Influence of season and pasture feeding on the content of α-tocopherol and β-carotene in milk from Holstein, Brown Swiss and Modicana cows in Sicily

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Dairy Sci. & Technol. (2012) 92:501–513
DOI 10.1007/s13594-012-0069-2

ORIGINAL PAPER

Influence of season and pasture feeding on the content of α-tocopherol and β-carotene in milk from Holstein, Brown Swiss and Modicana cows in Sicily

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Received: 30 November 2011 / Revised: 8 May 2012 / Accepted: 10 May 2012 /
Published online: 26 July 2012

Abstract It has been shown that several factors such as feed source and breed might influence milk fat-soluble antioxidants (AOs). This study investigated pasture feeding effects and dairy cattle breed on the content of α-tocopherol and β-carotene in cows' milk by monitoring two grazing seasons (spring and fall) as well as a summer no-pasture season. Four dairy farms located in Sicily were selected: two with both Holstein and Brown Swiss cows and two with only a Modicana (M) local breed cows. Bulk milk samples of each breed per farm were collected. Milk α-tocopherol and β-carotene were highest during spring (16.2 and 9.7 $\mu g.g^{-1}$ of fat, respectively), lowest during fall (11.2 and 0.8 $\mu g.g^{-1}$ of fat, respectively) and intermediate during summer (13.3 and 2.5 $\mu g.g^{-1}$ of fat, respectively). These results indicate that grazing pasture season has an important impact on milk fat-soluble antioxidant content. In particular, higher milk AO levels in spring compared to fall might be attributed to several factors such as differences in the quality and composition of pasture, differences in pasture intake and even the climate. Breed effect on milk AO contents was not so pronounced. Milk β-carotene levels did not differ significantly among breeds. Saturation of milk β-carotene may explain similar vitamin levels among breeds in spring despite different pasture intakes. It was interesting that significant levels of α-tocopherol were detected in milk from M cows during summer. The latter effect could be masked by the considerably higher pasture intake of M in spring compared to the other two breeds.

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季节和牧草对西西里Holstein, Brown Swiss 和 Modicana 奶牛乳中 α-生育酚和 β-胡萝卜素含量的影响

摘要：饲料和季节可以影响乳中脂溶性抗氧化剂的含量。本研究考察了春季和秋季(放牧季节)和夏季(非放牧季节)饲料以及奶牛品种对乳中α-生育酚和β-胡萝卜素含量的影响。选择西西里地区的四个牧场作为研究对象,其中两个农场有Holstein (H) 奶牛和Brown Swiss (BS)奶牛,另外两个牧场只有当地的Modicana (M)奶牛。分别对不同牧场和不同品种的奶牛进行采样。实验结果表明,春季乳中α-生育酚和β-胡萝卜素含量最高(分别为16.2和9.7 μg.g^{-1} 脂肪),秋季乳中α-生育酚和β-胡萝卜素含量最低(分别为11.2和 0.8 μg.g^{-1} 脂肪),夏季乳中含量处于春季和秋季中间(分别为13.3 和2.5 μg.g^{-1} 脂肪)。不同季节的牧草对乳中脂溶性抗氧化剂含量有显著的影响。春季比秋季乳中抗氧化剂含量高可能与牧草的组成和质量差异、牧草摄入量的差异、气候等因素有关。品种对乳中抗氧化剂含量的影响不显著,不同品种之间牛乳β-胡萝卜素含量差异不显著。β-胡萝卜素在乳中的饱和度与乳牛品种和所摄入饲料中维生素含量有关。在夏季, M牛乳中α-生育酚含量较高,但是春节M牛乳中,牧草摄入量对α-生育酚含量影响高于品种的影响。

Keywords α-Tocopherol · β-Carotene · Season · Pasture · Breed

关键词 α-生育酚 · β-胡萝卜素 · 季节 · 牧草 · 品种

1 Introduction

There is an increasing interest in functional foods, including milk, for the presence of biologically active molecules with beneficial effects on human health (Secchiari 2008; Steijns 2001). Alpha-tocopherol and β-carotene belong to this category of beneficial molecules. They are powerful antioxidants known to protect against oxidative stress by scavenging reactive oxygen species that, in excessive amounts, can cause deleterious effects to different biological molecules (Lindmark-Månsson and Åkesson 2000). In fact, a large number of studies have investigated a possible role of the antioxidants in prevention or treatment of disorders such as cancer, coronary heart disease, neurodegenerative diseases and immune and autoimmune diseases (Nicoletti et al. 2005; Nakamura and Omaye 2010). Moreover, there is also interest of the food industry in these molecules for their ability to inhibit rancidity and prolong the shelf-life of foods. Alpha-tocopherol and β-carotene are hydrophobic molecules and act in the lipid phase by delaying the oxidation of polyunsaturated fatty acids and by preventing biological membrane injuries as well as off-flavour development in milk (Havemose et al. 2006; Slots et al. 2007). Fat-soluble vitamins are not synthesised by cows but their presence in milk derives from feedstuff or diet-supplemented synthetic compounds (Slots et al. 2009). The main dietary fat-soluble antioxidant (AO) vitamin supplement for cows is fresh forage or pasture. La Terra et al. (2010) reported that milk from cows grazing on pasture is richer in fat-soluble antioxidants than milk from cows kept indoors and fed mainly on conserved forage. Plant maturity, botanical composition as well as climate may affect forage AO contents (Ballet et al. 2000). Reynoso et al. (2004) reported that β-carotene amount in green forages within the same plant species depends on temperature and solar radiation. The same authors also showed that forage β-carotene and α-tocopherol levels are higher in cool and humid summers than in dry and hot summers because of a higher proportion of leaves. Ballet et al. (2000) reported that the amount of β-carotene and vitamin E in fresh pasture is influenced by both climatic conditions and the origin and the maturity stage of forage, although the ratio of leaf to stem is the main factor responsible for the AO variations. As a consequence, feeding season as well as geographic location may also influence AO content in milk. There is a limited knowledge of AO contents in Mediterranean pastures or of milk from Mediterranean pastures since most studies regarding AO content in milk have been performed in the northern hemisphere. Sicilian pastures grow under subtropical climate. Pasture season is not interrupted by winter frost but by hot and dry summer. The objective of this study was, therefore, to evaluate α-tocopherol and β-carotene concentrations in cows' milk by monitoring two grazing seasons in Sicily (spring and fall) as well as the summer no-pasture season. Besides diet, other factors such as animal breed, stage of lactation and health status may influence fat-soluble antioxidant vitamin concentration in milk (Nozière et al. 2006). Nozière et al. (2006) reported differences in β-carotene content in milk of different breeds. The same author reported that the differences between breeds in butterfat β-carotene content were maximal under high carotene diets. The cows' breed effect on the milk carotenoid content could be explained by differences between breeds in: (a) cleavage activity of 15,15′-dioxygenase enzyme (Morales et al. 2007), (b) secretory capacity of β-carotene from plasma to milk (Jensen et al. 1999) and (c) β-carotene storage and mobilization (Arias et al. 2009). Lucas et al. (2006) showed that dioxygenase activity is higher under poor β-carotene diets. A few studies have previously reported milk α-tocopherol content experimentally comparing different dairy cattle breeds (Lucas et al. 2006; Morales et al. 2000). No knowledge is available in the literature regarding milk AO content of Modicana cow, a local breed reared in Sicily. Only milk chemical composition of Modicana cows has previously been reported by Chiofalo et al. (2000). Thus, the second objective of this study was to compare the milk fat-soluble vitamin content of Modicana cows with Holstein and Brown Swiss cows.

2 Materials and methods

2.1 Farms and experimental conditions

Farms producing Protected Denomination of Origin (PDO) Ragusano cheese were chosen because PDO Ragusano cheese disciplinary regulation implicates pasture feeding, allows feeding of only little supplementary hay and concentrate and completely omits silage feeding. Four farms were selected close to each other in order to increase the probability of most similar pasture qualities between farms. Two of the farms had both Holstein (H) and Brown Swiss (BS) cows and two exclusively Modicana (M) local breed cows. Bulk milk samples per breed per farm were collected four times with weekly intervals in spring (March/April), summer (June/July) and fall (November/December). Pasture was available in spring and fall but not in summer. Additional hay and concentrate were supplemented during all periods. No vitamin A and E supplementation was given with the concentrate to cows during the three experimental periods. Cows were, on average, in mid-late lactation in each experimental period (177±135 days in milk during spring, 189±123 during summer and 180±115 during fall). On average, M, BS and M cows were 148±109, 200±151 and 207±151 days in milk in spring, 174±96, 200±131 and 202±146 days in milk in summer and 161±104, 203±135 and 190±113 in fall, respectively (Table 1). Milk production of both BS and H cows was on average 22.3 and 23.8 kg.day^{-1} in spring, 20.3 and 24.3 kg.day^{-1} in summer and 18.5 and 23.2 kg.day^{-1} in fall, respectively; whereas M cows produced on average 13.9 kg.day^{-1} in spring, 8.9 kg.day^{-1} in summer and 11.2 kg.day^{-1} in fall, respectively (Table 1). Bulk milk samples were collected from the evening milking after cows' pasture feeding. Five hundred-millilitre bottles were wrapped in aluminium foil to protect from light and stored at 4 °C until next day for analysis.

2.2 Milk

2.2.1 Chemical analyses

Bulk milk samples per farm per breed were analysed for fat and protein contents by MilkoScan ™ Minor (FOSS, Italy).

Table 1 Days in milk, milk production and diet composition of cows in spring, summer and fall[1]

Season	Item	Breed		
		M	BS	H
Spring				
	Days in milk	147.7 (109.1)	200.0 (151.4)	206.9 (150.7)
	Milk production (kg/cow/day)	13.9 (4.6)	22.3 (0.7)	23.8 (1.1)
	Diet composition (% of DMI)			
	Pasture	69.0 (21.3)	20.5 (5.6)	22.5 (4.8)
	Hay	18.3 (10.4)	37.0 (6.1)	36.1 (5.6)
	Concentrate	12.6 (1.0)	42.4 (2.4)	41.4 (2.7)
Summer				
	Days in milk	174.1 (96.2)	199.6 (130.9)	201.6 (146.2)
	Milk production (kg/cow/day)	8.9 (1.6)	20.3 (1.5)	24.3 (1.7)
	Diet composition (% of DMI)			
	Pasture	0.0	0.0	0.0
	Hay	81.8 (19.5)	48.6 (4.2)	51.7 (4.0)
	Concentrate	18.6 (1.4)	51.4 (4.2)	48.3 (4.0)
Fall				
	Days in milk	160.6 (104.4)	202.7 (134.6)	190.5 (113.3)
	Milk production (kg/cow/day)	11.2 (4.5)	18.5 (1.7)	23.2 (2.5)
	Diet composition (% of DMI)			
	Pasture	55.3 (30.6)	12.4 (5.1)	19.0 (5.0)
	Hay	30.8 (16.2)	44.3 (7.1)	41.0 (6.8)
	Concentrate	13.9 (0.4)	43.3 (3.3)	40.0 (2.8)

Values represent the means of eight measurements and the standard deviations are in parenthesis

M Modicana, *BS* Brown Swiss, *H* Holstein, *DMI* dry matter intake

2.2.2 Determination of milk α-tocopherol and β-carotene

Both extractions of α-tocopherol and β-carotene were performed on fresh milk samples in darkness to avoid oxidation reactions and performed according to Palozza and Krinsky (1992) and Marino et al. (2010), respectively. Vitamin concentrations were determined by HPLC method by using an SB-C18 column (5 μm particle size, 4.6 nm ID×250 nm, Agilent Zorbax). The HPLC system (Waters 2695) was equipped with a multi λ fluorescence detector (Waters 2475) using an excitation wavelength of 297 nm and an emission of 340 nm for tocopherol isomer detection and with a dual λ absorbance detector (Waters 2487) setting at the wavelength of 450 nm for all-*trans*-β-carotene detection.

2.3 Forage and feeds

2.3.1 Botanical analysis

Pasture botanical composition was determined by using wooden frames. The frames were placed over a total surface of 3 m^2 close to cows' grazing places avoiding the *Carduus* species which are not selected by the cows (Carpino et al. 2003). Forage inside the frame was collected in paper bags, removed to the laboratory, where plant species and families were identified, sorted and weighed. Field botanical composition was calculated by dividing individual species weight by the total weight collected (wet basis).

2.3.2 Chemical analysis

Bulk pasture samples and all supplemental feeds were sampled at each experimental test day for chemical analyses. Pasture samples were dried at 60 °C to a constant weight. All feed samples were chemically analysed to determine dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and crude protein (CP). Feeds were dried overnight at 105 °C to obtain DM (ISO 6496:1999) and ashed in a muffle furnace at 550 °C for 4 h to obtain ash content and OM calculated by difference. The CP content was determined in accordance to the Kjeldahl method (EN ISO 5983–2:2009). Neutral detergent fibre was determined with a Fibretec™ (Foss, Denmark) fibre analyser using reagents and method described by Van Soest et al. (1991). Pasture intake levels were calculated based on feed, milk chemical composition and milk yield using CPM Dairy® version 3.0.8 (Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY).

2.4 Statistical analysis

Statistical analyses were carried out with SAS (SAS Institute Inc. 2004). Data were analysed using a MIXED model with season, breed and their interaction set as fixed effects, and the nested effect of herd within season as repeated measures. Differences in milk chemical composition and vitamin content were tested using pairwise least square means coupled with Bonferroni's adjustment and considered significant when $P<0.05$.

3 Results

3.1 Pasture quality and intake

Table 2 shows pasture botanical incidence in spring and fall. Pastures had heterogeneous botanical composition. Over 18 different plant families in spring pasture and 13 in fall pasture were identified. However, most plant families and species proportions were present in small quantities and their data were not statistically evaluated between spring and fall pastures. Leguminosae was the most abundant family and spring pasture contained significantly more Leguminosae relative to fall pasture.

Forages in spring were generally at a more advanced biological (mid-late bloom to seed stage) stage compared to plants growing in fall right after the dry summer period (early-vegetative to mid-bloom) and moisture content was lower in spring compared to the fall pasture (77 vs. 83%, respectively; Table 2). Pastures from different farms had similar chemical composition, but the availability of Leguminosae, Graminaceae and Cruciferae differed between farms even though pastures were located close to each other. Table 1 shows dry matter forage feeding intakes (in percent) of each breed in the three experimental periods. Cows ate more pasture during spring compared to fall. Brown Swiss and Holstein cows belonged to the same farms and had the same management and feeding regimen, but Modicana cows were on different farms and had higher pasture intakes. On average, pasture intakes during spring and fall of M cows were 69.0 and 55.3% DM, respectively, whereas pasture intakes of BS and H cows were 20.5 and 22.5% DM in spring, respectively, and 12.4 and 19.0% DM in fall, respectively. Interactions between breed and nutritional parameters such as intake and quality of pasture were probable; however, these combined effects could not be estimated in this study.

3.2 Milk α-tocopherol

Data on concentrations of milk α-tocopherol during spring, summer and fall are reported in Table 3. Milk α-tocopherol levels differed between seasons ($P<0.001$). Milk α-tocopherol was highest during spring, intermediate during summer and lowest during fall. Levels of α-tocopherol referred to gram of milk fat ranged from 13.3 to 18.0 μg in spring, from 12.6 to 13.9 μg in summer and from 10.6 to 11.7 μg in fall. Large differences in α-tocopherol contents were not observed between pasture and no pasture seasons but were observed between spring and fall pasture seasons ($P<0.001$). Data on concentrations of milk α-tocopherol of each breed are reported in Table 3. Milk α-tocopherol levels differed significantly between breeds ($P<0.001$). Alpha-tocopherol referred to gram of milk fat in M cows (17.7 μg) was higher compared to BS and H cows (12.3 and 10.9 μg, respectively). Furthermore, there was a significant effect of interaction season × breed for milk α-tocopherol ($P<0.05$; Fig. 1). The concentration of milk α-tocopherol of BS and H was significantly higher in spring ($P<0.05$), than summer and fall. In contrast, the concentration of milk α-tocopherol of M was significantly higher in spring and summer compared to fall

Table 2 Incidence of plant families/species in pasture and chemical composition of pasture

Item	Spring	Fall
Pasture incidence		
Plant families/species		
Boraginaceae	2.6	1.7
Caryophyllaceae[a]	0.3	0.0
Chenopodiaceae[b]	0.1	1.7
Compositae		
Anthemis arvensis L.	5.8	1.6
Calendula arvensis L.	2.4	7.1
Chrysantemum segetum	2.6	12.9
Other species	11.0	16.6
Cruciferae	3.6	7.8
Euphorbiaceae	1.9	4.0
Geraniaceae	3.9	3.5
Graminaceae	9.7	12.8
Labiatae	0.3	0.0
Leguminosae	47.3	24.9
Liliaceae	1.4	0.0
Malvaceae	1.6	3.3
Oxalidaceae	0.0	0.8
Plantaginaceae[c]	1.2	0.8
Polygonaceae[d]	0.2	0.0
Primulaceae[e]	0.4	0.0
Ranunculaceae[f]	0.5	0.5
Resedaceae	0.3	0.0
Umbelliferae	2.9	0.3
Chemical composition		
DM (%)	22.8	17.1
CP (% DM)	18.0	16.4
NDF (% DM)	37.7	33.8

During spring and fall, 16 pasture samples were analysed. The values are expressed as averages

DM dry matter, *CP* crude protein, *NDF* neutral detergent fibre

[a] Mainly *Silene colorata*

[b] Mainly *Beta vulgaris*

[c] Mainly *Plantago cupani*

[d] Mainly *Anagallis arvensis*

[e] Mainly *A. arvensis*

[f] Mainly *Adonis annua*

(P<0.05). Milk α-tocopherol of M was significantly higher compared to BS and H relative to summer (P<0.001).

Table 3 Chemical composition and fat-soluble vitamins of milk

Item	Season[a]			SE	Breed[b]			SE	Effects		
	Spring	Summer	Fall		M	BS	H		S	B	S × B
Chemical composition (g.100 g^{-1})											
Milk fat	3.6	3.9	3.6	0.07	3.9	3.7	3.5	0.07	–*	–*	NS
Milk protein	3.4	3.6	3.2	0.03	3.6	3.4	3.3	0.03	–**	–**	NS
Fat-soluble antioxidants (μg.g^{-1} of milk fat)											
All-*trans*-β-carotene	9.7	2.5	0.8	0.07	3.7	3.9	2.9	0.07	–**	NS	–*
α-Tocopherol	16.2	13.3	11.2	0.05	17.7	12.3	10.9	0.05	–**	–**	–*

SE standard error, *M* Modicana, *BS* Brown Swiss, *H* Holstein, *S* season, *B* breed, *NS* not significant

*$P<0.05$, **$P<0.0001$, $P>0.05$

[a] The values are least square means. During each season, 24 bulk milk samples were analysed

[b] The values are least square means. Per breed, 24 total bulk milk samples were analysed

3.3 Milk β-carotene

Data on concentrations of milk all-*trans*-β-carotene during spring, summer and fall are reported in Table 3. Milk all-*trans*-β-carotene levels differed between seasons ($P<0.001$). Milk all-*trans*-β-carotene was highest during spring, intermediate during summer and lowest during fall. Levels of milk all-*trans*-β-carotene, referred to gram of milk fat, ranged from 8.3 to 11.0 μg in spring, from 2.1 to 2.8 μg in summer and from 0.5 to 2.3 μg in fall. Data on concentrations of milk all-*trans*-β-carotene of each breed are reported in Table 3. Milk β-carotene levels did not differ significantly between breeds. However, Modicana and BS cows had higher milk all-*trans*-β-carotene (3.7 and 3.9 μg.g^{-1} of milk fat, respectively) compared to H cows

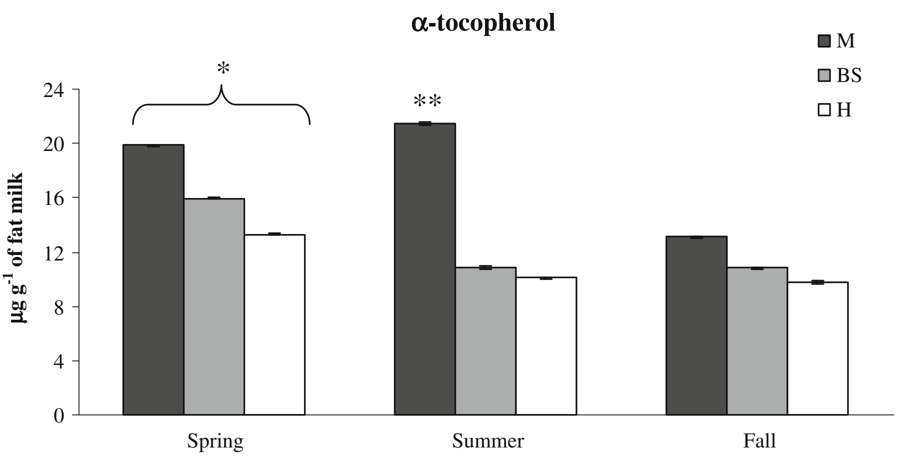

Fig. 1 Milk α-tocopherol concentration in spring, summer and fall seasons. *Asterisk*: α-Tocopherol concentration in spring is significant ($P<0.001$) vs. summer and fall seasons. *Double asterisks*: α-Tocopherol concentration in M is significant ($P<0.001$) vs. BS and H milk. *Bars* represent least square means of eight measurements and *error bars* indicate standard error. *M* Modicana, *BS* Brown Swiss, *H* Holstein

(2.9 μg.g^{-1} of milk fat). Furthermore, there was a significant effect of interaction season × breed for milk all-*trans*-β-carotene ($P<0.05$; Fig. 2). The concentration of milk all-*trans*-β-carotene of M, BS and H was significantly higher in spring compared to summer and fall ($P<0.001$).

4 Discussion

4.1 Season and pasture feeding effects

4.1.1 Spring and summer

Higher α-tocopherol and β-carotene levels of milk in response to spring grazing compared to summer agreed with Butler et al. (2008) and Calderón et al. (2006) who emphasised the importance of fresh forage on milk AO content. According to Nozière et al. (2006), fresh forage-based diets have a higher impact on total AO content of milk compared to preserved forages that undergo AO substantial losses (30 to 80%) when they are cut, dried, processed and stored. Levels of α-tocopherol and β-carotene in cows' milk reported in literature vary greatly among studies. In the current study, milk β-carotene values obtained in spring were twice as high as those found by Martin et al. (2004) (0.35 vs. 0.18 μg.mL^{-1}, respectively) and by Agabriel et al. (2007) (9.7 vs. 4.9 μg.g^{-1} of milk fat, respectively) observed when cows were grazed, whereas milk α-tocopherol values were similar. Milk β-carotene values measured in spring were comparable with those found by Butler et al. (2008) in milk during fresh forage-based feeding period, whereas α-tocopherol values were lower in the same report.

Low milk AO levels determined in summer were consistent with those observed by Martin et al. (2004), Agabriel et al. (2007) and Nozière et al. (2006), when cows were fed with a diet consisting of hay and concentrates. Milk α-tocopherol values were higher compared to values measured by Calderon et al. (2007) in milk when

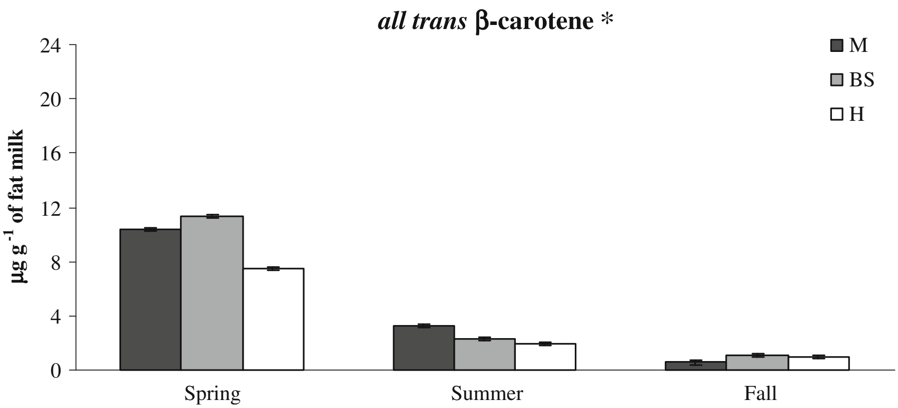

Fig. 2 Milk β-carotene concentration in spring, summer and fall seasons. *Asterisk*: β-Carotene concentration is significant ($P<0.001$) in spring vs. summer and fall; summer vs. fall seasons. *Bars* represent least square means of eight measurements and *error bars* indicate standard error. *M* Modicana, *BS* Brown Swiss, *H* Holstein

cows were fed with a preserved forage diet (13.3 vs. 11.3 μg.g^{-1} of milk fat, respectively). In contrast, Butler et al. (2008) showed both higher β-carotene and α-tocopherol levels in milk fat during the indoor conserved forage-based feeding period compared to our summer values (6.3 vs. 2.5 μg.g^{-1} of milk fat for β-carotene; 23.1 vs. 13.3 μg.g^{-1} of milk fat for α-tocopherol, respectively). Higher values reported in the latter study may be explained by different physiological stage of cows compared to cows used in this study. Butler et al. (2008) used cows in early lactation whereas cows in the present study were in mid-late lactation. According to Calderon et al. (2007), the efficiency of β-carotene transfer from plasma to milk decreases through stage of lactation, whereas the efficiency of α-tocopherol was comparable between early and mid-lactation. During early lactation, the demand for energy is higher than the amount of energy available from feed, and therefore, cows in mid-late lactation could replenish the adipose tissue lost before during early lactation.

4.1.2 Spring and fall

Despite milk samples collected during fall derived from animals fed with fresh pasture, AO levels in fall milk were surprisingly lower than spring milk. Several factors could be responsible for differences in milk β-carotene and α-tocopherol levels between spring and fall. First of all, during spring, cows tend to select feed based on palatability since pasture is lush and heterogeneous compared to fall. Moreover, in fall, young forage had a higher moisture content that could in part explain the lower dry matter pasture intake compared to spring (29.4 vs. 37.6% DM, respectively). Feed botanical composition was also different between the two grazing periods. Spring pasture contained more Leguminosae than fall pasture. Leguminosae are more favourite feed by cows because of their minor fibre content than grasses. In addition, several authors showed that Leguminosae in dairy cows diet increase the concentration of bioactive substances in milk such as AO content (Bolstad et al. 2007; Danielsson et al. 2008). Ballet et al. (2000) showed that Leguminosae contain more carotenes compared to Graminaceae at advanced plant maturity stage, probably because of the higher proportion of leaves. Thus, forages in spring were generally at a more advanced biological stage compared to plants growing in fall right after the dry summer period. Daley et al. (2010) highlighted seasonal shifts in plant vitamin content. Arizmendi-Maldonado et al. (2003) showed that both α-tocopherol and β-carotene concentrations in subtropical forages were low from October to January. Longer daylight in spring compared to fall increases the synthesis of carotenoids which act in leaves' chloroplasts as accessory photosyntetic pigments and increase the efficiency of chlorophyll (Albrecht and Sandmann 1994). In addition, spring climate promotes the active conversion of chloroplasts to chromoplasts, the main synthesis and storage organs of carotenoids in fruit and flowers (Ljubešić et al. 1991).

4.1.3 Fall and summer

Different botanical composition, forage maturity and climate conditions may explain different AO levels between spring and fall milk, but they do not explain lower AO levels in fall milk than summer milk. Physiological factors may give an explanation. Nozière et al. (2006) showed that fat-soluble vitamins are stored for long periods in cows' body tissues when the intake is in excess. Adipose tissue releases stored vitamins, though slowly, when dietary supply of AO is reduced, independently of lipid mobilization (Dunne et al. 2008).

Higher levels of both β-carotene and α-tocopherol found in summer milk compared to fall suggest vitamin mobilization from body tissue resources previously filled up by the spring diet. During fall, the body resources may first need to be restored before vitamins can be released into milk. Calderon et al. (2007) reported that there may be a β-carotene saturation in milk under high carotenoid diets due to mechanisms which limit the secretion from plasma to milk including a limited uptake by the mammary gland or limited transport by β-lactoglobulin.

During summer, compared to spring, β-carotene level (74%) was more reduced relative to α-tocopherol (18%). The drastic decrease of milk β-carotene in summer and fall could be explained by the interference between α-tocopherol and β-carotene absorption and deposition in tissues. Yang et al. 2002 showed that more vitamin E was deposited in tissues than β-carotene from pasture-fed cattle. Thus, high vitamin E intake from fresh pasture during spring may have reduced storage of β-carotene, which is supported by Jensen et al. (1999) and La Terra et al. (2010), and its release during summer as a consequence.

4.2 Breed effect

Milk of Modicana cows had higher levels of α-tocopherol compared to both H and BS milk samples ($P<0.001$). A significant breed effect was observed in summer period for α-tocopherol, in particular, M milk was approximately twice as high as levels in both H and BS milk ($P<0.05$). The latter findings could be explained by higher pasture intake of M in spring and as a consequence of a major mobilization of α-tocopherol from body tissue resources in response to stress-inducing factors (heat stress, pasture–no-pasture dietary shift). Nozière et al. (2006) reported that in dairy cows, α-tocopherol uptake by the mammary gland increased under stress conditions including micronutrient deficiency, diet restriction and oxidative stress-inducing diets. Although the M breed effect was masked by the pasture intake considerably higher compared to the other two breeds but, it was interesting to highlight that, during summer, when AOs were lacking in the diet, milk of M cows kept significant α-tocopherol levels. Milk β-carotene levels did not differ significantly among breeds. Saturation of milk β-carotene may explain similar vitamin levels among breeds in spring despite different pasture intakes.

5 Conclusion

This study investigated pasture feeding effects and dairy cattle breed on α-tocopherol and β-carotene content in cows' milk by monitoring two grazing seasons in Sicily (spring and fall) as well as a summer no-pasture season. Milk AO levels were generally higher during spring, intermediate during summer and lower during fall. These results indicate that grazing pasture season has an important impact on milk AO content. In particular, higher milk AO levels in spring compared to fall might be attributed to several factors such as different quality and composition of pasture, different pasture intakes and even the climate. Spring pasture contained more Leguminosae than fall pasture that might increase the concentration of AO in milk. The effects of breed on milk AO content were not so pronounced. Further studies are needed to investigate on factors responsible for differences in summer AO milk composition and in particular to verify whether higher AO content in summer M milk was a breed effect or linked to the traditional farming system with higher levels of pasture intake. Thus, differences in AO content in milk between summer and fall could be partially linked to their storage in cow body filled up by spring diet. During fall, AO provided by diet could partially meet the cows' physiological requirements and feeding strategies need to be optimised relative to milk AO compounds with positive health impacts.

Acknowledgments This research was funded by 'Assessorato Agricolture e Foreste' of the Sicilian Region, (Palermo, Italy).

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