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Original article

Effect of diet on the fatty acid pattern of milk from dairy cows

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Abstract – Twelve dairy cows 130 days in milk were sorted by milk production and body weight and assigned to three feeding regimens in a 3 × 3 Latin-square design, in order to study the effects of diet on milk fatty acid (FA) composition. The cows were fed a total mixed ration (TMR) consisting of corn silage (60%) and concentrate (40%) on dry matter basis, or grazed pasture, without (P) or with 5 $kg \cdot d^{-1}$ concentrate as a supplement (SP). Supplemented grazing dairy cows produced significantly more milk than the cows on the TMR and P diets ($P < 0.05$). The supplementation of grazing dairy cows with a low fat concentrate did not significantly affect the milk fat FA profile. The pasture diet, with a supplement or not, decreased the concentration of saturated FA ($P < 0.05$) and increased the concentration of unsaturated FA ($P < 0.05$), of milk fat as compared to the TMR diet. The reduction in medium-chain FA was offset in large part by increases in long-chain FA (mainly oleic acid). The concentrations of conjugated linoleic acid (CLA) ($P < 0.05$) and *trans*-vaccenic acid were higher ($P < 0.05$) in the milk fat from the grazing cows. The results showed substantial variation among individual cows within treatments on milk fat content of CLA. Significant correlations were found for individual cow's milk fat CLA content across diets. Overall, this study indicates that the concentration of CLA in milk fat is enhanced by the dietary intake of pasture and that moderate low fat concentrate supplementation of grazing dairy cows increases performance without compromising the FA profile of milk fat.

conjugated linoleic acid / dairy cow / fatty acid / milk fat / pasture / total mixed ration

Résumé – **Effet du régime alimentaire sur la composition en acides gras de la matière grasse du lait chez la vache.** Douze vaches laitières, en lactation depuis 130 jours, ont été réparties en trois lots sur la base de la production laitière et du poids vif. Elles ont reçu trois régimes alimentaires, selon un carré latin 3 × 3, afin d'étudier leurs effets sur la composition en acides gras (AG) du lait. Les trois régimes étaient une ration complète (TMR) composée d'ensilage de maïs et de concentré (respectivement 60 % et 40 % sur la base de la matière sèche), de l'herbe pâturée sans

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complémentation (P), ou de l'herbe pâturée complémentée avec 5 kg par jour de concentré (SP). Les vaches laitières complémentées au pâturage (SP) ont produit significativement plus de lait que celles qui ne disposaient que du pâturage ou qui recevaient la ration complète. Le pâturage, complémenté ou non, a diminué la concentration de la somme des AG saturés et a augmenté celle des AG insaturés ($P < 0,05$) dans les lipides du lait. La réduction de la concentration des AG à chaîne moyenne a principalement été compensée par une augmentation de celle des AG à chaîne longue (acide oléique surtout). La concentration de l'acide linoléique conjugué (CLA) dans les lipides du lait, et celle de l'acide *trans*-vaccénique ont été plus élevées ($P < 0,05$) quand les vaches étaient au pâturage que quand elles recevaient le TMR. Les résultats ont montré une variation notable entre vaches et intra régime de la teneur en CLA des lipides du lait, ainsi que des liaisons significatives individuelles entre les régimes. Notre étude a montré que la teneur en CLA des lipides du lait est accrue par l'ingestion d'herbe fraîche et qu'une complémentation modérée en concentré augmente la production laitière sans compromettre le profil en AG.

acide linoléique conjugué / vache laitière / acide gras / pâturage / ration totale mixte

1. INTRODUCTION

In the Azores, the temperate Atlantic climate presents excellent conditions for the implantation of improved pastures allowing milk production to be heavily based on grazing almost all year round. The milk fat produced from the pasture has long been known to be rich in unsaturated fatty acids including *trans*-octadecenoic acids and conjugated isomers of linoleic acid (CLA) [21]. Fatty acid (FA) composition allied with higher contents of carotene [18] and its association by consumers with ecological and safe natural products may potentially increase the health and market value of these pasture produced milks.

Ruminant milk and fat tissues are the richest sources of the isomer $C_{18:2}$ *cis*-9, *trans*-11, the main CLA isomer, which is produced by rumen bacteria during the biohydrogenation of linoleic acid [2] and from Δ^9 desaturation of *trans*-vaccenic acid ($C_{18:1}$ *trans*-11) in the tissues of rodents, ruminants and humans [9]. *Trans*-vaccenic acid is also produced in the rumen during the biohydrogenation of linoleic and linolenic acids [8]. In recent years, the biological proprieties of CLA have been under intensive investigation and it is now well established that they have potent anticarcinogenic effects, modulate immunity, cell differentiation and lipid metabolism [17]. Ip et al. [10] demonstrated that the isomer $C_{18:2}$ *cis*-9, *trans*-11, supplied as a natural constituent of butter manufactured from CLA enriched milks, reduces by over 50% the incidence of mammary tumours in rats. Therefore, the increase in the concentration of this FA in milk can probably exert a beneficial effect on public health.

The concentration of CLA in cow milk fat can be increased by nutritional manipulation, particularly by polyunsaturated lipid supplementation [4]. The reason why the pasture increases the CLA content of milk so clearly is not fully understood. However, it should be considered that pasture contains high levels of polyunsaturated FA (mainly linolenic acid), that certainly contribute as precursors of CLA and $C_{18:1}$ *trans*-11.

The supplementation of grazing dairy cows with concentrates is a common practice and it is recommended when pasture quantity or quality are limiting factors. Feeding dairy cows on pasture instead of feeding a total mixed ration (TMR), enhances the concentration of CLA in milk fat [12]. However, the information on the effect of a low fat concentrate, usually used in the supplementation of grazing dairy cows on milk FA pattern and particularly on CLA is not well documented. The objective of this study was, therefore, to investigate the effect of feeding regime (TMR vs. pasture with or without concentrate supplementation) on the milk fat FA profile of dairy cows.

2. MATERIALS AND METHODS

2.1. Animals, feeds and management

The experiment was conducted at the Department of Agriculture Sciences, University of Azores with the European Union animal welfare directive number 86/609/EEC. Twelve multiparous Holstein cows in lactation were blocked based on daily milk yield (33 ± 7.9 kg), body weight (542 ± 56 kg) and days in milk (130 ± 72 d) and were randomly assigned to three treatments, in a 3 × 3 Latin-square design. Experimental periods lasted 22 days during which the cows were submitted to 3 feeding regimes: (1) TMR – total mixed ration consisting of 60% corn silage and 40% concentrate A on a DM basis, (2) P – allowed to graze pasture alone, (3) SP – allowed to graze pasture supplemented with 5 kg per day of concentrate B. The ingredients ($g \cdot 100\ g^{-1}$ fresh weight) of the concentrates A and B were respectively 40 and 35 for barley, 5 and 24 for corn, 43 and 16 for sunflower meal, 0 and 20 for corn gluten feed, 7 and 0 for fish meal and 5 and 5 for a mineral and vitamin premix. The collection of samples and data were made during the last 7 days of each experimental period. In the TMR treatment group, the animals were fed twice daily in individual pens with ad libitum access to feed, allowing 10% orts. The cows on treatments P and SP grazed as a group (stocking rate at 2.5 cows per ha) on a leafy spring ryegrass and white clover pasture. The cows on the SP treatment received 2.5 kg of concentrate B twice a day in the milking room. Pasture intake was estimated from animal performance [16]. The cows were weighed on two consecutive days following a.m. milking at the end of each period. Twenty plucked samples per hectare of pasture were randomly taken daily over the last 7 days of each period and mixed in order to obtain a composite sample. These samples were then stored at –20 °C until assay. Similar procedures were applied to TMR samples. The samples of feed were analysed for DM, crude protein (CP), crude fat (CF) and ash according to AOAC [1] and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Robertson and Van Soest [22]. In vitro DM digestibility was analysed by the two-stage technique of Tilley and Terry [27]. The chemical composition, in vitro DM digestibility and FA composition of all feeds are presented in Table I.

Table I. Chemical and FA composition of concentrates A and B, total mixed ration (TMR) and pasture (P).

	Conc. A	Conc. B	TMR	Pasture
Chemical composition	(% DM)			
CP	24.6	16.8	15.6	26.4
ADF	16.0	10.8	24.6	22.3
ADL	–	–	4.2	2.0
CF	2.2	2.4	2.6	4.4
In vitro DMD	–	–	75.4	80.8
Fatty acids (FA)	($g\ 100 \cdot g^{-1}$ FA)			
$C_{14:0}$	2.4	2.0	2.7	5.5
$C_{16:0}$	16.8	15.3	17.7	8.7
*cis*9-$C_{16:1}$	–	–	0.7	1.6
$C_{18:0}$	2.8	3.2	3.4	1.4
*cis*9-$C_{18:1}$	20.8	22.7	15.3	2.2
$C_{18:2}$ n-6	47.2	48.6	38.1	6.7
$C_{18:3}$ n-3	3.0	2.6	6.3	48.9

DMD: dry matter digestibility; ADF: acid detergent fibre; ADL: acid detergent lignin; FA: fatty acid; CP: crude protein; CF: crude fat; Conc.: concentrate.

Milk samples were collected from two consecutive milkings on the last day of each period and mixed according to milk production. Milk composition (fat, protein) was determined by automated infrared analysis using a Milkoscan 605 (Foss Electric, Hillerod, Denmark). Another set of feed and milk samples were stored at – 20 °C until analysis for FA composition.

2.2. Fatty acid analysis

Feed samples were weighed into a culture tube, and FA methyl esters were prepared by the one-step extraction-methylation method of Sukhija and Palmquist [26]. Quantification of FA was done using 4 mg of 17:0 as the internal standard.

The milk samples were lyophilised and 125 mg of dry solids were then extracted by chloroform:methanol (2:1) according to Folch et al. [7]. After lipid extraction and evaporation of solvents by nitrogen flow in a 30 °C dry bath, fatty acid methyl esters (FAME) were prepared by alkaline transesterification with methanolic KOH [5]. FAME were analysed by gas chromatography (GC), using a 60-m fused silica capillary column SP-2380 (Supelco, Bellefonte, PA, USA) with 0.25-mm internal diameter and 0.20-µm film thickness. A HP5890A series II chromatograph (Hewlett-Packard, Avondale, PA, USA) working with nitrogen as the carrier gas and a flame ionisation detector was used. The initial column temperature of 130 °C was held for 10 min, increased to 165 °C at 5 °C per min and held for 5 min. Then, the temperature was increased to 180 °C at 3 °C per min and held for 6 min. Then the temperature was increased at a rate of 1.5 °C per min until 230 °C, where it remained for 25 minutes. The injector and detector temperatures were 230 °C and 280 °C, respectively. Peak identification was based on co-chromatography with known standards of FAME (Sigma, St. Louis, USA). The *trans*-$C_{18:1}$ isomers are reported as one value, since this column incompletely resolves them, and we cannot exclude some minor contamination with other 18:1 isomers. The CLA was computed as the major peak in the conjugated octadecadienoic region of the chromatogram that had an elution time consistent with *cis*-9,*trans*-11 octadecadienoic FAME (Sigma, St. Louis, USA). FA were expressed as g·100 g^{-1} of reported fatty acids.

Table II. Dry matter intake, milk production, milk composition and body weight of cows fed total mixed ration (TMR), pasture (P) and concentrate supplemented pasture (SP).

	Diet			
	TMR	P	SP	SEM
DMI (kg·d^{-1})	21.0	17.8	19.5	0.61
Milk yield (kg·d^{-1})	25.0^{a}	24.1^{a}	28.5^{b}	0.44
Milk fat (g·kg^{-1})	41.7^{a}	41.1^{a}	38.1^{b}	0.40
Milk fat yield (kg·d^{-1})	1.04	0.99	1.09	0.02
Milk protein (g·kg^{-1})	32.9^{ab}	32.0^{b}	33.7^{a}	0.21
Milk protein yield (kg·d^{-1})	0.82^{a}	0.77^{a}	0.96^{b}	0.01
Body weight (kg)	580	552	561	7.44

On each line, the values with different letters are significantly different ($P < 0.05$).
DMI: dry matter intake.

2.3. Statistical analyses

Data were analysed as a replicated 3 × 3 Latin-square using the GLM procedure of SAS [23] with the following model: $Y_{ijk} = \mu + T_I + P_j + A_k + \varepsilon_{ijk,}$ where Y_{ijk} is the dependent variable, μ is the global mean, T is the treatment effect, P is the period effect, A is the animal effect and ε_{ijl} is the residual error. The values presented are least square means followed by standard error of mean (SEM).

3. RESULTS AND DISCUSSION

Concentrates A and B had very similar FA profiles, despite the differences in formulation and chemical composition (Tab. I). The in vitro DM digestibility of pasture was 5% higher than the TMR diet.

Data of DM intake, milk production, milk composition, and body weight of cows have been previously presented and discussed by Sousa et al. [25] and can be seen in Table II. Dry matter intake did not differ significantly between treatments. The estimated intake of metabolisable energy (ME) was 220, 206 and 232 MJ per day, respectively, for TMR, P and SP cows, which can explain the differences in animal performance. Supplemented grazing dairy cows produced significantly ($P < 0.05$) more milk (16% with a response of 0.88 kg milk·kg^{-1} concentrate) with lower fat (resulting in no effect on fat yield) and higher protein contents. These results are consistent with the greater nutritive value (more ME·kg DM^{-1}) of grazed pasture relative to the TMR diet (Tab. I), but contrast with the results of Kolver and Muller [13].

The effect of the treatments on the FA profile of milk fat is present in Table III. The concentrate supplementation of grazing dairy cows (SP) did not exert a significant effect on the composition of principal FA of milk fat, including CLA and $C_{18:1}$ *trans*-11, relative to the P treatment (Tab. III). This can be explained by the fact that the low fat concentrate (Tab. I) supplemented to pasture in the SP treatment group represented only a small fraction (about 12%) of total lipid intake. Probably this amount of supplemented lipids, mainly as linoleic acid, is not enough to modify the rumen environment and FA biohydrogenation pattern. Pasture based diets (P and SP) decreased by 32% the amount of hypercholesterolemic saturated FA on milk fat (sum of $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) and increased the amount of monounsaturated (sum of $C_{16:1}$ *cis*-9, $C_{18:1}$ *trans*-11 and $C_{18:1}$ *cis*-9) and polyunsaturated FA (sum of $C_{18:2}$ n-6, CLA and $C_{18:3}$) respectively by 33 and 43%, relative to the TMR diet ($P < 0.05$). The intake of pasture increased ($P < 0.05$) the linolenic acid content of milk fat almost 2.5 fold, in agreement with Kelly et al. [12] and Dhiman et al. [6]. Increases in concentrations of $C_{18:0}$ and $C_{18:1}$ *trans*-11 and $C_{18:1}$ *cis*-9 in milk fat and decreases in $C_{16:0}$ are typically observed in grazing cows, when compared to those fed TMR diets or conserved forages [3]. Rego and Almeida [20] observed that replacing pasture by maize and grass silage induces an increase of short and medium-chain FA in milk fat and a decrease of long-chain FA, particularly of oleic acid.

Table III. Fatty acid (g 100·g^{-1} FA) of milk fat from cows fed total mixed ration (TMR), pasture (P) and concentrate supplemented pasture (SP).

Fatty acids (FA)	Diet			SEM
	TMR	P	SP	
$C_{10:0}$	1.73^{a}	1.36^{b}	1.62^{ab}	0.06
$C_{12:0}$	2.92^{a}	2.24^{b}	2.66^{ab}	0.11
$C_{14:0}$	11.6^{a}	9.5^{b}	10.3^{ab}	0.29
$C_{15:0}$	1.17	1.05	1.28	0.04
$C_{16:0}$	37.1^{a}	27.4^{b}	26.0^{b}	1.37
*cis*9-$C_{16:1}$	1.53	1.29	1.23	0.05
$C_{17:0}$	0.77^{a}	0.93^{b}	0.95^{b}	0.02
$C_{18:0}$	11.4^{a}	14.5^{b}	13.7^{ab}	0.54
*cis*9- $C_{18:1}$	18.7^{a}	25.3^{b}	23.7^{b}	0.79
trans-$C_{18:1}$	2.07^{a}	3.74^{ab}	3.94^{b}	0.33
$C_{18:2}$ n-6	1.35^{a}	0.99^{b}	1.18^{b}	0.04
CLA	0.51^{a}	1.25^{b}	1.26^{b}	0.11
$C_{18:3}$ n-3	0.29^{a}	0.72^{b}	0.75^{b}	0.05
UFA	24.4^{a}	33.3^{b}	32.1^{b}	1.09
SFA	66.7^{a}	57.0^{b}	56.4^{b}	1.29
UFA:SFA ratio	0.37^{a}	0.58^{b}	0.57^{b}	0.03
MUFA	22.3^{a}	30.3^{b}	28.9^{b}	0.95
PUFA	2.15^{a}	2.96^{ab}	3.19^{b}	0.15

On each line, the values with different letters are significantly different ($P < 0.05$).
CLA: *cis*9,*trans*11-$C_{18:2}$ isomer;
UFA: sum of unsaturated fatty acids ($C_{16:1}$, *cis*9-$C_{18:1}$, *trans*-$C_{18:1}$, $C_{18:2}$, CLA, $C_{18:3}$);
SFA: sum of saturated fatty acids ($C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$);
MUFA: sum of monunsaturated FA ($C_{16:1}$, *cis*9-$C_{18:1}$, *trans*-$C_{18:1}$);
PUFA: sum of polyunsaturated FA ($C_{18:2}$, CLA, $C_{18:3}$).

Milk fat from cows grazing pasture (supplemented or not) had 2.5 times more CLA *cis*-9,*trans*-11 isomer than milk fat from the TMR diet (Tab. III). The effects of intake of pasture on CLA concentration in milk fat are now well established [4, 8]. Kelly et al. [12] reported that a diet exclusively based on pasture increases the levels of CLA in milk fat by 2.5 fold relative to TMR. In agreement with this, Dhiman et al. [6] found a linear increase in CLA levels in milk fat

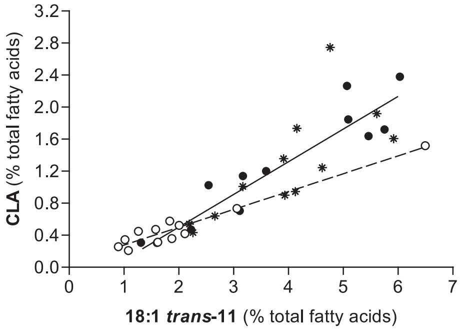

Figure 1. Relationships between the concentrations of $C_{18:1}$ *trans*-11 and CLA in milk fat from cows fed either TMR (o) or P (•) and SP (*).
Regression equations: TMR: $y = 0.22 \pm 0.017x + 0.05 \pm 0.044$, $n = 12$, $r^2 = 0.94$, $P < 0.0001$; P and SP: $y = 0.41 \pm 0.052x - 0.32 \pm 0.214$, $n = 24$, $r^2 = 0.74$, $P < 0.0001$.

when pasture is increased in the diet of dairy cows. A survey study of CLA content in the milk fat of ruminants, involving several farms, showed a typical seasonal variation, with a maximum concentration in the spring-summer, which coincides with the grazing period, and a minimum concentration in the winter when animals are kept indoors [11]. The reason why pasture grazing increases milk CLA so clearly is not fully understood. Fresh grass contains 1 to 3% FA, of which 55 to 65% is α-linolenic acid [3]. Linolenic acid pathways in the rumen are not known to involve CLA as an intermediate, although $C_{18:1}$ *trans*-11 is produced [8]. This FA is a known precursor of endogenous synthesis of $C_{18:2}$ *cis*-9, *trans*-11 in the lactating mammary gland [9]. Other specific factors affecting the rumen microbial ecology may contribute to CLA production when cows are switched to pasture.

A close relationship between concentrations of CLA (*cis*-9, *trans*-11) isomer and $C_{18:1}$ *trans*-11 in milk fat was observed in this study (Fig. 1). Slopes of the regression lines for P and SP treatments did not differ statistically and the data from both treatments were pooled together. The slope of the regression line obtained from TMR data was different from that obtained for P and for SP. The close relationship between CLA/$C_{18:1}$ *trans*-11 has been reported by several authors, using a great spectrum of diets, as reviewed by Chilliard et al. [4].

Despite the limitation of the experimental design, a high variation in milk fat CLA content can be inferred for cows within each treatment. The variation limits were, 0.21 to 1.52, 0.31 to 2.38 and 0.43 to 2.75 $g \cdot 100\ g^{-1}$ respectively for TMR, P and SP diets. Several studies [12, 14, 24], report a wide variation of CLA levels of milk fat among cows. The correlations between the milk fat CLA contents for individual cows across diets are shown in Table IV. The correlations were statistically significant for the TMR × P and TMR × SP diets and close to significance for the P × SP diets. Lawless et al. [15] showed significant correlations between levels of CLA in milk fat from individual cows within the same breed, in two distinct samplings, on pasture. On the contrary, Solomon et al. [24] observed that individual cows did not maintain the same relative rank in milk CLA concentration across diets. However, Peterson et al. [19] reported that the individual hierarchy for milk fat CLA content was maintained to a large extent over a 12-week study even in the variable treatment group that alternated between two diets. The physiological basis for the variation between individuals in CLA levels of milk fat and the individual rank kept

Table IV. Correlation coefficients between the CLA contents of milk fat of the individual cows across diets (n = 36).

Diets	SP	TMR
P	0.51 ($P < 0.09$)	0.65 ($P < 0.02$)
SP		0.75 ($P < 0.01$)

SP: concentrate supplemented pasture, TMR: total mixed ration, P: pasture.

for different diets has not been established, but may include differences related to both feeding patterns, rumen ecology and Δ^9 desaturase activity in the mammary gland. The results of the present study indicate that while diet is a major determinant of the CLA content in milk fat, individual cow variations should also be considered.

4. CONCLUSIONS

Milk fat produced from pasture had a FA profile that might be deemed more favourable by consumers, particularly the higher CLA and linolenic acid content and lower concentrations of saturated hypercholesterolemic fatty acids. The supplementation of grazing cows with a low fat concentrate at a moderate level simultaneously allowed the maintenance of the desirable milk fatty acid profile and an improvement in milk yield. It was concluded that concentrate supplementation of grazing cows may increase performance without compromising the fatty acid profile of milk fat, relative to milk fat produced exclusively from pasture.

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