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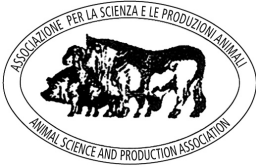
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Effect of unsaturated fatty acid supplementation on performance and milk fatty acid profile in dairy cows fed a high fibre diet

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ABSTRACT

The influence of unsaturated fatty acid (UFA) supplement on productive performance, physiochemical properties and fatty acid (FA) profile of milk, was investigated in lactating dairy cows fed with high fibre diets. According to a cross-over design, twelve cows were assigned to two experimental settings characterized by different FA profiles. Cows received a high fibre diet (~42% NDF on DM basis) supplemented with soybean based mixtures with these FA compositions: 92.0% of saturated FA (SFA), 2.8% of monounsaturated FA (MUFA) and 5.2% of polyunsaturated FA (PUFA) in the control diet (C-diet); 19.1% of SFA, 20.9% of MUFA and 60.0% of PUFA in the experimental diet (E-diet).

The E-diet did not affect dry matter intake nor milk yield. Milk composition and coagulation traits resulted similar between treatments, except for the lactose level, which was lower in the E-diet (5.0 vs 4.8%; $P < 0.05$) and the freezing point (-0.546 vs -0.535 °C; $P < 0.05$). As respects the milk FA profile, the E-diet significantly increased the percentage of UFA because of their greater amount in the ration; however the "transfer" of UFA in milk was limited by the high level of FA biohydrogenation (BH) at the ruminal level. UFA showed low values of carry over in milk (67.5 vs 39.7%; $P < 0.001$) due to the saturation process; on the contrary SFA had a threefold increment (124 vs 323%; $P < 0.001$), mostly due to a peak in the production of stearic acid. In this study, the percentage of CLA in milk (0.50 vs 0.62%; $P < 0.05$) was quite low for both diets, if compared with other studies, and this was probably due to a low vaccenic acid supply at duodenal level.

Key words: Dairy cow, Milk quality, Biohydrogenation, Fatty acid composition.

RIASSUNTO

EFFETTO SULLA PRODUZIONE E DESTINO DI UN'INTEGRAZIONE A BASE DI ACIDI GRASSI
INSATURI IN BOVINE DA LATTE CON UNA DIETA AD ELEVATO TENORE DI FIBRA

È stato valutato l'effetto di un'integrazione alimentare con acidi grassi (FA) insaturi sulle prestazioni pro-

duttive, sulle caratteristiche chimico-fisiche e sul profilo acido del latte, in bovine lattifere alimentate con diete ad elevato tenore di fibra. Adottando un modello sperimentale a cross-over, 12 bovine di razza Frisona sono state assegnate a due tesi aventi un differente profilo alimentare di FA; a una razione base caratterizzata da un elevato tenore fibroso (~42% di NDF SS) sono stati addizionati due nuclei a base di soia, differenti per la composizione in FA: il nucleo della dieta controllo (C-diet) era composto dal 92,0% di acidi grassi saturi (SFA), dal 2,8% di acidi grassi monoinsaturi (MUFA) e dal 5,2% di polinsaturi (PUFA), mentre il nucleo della razione sperimentale (E-diet) presentava il 19,1% di SFA, il 20,9% di MUFA ed il 60,0% di PUFA. La dieta sperimentale (E-diet) non ha influenzato l'ingestione volontaria e la produzione di latte; la composizione del latte è risultata simile nei due trattamenti, ad eccezione della concentrazione di lattosio, per il quale si è osservato un contenuto inferiore nella E-diet (5,00 vs 4,81%; $P < 0,05$), e del punto crioscopico (-0,546 vs -0,535 °C; $P < 0,05$). La maggiore inclusione di FA nella dieta sperimentale ha determinato un lieve ma significativo aumento della percentuale di UFA del latte. In riferimento alla dieta sperimentale, il trasferimento degli UFA nel latte è risultato limitato (67,5 vs 39,7%; $P < 0,001$); ciò sembrerebbe dovuto ad un intenso processo di bioidrogenazione (BH) che ha determinato la saturazione degli UFA. Il carry over dei saturi è risultato pari a 3 (124 vs 323%; $P < 0,001$) ed è stato caratterizzato da un picco di produzione di acido stearico. In tale studio la percentuale di CLA nel latte è risultata relativamente bassa, se paragonata ai risultati di altri lavori (0,50 vs 0,62%; $P < 0,05$); tale risultato potrebbe essere riconducibile ad una ridotta concentrazione di acido vaccenico a livello duodenale.

Parole chiave: Bovine da latte, Qualità latte, Bioidrogenazione, Profilo acidi grassi.

Introduction

Recent studies (Bailoni *et al.*, 2004; Eger *et al.*, 2007) on high productive dairy cow nutrition pointed out that dietary unsaturated fatty acids (UFA) may influence the milk fatty acids (FA) profile, lowering the content of saturated FA (SFA), such as lauric (C12:0), miristic (C14:0) and palmitic acid (C16:0). Moreover, the high level of dietary polyunsaturated FA (PUFA) has been demonstrated increasing the milk content in PUFA and, among them, in linoleic conjugated acids (CLA) (Kelly *et al.*, 1998; Dhiman *et al.*, 1999; Khanal and Dhiman, 2004; Ponter *et al.*, 2006). Several authors suggested positive effects of CLA in human beings such as antiatherogenic, anticarcinogenic and antiobesity properties (Pariza *et al.*, 2001; Wahle *et al.*, 2004).

Dietary FA are only partially absorbed and transferred to milk because of the UFA biohydrogenation occurring in rumen (Bauman *et al.*, 1999; Griinari and Bauman, 2006) and FA desaturation in the mammary gland (Corl *et al.*, 2001; Piperova *et al.*, 2002).

Recent papers (Piperova *et al.*, 2002;

Cabrita *et al.*, 2003; Sackmann *et al.*, 2003) showed that diets high in fibre support ruminal biohydrogenation (BH) phenomena, but the production degree of BH intermediate compounds among different feeds and rumen conditions is still under investigation. Piperova *et al.* (2002) reported that in comparison with rations rich in concentrates, fibrous diets led to a high degree of complete UFA conversion, resulting in a bigger production of stearic acid (C18:0) and a lower presence of intermediate compounds in milk. Moreover, Sackmann *et al.*, (2003) found that the forage level can influence the production of different trans FA (tFA) during the BH process, underlying a positive relation between diet forage content and ratio between C18:1, *trans*-11 (vaccenic acid, the cis-9, *trans*-11 CLA precursor) and C18:1, *trans*-10 acid in duodenum.

Taking those results into account our purpose was an evaluation of productive performance, milk quality and FA profile in high productive dairy cows fed a high fibre-based diet supplemented with a source (soybean) rich in linoleic acid (C18:2 n-6). In addition, the dietary fatty acid conversion and

their carry-over in milk were calculated.

Materials and methods

Animals, experimental design and dietary intake

According to a cross-over design, twelve Friesian cows (730 ± 78 kg body weight) were assigned to two experimental groups balanced for milk yield (36.9 ± 5.8 kg/d), days of lactation (167 ± 80 d) and parity (2.2 ± 1.3). The trial lasted two months and each period was preceded by a three-week adjustment time in which cows were gradu-

ally accustomed to the diet composition of the subsequent experimental period. Cows were offered one of two dietary treatments which were formulated as reported in Table 1. The total mixed rations (TMR) were differentiated by using two blends (Table 2). In comparison to the control diet (C-diet), the experimental one (E-diet) was formulated in order to increase the UFA content (Table 3). Cows were fed *ad libitum* and dry matter intake (DMI) was individually and continuously recorded through the BC40 automated feeding control system (Biocontrol System A/S, Grimstad Gred, Norway).

Table 1. Formulation and diet composition.

		Treatment	
		C-diet	E-diet
Ingredients:			
Maize silage	% DM	50.9	50.7
Permanent meadow 1 st crop	"	12.1	12.0
Cereal mix ¹	"	12.0	11.9
Control supplement	"	10.8	-
Experimental supplement	"	-	11.1
Dehydrated alfalfa	"	4.8	4.8
Permanent meadow 3 rd crop	"	4.8	4.8
Beet dry pulps	"	2.4	2.4
Straw	"	1.2	1.2
Crushed linseed	"	0.8	0.8
Brewer's yeast	"	0.2	0.2
Diet Composition:			
Crude protein	% DM	14.5 ± 0.7	14.7 ± 0.8
Ether extract	"	3.2 ± 0.3	3.0 ± 0.2
Crude ash	"	7.6 ± 0.8	8.2 ± 0.3
NDF	"	41.0 ± 2.0	43.3 ± 2.2
ADF	"	25.0 ± 1.5	26.5 ± 1.4
NFC	"	33.7 ± 2.3	30.9 ± 1.6
Milk FU	/kg DM	0.82	0.82

¹70% maize meal and 30% barley meal.

NDF: neutral detergent fibre; ADF: acid detergent fibre; NFC: non-fibre carbohydrates, $NFC = 100 - (CP + EE + CA + NDF)$.

Table 2. Blend ingredients and composition.

		Treatment	
		C-blend	E-blend
Ingredients:			
Soybean meal	% DM	80.0	42.3
Cracked and toasted soybean seeds	"	-	44.2
Vit. - minerals premix	"	10.5	10.5
Hydrogenated fat mixture	"	8.5	-
Extruded ground maize	"	1.0	3.0
Composition:			
Crude protein	% DM	40.2	39.2
Ether Extract	"	8.9	9.3
Crude ash	"	16.2	17.1
NDF	"	8.0	9.0
NFC	"	26.7	25.4

Table 3. Fatty acid profile of supplements and diets (% of the total detected fatty acids).

		Treatment			
		C-blend	E-blend	C-diet	E-diet
Fatty acid (FA):					
C16:0	% total FA	46.8	13.5	31.7	16.1
C18:0	"	42.9	4.60	23.2	3.50
C18:1 n-9	"	1.77	19.1	8.98	16.6
C18:1 n-7	"	-	-	0.46	0.93
C18:2 n-6	"	4.61	52.7	21.9	41.4
C18-3 n-3	"	0.51	7.19	9.07	13.2
Saturated fatty acids	"	92.0	19.1	57.5	21.3
Monounsaturated fatty acids	"	2.78	20.9	10.2	18.6
Polyunsaturated fatty acids	"	5.22	60.0	32.3	60.1
Total n-3	"	0.53	7.19	9.07	13.9
Total n-6	"	4.68	52.8	22.0	43.5

Sampling and chemical analyses

Twice a week throughout the trial, samples of TMR and of 24h residuals of each diet were collected. The degree of selection

was evaluated by Pennsylvania State University separator and a selection index for each fraction (>19 mm, 19-8 mm and <8 mm) was calculated as the rate of the per-

centage of each fraction between TMR and 24h residuals. All samples of TMR and uneaten food were analyzed for DM, crude protein (CP), ether extract (EE) in accordance with AOAC (2000). The content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods proposed by Mertens (2002) and modified by using a Fibre Analyzer (ANKOM/2000, ANKOM Technology, New York, USA). After the morning milking, samples of Li-heparinized blood were collected from the jugular vein twice, on the 2nd and on the 5th day for each experimental week. Plasma was analyzed by a biochemical auto-analyzer (Hitachi 911, Roche Diagnostics GmbH Mannheim) in order to determine the metabolic profile (total protein, albumin, urea, glucose, total cholesterol, triglycerides, AST, GGT, CK, Ca, P, Mg), except for non-esterified fatty acids (NEFA) which was assessed by a commercial kit procedure (Novatech Diagnostics, Randox).

Cows were milked twice a day and milk yield was manually recorded during the final 5 days of each period. A milk sample, containing morning and afternoon milk at a 1:1 ratio was collected twice, on the 2nd and on the 5th day of each experimental week; milk samples were refrigerated at 4°C, conserved using azidiol and analysed for fat, protein, lactose and freezing point by a Milk-o-Scan 4000 infrared analyzer (Fossomatic, Fosselectric, Hillerød, Denmark). Somatic cells count (SCC) was detected by Fluoro-opto-electronic cell counting (Fossomatic, Fosselectric, Hillerød, Denmark). The urea content was determined using differential pH-metry method (EUROCHEM CL 10 plus, Microlab EFA). Coagulation properties (rennet clotting time, r; rate of curd firming, k_{20} and curd firmness after 30 min, a_{30}) were assessed according to the method of Zannoni *et al.*

(1981) on a Formagraph apparatus (CRM 48, Polo Trade, Padova, Italy).

For the determination of the milk FA composition, samples were stored at -80°C until analyses. FA composition of feed and diets were determined after lipid extraction by chromatography, using a dichloromethane/methanol solution (2:1 v/v) as described by Nourooz-Zadeh and Appelqvist (1988). Aliquots of the extracts were then trans-esterified according to the Christie (1982) procedure and fatty acid methyl esters (FAME) were detected as described. An aliquot of 100 ml was homogenised in a 100 ml solution of anhydrous sodium sulphate (0.47 M), centrifuged for 10 min at 4000 xg, and 100 mg of surfaced fat was mixed with 4 ml methanol and 4 ml n-heptane and centrifuged again (4000 xg, 5 min, 4°C). Two ml of the upper phase containing the ether extract was trans-esterified with sodium methoxide and FAME were quantified by gas chromatography (Shimatzu GC17A, equipped with FID detector, using an Omegawax 250 column 30 m x 0.25 µm x 0.25 µm). Obtained data were reported as percentage of the total detected fatty acids.

In order to evaluate the degree of ruminal biohydrogenation of unsaturated FA, we measured the carry over of long chain fatty acids (LCFA); first, the ratio between each FA and the total amount of FA having the same number of carbon atoms was calculated for both milk and feed; carry over, expressed as percentage, was calculated dividing the two ratios (modified from Sackmann *et al.*, 2003). The amount of dietary and milk FA were expressed as g/100 g of lipids, by using coefficients estimating the proportion of FA in feed and milk fat, respectively (Greenfield and Southgate, 1992; Schauff *et al.*, 1992). The carry over (%) of C18:0 is determined for example as $[100 \times (C18:0 / \sum C18 \text{ in milk}) / (C18:0 / \sum C18 \text{ in feed})]$. As suggested by Bailoni *et al.* (2004), the Δ^9 -desaturase

activity index (D9DI) in the mammary gland was calculated according to the formula:

$$D9DI = 100 \times [(C_{14:1} + C_{16:1} + C_{18:1} + CLA) / (C_{14:1} + C_{16:1} + C_{18:1} + CLA + C_{14:0} + C_{16:0} + C_{18:0} + \text{trans-11 } C_{18:1})]$$

Statistical analysis

After verifying the normality and variance homogeneity (PROC UNIVARIATE and Shapiro-Wilk test), data were analyzed using variance component (VC) ANOVA within PROC MIXED (SAS, 2002). The linear model used for data processing of productive and qualitative variables was

$$Y_{ijkl} = \mu + C_i + P_j + D_k + PD_{jk} + e_{ijkl}$$

Where: μ =overall mean, C_i =random effect of cow ($i=1$ to 6), P_j =fixed effect of period ($j=1$ to 2), D_k =fixed effect of diet ($k=1$ to 2), PD_{jk} =interaction of period and diet, and e_{ijkl} =random residual error.

Results and discussion

Feed intake, productive performance and metabolic profile

The dietary treatment did not affect DMI

which resulted 18.6 kg/d on average (Table 4). As expected, this level of DMI was slightly lower than data observed in other comparable studies (Abel-Caines *et al.*, 1998; Bailoni *et al.*, 2004) and most likely this was related to the high level of NDF as reported by Egger *et al.* (2007). The NDF intake was not affected and averaged 1.06% of BW; this finding was lower than the value reported by Rayburn and Fox (1993) and the level of intake (1.1-1.2 NDF Intake %BW) indicated by Mertens (1987). We do not know exactly the cause of this lower than expected intake, but the trial was run in late spring - early summer and cows had started showing signs of moderate heat stress. The degree of selection between diets did not show any variations, confirming the preference for smaller particles, *i.e.* concentrates (Figure 1). The dietary treatment significantly modified some blood parameters (Table 5); in comparison to the C-diet, the E-diet led to lower content of albumin (35.4 *vs* 34.1 g/l; $P < 0.05$), urea (4.48 *vs* 4.12 mmol/l; $P < 0.05$), GGT (24.5 *vs* 22.6 U/l; $P < 0.05$) and Mg (1.05 *vs* 1.01 mmol/l; $P < 0.05$). These differences were negligible; in fact the metabolic profile was in agreement with the values related to healthy lactating dairy

Table 4. Feed intake and milk yield.

		Treatment		SEM	P
		C-diet	E-diet		
Intake (day ⁻¹ cow ⁻¹):					
DM	kg	18.6	18.5	0.6	ns
NDF	%BW	1.04	1.09	0.04	ns
Yield (day ⁻¹ cow ⁻¹):					
Milk	kg	31.3	31.8	1.1	ns
FCM 3.5%	"	31.8	30.8	1.2	ns
Crude Protein	g	1044	1044	38	ns
Fat	"	1125	1055	51	ns

FCM: fat corrected milk (3.5%); ns: not significant.

NDF intake=(DM intake x NDF %DM)/BW.

Figure 1. Selection Index (>1 active selection; =1 no selection; <1 active avoidance).

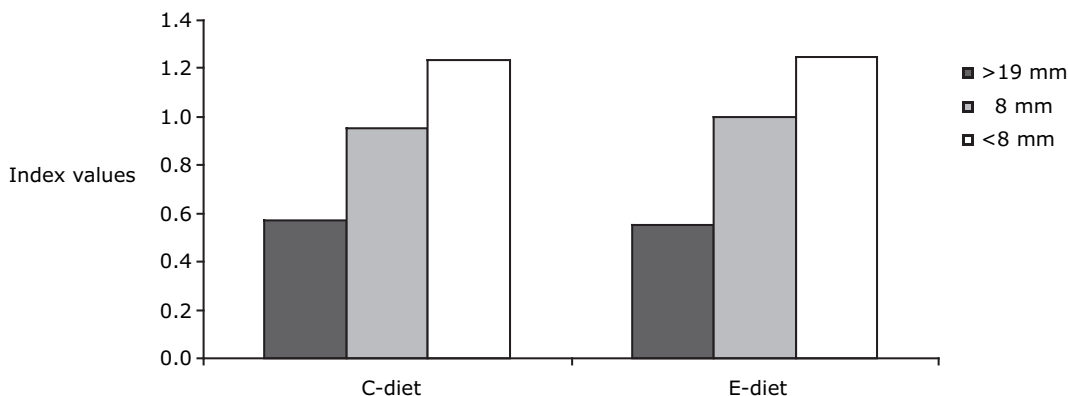


Table 5. Plasma metabolic profile.

		Treatment		SEM	P
		C-diet	E-diet		
Total protein	g/l	76.3	75.3	1.3	ns
Albumin	"	35.4	34.1	0.6	*
Urea	mmol/l	4.48	4.12	0.16	*
Glucose	"	2.72	2.80	0.07	ns
Total cholesterol	"	4.50	4.40	0.28	ns
Triglycerides	"	0.14	0.16	0.02	ns
NEFA	"	0.22	0.23	0.02	ns
AST	U/l	79.1	76.6	3.9	ns
GGT	"	24.5	22.6	1.7	*
CK	"	169	152	10	ns
Ca	mmol/l	2.47	2.47	0.02	ns
P	"	1.55	1.66	0.07	ns
Mg	"	1.05	1.01	0.01	*

*: $P < 0.05$; ns: not significant.

cow conditions (Aubadie-Ladrix, 2004). In addition, the higher plasma urea content of C-diet fed cows did not result in any differences in milk urea nitrogen (MUN). As expected, the fixed effect period in the sec-

ond phase of the trial significantly reduced the fat corrected milk (FCM) as result of a lower DMI. The diet per period interaction did not affect production, metabolic parameters nor milk quality.

Table 6. Effect of the experimental diets on milk components and cheese-making traits.

		Treatment		SEM	P
		C-diet	E-diet		
Milk composition:					
Fat	%	3.61	3.32	0.12	ns
Protein	"	3.35	3.30	0.07	ns
Casein	"	2.59	2.46	0.06	ns
Lactose	"	5.00	4.81	0.06	*
Urea	mg/100 ml	24.42	24.08	1.32	ns
Clotting parameters:					
Freezing point	°C	-0.546	-0.535	0.003	*
Clotting time, r	min	18.0	19.3	1.0	ns
Curd firming time, K ₂₀	"	4.50	4.77	0.35	ns
Curd firmness, a ₃₀	mm	35.5	31.8	2.3	ns
Titrate acidity	°SH/50 ml	4.10	4.07	0.14	ns

*: $P < 0.05$; ns: not significant.

Milk yield and composition and parameters related to cheesemaking potential

Milk yield and milk composition were not significantly affected by treatments (Table 4 and 6), confirming results found by other studies (Abel-Caines *et al.*, 1998; Kelly *et al.*, 1998; Thangavelu *et al.*, 2007) that did not reveal any changes in productivity in cows fed with different fat sources.

The only exception was the lactose level, which was lower in the E-diet (5.00 vs 4.81%; $P < 0.05$); the cause of this difference is not clear. Regardless of diet treatment milk was characterized by a moderate fat concentration possibly because of the low energy level of diets (0.82 milk FU/kg DM). As respects milk technological properties, the treatment had no significant effect except for the freezing point (-0.546 vs -0.535 °C; $P < 0.05$). Freezing point of milk is an indirect measure of the osmotic pressure and is dependent on the concentration of water-soluble constituents; the addition of a solute

to any solvent depresses the freezing point. As respects milk, lactose and chlorides account for almost 75-80% of the total freezing point depression. The freezing point of milk is determined primarily to prove milk adulteration with water and/or to determine the amount of water added; in compliance with EU regulations its limit value should be ≤ -0.520 °C (Navrátilová *et al.*, 2006).

Milk fatty acid profile

Significant differences between treatments were found in the FA milk fat composition (Table 7). As expected the highest content in UFA of E-diet had a marked effect on FA profile leading to a decrease in SFA (71.3 vs 68.8%; $P < 0.01$) and consequently to an increase in PUFA (4.36 vs 5.90%; $P < 0.01$). With regard to the specific groups, the E-diet significantly increased the amount of CLA (0.50 vs 0.62%; $P < 0.05$), PUFA of n-3 (0.76 vs 0.93%; $P < 0.001$) and n-6 series (2.80 vs 3.87%; $P < 0.01$).

Table 7. Effect of the experimental diet on fatty acid composition of milk (% of the total detected FA).

		Treatment		SEM	P
		C-diet	E-diet		
Fatty acid (FA):					
C4:0	% total FA	2.38	2.35	0.13	ns
C6:0	"	1.88	1.91	0.06	ns
C8:0	"	1.15	1.16	0.07	ns
C10:0	"	2.60	2.78	0.09	ns
C12:0	"	3.38	3.65	0.08	*
C14:0	"	11.6	12.0	0.17	ns
C14:1	"	1.06	1.11	0.06	ns
C16:0	"	36.2	32.5	0.9	**
C16:1	"	1.43	1.30	0.06	ns
C18:0	"	11.4	11.5	0.4	ns
C18:1 n-9	"	19.9	20.6	0.37	ns
C18:1 n-7	"	1.54	1.95	0.06	***
C18:2 n-6	"	2.54	3.68	0.16	***
C18:3 n3	"	0.69	0.88	0.02	***
CLA	"	0.50	0.62	0.03	*
Saturated fatty acids	"	71.3	68.8	0.6	**
Monounsaturated fatty acids	"	24.3	25.3	0.5	ns
Polyunsaturated fatty acids	"	4.36	5.90	0.21	***
Total n-3	"	0.76	0.93	0.02	***
Total n-6	"	2.80	3.87	0.16	**
n-6/n-3		3.68	4.16	0.09	**
D9DI		27.4	28.9	0.61	*

D9DI: $\Delta 9$ -desaturase activity index.

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

According to previous studies (Kelly *et al.*, 1998; Dhiman *et al.*, 1999; Petit, 2002; Ponter *et al.*, 2006; Thangavelu *et al.*, 2007), feeding a supplement of UFA and, in particular, rich in oleic, linoleic and linolenic acids lead to an increase in UFA in milk. In our study the milk FA profile was also influenced by the

use of cracked and toasted soybean seeds as a partial source of fat in the E-diet; the natural protection of seeds and heat treatment could be adequate to prevent a rapid release of oil and thus biohydrogenation of long-chain fatty acids (Abel-Caines *et al.*, 1998; Petit, 2002; Petit, 2003; Bailoni *et al.*,

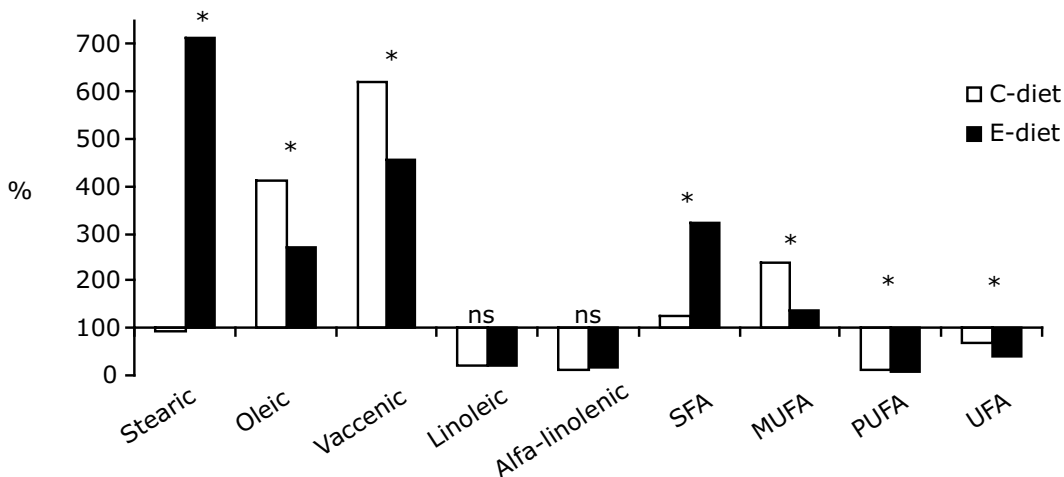
2004; Petit and Twagiramungu, 2006).

However the amount of UFA in milk in the experimental thesis resulted lower than our expectation showing a high level of FA BH. In order to evaluate the amount of UFA lost during the rumen BH process the degree of dietary FA carry over in milk was calculated (Sackmann *et al.*, 2003). The dietary FA metabolism involves two steps: the BH in rumen and the desaturation at mammary gland level (Kelly *et al.*, 1998; Baumann *et al.*, 1999; Corl *et al.*, 2001). The first is responsible for UFA saturation and *trans* FA (tFA) production, the second leads to the presence in milk of many MUFA, especially oleic and vaccenic acids, and of *cis*-9, *trans*-11 CLA.

As reported in Figure 2, in comparison to the control diet, the experimental one was associated with almost a threefold increment of SFA (124 vs 323%; $P < 0.001$), mostly due to a peak in the production of stearic acid (91 vs 714%; $P < 0.001$), which is the fi-

nal product of ruminal BH of UFA and in particular of linoleic acid. In the experimental thesis there was also an increase of the percentage of palmitic acid (Table 7), but this result is partially related to its *de novo* synthesis. PUFA showed low values of carry over (<100%) due to the saturation process (13.5 vs 9.8%; $P < 0.001$), among them only linoleic acid presented a slight difference between diets (21.5% vs 19.3%; $P = 0.07$). MUFA showed a consistent carry over; oleic acid increased 4.1- and 2.7-fold ($P < 0.001$) in the C- and E-diet, because of the stearic acid desaturation at the mammary gland level, which contributed more than 50% (Bailoni *et al.*, 2004) and because of direct adsorption from *duodenum* (Petit, 2002). Vaccenic increased 6.2- and 4.5-fold ($P < 0.001$); its concentration in milk depended both on its level in the gut and on its conversion in CLA in the udder (Corl *et al.*, 2001; Antongiovanni *et al.*, 2003). In this study, the percentage of CLA in milk was quite low for both diets

Figure 2. Fatty acid carry over (%) in milk.



*: $P < 0.001$; ns: not significant.

(Table 7) when compared with other studies (0.7-1.3%) (Kay *et al.*, 2005; Pottier *et al.*, 2006; Egger *et al.*, 2007). This was probably due to the high level of dietary fibre which leads to a high ruminal pH which, as suggested by Piperova *et al.* (2002), is related to a lower production of BH intermediate compounds; this means a low vaccenic acid supply at duodenal level. Griinari *et al.* (2000) and Corl *et al.* (2001) stated that 64% to 78% of CLA in milk fat originates from desaturation of *trans*-11-vaccenic acid present in the gut and this finding is possibly related to a consistent reduction of *trans*-octadecenoic acids (e.g. vaccenic) to stearic acid, the last of the three main reactions of FA biohydrogenation present at the ruminal level (Trogeler-Meynadier *et al.*, 2006).

The E-diet showed a slight but significantly higher value of Δ^9 desaturation index (D9DI) than C-diet (27.4 *vs* 28.9; $P < 0.05$) (Table 7), partially explaining both the higher content of CLA and the lower carry over of vaccenic acid in milk of the experimental thesis; however according to Fievez *et al.* (2003) the variation of the mammary gland Δ^9 -desaturase activity resulted less relevant

than vaccenic acid supply among factors influencing CLA synthesis.

Conclusions

According to our results it could be stated that feeding high productive dairy cows with a supplement based on cracked and toasted soybean seeds, in comparison with a supplement rich in SFA, did not affect milk yield and milk composition, but led to a higher concentration of PUFA and CLA.

The UFA and, in particular, the PUFA and CLA levels in milk were, however, below our expectation probably because the biohydrogenation process resulted in their low carry over and in a high production of stearic acid. Feeding cracked and toasted soybean seeds in the presence of high fibre diets did not seem to give a high level of protection against UFA biohydrogenation in the rumen.

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