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Inclusion of fresh forage in the ration for dairy cows: effects on CLA and *trans* C18:1 isomers content of milk fat

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RIASSUNTO – Inclusione di foraggio fresco in razioni miscelate per vacche da latte: effetto sul contenuto di CLA e di isomeri *trans* C18:1 nel grasso del latte. *In un disegno sperimentale a quadrato latino 2X2, sei vacche pluripare di razza Frisona Italiana al sesto mese di lattazione sono state alimentate con una dieta basata su foraggio fresco e un'integrazione di farina di mais (dieta F) oppure con una dieta basata su fieno di erba medica e insilato di mais, integrati con farina di mais, farina di estrazione di soia e semi interi di soia estrusi (dieta S). La dieta S ha consentito di ottenere livelli medio-alti di acido linoleico, CLA, acido vaccenico e acido oleico (rispettivamente 3,31, 0,91, 1,77, 16,54 g/100 g lipidi totali). La dieta F, invece, non ha dato i risultati attesi. I contenuti di CLA, di acido vaccenico e di acido linolenico, infatti, sono risultati bassi (rispettivamente 0,24, 0,64 e 1,06 g/100 g lipidi totali).*

Key words: milk, cows, quality, soybean oil.

INTRODUCTION – Milk fat is the richest natural source of conjugated linoleic acid (CLA) isomers. The 9-*cis*, 11-*trans* CLA isomer (rumenic acid, RA) originates from two pathways: as an intermediate of the rumen biohydrogenation process of linoleic acid or as the product of the activity of mammary Stearoyl Co-A desaturase enzyme (SCD) with *trans*-11, C18:1 (vaccenic acid, VA) as the precursor, another intermediate in the biohydrogenation of linoleic and linolenic acid. Depending on dietary regimen based on preserved forages, pasture, concentrates and plant oil supplement the range of milk CLA concentration is very large (Schroeder *et al.*, 2004). This variability is due to several factors: source of dietary fat (plant oil rich in linoleic acid or pasture rich in linolenic acid), dietary forage/concentrate ratio, individual difference in SCD activity and so on (Kelsey *et al.*, 2003). Among dietary factors that affect milk fat composition, pasture based diets resulted in milk with higher levels of CLA, in comparison to milk from cows fed total mixed ration (TMR) (Schroeder *et al.*, 2004). Few data are available about the effects of fresh forage on milk fatty acid composition offered to dairy cows without grazing. Aim of this paper was to compare a fresh forage based diet with a TMR diet containing extruded whole soybean seeds as the major source of dietary lipids.

MATERIAL AND METHODS – Six multiparous Italian Friesian cows at their sixth month of lactation were randomly allocated to two treatments in a 2x2 Latin square design with three replications. Treatments were: Lucerne + Oats + White Clover offered as fresh forage + maize meal and a mineral-vitamins integrator (diet F) and maize meal + soybean meal + extruded whole soybean seed + Lucerne hay + maize silage + a mineral-vitamins integrator (diet S), offered as TMR. Fresh pasture was cut once daily and offered to cows. The

composition and chemical analysis of experimental diets are reported in table 1. At the end of each experimental period, individual milk samples were collected. Milk samples were analysed for fat content and fatty acid composition. Fat of milk samples was extracted and *trans*-methylated, then methyl esters of fatty acids were analysed by a chromatograph fitted with a FID detector and equipped with a 100 m fused silica capillary column. The analysis involved a programmed run with temperature ramps. Nonadecanoic acid methyl ester was used as the internal standard. Statistical analysis was performed using a linear model including the fixed effects of: diet, replicate, period within replicate and cow within replicate.

Table 1. Composition and chemical analysis of the experimental diets (as% DM).

	Diet F	Diet S
Maize meal kg	42.3	17.5
Soybean meal		4.4
Extruded whole soybean seeds		17.2
Lucerne hay		26.8
Maize silage		31.7
Oat + White Clover	23.2	
Lucerne	32.0	
Mineral and vitamin integrator	2.5	2.4
Crude protein	17.9	18.0
Crude fat	3.7	5.3
Neutral detergent fibre	26	39
Non fibrous carbohydrates	46.1	34.3

RESULTS AND CONCLUSIONS – Several studies report that pasture based diets increase the linolenic acid (LNA), VA and CLA content, although the total lipid content of these diets is quite low (Schroeder *et al.*, 2004). In this case, the increase in milk CLA concentration is probably due to high ruminal production of VA derived from LNA in pasture and its endogenous conversion to CLA by mammary SCD (Kay *et al.*, 2004). High levels of milk CLA content were reported also when cows were fed TMR diets added with full fat extruded soybeans (ESB) (Chouinard *et al.*, 2001). In our study we compared a fresh forage based diet rich in LNA (diet F) with a TMR diet based on preserved forage and ESB, rich in linoleic acid (LA). As expected, the milk fatty acid composition of cows fed diet S resulted in higher levels of LA than milk from cows fed diet F (table 2). Moreover, when cows were fed diet S, the CLA and VA content of milk agreed with literature data (Chouinard *et al.*, 2001). Unexpectedly, diet F did not allow to increase LNA content of milk and resulted in low levels of LNA, VA and CLA in milk. In particular, the level of CLA in milk from the cows fed diet F was lower than that reported either for cows feeding only on pasture (2.21 g/100 fatty acids), or for cows fed one-third pasture with supplemental feed (0.89 g/100 g fatty acids; Dhiman *et al.*, 1999).

Similar levels of milk CLA have been reported for dairy cows reared in the Parmigiano Reggiano area, where, during Spring season, cows are fed diets based partly on fresh forage (Secchiari *et al.*, 2003). These results, however, do not agree with those reported by Kay *et al.* (2004), who obtained higher level of CLA in milk from cows fed pasture only (1.21 g/100 g fatty acids), by offering twice daily fresh forage for *ad libitum* intake to cows. This difference may be related to cut and carry method used in our study, which may have favoured oxidation processes after the pasture cut. In fact, LNA, which is the main fatty acid in plant leaves, represents the most common substrate of plant lipoxygenases (Porta and Rocha-Sosa, 2002). If the interval between cut and feed supply is too large, these enzymes may oxidize an aliquot of LA and LNA and reduce the amount of ruminal precursor for the biohydrogenation process that leads to VA accumulation. As a consequence, the fatty acid profile of milk from cows fed diet F was similar to that of milk from cows fed diets based on preserved forage. In a similar way to what reported for RA, also the other CLA isomers were higher in milk from cows fed diet S, with the exception of *trans*, 10, *cis* 12 CLA that resulted higher in milk of cows fed diet F (table 2). The high level of maize meal in diet F may contribute to explain this result, because it may have affected ruminal

Table 2. Selected fatty acids composition of milk from cows fed experimental diets.

	Fatty acids	Diet F	Diet S	SD	P
C4:0	g / 100 g total lipids	3.55	3.66	0.069	
C6:0	""	0.77	0.72	0.02	
C8:0	""	1.52	1.44	0.046	
C10:0	""	4.92	4.42	0.362	
C12:0	""	5.27	4.6	0.18	
C14:0	""	14.99	13.4	0.345	*
C14:1	""	1.55	1.43	0.106	
C15:0	""	1.58	1.17	0.095	*
C16:0	""	29.3	24.93	1.25	*
C16:1	""	1.32	1.24	0.144	
C18:0	""	6.66	7.6	0.162	*
C 18:1 t9	""	0.13	0.32	0.019	*
C 18:1 t11	""	1.06	1.77	0.11	*
C18:1 c9	""	14.68	16.54	0.249	*
C18:2 c9/c12	""	1.43	3.31	0.141	*
C18:3 w 3	""	0.24	0.35	0.012	*
CLA 7c/9t	mg / g total lipids	0.22	0.68	0.05	*
CLA 9c/11t	""	5.9	8.2	0.085	*
CLA 10c/12t	""	0.17	0.05	0.02	*
CLA 10t/12t	""	0.05	0.08	0.003	*
CLA 11t/13t	""	0.09	0.07	0.009	
Total CLA	""	6.43	9.08	0.167	*

* P<0.05.

environment and caused a shift of biohydrogenation process of LA toward *trans*, 10, *cis*, 12 CLA accumulation (Loor *et al.*, 2002). In conclusion, in order to obtain a modification of milk fatty acid composition by including fresh forage in diet, high attention should be dedicated to the cut and carry methods adopted, during both the harvest and supply of fresh pasture. The inclusion of ESB in diet for dairy cows confirms its effectiveness in order to improve milk fatty acid composition toward more desirable characteristics for human health.

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