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Original article

Increasing pasture intakes enhances polyunsaturated fatty acids and lipophilic antioxidants in plasma and milk of dairy cows fed total mix ration

Stefania La Terra[1]*, Vita Maria Marino[1], Mario Manenti[1], Giuseppe Licitra[1,2], Stefania Carpino[1]

[1] CoRFiLaC, Regione Siciliana, Ragusa, Italy
[2] DACPA Catania University, Catania, Italy

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Abstract – Polyunsaturated fatty acids and lipo-soluble vitamins in the milk are considered as neutraceutical compounds due to their beneficial effects on human health. The aim of the present study was to evaluate the changes in fatty acid composition and fat-soluble antioxidant content in plasma and milk from cows fed with different dietary proportions from pasture. Cows from a farm in the Hyblean mountain region in Italy were randomly divided into three groups (12 animals per group): CTRL fed only a total mix ration (TMR); 30P fed a TMR supplemented with 30% dry matter (DM) from pasture and 70P fed a TMR supplemented with 70% DM of pasture. Blood and milk samples were collected, stored and analysed for their content of fatty acids and fat-soluble antioxidants. Fatty acid profiles were significantly modified by different diets. CLA, vaccenic acid (VA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) significantly ($P < 0.05$) increased in plasma as a function of the proportion of pasture added to the diet. In agreement with these data, a progressively significant ($P < 0.05$) increase in concentrations of VA, CLA and EPA was observed in the milk. Such changes in fatty acid composition were accompanied by a concomitant increase in the concentrations of α-tocopherol and β-carotene in both plasma and milk. The increase in EPA, DHA and CLA, β-carotene and α-tocopherol in plasma may not only have a beneficial impact for milk and meat quality, but may also result in an increased protection against inflammatory events.

pasture / plasma / milk / PUFA / fat-soluble vitamins

摘要 – 增加牧草摄取量改善全混合日粮饲养奶牛血浆和乳中多不饱和脂肪酸和脂溶性抗氧化物质的含量。乳中的多不饱和脂肪酸 (PUFA) 和脂溶性维生素是对人体健康有益的微量化合物。 该研究主要评价饲喂不同百分比牧草的奶牛血浆和乳中脂肪酸和脂溶性抗氧化物质的含量变化。 将来自海布伦山脉地区的奶牛随机分为 3 组(每组 12 头)。 对照组 (CTRL) 仅仅喂养全混合日粮(TMR);30P 组喂养全混合日粮 (TMR) 还补充含 30% 干物质 (DM) 的牧草; 70P 组喂养全混合日粮 (TMR) 还补充 70% 干物质 (DM) 的牧草。 采集每头奶牛的血浆和乳样，贮藏后用于脂肪酸和脂溶性抗氧化物质含量的分析。 实验结果发现: 脂肪酸组成随着不同喂养条件发生显著变化。 当用不同比例干物质 (DM) 的牧草喂养奶牛后，奶牛血浆中共轭亚油酸 (CLA)，十八烷烯酸 (VA)，二十碳五烯酸 (EPA) 和

*Corresponding author (通讯作者): laterra@corfilac.it

二十二碳六烯酸 (DHA) 会显著性 ($P < 0.05$) 增加。 与这些数据一致的是，乳中的十八烷烯酸 (VA)，共轭亚油酸 (CLA) 和二十碳五烯酸 (EPA) 也显著的 ($P < 0.05$) 增加。 奶牛血浆和乳中的脂肪酸变化与其 α-维生素 E 和 β-胡萝卜素浓度的增加保持同步。 血浆中二十碳五烯酸 (EPA)、二十二碳六烯酸 (DHA)、共轭亚油酸 (CLA)、α-维生素 E 和 β-胡萝卜素含量的增加不仅对乳和奶牛肉的质量有益，而且对奶牛的健康也有益。 特别是奶牛组织中的共轭亚油酸 (CLA) 和 n-3 PUFA含量的增加能够阻止奶牛炎症疾病的发生。

牧草 / 血浆 / 乳 / 多不饱和脂肪酸 / 脂溶性维生素

Résumé – L'augmentation de la teneur en herbe de la ration améliore la teneur en acides gras polyinsaturés et en antioxydants lipophiles dans le plasma et le lait des vaches laitières en ration complète. Les acides gras polyinsaturés (AGPI) et les vitamines liposolubles du lait sont considérés comme des composés nutraceutiques en raison de leurs effets bénéfiques pour la santé. Le but de cette étude était d'évaluer les changements de composition en acides gras et de teneur en antioxydants liposolubles dans le plasma et le lait de vaches laitières recevant différentes proportions de pâture. Les vaches provenant d'une ferme de la région du mont Iblei en Italie, ont été réparties aléatoirement en trois groupes (12 animaux par groupe) : un groupe témoin recevant une alimentation en ration complète (TMR) ; un groupe recevant une alimentation TMR supplémentée à hauteur de 30 % de matière sèche en herbe ; un groupe recevant une alimentation TMR supplémentée à hauteur de 70 % de matière sèche en herbe. Des échantillons sanguins et du lait ont été collectés, conservés et analysés pour leur teneur en acides gras et en antioxydants liposolubles. Les profils en acides gras étaient modifiés de façon significative par les différents régimes. L'acide linoléique conjugué (CLA), l'acide vaccénique (VA), l'acide eisapentanoïque (EPA) et l'acide docohexanoïque (DHA) augmentaient significativement ($P < 0,05$) dans le plasma en fonction de la proportion de pâture. En accord avec ces résultats, une augmentation progressivement significative ($P < 0,05$) des concentrations en VA, CLA et EPA était observée dans le lait. De tels changements dans la composition en acides gras étaient accompagnés d'une augmentation concomitante des concentrations en α-tocophérol et en β-carotène à la fois dans le plasma et le lait. L'augmentation en EPA, DHA et CLA, α-tocophérol et β-carotène dans le plasma pourrait avoir un effet bénéfique en ce qui concerne non seulement la qualité du lait et de la viande, mais aussi de possibles effets contre les évènements inflammatoires.

pâture / lait / plasma / acide gras polyinsaturé / vitamine liposoluble

1. INTRODUCTION

Milk and dairy products have always played an important role in human nutrition and, more recently, have also been described as an important source of a variety of relevant biologically active molecules [46]. Recently, the potential human health benefits of specific fatty acid (FA), including benefits for conjugated linoleic acid (CLA) [48], docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been identified. CLA comprises a group of unsaturated fatty acid isomers with a variety of healthy biological effects, primarily associated to cis-9, trans-11 CLA and trans-10, cis-12 CLA [46]. DHA and EPA are essential for normal growth, brain development, vision and immunity, and also play a vital role in the prevention and treatment of human diseases [47, 53].

The lipid fraction of dairy products has been often treated as a health concern because of the relatively high content of saturated and trans fatty acids, which adversely influence plasma cholesterol. However, studies have shown that whole milk was more effective in protecting against cardiovascular disease than skimmed milk [51].

The reported polyunsaturated fatty acids (PUFAs) CLA, EPA and DHA, and fat-soluble-antioxidants α-tocopherol,

β-carotene and retinol, could be envisaged as main players. Changes in plasma and milk content of these compounds are influenced by forage species, the breed, the parity, the physiological stage, type of diet, the animal production level and sanitary state [16]. However, several reports suggest that the nature of the diet strongly influences the content and the composition of milk fatty acids [11], and the fat-soluble micronutrient fraction of dairy products, in particular β-carotene, α-tocopherol and retinol [14, 31, 32, 35].

Supplementing diets with oils, crushed seeds or rumen-protected lipids is a common nutritional means for manipulating milk fatty acid composition [12] despite forages often being the major source of fatty acids in the diet [23]. On the other hand, ruminant ingestion of plant oils rich in linoleic acid, including soybean oil [34] and sunflower oil [27], has increased milk fat CLA concentrations effectively.

Studies have also confirmed that pasture feeding significantly increases milk fat CLA, EPA and fat-soluble constituents, such as α-tocopherol and β-carotene. Dhiman et al. [15] have shown that grazing cows had a 5.7 times higher concentration of CLA in milk than cows fed diets containing preserved forage and grain at 50:50. Grass-based diets, especially pasture, also lead to higher milk β-carotene concentrations than diets rich in concentrates or corn silage. The α-tocopherol concentration in fresh pasture is 4–5 times higher than that found in a typical total mix ration (TMR) based on National Research Council (NRC, 2001) [42] values. However, pasture is unique in terms of increase of PUFAs and fat-soluble antioxidants.

The aim of the present study was to evaluate the changes in fatty acid composition and fat-soluble antioxidant content in plasma and milk from cows fed with different proportions of pasture. These findings could have an important outcome in terms of maximising the levels of bioactive compounds in milk, keeping the animal management as natural as possible.

2. MATERIALS AND METHODS

2.1. Cows and design

The experiment was conducted in one farm of the Hyblean region when native pasture was available. This farm had all the typical characteristics of a farmstead cheese producer: native pasture, TMR facility and a sufficient number of cows to select a similar stage lactation group for each feeding treatments.

The experiment was conducted between March and April 2004, with sampling collections every two weeks. Animals started grazing on native pasture about two weeks prior to the first sampling date, in order to allow adaptation of selective behaviour to the nutritional situation. Hyblean pasture availability is optimum between February and April determined by the sub-humid Mediterranean climate with mild wet winter and hot dry summers. The most common plants in the Hyblean pasture in terms of occurrence were 39.7% of Calendula (various species), 23.4% of Geraniaceae (various species), 14.2% of Graminaceae (various species), unspecified short Asteraceae, and Fabaceae (various species) and other minor families [8]. In the selected farm 36 Friesian cows were chosen and randomly divided into three groups. Cows were in late lactation, with a mean body weight of 660 kg, body condition score 3.25 and producing an average on 23.93 $kg \cdot d^{-1}$ of milk with a fat content of about 3.7% and CP content 3.14%.

Of the three experimental groups one of 12 cows was fed only a TMR (CTRL); another group of 12 cows received some TMR after the evening milking, plus grazing on native pasture approximately for 6 h (30P) starting after the morning

Table I. Average daily feed consumption data for the cows fed TMR and TMR plus pasture diet.

	TMR[1] (CTRL)	TMR + pasture (30P)	TMR + pasture (70P)
Diet (TMR + pasture) in kg			
Triticale silage	15.2	9.6	4.0
Corn silage	9.1	5.8	2.4
Citrus pulp	9.1	5.8	2.4
Hay graminacee	4.6	2.9	1.2
Soybean F.E. 44	3.0	1.9	0.9
Soybean roast	0.5	0.3	0.1
Barley	1.0	0.6	0.3
Corn meal	5.0	3.1	1.3
Megalac	0.2	0.2	0.1
Concentrate supplement	1.5	1.0	0.4
Intake TMR (DM)	21.8	13.8	5.7
Intake pasture (DM)	–	6.0	12.2
Diet composition from CNCPS[2]			
NDF[3] (% DM)	39.7	39.3	43
NE_L[4] (Mcal·kg^{-1} DM)	1.6	1.5	1.3
CP[5] (% DM)	16.1	17.3	16.4
PS[6] (% CP)	27.2	30.1	32.9
RUP[7] (% CP)	37.4	33.8	31.8
NFC[8] (% DM)	34.7	33.2	28.7

[1]TMR = total mix ration; [2]CNCPS = cornell net carbohydrate protein system; [3]NDF = neutral fibre detergent; [4]NE_L = net energy for milk production; [5]CP = crude protein; [6]PS = soluble protein; [7]RUP = ruminal degradable protein; [8]NFC = non fibre carbohydrate.

milking, and the other group of 12 cows received some TMR after the evening milking, plus grazing on native pasture approximately for 16 h·d^{-1} (70P) starting after the morning milking. Daily grazing time was approximately 6 and 16 h·d^{-1}, respectively, for each experimental animal group, between the two milking times.

TMR intake was estimated on each sampling day by calculating the differences between TMR offered minus TMR remaining in the morning before milking time; pasture intakes were estimated using Cornell net carbohydrate protein system model (CNCPS) [19] elaborating the data for TMR intake as well as chemical analysis parameters for TMR, pasture samples, milk and milk yield for each group. The CNCPS model is in fact based on the energy and protein requirements of the dairy cows correlated to daily milk production and quality [9].

The DM intake of the control group (CTRL) fed only TMR was 21.8 kg·d^{-1}. The second group of cows (30P) consumed 13.8 kg of DM of TMR and 6 kg·d^{-1} of pasture. The third group of cows (70P) consumed 5.7 kg of DM of TMR and 12.2 kg·d^{-1} of pasture.

Feed composition and nutritive values of CTRL, 30P, 70P, diets are given in Table I and effective nutrient content (NDF, NE_L, CP, PS, RUP and NFC) of the three diets was also calculated.

2.2. Sampling

Cows were milked twice daily. Blood samples from the jugular vein were collected from each cow before evening milking once a fortnight for two months. Samples were collected in evacuated foiled containers containing heparin as

anticoagulant, placed on ice and then promptly transported to the laboratory. They were centrifuged at 2000 rpm 4 °C × 10 min in the dark. Plasma samples were frozen and stored at −80 °C to be analysed for fatty acids, fat-soluble antioxidants. Bulk milk samples from each (cow) group were collected every two weeks for two months and were protected from light, refrigerated immediately and then brought directly to the laboratory where they were split in two portions: the first one was analysed with milkoscan (MilkoScan Minor, Foss Systems, Hillerød, Denmark) to determine milk fat and protein contents and the second one was stored at −80 °C to determine fatty acid profiles, fat-soluble antioxidant content. All the analyses were performed in ice and in the dark.

2.3. Chemical analysis

2.3.1. Conjugated linoleic acid, unsaturated fatty acids and retinol

Total lipids were extracted from plasma and milk by the method of Folch et al. [18]. Aliquots were saponified as described by Banni et al. [4], in order to obtain free fatty acids (FFA) for HPLC analysis. Particular attention was paid at each stage of analysis to avoid heating or excessive exposure to air and light in order to prevent oxidation of the samples.

Separation of PUFAs and MUFAs (monounsaturated fatty acids) was carried out with a Hewlett-Packard 1100 HPLC system (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector [38]. AC-18 Inertsil 5 ODS-2 Chrompack column, (Chrompack International BV, Middleburg, The Netherlands), 5 μm particle size, 150 × 4.6 mm, was used with a mobile phase of $CH_3CN/H_2O/CH_3COOH$ (70/30/0.12, v/v/v) at a flow rate of 1.5 mL·min^{-1}. Total CLA was detected at 234 nm and at 200 nm the following unsaturated fatty acids: oleic acid (OA), vaccenic acid (VA), linoleic acid (LA), total CLA, alpha-linolenic acid (ALA), 18:3n6, arachidonic acid (AA), EPA, DHA.

Separation of CLA isomers as FFA was carried out with the same HPLC system using two silver-ion in series ChromSpher 5 lipid Chrompack columns (Chrompack International BV, Middelburg, The Netherlands), 5-mm particle size, 250 × 34.6 mm; the mobile phase was *n*-hexane with 0.5% ether and 0.1% CH_3CN at a flow rate of 1 mL·min^{-1}. Plasma and milk retinol were measured as previously described [3]. Retinol was determined simultaneously from aliquots of total lipid extract, using a Hewlett-Packard 1100 liquid chromatograph equipped with a diode array detector. An Inertsil ODS-3 Chrompack\column (Chrompack International BV), 5 mm particle size, 150 × 3 mm was used with 100% methanol as a mobile phase, at a flow rate of 0.7 mL·min^{-1}. Retinol was detected at 324 nm. UV spectra of the eluate, generated by the Phoenix 3D HP Chemstation software, was obtained at every 1.28 s and electronically stored. The spectra were taken to confirm the identification of the peaks [2].

2.3.2. β-Carotene

The extraction of β-carotene from plasma and milk was performed as indicated by Palozza and Krinsky [45]. Sample was dissolved in methanol, and 20-μL aliquot was analysed by reverse phase HPLC with spectrophotometric detection on a Perkin-Elmer LC-295 detector at 450 nm (β-carotene content) and at 350 nm (β-carotene 5,6-epoxide). The column was packed with Alltech C18 Adsorbosphere HS material, 3-μm particle size, in a 15 × 0.46-cm cartridge format (Alltech Associates, Deerfield, IL). A 1-cm cartridge precolumn containing 5-μm C18 Adsorbosphere packing was used. Analyses were done by gradient elution, the initial mobile phase was 85% acetonitrile/15% methanol, with the addition at 8 min of

30% 2-propanol. Ammonium acetate, HPLC grade, 0.01%, was added to the initial mobile phase.

2.3.3. α-Tocopherol

Plasma tocopherol was extracted according to Palozza and Krinsky [45] where 500 μL of plasma were mixed with 500 μL of distilled water and extracted with ethanol 1 and 3 mL of hexane. Both α-tocopherol and the internal standard (tocopherol acetate; 150 μL of a 60 $\mu g \cdot mL^{-1}$ ethanol solution) contained in the hexane phase were extracted by centrifugation (10 min at 710× *g*). A second extraction with 3 mL of hexane was subsequently performed. Concentration of α-tocopherol in milk was determined according to Noziere et al. [41]. Two millilitres of milk were combined in a test tube with BHT, 14.6 mL of saponification solution containing 11% KOH (wt/v), 55% ethanol (v/v) and 45% deionised water (v/v) plus 0.4 mL of internal standard: 2.25% α-tocotrienol (wt/v); 97.75% ethanol (v/v). The tubes were then placed in a shaking water bath for 20 min at 80 °C.

After cooling in an ice water bath for 10 min, α-tocopherol was extracted using a hexane and water mixture (2/1, v/v). The hexane phase, of both plasma and milk samples, was evaporated under a stream of N_2 and redissolved in 60 μL methanol and a 20 μL aliquot was analysed by reverse phase HPLC with fluorescence detection on a Perkin Elmer 650-LC fluorescence detector with excitation at 295 nm and emission at 340 nm. Alpha-tocopherol was eluted with 100% methanol on an Alltech C18 3 μm column (Alltech Associates, Deerfield, IL).

3. STATISTICAL ANALYSIS

Statistical analysis was carried out using the SAS-software GLM procedure (SAS, 1999) [49]. Data for PUFAs, MUFAs and fat-antioxidants in plasma and milk sample were analysed using ANOVA model with factorial term for diet composition. Means were tested for differences between diets using *t* test (LDS, $P < 0.05$).

4. RESULTS

The results clearly show a relationship between amount of native pasture in the diet and level of fatty acids and fat-soluble antioxidants in plasma and milk produced from grazing animals. Table II shows the results of beneficial fatty acids, expressed in $mg \cdot g^{-1}$ of lipids, contents in plasma in the three groups. In particular, total CLA and ALA increased almost two and threefold in 30P and 70P, respectively, compared to CTRL as a function of the proportion of pasture intake. In addition, VA increased slightly in 30P (17%) and much more in 70P (80%) compared to CTRL. Linoleic acid showed a slight increase (16%) only at higher pasture intake compared to CTRL. A progressive significant increase was observed for EPA and DHA in 30P and 70P. In contrast no significant differences in AA concentrations were found among three groups.

Table III shows the results of fatty acids contents in milk in the three groups. A progressive significant increase of two and threefold was only observed for ALA, in 30P and 70P, respectively, compared to CTRL. Oleic acid, VA, LA and total CLA increased on average 55% in 70P, respectively, compared to CTRL.

Arachidonic acid significantly increased at both pasture intake versus CTRL, whereas EPA significantly increased at higher pasture intake versus CTRL.

Moreover, the ratio between total CLA and VA calculated for the three groups of samples was much lower in plasma (0.008) than in milk (0.48).

Table II. Fatty acid and fat-soluble antioxidant profile in plasma.

	Fatty acid profile (mg·g^{-1}of lipids)					
	TMR[1] (CTRL)	SEM	TMR + pasture (30P)	SEM	TMR + pasture (70P)	SEM
OA[2]	36.87[c]	1	43.37[b]	1.69	48.85[a]	2.65
VA[3]	59.00[c]	2.05	73.75[bc]	2.07	106.90[a]	3.03
LA[4]	289.89[b]	48.98	268.07[b]	108.30	311.27[a]	34.01
Tot CLA[5]	0.40[c]	0.21	0.70[b]	0.20	1.04[a]	0.21
ALA[6]	0.24[c]	0.11	0.41[b]	0.12	0.75[a]	0.12
GLA[7]	0.24	0.01	0.27	0.03	0.22	0.01
AA[8]	0.17	0.03	0.15	0.02	0.20	0.02
EPA[9]	2.47[c]	0.80	3.90[b]	0.99	6.10[a]	1.56
DHA[10]	13.36[c]	1.56	19.82[b]	0.56	29.83[a]	2.56
	Fat-soluble antioxidants (μg·mL^{-1})					
β-Carotene	1.74[c]	0.35	2.89[b]	0.38	4.39[a]	0.45
Retinol	0.41	0.10	0.53	0.08	0.46	0.22
α-Tocopherol	3.32[c]	0.37	4.55[b]	0.20	5.60[a]	0.50

[a–c] Means on the same line, not sharing the same superscript are different ($P < 0.05$).
[1]TMR = total mix ration; [2]OA = oleic acid; [3]VA = vaccenic acid; [4]LA = linoleic acid; [5]Tot CLA = conjugated linoleic acid; [6]ALA = alpha-linolenic acid; [7]GLA = gamma-linolenic acid; [8]AA = arachidonic acid; [9]EPA = eicosapentaenoic acid; [10]DHA = docosahexaenoic acid.

Tables II and III show the content of fat-soluble antioxidants in plasma and in milk. A progressive increase of β-carotene and α-tocopherol was found in plasma by increasing pasture intakes, whereas no variation in retinol concentration was found (Tab. II). In contrast, in milk samples β-carotene significantly increased at lower pasture intake compared to CTRL without significative differences between 30P and 70P, whereas retinol and α-tocopherol significantly increased at higher pasture intake versus CTRL (Tab. III).

5. DISCUSSION

The results clearly showed that fatty acid composition and fat-soluble antioxidant content in plasma and milk from cows were deeply influenced by the percentage of pasture in the diet. In particular, a remarkable increase in the content of CLA and VA was observed in plasma and milk following an increased ratio pasture/TMR. Many authors report that milk fat from cows grazing pasture shows a CLA increase compared to milk fat from cows fed by dry forage [14, 32, 52]. Dhiman et al. [15] reported that cows grazing pasture had three times higher CLA content in milk fat (2.21% of total FA) compared to cows fed a diet containing 50% conserved forage (hay and silages) and 50% grain (0.38% of total FA). It has been also shown that milk fat CLA increased linearly as the proportion of fresh pasture increased in the dairy cow diet [11]. Fresh grass contains approximately 1–3% FA on a DM basis, depending on the variety of the pasture, with the highest FA contents usually occurring in the spring and fall seasons [13, 14].

Ruminant feeds are rich in PUFA such as LA and ALA rapidly hydrogenated by rumen bacteria to produce more highly saturated end products. However, ALA is the predominant fatty acid in fresh pasture, representing 48–56% of total fatty acids, and in

Table III. Fatty acid and fat-soluble antioxidant profile in milk.

	Fatty acid profile (mg·g^{-1} of lipids)					
	TMR[1] (CTRL)	SEM	TMR + pasture (30P)	SEM	TMR + pasture (70P)	SEM
OA[2]	202.00[b]	16.06	237.94[b]	16.06	323.26[a]	14.67
VA[3]	7.32[b]	0.70	8.96[ab]	0.70	11.56[a]	0.65
LA[4]	24.30[c]	1.0	29.68[b]	1.18	35.23[a]	1.08
Tot CLA[5]	3.60[b]	0.31	4.20[b]	0.30	5.60[a]	0.28
ALA[6]	4.1[c]	0.32	8.6[b]	0.32	11.98[a]	0.29
GLA[7]	0.16[c]	0.06	0.53[a]	0.06	0.38[b]	0.05
AA[8]	0.08[b]	0.03	0.22[a]	0.03	0.18[ab]	0.03
EPA[9]	0.41[b]	0.04	0.49[b]	0.04	0.81[a]	0.04
DHA[10]	ND		ND		ND	
	Fat-soluble antioxidants (μg·g^{-1} of lipids)					
β-Carotene	3.02[b]	0.50	15.21[a]	0.50	16.18[a]	0.80
Retinol	2.50[b]	0.40	2.71[a]	0.60	3.50[a]	0.20
α-Tocopherol	12.9[b]	0.20	13.20[ab]	0.50	16.05[a]	0.50

[a–c] Means on the same line, not sharing the same superscript are different ($P < 0.05$).
ND = not detected.
[1]TMR = total mix ration; [2]OA = oleic acid; [3]VA = vaccenic acid; [4]LA = linoleic acid; [5]Tot CLA = conjugated linoleic acid; [6]ALA = alpha-linolenic acid; [7]GLA = gamma-linolenic acid; [8]AA = arachidonic acid; [9]EPA = eicosapentaenoic acid; [10]DHA = docosahexaenoic acid.

the bovine rumen may contribute to VA production [29]. Moreover, changes of VA and CLA content in milk also depend on other factors such as ruminal pH and bacteria population [25, 30]. It is known that cows grazing on pasture have a higher ruminal pH that is favourable for cellulolytic bacteria growth in the rumen, responsible for CLA and VA production [36].

In the current study the ratio CLA/VA was much lower in plasma than in milk, suggesting the important contribution of plasma VA to CLA synthesis in the mammary gland by the epithelial tissue Δ9-desaturase [22]. In fact, it is known by literature that CLA in milk originates as an intermediate product from either ruminal biohydrogenation of ALA and LA and from the endogenous synthesis in mammary gland that is the major pathway of CLA synthesis from VA in dairy cows [22]. According to this theory of endogenous synthesis of CLA, our results showed a higher content of CLA in milk than in plasma [21, 22].

Apart from the increase of total CLA and VA in plasma and milk from cows with higher intake of pasture, our data also show a consistent increase of ALA and EPA. LA and ALA are the main (n-6 and n-3) PUFAs, respectively, in milk and the precursors of AA and of EPA and DHA that are further converted to eicosanoids. In particular, EPA is an important long n-3 fatty acid because it is able to inhibit the conversion of n-6 fatty acids to harmful eicosanoids, thereby protecting against cardiovascular diseases and cancer. In this study the increase of n-3 fatty acids (ALA and EPA) especially in milk derived from group 70P shows the importance of fresh pasture on these molecules that have a beneficial impact on human health. Thus, our results show how the amount of fresh pasture affects the content of total CLA and the ratio n-6 and n-3 in milk, and also the content of OA that is considered to be favourable for health.

According to the above considerations, milk derived from cows fed pasture instead

of conserved forages may be considered a healthier food than dairy products derived from more intensive feeding regimes, although this does not address the potential impact of oilseed supplementation.

Studies have shown that enhancing the PUFA content of plasma and milk is associated with increased susceptibility to auto-oxidation and development of milk off-flavours [33]. PUFAs in plasma and milk are protected from oxidation by natural antioxidants. Among the fat-soluble antioxidants, the most important are α-tocopherol and β-carotene, the precursor of retinol. α-tocopherol is a potent peroxyl radical scavenger [6] and it can protect PUFA within phospholipids of biological membranes [7] and in plasma lipoproteins.

The plasma levels of α-tocopherol and β-carotene are extremely dependent on the diet. In the present study, the plasma concentrations of the fat-soluble α-tocopherol and β-carotene differed between pasture and TMR-fed cows. The levels obtained of β-carotene and retinol are in the same range reported in a review article from Noziere et al. [41].

Plasma concentrations of α-tocopherol and β-carotene were significantly increased by enhancing dietary pasture/TMR ratio (30P versus 70P), suggesting that pasture naturally supplies high levels of both antioxidants in cows. It has been reported that α-tocopherol concentration in fresh pasture (approximately 106 i.u.·kg^{-1} DM) is 4–5 times greater than that found in a typical TMR based on NRC (2001) values (15 i.u.·kg^{-1}) [29, 42]. Similarly, native pasture is very rich in plants that contain β-carotene and especially *Calendula* [5]. The progressive increase of the two fat-soluble antioxidants in plasma observed by increasing dietary pasture/TMR ratio is particularly interesting in view of the possible metabolic interactions occurring among the fat-soluble antioxidants. It has been shown that plasma α-tocopherol concentrations were depressed in animals supplemented with β-carotene [44] or vitamin A [40] by mechanisms involving competition in intestinal absorption [20] or oxidative interactions [44]. Our results indicate that pasture obviously provides high amounts of both α-tocopherol and β-carotene in plasma for milk synthesis.

Although plasma β-carotene concentration was remarkably higher in pasture-fed cows, plasma retinol concentrations did not statistically exceed those in TMR-fed cows. Our results and those from studies in humans [37], in which β-carotene was given as a supplement, indicate that an increase in plasma of β-carotene concentrations has minimal impact on plasma retinol concentrations. Chew et al. [10] also reported that oral administration of β-carotene to calves fed a diet containing normal concentrations of vitamin A does not affect plasma retinol concentration.

Even though transport of α-tocopherol and β-carotene from plasma lipoproteins into mammary gland conforms to Michaelis-Menten kinetics, concentrations of α-tocopherol and β-carotene in milk are thought to be a function of the dietary intake [17]. In agreement with this, in the present study, the concentrations of α-tocopherol and β-carotene in milk paralleled those observed in plasma. A clear relationship between plasma concentrations and secretion into milk of α-tocopherol and β-carotene has been also reported by other authors [24, 26, 39]. The concentrations of the two fat-soluble antioxidants in milk derived from pasture supplemented groups were much higher than the minima suggested by Al-Mabruk et al. [1] to ensure a favourable oxidative stability of milk.

Milk from cows fed a higher percentage of pasture (70P) had a more favourable fatty acid pattern, high total CLA and n-3 PUFA content and lower n-6:n-3 ratio, than milk from cows fed a conventional TMR diet. This may have several implications in the prevention of mastitis and problems of immune depression in cows [43]. On the other hand, changes in milk fatty acid

composition and fat-soluble antioxidant concentration may have several implications for nutrient content, organoleptic attributes and shelf life of milk.

6. CONCLUSION

In conclusion, it is the consumption of native pasture that increases these parameters in plasma and milk.

The observed variations in milk may have a great potential for human consumption as a source of PUFAs with important beneficial activities. Taking into account that cow milk is generally used for cheese making and that milk transformation does not influence the CLA content of dairy products [16, 50], pasture management could become a useful strategy to naturally manipulate dietetic characteristics of milk and dairy products.

Further studies are needed to verify whether changes in fatty acid profile and other lipid soluble compounds in milk could be used as quantitative and qualitative biomarkers of PDO (products with Denominations of Origin).

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