J. Dairy Sci. 106:2347–2360
https://doi.org/10.3168/jds.2022-22327

Effects of replacement of barley with oats on milk fatty acid composition in dairy cows fed grass silage-based diets

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ABSTRACT

This study consists of milk fatty acid (FA) data collected during 2 in vivo experiments. For this study, 8 cows from each experiment were included in a replicated 4 × 4 Latin square design. At the start of experiment 1 (Exp1) cows were at (mean ± standard deviation) 87 ± 34.6 d in milk, 625 ± 85.0 kg of body weight, and 32.1 ± 4.17 kg/d milk yield and at the start of experiment 2 (Exp2) cows were at 74 ± 18.2 d in milk, 629 ± 87.0 kg of body weight, and 37.0 ± 3.2 kg/d milk yield. In Exp1, we examined the effects of gradual replacement of barley with hulled oats (oats with hulls) on milk FA composition. The basal diet was grass silage and rapeseed meal (58 and 10% of diet DM, respectively), and the 4 grain supplements were formulated so that barley was gradually replaced by hulled oats at levels of 0, 33, 67, and 100% on dry matter basis. In Exp2, we examined (1) the effects of replacing barley with both hulled and dehulled oats (oats without hulls) and (2) the effects of gradual replacement of hulled oats with dehulled oats on milk FA composition. The basal diet was grass silage and rapeseed meal (60 and 10% of diet DM, respectively), and the 4 pelleted experimental concentrates were barley, hulled oats, a 50:50 mixture of hulled and dehulled oats, and dehulled oats on dry matter basis. In Exp1, gradual replacement of barley with hulled oats decreased relative proportions of 14:0, 16:0, and total saturated FA (SFA) in milk fat linearly, whereas proportions of 18:0, 18:1, total monounsaturated FA, and total *cis* unsaturated FA increased linearly. Transfer efficiency of total C18 decreased linearly when barley was replaced by hulled oats in Exp1. In Exp2, relative proportions of 14:0, 16:0, and total SFA were lower, whereas proportions of 18:0, 18:1, monounsaturated FA, and *cis* unsaturated FA were higher in milk from cows fed the oat diets than in milk from cows fed the barley diet. Moreover, in Exp2, gradual replacement of hulled oats with dehulled oats slightly decreased the relative proportion of 14:0 in milk fat but did not affect the proportions of 16:0, 18:0, 18:1, total SFA, monounsaturated FA, *trans* FA, or polyunsaturated FA. In Exp2, transfer efficiency of total C18 was lower when cows were fed the oat diets than when fed the barley diet and decreased linearly when hulled oats were replaced with dehulled oats. Predictions of daily CH_4 emissions (g/d) using the on-farm available variables energy-corrected milk yield and body weight were not markedly improved by including milk concentrations of individual milk FA in prediction equations. In conclusion, replacement of barley with oats as a concentrate supplement for dairy cows fed a grass silage-based diet could offer a practical strategy to change the FA composition of milk to be more in accordance with international dietary guidelines regarding consumption of SFA.

Key words: nutrition, milk quality, concentrate supplement, transfer efficiency, methane emission

INTRODUCTION

Intake of SFA has long been linked to increased risk of developing cardiovascular disease (**CVD**) in humans (Kromhout et al., 1995; Zong et al., 2016). Although there is emerging evidence suggesting that intake of SFA alone is not a cause of CVD (Virtanen et al., 2014; de Souza et al., 2015), most public health policies still recommend a restricted dietary intake of these fatty acids (**FA**). The World Health Organization and the Food and Agriculture Organization of the United Nations recommends less than 10% of the daily total energy intake to be in the form of SFA, and that SFA should be replaced with PUFA, and to some extent with MUFA (FAO, 2010; WHO, 2020). Most European countries exceed the recommended limit for SFA intake with SFA representing up to 16% of total energy intake in some countries (Eilander et al., 2015). Because dairy products are one of the major sources for SFA in the

Received May 23, 2022.
Accepted October 24, 2022.
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diet (17–41% of total SFA intake; Eilander et al., 2015), modulating milk FA composition could contribute to keeping SFA intake below the recommended limit without changing consumer behavior.

Milk FA composition can be modulated through different dietary strategies, and it has been the focus of substantial research efforts during many years (Kliem and Shingfield, 2016). Studies showed that supplementing dairy cow diets with plant oils (Bayat et al., 2018), oilseeds (Muñoz et al., 2019), and fish and sunflower oil (Shingfield et al., 2006) decreased milk fat concentrations of SFA while increasing MUFA and PUFA. Although efficient, plant oil and oilseed supplements can increase farmer's feed costs. An alternative to oil supplementation could be to increase the fat content of the basal diet by replacing one ingredient with another.

In the Nordic countries, dairy cows are commonly fed barley (*Hordeum vulgare*) as a grain supplement. Oats (*Avena sativa*) used to be a popular ingredient in dairy cow diets but its use has declined during recent years, mostly due to higher tabulated energy and MP values of barley compared with oats (NorFor, 2022). However, as oats have a higher crude fat content than barley (NorFor, 2022), replacing barley with oats would increase the fat content of the diet. In addition, the FA composition differs between oat and barley grain. Oats have slightly lower relative concentrations of C16:0, C18:0, C18:2, and C18:3 than barley (Liu, 2011). However, the relative concentration of C18:1 is approximately 3 times higher in oats than in barley (Liu, 2011). Because most of the crude fat in oat grain is contained in the groat, oats with the hulls removed (dehulled oats) have a higher crude fat content than oats with hulls (hulled oats; 52.8–63.0 g/kg DM vs. 75.7–93.0 g/kg DM; Biel et al., 2014; LUKE, 2022). Several studies showed that replacement of barley with hulled oats decreased the concentration of total SFA and increased the concentration of total UFA in milk fat, while also maintaining or even increasing milk yields (Heikkilä et al., 1988; Ekern et al., 2003; Vanhatalo et al., 2006). In the previously mentioned studies, milk FA concentrations were not analyzed in detail, and to the best of our knowledge, no study has investigated how replacement of hulled oats with dehulled oats affects milk FA composition.

Adjustment of diets and subsequent mitigation of CH_4 emissions from dairy cows also requires that the emissions can be accurately measured on the farm. Measurements of enteric CH_4 emissions calls for expensive equipment and increases the workload of the farmer. To avoid this, enteric CH_4 emissions could potentially be predicted by using variables that are already available on farm, such as ECM, BW, and milk composition. There is a close connection between the metabolic pathways producing VFA in the rumen and enteric CH_4 production (Dijkstra et al., 2011; Bougouin et al., 2019). Because VFA are used as precursors for de novo FA synthesis in the mammary gland it has been suggested that milk FA composition could be used to predict CH_4 emissions from dairy cows (Dijkstra et al., 2011; Bougouin et al., 2019).

The primary objective of this study was to investigate the effect of replacing barley with hulled oats and dehulled oats and the effect of replacing hulled oats with dehulled oats in the diet of dairy cows fed a grass silage-based diet on milk FA composition. For this purpose, we used milk samples and data collected during 2 in vivo experiments: experiment 1 (**Exp1**; Ramin et al., 2021) and experiment 2 (**Exp2**; Fant et al., 2021). For Exp1, we hypothesized that gradual replacement of barley with hulled oats would decrease milk fat concentrations of SFA and increase concentrations of UFA because of the higher crude fat content in oats than in barley. For Exp2, we hypothesized that (1) replacing barley with different types of oats (hulled, dehulled, mix of hulled and dehulled) would decrease milk SFA and increase milk UFA and that, because dehulled oats has an even higher crude fat content than hulled oats, (2) replacing hulled oats with dehulled oats would lead to similar changes as replacing barley with oats. The secondary objective of this study was to examine whether the predictions of daily CH_4 emissions (g/d) using the on-farm available variables ECM and BW could be improved by including milk concentrations of individual FA.

MATERIALS AND METHODS

This study consists of 2 in vivo experiments which were conducted in 2016 (Exp1) and in 2018 (Exp2) at Röbäcksdalen experimental farm of the Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences in Umeå, Sweden (63°45′N; 20°17′E). These 2 experiments are published, and more details are found in Ramin et al. (2021) for Exp1 and Fant et al. (2021) for Exp2. All experimental procedures were approved by the Swedish Ethics Committee on Animal Research (Umeå, Sweden) and were in accordance with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

Regarding terminology, the term "hulled oats" will be used when referring to oats with hulls, whereas the term "dehulled oats" will be used when referring to oats with the hulls removed through dehulling.

Experimental Design, Animals, and Diets

Sixteen lactating Nordic Red dairy cows were included in each experiment and the experimental design was a replicated 4 × 4 Latin square design including 4 dietary treatments, 4 blocks, and 4 periods. The cows were blocked according to parity and milk yield and randomly allocated to treatment within block. However, out of these 16, only 8 cows from 2 blocks were sampled for milk FA composition and included in this study. In Exp1, periods were 21 d each, with 11 d of adaptation and 10 d of sampling and data collection. At the start of the experiment, cows were at 87 ± 34.6 (mean ± SD) DIM, 625 ± 85.0 kg of BW, and 32.1 ± 4.17 kg/d milk yield. The cows were fed grass silage (58% of diet DM), rapeseed meal (10% of diet DM), minerals (2% of diet DM), and 1 of 4 grain supplements (30% of diet DM, forage-to-concentrate ratio 58:42 on DM basis). The grass silage was prepared from a primary growth perennial ley of timothy (*Phleum pratense*). The experimental grain supplements comprised 100% barley, 67% barley and 33% hulled oats, 33% barley and 67% hulled oats, and 100% hulled oats.

In Exp2, periods were 28 d each with 18 d of adaptation and 10 d of sampling and data collection. At the start of the experiment, cows were at 74 ± 18.2 (mean ± SD) DIM, 629 ± 87.0 kg of BW, and 37.0 ± 3.2 kg/d milk yield. The cows were fed grass silage and 1 of 4 pelleted experimental concentrates (forage-to-concentrate ratio 60:40 on DM basis). The grass silage was prepared from a primary growth perennial ley of timothy (*Phleum pratense*). The concentrates were composed of the experimental grain component (78.8%), rapeseed meal (18.0%), $CaCO_3$ (1.6%), NaCl (1.0%), MgO (0.4%), and a premix (0.2%), and were produced by Raisioagro Oy (Ylivieska, Finland). The 4 experimental grain components comprised barley, hulled oats, an oat mixture consisting of hulled oats and dehulled oats 50:50 on DM basis, and dehulled oats.

In both experiments, the cows were housed in an insulated free stall barn with free access to water and salt blocks throughout the experiment. Cows were fed ad libitum a TMR that was delivered to the feed troughs 4 times per day (0300, 0800, 1400, and 1800 h) by an automatic feeding wagon. Feed intake was recorded individually throughout the experiments by Roughage Intake Control feeders (Insentec B.V.). Cows were milked twice daily at 0600 and 1600 h.

Measurements, Sampling, and Chemical Analyses

Recordings of feed intake, BW, milk yield, as well as detailed collection procedures for feed and milk samples in Exp1 and Exp2 have been previously reported in Ramin et al. (2021) and Fant et al. (2021), respectively. In Exp1, grass silage samples were collected twice a week and concentrate samples once a week during the last 2 wk of each period. In Exp2, grass silage samples were collected 3 times and concentrate samples once during each sampling period. Samples of feed ingredients were determined for DM, ash, CP, crude fat, starch, NDF, and indigestible NDF (**iNDF**) concentration.

Milk samples were determined for fat, protein, and lactose concentrations. All analytical methods are described in Ramin et al. (2021) and Fant et al. (2021). In addition, samples for determination of milk FA composition were collected from 8 cows during 4 consecutive milking times, starting from evening milking on d 19 until morning milking on d 21 in Exp1 and from d 26 until d 28 in Exp2. Samples were pooled within cow and a subsample of 50 mL was collected and stored in Falcon tubes (50 mL) at −18°C until further analysis.

Emissions of CH_4 and CO_2, and consumption of O_2, were recorded throughout the experiments by the GreenFeed system (C-Lock Inc.) described by Huhtanen et al. (2015), but only data collected during the last 10 d of each period were used for statistical analysis. The calibrations of the GreenFeed system, gas recordings, and calculations are described in Ramin et al. (2021). The cows had free access to the GreenFeed but were only allowed to visit at a minimum of 5-h intervals. During each visit, cows received a maximum of 8 drops of concentrate every 40 s and each drop contained 50 g of a commercial concentrate. In Exp1, the commercial concentrate consisted of barley, Distillers dried grains, wheat bran, oats, beet molasses, beet pulp, $CaCO_3$, NaCl, and MgO, whereas in Exp2, it consisted of rapeseed meal, triticale, wheat bran, wheat, beet molasses, beet pulp, $CaCO_3$, NaCl, and MgO. The intake of the commercial concentrate was accounted for in the calculations of total DMI.

Fatty acid methyl esters of lipid in all feed ingredients (silage, barley, oats, rapeseed meal, and commercial concentrate used in GreenFeed in Exp1, and silage, experimental concentrates, and commercial concentrate used in GreenFeed in Exp2) and milk samples were prepared as described by Shingfield et al. (2003). Total FAME profile was determined by GC (6890N, Agilent Technologies). The gas chromatograph was equipped with a CP-Sil 88 column (100 m × 0.25 mm i.d., 0.2-μm film thickness, Agilent Technologies) and flame ionization detector, and a temperature gradient program was used (Shingfield et al., 2003). Tritridecanoin (T-3882, Sigma-Aldrich) was used as an internal standard in calculating the FA content in feed samples. Peaks were identified by comparing retention times with authentic FAME standards (Larodan Fine Chemicals AB, Malmö, Sweden; Nu-Chek Prep Inc., Elysian, MN; Sigma-Aldrich) and verified by GC-MS (6890 and 5973, Agilent Technologies) in positive electron ionization mode for FAME and corresponding 4,4-dimethyloxazoline derivatives (Shingfield et al., 2003).

Calculations

Concentration of neutral detergent solubles was calculated as a difference between OM and NDF. Potentially digestible NDF concentration was calculated as a difference between NDF and iNDF concentration. The output of milk FA (g/d) was calculated based on the assumption that all fat in milk is present as triacylglycerol according to

$$\text{FA output of X (g/d)} = \text{X (g/100 g of FA)} \times 0.9446/100 \times \text{Milk fat yield (g/d)},$$

where X is a specific milk FA and 0.9446 is the ratio of FA in milk triacylglycerol. Transfer efficiency was calculated as milk FA output (g/d)/FA intake (g/d) × 100. Energy-corrected milk was calculated as described by Sjaunja et al. (1990) according to

$$\text{ECM (kg/d)} = \text{Milk yield (kg/d)} \times [38.3 \times \text{Fat (g/kg)} + 24.2 \times \text{Protein (g/kg)} + 16.54 \times \text{Lactose (g/kg)} + 20.7]/3{,}140.$$

Statistical Analysis

For both experiments, the experimental data were subjected to ANOVA using the MIXED procedure of SAS and analyzed as a replicated 4 × 4 Latin square design according to the following statistical model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_l(S_k) + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable and μ is the population mean, T_i is the fixed effect of diet i, P_j is the fixed effect of period j, S_k is the fixed effect of square k, $C_l(S_k)$ is the random effect of cow l within square k, and ε_{ijkl} is the random residual error. Interactions $T_i \times S_k$ and $P_j \times S_k$ were excluded from the final model because they were nonsignificant ($P > 0.10$). In Exp1, we used linear, quadratic, and cubic contrasts to test the effects of gradual replacement of barley with oats in the diet. In Exp2, 1 observation was excluded from period 4 due to an unexplained drop in milk yield, which resulted in an unbalanced data set and the use of Kenward-Roger approximation for estimating degrees of freedom. Presented mean values are least squares means obtained by the LSMEANS statement in SAS. The barley diet was compared with the overall mean of the hulled oat, oat mixture, and dehulled oat diet, and linear and quadratic contrasts were used to investigate the effects of gradual replacement of hulled oats with dehulled oats. Differences were declared significant if $P \leq 0.05$, highly significant if $P \leq 0.01$, and a tendency toward significant was declared if $0.05 < P \leq 0.10$.

Relationships between intake and output of FA were investigated for the FA groups total C16:0, total C16, total C18:0, total C18:1, total C18:2, total C18:3, and total C18. For this purpose, data from Exp1 and Exp2 were combined, and we applied a linear regression using the REG procedure in SAS according to the following statistical model:

$$Y_i = B_0 + B_1X_{1i} + \varepsilon_i,$$

where Y_i is output of FA in milk, B_0 is the intercept, B_1 is the slope for X_1, X_{1i} is intake of FA, and ε_i is the error term. We also investigated whether the predictions of daily CH_4 emissions (g/d) using the on-farm available variables ECM and BW could be improved by including milk proportions of specific FA. This analysis included all the milk FA presented in Tables 3 and 4, as well as 15:0, *anteiso*-15:0, *iso*-15:0, and 17:0. Combined data from Exp1 and Exp2 were applied to a mixed model linear regression using the MIXED procedure in SAS according to the following statistical model:

$$Y_{ijk} = B_0 + B_1X_{1i} + B_2X_{2j} + B_3X_{3k} + \varepsilon_{ijk},$$

where Y_{ijk} is daily CH_4 emissions; B_0 is the intercept; X_{1i}, X_{2j}, and X_{3k} are ECM, BW, and milk FA proportions; B_1, B_2, and B_3 are the corresponding slopes; and ε_{ijk} is the error term. The models included 3 random statements: a random intercept with SUBJECT = Period (Exp), a random intercept with SUBJECT = Exp, and a random intercept with SUBJECT = Diet (Exp) with TYPE = VC as covariance structure in all 3 statements.

RESULTS

Feed and FA Intake

Ingredient composition of formulated diets, chemical composition of dietary ingredients, and chemical composition of experimental diets for Exp1 and Exp2 are reported in Ramin et al. (2021) and Fant et al. (2021), respectively.

The DMI and FA intake for the 8 cows that were sampled for milk FA in each experiment are presented in Table 1 and Table 2 for Exp1 and Exp2, respectively. In Exp1, gradual replacement of barley with hulled oats increased the intake of total FA linearly ($P < 0.01$). The greatest linear increase for individual FA was for intake of *cis*-9 18:1, which increased from 78 to 174 g/d when barley was replaced with hulled oats ($P < 0.01$).

Table 1. Effects of gradual replacement of barley with hulled oats in experiment 1 on DM and fatty acid intake (n = 32) in lactating cows

Intake	Diet[1]				SEM	*P*-value[2]	
	O0	O33	O67	O100		Linear	Quadratic
DM, kg/d	21.6	22.4	21.9	21.8	0.42	0.95	0.22
Fatty acids, g/d							
12:0	8.73	9.43	9.72	9.46	0.354	0.05	0.08
14:0	5.27	5.89	6.23	6.41	0.151	<0.01	0.08
16:0	103	116	123	131	2.2	<0.01	0.15
16:1 *cis*-9	2.76	3.03	3.13	3.28	0.056	<0.01	0.21
16:1 *trans*-3	3.45	3.55	3.48	3.46	0.068	0.90	0.28
18:0	8.8	10.6	11.7	12.9	0.20	<0.01	0.14
18:1 *cis*-9	78	113	143	174	2.5	<0.01	0.28
18:1 *cis*-11	15.2	16.6	17.1	17.8	0.31	<0.01	0.19
18:2n-6	158	178	189	202	3.3	<0.01	0.22
18:3n-3	144	147	143	141	2.8	0.15	0.28
20:0	3.21	3.40	3.42	3.48	0.063	<0.01	0.21
20:1 *cis*-11	2.06	2.58	2.97	3.38	0.051	<0.01	0.22
22:0	4.14	4.23	4.11	4.04	0.080	0.17	0.25
24:0	3.02	3.14	3.10	3.10	0.059	0.35	0.24
Σ SFA	151	168	177	186	3.1	<0.01	0.13
Σ MUFA	105	144	175	208	3.0	<0.01	0.25
Σ PUFA	304	327	334	345	6.2	<0.01	0.24
Total	560	639	686	739	11.9	<0.01	0.18

[1]O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.
[2]Cubic effects were not significant ($P > 0.32$).

In Exp2, total FA intake was higher ($P < 0.01$) on the oat diets than on the barley diet. The greatest differences in intake of individual FA between the barley diet and the oat diets were for *cis*-9 18:1 and 18:2-n6, for which the intakes were 97 and 75 g/d higher ($P < 0.01$) when cows were fed the oat diets than when fed the barley diet. When the hulled oats diet was gradually replaced by dehulled oats in Exp2, intake of total FA increased linearly ($P < 0.01$). The greatest increase in individual FA intake was for 18:2-n6, which increased by 89 g/d ($P < 0.01$), whereas the intake of *cis*-9 18:1 increased by 54 g/d ($P < 0.01$).

Fatty Acid Composition of Milk

In Exp1, gradual replacement of barley with hulled oats decreased ($P < 0.01$) the proportions of 10:0, 12:0, 14:0, and 16:0 in milk fat linearly (Table 3). The proportion of 18:0 increased linearly ($P < 0.01$) but the proportions of total SFA and SFA + *trans* FA (**TFA**) decreased linearly ($P < 0.01$) with increasing inclusion of hulled oats in the diet. Increasing inclusion of hulled oats in the diet also linearly increased ($P < 0.01$) the proportion of total 18:1 in milk fat, mostly due to the linear increase ($P < 0.01$) in the proportion of *cis*-9 18:1. The proportions of total TFA, MUFA, and *cis* UFA increased linearly with increasing proportion of hulled oats in the diet ($P < 0.01$), whereas total PUFA decreased slightly ($P < 0.01$).

In Exp2, the oat diets resulted in lower ($P < 0.01$) proportions of 8:0, 10:0, 12:0, 14:0, and 16:0 but higher ($P < 0.01$) proportions of 18:0 and 20:0 in milk fat compared with the barley diet (Table 4). Proportions of total SFA and SFA + TFA were lower ($P < 0.01$) on the oat diets than on the barley diet, although the sum of TFA was higher ($P < 0.01$) on the oat diets. Feeding the oat diets led to a higher ($P < 0.01$) proportion of *cis*-9 18:1 in milk fat, as well as a higher ($P < 0.01$) proportion of total 18:1. Overall, feeding oats instead of barley led to higher ($P < 0.01$) relative proportions of total MUFA and *cis* UFA in milk fat but total PUFA was not affected ($P = 0.39$).

In Exp2, gradual replacement of hulled oats with dehulled oats increased the relative milk fat proportions of 4:0 ($P = 0.04$), total *cis* 18:2 ($P < 0.01$), total 18:2 ($P = 0.02$), and 18:2*n*-6 ($P < 0.01$) and decreased the proportion of 14:0 ($P = 0.01$) linearly. Increasing inclusion of dehulled oats had a quadratic effect on milk fat proportions of total *cis* 16:1 ($P = 0.04$) and 20:0 ($P = 0.03$).

Relationships Between FA Intake and Milk FA Output

Intake and output in milk fat (g/d) as well as mean apparent transfer efficiency of total C18 for Exp1 and Exp2 are presented in Figure 1. Mean apparent transfer efficiency of total C18 decreased linearly ($P < 0.01$) with increasing inclusion of hulled oats in Exp1. In

Table 2. Effects of replacing barley with hulled and dehulled oats and gradual replacement of hulled oats with dehulled oats in experiment 2 on DM and fatty acid intake (n = 31) in lactating cows

	Diet[1]					P-value[2]		
Intake	Barley	Hulled oats	Oat mixture	Dehulled oats	SEM	Barley vs. oats	Linear	Quadratic
DM, kg/d	23.6	24.4	23.7	23.3	0.80	0.71	0.10	0.81
Fatty acids, g/d								
12:0	4.57	4.68	4.59	4.54	0.230	0.72	0.19	0.84
14:0	4.53	4.66	4.64	4.66	0.155	0.12	0.98	0.80
16:0	129	142	144	147	4.5	<0.01	0.11	0.82
16:1 *cis*-9	3.33	3.54	3.66	3.82	0.117	<0.01	0.00	0.84
16:1 *trans*-3	3.76	3.88	3.77	3.71	0.130	0.74	0.10	0.81
18:0	11.0	14.4	14.6	14.9	0.43	<0.01	0.11	0.83
18:1 *cis*-9	68.4	138	165	192	4.4	<0.01	<0.01	0.99
18:1 *cis*-11	13.0	14.0	15.2	16.4	0.46	<0.01	<0.01	0.86
18:2n-6	104	135	179	224	5.2	<0.01	<0.01	0.99
18:3n-3	156	162	160	159	5.4	0.24	0.43	0.82
20:0	4.31	4.63	4.46	4.34	0.148	0.08	0.02	0.81
20:1 *cis*-11	1.78	2.86	3.19	3.53	0.090	<0.01	<0.01	0.91
22:0	5.29	5.46	5.27	5.13	0.180	0.98	0.03	0.81
24:0	3.75	3.87	3.81	3.79	0.128	0.36	0.41	0.82
Σ SFA	181	200	201	204	6.3	<0.01	0.42	0.82
Σ MUFA	94.6	169	196	224	5.33	<0.01	<0.01	0.96
Σ PUFA	262	300	341	384	10.5	<0.01	<0.01	0.90
Total	538	669	738	812	21.9	<0.01	<0.01	0.88

[1]Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2]Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were barley vs. oats = barley vs. hulled oats, oat mixture, and dehulled oats, Linear = linear effect of replacement of hulled oats with dehulled oats, Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

Exp2, mean apparent transfer efficiency was lower ($P < 0.01$) on the oat diets than on the barley diet and it decreased linearly ($P < 0.01$) with increasing inclusion of dehulled oats in the diet. In addition, in Exp2 the output of total C18 in milk fat was numerically ($P = 0.39$) higher than the intake of total C18 on the barley diet.

The output of C16:0 and total C16 FA (Figure 2), and the output of C18:0, C18:1, C18:2, and C18:3 (Figure 3) in milk fat were all positively related ($P < 0.01$) to the intake of the corresponding FA groups. All the investigated FA groups expressed weak relationships between intake and output in milk fat. The strongest relationship was expressed between intake and output of the C18:1 group ($R^2 = 0.28$), and the weakest relationship was expressed between intake and output of the C18:3 group ($R^2 = 0.13$). The mean response in output of the C18:1 group to increased C18:1 intake was 0.42 g/d for every 1 g/d increase in C18:1 intake. The greatest mean response to increased intake was for the C18:0 group, where the output of C18:0 increased by 4.55 g/d for every 1 g/d increase in C18:0 intake.

Relationships Between Milk FA Composition and Ruminal CH_4 Emissions

The bivariate model with ECM and BW as predictors explained 65% of the variation in daily CH_4 emissions (Table 5). Both ECM and BW were positively related to daily CH_4 emissions. Out of all the specific FA tested, only 4:0, *cis*-9 12:1, *cis*-9 14:1, and *cis*-11 18:1 had significant slopes ($P < 0.05$) and improved the R^2 slightly when added separately to the model. Both 4:0 and *cis*-11 18:1 expressed negative relationships, whereas *cis*-9 12:1 and *cis*-9 14:1 expressed positive relationships with daily CH_4 emissions. The model including *cis*-9 14:1 had the lowest root mean square error (35.4). Inclusion of several of the significant FA in the same model did not improve predictions (data not shown).

DISCUSSION

The aims of this study was to investigate the effect of gradual replacement of barley with hulled oats in Exp1 and the effect of replacement of barley with both hulled and dehulled oats and replacement of hulled oats with dehulled oats in Exp2 on milk FA composition. Our hypothesis for Exp1 was that gradually replacing barley with hulled oats would decrease concentrations of SFA and increase concentrations of UFA in milk fat. Because the relative proportion of total SFA decreased and the relative proportion of total *cis* UFA and total MUFA in milk fat increased when barley was replaced by hulled oats, we accept our first hypothesis. Our first hypothesis for Exp2 was that milk fat concentrations

Table 3. Effects of gradual replacement of barley with hulled oats in experiment 1 on selected milk fatty acids (n = 32) in lactating cows

	Diet[1]					*P*-value[2]	
Fatty acid, g/100 g	O0	O33	O67	O100	SEM	Linear	Quadratic
4:0	3.19	3.15	3.24	3.24	0.041	0.02	0.36
6:0	2.22	2.17	2.22	2.20	0.049	0.90	0.59
8:0	1.45	1.43	1.45	1.42	0.049	0.29	0.86
10:0	3.75	3.64	3.61	3.48	0.162	0.01	0.83
10:1 *cis*-9	0.35	0.36	0.36	0.33	0.018	0.34	0.20
12:0	4.78	4.64	4.53	4.31	0.204	<0.01	0.70
12:1 *cis*-9	0.13	0.13	0.13	0.12	0.010	0.13	0.42
14:0	13.4	13.2	13.0	12.7	0.26	<0.01	0.68
14:1 *cis*-9	1.08	1.09	1.10	1.00	0.079	0.23	0.23
16:0	34.0	32.7	31.9	30.8	0.76	<0.01	0.71
Σ 16:1 *cis*	1.96	1.98	1.94	1.83	0.099	0.05	0.18
Σ 16:1 *trans*	0.36	0.37	0.36	0.37	0.007	0.34	0.73
Σ 16:1	2.32	2.34	2.30	2.20	0.097	0.07	0.17
18:0	8.2	8.8	9.4	10.3	0.47	<0.01	0.51
18:1 *cis*-9	13.0	14.2	14.7	15.7	0.22	<0.01	0.50
18:1 *cis*-11	0.52	0.54	0.53	0.55	0.009	0.08	0.44
18:1 *trans*-11	0.90	0.84	0.90	0.91	0.049	0.49	0.33
Σ 18:1 *cis*	13.9	15.1	15.6	16.7	0.23	<0.01	0.54
Σ 18:1 *trans*	3.28	3.39	3.63	3.78	0.073	<0.01	0.69
Σ 18:1	17.2	18.5	19.3	20.4	0.26	<0.01	0.61
18:2 *trans*-11,*cis*-15	0.14	0.14	0.13	0.13	0.011	0.05	0.73
18:2n-6[3]	1.03	1.04	0.96	0.98	0.034	<0.01	0.77
Σ 18:2 *cis*	1.15	1.15	1.08	1.10	0.035	0.01	0.40
Σ 18:2 *trans*	1.11	1.11	1.12	1.13	0.047	0.48	0.70
Σ 18:2[4]	1.77	1.78	1.72	1.75	0.036	0.10	0.55
CLA *cis*-9,*trans*-11	0.40	0.38	0.38	0.38	0.031	0.25	0.45
Σ CLA	0.50	0.48	0.48	0.48	0.034	0.31	0.55
18:3n-3[5]	0.42	0.42	0.40	0.40	0.016	0.01	0.79
20:0	0.16	0.16	0.17	0.18	0.006	<0.01	0.29
Σ 20:1 *cis*	0.12	0.12	0.13	0.13	0.004	<0.01	0.46
Σ 20:1 *trans*	0.05	0.04	0.05	0.05	0.002	0.37	0.05
Σ 20:1	0.16	0.17	0.17	0.18	0.004	<0.01	0.78
Σ Unidentified	0.30	0.26	0.28	0.26	0.0142	0.02	0.41
Σ *trans* fatty acids	5.07	5.19	5.43	5.58	0.094	<0.01	0.65
Σ SFA	74.5	73.2	72.5	71.6	0.37	<0.01	0.38
Σ SFA + *trans* fatty acids	79.6	78.4	78.0	77.2	0.33	<0.01	0.35
Σ MUFA	22.0	23.4	24.1	25.0	0.34	<0.01	0.32
Σ *cis* UFA	20.1	21.3	21.7	22.5	0.33	<0.01	0.35
Σ PUFA	3.22	3.22	3.10	3.13	0.052	<0.01	0.37
Σ n-6	0.23	0.23	0.21	0.21	0.007	<0.01	0.72
Σ n-3	0.61	0.62	0.58	0.59	0.022	0.01	0.88

[1]O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2]Cubic effects were not significant ($P > 0.39$).

[3]Co-elutes with *cis*-9,*cis*-15 18:2. Sum of 18:2 fatty acids excluding isomers of CLA.

[4]Sum of 18:2 fatty acids excluding isomers of CLA.

[5]Co-elutes with *cis*-11 20:1 and *trans*-16 20:1.

of SFA and UFA would be lower and higher, respectively, when cows are fed different types of oats (hulled, dehulled, mix of hulled and dehulled) than when cows are fed barley. As the average proportion of total SFA was lower and the average proportion of total *cis* UFA and total MUFA were higher in milk fat from cows fed the oat diets compared with the barley diet, we accept our first hypothesis for Exp2. Our second hypothesis for Exp2 was that gradually replacing hulled oats with dehulled oats would decrease milk fat concentrations of SFA and increase concentrations of UFA. As replacement of hulled oats with dehulled oats did not affect milk fat proportions of total SFA, *cis* UFA, or MUFA, we reject the second hypothesis for Exp2.

Fatty Acid Composition of Milk

In our study, the treatment mean proportion of SFA in milk fat varied between 71.6 (100% oats in grain supplement) and 74.5 g/100 g FA (100% barley in grain supplement) in Exp1 and between 70.9 and 73.8 g/100 g of FA in Exp2, which is within the normal variation of

Table 4. Effects of replacing barley with hulled and dehulled oats and gradual replacement of hulled oats with dehulled oats in experiment 2 on selected milk fatty acids (n = 31) in lactating cows

	Diet[1]					*P*-value[2]		
Fatty acid, g/100 g	Barley	Hulled oats	Oat mixture	Dehulled oats	SEM	Barley vs. oats	Linear	Quadratic
4:0	3.11	3.14	3.20	3.27	0.112	0.07	0.04	0.97
6:0	2.23	2.18	2.22	2.22	0.073	0.39	0.10	0.37
8:0	1.46	1.40	1.42	1.41	0.052	0.01	0.67	0.40
10:0	3.75	3.48	3.47	3.43	0.133	<0.01	0.50	0.75
10:1 *cis*-9	0.37	0.35	0.35	0.34	0.019	0.07	0.48	0.69
12:0	4.65	4.26	4.22	4.15	0.157	<0.01	0.29	0.86
12:1 *cis*-9	0.13	0.11	0.11	0.11	0.009	0.01	0.33	0.86
14:0	13.8	13.3	13.4	12.8	0.22	<0.01	0.01	0.05
14:1 *cis*-9	1.24	1.18	1.17	1.10	0.119	0.05	0.14	0.64
16:0	33.2	30.0	30.8	30.1	0.64	<0.01	0.92	0.13
Σ 16:1 *cis*	2.11	1.98	1.87	1.93	0.109	<0.01	0.17	0.04
Σ 16:1 *trans*	0.15	0.16	0.16	0.15	0.007	0.10	0.76	0.29
Σ 16:1	2.25	2.13	2.04	2.08	0.110	<0.01	0.18	0.08
18:0	7.96	9.77	9.69	10.1	0.375	<0.01	0.20	0.30
18:1 *cis*-9	14.5	17.1	16.6	17.4	0.44	<0.01	0.46	0.07
18:1 *cis*-11	0.54	0.53	0.49	0.53	0.034	0.35	0.96	0.21
18:1 *trans*-11	0.91	1.04	0.98	0.95	0.054	0.10	0.11	0.74
Σ 18:1 *cis*	15.4	18.1	17.5	18.4	0.47	<0.01	0.47	0.08
Σ 18:1 *trans*	2.16	2.53	2.57	2.54	0.076	<0.01	0.96	0.73
Σ 18:1	17.6	20.6	20.1	21.0	0.50	<0.01	0.51	0.13
18:2 *trans*-11,*cis*-15	0.12	0.13	0.11	0.11	0.009	0.40	0.03	0.64
18:2n-6[3]	1.29	1.19	1.25	1.35	0.040	0.44	<0.01	0.70
Σ 18:2 *cis*	1.32	1.22	1.29	1.38	0.041	0.57	<0.01	0.81
Σ 18:2 *trans*	1.12	1.21	1.15	1.14	0.067	0.32	0.28	0.63
Σ 18:2[4]	1.92	1.87	1.91	2.00	0.042	0.91	0.02	0.63
CLA *cis*-9,*trans*-11	0.43	0.48	0.46	0.44	0.039	0.26	0.26	0.90
Σ CLA	0.51	0.56	0.54	0.53	0.043	0.34	0.27	0.78
18:3n-3[5]	0.52	0.47	0.45	0.44	0.024	<0.01	0.09	0.96
20:0	0.13	0.15	0.14	0.15	0.005	<0.01	0.16	0.03
Σ 20:1 *cis*	0.17	0.19	0.18	0.18	0.005	<0.01	0.03	0.25
Σ 20:1 *trans*	0.02	0.03	0.03	0.03	0.003	0.39	1.00	0.86
Σ 20:1	0.19	0.22	0.21	0.21	0.006	<0.01	0.21	0.60
Σ Unidentified	0.20	0.18	0.19	0.19	0.007	0.19	0.71	0.71
Σ *trans* fatty acids	3.72	4.19	4.16	4.11	0.136	<0.01	0.64	0.96
Σ SFA	73.8	71.0	71.8	70.9	0.70	<0.01	0.78	0.13
Σ SFA + *trans* fatty acids	77.5	75.2	75.9	75.0	0.60	<0.01	0.61	0.06
Σ MUFA	22.5	25.4	24.7	25.5	0.64	<0.01	0.85	0.11
Σ *cis* UFA	22.2	24.6	23.9	24.8	0.60	<0.01	0.60	0.06
Σ PUFA	3.45	3.38	3.35	3.43	0.081	0.39	0.55	0.51
Σ n-6	0.19	0.19	0.18	0.19	0.011	0.19	0.29	0.07
Σ n-3	0.73	0.67	0.64	0.63	0.026	<0.01	0.06	0.65

[1]Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2]Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were Barley vs. oats = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of hulled oats with dehulled oats; Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

[3]Co-elutes with *cis*-9,*cis*-15 18:2.

[4]Sum of 18:2 fatty acids excluding isomers of CLA.

[5]Co-elutes with *trans*-15 20:1 and *trans*-16 20:1.

67.1 to 74.4 g of SFA/100 g of FA in Swedish milk collected during 2001 as reported by Lindmark Månsson (2008). The variation in treatment means proportion of milk fat MUFA in Exp1 (22.0–25.0 g/100 g of FA) and in Exp2 (22.5–25.5 g/100 g of FA) are also within the normal variation of 22.2 to 26.7 g of MUFA/100 g of FA in Swedish milk (Lindmark Månsson, 2008).

In agreement with the results of this study (Exp1 and Exp2), several earlier studies have reported lower relative proportions of SFA with chain length ≤16:0 (except for 4:0) in milk fat from cows fed oat concentrate than from cows fed barley concentrate. Heikkilä et al. (1988) reported decreased milk fat proportion of 16:0, whereas Martin and Thomas (1988) reported decreased milk fat proportions of 10:0, 12:0, 14:0, and 16:0 when barley was replaced by hulled oats. In addition, both Ekern et al. (2003) and Vanhatalo et al. (2006) reported lower proportions of 12:0, 14:0, and 16:0 in milk fat when barley was completely replaced by hulled oats on grass silage-based diets with approximately 50:50 forage-to-

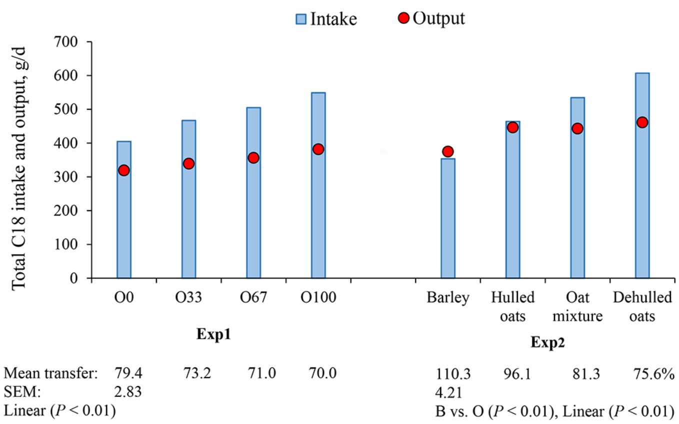

Figure 1. Intake (bars) and output (dots) of total C18 fatty acids in milk (g/d) and mean net transfer rates (%) from diet to milk in experiment 1 (Exp1) and experiment 2 (Exp2). O0 = 100% barley; O33 = 67% barley and 33% hulled oats; O67 = 33% barley and 67% hulled oats; O100 = 100% hulled oats; Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis. B vs. O = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of barley with oats (Exp1) and replacement of hulled oats with dehulled oats (Exp2). Linear effect on total C18 intake and output in Exp1 ($P < 0.01$), effect of B vs. O on total C18 intake and output in Exp2 ($P < 0.01$), Linear effect on total C18 intake in Exp2 ($P < 0.01$).

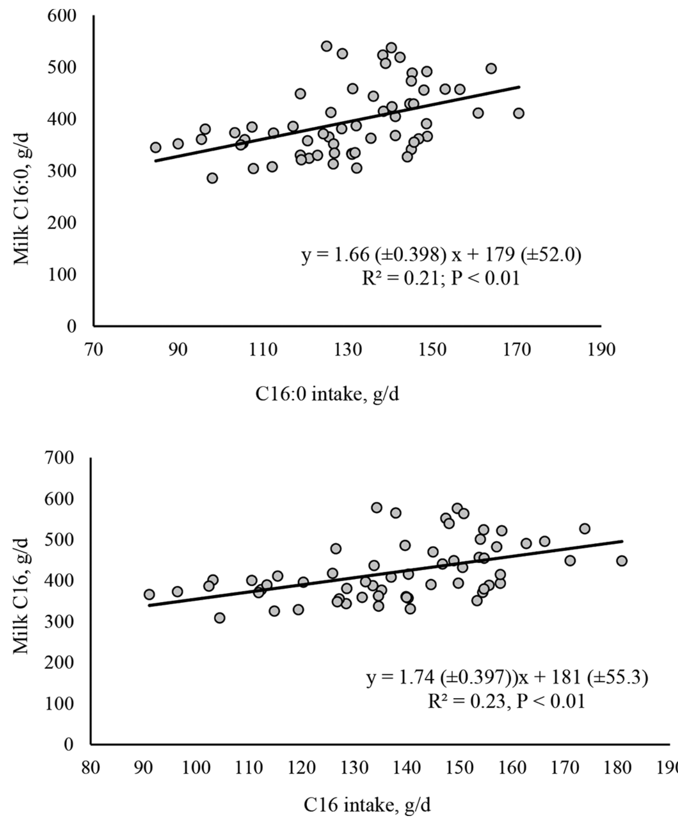

Figure 2. Relationships between intake of C16 fatty acid groups (g/d) and their output in milk (g/d) predicted by mixed model regression analysis with combined data from experiment 1 and experiment 2. The C16 intake group (lower panel) includes 16:0, *trans*-3 16:1, and *cis*-9 16:1; and the Milk C16 group (upper panel) includes 16:0, *iso* 16:0, *trans*-4 16:1, *trans*-5 16:1, *trans*-6 16:1, *trans*-7 16:1, *trans*-8 16:1, *trans*-9 16:1, *trans*-10 16:1, *trans*-11 16:1, *trans*-12 16:1, *trans*-13 16:1, *cis*-6 16:1, *cis*-7 16:1, *cis*-9 16:1, *cis*-10 16:1, *cis*-11 16:1, *cis*-12 16:1, *cis*-13 16:1, and *cis*-15 16:1.

concentrate ratio in the study by Ekern et al. (2003) and 60:40 in the study by Vanhatalo et al. (2006). In the study by Vanhatalo et al. (2006), barley and oats were the sole concentrates fed to the cows.

According to Palmquist (2006), 12:0, most of the 14:0, and about 50% of the 16:0 FA present in milk fat originate from de novo synthesis in the mammary gland (Palmquist, 2006). Although, based on intake and output of 16:0 in milk (Figure 2), de novo synthesis might have contributed with 60 to 70% of milk 16:0 depending on dietary treatment in our study. Studies show that de novo synthesis of milk FA can be inhibited by an increased supply of 16:0, and especially of C18 FA to the epithelial cells in the mammary gland (Souza and Williamson, 1993; Wright et al., 2002), thereby decreasing the concentrations of ≤16 carbon FA in milk fat. As intake of both 16:0 and C18 increased when barley was replaced by hulled oats (Exp1) and by hulled and dehulled oats (Exp2), reduced de novo synthesis could explain lower concentrations of 12:0, 14:0, and 16:0 FA in milk fat on oat diets.

Increases in milk fat proportion of 18:1 when barley is replaced by oats were also reported in previous studies (Heikkilä et al., 1988; Martin and Thomas, 1988; Ekern et al., 2003; Vanhatalo et al., 2006), although the magnitude of these increases varied depending on basal diet, forage-to-concentrate ratio, and the additional amount of fat provided by oats. In the study by Vanhatalo et al. (2006), with similar basal diet and forage-to-concentrate ratio as in our study, the proportion of 18:1 in milk fat increased by 6.1 g/100 g FA, compared with increases of 3.3 and 3.0 g/100 g FA in Exp1 and Exp2, respectively. The incremental level of fat supplied by oats in the study of Vanhatalo et al. (2006) (12 g/kg DM) was similar to the levels in our study (9.3 g/kg DM in Exp1 and 11.7 g/kg DM in Exp2), and thus cannot explain the smaller increase of total 18:1 observed in our study. The FA composition of the barley and oat varieties used in the study by Vanhatalo et al. (2006) was not analyzed but could explain the greater increase of milk 18:1 in their study compared with our study.

The previous studies have not analyzed specific isomers of 18:1 in milk fat but based on the proportional increases of 18:1 and *cis*-9 18:1 in milk fat in our study (Exp1 and Exp2), the increase in 18:1 is mostly due to the increase in the *cis*-9 18:1 isomer, although there was a slight increase in total *trans* 18:1 as well. As there is not de novo synthesis of C18 or longer chain

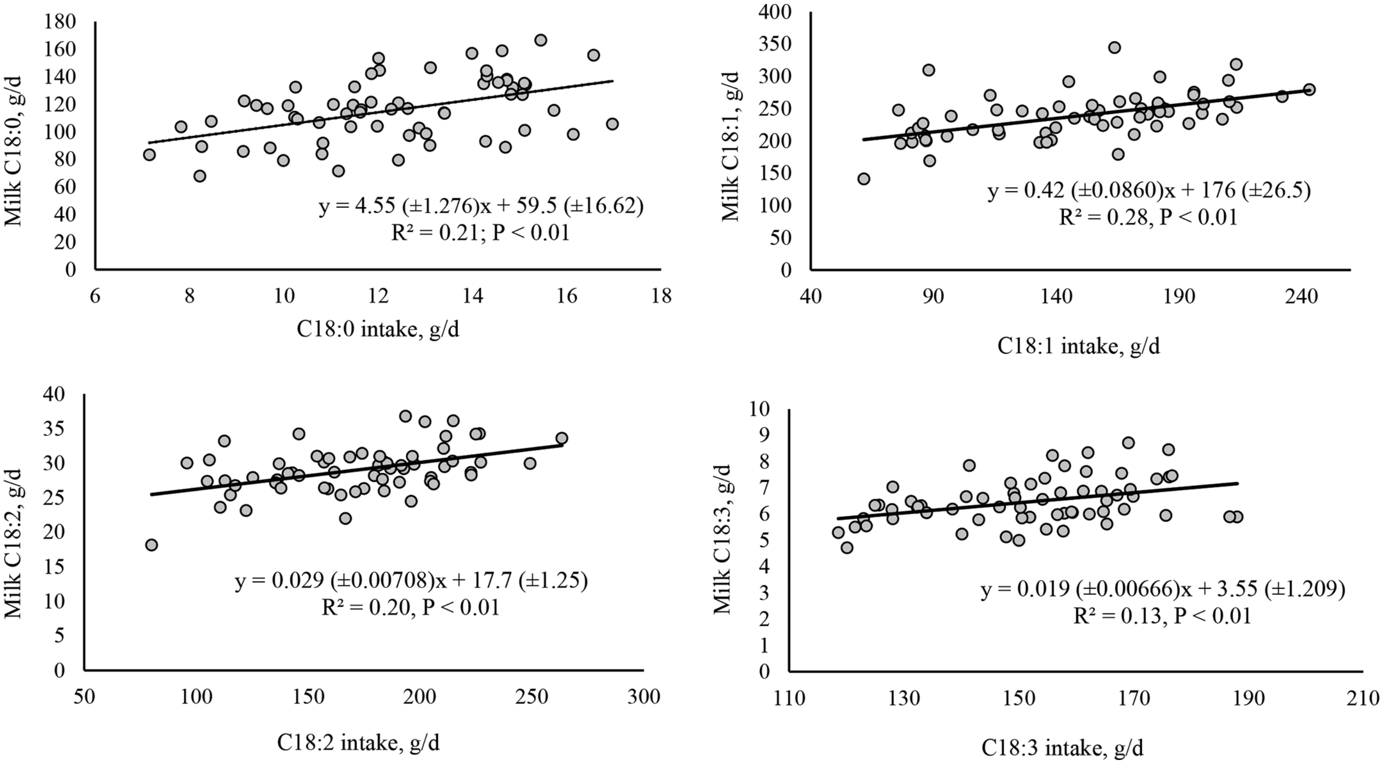

Figure 3. Relationships between intake of selected C18 fatty acid groups (g/d) and their output in milk (g/d) predicted by mixed model regression analysis with combined data from experiment 1 and experiment 2.

FA in the mammary gland, all the *cis*-9 18:1 present in milk fat originate from the diet, directly or indirectly through the action of mammary Δ^9-desaturase on 18:0, or from the mobilization of body fat reserves. The effects reported in the literature of replacing barley with oats on milk proportions of 18:2 and 18:3 have been inconsistent and small or absent, which agrees with the results of our study. Martin and Thomas (1988) reported slightly decreased concentration of milk 18:2 and no effect on milk 18:3, whereas Vanhatalo et al. (2006) reported no effect on 18:2 nor 18:3.

The lower proportions of 12:0, 14:0, 16:0, and total SFA in milk fat observed with oat diets in our study can be regarded as favorable changes for human nutrition and to be in line with the guidelines provided by FAO (2010) and WHO (2020). It has been shown that consumption of 12:0 and 16:0 compared with 18:0 raises low-density lipoprotein cholesterol levels in blood, which is generally considered a risk factor for CVD (Temme et al., 1996). Therefore, the increase in the proportion of 18:0 in milk fat from cows fed the oat diets is of less concern. Increases in the proportion of total UFA in milk fat occurs simultaneously with an increase in milk TFA (Shingfield et al., 2006; Bayat et al., 2018; Muñoz et al., 2019; our study), which may be explained by the biohydrogenation of feed UFA in the

Table 5. Mixed regression analysis for prediction of daily methane emissions (g/d) using combined data from experiment 1 and experiment 2

X_1	X_2	X_3	Intercept	SE	$Slope_1$	SE	$Slope_2$	SE	$Slope_3$	SE	RMSE[1]	Adjusted R^2
ECM[2]	BW		−1	48.9	5.65	1.173	0.44	0.045			37.5	0.65
ECM	BW	C4:0[3]	178	95.1	5.78	1.132	0.39	0.048	−48.1	22.39	36.1	0.68
ECM	BW	C12:1 *cis*-9	−34	49.1	6.01	1.128	0.39	0.045	435	174.3	35.2	0.68
ECM	BW	C14:1 *cis*-9	21	46.6	4.67	1.153	0.37	0.050	49.8	19.50	35.4	0.69
ECM	BW	C18:1 *cis*-11	149	76.4	4.91	1.182	0.42	0.045	−216	88.1	36.9	0.67

[1]RMSE = root mean square error.

[2]Calculated as described by Sjaunja et al. (1990): ECM (kg/d) = Milk yield (kg/d) × [38.3 × Fat (g/kg) + 24.2 × Protein (g/kg) + 16.54 × Lactose (g/kg) + 20.7]/3,140.

[3]Milk fatty acids (g/100 g of fatty acid).

rumen. Although dietary TFA are reported to increase the risk of CVD (Mensink et al., 2003), one should also consider that the different TFA isomers may differ in their effects on the human body. For example, the most common isomers of TFA present in ruminant-derived food, mainly *trans*-11 18:1, do not seem to be associated with increased CVD risk (Gebauer et al., 2011). In addition, a meta-analysis of 29 prospective cohort studies reported that consumption of dairy products and milk did not increase the risk of developing CVD (Guo et al., 2017), which even questions the need for modulating milk FA composition at all.

The effects of replacement of barley with oats on milk FA composition observed in this study are similar to the results obtained when supplementing dairy cow diets with plant oils and oil seeds, although the magnitude of the effect was smaller due to the smaller incremental levels of fat (9.3 and 11.7 g of crude fat/kg of DM in Exp1 and Exp2, respectively). For example, supplementing dairy cow diets with 50 g of lipid per kilogram of diet DM in the form of rapeseed oil, safflower oil, and linseed oil decreased the proportions of 12:0, 14:0, and 16:0, and increased the proportions of 18:0 and 18:1 in milk fat more substantially than in the present study, which also led to much lower proportions of total SFA and higher proportions of total MUFA and total TFA in milk (Bayat et al., 2018). Although the changes in milk FA composition observed in our study were relatively small compared with oil supplementation, replacement of barley with oats in dairy cow diets could offer a more practicable and cost-effective way of improving the nutritional quality of cow milk in regions where oats grow well.

Contrary to our hypothesis, replacement of hulled oats with dehulled oats in the diet did not decrease relative proportion of total SFA in milk fat (Exp2). Even though most of the oil in oat grain is contained in the groat, dehulled oat displays slightly lower concentrations (% of total FA) of C16:0 and total SFA but slightly higher concentrations of C18:2, C18:3, and total PUFA than hulled oats, whereas the concentration of total MUFA is similar (Biel et al., 2014). In our study, the concentrations of C18:1, C18:2, total MUFA, and total PUFA were higher in the concentrate containing dehulled oats than in the concentrate containing hulled oats, whereas concentrations of C16:0, C18:3 and total SFA were similar. The incremental supply of 16:0 when the hulled oats diet was replaced with dehulled oats was only numerical. In contrast to the supply of 16:0, the supply of C18 increased by approximately 150 g/d when the hulled oats diet was replaced with dehulled oats, compared with 180 g/d increase when barley was replaced with both hulled and dehulled oats in Exp2. As mentioned earlier, increased supply of C18 has an inhibiting effect on de novo FA synthesis in the mammary gland (Souza and Williamson, 1993; Wright et al., 2002). Although intake of total C18 increased with increasing inclusion of dehulled oats in the present study, output of de novo FA in milk was not affected. This indicates that the increased supply of C18 with increasing inclusion of dehulled oats was directed toward body fat stores instead of the mammary gland, thereby not affecting de novo FA synthesis to the same extent as with replacement of barley with oats.

Relationships Between FA Intake and Milk FA Output

In Exp1, apparent transfer efficiency of total C18 into milk decreased linearly by 9.4 percentage points when barley was replaced by hulled oats and in Exp2, apparent transfer efficiency was on average 26 percentage points lower on the oat diets than on the barley diet and decreased linearly by 21 percentage points when the hulled oats diet was replaced with dehulled oats. Most previous studies have not reported FA composition of feeds and FA intake and thus, transfer efficiencies cannot be accurately calculated. However, similarly to the results of our study, apparent transfer efficiency of total C18 was lower on oat diets than on barley diets (141 vs. 76.9% based on reported FA intake and output in milk) in the study by Martin and Thomas (1988).

Digestibility of FA, and especially the digestibility of C18:0 decreases with increasing FA intake (Weisbjerg et al., 1992; Boerman et al., 2015). However, in this study, no major changes in true FA digestibility can be expected as crude fat concentrations were low (<50 g/kg DM; Fant et al., 2021; Ramin et al., 2021) in all experimental diets and the differences between diets were small. The most likely explanation for the observed effects on transfer efficiency is a change in the partitioning of total C18 between the mammary gland and adipose tissue after absorption from the digestive tract. This is especially evident in the case of gradual replacement of hulled oats with dehulled oats in Exp2, where total C18 output remained unchanged although total C18 intake increased from 462 to 607 g/d. In addition, prediction of energy utilization parameters in the study by Fant et al. (2021) showed a linear increase in energy balance but no change in milk energy with increased inclusion of dehulled oats. Thus, the incremental level of total C18 that cows received on the dehulled oat diet was most likely used for body fat synthesis rather than milk fat synthesis.

Apparent transfer efficiency of total C18 on the barley diet in Exp 2 was very high (110%), considering the fact that the digestibility of C18 is not 100%. Therefore, the transfer efficiency of total C18 was likely higher than 110%. In the study by Martin and Thomas

(1988), transfer efficiency of total C18 on the barley diet was even higher (141%) than observed in our study. However, their diets consisted of 70% concentrate and the intake of total C18 on the barley diet was less than half of the corresponding intake in our study (149 vs. 354 g/d). High transfer efficiency of total C18 into milk indicates some degree of de novo FA synthesis in adipose tissue with acetyl-CoA (from acetate) as the main substrate (Hanson and Ballard, 1967).

Smaller transfer efficiencies for C18:2 and C18:3 FAs than for C18:1 FAs are expected because C18:2 and C18:3 are extensively biohydrogenated in the rumen and also tend to be incorporated into plasma phospholipids and cholesterol esters instead of triacylglycerols (Kliem and Shingfield, 2016). In contrast, C18:1 in milk is derived not only from feed but also from desaturation of C18:2 and C18:3. Therefore, apparent transfer efficiencies of C18:2 and C18:3 better reflect true transfer efficiency, as most of these FA isomers are directly derived from the diet.

Relationships Between Milk FA Composition and Ruminal CH_4 Emissions

In this study, we also investigated whether the predictions of daily CH_4 emissions (g/d) using the on-farm available variables ECM and BW could be improved by including milk concentrations of individual FA. Although DMI is a good predictor of CH_4 emissions (Ramin and Huhtanen, 2013; Bougouin et al., 2019), it is not available on commercial farms. Instead, ECM yield could be used because it is closely related to DMI. Positive relationships between daily CH_4 emissions and ECM yield, as well as between daily CH_4 emissions and BW, were expected and were also reported in the study by Bougouin et al. (2019). In contrast to our results, de Souza et al. (2020) reported positive relationships between predicted daily CH_4 emissions and milk concentration of 4:0, whereas Dijkstra et al. (2011) reported no association between CH_4 yield and milk 4:0. In agreement with the findings of our study, a negative relationship between milk *cis*-11 18:1 and daily CH_4 emissions (g/d) was reported in the meta-analyses by Bougouin et al. (2019) and de Souza et al. (2020). In addition, CH_4 emissions expressed as CH_4 yield (g/kg DMI) were negatively related to milk *cis*-11 18:1 in the studies by Dijkstra et al. (2011), van Lingen et al. (2014), and Bougouin et al. (2019). In de Souza et al. (2020), the between-cow variation and repeatability of FA reported to be correlated with CH_4 emissions were low in both omasal and milk FA. In our study, the minor improvements in predictions of daily CH_4 emissions when specific milk FA were included in the model in addition to ECM and BW suggest that the potential for using milk FA composition to predict CH_4 emissions on-farm is low.

CONCLUSIONS

In this study, replacement of barley with hulled oats decreased the relative proportion of total SFA and increased the proportion of total MUFA in milk fat. In addition, replacement of barley with both hulled and dehulled oats decreased milk fat proportion of total SFA and increased the proportion of total MUFA. Replacement of hulled oats with dehulled oats did not affect relative proportion of MUFA in milk fat. We conclude that replacing barley grain with oat grain (hulled or dehulled) on a grass silage-based diet to dairy cows offers a practical strategy to slightly change the FA composition of milk fat to be more in accordance with international recommendations. It should be noted, however, that recent evidence suggests that there is no association between intake of milk SFA and CVD. Although this feeding strategy might decrease milk fat concentrations, milk yield and yield of milk fat are not compromised.

ACKNOWLEDGMENTS

This study was funded by the Swedish Research Council, Formas (Stockholm, Sweden), and conducted using data and material from Röbäcksdalen, SITES (Swedish Infrastructure for Ecosystem Science), a national coordinated infrastructure, supported by the Swedish Research Council. We acknowledge the laboratory staff at the Natural Resources Institute Finland, Jokioinen, for their assistance during the analysis of milk FA composition. The authors have not stated any conflicts of interest.

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