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Original article

Effect of silage type and concentrate level on conjugated linoleic acids, trans-C18:1 isomers and fat content in milk from dairy cows

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Abstract – The objective of the study was to examine how the fatty acid composition of milk especially concentrations of conjugated linoleic acids (CLA) and *trans*-C18:1 isomers and milk fat percentage were affected by silage type and concentrate level. Forty dairy cows were blocked and randomly assigned to one of four diets in a 2 × 2 factorial arrangement of treatments and a six week experimental period. Treatments were total mixed rations with maize (M) or grass (G) silage differing in polyunsaturated fatty acid (PUFA) profile and starch content, combined with a high (H) or a low (L) level of concentrate (with or without grain). Treatments had no significant effect on milk, protein and lactose yield, but energy corrected milk yield, milk fat percentage and fat yield was lower and protein percentage higher for maize compared with grass silage diets. Overall, maize silage diets resulted in higher concentrations of CLA isomers compared with grass silage diets, but there was a significant interaction between silage type and concentrate level for concentrations of *cis*9,*trans*11-CLA; *trans*10,*cis*12-CLA; *trans*11-C18:1 and *trans*10-C18:1. A high level of concentrate increased *trans*10,,*cis*12-CLA and *trans*10-C18:1 and reduced *cis*9,*trans*11-CLA and *trans*11-C18:1 when maize but not grass silage was provided. The results suggest that high levels of concentrate (grain) do not significantly alter the pattern of PUFA biohydrogenation in the rumen, the concentration of CLA and *trans*-C18:1 isomers in milk or cause milk fat depression unless combined with forage naturally high in starch and C18:2*n*-6 such as maize silage.

CLA / *trans*-C18:1 isomers / milk fat / silage type / concentrate level

1. INTRODUCTION

Numerous studies have indicated that consumption of conjugated linoleic acid (CLA) may be associated with positive effects on human health [1], and since milk and dairy products represent the primary source of CLA in the human diet [2] there has been widespread interest in increasing milk fat CLA.

The major CLA isomer in milk fat is *cis*9,*trans*11, which originates mainly by endogenous synthesis in the mammary gland from *trans*11-C18:1 via the enzyme Δ^9-desaturase, whereas a minor proportion comes from escaped and absorbed *cis*9,*trans*11-CLA produced in the rumen in the biohydrogenation of C18:2*n*-6 [1].

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*Trans*11-C18:1 is the predominant *trans*-C18:1 fatty acid produced by incomplete biohydrogenation of dietary polyunsaturated fatty acids (C18:2*n*-6 and C18:3*n*-3; PUFA) under most dietary conditions [3]. However, diets that provide large amounts of readily digestible carbohydrates such as starch, with [4, 5] or without [6, 7] a supplement of vegetable oil often lead to increased proportions of *trans*10-C18:1 and *trans*10,*cis*12-CLA in milk fat. This response is usually caused by a decline in rumen pH [4, 6] and shifts in the bacterial populations [8] whereby the biohydrogenation pathway of C18:2*n*−6 is altered [9]. Since mammals lack Δ^{12}-desaturase enzyme activity in tissues [10], all *trans*10,*cis*12-CLA in milk originates from ruminally produced *trans*10,*cis*12-CLA with *trans*10-C18:1 being the subsequent product of hydrogenation.

Several studies have shown that increased proportions of *trans*10,*cis*12-CLA and *trans*10-C18:1 in milk are associated with milk fat depression [4, 7, 11]. Sufficient amounts of C18:2*n*−6 in the diet as substrate for potential *trans*10-C18:1 and *trans*10,*cis*12-CLA production appears to be a prerequisite for milk fat depression to develop when high concentrate/low roughage diets are fed [4].

The effects of including oilseeds and plant oils with different PUFA profiles in high concentrate/low roughage diets on milk fat CLA concentration and milk fat depression have been thoroughly investigated [4, 5, 12]. Less well studied are the effects of commonly used forages differing in PUFA composition and starch content in combination with different levels of concentrate. Therefore the objective of the present study was to investigate how grass and maize silage with different PUFA profiles and starch content affected the fatty acid composition of milk, CLA isomers and certain *trans*-C18:1 fatty acids in particular, as well as milk fat percentage, when combined with a high or low level of concentrate.

2. MATERIALS AND METHODS

2.1. Experimental design, animals and management

Forty Holstein Friesian cows were grouped in 10 blocks according to parity, stage of lactation (i.e. days in milk post partum; DIM) and actual milk yield. Cows within each block were randomly assigned to one of four diets in a 2 × 2 factorial arrangement of treatments. In the beginning of the 6-week experimental period cows were on average 178 DIM (SD = 59, range 17 to 231 d) and yielding 25.5 kg.d^{-1} of milk (SD = 3.5, range 20.9 to 32.5 kg.d^{-1}). The cows were housed in individual tie stalls and fresh feed was offered ad libitum twice daily at 7.30 and 14.00 h. Milking took place at 04.00 and 16.00 h. During the first experimental week cows were switched from the herd diet to the experimental diets over a period of 3 days for adaptation. The experiment was carried out at the Foulum Research Centre from September to October 2003, and all experimental procedures were in compliance with Danish laws and regulations for the human care and use of animals in research (The Danish Ministry of Justice, Animal Testing Act [Consolidation Act No. 726, 1993, as amended by Act No. 1081, 1995]).

2.2. Experimental diets

Cows were offered total mixed rations containing grass (G) or maize (M) silage, as the only forage source, and a high (H) or a low (L) level of concentrate resulting in the following experimental diets; GL, GH, ML and MH. Rolled barley, which is the most commonly used concentrate in

Table I. Dry matter (DM) content and chemical composition (% of DM) of silages and concentrates.

Dietary component	Maize silage	Grass silage	Rolled barley	Rapeseed cake
DM, %	30.1	28.5	86.6	91.2
Crude protein	8.4	16.9	10.6	31.9
Crude fat	3.0	2.9	2.8	16.0
NDF	40.5	39.6	15.3	18.6
Starch	39.9	0.3	53.1	0.7
*cis*9-C18:1	0.6	0.07	0.4	8.5
C18:2*n*-6	1.2	0.3	1.6	3.3
C18:3*n*-3	0.2	0.8	0.2	1.4
Total FA	2.5	1.7	3.0	14.7

Danish dairy farming, was included in the two H diets at the expense of silage dry matter (DM). Rapeseed cake was included in all experimental diets as an additional source of protein, and in order to increase the overall amount of PUFA in the diets.

Grass silage was based on a mix of perennial ryegrass and clover, which was cut on June 3, 2003 and prewilted for 48 h, then picked up by a precision-chop forage harvester and ensiled in a walled pit. Before closing the stack the surface was treated with an additive (GrasAAT Plus, Yara Formates, Lysaker, Norway) containing 9% propionic acid and 2% benzoic acid at 2 liter/m^2. Forage maize (variety Passat) was harvested on September 19, 2002 using a forage harvester fitted with grain crackers (4.5 mm) in a cutting length of 9.6 mm and ensiled directly without additives in a walled pit. The DM content and chemical composition of silages and concentrates are presented in Table I. Compared with grass silage, maize silage had a substantially higher starch and lower crude protein content, but silages were quite similar in neutral-detergent fiber (NDF) content. Grass silage contained relatively high proportions of C18:3*n*-3 whereas C18:2*n*-6 was the predominant C18 fatty acid in maize silage and barley (Tab. I).

Dietary composition, DM content and chemical composition of the four experimental diets are given in Table II. The DM content and chemical composition of the experimental diets was calculated from the DM content and chemical composition of individual feedstuffs (Tab. I). The diets were formulated to have the same fill and amount of fatty acids per Scandinavian feed unit [13], and to meet requirements for protein (the AAT/PBV evaluation system) and other nutrients and minerals according to Danish recommendations [13]. However, compared with G diets, M diets had a slightly higher content of total fatty acids (Tab. II), which was due to a higher than expected level of total fatty acids in maize silage. The DM content of silages was determined weekly in order to adjust dietary formulations to account for small changes in the DM content if necessary.

2.3. Recordings, sampling and analysis

The feed intake of individual cows was recorded daily using feed weighbacks after the morning milking, but only data collected during the last 4 weeks of the experimental period were included in the statistical analysis, due to the temporal pattern in milk fat percentage (Fig. 1). Each week, samples of silages, rolled barley and rapeseed cake were obtained and frozen. Samples of individual feedstuffs from weeks 1 to 3 and 4 to 6 of the experiment were mixed, and a representative

Table II. Ingredients and chemical composition of grass (G) and maize (M) silage diets with a low (L) or a high (H) level of concentrate.

	Dietary treatment			
Silage type	G		M	
Concentrate level	L	H	L	H
Ingredient (% of DM)				
Maize silage	–	–	68.8	52.0
Grass silage	72.7	52.7	–	–
Rolled barley	–	18.3	–	16.2
Rapeseed cake	27.0	28.3	30.2	30.6
Mineral mix[1]	0.19	0.54	0.89	1.14
Vitamin mix[2]	0.16	0.14	0.14	0.12
Chemical composition (% of DM)				
DM, %	35.0	41.9	38.9	45.5
Crude protein	20.9	19.9	15.4	15.8
Crude fat	6.5	6.6	6.9	6.9
Starch	0.38	10.1	20.8	24.4
NDF	33.8	29.0	33.4	29.2
C18:0	0.10	0.10	0.12	0.11
*cis*9-C18:1	2.35	2.51	2.99	2.98
C18:2*n*−6	1.11	1.40	1.80	1.88
C18:3*n*−3	0.96	0.85	0.52	0.53
Total fatty acids	5.2	5.6	6.2	6.3
NE[3], MJ/kg DM	7.57	7.97	7.18	7.57

[1] Contained 20% Ca, 10% P, 4% Mg, 0.3% Zn, 0.001% Se, 400 IU of vit. A, 70 IU of vit. D.g^{-1}.
[2] Contained 5000 IU of vit. A, 200 IU vit. D_3, 1% vit. E.g^{-1}.
[3] Net energy (NE) for lactation is based on Scandinavian Feed Units (SFU) (7.89 MJ NE = 1 SFU).

sample from the two periods was taken for determination of DM and for chemical analysis. Since the chemical composition of feedstuff in the two periods showed very little variation, an average of results was used for further calculations.

Milk yield was recorded twice weekly on two consecutive days where milk samples were obtained and stored at 4 °C with a preservative tablet (Microtabs 2; D&F Control System, San Ramon, CA, USA) until analysis at the Sønderjysk Kontrolforening laboratory (Vojens, Denmark) for fat, protein and lactose with near infrared procedures using a Milko Scan 54 analyser (Foss Electrics, Hillerød, Denmark). Milk samples for fatty acid analysis were collected in week 0 (before cows were switched to the experimental diets) and in weeks 2, 4 and 6 of the experimental period from two consecutive morning and afternoon milkings. The morning and afternoon milk samples were mixed and stored at −20 °C until fatty acid analysis.

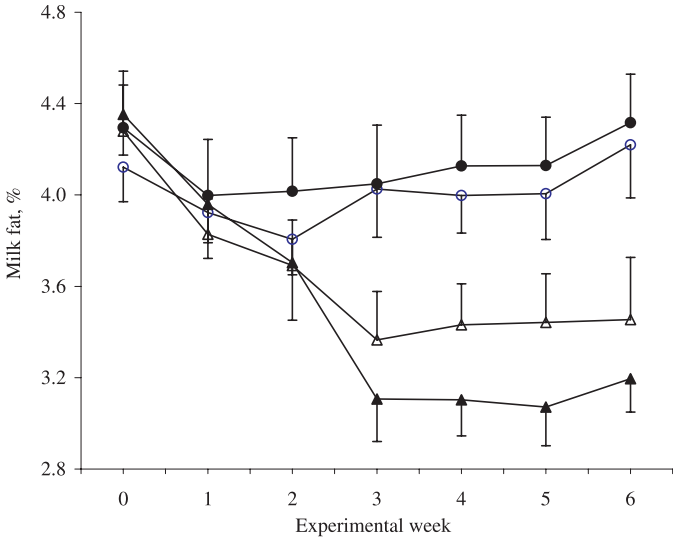

Figure 1. Temporal pattern of milk fat percentage. Dietary treatments were GL (○) GH (●), ML (△) and MH (▲). Values represent means ± SE for each treatment group (n = 10).

Lipid extraction of milk fat was performed according to methods described earlier [14], and methyl esters of the fatty acids were prepared by transesterification with KOH [15]. Fatty acid methyl esters (FAME) were separated and identified by a gas-liquid chromatograph (HP 6890 Hewlett-Packard GmbH, Waldbronn, Germany) equipped with a CP Select CB for FAME capillary column (100 m × 0.25 mm i.d. with 0.25 μm film thickness) (Chrompack, Middleburg, The Netherlands). The oven temperature was initially 50 °C for 5 min then raised at 10 °C.min^{-1} to 165 °C (held for 40 min). The temperature was then raised again at 1 °C.min^{-1} to 180 °C and at 10 °C.min^{-1} to 200 °C (held for 6.5 min). Injection and detection temperatures were maintained at 270 °C. The flow rate for the helium carrier gas was 2 mL.min^{-1}, and a split/splitless injector was used with a split ratio of 1:14. Fatty acids were identified and quantified using pure methyl ester standards, including CLA isomers (Nu-Chek Prep Inc., Elysian, MN). The chromatogram areas of C4:0, C6:0, C8:0 and C10:0 FAME were corrected by response factors (C4:0-2.01; C6:0-1.40; C8:0-1.16; C10:0-1.04).

2.4. Statistics

Data were analysed statistically as a 2 × 2 factor design with blocks using the GLM procedure of SAS (Version 5.1, Release 8.02 SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + S_i + E_j + (S \times E)_{ij} + B_k + \varepsilon_{ijk}$$

where Y_{ijk} is the observation, μ is the overall mean, S_i is the silage type (i = 1, 2), E_j is the energy level (j = 1, 2), $(S \times E)_{ij}$ the interaction between silage type and energy level, B_k is the block (k = 1, 2,. . . , 10), and ε_{ijk} is the residual error.

The primary focus of the experiment was on the maximal effects of treatments rather than on the change in milk fatty acid composition and milk fat percentage over time. During the third experimental

week, treatment effects on milk fat percentage became evident and remained stable throughout the experiment (Fig. 1). Because milk fat percentage and the fatty acid composition of milk were assumed to be related, data on all parameters from weeks three to six of the experiment were included in the statistical analysis in order to be consistent in the use of data. In the analysis of milk yield, energy corrected milk yield (ECM) and milk constituents, the pre-experimental value (week 0) of the parameter was included in the model as a covariate. Regression procedures were employed to explore the form of the relationship between milk fat percentage and individual CLA isomers of interest by the non linear (NLIN) procedure of SAS. All model fits were evaluated using mean square errors and examination of studentised residual plots.

3. RESULTS

Feed intake, milk yield and milk composition data are presented in Table III. Corresponding to the higher DM content of H diets (Tab. I), DM intake was higher for cows receiving H diets. There was a silage type by concentrate level interaction for the intake of starch and crude protein reflecting the differences in starch and crude protein content of the four diets (Tab. II), as well as differences in DM intake (Tab. III). As expected, the intake of C18:2*n*-6 was higher and the intake of C18:3*n*-3 was lower for M compared with G diets, but there was an interaction between silage type and concentrate level for the intake of C18:3*n*-3. H diets also resulted in higher intakes of C18:2*n*-6 than L diets. The intake of total C18 fatty acids was increased for M and H diets compared with G and L diets.

Milk yield was unaffected by treatment, but feeding M compared with G diets was associated with a marked decrease in milk fat percentage (mean 3.23 and 4.15%, respectively) and a mean decrease in milk fat yield of 272 $g.d^{-1}$. Although not significant ($P = 0.15$), milk fat percentage tended to decrease with increased concentrate level, especially when maize silage was fed. The lower secretion of milk fat resulted in a significantly lower ECM for M compared with G diets (21.9 and 26.6 $kg.d^{-1}$, respectively) (Tab. III), but no effect of concentrate level was observed. The protein percentage of milk was lower for cows fed G and L diets compared with M and H diets, and the yield of protein tended ($P = 0.06$) to increase as the concentrate level increased. Lactose contents and yields were unaffected by treatment (Tab. III).

The fatty acid composition of milk fat is presented in Table IV. G diets resulted in higher concentrations of short and medium-chain fatty acids (≤ C16:0) than M diets. Except for C4:0, the concentrate level did not affect short and medium-chain fatty acids. Relative to M diets, the G diets increased stearic acid (C18:0) and decreased levels of the monounsaturated fatty acids palmitoleic acid (*cis*9-C16:1) and oleic acid (*cis*9-C18:1).

Overall, M diets resulted in higher concentrations of *cis*9,*trans*11 and *trans*10,*cis*12-CLA compared with the G diets, but there was a significant interaction between silage type and concentrate level for CLA-isomers and also for *trans*11-C18:1 and *trans*10-C18:1. The ML diet resulted in the significantly highest *cis*9,*trans*11-CLA and *trans*11-C18:1 concentration, whereas *trans*10,*cis*12-CLA and *trans*10-C18:1 concentrations were the highest in MH milk. *Cis*9,*trans*11-CLA, *trans*10,*cis*12-CLA, *trans*11-C18:1 and *trans*10-C18:1 were unaffected by the concentrate level when grass silage was fed (Tab. IV). There was a positive linear relationship between *cis*9,*trans*11-CLA and *trans*11-C18:1 ($y = 0.34x + 0.45$, $R^2 = 0.60$, $P < 0.001$) and between *trans*10,*cis*12-CLA and *trans*10-C18:1 ($y = 0.003x + 0.012$, $R^2 = 0.91$,

Table III. Feed intake, milk yield and composition of cows fed grass (G) or maize (M) silage diets with a low (L) or high (H) level of concentrate.

	Dietary treatment[1]							
Silage type (S)	G		M			P^1		
Concentrate level (C)	L	H	L	H	SEM	S	C	S × C
Intake/d								
DM, kg	18.4	19.4	18.4	20.0	0.4	0.49	**	0.43
NE[2], MJ	139	154	132	151	3	0.11	***	0.49
Starch, g	70 a	1948 b	3818 c	4874 d	80	***	***	***
Crude protein, g	3845 a	3848 a	2826 b	3164 c	74	***	*	*
C18:2*n*-6, g	211	275	345	390	7	***	***	0.19
C18:3*n*-3, g	180 a	167 b	105 c	114 c	3	***	0.50	**
Total C18 FA, g	842	948	1023	1123	22	***	***	0.88
Total FA, g	957	1085	1139	1260	25	***	***	0.90
Yield/d								
Milk, kg	24.4	25.6	23.4	23.7	1.0	0.17	0.49	0.67
ECM[3], kg	24.8	26.3	22.0	21.7	1.0	***	0.54	0.36
Fat, g	1003	1053	786	726	45	***	0.91	0.23
Protein, g	842	933	864	917	36	0.92	0.06	0.60
Lactose, g	1175	1222	1106	1124	52	0.12	0.53	0.79
Concentration, %								
Fat	4.16	4.13	3.41	3.05	0.13	***	0.15	0.22
Protein	3.48	3.67	3.76	3.90	0.07	**	*	0.76
Lactose	4.80	4.80	4.81	4.80	0.04	0.81	0.88	0.98

[1] Statistical probabilities of treatment differences, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values represent means of the last 4 measurements in the experimental period and means within row with different superscripts differ according to level of significance indicated. *P* values for non-significant effects are shown.

[2] Calculated on the basis of content of NE in the four diets (Tab. II).

[3] ECM = milk yield × (383 × fat% + 242 × protein% + 157.1 × lactose% + 20.7)/3140.

$P < 0.001$). Milk fat percentage decreased in a curvilinear manner with increasing concentrations of *trans*10,*cis*12-CLA and *cis*9,*trans*11-CLA (Fig. 2).

4. DISCUSSION

To our knowledge, only one study previously compared the effects of maize and grass silage and the proportion of concentrate in the diet on milk production and concentrations of different CLA and *trans*-C18:1 isomers in milk [16]. However, in that study, diets were supplemented with 3% fish and sunflower oil (2:3 w/w), so the results should be compared with caution although we included rapeseed cake (27−31% of DM) in all four experimental diets to increase the overall content of PUFA. The increase in *trans*10,*cis*12-CLA and *trans*10-C18:1 concentration when maize silage was substituted for grass silage was in line with Shingfield et al. (2005) [16], but on the contrary to our results they did not observe any significant silage type effect on *cis*9,*trans*11-CLA.

Table IV. Effect of grass (G) or maize (M) silage and a low (L) or high (H) level of concentrate on milk fatty acid composition.

	Dietary treatment							
Silage type (S)	G		M			P^1		
Concentrate level (C)	L	H	L	H	SEM	S	C	S × C
FA, g/100 g FA								
C4:0	3.73	3.59	3.22	2.69	0.13	***	*	0.15
C6:0	1.94	1.97	1.51	1.36	0.08	***	0.50	0.31
C8:0	1.09	1.17	0.93	0.87	0.05	***	0.82	0.20
C10:0	2.16	2.29	1.68	1.78	0.13	**	0.30	0.78
C12:0	2.39	2.64	2.12	2.34	0.14	0.05	0.10	0.90
C14:0	9.73	9.79	8.71	8.59	0.28	**	0.92	0.76
C16:0	22.1	21.8	19.1	18.6	0.63	***	0.51	0.90
*cis*9-C16:1	1.30	1.42	1.70	1.94	0.16	**	0.29	0.70
C18:0	13.7	13.0	11.9	11.0	0.61	**	0.22	0.87
*cis*9-C18:1	25.5	25.3	27.5	27.2	0.77	*	0.77	0.99
*trans*11-C18:1	1.74 a	1.79 a	2.80 b	1.55 a	0.25	0.11	*	*
*trans*10-C18:1	0.60 a	0.66 a	3.14 b	6.05 c	0.55	***	*	*
Total *trans*-C18:1[2]	4.86	5.08	9.25	10.53	0.46	***	0.11	0.26
*cis*9,*trans*11-CLA	0.89 a	0.92a	1.61 b	1.17 a	0.10	***	*	*
*trans*10,*cis*12-CLA	0.013 a	0.014 a	0.024 b	0.034 c	0.002	***	**	*
*cis*9,*cis*12-C18:2	1.17	1.56	1.59	1.93	0.06	***	***	0.66
*cis*9,*cis*12,*cis*15-C18:3	0.63	0.64	0.36	0.41	0.02	***	0.09	0.29
≥ C20:0	0.84	0.91	0.74	0.80	0.03	**	*	0.94
Others	7.50	7.47	7.66	8.24	0.19	*	0.15	0.11

[1] Statistical probabilities of treatment differences, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values represent the mean of the samples collected at the end of weeks 3, 4, 5 and 6 in the experimental period and means within row with different superscripts differ according to level of significance indicated. *P* values for non-significant effects are shown.

[2] Total *trans*-C18:1 is the sum of; *trans* C18:1*n*-2, *trans* C18:1*n*-6, *trans* C18:1*n*-7, *trans* C18:1*n*-8, *trans* C18:1*n*-9 and *trans* C18:1*n*-10.

A number of studies have demonstrated that increasing amounts of readily digestible carbohydrates in the diet are associated with increased proportions of *trans*10,*cis*12-CLA and *trans*10-C18:1 in milk fat [4–6, 16]. The fact that the concentrate level only affected *trans*10,*cis*12-CLA and *trans*10-C18:1 but also the *cis*9,*trans*11-CLA and *trans*11-C18:1 concentration in milk when maize silage was fed, suggests that increased proportions of concentrate (starch) in the diet does not severely affect the ruminal biohydrogenation pattern unless combined with a forage naturally high in starch. An increased ratio of starch to fibre in the diet has been shown to impair the rate of lipolysis of dietary lipids in vitro [17], and is an important factor controlling the formation of

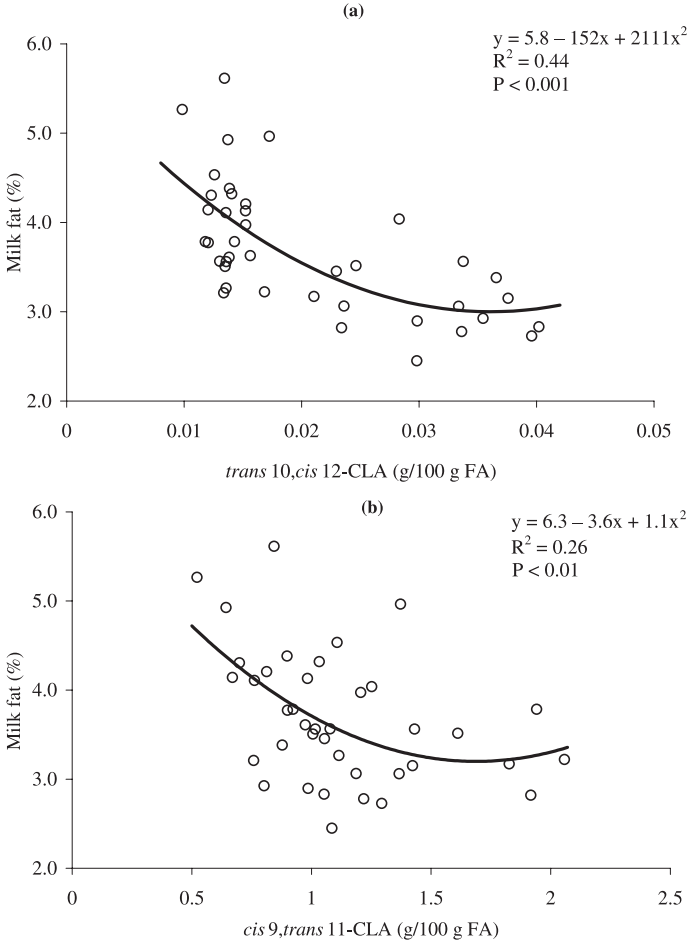

Figure 2. The relationship between milk fat percentage and milk fat content of *trans*10,*cis*12-CLA **(a)** and *cis*9,*trans*11-CLA **(b)**. Regression equations, R^2 and probability are presented for each plot.

individual biohydrogenation intermediates in the rumen [9]. In this experiment, the daily intake of starch accounted for 67 and 54% of the variation in milk fat *trans*10,*cis*12-CLA and *trans*10-C18:1, respectively (Figs. 3a and 3b) and the concentration of total *trans*-C18:1 increased with starch intake (Fig. 3c). High grain diets promote the growth of the bacterial strain *Megasphera elsdenii* YJ-4 [18] due to a decrease in rumen pH and cause a rapid decline in the main cellulose digesting bacteria *Butyrivibrio fibrisolvens* [19]. *Megasphera elsdenii* YJ-4 exhibits *cis*9,*trans*10-isomerase activity [20], which can convert 18:2*n*-6 to *trans*10,*cis*12-CLA and *trans*10-C18:1, whereas *Butyrivibrio fibrisolvens* produces *cis*9,*trans*11-CLA and *trans*11-C18:1 as intermediates in the hydrogenation of 18:2*n*-6 [21]. The large variability in *trans*10-C18:1 and *trans*10,*cis*12-CLA concentration in milk from cows fed maize silage diets (Figs. 3a and 3b) may

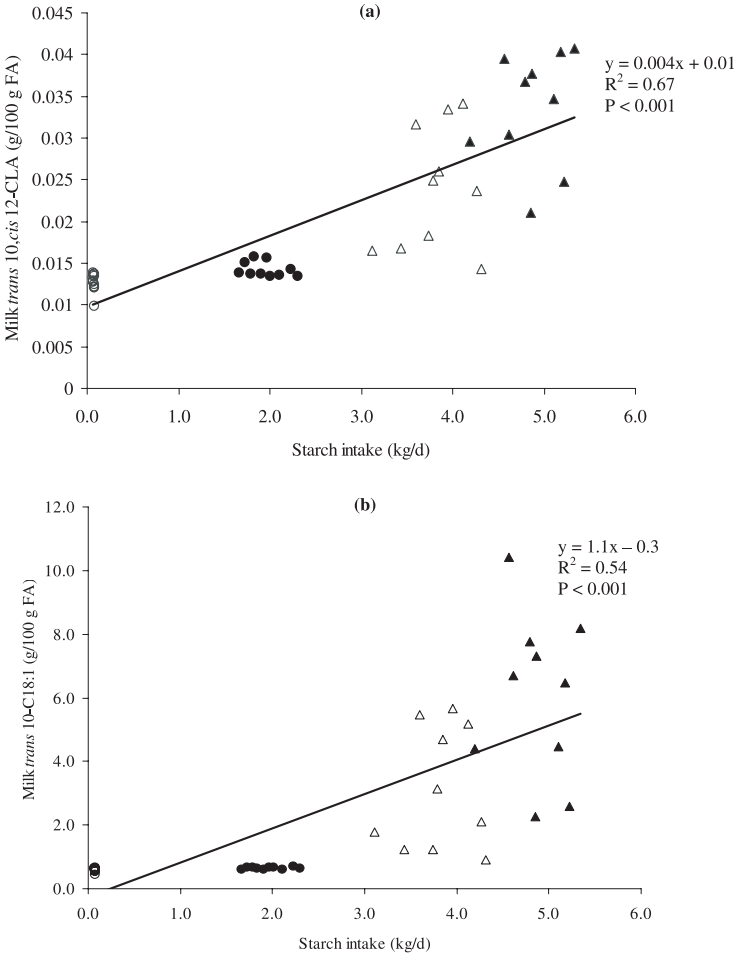

Figure 3. The relationship between the intake of starch across all dietary treatments and concentrations of *trans*10,*cis*12-CLA **(a)**, *trans*10-C18:1 **(b)**, total *trans*-C18:1 **(c)** and milk fat **(d)**, for cows given the GL (○) GH (●), ML (△) and MH (▲) diets. Regression lines, R^2 and probability are presented for each plot.

suggest that cows respond differently to high intakes of starch. According to Kim et al. [18], it is conceivable that individual cows may harbour different *Megasphera elsdenii* strains with different capacities to produce *trans*10-C18:1 and *trans*10,*cis*12-CLA, and this may contribute to the larger variability observed on high starch diets.

In accordance with Griinari et al. [4], the results also indicate that relatively large amounts of 18:2*n*-6 as provided by maize silage diets, is a prerequisite for increased proportions of *trans*10,*cis*12-CLA and *trans*10-C18:1 in milk fat. However, we can not exclude that some of the silage type and concentrate level effects on

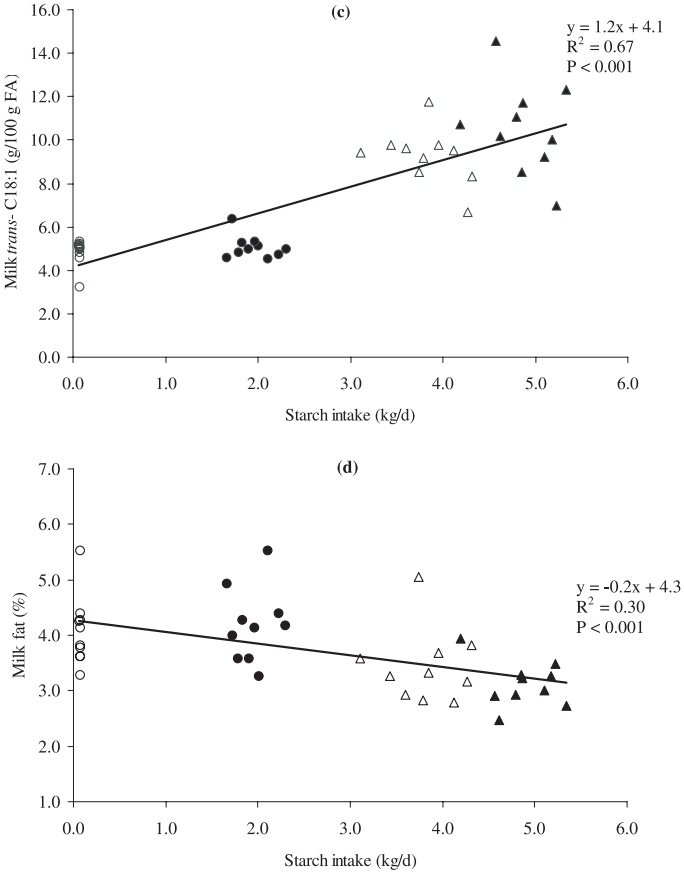

Figure 3. Continued.

trans-C18:1 and CLA isomers in milk to some extent might to some extent be related to the unexpected higher intakes of total C18 fatty acids for cows fed maize silage (Tab. III), and perhaps also to differences in crude protein contents of maize and grass silage diets (Tab. II).

C18:0 is the end product in the biohydrogenation of PUFA in the rumen, but apart from absorption, the C18:0 concentration in milk also reflects the relative activity of Δ^9-desaturase in the mammary gland. The lower C18:0 and the substantially higher total *trans*-C18:1 in milk from cows fed maize silage may therefore indicate a less extensive complete biohydrogenation of PUFA in the rumen and/or a lower Δ^9-desaturase activity in the mammary gland, since the differences are not related to a lower intake of total C18 fatty acid for cows fed maize silage (Tab. III).

On the ontrary to our results, feeding maize compared with grass silage is not always associated with significant reductions in milk fat percentage [22, 23]. The different results can probably be ascribed to variations in chemical composition of maize silages especially in NDF and starch content, and physical characteristics such as particle size of the silages may also be

important in terms of development of milk fat depression [9] together with the overall composition and amount of PUFA in the total mixed rations.

Concentrate level did not significantly affect milk fat percentage, but compared with studies where milk fat depression has been reported [6, 7], the concentrate:forage ratio in our study was rather low for both high and low concentrate diets (50:50 and 30:70, respectively). However, the total intake of starch appeared to be a major cause of variation in milk fat percentage (Fig. 3d) in agreement with the results by Shingfield et al. [16].

In accordance with previous studies [4, 5, 7], the reduction in milk fat percentage was associated with an increase in milk fat *trans*10,*cis*12-CLA concentration. *Trans*10,*cis*12-CLA is a potent inhibitor of milk fat synthesis in the mammary gland [11, 24], especially by reducing the de novo synthesis of short and medium-chain fatty acids [11]. In agreement with this, maize silage diets resulted in lower short and medium-chain fatty acids (C4:0-C16:0) in milk, compared with grass silage diets. However, Griinari and Bauman [9] summarised that *trans*10,*cis*12-CLA only accounts for up to 50% of the reduction in milk fat secretion when dietary milk fat depression is induced, and recently, Loor et al. [25] showed that milk fat *trans*10,*cis*12-CLA increased minimally in milk fat depressed cows offered a low forage diet supplemented with linseed oil. This suggests that in addition to *trans*10,*cis*12-CLA, other inhibitors of milk fat synthesis are formed in the rumen when dietary milk fat depression is induced. *Cis*9,*trans*11-CLA appeared to be related to the decrease in milk fat percentage in our study, but the correlation between *cis*9,*trans*11 CLA and milk fat was substantially lower ($R^2 = 0.26$, $P < 0.01$) than between *trans*10,*cis*12-CLA and milk fat ($R^2 = 0.44$, $P < 0.001$) (Fig. 2). Furthermore, Lin et al. [26] found that *trans*10,*cis*12-CLA is a much more potent inhibitor of de novo fatty acid synthesis and desaturation than *cis*9,*trans*11-CLA in the mammary gland of lactating mice. The *trans*10-C18:1 isomer is another obvious candidate due to the close relationship with *trans*10,*cis*12-CLA ($y = 0.003x + 0.012$, $R^2 = 0.91$, $P < 0.001$), but *trans*9,*cis*11-CLA may also be involved [27] as well as other not yet identified intermediates of ruminal biohydrogenation of PUFA.

In summary, maize silage diets may result in higher concentrations of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA as well as *trans*10-C18:1 in milk fat and a lower milk fat percentage than grass silage based diets. Furthermore, the results show that a high level of concentrate (grain) does not significantly alter the pattern of PUFA biohydrogenation in the rumen, the concentration of CLA and *trans*-C18:1 isomers in milk or cause milk fat depression unless provided in combination with forage naturally high in starch and C18:2*n*-6 such as maize silage.

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