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

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ABSTRACT

The effects of forage conservation method on plasma lipids, mammary lipogenesis, and milk fat were examined in 2 complementary experiments. Treatments comprised fresh grass, hay, or untreated (UTS) or formic acid treated silage (FAS) prepared from the same grass sward. Preparation of conserved forages coincided with the collection of samples from cows fed fresh grass. In the first experiment, 5 multiparous Finnish Ayrshire cows (229 d in milk) were used to compare a diet based on fresh grass followed by hay during 2 consecutive 14-d periods, separated by a 5-d transition during which extensively wilted grass was fed. In the second experiment, 5 multiparous Finnish Ayrshire cows (53 d in milk) were assigned to 1 of 2 blocks and allocated treatments according to a replicated 3 × 3 Latin square design, with 14-d periods to compare hay, UTS, and FAS. Cows received 7 or 9 kg/d of the same concentrate in experiments 1 and 2, respectively. Arterial concentrations of triacylglycerol (TAG) and phospholipid were higher in cows fed fresh grass, UTS, and FAS compared with hay. Nonesterified fatty acid (NEFA) concentrations and the relative abundance of 18:2n-6 and 18:3n-3 in TAG of arterial blood were also higher in cows fed fresh grass than conserved forages. On all diets, TAG was the principle source of fatty acids (FA) for milk fat synthesis, whereas mammary extraction of NEFA was negligible, except during zero-grazing, which was associated with a lower, albeit positive calculated energy balance. Mammary FA uptake was higher and the synthesis of 16:0 lower in cows fed fresh grass than hay. Conservation of grass by drying or ensiling had no influence on mammary extraction of TAG and NEFA, despite an increase in milk fat secretion for silages com-

pared with hay and for FAS than UTS. Relative to hay, milk fat from fresh grass contained lower 12:0, 14:0, and 16:0 and higher S3,R7,R11,15-tetramethyl-16:0, *cis*-9 18:1, *trans*-11 18:1, *cis*-9,*trans*-11 18:2, 18:2n-6, and 18:3n-3 concentrations. Even though conserved forages altered mammary lipogenesis, differences in milk FA composition were relatively minor, other than a higher enrichment of S3,R7,R11,15-tetramethyl-16:0 in milk from silages compared with hay. In conclusion, differences in milk fat composition on fresh grass relative to conserved forages were associated with a lower energy balance, increased uptake of preformed FA, and decreased synthesis of 16:0 de novo in the mammary glands, in the absence of alterations in stearyl-coenzyme A desaturase activity.

Key words: forage conservation, plasma lipid, milk fat composition, mammary lipogenesis

INTRODUCTION

Milk fat is a major source of SFA and contributes to *trans* FA (TFA) in the human diet (Hulshof et al., 1999; Kris-Etherton et al., 2000). A high consumption of SFA and TFA are known risk factors for cardiovascular disease (WHO, 2003), with the risk associated with TFA being higher than SFA (Shingfield et al., 2008). However, evidence on the role of specific isomers of TFA on the onset and development of cardiovascular diseases remains inconclusive (Shingfield et al., 2008; Bendtsen et al., 2011). Furthermore, excessive intakes of SFA may also be associated with lowered insulin sensitivity, which is a key factor in the development of the metabolic syndrome and diabetes (Funaki, 2009). In an attempt to lower the economic and social burden of chronic diseases, considerable interest exists in developing sustainable nutritional strategies to lower SFA in milk, 12:0, 14:0, and, 16:0 in particular, and increase *cis*-9 18:1, *cis*-9,*trans*-11 conjugated linoleic acid (CLA), and 18:3n-3 concentrations to improve

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long-term human health (Gebauer et al., 2006; Shingfield et al., 2008). Ruminant milk also contains diastereomers of 3,7,11,15-tetramethyl-16:0 derived from the metabolism of chlorophyll in the rumen (Schröder and Vetter, 2011) that may be beneficial in the treatment of diabetes and obesity in humans (Schlüter et al., 2002).

Depending on the production system, fresh or conserved forages typically contribute to 25 to 100% of the energy requirements of lactating cows. Despite relatively low lipid content, forages contain high proportions of 18:2n-6 and 18:3n-3, and may often represent the principle source of FA in the diet (Harfoot and Hazlewood, 1988). Milk from grazing cows is known to contain lower proportions of medium-chain SFA and higher unsaturated FA concentrations compared with that from cows fed diets based on dried or ensiled forages (Dewhurst et al., 2006; Elgersma et al., 2006; Chilliard et al., 2007). Available data suggest that the effects of forage conservation on milk fat composition are not explained solely by differences in dietary FA intake. Compared with silage, milk from hay-based diets often has higher concentrations of 18:2n-6 and 18:3n-3, in spite of much lower intakes of 18-carbon PUFA (Dewhurst et al., 2006). On the basis of changes in milk fat from pasture compared with diets based on grass or maize silage, it has been suggested that metabolic and hormonal factors may also contribute to the effects of forage conservation on milk FA composition via direct effects on mammary lipid metabolism (Lock and Garnsworthy, 2003).

The aim of the present study was to examine the influence of forage conservation method on circulating plasma lipids, mammary lipogenesis, and milk fat composition in lactating cows fed diets based on fresh or conserved grasses prepared simultaneously from the same swards of timothy and meadow fescue. Chemical composition of dietary ingredients and treatment effects on intake, rumen fermentation characteristics, FA flow at the omasum, and rumen microbial ecology are reported in a companion paper (Halmemies-Beauchet-Filleau et al., 2013).

MATERIALS AND METHODS

Animals, Experimental Design, and Experimental Diets

All procedures involving animals were approved by the Animal Experiment Committee of MTT Agrifood Research Finland in accordance with the Use of Vertebrates for Scientific Purposes Act of 1985. Five multiparous lactating Finnish Ayrshire cows (605 ± 25.7 kg of BW and 229 ± 31.7 DIM) were used in the first experiment. Another group of 5 Finnish Ayrshire cows

(617 ± 25.8 kg of BW and 53 ± 5.3 DIM) were recruited to the second experiment. In experiment 1, 3 cows were pregnant (60, 76, and 87 d of gestation), whereas none recruited to experiment 2 were pregnant. Cows were housed in a dedicated metabolism unit fitted with individual stalls with continuous access to fresh water and milked twice daily at 0700 and 1645 h.

A detailed description of the experimental design and treatments is provided in a companion paper (Halmemies-Beauchet-Filleau et al., 2013). In brief, experiment 1 comprised 2 consecutive 14-d experimental periods separated by a 5-d transition between diets. In the first period, cows were allowed to strip graze timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards for 7 d before being housed indoors and offered chopped grass harvested from the same pasture. Following a 5-d adaptation period, cows were offered hay also prepared from the same grass swards. For experiment 2, cows were assigned to 1 of 2 blocks according to stage of lactation and milk yield and allocated to treatments according to a replicated 3×3 Latin square with 14-d experimental periods. Experimental treatments comprised grass hay, grass silage prepared without additive (untreated, **UTS**), or grass silage prepared with formic acid-based additive (**FAS**; 6 L/t of grass; AIV 2Plus; Kemira Ltd., Helsinki, Finland) prepared simultaneously from the same experimental pasture. For both experiments, forages were offered ad libitum in amounts to ensure 10% refusals and supplemented with the same standard concentrate distributed as equal meals at 0600, 0800, 1800, and 2000 h at a rate of 7 or 9 kg/d, for experiments 1 and 2, respectively.

Measurements, Sampling, and Chemical Analysis

Milk yield was recorded daily throughout the experiment, but only measurements between d 12 to 14 of each period in both experiments were used for statistical analysis. Samples of milk were collected from each cow over 4 consecutive milkings, starting at 1645 h on d 12. Milk samples treated with preservative (bronopol; Valio Ltd., Helsinki, Finland) were stored at 4°C, until analyzed for fat, CP, and lactose (MilkoScan 133B analyzer; Foss Electric A/S, Hillerød, Denmark). Unpreserved milk samples were also collected at the same time, stored immediately at -20°C , and composited according to milk yield until analyzed for FA composition. In addition, samples of unpreserved milk were collected at 1645 h on d 13 and 0700 h on d 14, stored at 4°C, composited according to yield, and submitted for the determination of urea concentrations (Shingfield et al., 2002a).

Samples of blood were collected simultaneously from coccygeal vessels and caudal superficial epigastric veins

into 3×10 -mL collection tubes containing EDTA (Venoject; Terumo Europe Ltd., Leuven, Belgium) at 0600, 0900, and 1200 h on d 14 of each experimental period. Blood samples were collected 3 times daily to minimize the influence of diurnal variation in plasma lipid composition. Coccygeal vessels and superficial epigastric veins were used as convenient sites for the sampling of arterial blood and mammary gland venous blood, respectively (Cant et al., 1993). Once collected, tubes were placed on ice. Plasma was recovered by centrifugation at $3,000 \times g$ for 15 min at 4°C, frozen immediately at -20°C, and composited across sampling times for each cow. Once plasma from the third blood sample had been recovered, composite samples were stored at -80°C until analyzed for lipid composition. Plasma glucose and BHBA were analyzed using standard methods (Shingfield et al., 2002b). Plasma amino acid concentrations were determined following the precipitation of protein with 12% sulfosalicylic acid (1:2, vol/vol) using an autoanalyzer (Biochrom 20; Pharmacia Biotech, Cambridge, UK) equipped with 90×4.6 -mm PEEK lithium prewash column and 250×4.6 mm Bio PEEK lithium high-performance column (Pharmacia Biotech) in accordance with the procedures recommended by the manufacturer.

Lipid Analysis

Lipid in 3 mL of plasma was extracted in duplicate using a mixture of hexane and isopropanol (3:2, vol/vol). Organic extracts were combined, evaporated to dryness at 45°C under nitrogen, and dissolved in 0.5 mL of chloroform. Phospholipid (PL), cholesterol ester (CE), triacylglycerol (TAG), and NEFA were separated by solid-phase extraction using Bond Elut NH₂ aminopropyl-bonded silica cartridges (500 mg; Varian Inc., Lake Forest, CA) loaded on a Visiprep (Supelco Inc., Bellefonte, PA) vacuum filtering unit operated under atmospheric pressure. Plasma PL, CE, TAG, and NEFA were recovered by eluting columns with methanol, hexane, a mixture of 1% diethyl ether, and 10% methylene chloride in hexane (vol/vol), or 2% acetic acid in diethyl ether (vol/vol), respectively (Kaluzny et al., 1985). Concentrations of PL, CE, TAG, and NEFA in plasma samples were determined using 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylcholine (37-1700-11; Larodan Fine Chemicals AB, Malmö, Sweden), cholesteryl pentadecanoate (CH-814; Nu-Chek Prep Inc., Elysian, MN), tritridecanoin (T-135; Nu-Chek Prep Inc.), and tricosanoic acid (N-23A; Nu-Chek Prep Inc.) as internal standards, respectively. Internal standard solutions were prepared to contain 100 mg of CE, TAG, or NEFA in 100 mL of hexane or 100 mg of PL in 100 mL of chloroform with 1 mL of each added to

plasma samples for quantitative determinations. Fatty acids in TAG and PL fractions were transesterified to FA methyl esters (FAME) by incubation with 2 mL of hexane and 40 µL of fresh methylation reagent (1.75 mL methanol and 0.4 mL of Na-methoxide solution; Fluka reagent 71748) at 20°C for 5 min. Methyl esters of NEFA and CE were prepared by incubation with 1.5 mL of 1% (vol/vol) sulfuric acid in methanol at 50°C for 30 min and 24 h, respectively. Lipid in 1-mL milk samples was extracted 3 times with a mixture of ammonia, methanol, diethyl ether, and hexane (0.2:1:2.5:2.5, vol/vol/vol, respectively) and converted to FAME using methanolic sodium methoxide as a catalyst (Halmemies-Beauchet-Filleau et al., 2011).

Samples of FAME were quantified using a gas chromatograph (model 6890; Hewlett-Packard, Wilmington, DE) equipped with a flame-ionization detector, automatic injector, split injection port, and a 100-m fused silica capillary column (0.25-mm i.d.) coated with a 0.2-µm film of cyanopropyl polysiloxane (CP-SIL, Chromopack 7489; Chromopack, Middelburg, the Netherlands). The total FAME profile in a 2-µL sample at a split ratio of 1:50 was determined using a temperature gradient program and hydrogen as a carrier gas operated at constant pressure (137.9 kPa) at a flow rate of 0.5 mL/min. Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C. Peaks were identified by comparison of retention times with authentic FAME standards and by GC-MS analysis of 4,4-dimethyloxoline derivatives prepared from FAME. Preparation of 4,4-dimethyloxazoline (DMOX) derivatives, parameters used for GC-MS analysis, and interpretation mass spectra were in accordance with earlier reports (Halmemies-Beauchet-Filleau et al., 2011). Relative retention time and order of elution was used to differentiate between diastereomers of 2,6,10,14-tetramethyl-15:0 (Ackman and Hansen, 1967) and 3,7,11,15-tetramethyl-16:0 (Schröder and Vetter, 2011). The distribution of CLA isomers in milk samples was determined using an HPLC system (model 1090; Hewlett-Packard) equipped with 4 silver-impregnated silica columns (ChromSpher 5 Lipids, 250×4.6 mm, 5-µm particle size; Varian Ltd., Walton-on-Thames, UK) coupled in series. Methyl esters of CLA were separated under isothermal conditions at 22°C using 0.1% (vol/vol) acetonitrile in heptane at a flow rate of 1 mL/min and monitoring column effluent at 233 and 210 nm (Halmemies-Beauchet-Filleau et al., 2011).

Plasma and milk FA composition were expressed as a weight percentage of total FA using theoretical relative response factors (Halmemies-Beauchet-Filleau et al., 2011) to account for the carbon deficiency in the flame ionization detector response for FAME containing 4- to 10-carbon atoms. Concentrations of specific

conjugated isomers were calculated based on proportionate peak area responses determined by HPLC and the sum of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA weight percentage determined by GC.

Calculations

Metabolizable energy content of experimental diets was calculated from the concentration of digestible nutrients [$0.016 \times$ digestible OM in DM (g/kg); Ministry of Agriculture, Fisheries and Food, 1975] determined by total fecal collection (Halmemies-Beauchet-Filleau et al., 2013). The energy requirement (MJ/d) for maintenance and milk production was calculated as $[BW (kg^{0.75}) \times 0.515 + ECM \text{ yield (kg/d)} \times 5.15]$; MTT Agrifood Research Finland, 2010].

Extraction (%) of lipid and metabolites by the mammary gland from arterial plasma was calculated as (arterial-venous concentration)/arterial concentration $\times 100$. Mammary plasma flow was calculated according to the Fick principle based on the stoichiometric transfer of mammary Phe and Tyr uptake into milk (Cant et al., 1993). Secretion of Phe and Tyr in milk was estimated from the difference in milk CP and urea output, known amino acid content of milk true protein, and assuming a 3.5% contribution originating from the transfer from blood-borne albumin and immunoglobulins (Cant et al., 1993), but excluding free Phe and Tyr concentrations:

$$\begin{aligned} \text{Mammary plasma flow} &= (\text{milk Phe} + \text{Tyr}) \\ &\times 0.965 / (\text{arteriovenous difference of Phe} + \text{Tyr}). \end{aligned}$$

Mammary uptake (mmol/d) of individual FA was calculated as the product of arteriovenous differences in plasma TAG (mmol/L) $\times$ mammary plasma flow (L/d). Owing to the positive extraction of NEFA across the mammary glands during zero grazing, the contribution of both TAG and NEFA was included in the calculation of total mammary FA uptake for cows fed diets based on fresh grass in experiment 1. Mammary uptake (mmol/d) of metabolites was calculated as arteriovenous differences in plasma (mmol/L) $\times$ mammary plasma flow (L/d).

Fatty acid balance across the mammary glands was calculated as secretion in milk (mmol/d) – mammary gland uptake (mmol/d), assuming negligible oxidation of ≥ 16 -carbon FA (Annison et al., 1968) and that FA synthesized de novo were not utilized for processes other than desaturation or milk TAG synthesis. Relative proportions of SFA in milk fat synthesized de novo (%) was calculated as $[\text{secretion in milk (mmol/d)}]$

– mammary uptake (mmol/d)]/[secretion in milk (mmol/d)] $\times 100$. Desaturation of FA (%) in the mammary glands was calculated as $[\text{mammary balance of product (mmol/d)}]/[\text{mammary availability of substrate (mmol/d)}] \times 100$, taking into account that 10:0 is synthesized exclusively de novo, 18-and-longer-carbon FA originated from plasma, whereas 12:0, 14:0, and 16:0 are derived from both sources.

Statistical Analysis

Treatment means in experiment 1 were evaluated by a paired *t*-test (SAS version 9.2; SAS Institute Inc., Cary, NC). In all cases, a 95% confidence interval was used as the default for the hypothesis test, whereas assumptions of normality were validated using the univariate procedure. Differences between treatment means were considered to reflect the effect of forage conservation by drying.

For experiment 2, data were analyzed by ANOVA for a replicated 3×3 Latin square with 5 cows to evaluate 3 treatments over 3 experimental periods, with a model that included the fixed effects of treatment and period and random effects of block and cow within block using PROC MIXED of SAS. Sums of squares were further separated into single-degree-of-freedom contrasts to test for the significance of forage conservation by drying versus ensiling (method = hay vs. UTS and FAS) and extent of silage fermentation (UTS vs. FAS). Least squares means are reported, with treatment effects declared significant at $P \leq 0.05$. Differences between treatments with *P*-values between 0.05 and 0.10 were considered a trend toward significance. Relationships between FA flows at the omasum (Halmemies-Beauchet-Filleau et al., 2013), arterial plasma lipid composition, and secretion of FA in milk were evaluated by linear regression analysis using PROC REG of SAS.

RESULTS

Milk Production

Fresh grass in the diet had no effect ($P > 0.10$) on milk yield, or milk fat or protein content, but increased ($P < 0.03$) milk fat secretion compared with hay (Table 1). Relative to hay, diets based on silage resulted in higher ($P < 0.04$) milk and milk fat yield, but had no effect ($P > 0.10$) on milk constituent concentrations (Table 1). Compared with UTS, FAS increased ($P < 0.04$) the yield of milk fat and milk protein (Table 1). Cows were in positive energy balance across all treatments. However, calculated energy balance tended ($P = 0.08$) to be lower in cows offered fresh grass compared with hay (Table 1).

Table 1. Effect of fresh grass or conserved forages on energy intake and milk production in lactating cows

Item	Experiment 1				Experiment 2				P-value ⁵	
	Grass	Hay	SEM ¹	P-value	Hay	UTS ²	FAS ³	SEM ⁴	Method	Extent
ME intake, MJ/d	199	209	10.2	0.36	221	229	247	11.9	0.012	0.017
ME balance, MJ/d	0.53	20.7	8.48	0.08	10.5	11.8	16.1	5.57	0.53	0.50
Yield, kg/d										
Milk	24.7	23.3	1.12	0.28	29.3	31.2	32.7	2.14	0.036	0.23
ECM	26.3	24.4	0.70	0.06	28.5	29.8	32.4	1.97	0.002	0.004
Fat	1.10	1.01	0.025	0.022	1.13	1.20	1.32	0.090	<0.001	<0.001
Protein	0.89	0.85	0.049	0.46	0.91	0.90	1.01	0.059	0.19	0.032
Lactose	1.11	1.03	0.053	0.22	1.44	1.52	1.58	0.102	0.13	0.45
Concentration in milk, %										
Fat	4.49	4.38	0.240	0.67	3.83	3.88	4.04	0.119	0.44	0.39
Protein	3.62	3.68	0.050	0.25	3.12	2.97	3.13	0.194	0.53	0.26
Lactose	4.47	4.40	0.045	0.18	4.91	4.87	4.84	0.045	0.31	0.67
Urea, mmol/L	2.52	3.15	0.244	0.07	3.90	3.79	3.58	0.364	0.53	0.60

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).

Concentration and Extraction of Plasma Lipids and Metabolites

Circulating PL and CE represented the major lipid fractions in arterial and venous blood, whereas concentrations of TAG and NEFA were several-fold lower (Table 2). On all diets, TAG in arterial blood was the major source of preformed FA for milk fat synthesis (Table 2). With the exception of cows fed fresh grass, arteriovenous differences indicated a net efflux of NEFA from the mammary gland (Table 2). In most cases, mammary extraction of arterial CE and PL was negligible, whereas forage conservation had often significant, but quantitatively small effects (Table 2). Associations between the flow of specific FA at the omasum with their concentration in arterial blood, uptake across the mammary glands, and secretion in milk are presented in Supplemental Table S4 (available online at <http://www.journalofdairyscience.org/>).

Treatments had no effect ($P > 0.10$) on glucose and urea levels in arterial or venous plasma, or on the rate of mammary extraction of BHBA, glucose, and urea across the mammary glands (Table 2). Mammary uptake of BHBA and urea was unaffected by treatment, whereas the uptake of glucose tended to be higher ($P \leq 0.07$) for fresh grass compared with hay and for silages relative to hay (Table 2).

Fresh Grass Versus Grass Hay. Fresh grass resulted in higher ($P < 0.03$) PL and NEFA concentrations in arterial blood, elevated PL and CE in venous blood, and tended ($P = 0.08$) to increase TAG in arte-

rial blood compared with hay (Table 2). Plasma BHBA concentrations did not differ ($P > 0.10$) between cows offered fresh grass or grass hay (Table 2).

Grass Hay Versus Grass Silage. Relative to hay, diets based on grass silage increased ($P < 0.04$) PL and TAG concentrations in arterial blood, TAG and NEFA in venous blood, and BHBA in arterial and venous blood (Table 2).

Extent of Silage Fermentation. Compared with UTS, FAS elevated ($P < 0.05$) NEFA in arterial and venous blood, and increased ($P < 0.05$) arterial CE and BHBA concentrations (Table 2).

FA Composition of Plasma Lipids

Circulating PL in arterial blood were relatively abundant in 3,7,11,15-tetramethyl-16:0 (Supplemental Figure S1, available online at <http://www.journalofdairyscience.org/>), 16:0, 18:0, *cis*-9 18:1, and 18:2n-6, 20- and 22-carbon PUFA (Table 3). Cholesterol esters were rich in 18:2n-6, with 18:3n-3 as a major component (Table 4). In contrast, 16:0 and 18:0 predominated in TAG (Table 5), whereas the FA composition of NEFA resembled that of TAG (Table 6). Triacylglycerols were the main repository for the transport of 2,6,10,14-tetramethyl-15:0 in arterial and venous blood (Tables 3 to 6).

Fresh Grass Versus Grass Hay. Relative concentrations of 18:3n-3 in CE and TAG fractions in arterial blood were higher ($P < 0.02$) in cows fed fresh grass

Table 2. Effect of fresh grass or conserved forages on the concentration and mammary extraction of plasma lipid fractions and metabolites in lactating cows

Item ¹	Experiment 1				Experiment 2				P-value ⁶	
	Grass	Hay	SEM ²	P-value	Hay	UTS ³	FAS ⁴	SEM ⁵	Method	Extent
Arterial concentration, mg/dL										
PL	104	91.1	3.00	0.013	122	131	145	9.5	0.023	0.06
CE	92.7	83.9	11.10	0.48	124	125	137	7.5	0.11	0.041
TAG	8.69	7.19	0.632	0.08	5.30	6.36	6.91	0.325	0.011	0.25
NEFA	8.22	3.06	1.532	0.028	3.20	3.30	4.35	0.549	0.11	0.041
BHBA, mmol/L	0.72	0.73	0.054	0.89	0.74	0.88	1.06	0.087	0.007	0.036
Glucose, mmol/L	3.85	3.71	0.128	0.33	3.64	3.67	3.63	0.114	0.89	0.72
Urea, mmol/L	3.84	3.84	0.311	1.00	4.59	4.56	4.94	0.633	0.81	0.61
Venous concentration, mg/dL										
PL	104	88.3	1.77	<0.001	129	132	143	9.2	0.16	0.15
CE	98.1	85.2	3.81	0.028	128	130	137	8.8	0.20	0.18
TAG	3.99	4.03	0.327	0.89	2.28	3.33	3.54	0.341	0.033	0.69
NEFA	6.96	3.11	1.600	0.89	3.47	3.88	4.65	0.428	0.024	0.045
BHBA, mmol/L	0.45	0.48	0.044	0.49	0.46	0.59	0.73	0.074	0.007	0.06
Glucose, mmol/L	3.13	3.07	0.123	0.63	2.97	2.96	2.85	0.138	0.57	0.38
Urea, mmol/L	3.96	3.76	0.289	0.53	4.84	4.65	5.11	0.635	0.96	0.57
Mammary extraction rate, %										
PL	-0.3	3.1	2.50	0.25	-5.1	-0.7	1.6	2.43	0.041	0.40
CE	-5.9	-5.2	12.32	0.96	-3.6	-4.3	0.9	1.71	0.32	0.043
TAG	53.2	44.2	4.98	0.15	56.8	48.3	47.9	4.58	0.18	0.95
NEFA	17.1	-9.9	9.84	0.06	-13.9	-18.0	-9.8	7.58	1.00	0.46
TAG + NEFA	35.4	30.0	2.96	0.14	31.5	25.3	27.2	2.04	0.06	0.49
BHBA	36.5	33.5	5.30	0.60	38.7	32.1	32.1	3.38	0.17	1.00
Glucose	18.3	17.2	0.96	0.35	18.3	19.4	21.5	2.18	0.37	0.44
Urea	-1.8	1.8	5.52	0.56	-5.1	-3.0	-3.7	3.33	0.69	0.90
Mammary uptake, mol/d										
BHBA	3.08	2.83	0.217	0.31	3.60	4.18	5.03	0.579	0.20	0.34
Glucose	8.09	7.18	0.329	0.06	8.57	9.65	11.7	1.133	0.07	0.11
Urea	-1.65	1.06	2.328	0.31	-3.44	-2.12	-2.69	2.026	0.70	0.86

¹PL = phospholipid; CE = cholesterol ester; TAG = triacylglycerol.²Standard error of the mean for n = 10 measurements; error degree of freedom = 4.³Untreated silage.⁴Silage ensiled using a formic acid-based additive.⁵Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁶Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).

compared with hay (Tables 4 and 5). Furthermore, fresh grass in the diet increased ($P < 0.01$) the abundance of 18:2n-6 in arterial TAG (Table 5) compared with hay. Relative to hay, fresh grass had no effect ($P > 0.05$) on 18:0 concentrations of arterial lipid fractions (Tables 3 to 6), but resulted in a marked increase ($P < 0.04$) in the relative proportions of 16:0 in circulating NEFA (Table 6), and *cis*-9 18:1 of PL and NEFA fractions (Tables 3 and 6). Furthermore, incorporation of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA in plasma PL tended ($P \leq 0.07$) to be higher for fresh grass compared with hay (Table 3), whereas enrichment in arterial TAG and NEFA did not differ ($P > 0.10$; Tables 5 and 6).

Grass Hay Versus Grass Silage. Differences in the composition of major FA in plasma lipids of cows fed grass silages or hay were relatively minor (Tables 3 to 6). Relative to hay, silage increased ($P < 0.02$)

18:3n-3 concentrations of PL and CE in arterial blood (Tables 3 and 4). Even though hay decreased ($P < 0.01$) the relative proportions of 18:2n-6 in arterial PL and CE compared with silages, absolute concentrations of 18:2n-6 transported by both lipid fractions in arterial blood were similar across diets. Compared with hay, feeding silage resulted in higher ($P < 0.02$) concentrations of 3S,7R,11R,15-tetramethyl-16:0 in PL, TAG, and NEFA fractions in arterial blood and enrichment of 3R,7R,11R,15-tetramethyl-16:0 in arterial PL (Tables 3, 5, and 6).

Extent of Silage Fermentation. Restricting fermentation in silo had no effect ($P > 0.10$) on the enrichment of 16:0, 18:0, *cis*-9, *trans*-11 CLA, 18:2n-6, or 18:3n-3 in lipids circulating in arterial blood, other than a trend toward decreasing ($P = 0.08$) 18:3n-3 concentrations in PL and ($P = 0.09$) increasing the

Table 3. Effect of fresh grass or conserved forages on the FA composition of phospholipids in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 1				Experiment 2					
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	<i>P</i> -value ⁵	
									Method	Extent
12:0	0.01	0.01	0.003	0.27	0.02	0.03	0.03	0.013	0.47	0.75
14:0	0.90	0.80	0.162	0.59	1.16	1.46	1.37	0.720	0.72	0.93
15:0	0.32	0.51	0.030	0.003	0.52	0.44	0.47	0.023	0.019	0.30
<i>anteiso</i> 15:0	0.11	0.20	0.018	0.006	0.20	0.19	0.25	0.040	0.65	0.32
<i>iso</i> 15:0	0.08	0.12	0.010	0.013	0.13	0.12	0.14	0.011	0.94	0.15
16:0	14.6	13.3	0.664	0.13	12.2	11.3	12.8	0.724	0.88	0.19
<i>iso</i> 16:0	0.20	0.27	0.018	0.021	0.29	0.26	0.39	0.077	0.77	0.29
<i>cis</i> -9 16:1	0.29	0.17	0.037	0.030	0.16	0.14	0.34	0.075	0.43	0.11
<i>trans</i> -6+7+8 16:1	0.02	0.03	0.003	0.008	0.03	0.04	0.05	0.007	0.16	0.70
<i>trans</i> -9 16:1	0.04	0.02	0.006	0.032	0.02	0.03	0.04	0.005	0.07	0.15
<i>trans</i> -12 16:1	0.15	0.15	0.010	0.98	0.16	0.17	0.16	0.012	0.88	0.61
<i>anteiso</i> 17:0	0.57	0.75	0.061	0.043	0.86	0.69	0.75	0.170	0.39	0.74
<i>iso</i> 17:0	0.36	0.45	0.023	0.015	0.46	0.38	0.41	0.048	0.25	0.60
<i>cis</i> -6+7 17:1	0.03	0.08	0.025	0.010	0.08	0.08	0.08	0.010	0.98	0.94
<i>cis</i> -8 17:1	0.12	0.16	0.008	0.006	0.16	0.20	0.20	0.012	0.006	0.82
<i>cis</i> -9 17:1	0.21	0.20	0.008	0.25	0.18	0.19	0.17	0.014	0.87	0.45
18:0	24.1	25.2	0.44	0.08	24.2	23.1	22.6	0.543	0.06	0.54
11-cyclohexyl 11:0	0.16	0.25	0.015	0.004	0.24	0.24	0.23	0.020	0.86	0.91
<i>iso</i> 18:0	0.05	0.07	0.003	0.004	0.08	0.05	0.05	0.006	0.008	0.40
<i>cis</i> -9 18:1	12.6	8.89	1.226	0.038	8.83	9.24	8.82	0.427	0.72	0.52
<i>cis</i> -11 18:1	0.92	0.71	0.048	0.013	0.72	0.62	0.62	0.058	0.09	0.99
<i>cis</i> -12 18:1	0.28	0.29	0.016	0.60	0.35	0.46	0.45	0.030	0.002	0.68
<i>cis</i> -13 18:1	0.09	0.08	0.012	0.48	0.07	0.09	0.16	0.058	0.48	0.40
<i>cis</i> -15 18:1 ⁶	0.16	0.32	0.013	<0.001	0.30	0.25	0.28	0.031	0.41	0.50
<i>cis</i> -16 18:1	0.03	0.03	0.004	0.29	0.02	0.04	0.04	0.005	0.09	0.83
<i>trans</i> -6+7+8 18:1	0.06	0.07	0.007	0.12	0.08	0.10	0.11	0.010	0.024	0.11
<i>trans</i> -9 18:1	0.06	0.07	0.004	0.041	0.07	0.08	0.08	0.008	0.69	0.71
<i>trans</i> -10 18:1	0.04	0.03	0.006	0.11	0.04	0.04	0.05	0.004	0.13	0.039
<i>trans</i> -11 18:1	0.69	0.46	0.091	0.07	0.53	0.51	0.60	0.061	0.34	0.036
<i>trans</i> -12+13+14 18:1	0.29	0.51	0.073	0.039	0.63	0.65	0.68	0.063	0.66	0.77
<i>trans</i> -15 18:1	0.45	0.35	0.048	0.11	0.39	0.43	0.40	0.058	0.60	0.61
<i>trans</i> -16 18:1 ⁷	0.11	0.09	0.009	0.16	0.10	0.18	0.24	0.039	0.043	0.31
18:2n-6	23.3	27.0	0.68	0.006	28.8	24.9	25.7	0.803	0.003	0.35
<i>cis</i> -9, <i>trans</i> -12 18:2	0.06	0.04	0.007	0.10	0.03	0.04	0.03	0.004	0.16	0.77
<i>trans</i> -9, <i>cis</i> -12 18:2	0.07	0.09	0.013	0.15	0.07	0.08	0.16	0.052	0.47	0.31
<i>cis</i> -9, <i>trans</i> -11CLA ⁸	0.12	0.05	0.007	<0.001	0.08	0.08	0.08	0.010	0.58	0.88
18:3n-3	3.66	3.68	0.281	0.97	3.57	4.34	3.94	0.174	0.012	0.08
18:3n-6	0.17	0.36	0.050	0.020	0.27	0.23	0.23	0.027	0.12	1.00
18:4n-3	0.05	0.07	0.010	0.12	0.06	0.08	0.06	0.004	0.12	0.017
20:0	0.04	0.06	0.006	0.025	0.07	0.07	0.06	0.006	0.91	0.48
3S,7R,11R,15-tetramethyl-16:0	1.37	1.77	0.274	0.23	2.83	6.66	5.36	0.726	0.013	0.26
3R,7R,11R,15-tetramethyl-16:0	0.66	0.70	0.057	0.47	0.65	0.92	0.83	0.065	0.004	0.16
<i>cis</i> -11 20:1	0.05	0.06	0.007	0.14	0.08	0.10	0.09	0.007	0.18	0.69
20:2n-6	0.13	0.16	0.014	0.06	0.19	0.17	0.15	0.008	0.07	0.12
20:3n-6	3.21	3.06	0.301	0.65	2.63	2.61	2.50	0.205	0.59	0.54
20:4n-3	1.29	1.26	0.183	0.92	1.05	1.24	1.13	0.107	0.06	0.14
20:4n-6	2.31	2.03	0.110	0.07	1.88	1.96	1.83	0.091	0.93	0.33
20:5n-3	1.43	1.17	0.039	0.003	0.95	1.10	1.09	0.084	0.010	0.81
21:0	0.01	0.02	0.001	0.010	0.02	0.02	0.03	0.005	0.48	0.19
22:0 ⁹	0.08	0.08	0.009	0.57	0.09	0.11	0.11	0.005	0.014	0.73
22:4n-3	0.07	0.08	0.018	0.72	0.06	0.05	0.06	0.006	0.53	0.49
22:4n-6	0.22	0.19	0.018	0.15	0.17	0.18	0.15	0.011	0.52	0.11
22:5n-3	1.83	1.41	0.077	0.006	1.29	1.28	1.14	0.107	0.41	0.21
22:5n-6	0.04	0.04	0.004	0.88	0.03	0.02	0.03	0.004	0.75	0.29
22:6n-3	0.13	0.11	0.013	0.12	0.10	0.10	0.09	0.013	0.53	0.31
23:0	0.15	0.19	0.070	0.60	0.11	0.15	0.15	0.070	0.64	0.96
24:0	0.03	0.04	0.003	0.011	0.03	0.03	0.04	0.003	0.76	0.40

Continued

Table 3 (Continued). Effect of fresh grass or conserved forages on the FA composition of phospholipids in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 1				Experiment 2					
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	<i>P</i> -value ⁵	
									Method	Extent
26:0	0.01	0.01	0.002	0.59	0.01	0.02	0.03	0.010	0.38	0.46
28:0	0.02	0.01	0.006	0.11	0.02	0.02	0.02	0.002	0.13	0.83
Σ Unidentified	1.28	1.61	0.119	0.06	1.61	1.73	1.80	0.114	0.30	0.65

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.

²Untreated silage.

³Silage ensiled using a formic acid-based additive.

⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.

⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).

⁶Co-elutes with 19:0.

⁷Co-elutes with *cis*-14 18:1.

⁸CLA = conjugated linoleic acid.

⁹Co-elutes with an unidentified FA.

proportion of 16:0 in NEFA. However, compared with UTS, FAS increased ($P < 0.04$) *trans*-11 18:1 abundance in PL and NEFA fractions (Tables 3 and 6) and the proportions of *cis*-9 18:1 in NEFA of arterial blood (Table 6).

Mammary FA Metabolism

The uptake of individual FA from arterial blood and the overall balance of FA across the mammary glands are shown in Table 7. On average, 16:0, 18:0, and *cis*-9 18:1 accounted for 19 to 23, 34 to 47, and 7 to 22% of total FA uptake across the mammary glands, respectively. For diets based on hay or silages, 16:0 was the major FA synthesized de novo, whereas synthesis of 4:0, 14:0, and 16:0 in the mammary glands were of a similar magnitude on fresh grass. Across all treatments, synthesis de novo accounted for, on average 98, 95, and 74% of mammary 12:0, 14:0, and 16:0 secretion, respectively (Table 8). Furthermore, on average, 54 and 24% of 18:0 and *trans*-11 18:1 extracted from arterial plasma were desaturated in the mammary glands, respectively (Table 8). Conversion of 18:0 to *cis*-9 18:1 accounted for, on average, 71% of total FA desaturation in the mammary glands, with the formation of *cis*-9 14:1 and *cis*-9 16:1 accounting for 10 and 11%, respectively, whereas that associated with the synthesis of *cis*-9 10:1, *cis*-9 12:1, *cis*-9, *trans*-11 CLA, and *cis*-9 20:1 were marginal (4, <0.1, 2, and 1%, respectively). For fresh grass, 53% of *cis*-9 18:1 secreted in milk originated from the desaturation of 18:0 in the mammary glands, whereas the contribution of stearoyl CoA desaturase to *cis*-9 18:1 output in milk was higher (ca. 76%) for hay and grass silages. Overall, ca. 78% of *cis*-9, *trans*-11 CLA

secreted in milk originated from the desaturation of *trans*-11 18:1 in the mammary glands, with no evidence of differences among treatments (data not presented).

Fresh Grass Versus Grass Hay. Mammary FA uptake was ca. 70% higher for fresh grass compared with hay. Compared with hay, fresh grass resulted in higher ($P < 0.01$) uptakes of *trans*-11 18:1 and 18:3n-3 across the mammary glands, and numerical ($P > 0.10$) increases in mammary uptake of *cis*-9, *trans*-11 CLA and 18:2n-6 (Table 7). Zero grazing was also associated with greater ($P < 0.03$) uptakes of 16:0, 18:0, and *cis*-9 18:1 across the mammary glands compared with hay. Furthermore, mammary uptake of 16:0 + *cis*-9 16:1, 18:0 + *cis*-9 18:1, and total FA for fresh grass exceeded the corresponding flows at the omasum by 146, 70, and 199 g/d, respectively (Supplemental Figure S2, available online at <http://www.journalofdairyscience.org/>).

Mammary synthesis of 16:0 de novo was markedly lower ($P < 0.01$), and that of 12:0 tended ($P = 0.08$) to be lower in cows fed fresh grass relative to hay (Table 7). Compared with hay, fresh grass decreased ($P \leq 0.04$) the desaturation of 14:0 and 18:0 in the mammary glands, but had no effect ($P > 0.10$) on the conversion of *trans*-11 18:1 to *cis*-9, *trans*-11 CLA (Table 8). However, the amount of *cis*-9, *trans*-11 CLA synthesized in the mammary glands was higher ($P = 0.03$) for cows during zero grazing compared with hay (Table 7).

Grass Hay Versus Grass Silage. Feeding extensively or restrictively fermented silages had no effect ($P > 0.10$) on mammary *cis*-9 18:1, 18:2n-6, or 18:3n-3 uptake compared with hay, whereas grass silage diets tended ($P \leq 0.10$) to increase the uptake of 18:0 and *trans*-11 18:1 across the mammary glands (Table 7). Relative to hay, silages increased ($P < 0.02$) the syn-

Table 4. Effect of fresh grass or conserved forages on the FA composition of cholesterol esters in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 1				Experiment 2				P-value ⁵	
	Grass	Hay	SEM ¹	P-value	Hay	UTS ²	FAS ³	SEM ⁴	Method	Extent
12:0	0.13	0.11	0.018	0.46	0.10	0.11	0.10	0.015	0.73	0.54
13:0	0.04	0.06	0.017	0.21	0.03	0.03	0.03	0.006	0.99	0.73
<i>anteiso</i> 13:0	0.01	0.02	0.003	0.07	0.01	0.02	0.01	0.004	0.70	0.13
<i>iso</i> 13:0	0.03	0.03	0.008	0.70	0.02	0.02	0.02	0.003	0.57	0.47
14:0	1.25	1.41	0.104	0.19	0.97	1.04	1.06	0.095	0.53	0.91
<i>iso</i> 14:0	0.14	0.16	0.010	0.16	0.14	0.13	0.12	0.008	0.20	0.47
<i>cis</i> -9 14:1	0.07	0.05	0.007	0.06	0.04	0.04	0.04	0.006	0.50	0.52
<i>anteiso</i> 15:0	1.55	1.42	0.169	0.46	1.28	1.37	1.27	0.076	0.51	0.19
<i>iso</i> 15:0	0.81	0.96	0.080	0.14	0.90	0.93	0.90	0.034	0.47	0.42
<i>trans</i> -5 15:1	0.17	0.20	0.024	0.34	0.22	0.22	0.24	0.005	0.09	0.009
<i>trans</i> -7 15:1	0.13	0.09	0.012	0.022	0.10	0.12	0.13	0.010	0.031	0.27
16:0	3.54	3.98	0.028	0.19	3.08	2.96	2.92	0.140	0.12	0.61
<i>iso</i> 16:0	0.34	0.39	0.047	0.39	0.35	0.33	0.31	0.017	0.17	0.37
<i>cis</i> -9 16:1	1.84	1.36	0.079	0.004	1.11	1.11	1.09	0.083	0.89	0.76
<i>cis</i> -11 16:1	0.16	0.13	0.011	0.037	0.11	0.10	0.11	0.016	0.53	0.47
<i>cis</i> -13 16:1	0.10	0.07	0.023	0.23	0.07	0.08	0.07	0.004	0.27	0.44
<i>trans</i> -6+7+8 16:1	0.12	0.14	0.017	0.53	0.12	0.14	0.15	0.011	0.035	0.63
<i>trans</i> -9 16:1	0.14	0.06	0.019	0.014	0.07	0.07	0.10	0.007	0.11	0.014
<i>trans</i> -12 16:1 ⁶	0.85	1.00	0.108	0.26	0.81	0.86	0.86	0.040	0.09	0.99
17:0	0.05	0.07	0.011	0.23	0.07	0.06	0.07	0.012	0.93	0.57
<i>anteiso</i> 17:0	0.46	0.44	0.059	0.77	0.34	0.34	0.36	0.025	0.91	0.46
<i>iso</i> 17:0	0.10	0.09	0.013	0.79	0.08	0.08	0.09	0.010	0.61	0.42
<i>cis</i> -6+7 17:1	0.05	0.13	0.015	0.008	0.11	0.15	0.13	0.010	0.07	0.21
<i>cis</i> -8 17:1	0.31	0.35	0.033	0.37	0.35	0.41	0.42	0.019	<0.001	0.54
<i>cis</i> -9 17:1	0.27	0.26	0.024	0.64	0.23	0.25	0.25	0.014	0.18	0.87
<i>trans</i> -6 17:1	0.26	0.46	0.093	0.10	0.34	0.37	0.34	0.027	0.50	0.29
18:0	0.46	0.86	0.181	0.10	0.47	0.51	0.73	0.118	0.32	0.23
11-cyclohexyl 11:0	0.30	0.39	0.061	0.24	0.41	0.45	0.42	0.026	0.35	0.38
<i>cis</i> -9 18:1	3.56	4.39	0.577	0.22	3.11	3.34	3.38	0.263	0.18	0.82
<i>cis</i> -11 18:1	0.32	0.34	0.027	0.49	0.27	0.27	0.26	0.020	0.77	0.78
<i>cis</i> -12 18:1	0.12	0.11	0.011	0.33	0.13	0.16	0.16	0.014	0.06	0.88
<i>cis</i> -16 18:1	0.04	0.03	0.006	0.38	0.04	0.05	0.05	0.003	0.039	0.88
<i>trans</i> -11 18:1 ⁷	0.09	0.06	0.005	0.009	0.08	0.09	0.09	0.006	0.019	0.35
<i>trans</i> -12+13+14 18:1 ⁸	0.08	0.10	0.025	0.48	0.11	0.12	0.13	0.011	0.23	0.62
18:2n-6	53.1	54.4	2.05	0.54	60.4	56.2	56.5	1.06	<0.001	0.63
<i>cis</i> -9, <i>trans</i> -12 18:2	0.07	0.05	0.016	0.32	0.03	0.04	0.04	0.004	0.15	0.99
<i>trans</i> -9, <i>cis</i> -12 18:2	0.06	0.05	0.015	0.44	0.04	0.05	0.04	0.004	0.09	0.47
<i>cis</i> -9, <i>trans</i> -11 CLA ⁹	0.06	1.33	0.563	0.09	0.38	0.44	0.44	0.081	0.36	0.99
18:3n-3	21.8	15.8	1.49	0.016	16.4	19.6	19.3	0.827	<0.001	0.49
18:3n-6	1.47	2.55	0.356	0.040	1.70	1.72	1.65	0.083	0.86	0.52
18:4n-3	0.57	0.93	0.172	0.11	0.66	0.84	0.81	0.064	0.019	0.59
20:3n-6	0.40	0.36	0.039	0.42	0.28	0.28	0.28	0.027	0.73	0.85
20:4n-3	0.61	0.47	0.080	0.16	0.42	0.48	0.48	0.051	0.012	0.98
20:4n-6	0.89	0.92	0.070	0.67	0.90	0.84	0.84	0.046	0.15	0.97
20:5n-3	1.01	0.96	0.182	0.82	1.18	1.13	1.23	0.155	0.90	0.08
23:0	0.07	0.06	0.011	0.54	0.02	0.01	0.01	0.002	0.019	0.84
Σ Unidentified	2.02	2.35	0.281	0.30	1.83	2.03	1.89	0.111	0.25	0.23

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).⁶Co-elutes with *cis*-6 16:1.⁷Co-elutes with *cis*-5 18:1.⁸Co-elutes with *cis*-6+7+8 18:1.⁹CLA = conjugated linoleic acid.

Table 5. Effect of fresh grass or conserved forages on the FA composition of triacylglycerols in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 2									
	Experiment 1									
	Grass	Hay	SEM ¹	P-value	Hay	UTS ²	FAS ³	SEM ⁴	P-value ⁵	
									Method	Extent
12:0	0.21	0.31	0.011	<0.001	0.33	0.35	0.25	0.047	0.67	0.20
14:0	1.54	2.16	0.069	<0.001	2.00	1.75	2.06	0.127	0.57	0.14
<i>iso</i> 14:0	0.20	0.44	0.036	0.003	0.39	0.27	0.28	0.028	0.020	0.73
<i>cis</i> -9 14:1	0.11	0.14	0.021	0.26	0.15	0.17	0.19	0.053	0.54	0.71
15:0	4.19	5.14	1.521	0.57	3.60	3.20	2.71	0.325	0.15	0.31
<i>anteiso</i> 15:0	1.01	1.73	0.082	<0.001	1.65	1.32	1.33	0.067	0.007	0.92
<i>iso</i> 15:0	0.70	1.40	0.132	0.006	1.22	1.13	1.10	0.164	0.55	0.88
16:0	16.0	18.6	0.85	0.039	18.4	17.9	18.2	0.543	0.59	0.74
<i>iso</i> 16:0	0.56	0.76	0.083	0.07	0.67	0.53	0.49	0.042	0.002	0.31
<i>cis</i> -9 16:1	0.81	0.57	0.168	0.23	0.32	0.21	0.31	0.061	0.45	0.31
<i>trans</i> -6+8 16:1	0.19	0.23	0.033	0.30	0.15	0.14	0.17	0.008	0.71	0.039
<i>trans</i> -9 16:1	0.14	0.08	0.034	0.15	0.11	0.14	0.15	0.033	0.36	0.88
<i>trans</i> -12 16:1	0.66	0.80	0.059	0.09	0.67	0.59	0.64	0.057	0.31	0.38
17:0	0.73	1.38	0.071	<0.001	1.11	0.90	0.87	0.046	0.004	0.65
<i>anteiso</i> 17:0	1.00	1.21	0.092	0.09	1.24	0.97	0.87	0.036	<0.001	0.06
<i>iso</i> 17:0	0.60	0.82	0.077	0.045	0.77	0.64	0.59	0.040	0.003	0.19
<i>cis</i> -8 17:1	0.16	0.12	0.025	0.19	0.13	0.17	0.18	0.043	0.35	0.75
<i>cis</i> -9 17:1	0.15	0.12	0.024	0.33	0.12	0.11	0.10	0.023	0.56	0.69
18:0	25.9	32.3	2.79	0.09	35.5	36.5	36.7	1.73	0.63	0.94
11-cyclohexyl 11:0	0.24	0.30	0.072	0.40	0.33	0.31	0.30	0.027	0.29	0.93
<i>cis</i> -9 18:1	7.87	6.69	1.318	0.42	6.59	6.57	6.52	0.583	0.96	0.95
<i>cis</i> -11 18:1	0.74	0.76	0.134	0.90	0.94	0.72	0.74	0.074	0.009	0.77
<i>cis</i> -12 18:1	0.11	0.14	0.035	0.44	0.23	0.26	0.24	0.023	0.58	0.65
<i>cis</i> -13 18:1	0.60	0.78	0.216	0.45	0.37	0.67	0.43	0.198	0.42	0.35
<i>cis</i> -15 18:1 ⁶	0.23	0.48	0.037	0.003	0.51	0.42	0.60	0.080	0.95	0.17
<i>trans</i> -6+7+8 18:1	0.13	0.16	0.012	0.07	0.24	0.21	0.27	0.029	1.00	0.22
<i>trans</i> -9 18:1	0.10	0.11	0.016	0.39	0.14	0.15	0.16	0.018	0.40	0.68
<i>trans</i> -10 18:1	0.16	0.18	0.026	0.47	0.26	0.23	0.31	0.040	0.81	0.17
<i>trans</i> -11 18:1	1.61	1.61	0.169	0.99	1.97	1.87	2.13	0.231	0.86	0.25
<i>trans</i> -12+13+14 18:1	0.37	0.78	0.094	0.013	0.80	0.88	0.87	0.056	0.31	0.89
<i>trans</i> -15 18:1	0.36	0.32	0.025	0.17	0.38	0.63	0.57	0.044	<0.001	0.07
<i>trans</i> -16 18:1 ⁷	0.23	0.34	0.013	<0.001	0.33	0.59	0.60	0.083	0.029	0.96
18:2n-6	13.3	4.59	0.928	<0.001	6.09	4.69	4.86	1.249	0.40	0.93
<i>cis</i> -9, <i>trans</i> -11 CLA ⁸	0.16	0.72	0.261	0.10	0.27	0.21	0.25	0.040	0.43	0.55
18:3n-3	8.57	2.07	0.473	<0.001	2.42	2.10	2.10	0.445	0.57	1.00
18:3n-6	0.49	0.54	0.238	0.84	0.21	0.18	0.16	0.037	0.30	0.60
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.15	0.05	0.035	0.045	0.05	0.04	0.04	0.014	0.43	0.78
18:4n-3	0.42	0.36	0.135	0.68	0.10	0.08	0.08	0.023	0.53	0.89
3S,7R,11R,15-tetramethyl-15:0	0.26	0.46	0.030	0.003	0.49	0.55	0.58	0.070	0.43	0.80
3R,7R,11R,15-tetramethyl-15:0	0.19	0.23	0.030	0.15	0.23	0.18	0.20	0.030	0.26	0.58
<i>cis</i> -10 19:1	0.29	0.28	0.073	0.86	0.31	0.25	0.25	0.032	0.17	1.00
20:0	0.37	0.62	0.064	0.020	0.61	0.49	0.51	0.024	0.010	0.53
3S,7R,11R,15-tetramethyl-16:0	1.47	2.17	0.288	0.08	2.53	6.25	5.19	0.655	0.008	0.30
3R,7R,11R,15-tetramethyl-16:0	1.62	1.66	0.274	0.90	1.41	1.27	1.49	0.211	0.91	0.46
20:3n-6	0.24	0.20	0.043	0.44	0.14	0.10	0.14	0.030	0.53	0.30
20:4n-3	0.41	0.24	0.065	0.07	0.18	0.15	0.17	0.042	0.61	0.69
20:4n-6	0.57	0.32	0.133	0.13	0.26	0.18	0.20	0.028	0.09	0.63
20:5n-3	1.46	0.66	0.251	0.034	0.29	0.19	0.20	0.049	0.16	0.93
21:0	0.11	0.16	0.011	0.018	0.15	0.09	0.13	0.025	0.23	0.36
22:0	0.23	0.42	0.044	0.012	0.36	0.27	0.30	0.024	0.006	0.18
22:5n-3	0.25	0.22	0.056	0.59	0.27	0.20	0.22	0.025	0.07	0.42
23:0	0.20	0.33	0.013	<0.001	0.27	0.22	0.24	0.043	0.47	0.76
<i>cis</i> -14 23:1	0.18	0.31	0.042	0.045	0.28	0.32	0.33	0.027	0.18	0.79
24:0	0.35	0.50	0.065	0.09	0.34	0.30	0.30	0.043	0.26	0.99
<i>cis</i> -15 24:1	0.11	0.17	0.020	0.033	0.14	0.10	0.13	0.017	0.16	0.15
25:0	0.06	0.08	0.014	0.22	0.07	0.07	0.06	0.021	0.80	0.75
26:0	0.26	0.54	0.075	0.022	0.51	0.50	0.48	0.041	0.75	0.77

Continued

Table 5 (Continued). Effect of fresh grass or conserved forages on the FA composition of triacylglycerols in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 1				Experiment 2					
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	<i>P</i> -value ⁵	
									Method	Extent
28:0	0.11	0.19	0.031	0.047	0.15	0.14	0.13	0.018	0.55	0.41
Σ Unidentified	0.56	0.33	0.131	0.96	0.16	0.28	0.31	0.072	0.12	0.48

¹Standard error of the mean for *n* = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for *n* = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).⁶Co-elutes with 19:0.⁷Co-elutes with *cis*-14 18:1.⁸CLA = conjugated linoleic acid.

thesis of short- and medium-chain SFA (4-, 6-, 8-, and 14-carbon) de novo (Table 7). Furthermore, synthesis of 16:0 in the mammary glands tended to be higher ($P = 0.06$) in cows fed UTS and FAS than hay (Table 7). Compared with hay, silages had no effect ($P > 0.10$) on the extent of FA desaturation in the mammary glands, other than a tendency ($P = 0.07$) for lower conversion of 16:0 to *cis*-9 16:1 (Table 8).

Extent of Silage Fermentation. Restricting fermentation in silo had no effect ($P > 0.10$) on mammary FA uptake, other than increasing ($P < 0.02$) 18:3n-3 uptake from TAG in arterial blood (Table 7). Synthesis de novo of 4- to 10-even-carbon SFA, and 14:0 was also increased ($P < 0.04$) in the mammary glands of cows fed FAS compared with UTS (Table 7). However, the extent of fermentation in silo had no effect ($P > 0.10$) on the extent of FA desaturation in the mammary glands (Table 8).

Milk FA Composition and Secretion

Fresh Grass Versus Grass Hay. Milk from fresh grass contained lower ($P \leq 0.05$) 12:0, 14:0, 16:0, and total SFA concentrations compared with hay (mean responses of -0.9, -2.0, -9.6, and -10.4 percentage units, respectively; Table 9). Changes in milk fat composition were associated with a substantial decrease ($P < 0.01$) in the synthesis of 16:0 de novo in the mammary glands during zero grazing (Table 7). Overall, fresh grass resulted in higher ($P < 0.05$) milk fat 18:0, *cis*-18:1 ($\Delta 9$ -16), *trans*-18:1 ($\Delta 6$ -16), *cis*-9,*trans*-11 CLA, 18:2n-6, and 18:3n-3 concentrations compared with hay (Table 9; Supplemental Table S1, available online at <http://www.journalofdairyscience.org/>). Milk from fresh grass also contained higher ($P < 0.05$) concentrations of other PUFA, including *trans*-7,*cis*-9 CLA, $\Delta 11,13$ CLA, $\Delta 13,15$ CLA, $\Delta 9,12$ 18:2, $\Delta 11,15$

18:2, *cis*-9,*trans*-11,*cis*-15 18:3, 20:3n-3, 20:3n-6, and 22:5n-3 (Table 9; Supplemental Table S1) and lower ($P \leq 0.02$) 18-carbon oxygenated FA content (Supplemental Table S3) relative to hay. However, fresh grass had variable effects ($P \leq 0.05$) on the abundance of milk fat odd- and branched-chain FA compared with hay (Supplemental Table S3).

Relative to hay, fresh grass lowered ($P < 0.05$) 16:0 secretion and increased ($P < 0.05$) the output of 18:0, *cis*-9 18:1, *trans*-11 18:1, *cis*-9,*trans*-11 CLA, 18:2 n-6, and 18:3n-3 in milk (Figure 1). Fresh grass also increased ($P < 0.03$) the secretion of 3S,7R,11R,15-tetramethyl-16:0 and unresolved *cis*-11 16:1 and 3R,7R,11R,15-tetramethyl-16:0 in milk relative to hay [0.9 vs. 0.6 (SE = 0.09) and 4.4 vs. 2.7 (SE = 0.35) mmol/d, respectively].

Grass Hay Versus Grass Silage. Feeding hay or grass silages of different fermentation characteristics resulted in relatively minor, albeit often significant differences in milk FA composition. Relative to hay, grass silages increased ($P < 0.01$) 4:0 and 6:0 concentrations and decreased ($P < 0.05$) 12:0 and 14:0 content (Table 9). Forage conservation method had no effect ($P > 0.10$) on milk 16:0 concentration (Table 9), whereas grass silages lowered ($P < 0.03$) the concentrations of *cis*-9 16:1 and several *trans*-16:1 ($\Delta 9,10,12$) isomers compared with hay (Supplemental Table S2, available online at <http://www.journalofdairyscience.org/>). Relative to hay, grass silage decreased ($P < 0.01$) milk fat 18:2n-6 content and tended ($P = 0.08$) to lower 18:3n-3 concentrations (Table 9). Grass silages also increased ($P < 0.05$) milk fat 18:0 (Table 9) and *trans*-18:1 ($\Delta 12$ -16; Supplemental Table S1) content and the concentration of several 18-carbon PUFA including *cis*-9,*trans*-13 + *cis*-10,*trans*-14 18:2, *trans*-11,*cis*-15 18:2, $\Delta 9,12$ 18:2, $\Delta 12,15$ 18:2, and *cis*-9,*trans*-11,*cis*-15 18:3 compared with hay (Table 9; Supplemental Table S1). Treatments

Table 6. Effect of fresh grass or conserved forages on the FA composition of NEFA in arterial blood of lactating cows

FA, g/100 g of FA	Experiment 2									
	Experiment 1				Experiment 2					
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	<i>P</i> -value ⁵	
									Method	Extent
12:0	0.16	0.34	0.074	0.08	0.38	0.22	0.29	0.080	0.25	0.58
13:0	1.40	2.60	0.281	0.013	1.03	0.91	0.77	0.055	0.032	0.12
14:0	0.97	1.11	0.130	0.35	1.13	1.19	1.27	0.087	0.35	0.53
<i>cis</i> -9 14:1	0.20	0.28	0.026	0.038	0.24	0.26	0.32	0.081	0.49	0.47
15:0	5.16	11.3	1.431	0.013	4.21	4.02	3.28	0.440	0.32	0.27
<i>anteiso</i> 15:0	0.42	0.65	0.078	0.042	0.52	0.52	0.61	0.069	0.43	0.26
<i>iso</i> 15:0	0.42	0.59	0.070	0.07	0.55	0.54	0.48	0.071	0.56	0.40
16:0	15.3	11.1	1.23	0.027	12.7	12.7	14.4	0.580	0.30	0.09
<i>cis</i> -9 16:1	1.13	0.72	0.167	0.07	0.55	0.43	0.69	0.073	0.95	0.045
17:0	0.65	0.58	0.110	0.58	1.56	0.81	0.98	0.398	0.15	0.71
<i>anteiso</i> 17:0	0.74	0.63	0.132	0.45	0.67	0.54	0.67	0.057	0.35	0.13
<i>iso</i> 17:0	0.26	0.35	0.082	0.38	0.34	0.42	0.38	0.091	0.23	0.39
18:0	25.0	20.5	2.76	0.18	30.7	32.5	31.7	1.84	0.57	0.77
<i>cis</i> -9 18:1	21.8	8.45	2.394	0.005	9.14	8.44	12.5	1.535	0.24	0.011
<i>cis</i> -11 18:1	0.82	0.43	0.081	0.009	0.43	0.37	0.50	0.097	0.97	0.40
<i>trans</i> -11 18:1	0.59	0.42	0.106	0.20	0.67	0.49	0.83	0.093	0.85	0.011
18:2n-6	11.0	19.7	3.35	0.07	13.6	12.4	10.4	1.76	0.30	0.41
<i>cis</i> -9, <i>trans</i> -11 CLA ⁶	0.22	0.60	0.230	0.17	1.66	1.74	1.48	0.190	0.81	0.31
18:3n-3	4.33	5.53	1.030	0.31	3.84	3.83	3.11	0.600	0.64	0.43
18:3n-6	0.25	0.65	0.076	0.007	0.37	0.39	0.31	0.037	0.70	0.19
18:4n-3	0.00	0.33	0.094	0.026	0.20	0.23	0.16	0.036	0.92	0.19
20:0	0.92	1.36	0.196	0.10	1.55	1.54	1.38	0.098	0.49	0.30
3S,7R,11R,15-tetramethyl-16:0	0.54	0.57	0.085	0.75	0.82	2.13	1.34	0.228	0.016	0.048
3R,7R,11R,15-tetramethyl-16:0	0.99	0.75	0.237	0.35	1.04	1.22	0.92	0.238	0.86	0.20
20:3n-6	0.23	0.39	0.080	0.13	0.32	0.29	0.20	0.065	0.42	0.37
20:4n-3	0.20	0.28	0.015	0.007	0.21	0.20	0.05	0.028	0.045	0.010
20:4n-6	0.27	0.42	0.056	0.06	0.33	0.29	0.25	0.052	0.42	0.60
20:5n-3	0.32	0.47	0.069	0.10	0.36	0.31	0.42	0.010	0.95	0.25
21:0	0.48	0.79	0.191	0.17	1.04	1.09	1.04	0.159	0.80	0.73
22:0	0.94	1.39	0.234	0.13	1.46	1.64	1.54	0.142	0.40	0.54
<i>cis</i> -14 23:1	0.25	0.25	0.073	0.96	0.52	0.62	0.63	0.125	0.10	0.79
24:0	0.86	1.51	0.236	0.06	1.77	1.69	1.54	0.216	0.45	0.53
<i>cis</i> -15 24:1	0.24	0.43	0.084	0.09	0.50	0.49	0.48	0.088	0.90	0.84
25:0	0.64	1.05	0.157	0.06	1.09	1.09	1.16	0.172	0.82	0.76
26:0	0.64	1.24	0.203	0.041	1.73	1.83	1.58	0.183	0.85	0.19
28:0	0.21	0.33	0.055	0.09	0.46	0.49	0.47	0.072	0.90	0.85
Σ Unidentified	1.38	1.44	0.619	0.93	0.83	0.69	0.67	0.336	0.73	0.98

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).⁶CLA = conjugated linoleic acid.

had no effect ($P > 0.10$) on milk *cis*-9,*trans*-11 CLA concentrations (Table 9). Relative to hay, silages resulted in higher ($P < 0.01$) milk fat *trans*-12,*trans*-14 CLA, *trans*-13,*cis*-15 CLA, and Δ 11,13 CLA concentrations, and lower ($P < 0.02$) *trans*-8,*cis*-10 CLA, and *trans*-10,*cis*-12 CLA content (Supplemental Table S1). Grass silages also decreased ($P \leq 0.05$) the concentration of several 20- and 22-carbon PUFA in milk fat (Table 9), and the majority of 13- to 18-carbon *iso* and *anteiso* FA compared with hay (Supplemental Table S3).

Relative to hay, grass silages had no effect on the secretion of 18:2n-6 and 18:3n-3 in milk ($P > 0.05$),

but increased ($P \leq 0.05$) 14:0, 16:0, 18:0, *trans*-11 18:1, and *cis*-9,*trans*-11 CLA output (Figure 2). Grass silages also increased ($P < 0.01$) the secretion of 3S,7R,11R,15-tetramethyl-16:0 in milk compared with hay [0.9, 2.7, and 2.9 (SE = 0.41) mmol/d, for hay, UTS, and FAS, respectively].

Extent of Silage Fermentation. Restricting fermentation in silo resulted in higher ($P < 0.04$) milk 18:0 and *trans*-11 18:1 content, but had no effect ($P > 0.10$) on *cis*-9,*trans*-11 CLA, 18:2n-6, and 18:3n-3 concentrations (Table 9). Compared with UTS, FAS increased ($P \leq 0.05$) *trans*-9,*cis*-12 18:2, *trans*-11,*cis*-15 18:2,

Table 7. Effect of fresh grass or conserved forages on the uptake and balance of FA across the mammary glands of lactating cows

Item	Experiment 1				Experiment 2				<i>P</i> -value ⁵	
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	Method	Extent
Mammary FA uptake, mmol/d										
12:0	2.97	4.29	1.568	0.45	3.05	4.91	4.23	0.837	0.31	0.17
14:0	28.6	35.2	4.59	0.23	28.6	21.2	26.7	4.59	0.42	0.41
<i>cis</i> -9 14:1	1.79	0.85	0.852	0.34	0.29	2.07	2.82	1.433	0.16	0.65
16:0	508	307	30.0	0.003	316	333	348	34.7	0.47	0.71
<i>cis</i> -9 16:1	28.7	11.8	7.15	0.08	4.53	3.63	3.44	1.773	0.67	0.95
18:0	793	555	70.0	0.028	671	769	883	96.0	0.07	0.21
<i>cis</i> -9 18:1	506	107	67.0	0.004	111	149	133	19.7	0.27	0.60
Σ <i>cis</i> -18:1	545	136	72.7	0.005	134	188	174	21.5	0.13	0.66
<i>trans</i> -10 18:1	3.67	2.41	0.900	0.24	4.20	4.83	7.78	1.149	0.18	0.12
<i>trans</i> -11 18:1	61.0	28.4	6.94	0.010	36.7	44.0	52.5	5.98	0.10	0.26
18:2n-6	39.3	33.1	4.81	0.27	43.8	39.1	45.4	7.97	0.89	0.60
<i>cis</i> -9, <i>trans</i> -11 CLA ⁶	3.22	0.73	1.226	0.12	3.38	2.45	4.18	1.134	0.97	0.33
18:3n-3	28.6	13.8	0.533	<0.001	18.9	15.5	20.9	1.48	0.65	0.019
20:0	8.85	9.08	3.421	0.95	10.6	8.68	9.63	1.351	0.36	0.58
Σ FA										
mmol/d	2,302	1,351	110.4	0.001	1,532	1,654	1,879	175.8	0.16	0.22
g/d	636	371	44.7	0.005	426	459	518	50.6	0.18	0.26
Mammary FA balance, mmol/d										
4:0	346	312	16.5	0.11	390	458	549	49.2	<0.001	<0.001
6:0	161	159	10.0	0.84	195	216	251	21.4	<0.001	0.001
8:0	82.3	83.1	5.87	0.89	97.2	104	119	0.81	0.002	0.003
10:0	161	176	10.4	0.23	199	200	218	20.9	0.14	0.038
<i>cis</i> -9 10:1	17.4	21.2	2.17	0.16	21.2	23.3	25.6	2.37	0.05	0.19
12:0	163	190	11.1	0.08	205	201	217	21.3	0.62	0.12
<i>cis</i> -9 12:1	0.00	0.01	0.001	0.07	0.01	0.01	0.01	0.001	0.92	0.94
14:0	467	506	32.9	0.31	589	608	651	54.1	0.015	0.021
<i>cis</i> -9 14:1	45.0	56.8	4.98	0.08	56.0	55.6	55.6	4.95	0.93	0.99
16:0	601	1,072	67.2	0.003	1,225	1,295	1,364	130.4	0.06	0.23
<i>cis</i> -9 16:1	44.8	59.2	4.89	0.043	60.4	59.0	60.4	5.98	0.80	0.66
18:0	-437	-340	54.5	0.15	-359	-405	-448	72.2	0.32	0.58
<i>cis</i> -9 18:1	450	390	75.9	0.47	345	366	412	32.0	0.10	0.12
Σ <i>cis</i> -18:1	453	386	86.2	0.48	343	316	407	51.6	0.37	0.007
<i>trans</i> -10 18:1	1.99	1.11	0.794	0.33	2.46	2.48	1.25	0.941	0.63	0.40
<i>trans</i> -11 18:1	-20.2	-8.81	7.48	0.21	-9.03	-13.8	-11.9	3.754	0.44	0.74
18:2n-6	17.1	11.5	4.08	0.25	6.31	5.68	5.05	8.615	0.93	0.96
<i>cis</i> -9, <i>trans</i> -11 CLA	17.1	9.99	2.156	0.030	6.76	8.59	9.80	1.261	0.11	0.45
18:3n-3	-3.50	3.55	1.001	0.003	-1.69	1.84	-1.90	2.401	0.35	0.10
20:0	-4.74	-5.18	3.218	0.90	-5.53	-3.73	-4.89	1.710	0.57	0.64
<i>cis</i> -9 20:1	4.04	4.25	0.168	0.30	3.86	3.67	4.21	0.316	0.37	0.002
Σ ≤16-carbon	2,123	2,671	139.6	0.018	3,174	3,400	3,631	280.4	0.003	0.029
Σ 18-carbon	-10.3	86.2	86.4	0.33	36.6	-17.7	33.7	64.48	0.66	0.50
Σ SFA	1,514	2,141	131.2	0.009	2,501	2,690	2,872	263.2	0.042	0.20
Σ MUFA	526	574	70.1	0.54	551	523	626	62.5	0.48	0.026
Σ PUFA	52.0	41.5	20.07	0.63	13.9	33.7	32.6	24.0	0.54	0.98
Σ FA										
mmol/d	2,087	2,753	120.5	0.006	3,060	3,237	3,516	334.5	0.031	0.08
g/d	402	579	24.1	0.002	620	651	696	71.7	0.15	0.26

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).⁶CLA = conjugated linoleic acid.

and *trans*-11,*cis*-13 CLA concentrations (Supplemental Table S1, available online at <http://www.journalofdairyscience.org/>), but had relatively minor effects on the abundance of odd- and branched-chain FA in

milk (Supplemental Table S3). Relative to UTS, FAS resulted in higher ($P < 0.05$) secretion of *trans*-11 18:1, *cis*-9,*trans*-11 CLA, 18:2n-6, and 18:3n-3 in milk (mean responses +35, +26, +13, and +10%, respectively; Fig-

Table 8. Effect of fresh grass or conserved forages on the proportion of medium-chain FA secreted in milk fat originating from synthesis de novo and desaturation in the mammary glands of lactating cows

FA	Experiment 1				Experiment 2				P-value ⁵	
	Grass	Hay	SEM ¹	P-value	Hay	UTS ²	FAS ³	SEM ⁴	Method	Extent
De novo synthesis, %										
12:0	98.0	97.8	0.903	0.77	98.5	97.3	98.6	0.52	0.42	0.10
14:0	93.9	93.5	1.383	0.80	95.3	96.4	96.1	0.80	0.21	0.73
16:0	53.6	77.5	3.596	0.003	79.2	79.1	79.8	2.00	0.91	0.79
Desaturation, %										
10:0	9.88	10.9	0.548	0.15	9.83	10.6	10.6	0.66	0.17	0.97
12:0	0.0027	0.0032	0.00024	0.11	0.0028	0.0028	0.0026	0.00011	0.61	0.31
14:0	8.31	9.55	0.411	0.040	8.40	8.16	7.66	0.479	0.40	0.45
16:0	3.94	4.18	0.330	0.51	3.78	3.49	3.41	0.205	0.07	0.65
18:0	55.1	68.5	4.02	0.029	53.1	48.9	43.6	4.02	0.22	0.39
<i>trans</i> -11 18:1	28.1	34.1	4.82	0.28	19.5	19.4	18.6	2.97	0.91	0.86
20:0	45.1	51.8	16.5	0.71	37.5	42.0	43.9	4.87	0.31	0.76

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).

ure 2), but had no effect ($P > 0.10$) on the output of 3,7,11,15-tetramethyl-16:0 in milk (data not presented).

DISCUSSION

Numerous studies have demonstrated that the conservation of forage alters milk fat composition (Dewhurst et al., 2006; Elgersma et al., 2006; Chilliard et al., 2007). However, in most cases, investigations of the influence of conservation method are confounded with differences in forage maturity that alter the distribution and abundance of lipid in forage crops (Dewhurst et al., 2006; Elgersma et al., 2006). Novel aspects of this experiment included measurements of plasma lipids, mammary lipid metabolism, and milk fat composition in cows fed diets based on fresh grass or conserved forages prepared simultaneously from the same grass leys. Furthermore, the same grass hay was fed in both experiments, allowing for indirect inferences on the effects of silages relative to fresh grass in the absence of differences in forage maturity or chop length. Nevertheless, the experimental design used in experiment 1 can be criticized due to the confounding effects of treatment with time. However, during established lactation, time-dependent effects on milk FA composition in cows fed the same diet are marginal (Roy et al., 2006; Mohammed et al., 2011) relative to the effects that can be expected due to forage conservation.

Milk Production

Fresh Grass Versus Grass Hay. Forage conservation method tends to have inconsistent effects on milk

production and composition, with differences often being related to the effects on energy intake, rumen metabolism, and nutrient partitioning (Huhtanen, 1998; Lock and Shingfield, 2004). Energy intake and milk yield did not differ between diets based on fresh grass or hay, whereas zero grazing resulted in a higher output of fat in milk. Differences in milk fat secretion were not accompanied by alterations in the mammary blood flow (11,320 and 11,323 L/d for fresh grass and hay, respectively), suggesting that the increase in mammary lipogenesis associated with zero grazing was related to a greater uptake of preformed FA across the mammary glands rather than differences in milk volume per se.

Grass Hay Versus Grass Silage. Grass silages increased energy intake, milk yield, and milk fat output compared with hay. Increased synthesis of FA de novo in the mammary glands for silages was associated with higher BHBA concentrations in the peripheral circulation, which is often the case in cows fed diets based on grass silage compared with grass hay (Shingfield et al., 2002b; Ferlay et al., 2006). Under the specified conditions of this experiment, higher concentrations of BHBA in arterial blood of cows fed silages compared with hay were accompanied by increases in the molar proportions of butyric acid in rumen VFA (Halmemies-Beauchet-Filleau et al., 2013).

Extent of Silage Fermentation. Restricting fermentation in silo increased milk fat yield, principally due to greater synthesis of 4:0 to 10:0 and 14:0 in the mammary glands. For grasses grown in Northern European climates, ensilage of grass using a formic acid-

Table 9. Effect of fresh grass or conserved forages on milk FA composition in lactating cows

FA, g/100 g of total FA	Experiment 2									
	Experiment 1									
	Grass	Hay	SEM ¹	<i>P</i> -value	Hay	UTS ²	FAS ³	SEM ⁴	<i>P</i> -value ⁵	
4:0	3.18	3.14	0.138	0.79	3.55	3.93	4.26	0.149	<0.001	0.008
6:0	1.90	2.05	0.120	0.30	2.25	2.35	2.48	0.063	0.005	0.025
8:0	1.19	1.31	0.087	0.25	1.36	1.37	1.43	0.054	0.13	0.10
10:0	2.76	3.27	0.189	0.06	3.26	3.09	3.09	0.203	0.10	0.99
<i>cis</i> -9 10:1	0.29	0.39	0.041	0.09	0.35	0.36	0.36	0.020	0.54	0.95
12:0	3.29	4.17	0.254	0.026	3.92	3.66	3.58	0.247	0.042	0.58
<i>cis</i> -9 12:1	0.09	0.13	0.015	0.05	0.11	0.10	0.09	0.007	0.16	0.31
<i>trans</i> -9 12:1	0.08	0.12	0.014	0.034	0.10	0.10	0.09	0.006	0.22	0.34
14:0	11.0	13.0	0.72	0.05	13.2	12.6	12.4	0.43	0.021	0.45
<i>cis</i> -9 14:1	1.03	1.38	0.131	0.06	1.20	1.16	1.07	0.051	0.20	0.26
<i>trans</i> -9 14:1	0.01	0.02	0.003	0.05	0.02	0.02	0.02	0.001	0.28	0.44
16:0	27.2	36.8	1.53	0.004	36.8	36.7	34.9	1.39	0.17	0.07
Σ <i>cis</i> -16:1	2.09	2.25	0.107	0.21	1.83	1.66	1.53	0.090	0.008	0.10
Σ <i>trans</i> -16:1	0.66	0.50	0.010	<0.001	0.48	0.43	0.44	0.021	0.027	0.79
<i>cis</i> -9, <i>cis</i> -12 16:2	0.007	0.005	0.0009	0.22	0.008	0.005	0.006	0.0008	0.026	0.86
18:0	9.57	6.21	0.603	0.006	8.25	9.05	9.82	0.363	0.004	0.038
<i>cis</i> -9 18:1	23.1	14.5	2.59	0.030	12.0	11.9	12.4	0.45	0.79	0.43
Σ <i>cis</i> -18:1	24.2	15.2	2.646	0.028	12.8	12.7	13.3	0.477	0.77	0.41
<i>trans</i> -11 18:1	1.11	0.58	0.075	0.003	0.73	0.76	0.92	0.079	0.06	0.025
Σ <i>trans</i> -18:1	2.51	1.55	0.070	<0.001	2.46	3.00	3.24	0.156	<0.001	0.038
18:2n-6	1.51	1.30	0.069	0.039	1.33	1.11	1.15	0.045	0.003	0.44
Σ 18:2 ⁶	2.06	1.63	0.062	0.003	1.63	1.54	1.66	0.067	0.68	0.20
<i>cis</i> -9, <i>trans</i> -11 CLA	0.55	0.32	0.079	0.041	0.27	0.28	0.32	0.025	0.16	0.11
Σ CLA	0.66	0.38	0.082	0.030	0.33	0.36	0.41	0.031	0.049	0.10
18:3n-3	0.66	0.50	0.013	<0.001	0.45	0.42	0.43	0.011	0.08	0.73
18:3n-6	0.02	0.03	0.002	0.028	0.02	0.01	0.01	0.001	<0.001	0.83
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.05	0.03	0.004	0.010	0.03	0.04	0.04	0.003	0.003	0.17
18:4n-3	0.010	0.013	0.002	0.14	0.01	0.01	0.01	0.001	0.11	0.43
20:0	0.12	0.13	0.005	0.51	0.15	0.13	0.12	0.016	0.33	0.45
3S,7R,11R,15-tetramethyl-16:0	0.03	0.02	0.003	0.06	0.03	0.07	0.07	0.009	0.004	0.88
Σ <i>cis</i> -20:1	0.22	0.22	0.012	0.39	0.19	0.17	0.18	0.005	0.024	0.45
20:2n-6	0.02	0.02	0.001	0.83	0.025	0.021	0.020	0.0016	0.002	0.24
20:3n-3	0.013	0.009	0.0009	0.007	0.01	0.01	0.01	0.0001	0.15	0.36
20:3n-6	0.068	0.060	0.0024	0.021	0.04	0.04	0.04	0.005	0.23	0.99
20:4n-3	0.06	0.05	0.006	0.18	0.04	0.03	0.03	0.004	0.007	0.34
20:4n-6	0.08	0.07	0.003	0.07	0.07	0.06	0.06	0.0014	<0.001	0.12
20:5n-3 ⁷	0.11	0.10	0.008	0.49	0.09	0.07	0.07	0.006	0.002	0.46
22:0	0.05	0.06	0.004	0.06	0.064	0.053	0.058	0.0019	0.004	0.06
Σ <i>cis</i> -22:1	0.03	0.04	0.001	0.018	0.032	0.028	0.029	0.0015	0.032	0.36
22:4n-3	0.007	0.005	0.0008	0.07	0.01	0.02	0.01	0.001	0.007	0.002
22:4n-6 ⁸	0.015	0.012	0.0014	0.08	0.01	0.01	0.03	0.010	0.43	0.35
22:5n-3	0.08	0.06	0.004	0.010	0.055	0.049	0.048	0.0019	0.008	0.55
22:6n-3	0.005	0.003	0.0007	0.07	0.001	0.001	0.002	0.0003	0.52	0.31
<i>cis</i> -15 24:1	0.015	0.017	0.0017	0.29	0.01	0.01	0.001	0.0022	0.68	0.44
26:0	0.02	0.03	0.004	0.08	0.03	0.03	0.03	0.004	0.29	0.66
28:0	0.004	0.004	0.0003	0.240	0.004	0.005	0.004	0.0007	0.83	0.87
Σ Unidentified	0.25	0.27	0.015	0.25	0.28	0.29	0.30	0.014	0.38	0.40
Σ SFA	63.8	74.2	2.38	0.012	76.8	76.5	75.4	0.71	0.37	0.33
Σ MUFA	32.0	22.5	2.40	0.017	20.1	20.3	20.9	0.61	0.54	0.50
Σ PUFA	3.94	3.00	0.068	<0.001	2.81	2.71	2.88	0.093	0.82	0.12
Σ FA, g/100 g of fat	94.1	94.0	0.06	0.07	94.1	93.9	93.6	0.118	<0.001	0.007

¹Standard error of the mean for n = 10 measurements; error degree of freedom = 4.²Untreated silage.³Silage ensiled using a formic acid-based additive.⁴Standard error of the mean for n = 15 measurements; error degree of freedom = 6.⁵Significance of effect due to forage conservation method (drying vs. ensiling; method) or extent of silage fermentation (extensively vs. restrictively fermented silage; extent).⁶Total 18:2 excluding isomers of conjugated linoleic acid (CLA).⁷Co-elutes with 24:0.⁸Co-elutes with 25:0.

Figure 1. Effect of fresh grass or grass hay on the secretion of specific FA in milk of lactating cows. Bars without a common letter (a and b) differ ($P < 0.05$). CLA = conjugated linoleic acid.

based additive typically increases milk protein yield through improvements in DMI and the energy efficiency of microbial protein synthesis in the rumen (Huhtanen 1998; Lock and Shingfield, 2004). In the present study, increases in milk protein yield for FAS compared with UTS were associated with higher ME intakes, in the absence of higher flows of microbial protein at the omasum (data not presented).

Concentration of Plasma Lipids and Metabolites

Plasma glucose concentrations were relatively constant within and between experiments, whereas BHBA and urea concentrations exhibited more variation, with all metabolites being in the range expected for cows offered diets containing relatively high proportions of forage (Miettinen and Huhtanen, 1997; Kay et al.,

Figure 2. Effect of grass hay and restrictively or extensively fermented grass silage on the secretion of specific FA in milk of lactating cows. Bars without a common letter (a-c) differ ($P < 0.05$). CLA = conjugated linoleic acid.

2005; Ferlay et al., 2006). Consistent with the known distribution of lipid fractions in bovine plasma (Offer et al., 2001; Tyburczy et al., 2008), circulating CE and PL represented the major FA repositories, whereas the concentrations of FA in TAG and NEFA were more than an order of magnitude lower. It is well established that the profile of lipoproteins in bovine plasma is characterized by extremely low concentrations of chylomicrons, very low-density lipoproteins, and total TAG, whereas more than 90% of FA in plasma are transported principally as CE and PL components of high-density lipoproteins (Palmquist, 1976; Moore and Christie, 1979).

Across all diets, concentrations of PL, CE, and total FA in arterial blood were associated with changes in the flow of total FA at the omasum (r^2 values 0.27 to 0.34), whereas no relation existed for arterial NEFA and TAG fractions. Earlier investigations in lactating cows also reported a close relationship between the amount of FA absorbed in the intestine and total FA content of venous blood in lactating cows, but not for individual NEFA, TAG, CE, or PL fractions (Glasser et al., 2007). Similarly, dietary oilseed supplements were found to progressively increase CE and PL concentrations in the peripheral circulation of growing cattle, but have no influence on plasma TAG or apolipoprotein B (Scislowski et al., 2005). These findings highlight that the regulation of circulating lipid fractions is not solely dependent on the absorption of FA, but involves the co-ordinate and highly regulated catabolism and synthesis of FA in body tissues, and the overall balance between the entry and removal of lipids from the circulatory system (Christie, 1981; Bauchart, 1993).

Fresh Grass Versus Grass Hay. Zero grazing resulted in higher concentrations of PL and TAG in arterial blood, increases that were of a similar magnitude to numerical differences in the flow of total FA at the omasum (437 and 365 g/d for fresh grass and hay, respectively; Halmemies-Beauchet-Filleau et al., 2013), suggesting that changes in the supply of medium- and long-chain FA may have contributed to the alterations in the concentration of circulating lipid fractions associated with forage conservation method. Even though all lipid fractions in blood are influenced by changes in FA supply within a few days, changes in the concentrations of TAG and NEFA are far more transitory compared with PL and CE fractions (Moore and Christie, 1979). In lactating cows, an interval of 45 d or more may be required for plasma PL and CE concentration responses to increases in FA supply to reach a plateau (Scislowski et al., 2005). It therefore follows that forage conservation may have a greater influence on the concentration and composition of plasma CE and PL fractions when diets are fed over an interval longer than the 14-d experimental periods implemented in the pres-

ent study. Furthermore, the much slower turnover of FA transported in the blood within CE and PL fractions serves to highlight the importance of previous nutritional status in attempts to define the relation between FA supply and plasma lipid content and composition.

Relative differences in arterial NEFA concentrations in cows fed fresh grass or hay were much larger compared with the differences in total FA at the omasum (Halmemies-Beauchet-Filleau et al., 2013). Several studies have reported that grazing results in higher plasma NEFA concentrations compared with conserved forages, differences that have been attributed to lower circulating insulin concentrations (Agenäs et al., 2002; Kay et al., 2005). However, circulating NEFA concentrations are also elevated during periods of nutrient restriction in lactating cows as a consequence of more extensive lipolysis and lowered lipogenesis in adipose (Chilliard et al., 2000). Intakes of ME were similar for diets based on fresh grass and hay, whereas zero grazing resulted in a 9.8 MJ/d increase in milk energy secretion, differences that were only partially compensated for by +2.9 and +3.5 percentage unit improvements in total-tract OM and NDF digestibility, respectively (Halmemies-Beauchet-Filleau et al., 2013). As a result, fresh grass resulted in a much lower, albeit slightly positive calculated energy balance compared with hay. Even though experimental periods were relatively short, the differences in energy secretion in milk, calculated energy balance, and higher uptakes of 16:0 + *cis*-9 16:1 and 18:0 + *cis*-9 18:1 across the mammary glands relative to the flow of these FA at the omasum suggest that alterations in the metabolism of lipids in peripheral tissues also contributed to the effects of forage conservation on mammary lipid metabolism and milk FA composition. Direct comparisons of FA uptake across the mammary glands with the flow of total FA at the omasum (mean +199 and +6 g/d for fresh grass and hay, respectively), indicate that medium- and long-chain FA used for the synthesis of milk fat also originated from body tissues, with the apparent contribution being much greater during zero grazing. More extensive mobilization of adipose also offers an explanation for the elevated concentrations of PL, TAG, and NEFA in arterial blood for fresh grass compared with hay.

Grass Hay Versus Grass Silage. Compared with hay, grass silages resulted in higher concentrations of PL and TAG in arterial blood that were accompanied by higher flows of total FA at the omasum (422, 551, and 587 g/d, for hay, UTS, and FAS, respectively; Halmemies-Beauchet-Filleau et al., 2013), with the implication that alterations in the amount of FA available for absorption may contribute to the influence of conservation method on plasma lipid fractions. However, drying rather than ensiling of grass had no influence

on plasma NEFA or glucose concentrations, which are often similar in lactating cows fed grass hay and grass silage (Shingfield et al., 2002b; Ferlay et al., 2006).

Extent of Silage Fermentation. Restricting fermentation in silo increased NEFA concentrations in the peripheral circulation, whereas in earlier reports, methods used for the ensilage of a mixture of timothy and meadow fescue had little an influence on arterial TAG or NEFA concentrations of lactating cows (Miettinen and Huhtanen, 1997). Diets based on restrictively fermented silage typically supply lower amounts of gluconeogenic precursors than extensively fermented grass silages (Huhtanen, 1998), whereas differences in the extent of fermentation in silo in this study had no influence on plasma glucose concentrations.

FA Composition of Plasma Lipids

The amount and relative abundance of FA is known to differ between isolated plasma lipid fractions in ruminants (Christie, 1981; Bauchart, 1993). Plasma TAG and NEFA fractions contained relatively high proportions of 16:0 and 18:0, and *cis*-9 18:1, 16:0, 18:0, *cis*-9 18:1, and 18:2n-6 were abundant in PL, whereas 18:2n-6 and 18:3n-3 were the major constituents of CE fractions, a distribution of FA in plasma lipids typical for lactating cows (Offer et al., 2001; Tyburczy et al., 2008). Across both experiments, differences in the relative proportions of *trans* 18:1 isomers in arterial lipid fractions were associated with changes in the flow at the omasum, consistent with previous reports examining the relation between FA supply and plasma lipid concentrations in lactating cows (Glasser et al., 2007). Comparison of the flow at the omasum and absolute concentrations in arterial blood indicated that the majority of *trans*-11 18:1 was transported in PL and TAG fractions, whereas CE was apparently the major repository for *cis*-9,*trans*-11 CLA. Recovery of ¹³C-labeled *trans*-11 18:1 administered at the duodenum also indicated that a substantial proportion of absorbed *trans*-11 18:1 was sequestered into plasma PL fractions in lactating cows (Mosley et al., 2006).

On all diets, 18:2n-6 and 18:3n-3 represented, on average, 39 and 11 g/100 g of total FA in arterial blood, respectively, but only 3.8 and 1.7 g/100 g of FA at the omasum. No significant associations were identified between 18:2n-6 and 18:3n-3 at the omasum and enrichment in lipid fractions or total concentrations in arterial blood, other than the amount of 18:3n-3 transported in TAG fractions. These findings reflect both the limited influence of treatments on the availability of 18:2n-6 and 18:3n-3 for absorption (Hallemies-Beauchet-Filleau et al., 2013), as well as the sparing of essential FA for vital biological functions and possible

remodeling of FA components of plasma lipid fractions in the liver (Noble, 1984; Christie et al., 1986; Chilliard et al., 2007).

Concentrations of 16:0, 18:0, and *cis*-9 18:1 in arterial blood for all cows were associated with changes in the flow of these FA at the omasum. However, significant positive intercepts for these relationships indicate that dietary intake and synthesis of FA *de novo* by rumen bacteria are not the sole determinants of these FA in arterial blood of lactating cows in positive calculated energy balance. Other factors including selective retention, *de novo* synthesis, elongation, desaturation, deposition, and mobilization of these FA in several tissues including adipocytes and enterocytes (Chilliard et al., 2007; Glasser et al., 2007; Shingfield et al., 2013), as well as differences in the relative entry and removal rate from the circulation may also influence plasma 16:0, 18:0, and *cis*-9 18:1 concentrations.

Plasma CE were devoid of 3,7,11,15-tetramethyl-16:0, whereas limited amounts of both diastereomers were incorporated into other lipid fractions, with PL being the major repository in arterial blood, which is in agreement with much earlier reports (Lough, 1977). Diastereomers of 2,6,10,14-tetramethyl-15:0 were recovered exclusively in TAG with a SRR/RRR ratio similar to that of 3,7,11,15-tetramethyl-16:0, suggesting that 2,6,10,14-tetramethyl-15:0 is formed during α -oxidation of 3,7,11,15-tetramethyl-16:0 in the bovine liver, as is known to occur in humans (Verhoeven et al., 1997).

Fresh Grass Versus Grass Hay. Fresh grass increased the abundance of 18:2n-6 in TAG and 18:3n-3 in TAG and CE compared with hay, despite similar flows of 18:2n-6 and 18:3n-3 at the omasum (Hallemies-Beauchet-Filleau et al., 2013). Changes in the availability of 18:2n-6 and 18:3n-3 are known to rapidly influence the composition of TAG and NEFA in plasma, whereas incorporation into PL and CE occurs at a lower rate, although enrichment in these fractions persists over a longer period (Christie, 1981; Noble, 1984). Apparent transfer of 18:2n-6 and 18:3n-3 from the omasum into milk was numerically higher for cows fed fresh grass (89 and 71%, respectively) compared with hay (corresponding values 74 and 64%), which, along with more extensive total-tract OM digestion (Hallemies-Beauchet-Filleau et al., 2013), may indicate higher bioavailability of PUFA for diets based on fresh grass than hay. Based on responses to postruminal infusions determined in several experiments, ca. 50% of 18:2n-6 or 18:3n-3 administered is recovered in milk (Shingfield et al., 2013). It is possible that release of PUFA into the peripheral circulation due to mobilization of tissue lipids may also explain, or at least contribute to the relatively high apparent transfer of PUFA into milk in the present study, particularly during zero grazing.

In spite of a higher flow at the omasum, enrichment of *trans*-11 18:1 in all arterial lipid fractions was similar in cows fed fresh grass and hay, whereas absolute concentrations of *trans*-11 18:1 transported in arterial blood were 65% higher during zero grazing (0.99 vs. 0.60 mg/dL, for fresh grass and hay, respectively). Several studies have reported higher enrichment of *trans*-11 18:1 in total plasma lipids of lactating cows during grazing compared with diets based on conserved forages (Kay et al., 2005; Mohammed et al., 2009). Current data indicated that increases in supply resulted in the enrichment of *trans*-11 18:1 in PL, TAG, and NEFA fractions, with PL, and to a lesser extent TAG, being the main repositories in lactating cows (Mosley et al., 2006; Tyburczy et al., 2008). In contrast, dietary supplements of fish oil have been reported to increase *trans*-11 18:1 concentrations of CE in bovine plasma (Offer et al., 2001), but no evidence existed from the present or earlier investigations (Mosley et al., 2006; Tyburczy et al., 2008) to corroborate these findings.

Relative proportions of *cis*-9 18:1 and 16:0 in arterial NEFA fractions were 158 and 38% higher in cows fed fresh grass compared with hay. When considered in conjunction with an overall 169% increase in arterial NEFA concentrations and higher uptake of 16:0 + *cis*-9 16:1 and 18:0 + *cis*-9 18:1 across the mammary glands relative to flow at the omasum, this tends to suggest that mobilization of body fat was a significant source of FA entering the peripheral circulation during zero grazing in cows with a near-zero calculated energy balance.

Differences in the relative abundance of diastereomers of 3,7,11,15-tetramethyl-16:0 in plasma PL, TAG, or NEFA between fresh grass and hay were relatively minor. However, absolute concentrations in plasma NEFA fractions were 211% higher during zero grazing, which could account for the higher secretion of 3,7,11,15-tetramethyl-16:0 diastereomers in milk from fresh grass compared with hay. More extensive uptake across the mammary glands also appears to explain the higher enrichment of 3,7,11,15-tetramethyl-16:0 in milk from pasture or during zero grazing compared with conserved forages (Leiber et al., 2005; Baars et al., 2012).

Grass Hay Versus Grass Silage and Extent of Silage Fermentation. Composition of plasma lipid fractions were similar in cows fed grass hay or grass silages, which is in broad agreement with minimal changes in the flow of total FA at the omasum (Halmemies-Beauchet-Filleau et al., 2013). However, incorporation of 3,7,11,15-tetramethyl-16:0 diastereomers in plasma PL, TAG, and NEFA fractions were higher for cows fed silage compared with hay that reflected the differences in the amount of these FA at the omasum (Halmemies-Beauchet-Filleau et al., 2013). Appearance of 3,7,11,15-tetramethyl-16:0 diastereomers originate

from the sequential reduction and oxidation of the phytol moiety of chlorophyll a and b released during microbial digestion of cell walls (Schröder and Vetter, 2011). Current measurements are consistent with ensiling rather than extensive wilting and drying of grass increasing the supply of 3,7,11,15-tetramethyl-16:0 diastereomers available for absorption in lactating cows.

Mammary Lipid Metabolism

Mammary FA Uptake. Application of the Fick principle to estimate blood flow and uptake of substrates across the mammary glands has the implicit assumption that steady state conditions are reached, whereby blood flow, arterial concentrations, and rate of uptake remain constant (Cant et al., 1993). The technique also assumes that Phe is not metabolized within the mammary glands further than the hydroxylation to Tyr and is reliant on the collection of blood samples that are representative of arterial supply and venous drainage of the mammary glands. Even though the stoichiometric transfer of Phe + Tyr uptake to milk protein appears to hold true, a recent meta-analysis highlighted that the analysis of plasma samples composited over sampling times to estimate arteriovenous differences for Phe and Tyr may introduce errors in the estimation of mammary plasma flow (Lapierre et al., 2012). Despite these limitations and potential sources of error, the technique does allow for quantitative estimates of mammary FA uptake, which, when used in conjunction with the secretion of FA in milk, provides an insight into mammary lipid metabolism.

Measurements of arteriovenous differences and uptake of FA across the mammary glands indicated that, irrespective of forage conservation method, TAG in arterial blood was the major source of preformed FA for milk fat synthesis. Extensive evidence exists from measurements of arteriovenous differences, mammary gland perfusions, and administration of radioactively labeled lipids that the ruminant mammary gland obtains the FA required for the assembly of milk lipids by the action of mammary lipoprotein lipase on the TAG fraction of chylomicrons and very low-density lipoproteins (Palmquist, 1976; Moore and Christie, 1979). Across both experiments, the concentration of TAG or TAG plus NEFA in arterial blood (range 4.3–10.6 and 1.6–11.7 mg/dL for TAG and NEFA, respectively) were correlated with mammary arteriovenous differences (*r* values of +0.74 and +0.91, respectively). The magnitude of these associations are similar to those when postruminal infusions have been used to increase FA supply (Enjalbert et al., 1998; Chilliard et al., 2000). Although the mechanisms involved in the transport of FA from the circulation into mammary secretory cells

have not been fully elucidated, arterial concentrations of specific FA in TAG, and to a lesser extent NEFA, were major determinants of uptake across the mammary glands, with the exception of 18:2n-6. Associations between relative concentrations in arterial CE and PL fractions and the uptake of individual long-chain FA across the mammary glands were much weaker, which may be related to the low affinity of CE and PL as substrates for lipoprotein lipase (Offer et al., 2001). Overall, current data reinforce the necessity to fractionate lipids in arterial blood for assessing the availability of preformed FA for milk fat synthesis (Palmquist, 1976).

Extraction of TAG or TAG + NEFA and the uptake across the mammary glands could quantitatively account for the secretion of 18:3n-3 in milk, but explained only 70 and 84% of 18:2n-6 output in milk from fresh grass and conserved forages, respectively. In cows receiving postruminal infusions of plant oils, mammary uptake of 18:2n-6 from arterial TAG and NEFA fractions has, on average, amounted to 56% of 18:2n-6 output in milk (Enjalbert et al., 1998). The mammary glands of ruminants are capable of extracting 18-, 20- and 22-carbon unsaturated FA of plasma CE and PL fractions in ruminants (Christie et al., 1986; Thivierge et al., 1998), which may explain the apparent shortfall between the uptake and secretion of 18:2n-6. Although extraction of PL and CE from arterial blood across the mammary glands was limited, small differences were detected between cows fed hay or grass silages. Given that the concentrations of PUFA in CE and PL are several orders of magnitude higher relative to TAG and NEFA, even minimal extraction of these fractions would constitute a significant contribution to total mammary PUFA uptake. However, accurate determinations of PUFA processed by the mammary glands that originate from limited hydrolysis of arterial CE and PL fractions may well exceed the sensitivity of the arteriovenous technique, as well as measurements of the FA composition of different PL species in arterial and venous blood. In other respects, it is conceivable that limited amounts of 18:2n-6 may well be synthesized endogenously in the bovine mammary glands, based on reports that *trans*-12 18:1 is desaturated to 18:2n-6 during incubations with microsomes prepared from the liver of rats (Mahfouz et al., 1980).

Mammary FA Balance. Measurements of arteriovenous differences indicated that de novo synthesis accounted for all 4- to 10-carbon FA secreted in milk fat and 98 and 95% of 12:0 and 14:0 secretion in milk, respectively, with no significant variation across treatments. In contrast, when compared with fresh grass, forage conservation method altered the relative proportions of 16:0 in milk derived from the peripheral circulation or synthesized in the mammary glands. For cows

fed hay or grass silage, de novo synthesis accounted for ca. 79% of 16:0 secreted in milk, whereas the contribution was much lower (ca. 53%) during zero grazing. Most accounts indicate that between 39 and 81% (Palmquist et al., 1969; Bickerstaffe et al., 1974; Enjalbert et al., 1998) of 16:0 in bovine milk fat is synthesized de novo. Although it is generally considered that ca. 50% of 16:0 in milk is synthesized de novo (Chilliard et al., 2000), the current investigation indicated that the synthesis of 16:0 in the mammary glands can be much higher in cows fed diets containing 2 to 3% FA, with a genetic potential for producing milk with a relatively high fat content.

A lower synthesis of SFA de novo in the mammary glands on fresh grass was associated with higher uptake of 18-, 20-, and 22-carbon unsaturated FA. Increases in the availability of long-chain FA are known to inhibit the activity of acetyl-CoA carboxylase in the ruminant mammary glands (Chilliard et al., 2000). For all diets, the balance of total 18-carbon FA across the mammary glands were close to zero (mean = $+26 \pm 5.8$ mmol/d), confirming negligible oxidation of long-chain FA in the mammary glands of ruminants fed according to energy requirements (Annison et al., 1968).

Mammary FA Desaturation. Between 44 and 69% of 18:0 extracted from the peripheral circulation was desaturated in the mammary glands, values that are within the range of previous estimates of between 47 and 78% in lactating cows (Bickerstaffe et al., 1974; Enjalbert et al., 1998; Mosley and McGuire, 2007). Desaturation of 14:0 and 18:0 in the mammary glands was higher in cows offered hay in experiment 1 compared with other treatments, but the reasons for this are not obvious. Overall, desaturation in the mammary glands contributed, on average, 97, 85, 72, and 78% of *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1, and *cis*-9,*trans*-11 CLA secretion in milk, respectively. Estimates in the present study are in agreement with values determined based on milk fat secretion responses to postruminal infusions of substrates or sterculic oil to inhibit stearoyl-CoA desaturase activity (Palmquist et al., 2005; Mosley et al., 2006; Mosley and McGuire, 2007), or extensive evaluation of the relations between FA at the duodenum and secretion in milk (Glasser et al., 2008).

Milk FA Composition and Secretion

Fresh Grass Versus Grass Hay. Compared with hay, fresh grass decreased the secretion of 16:0 synthesized de novo, which can be explained by a higher uptake of preformed FA across the mammary glands, which also offers an explanation for milk from pasture containing higher concentrations of 18-carbon FA compared with diets based on conserved forages (Dewhurst

et al., 2006; Chilliard et al., 2007; Mohammed et al., 2009). Similarly, more-extensive extraction of TAG containing higher proportions of PUFA in arterial plasma, combined with a net uptake of NEFA accounted for the increased abundance and secretion of 18:2n-6 and 18:3n-3 during zero grazing. Enrichment of 18:3n-3 in milk is typically higher from pasture compared with conserved forages, whereas the effects on 18:2n-6 are inconsistent (Dewhurst et al., 2006; Chilliard et al., 2007; Mohammed et al., 2009). However, comparisons of milk 18:2n-6 content between fresh and conserved forages are often confounded with differences in the forage-to-concentrate ratio of the diet, forage species, or forage maturity (Dewhurst et al., 2006; Mohammed et al., 2009). Overall, the efficiency of transfer of 18:2n-6 (13.7 to 14.4%) and 18:3n-3 (4.8 to 8.5%) from the diet into milk was higher for hay than fresh grass, which was related to lowered lipolysis and biohydrogenation in the rumen (Halmemies-Beauchet-Filleau et al., 2013), as well as possible differences in the digestion and absorption of FA in the small intestine.

Higher intakes of unsaturated FA combined with more extensive accumulation of 18-carbon biohydrogenation intermediates in the rumen during zero grazing (Halmemies-Beauchet-Filleau et al., 2013) resulted in higher enrichment of *cis* 18:1 (Δ 9–16), *trans* 18:1 (Δ 6–16), Δ 11,13 CLA, Δ 13,15 CLA, Δ 9,12 18:2, Δ 11,15 18:2, and *cis*-9,*trans*-11,*cis*-15 18:3 in milk compared with grass hay. Increases in the concentration and output of *cis*-9 18:1 in milk from fresh grass were associated with higher extraction and uptake of *cis*-9 18:1 across the mammary glands, at least part of which may be related to more extensive mobilization of tissue adipose.

Secretion of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA in milk was higher from diets based on fresh grass than hay as a consequence of increased uptake of *trans*-11 18:1 across the mammary glands. Numerous studies have reported higher concentrations of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA in milk from fresh than conserved forages (Dewhurst et al., 2006; Chilliard et al., 2007; Mohammed et al., 2009), and higher concentrations at pasture compared with zero grazing (Leiber et al., 2005; Chilliard et al., 2007; Mohammed et al., 2009). Increases in *trans*-6+7+8 18:1 at the omasum (Halmemies-Beauchet-Filleau et al., 2013) and uptake across the mammary glands also offers an explanation for the higher enrichment of *trans*-7,*cis*-9 CLA in milk on fresh grass compared with hay.

Grass Hay Versus Grass Silages. Diets based on silages resulted in marginally lower milk fat 18:2n-6 and 18:3n-3 concentrations relative to hay, but the secretion of these PUFA did not differ across treatments despite the higher flow of 18:3n-3 at the omasum in cows fed silage (Halmemies-Beauchet-Filleau et al.,

2013). In earlier studies, ensiling decreased (Shingfield et al., 2005) or resulted in similar (Ferlay et al., 2006) concentrations of 18:2n-6 and 18:3n-3 in milk compared with hay. Higher 18-carbon PUFA intakes and greater accumulation of biohydrogenation intermediates in the rumen (Halmemies-Beauchet-Filleau et al., 2013) for silages than for hay were associated with higher concentrations of 18:0, *trans* 18:1 (Δ 12–16), *trans*-11,*cis*-15, Δ 9,12 18:2, Δ 12,15 18:2, Δ 13,15 CLA, and *cis*-9,*trans*-11,*cis*-15 18:3 in milk fat in the absence of altered extraction rate or the extent of FA desaturation in the mammary glands.

Silages resulted in higher concentrations and secretion of 3S,7R,11R,15-tetramethyl-16:0 in milk relative to hay, which is in agreement with several recent reports (Baars et al., 2012; Schröder et al., 2012). In the absence of direct measurements, differences in the enrichment of 3S,7R,11R,15-tetramethyl-16:0 in plasma lipid fractions and milk fat suggest that ensiling results in much lower losses of chlorophyll and (or) phytol compared with the conservation of grass by extensive wilting.

Extent of Silage Fermentation. Restricting fermentation in silo had relatively minor effects on milk fat composition. Use of a formic acid-based additive increased 18:2n-6 and 18:3n-3 secretion by 13 and 10%, respectively, consistent with numerical, but not significant, increases in the uptake of PUFA across the mammary glands. Secretion of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA in milk fat was also increased for FAS compared with UTS, confirming earlier reports (Shingfield et al., 2005). Restricting fermentation in silo resulted in higher milk fat 18:0, *trans*-9,*cis*-12 18:2, *trans*-11,*cis*-15 18:2, and *trans*-11,*cis*-13 CLA concentrations, which were accompanied by higher flows of these FA at the omasum (Halmemies-Beauchet-Filleau et al., 2013), whereas increased enrichment of 18-carbon biohydrogenation intermediates appears to reflect the marginally higher intake of PUFA for FAS than UTS, rather than alterations in lipolysis or biohydrogenation in the rumen (Halmemies-Beauchet-Filleau et al., 2013).

CONCLUSIONS

Feeding diets based on fresh grass, grass hay, or grass silage altered the concentration and composition of plasma lipid fractions. Concentrations of TAG and PL in arterial blood were higher in cows fed fresh grass or grass silages compared with hay. Zero grazing tended to lower positive calculated energy balance, increased NEFA concentrations in arterial blood, and resulted in a higher relative abundance of 18:2n-6 and 18:3n-3 in plasma TAG. Fresh grass resulted in higher *cis*-9 18:1, *cis*-9,*trans*-11 CLA, 18:2n-6, and 18:3n-3, and lower 16:0 and total SFA concentrations in milk fat compared

with hay, changes that were associated with increased uptake of preformed FA and lowered synthesis of FA de novo in the mammary glands. Conservation of grass by ensiling rather than drying increased milk fat synthesis, but had relatively minor effects on the composition of plasma lipids, mammary FA uptake, and milk FA composition. Differences in milk FA composition with fresh grass compared with conserved forages were accompanied by alterations in the composition of plasma lipid fractions and uptake of FA across the mammary glands, in the absence of altered stearoyl-CoA desaturase activity.

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