method on dairy performance

The lower yield and protein concentration of the milk of the silage-fed cows, an indication for a lower supply of net energy, are consistent with their lower feed intake and the lower digestibility of the organic matter in relation with the higher ADF proportion in the silage compared with hay-fed cows. The low palatability of the silage can be explained by the low DM proportion and a limited fermentation quality, especially the relatively high concentration of acetic acid (Gerlach et al., 2021). The low digestibility of the organic matter of grass silage may cause limited microbial protein synthesis and consequently the lower supply with metabolisable proteins for milk protein synthesis. Additionally offering 2 kg DM/day of hay did not fully compensate for the lower intake of silage. A lower silage intake compared with that of barn-dried hay and fresh herbage (only estimated in the present study) was also reported by others with concentrate proportions similar to the present study (Haselmann et al., 2020). Owing to the late stage of lactation, body fat mobilisation is unlikely to occur so the lower plasma non-esterified FAs concentration in silage-fed cows might have resulted from a higher utilisation of plasma non-esterified FAs as a readily available energy source. The lower plasma β-hydroxybutyrate concentration and the lower *de novo* synthesis of FAs (i.e. sum of C10:0, C12:0, and C14:0) point towards a lower energy supply to the mammary gland in silage-fed cows compared with all other groups. However, the lower energy supply did not apparently affect BW and body condition score. The high milk and protein yields as well as the high plasma glucose level of the hay-fed group compared with those of groups fed fresh

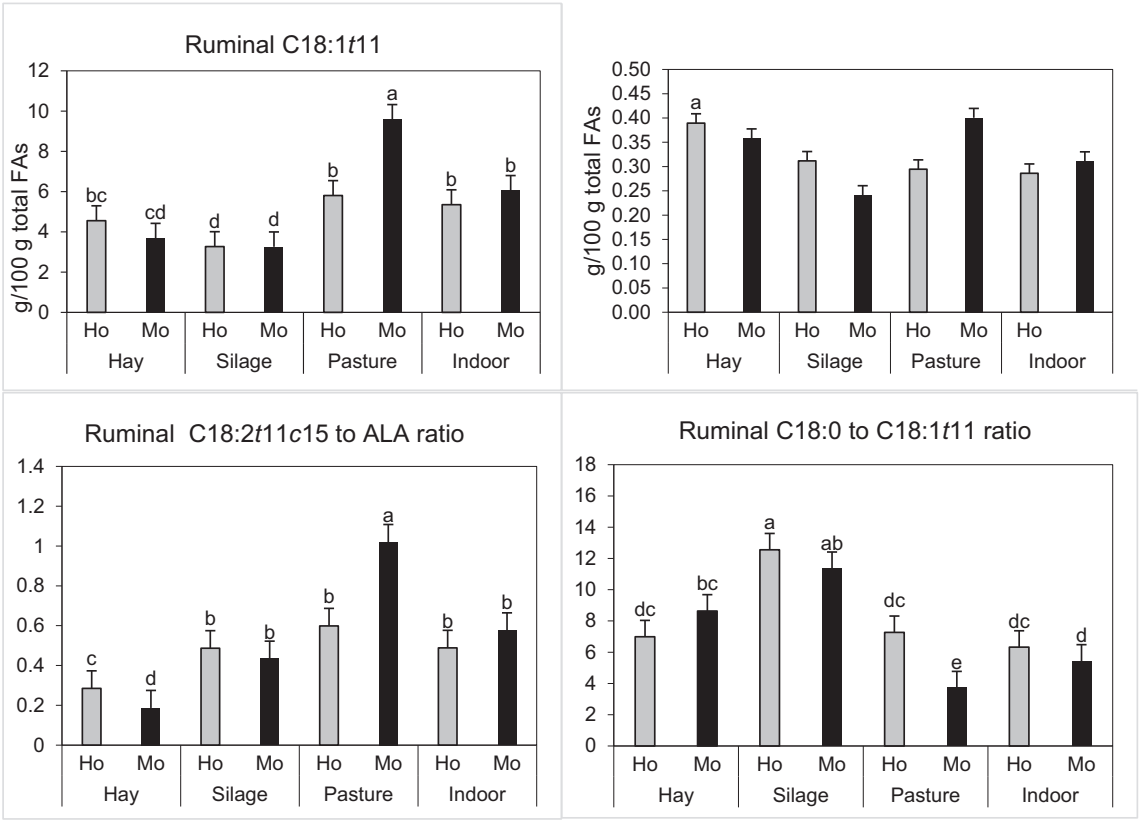

Fig. 1. Proportions of ruminal fatty acids and FA ratios to their precursors in Holstein (Ho) and Montbéliarde (Mo) dairy cows, in cases where there is an interaction of herbage utilisation method and breed (n = 6, except hay-fed Mo n = 5). Within the same variable, bars with different letters differ significantly at $P < 0.05$. Abbreviations; FAs = fatty acids; ALA = α-linolenic acid

herbage also point towards a higher nutritional quality of the hay compared with the other herbages.

Effect of the herbage utilisation method on the fatty acid composition of rumen fluid and milk

Minor fatty acids

The lower proportions of odd- and branched-chain FAs in rumen fluid and milk from the silage-fed cows compared with the hay-fed group are consistent with the lower proportion of hemicellulose and lower digestibility of the organic matter of the silage than the hay. The synthesis of odd- and branched-chain FAs is closely related to the proportions of dietary fibres (Vlaeminck et al., 2006). The branched-chain FAs are synthesised from isovaleric acid and isobutyric acid derived from the degradation of branched-chain amino acids along with dietary protein degradation (Vlaeminck et al., 2006). Their prevalence might have been reduced in the milk of the silage-fed cows due to the lower digestibility of the organic matter and partial degradation of amino acids, as reflected by the relatively high concentration of NH_3-N found in the silage. In previous reports, branched-chain FAs were lower in milk from silage-fed compared with grazing cows (Scherzer et al., 2020), and hay-fed cows (Shingfield et al., 2005). Bulk milk data from different farms also associated higher odd- and branched-chain FAs proportions in milk with prevalently hay-based diets compared with grasssilage-based diets (Ferlay et al., 2008).

Fatty acids related to ruminal biohydrogenation

In the present study, differences in the proportion of PUFAs in rumen and milk lipids found between herbage utilisation methods were mainly the result of the modified RBH pathways of the dietary PUFAs. For instance, the ratio of C18:2*t*11*c*15-to-ALA in rumen fluid was lower in hay-fed cows than in all other groups, at concomitantly the highest ALA and total PUFAs proportions in the rumen fluid. This may indicate that proportionately less ALA was converted by isomerisation and reduction to C18:2*t*11*c*15 in hay-fed cows and would mean that ALA is less biohydrogenated in hay than in silage or fresh herbage. However, the similar RA-to-LA ratio in rumen fluid of hay- and silage-fed cows suggests that the isomerisation of LA might have been similar in cows fed conserved herbages, pointing towards a difference in the steps of hydrogenation rather than in the reactions of isomerisation. Indeed, oxidation products (e.g. aldehydes) derived from the degradation of PUFAs, such as those formed during herbage drying for haymaking or due to non-enzymatic oxidation during hay storage, were shown to inhibit the *in vivo* RBH without altering the proportion of RA, but lowering the proportion of VA in rumen fluid and in milk fat (Kaleem et al., 2018). Furthermore, the rumen microbial colonisation of hay particles might be slower than those of fresh herbage and silage particles as was demonstrated *in vitro* by Belanche et al. (2017) with dried vs fresh ryegrass. However, the herbage's own lipolytic activity was shown to be lower in hay than in fresh herbage and direct-cut silage (Boufaïed et al, 2003). Also, the colonisation of silage particles in comparison to hay particles might be faster as their humidity is more favourable for microbes. However, this has never been reported in the literature. During ensiling, extensive lipolysis by plant-own lipases and from epiphytic bacteria (or inoculants) occurs, leading to up to 40% of the total PUFAs in silage being non-esterified and susceptible to RBH (Dewhurst et al., 2006). Accordingly, the C18:0-to-C18:1*t*11 ratio in rumen fluid was the highest in the silage-fed group that

Table 4
Effect of herbage utilisation method (HUM; n = 12, except for hay, with n = 11) on main individual fatty acids in milk fat (g/100 g of total FAs) of Holstein (Ho) and Montbéliarde (Mo) dairy cows (n = 24 and n = 23, respectively).

	Herbage utilisation method (HUM)									
Fatty acids (FAs)[1]			Fresh herbage		Breed (B)			*P*-values		
(g/100 g of total FAs)	Hay	Silage	Pasture	Indoor	Ho	Mo	SEM	HUM	B	HUM × B
C4:0	3.07	2.78	2.96	2.96	2.96	2.92	0.100	0.38	0.26	0.92
C6:0	1.83	1.81	1.72	1.72	1.80	1.73	0.055	0.36	0.47	0.83
C8:0	0.98[a]	0.93[ab]	0.89[b]	0.86[b]	0.93	0.91	0.025	0.01	0.37	0.85
C10:0 + C12:0 + C14:0	17.1[a]	15.8[b]	15.1[bc]	14.4[c]	15.7	15.5	0.440	<0.001	0.42	0.56
C10:1*c*9	0.28	0.29	0.26	0.25	0.29	0.25	0.017	0.27	0.02	0.91
C13:0	0.12[a]	0.11[a]	0.10[b]	0.08[c]	0.10	0.10	0.004	<0.001	0.35	0.20
C14:0 *iso*	0.22[a]	0.15[c]	0.20[ab]	0.20[b]	0.18	0.20	0.008	<0.001	0.002	0.28
C14:1*c*9	1.06	1.06	0.97	0.91	1.12	0.88	0.069	0.30	0.001	0.58
C15:0 *anteiso*	0.80[a]	0.63[c]	0.75[ab]	0.72[b]	0.70	0.74	0.021	<0.001	0.05	0.44
C15:0 *iso*	0.41[b]	0.35[c]	0.49[a]	0.47[a]	0.42	0.44	0.012	<0.001	0.08	0.63
C15:0	1.55[a]	1.49[ab]	1.42[b]	1.34[c]	1.42	1.47	0.027	<0.001	0.05	0.30
C16:0 *iso*	0.43[a]	0.35[c]	0.40[b]	0.40[b]	0.38	0.41	0.012	<0.001	0.006	0.16
C16:0	29.5[ab]	31.1[a]	28.8[b]	27.6[b]	30.5	28.0	0.77	0.02	0.002	0.88
C16:1*c*7	0.21[b]	0.17[c]	0.24[a]	0.23[a]	0.21	0.22	0.007	<0.001	0.04	0.48
C16:1*c*9	1.52	1.62	1.39	1.39	1.52	1.44	0.082	0.12	0.29	0.47
C16:1*t*9 + C17:0 *iso*	0.58[a]	0.47[b]	0.62[a]	0.64[a]	0.55	0.60	0.018	<0.001	0.007	0.04
C17:0 *anteiso*	0.59[a]	0.42[c]	0.45[bc]	0.49[b]	0.48	0.49	0.014	<0.001	0.29	0.71
C17:0	0.89[a]	0.73[c]	0.79[b]	0.79[b]	0.79	0.80	0.013	<0.001	0.52	0.89
C17:1*c*9	0.28[a]	0.25[b]	0.27[ab]	0.27[a]	0.26	0.27	0.008	0.04	0.04	0.14
C18:0	9.10	9.52	9.04	10.18	9.69	9.24	0.614	0.53	0.41	0.78
C18:1*c*9[2]	20.6	21.6	20.9	21.7	20.4	22.0	0.59	0.51	0.008	0.43
C18:1*c*11	0.67[a]	0.54[b]	0.66[a]	0.67[a]	0.59	0.68	0.024	<0.001	<0.001	0.29
C18:1*c*12	0.15	0.14	0.14	0.13	0.14	0.14	0.007	0.26	0.50	0.20
C18:1*c*15 + C19:0	0.15[b]	0.14[b]	0.17[a]	0.17[a]	0.16	0.16	0.006	0.001	0.99	0.56
C18:1*t*6/7/8	0.19[b]	0.21[ab]	0.21[ab]	0.23[a]	0.20	0.23	0.010	0.04	0.003	0.95
C18:1*t*9	0.17[bc]	0.16[c]	0.18[ab]	0.19[a]	0.16	0.19	0.007	0.006	<0.001	0.01
C18:1*t*10	0.27[ab]	0.21[b]	0.39[a]	0.35[a]	0.30	0.31	0.046	0.04	0.78	0.51
C18:1*t*11	1.68[b]	1.46[b]	2.39[a]	2.75[a]	1.82	2.35	0.177	<0.001	0.006	0.04
C18:1*t*12	0.19[c]	0.22[b]	0.24[ab]	0.26[a]	0.21	0.24	0.012	0.001	0.02	0.99
C18:1*t*16	0.19[b]	0.28[a]	0.25[a]	0.28[a]	0.25	0.25	0.014	<0.001	0.83	0.10
C18:2n-6	1.24[a]	1.06[b]	1.09[b]	1.05[b]	1.05	1.16	0.031	<0.001	<0.001	0.40
C18:2*c*9*t*13	0.12[c]	0.17[b]	0.20[a]	0.19[ab]	0.16	0.19	0.009	<0.001	0.005	0.58
C18:2*c*9*t*11	0.85[b]	0.79[b]	1.37[a]	1.42[a]	0.92	1.32	0.068	<0.001	<0.001	<0.001
C18:2*t*11*c*15	0.12[b]	0.16[b]	0.39[a]	0.35[a]	0.21	0.31	0.024	<0.001	<0.001	<0.001
C18:3n-3	0.65	0.55	0.64	0.61	0.58	0.64	0.040	0.26	0.14	0.98
C20:0	0.17	0.16	0.15	0.16	0.16	0.16	0.011	0.58	0.53	0.98
C20:1*c*9	0.15[a]	0.16[a]	0.14[b]	0.14[b]	0.14	0.15	0.004	0.003	0.03	0.89
C20:1*c*11	0.21	0.13	0.19	0.21	0.18	0.19	0.032	0.23	0.53	0.21
C20:4n-6	0.11[a]	0.10[ab]	0.09[b]	0.10[ab]	0.10	0.09	0.004	0.02	0.003	0.29
C22:5n-3	0.13[b]	0.12[b]	0.18[a]	0.19[a]	0.16	0.15	0.009	<0.001	0.03	0.83

Abbreviation: FAs = fatty acids.
[a-c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Not presented, when < 0.10 g/100g of total FAs. Minor FAs (< 0.10 g/100g of total FAs) are reported in Supplementary Table S2.

suggests a higher hydrogenation of C18:1t11-to-C18:0, namely the last RBH step. Furthermore, the ruminal ratio of C18:2*t*11*c*15-to-ALA in the silage-fed group of the present study was similar to that in the groups fed fresh herbage, with exception of the grazing Mo where this ratio was even higher. In the current study, the proportion of C18:2*t*11*c*15, the intermediate of the RBH of ALA, and that of C18:2*t*11*c*13, were lower in milk from hay-fed cows than in all other groups. As this happened at similar proportions of ALA in milk fat, ALA transfer rate from feed to milk was doubled in hay (6 vs 3% in hay vs silage).

Concerning LA, its lower ratio to RA in rumen fluid of cows fed fresh herbage compared with cows fed conserved herbage suggests that the isomerisation of LA to RA might have been lower in cows fed fresh herbage. The isomerisation of LA to RA and subsequent biohydrogenation of RA may be also very quick in fresh herbages (Buccioni et al., 2012). Similar to ALA, the apparent transfer rate of LA from feed to milk was higher with the hay than with the silage diet, confirming observations made by Shingfield et al. (2005) in cows fed hay or silage from a mixed timothy and meadow fescue sward. Ferlay et al. (2006) also reported higher transfer efficiencies of ALA and LA in milk from cows fed either ryegrass hay or mountain grassland hay compared with ryegrass silage.

Differences in transfer of linoleic acid and α-linolenic acid

One explanation for the difference between hay- and silage-fed cows in PUFAs recovery in milk is provided by the meta-analysis by Khiaosa-Ard et al. (2015) showing that transfer from feed to milk of LA and ALA decreases with increasing LA and ALA intake in grass-based diets. The FAs, LA or ALA, and their different locations in the plant tissues with different affinity to certain bacteria and RBH pathways seem also to play a role as this is suggested by the three-fold higher transfer rate of LA compared with ALA. It is known that ALA is more common in glyco- and phospholipids, whereas LA is more common in triacylglycerols in herbages, whereby different microbial lipases are hydrolysing different lipid fractions in the herbage (Buccioni et al., 2012). For instance, *Butyrivibrio fibrisolvens* was found to hydrolyse predominantly phospho- and galactolipids in the rumen, whereas also other bacteria such as *Anaerovibrio lipolyticus* are able to hydrolyse triacylglycerols (Enjalbert et al., 2017). Consequently, FAs esterified to phospholipids are less intensively biohydrogenated in the rumen compared to other lipid fractions (Lashkari et al., 2019). Eventually, the biohydrogenation of ALA, in particular its isomerisation to C18:3*c*9,*t*11*c*15, is faster than that of LA, as demonstrated *in vitro* by Meynadier et al. (2018),

Table 5
Effect of herbage utilisation method (HUM; n = 12, except for hay, with n = 11) on sums of fatty acids (FAs) in milk fat (g/100 g of total FAs) and indices of desaturation of Holstein (Ho) and Montbéliarde (Mo) dairy cows (n = 24 and n = 23, respectively), as well as on the apparent transfer rates of linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) from hay and silage to milk

	Herbage utilisation method (HUM)									
			Fresh herbage		Breed (B)			*P*-values		
Item	Hay	Silage	Pasture	Indoor	Ho	Mo	SEM	HUM	B	HUM × B
Saturated FAs	66.4[a]	66.3[a]	64.7[ab]	63.7[b]	66.7	63.9	0.712	0.04	<0.001	0.33
Odd-chain	2.70[a]	2.47[b]	2.42[bc]	2.31[c]	2.60	2.65	0.040	<0.001	0.12	0.31
Iso branched	1.19[a]	0.94[b]	1.21[a]	1.18[a]	1.09	1.17	0.031	<0.001	0.009	0.47
Anteiso branched	1.41[a]	1.07[c]	1.23[b]	1.22[b]	1.21	1.25	0.032	<0.001	0.08	0.70
cis MUFAs	25.3	26.1	25.4	26.1	25.0	26.4	0.577	0.63	0.02	0.48
trans MUFAs	3.27[b]	3.05[b]	4.39[a]	4.81[a]	3.56	4.24	0.200	<0.001	0.002	0.16
PUFAs	3.96[b]	3.64[c]	4.89[a]	4.68[a]	3.93	4.68	0.119	<0.001	<0.001	<0.001
n-3	0.94[a]	0.81[b]	1.00[a]	0.96[a]	0.91	0.94	0.045	0.03	0.37	0.97
n-6	1.57[a]	1.34[b]	1.38[b]	1.34[b]	1.35	1.45	0.034	<0.001	0.003	0.38
CLA[1]	1.01[b]	0.86[b]	1.35[a]	1.39[a]	1.03	1.45	0.078	<0.001	<0.001	<0.001
Desaturation index										
C14:1*c*9/C14:0	0.09	0.10	0.10	0.09	0.10	0.08	0.005	0.51	<0.001	0.61
Yield (g/d)										
C18:2n-6	6.71[a]	4.78[bc]	5.27[b]	4.59[c]	5.29	5.39	0.238	<0.001	0.57	0.79
C18:3n-3	3.41[a]	2.40[c]	2.97[ab]	2.68[bc]	2.83	2.90	0.215	<0.001	0.45	0.78
Apparent transfer rates										
C18:2n-6	0.18	0.10	(0.09)[2]	(0.10)	0.14	0.14	0.011	<0.001	0.62	0.80
C18:3n-3	0.06	0.03	(0.02)	(0.03)	0.05	0.04	0.004	<0.001	0.55	0.70

Abbreviations: MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
[a-c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Includes C18:2*c*9*t*11, C18:2*t*11*c*13, C18:2*t*11*t*13, and C18:2*t*9*t*11 as main isomers, as well as C18:2*t*7*c*9 C18:2*t*8*c*10 as minor isomers.
[2] Values in brackets are arithmetic means and were not considered for statistical analysis, as they are based on estimated feed intake

which therefore leads to a proportionally higher transfer of LA than ALA from forages to milk.

Grazing vs indoor green-feeding

The dietary selection behaviour of grazing cows concerning the different plant parts in the herbage could influence the proportions of the main FAs in the selected herbage compared with that offered to the indoor green-fed cows, which originated from the same meadow. Grazing cows select more nutritive leaves rather than stems on pasture, and leaves are richer in PUFAs (Coppa et al., 2015a). When offered green herbage indoors, cows typically ingest larger boluses compared with grazing cows and thus are not able to efficiently select for plant species (Boudon et al., 2006). Despite this, we observed no differences in the milk FAs composition between grazing and indoor green-fed cows, except for C13:0, C13 *iso*, and C15:0 that were lower in milk of the latter. Moreover, the strip-grazing management (4 days per strip) as practised in this experiment and the relatively low plant species diversity might have limited an extensive dietary selection (Coppa et al., 2015a) and therefore reduced at least the potential differences due to a differential intake of plant secondary compounds from the selected forbs.

Effect of breed and interactions with herbage utilisation method

The lower milk yield as well as the higher protein, casein, and urea concentrations of the milk of Mo compared with Ho is consistent with previous observations under similar grassland-based diets with low or without concentrate supplementation and is owed to the differences in genetic merit of the breeds obvious even at late lactation stages (Koczura et al., 2019).

In agreement with Lawless et al. (1999), the milk fat from Mo fed fresh herbage contained higher proportions of total CLA and especially of C18:2*c*9*t*11 compared with Ho, whereas this phenomenon was not observed when feeding conserved herbage. The opposite was observed when Ho and Mo were fed the same maize silage diet supplemented with linseed (Ferlay et al., 2010). Rumenic acid in milk mainly derives from the desaturation of C18:1*t*11 in the epithelial cells of the mammary gland through the action of the enzyme Δ^9-desaturase (Bernard et al., 2008). However, in the present study, this observation is not supported by the C14:1*c*9-to-C14:0 ratio, the best proxy of the Δ^9-desaturase activity (Bernard et al., 2008), which was lower in Mo than in Ho. The accumulation of RBH intermediates of ALA such as C18:2*t*11*c*15, C18:2*t*11*t*15, and C18:2*t*11*c*13 in milk fat from Mo fed fresh herbage suggests that some steps of the RBH of ALA were more extensively inhibited in Mo than in Ho. In fact, the ratio of C18:2*t*11*c*15-to-ALA was higher in the rumen fluid of grazing Mo at a similar ALA proportion in the rumen fluid. Moreover, the C18:0-to-C18:1*t*11 ratio in the rumenfluid lipids was the lowest in grazing Mo, which might indicate that also the last RBH step was more extensively inhibited in grazing Mo than in all other groups. A diverging dietary herbage selection behaviour between grazing cows of the two breeds might have played a role for these observations, as this behaviour may not be expressed as intensively in an indoor green-feeding system than during grazing (Boudon et al., 2006). Indeed, Mo cows were reported earlier to select less grasses compared with Ho on semi-permanent grasslands (Koczura et al., 2019). This, and the concomitantly higher selectivity for forbs that are generally richer in plant secondary compounds, could have promoted a partial inhibition of RBH in Mo compared with Ho and have led to the differences observed in rumen FAs profile. However, we found no clear differences between breeds in proportions of plant functional groups and proximate composition of selected bites (Supplementary Table S1). The botanical diversity of the meadow used in the present experiment was relatively low, limiting the selection of different plant species by the two breeds. Eventually, Lawless et al. (1999) also reported higher C18:2*c*9*t*11 proportions in milk fat from Mo compared with that from Dutch Ho grazing on the same pasture.

In the present experiment, ensiling reduced the proportion of PUFAs in the herbage and increased their RBH compared with feeding of fresh herbage, whereas drying reduced the proportion of PUFAs (both LA and ALA) in the herbage but slightly reduced their RBH compared with ensiling. Despite the putatively different pat-

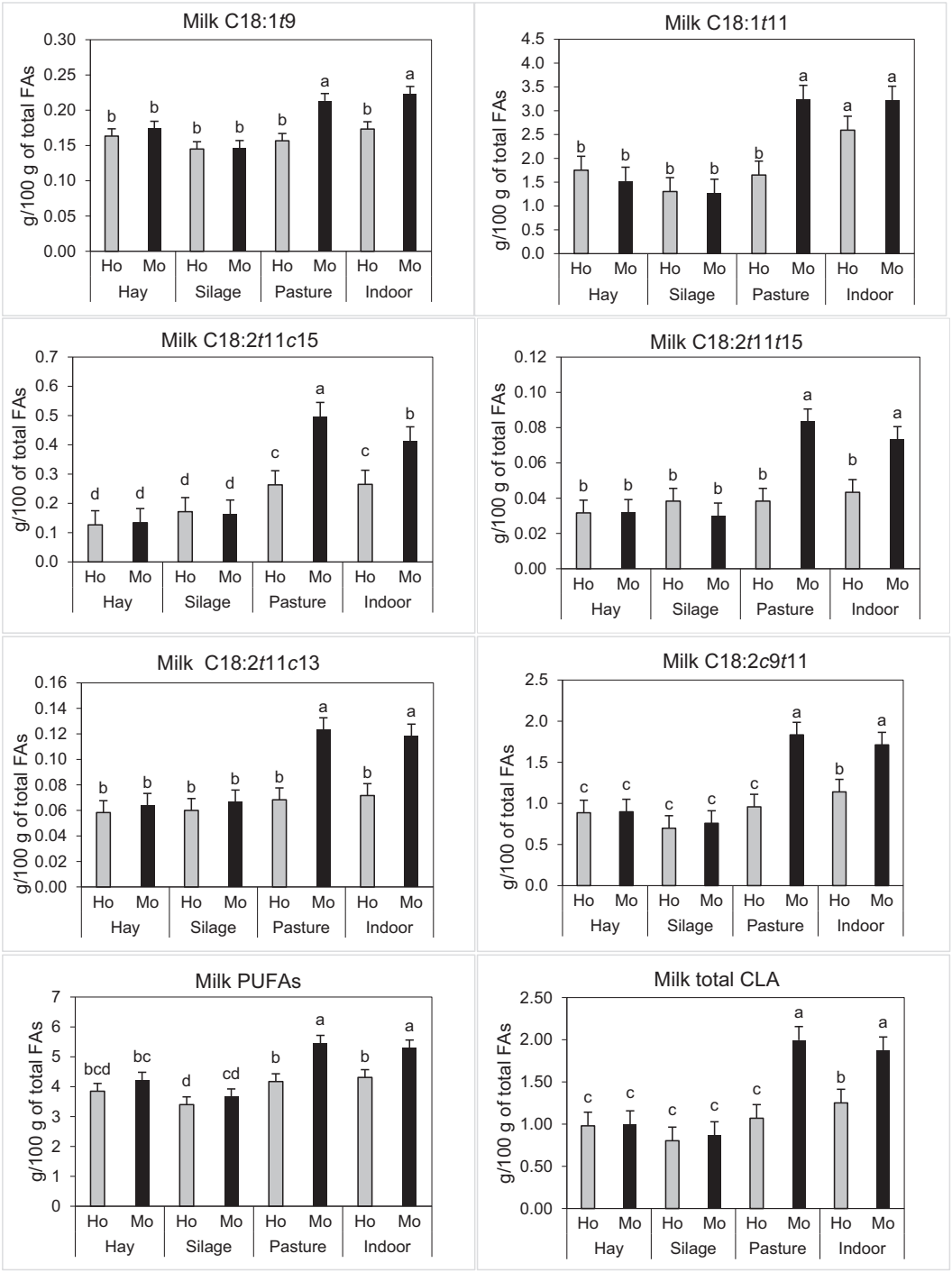

Fig. 2. Proportions of milk fatty acids of Holstein (Ho) and Montbéliarde (Mo) dairy cows in cases where there is an interaction of herbage utilisation method and breed (n = 6, except hay-fed Mo n = 5). Within the same variable, bars with different letters differ significantly at $P < 0.05$. Abbreviations: FAs = fatty acids; PUFAs = polyunsaturated fatty acids; CLA = conjugated linoleic acid

terns of RBH as evidenced by different FAs ratios in rumen fluid lipids, the milk FAs profile did not greatly differ between herbage conservation methods, except for a slightly higher n-3 FAs proportion in milk from the hay-fed cows compared with the silage-fed cows. Due to the limited possibility of plant selection under the practised strip-grazing conditions and the low botanical diversity of the investigated grassland, indoor green-feeding and pasture feeding did not differ in their effects on the milk FAs profile. Lastly, feeding fresh herbages has different effects on the RBH of dietary FAs and the milk FAs in Ho and Mo. Thereby, milk fat from grazing Mo contained more total CLA and total PUFAs, associated with a higher proportion of VA in the rumen fluid, than milk fat from Ho grazing on the same pasture.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100674.

Ethics approval

All animal-related procedures were conducted in accordance with the French guidelines for animal welfare and were approved by the local ethic committee (approval APAFIS#20190425141273).

Data availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon reasonable request to the corresponding author.

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Declaration of interest

None.

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