Chapter 15

Differential Characteristics of Milk Produced in Grazing Systems and Their Impact on Dairy Products

A.M. Descalzo, L.Rossetti, R. Páez, G. Grigioni,
P.T. García, L. Costabel, L. Negri, L. Antonacci, E. Salado,
G. Bretschneider, G. Gagliostro, E. Comerón and M.A. Taverna

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/ 50760

1. Introduction

Functional foods may exhibit health benefits beyond their nutritional value. Many traditional recommendations on food selection have included this view. In more recent years the interest in food with specific health benefits has greatly increased and stimulated the development of respective products for the food market. At the same time large efforts are made to substantiate health claims by validated experimental methods.

Dairy products have come to play an important role in this context. Regarding the nutritional and extra nutritional characteristics of milk, it can be considered a multifunctional food product. Milk is a source of proteins, lipids, vitamins and minerals, but also exerts other beneficial properties due to the presence of numerous bio-active molecules either through the direct consumption of milk or its derivatives (Guimont and others, 1997).

It is widely recognized that pasture, when efficiently grazed, is the cheapest feedstuff (Dillon, 2006) and that the cost of milk production is reduced as the proportion of grazed pasture in the diet of the cow increases (Dillon and others, 2008). Nevertheless, using pasture as the only source of food is inadequate for high milk producing cows. The lower intake of dry matter (DM) and energy would be the main cause of suboptimal production even under conditions of adequate quantity and quality of pasture (Kolver y Muller, 1998; Reis y Combs, 2000; Bargo and others, 2002).

Cows adapt to grazing through increased forage intake, but in order to achieve this, it is necessary to ensure around 60 kg DM/cow/day (Delagarde and others, 2004). Under these

conditions, the maximum yield obtainable on high quality pastures seems to be at around 22-25 kg/cow/day in spring and 18 kg/cow/day in autumn. However, at high levels of energy supplementation, rumen pH, the diurnal pattern of volatile fatty acid concentration and fiber digestion may be altered because of changes in rumen fermentation (Tamminga, 1993). These changes may provoke lower consumption of forage and a drop in the fat content of milk. In this regard, studies carried out in EEA INTA Rafaela suggest that the optimal level of supplementation that could maximize milk production without affecting the rumen of dairy cows grazing high quality lucerne, would be 7 kg of concentrate containing approximately 70% corn (Salado and others, 2010 a and b).

On the other hand, it is also known that the amount of conjugated linoleic acid (CLA) in milk fat increases according to the level of pasture included in the diet, with the highest CLA concentrations found in cattle fed 100 % pasture (Dhiman and others, 1999).

Alike other country, Argentina is moving forward to more complex livestock production systems. In this regard, component feeding systems based on the use of grazed pasture are gradually being replaced by confining ones, in which conserved forages (hay, silage) and concentrates (cereal grains, agro-industrial byproducts) are fed to dairy cattle as a total mixed ration (TMR). This phenomenon could be explained by the fact that argentinean dairy farmers are looking for ways to release land for agriculture (mainly for the cultivation of soybean [Glycine max]), which is considered a more profitable and simpler activity than dairy farming.

Nowadays, it is supported that the extreme intensification of dairy production systems would lead to lose not only the advantage that grazed pastures have on the reduction of milk production costs (Garcia and Fulkerson, 2005; Dillon and others, 2008; Macdonald and others, 2008, McEvoy and others, 2008) but also the marketing niches that look for dairy products from pasture-fed cattle due to their high CLA content (White and others, 2002). In this respect, pasture-fed dairy cows produced a significantly higher concentration of CLA (83%) than TMR fed-confined dairy cows (0.66 vs. 0.36% of the total fatty acids) (White and others, 2001).

For that purpose, grass-based dairy production should consider some productive aspects such as high-quality pasture and livestock adapted to a high-forage diet.

Pasture is a highly organized structure which changes with season and management. These changes can affect photosynthesis and hence dry matter accumulation, the degree of utilization and the feeding value of herbage. At the same time, these changes in pasture can affect the quality of milk and thereby the quality of dairy products.

2. Functional compounds characterized in diets for dairy cows

2.1. Natural antioxidants: Lipid-soluble vitamins

Plant secondary metabolites are incorporated into the tissues through dietary delivery. Among these, alpha- tocopherol is the main active form of vitamin E in animal tissues. It is selectively incorporated among eight isomers (alpha, beta, gamma and delta tocopherol and tocotrienol respectively) that are naturally found in plants. Vitamin E is the main fat-soluble

antioxidant incorporated into animal cell membranes and is also a regulator of cellular functions that are mediated by protein kinase C.

Carotenoids are lipophilic secondary plant and algae products consisting of eight isoprene units (C40). Among about 800 known carotenoids, up to ten of them have been determined in forages, namely oxygen-containing xanthophylls (lutein, epilutein, antheraxanthin, neoxanthin, violaxanthin and zeaxanthin), and carotenes (preferably β-carotene) of a hydrocarbon nature. Due to the high number of double bonds, carotenoids undergo oxidation and isomerisation (cis-isomers formation from all-trans-ones), in cut forage by the action of light. Carotenoids in cow's milk and consequently in milk products are important for human health and nutrition as natural antioxidants and some of them as precursors of vitamin A, among which all-trans-β-carotene is the main provitamin. Moreover, milk fat carotenoids cause the yellow color of butter and many cheeses, which is positively perceived by many consumers as ''green image" because of its association with grazing animals. This vitamin is implicated in reproduction, immunity and normal function of retinal cells. Lutein is another carotenoid which is abundant in plants and can be used as a tracer of pasture feeding, as animals are not able to synthesize this molecule and it is stored in the animal's fat after absorption and thus found in milk and meat (Prache 2005).

These molecules are highly variable in forages, silage and hay (Müller 2007; Hidiroglou 1996), and depend on the fermentation form as well as on the conservation method. Praché and others (2009) reported values between 40 and 123 microg/g DM for beta carotene (60 microg/g DM for lucerne) and from 167 to 437 microg/g DM for lutein (142 for lucerne) in pasture collected between Spring (May) and Summer (July).

Similar variation in carotenoid levels in mountain grazing was found by Calderón and others (2006) with values that ranged between 18 and 60.4 microg/g DM for beta carotene and between 96 and 262 microg/g DM for lutein.

Data from four separated experiments, which were carried out in INTA, also show a high variability. As shown in table 1, fresh pasture showed the highest input of alpha- tocopherol and beta-carotene among feed components. The variation of its composition is high, depending on seasonal variations, phenological stage and conservation technology, among other factors. Other components of feed are also sources of dietary antioxidants, but they showed less variation and their contribution in vitamins was less significant compared with pasture.

2.2. Natural antioxidants: Polyphenols

Polyphenols are antioxidants that may prevent various pathologies (cancer, cardiovascular diseases, immune deficiencies, etc.). Particularly, isoflavones may have a preventive effect against breast and prostate cancers and, to a certain extent, against some disorders related to menopause or andropause. The active molecules are plant-based isoflavones (directly absorbed) as well as a transformation component in the large intestine, equol. The main source of isoflavones in human diet are soy and soy-derived food. Among forage plants, other legumes (certain types of clover and lucerne) contain high levels of isoflavones. They may constitute up to 2% of dry matter in purple clover and are well preserved after silaging. Of the active isoflavones in forage, daidzein and formononetin were transformed in the rumen into

equol, which was secreted in the milk of goat and cow species in sufficient quantities to suggest they may exert a biological effect. Moreover, Besle and others (2010) found different phenolic compounds that can be used as tracers for the diet of dairy cows. Data shown in table 1, indicate that coumestrol was the main phytoestrogen found in lucerne (pasture and hay), corn silage, sunflower expeller and wheat bran. Concentrate mixture showed higher amounts of daizein, than other dietary compounds, probably derived from soy. In this experiment, the aglycone and glucoside forms were not differentiated. The extraction was performed in acid medium and therefore, the species were converted into the aglycone form.

Antioxidants (mg/Kg DM)[a]	*Lucerne pasture*	*Oat pasture*	*Concentrate mixture*	*Soy expeller*	*Sunflower expeller*
Gamma-tocopherol	19.679	9.10	67.231	101.538	3.519
Alfa-tocopherol	64.415	16.14	15.937	7.042	20.720
Beta-carotene	97.518	66.67	0.903	0.301	nd
Genistein	2.165	Not determined	1.110	Not determined	2.884
Lutein	481.92	1128.21	85.80	Not determined	Not determined
Daidzein	5.568	Not determined	14.061	Not determined	2.035
Coumestrol	25.821	Not determined	10.993	Not determined	11.943
% CV	61.98	17.15	34.51	nd	21.13
Antioxidants (mg/Kg DM)	***Corn silage***	***Sunflower pellets***	***Wheat Bran***	***Lucerne Hay***	
Gamma-tocopherol	20.111	1.521	48.809	3.345	
Alfa-tocopherol	17.702	2.445	27.533	24.612	
Beta-carotene	13.243	0.355	nd	5.493	
Genistein	1.593	Not determined	1.085	2.103	
Lutein	41.57	Not determined	Not determined	Not determined	
Daidzein	5.070	Not determined	1.665	1.962	
Coumestrol	22.731	Not determined	14.652	18.461	
% CV [b]	35.75	nd	9.95	38.68	

[a] For the determination of antioxidant vitamins, samples from individual components of the diet were extracted with hexane (after a saponification step) and quantified using high performance liquid chromatography. Carotenoids were monitored at 445 nm, tocopherols were detected by fluorescence at 296-330 nm (λ exc- λ em respectively) and retinol at 335 nm (Rossetti et al, 2010).

[b] Mean variation coefficient of all vitamins calculated for each dietary compound.

Table 1. Levels of fat-soluble antioxidants in dietary components used in INTA experiments with dairy cows.

2.3. Volatile compounds

Terpenes are direct biomarkers of animal diet as they are not modified by animal metabolism. In addition, the transfer of these compounds into milk fat is very fast and apparently exhibits no memory effect (Viallon and others, 2000). Therefore, their presence in milk and dairy products could be used to discriminate contrasting feeding conditions (Prache and others, 2005).

Lucerne pasture showed the highest volatile production (table 2). The most abundant terpene was d-limonene found in all dietary components. Only three minor compounds, nerol, menthol and linalyl acetate were found exclusively in lucerne. Terpinen-4-ol, was found in lucerne pasture and maize silage in a 2 to 1 proportion. In addition, ocimene was 4.5 times higher in lucerne pasture compared with the other components and linalool was 4 times higher than in maize silage and wheat bran. These results indicate that a group of terpenes, and their abundance and not a single compound could be an indicator of the different components of the diet.

Terpenoids (Relative Area Units)[a]	*Lucerne pasture*	*Oat pasture*	*Concentrate mixture*	*Sunflower expeller*	*Corn silage*	*Wheat Bran*
d-limonene	52.919	2.392	3.586	0.251	8.577	1.126
p-cymene	0.109	0.086	0.026	nd	0.009	nd
ocimene	0.164	nd	0.009	0.062	nd	0.077
linalool	0.985	nd	nd	0.072	0.116	0.136
menthol	nd	nd	nd	nd	nd	nd
terpinen-4-ol	nd	nd	nd	nd	0.002	nd
β-cyclocitral	0.412	0.051	0.004	nd	0.107	nd
nerol	0.052	nd	nd	nd	nd	nd
linalyl-acetate	0.021	nd	nd	nd	nd	nd
β-caryophyllene	0.061	nd	0.018	0.017	0.071	0.044
geranylacetone	0.089	nd	0.024	nd	0.122	0.028
%CV	36.10	12.01	14.29	17.45	18.07	8.83

[a] Volatile terpens were determined in individual feedstuff given to dairy cows in INTA experiments (2008-2009). For this assay, a fiber (CAR/PDMS/DVB 2cm-50/30mm) was used to extract/concentrate volatile compounds in the headspace (HS) of chopped samples. GC-FID was performed on a Shimadzu 14-B GC with a flame ionization detector (Negri et al., 2008).

[b] Mean variation coefficient of all volatiles calculated for each dietary compound.

Table 2. Volatile terpenoid compounds in dietary components used in INTA experiments with dairy cows.

Lucerne samples also presented a higher proportion of volatile aldehydes (table 3), at the expense of trans-2-hexenal, which was the most abundant volatile after d-limonene. Tava and Peccetti (1997) reported that trans-2-hexenal was the most frequent volatile in 13 genotypes of lucerne. This compound, originated in flowers, should have a physiological role for plant pollination by hymenopterans (Peccetti and others 2002).

Hexanal and trans-2 hexenal are end-products of the oxidation of C18:2 and C18:3 fatty acids respectively. In fact, fresh pasture shows the highest hexanal production, resulting from linoleic acid, whereas trans-2-hexenal indicates a high percentage of C18:3. These fatty acids are oxidized when fresh pasture is cut, producing the corresponding aldehydes. Both aldehydes and terpenes are responsible for the typical odour of different plant species and could be found in milk and dairy products.

Aldedhydes (Relative Area Units) [a]	*Lucerne pasture*	*Oat pasture*	*Concentrate mixture*	*Sunflower expeller*	*Corn silage*	*Wheat Bran*
3-methyl-butanal	0.161	0.022	0.026	nd	4.877	0.020
pentanal	0.363	0.016	0.008	nd	0.036	0.013
hexanal	0.889	0.360	0.130	0.192	0.129	0.190
trans-2-hexenal	6.689	1.911	nd	nd	0.302	0.030
heptanal	0.056	nd	0.018	nd	0.091	0.038
trans-2-heptenal	0.175	0.067	0.058	0.154	0.292	0.176
octanal	0.215	nd	0.041	nd	0.143	0.017
nonanal	0.235	0.039	nd	0.158	nd	0.205
trans-2-nonenal	0.292	0.019	nd	nd	0.022	0.081
%CV [b]	30.08	9.66	14.04	14.22	6.62	5.11

[a] Volatile aldehydes were determined as indicated in table 2.

[b] Mean variation coefficient of all volatiles calculated for each dietary compound.

Table 3. Volatile aldehydes in dietary components used in INTA experiments with dairy cows.

2.4. Fatty acids

Information available about the fatty acid composition of pasture lipids is scarce. Unsaturated fatty acids, particularly n-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) may be beneficial to human health. Therefore, maximizing C18:3 and CLA in milk and dairy products would benefit human health and nutrition. Milk and meat are the only significant source of CLA in the human diet and this appears to be related to the consumption by ruminant of fresh pastures (Elgersma and others, 2003).

Sources of variation in pasture lipid composition are plant species, growth stage, temperature and light intensity. Fresh grass contains a high proportion (50-75%) of its total fatty acid content in the form of n-3 linolenic acid. Levels of linolenic acid vary with plant factors such as stage of maturity and with light disponibility. Fatty acid profiles are distinctive to particular species, which confirm that fatty acid composition of grasses is under considerable genetic control (Dewhurst and others 2001). This offers the potential to select for grasses with higher concentrations or altered types of fatty acids.

Garcia et a. (2007) evaluated the range of fatty acid concentrations within various pastures and forage crops, at different cutting dates and season in Argentina for beef and milk production. Season affected ether extract (EE%) and fatty acid composition of lucerne cultivars (table 4). No interactions were detected between dormancy group and season ($p<0.344$). During the fall increased C18:2 and decreased C16:0, C16:1, C18:0. The differences between spring and summer were for C16:1, C18:0, C18:2 and C18:3. The EE% and contribution of C18:2 and C18:3 were higher in fall compared with the other seasons.

The fatty acid composition, percentage of ether extract and contribution (g/100g DM) of C18:2 and C18:3 of several cultivars are shown in tables 5 and 6. Cutting date has affected significantly these parameters.

SEASON	*C16:0*	*C16:1*	*C18:0*	*C18:1*	*C18:2*	*18:3*	*EE%*	*18:3 mg*	*18:2 mg*
Spring	29.4b	2.4c	5.6b	6.8	16.1a	39.4b	2.0a	0.80a	0.33a
Summer	31.0b	1.8b	6.6c	6.9	18.9b	33.9a	2.2a	0.75a	0.41b
Fall	20.0a	1.5a	3.2a	7.6	27.4c	40.8b	3.3b	1.33b	0.89c
SE	0.80	0.09	0.22	0.61	0.66	1.05	0.07	0.04	0.02

a b c Means in the same column with different letters differ ($p<0.05$)

Table 4. Fatty acid composition (%), ether extract (EE) and mg/g EE of C18:2 and C18:3 of different lucerne cultivars according to season.

		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	EE%
RGB	*Cut 1*	18.7c	2.0b	2.5c	4.5B	10.0 b CD	62.2a	23.3
	Cut 2	23.8ab	1.9bc	4.4ab	5.6B	11.8 a CD	52.4bc	5.5
RGF	*Cut 1*	19.6c	1.8bc	2.6c	4.7B	8.5b D	62.6a	22.7
	Cut 2	24.8ab	1.9bc	4.5ab	5.8B	10.9a D	51.6bcd	4.9
TC	*Cut 1*	21.3bc	2.0b	3.3abc	7.2AB	12.2b BC	53.8ab	22.4
	Cut 2	25.8a	1.8bc	4.9a	8.0AB	12.7a BC	46.1bcd	15.7
TG	*Cut 1*	24.7ab	2.5a	3.0bc	5.9AB	12.4b AB	51.6bcd	23.3
	Cut 2	25.4a	2.0b	4.0abc	8.6 AB	15.0a AB	45.3bcd	6.5
TDS	*Cut 1*	22.3abc	1.9b	3.5abc	7.3A	12.1b AB	52.8bc	21.9
	Cut 2	24.0ab	1.5cd	3.9abc	9.1A	15.7a AB	45.8bcd	16.5
CQ	*Cut 1*	25.4a	1.7bcd	4.8a	10.2A	13.4b A	43.1d	21.9
	Cut 2	25.9a	1.4d	3.4abc	8.3A	16.5a A	44.3cd	16.7
Pasture		***	***	NS	***	***	***	
Cut		***	***	***	NS	***	***	
Interaction		**	**	**	NS	NS	***	

Whole milk powder from pasture produced milk showed the same fatty acids profile as raw milk (table 5), and could constitute a natural source for n-3 fatty acids. In a similar way, antioxidants profile reproduced the composition of raw milk with estimated losses around 20 and 40% being higher for retinol and vitamin D3.

Higher natural antioxidants in whole milk powder lowered TBARS values in pasture (A70) samples compared to silage samples (A0) after 18-months shelf life assay (20ºC in sealed bi- laminated plastic pouches under nitrogen atmosphere).

M: Raw Milk; WMP: Whole Milk Powder; CH: Cheese (regianitto).

a: Thiobarbituric Reactive Substances

b: Relative Area Units

c: Ferric Reducing antioxidant activity

d: A0, A35 and A70, feed treatments with 0, 35 and 70% Lucerne pasture

ND: not done; nd: not determined, NS: Not significant

* p<0.05 **1 p<0.01 ***p<0.001

Table 5. Fatty acid composition (%) and ether extract (EE) (g/kg DM) of several pastures and forage crops. RGB (Rye grass Bill), Rye grass Florida (RGF), wheat Charrua (WC), wheat Guapo (WG), triticale Don Santiago® (TDS) and Centeno Quehue(CQ).

		C18:2	C18:3	C18:2+ C18:3	18:3/18:2
RGB	Cut 1	0.23b CD	1.5a	1.7a	6.4a
	Cut 2	0.18a CD	0.8cd	1.0e	4.5bc
RGF	Cut 1	0.19b D	1.4a	1.6ab	6.4a
	Cut 2	0.16a D	0.8cd	0.9e	4.8b
TC	Cut 1	0.27b BC	1.2b	1.5bc	4.4bc
	Cut 2	0.20a BC	0.7d	0.9e	3.7bcde
TG	Cut 1	0.29bAB	1.2b	1.5bc	3.9bcde
	Cut 2	0.25a AB	0.8d	1.0e	3.0e
TDS	Cut 1	0.26b AB	1.2b	1.4c	4.4bcd
	Cut 2	0.26a AB	0.8d	1.0e	3.1de
CQ	Cut 1	0.29b A	0.9c	1.2d	3.4cde
	Cut 2	0.28a A	0.7d	1.0e	2.7e
Pasture		***	***	***	***
Cut		***	***	***	***
Interaction		NS	*	***	**

* p<0.05 **p<0.01 ***p<0.001, NS: not significant

Table 6. Contribution (g/100 g DM) of C18:2 n-6 and C18:3 n-3 of several pastures and forage crops

As expected, fatty acids profile was different among dietary components. As shown in table 7, the most abundant fatty acid in lucerne pasture was C18:3 and it can be the molecule that originates branched aldehydes, especially trans-2-hexenal, in this assay.

Fatty Acid (%)[a]	Lucerne pasture	Lucerne hay	Concentrate mixture	Sunflower expeller	Corn silage	Wheat Bran
C16:0	33.21	38.42	16.84	15.72	27.97	18.02
C16:1	5.14	3.79	0.39	0.48	1.67	0.58
C18:0	9.06	8.73	2.57	4.78	7.89	1.69
C18:1	17.20	14.56	27.31	30.01	16.74	21.23
C18:2	13.21	18.41	49.58	48.57	37.14	54.21
C18:3	22.06	15.89	3.24	nd	8.51	4.03
18:3/18:2	1.69	0.82	0.06	nd	0.23	0.07
%CV [b]	14.57	32.88	13.69	13.29	28.29	8.25

[a] mean of samples from years 2007 and 2008.

[b] mean variation coefficient of all fatty acids calculated for each dietary compound, nd: not detected.

Table 7. Fatty acids in dietary components used in INTA experiments with dairy cows.

3. Incorporation of antioxidant vitamins into milk

3.1. Properties of milk produced on pasture

The nature of cow's forage diet, i.e. botanical composition, maturity stage and preservation mode, strongly influences milk composition in fatty acids, vitamins and carotenoids (Chillard 2001, Hartman 1965). The contents of retinol, α-tocopherol and β-carotene, lutein, xantophylls, saturated and polyunsaturated fatty acids profile in plasma, milk and milk fat are influenced by the diet. In addition, some of these parameters also differed according to sire and stage of lactation (Lucas 2006, Soren 1999).

Calderón and others (2007) reported that the incorporation of grass silage and lucerne protein (75:25), in an experimental diet designed for Montbéliarde cows in midlactation induced a rapid increase in plasma concentrations of beta carotene and vitamin E. The incorporation of these compounds varied in a linear form with the proportion of grass within the diet. Similar responses were observed in milk for vitamin E, whereas there was an apparent saturation in milk concentrations of beta carotene at high levels of carotenoid intake, i.e., when plasma beta carotene exceeded 5 microg/ml.

In an experiment conducted in INTA-Rafaela (Santa Fe, Argentina), two isoenergetic diets (1.55 Mcal EN/kgDM) of contrasting nature: 70% lucerne pasture (ALF) and grain sorghum silage (SS) were offered to Holstein cows on their second third of lactation. Milk and protein yields were higher ($P < 0.01$) in ALF than SS diet (31.78, 26.14 l/v/d and 1.019, 0.841 kg/v/d, respectively). Fat concentrations were lower on ALF than SS diet ($P < 0.01$). All remaining variables were not significantly different, with the only exception of total solid contents ($P < 0.05$). No significant differences were found on live weight variation (ALF= 0.084, SS= 0.271 kg/v/d).

As shown in figure 1, fat-soluble vitamins increased over the first 20 days after a dietary shift from silage to grazed pasture (70% lucerne), and the concentrations were stabilized at day 40 with persistence until day 60 (Rossetti and others, 2010). Similarly, Calderón and others (2007) observed a rapid increase during the first 14 days in antioxidant vitamins in plasma and milk of Montbéliarde dairy cows, after a dietary shift from a low-carotenoid diet based on hay and concentrates to a high-carotenoid diet based on grass silage that was prepared from perennial ryegrass (*Lolium perenne*). The concentration of α-tocopherol, 13-cis-β-carotene, all-trans-β-carotene and lutein reached a plateau between 21 and 28 days and persisted until day 42. The incorporation of zeaxanthine into plasma was reported but it was either not detected in milk (Calderón et al., 2007), or its concentration was around 1% of total carotenoids (Butler 2008).

3.2. Effect of increasing pasture content into a total mixed ration diet in relation to health promoting compounds

An increase of pasture in the diet, at the expense of a total mixed ration (TMR), has been shown to improve the incorporation of antioxidant vitamins into milk. Figure 2 shows the relationship between the percentage of pasture (grazed oat pasture) vs. α-tocopherol, retinol, all-trans-β-carotene and lutein in milk after 30-day treatments.

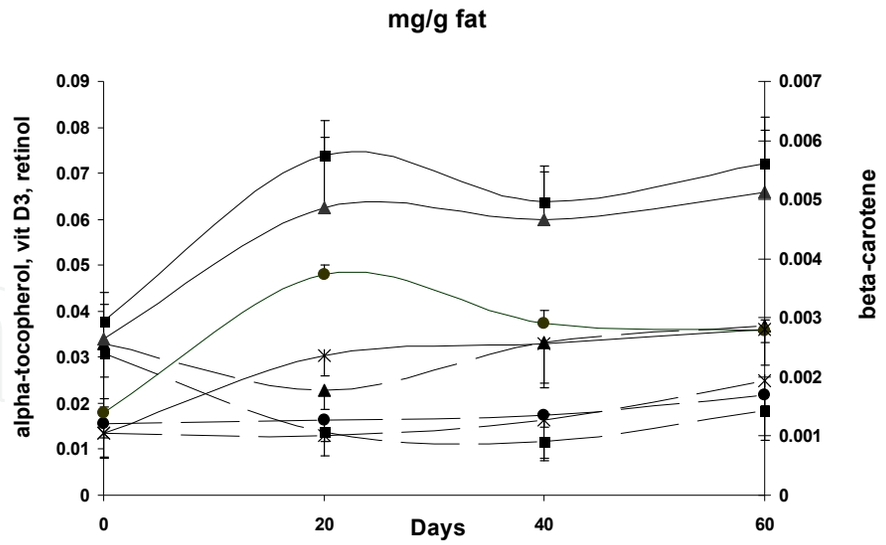

Figure 1. Lucerne or silage feeding. Evolution in α-tocopherol (●), all-*trans*-β-carotene (■), retinol (▲) and vitamin D3 (*) in milk of dairy cows fed diets differing in ALF (solid lines) or SS (dashed lines). All groups were fed sorghum silage during a pre-experimental period of 6 wk. Means ± SEM for 5 cows per group are presented.

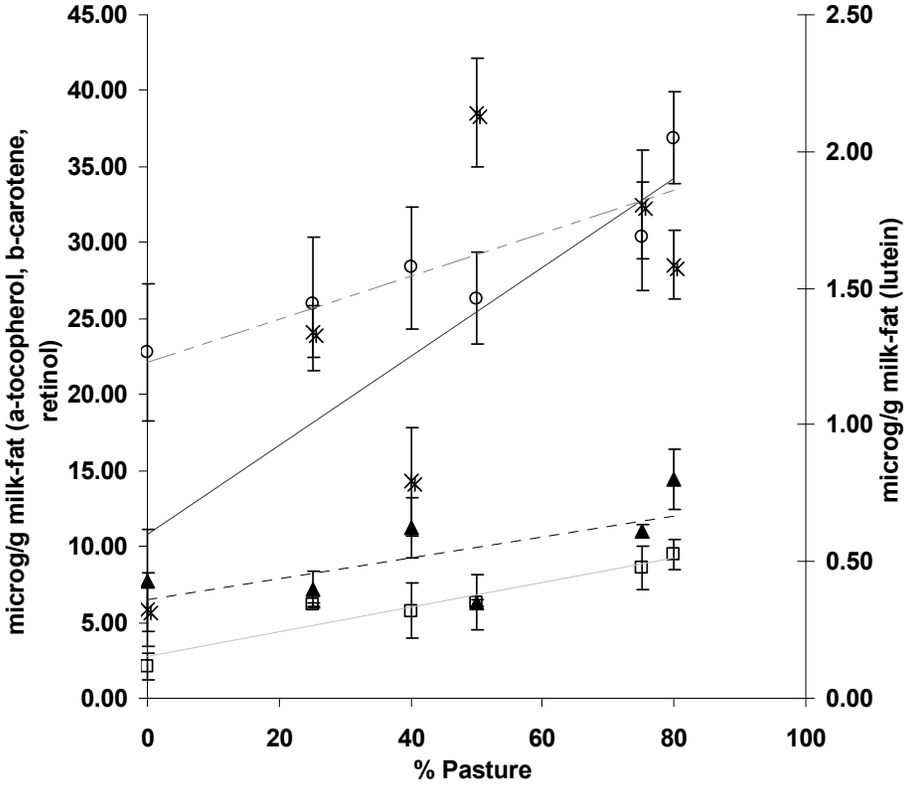

Figure 2. Oat pasture vs. TMR feeding. Concentration of α-tocopherol (○), all-*trans*-β-carotene (□), retinol (▲) and lutein (x) in milk-fat of dairy cows fed diets differing in the percentage of oat pasture. Linear tendency for α-tocopherol and retinol (dashed lines) and all-*trans*-β-carotene and lutein (solid lines) are shown. All groups were fed corn silage during a pre-experimental period of 6 wk. Each point represents eight cows per treatment. Data were compiled from two separated experiments with comparable diets.

Vitamin A in milk is incorporated under the form of retinol and carotenoids. Among these, all-trans-β-carotene was the mayor form recovered followed by lutein, which was consistent with what is generally reported. Although lutein was the major carotenoid found in pasture, its incorporation rate into milk was 10-fold lower than the incorporation of all-trans β-carotene. The same observation has been reported by Calderón (2007) and Butler (2008). Recovery of these species of vitamin A in milk was directly related to their concentration in feed. Pearson correlation coefficients for all-trans-β-carotene and lutein in feed and milk were R=0.8323 and R=0.7521 respectively. Concerning the lipoperoxyl radical-scavenging activity of all-trans retinol it behaves as a more effective antioxidant at a low partial oxygen pressure, low retinol concentration and high radical flux (Tesoriere 1997)

In a revision article, Nozière and others (2006), have discussed that the extent of carotenoid degradation by microorganisms in the rumen remains uncertain because of the wide range of results, mostly on β-carotene, from in vitro and in vivo studies. Whereas some authors reported no degradation (Dawson and Hemington, 1974; Cohen Fernandez and others, 1976a), others found moderate (10–25%; Davison and Seo, 1963; Potkanski and others,1974; Cohen Fernandez and others, 1976b; Mora and others, 1999) or higher β-carotene disappearance (40–55%; King and others, 1962). The carotenoid supplement form could explain discrepancies among experiments, because degradation rates were usually higher when carotenoids were supplied as purified products than when provided in forages. This hypothesis was recently confirmed in vitro at the INRA in a study where no apparent degradation was detectable for lutein provided in forage, whereas 50% of the initial amount disappeared when the same quantity of lutein was added as a pure commercially available source.

Vitamin E concentration ranged from 16.5 to 46 microg/g milk-fat for milk from silage or pasture diets respectively. Pasture values were even higher than concentrations reported by Al Mabruk (2004) for grass silage supplemented with vitamin E (22.26 microg/g milk fat). Calderón and others (2007) showed an increase from 8 to 14 microg/g milk fat depending on the proportions of experimental feed (75% grass silage + 25% lucerne protein concentrate) in the basal diet (from 0 to 66%).

Four different assays conducted in INTA (Argentina) indicate that after a shift from silage to pasture diet α-tocopherol augmented from 16.7 to 40.37 (70% grazed lucerne); from 11.59 to 26.05 and 28.53 (35 and 70% grazed lucerne pasture); 22.78 to 36.91 (80% grazed oat pasture) and 24.36 to 30.42 (75% oat pasture). In fact, Schingoethe and others (1978) reported a high variability in the concentrations of vitamin E in milk, depending on the grazing conditions. The concentration augmented from 20 to 50 microg/g milk fat at the time cows started grazing pasture. The same observation was reported by La Terra and others (2010) with an increase of 37% and 68% in α-tocopherol for cows fed 30:70 and 70:30 ratio for pasture:TMR, respectively in comparison to TMR diet. Therefore, results from INTA experiments showed the same tendency. However, one experiment conducted in autumn 2007, showed no differences between maize silage or pasture (lucerne) fed cows with an average of 27.5 microg/g milk fat, thus indicating that the quality of the diet and the pasture affects the incorporation of vitamin E into milk.

Vitamin D may be consumed in the diet as either ergocalciferol (D2) from plant sources or cholecalciferol (D3) from animal sources. With the exception of fatty fish, relatively few foods are naturally rich in vitamin D. In mammalians with ample sunlight exposure, the greatest source is endogenous vitamin D produced when 7-dehydrocholesterol in the epidermis and dermis of the skin is converted into vitamin D3 after exposure to ultraviolet B radiation. Vitamins D2 and D3 from dietary sources are transported to the liver, and from there to the tissues. Vitamin D3 from skin is transported through plasma. Therefore more evidence is needed to understand the factors that induce its variation in milk content. Consequently, for vitamin D, the dairy industry proposed technologically vitamin D-enriched dairy products to consumers (Graulet 2010). The vitamin D3 endocrine system has mostly been studied for its role in calcium and phosphorus metabolism and its possible role as an antioxidant has been neglected. Sardar and others (1996) demonstrated that D3 treatment brought about similar reduction in the extent of lipid peroxidation and induction in superoxide dismutase (SOD) activity, as with vitamin E supplementation in rats.

The concentration of vitamin D3 showed different tendencies among experiments. In one experiment, it ranged from 5.85±1.21; 8.01±2.13 and 10.29±2.34 microg/g milk-fat for diets containing 0, 40 and 80% oat pasture respectively, showing a linear increase with the proportion of pasture in the diet. This observation agreed with the described in figure 1, although values were lower (average of 30 microg/g milk-fat, in figure 1 with 70% of lucerne pasture diet vs. 10 microg/g milk-fat, with an 80% oat pasture diet). In contrast, in a second experiment the concentrations were 0.80; 0.91; 1,98 and 1.34, without significant differences ($P > 0.05$) for diets containing 0, 25, 50 and 75% of oat pasture. Therefore, a tendency for higher values of vitamin D3 with the increase of grazed pasture was observed but further assays are necessary to describe the productive parameters that may be used to improve the concentration of natural vitamin D3 in cow milk.

3.3. Effect of increasing concentrate content on grazed pasture.

The addition of concentrate on a pasture based feed was studied in two separated experiments: total feed assignment around 30 and 31.9 kg DM/head/day in experiments 1 and 2 respectively. Pasture consumption diminished with the increase of concentrate within the diet: 18.0; 16.1 and 14.2 kg DM/ head/day for 3.5; 7.0 and 10.5 kg of concentrate respectively in experiment 1 but not in experiment 2. In both cases, the addition of concentrate had no effect on the concentration of antioxidants in milk. The average concentrations were 5.34; 37.25; 1.09 and 6.51 microg/g milk fat for retinol, α-tocopherol, γ-tocopherol and β-carotene respectively. These values resulted similar to those obtained with pasture proportions above 40% in the diet (figures 1 and 2).

Calderón et. al, 2007 demonstrated that the transfer of β-carotene from plasma to milk reached a plateau when plasma levels exceeded 5 microg/ml and resulted a limiting factor in terms of the secretion of this antioxidant, that can be due to a limited uptake by the

mammary gland or limited transport by binding to transport proteins like β-lactoglobulin (Dufuor and Hartle, 1991) and/ or to saturation of milk fat globules.

It can be concluded that good quality pasture favored a linear increase of antioxidant vitamins in milk, and that this increment was not affected by the addition of concentrate in the feed.

3.4. Functional fatty acids in milk

There is a growing interest in CLA, considered to be beneficial in prevention of carcinogenesis (Ip and others, 1999), the n-6 to n-3 polyunsaturated fatty acids (PUFA) relationship, and the low percentage of saturated fatty acids, as these conditions are beneficial for the prevention of cardiovascular diseases. Their percentages in milk products can be increased through a suitable dietary regimen. Changes in milk fatty acids profile are possible due to the plasticity of milk fat (reviewed in Chillard and others 2000).

Dietary changes after shifting from silage to lucerne pasture diet induced significant changes in some fatty acids. Saturated fatty acids showed higher values in lucerne than in silage produced milk ($P<0.05$): C12 (2.79 ± 0,123 vs. 2,45 ± 0,132), C14 (10,79 ± 0,344 vs. 9,30 ± 0,359); C16 (29,63 ±0,617 vs. 26,26 ± 0,650); C17 (0,72 ± 0,012 vs. 0,64 ± 0,013) and C18 (8,84 ± 0,504 vs. 12,22 ± 0,563) respectively throughout the experiment. Conversely, silage produced milk showed higher values for C18:1c (21,00 ± 0,823 vs. 25,22 ± 0,840); C18:2c (2,63 ± 0,085 vs. 2,98 ± 0,098) and C18:2t (0,40 ± 0,029 0,15 ± 0,031) than lucerne produced milk. In addition, pasture diet favored the incorporation of C18:3 (1,03 ± 0,051 vs. 0,54 ± 0,054) into milk. In this experiment, C18:1t (3.80 ± 0,269 vs. 3.51 ± 0.285) and CLA (1.52 ± 0.096 vs.1.25 ± 0.107) values for lucerne and silage milk respectively showed similar results ($P>0.05$). This result was not expected as Castillo and others (2006) reported a positive association between proportion of lucerne pasture in the diet and content of cis9 trans11 CLA and C18:1t in milk under similar experimental conditions within the same geographical region.

A second experiment was conducted in INTA with increasing percentages of lucerne pasture. Twenty four Holstein cows from the experimental herd (6 heifers and 18 multiparous) in mid lactation, were randomly assigned to three different treatments. During a first pre-experimental period of 7 days, considered as co-variable, all cows were fed the same diet, in a second period each group was assigned to their treatment during 21 days and in the third period of 28 days, each group received the corresponding diet, with 0 (A0 or maize silage control diet), 35 (A35) and 70 % (A70) lucerne pasture. Diets were isoenergetic and isoproteic. Lucern pasture was cut daily and offered to the cows. Rations were fed as TMR (20.34 kg DM/cow/day, in average).

Milk production, protein and urea content showed lower values for lucerne groups compared with milk from silage-fed cows ($P<0.05$). For other parameters, milk from the three treatments showed a similar overall quality.

As mentioned above, the proportion of pasture in the diet influenced the composition of saturated fatty acids (SFA) in milk (table 8). C4, C15, C16 and C17 FAs were higher in A70

milk compared with A35 and A0 treatments ($P < 0.05$). C12 FA was higher in A0 than in A35 and A70 milk ($P < 0.05$). Total C18:2 FA was higher in A0 milk, but differences were observed for individual isomers. Trans-9, trans-12 C18:2 and cis-9, trans-12 C18:2 showed similar percentages in all milk samples. However, trans-9, cis-12 C18:2 increased with the higher content of lucerne in the diet (A70 compared with A35 and A0), and conversely, cis-9, cis-12 C18:2 decreased with an increase of lucerne ($P < 0.05$). These results differed from Schroeder and others (2003) that reported higher levels of trans-vaccenic and c9 t 11 CLA in milk from pasture (Avena sativa L.) fed cows compared to milk from TMR fed cows. The mean percentage of CLA in argentine milk from the central Region was reported to be 1.2 (Castillo and others, 2006; Páez and others, 2007). The nature of the differences with previous reports could rely on the quality of pasture and its highly variable composition, as discussed in the section above. The mean C18:3 n-3 FA content in lucerne milk was approximately 1.7 and 2.9 times higher (A35 and A70 respectively) than in silage milk ($P< 0.01$). In addition, C 20:5 n-3 showed an lucerne-dependant increase (A70> A35 > A0). Therefore, the incorporation of lucerne pasture in the diet was associated with a higher proportion of milk n-3 FA compared with maize silage diet. Indeed, the plot of C 18:2 vs. C 18:3 for milk samples showed a differential distribution of milk according to the diet and could therefore be used to differentiate the incorporation of lucerne into the diet of dairy cows (figure 3).

This result is consistent with other results shown for grazed systems (Chillard and others 2001; Castillo and others 2006; La Terra and others 2010). The source of the variability remains to be further studied and strategies different that using pasture as the unique factor to improve trans- vaccenic acid and CLA in milk continue under experimentation.

Low percentages of CLA (around 0.34 %) was found to be associated to cows fed fermented roughage and concentrates (most intensive production farm) whereas high percentages (around 0.80 %) was found in the ecologically produced milk fat. The concentration of CLA correlated positively with and trans-vaccenic acid (Jahreis 1997). The high C18:3 content of young grass and its low fibre content interact to increase the production of CLA or its trans C18:1 precursors (Chillard and others 2000). The presence of lipid precursors is one of the factors described by Griinari and Bauman (1999), to improve CLA biosynthesis. The second factor are the changes associated with the microbial activity associated with ruminal biohydrogenation, which is incomplete and leads to an accumulation of trans vaccenic acid, the precursor of CLA biosynthesis. In addition, factors that regulate these patways as well as the activity of the Δ-9 desaturase in the mammary gland and its regulation are a matter of investigation.

CLA isomers exert different biological activities. Juárez and others (2010) have found that feeding 1068 IU vitamin E, reduced the total trans-18:1 content in backfat ($P < 0.01$), as well as the percentage of trans 10-18:1 ($P < 0.001$), which are related to an increased risk for cardiovascular diseases. On the other hand, trans 11-18:1 (vaccenic acid) the precursor for cis9, trans 11-18:2 (CLA), increased ($P< 0.01$). Vitamin E could, therefore, be used to decrease trans-18:1 in beef and improve its isomeric profile.

Fatty acids (FA) percentages					
Fatty Acid (FA)	A0	A35	A70	SEM [a]	P [b]
C4	3.27 a	3.43 ab	3.59 b	0.248	0.0137
C6	2.16	2.17	2.3	0.172	0.096
C8	1.34	1.26	1.29	0.099	0.178
C10	2.97	2.74	2.76	0.262	0.063
C10:1	0.24	0.22	0.22	0.027	0.086
C12	3.29 a	2.97 b	2.96 b	0.286	0.0108
C14	10.92	10.66	10.96	0.577	0.371
C14:1	0.79	0.71	0.7	0.103	0.065
C15	0.89 a	0.96 a	1.11 b	0.072	<0.0001
C16	25.15 a	26.35 a	28.32 b	1.899	0.0019
C16:1	1.38	1.42	1.51	0.198	0.241
C17	0.60	0.62	0.71	0.037	< 0.0001
C18	13.04	13.28	11.9	1.209	0.066
trans total *C 18:1*	3.17	2.94	2.93	0.426	0.398
cis9 *C18:1*	20.56 a	20.00 a	18.95 b	0.958	0.0016
trans9, trans12 *C18:2*	0.15	0.15	0.18	0.056	0.228
cis9, trans12 *C18:2*	0.07	0.16	0.09	0.096	0.105
trans9, cis12 *C18:2*	0.09 a	0.09 a	0.17 b	0.069	0.0080
cis9, cis12 *C 18:2*	3.85 a	3.27 b	2.37 c	0.475	<0.0001
cis9, cis12, cis15 *C 18:3*	0.31 a	0.53 b	0.92 c	0.180	<0.0001
cis9, trans11 *18:2 (CLA)*	0.87	0.9	0.86	0.076	0.512
C20:5 n3	0.03a	0.04 a	0.07 b	0.029	0.0060
C24	0.04 a	0.04 ab	0.07 b	0.026	0.0153
C22:4 n6	0.04	0.03	0.04	0.066	0.8467
C22:5 n3	0.04	0.06	0.06	0.057	0.6473
SFA	63.86 a	64.68 ab	66.17 b	1.50	0.0036
MUFA	25.90 a	25.07 ab	24.09 b	1.06	0.0015
PUFA	5.82 a	5.53 a	5.07 b	0.44	0.0017
Total Trans FA	3.59	3.62	3.62	0.61	0.9806

Table 8. Fatty acids profile in milk produced on 70 (A70), 35 (A35) and 0 (A0) percentage of Lucerne pasture.

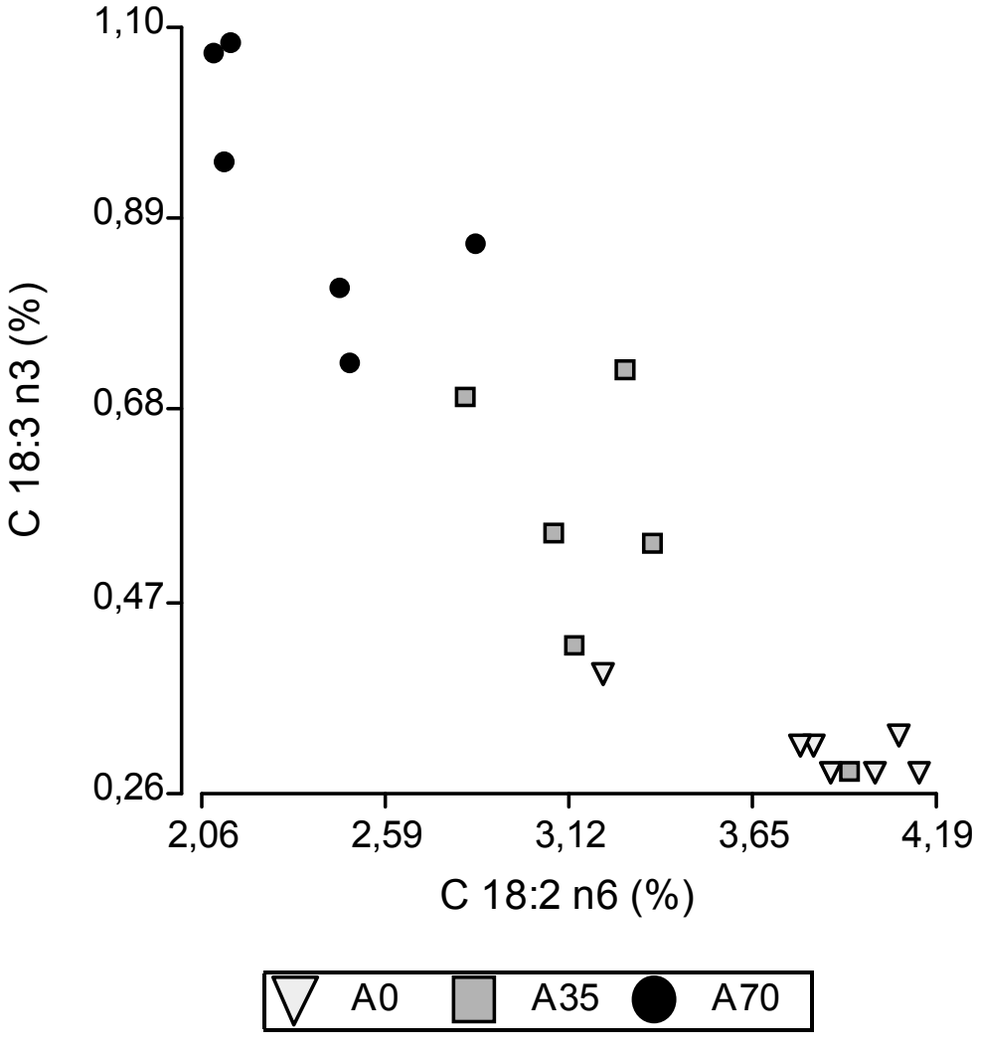

Figure 3. Relationship between linoleic acid (C18:2 n6) and linolenic acid (C18:3 n3) in milk from cows fed diets with different contents of lucerne (0, 35 and 70% for A0, A35 and A70 respectively) Different letters indicate significant differences (P<0.05).

4. Milk and dairy products

4.1. Oxidative status in milk and milk powder

Pasture feeding improved the antioxidant status in raw milk but also the peroxidative index at expenses of an increase of the n-3 fatty acids. However, feeding experiments carried out under the same production conditions showed quite dissimilar results depending on the seasonal variations.

Dry matter consumption differed between pasture and silage feeding regimes and also between Autumn and Spring. This feature is probably attributable to the differences between pasture qualities between assays. Total protein (TP) and butter fat (BF) were lower in pasture than in silage milk (3.38 vs. 3.71 % BF and 3.13 vs. 3.28 % TP respectively). α-tocopherol and β-carotene were significantly higher in pasture samples

(P<0.05), with a significant interaction between treatment and season for retinol and α-tocopherol, probably due to differences in pasture properties. β-carotene was highly correlated with the proportion of lucerne in the diet, as shown in figure 2 for oat pasture. As a consequence of the increase of cartonoids, milk, milk powder and cheese had higher b* values, which was traduced in a yellower fat colour that could differentiate dairy products from milk produced on pasture. Keenan and others., (1970) demonstrated that β-carotene was the major pigmented material encountered in bovine plasma membrane, and the plasma membrane origin of MFGM (milk globule fat membrane) would explain the origin of β-carotene in milk, which reflects the dietary origin of this provitamin.

Gamma-tocopherol was the minor isomer of vitamin E in milk and dairy products. Its incorporation into milk was not influenced either by seasonal variation or by dietary treatment. The significance of this minor form of vitamin E incorporated in milk should be further stated. Its concentration remains at least one order below α-tocopherol levels in vivo. However, its activity should be taken into account to prevent tissue damage. Particularly, γ-tocopherol has the ability to protect against nitrogen-based free radicals, whilst α-tocopherol cannot (Jiang and others, 2001).

Vitamin D3 was influenced by seasonal variation. The concentration in raw milk resulted 10-fold higher in spring than in autumn, probably due to degree of exposure to the sun which induces its biosynthesis in cows. Nevertheless, milk for human consumption is supplemented with 400 UI per quart (1 IU of vitamin D3 corresponds to 0.025 micrograms) to achieve the recommended mean intake of 5 micrograms per day (WHO). Natural vitamin D3 was not found in reggianito cheese.

Pasture feeding also modulated retinol content. Values were higher in pasture milk (p<0.05) with an interaction effect for seasonal variation. These differences were also found in milk powder and cheese, being enhanced in spring. . Therefore the susceptibility to peroxidation due to the number of double bonds in PUFAs in milk, increased with the proportion of pasture in the diet (table 9).

Oxidation in raw milk and milk powder was determined using the thiobarbituric acid reactive substances assay or TBARS, which is one of the oldest and most commonly used methods for assessing lipid oxidation in foods (Ladikos and others 1990).

This method is based on the spectrophotometric determination of extracted malonaldehyde, a minor product of oxidation, and can be performed either directly on the food product or on a steam distillate of the food. Due to the simple procedure and high correlation with sensory scores (Igene and others 1979), many studies have relied on TBARS for determination of oxidative status. The major disadvantage of the TBARS reaction is that it is not specific for malonaldehyde. No significant differences were found for TBARS (P>0.05), but this parameter showed consistently lower values in pasture milk, but was highly influenced by stationary variability.

Other method for the detection of intermediate peroxide compounds is the Peroxide Value. This parameter, as TBARS, was similar for all samples (data not shown).

Autumn	*A0*			*A35*			*A70*		
	M	MP	CH	M	MP	CH	M	MP	CH
L*	90.30	95.32	85.83	89.32	94.84	84.88	89.58	95.03	84.36
a*	-1.45	-1.24	3.46	-0.66	-0.77	5.20	-0.49	-0.90	5.48
b*	10.20	13.55	24.84	12.36	14.88	30.94	13.29	15.67	32.07
Beta carotene	0.08	0.72	0.41	0.25	1.56	0.78	0.27	1.58	0.74
Alfa tocoferol	1.13	6.68	5.44	0.97	8.07	6.54	1.06	7.04	5.93
Gamma tocoferol	0.06	0.30	0.24	0.02	0.19	0.15	0.02	0.09	0.04
Vit. D3	0.01	0.29	nd	0.02	0.36	nd	0.02	0.34	nd
Retinol	0.30	3.53	1.53	0.42	5.14	1.88	0.46	5.15	1.24
TBA (ppb)	44.01	1401.26		32.63	1165.21		47.19	1102.76	
Hexanal (RU/1000)	40.19	400		33.70	343		48.42	273	
FRAP (microM Fe+2)	355.1	291.43		374.5	319.85		390.2	310.22	
Spring	*A0*			*A35*			*A70*		
	M	MP	CH	M	MP	CH	M	MP	CH
L*	89.49	94.82	85.90	89.24	94.50	84.74	89.24	94.26	84.98
a*	-2.09	-1.52	4.11	-0.85	-0.92	6.43	-0.85	-0.95	5.97
b*	8.84	12.68	21.12	11.56	14.81	26.72	11.56	14.05	28.92
Beta carotene	0.02	0.23	0.17	0.19	0.78	0.51	0.19	0.87	0.84
Alfa tocoferol	0.35	2.82	3.23	0.75	3.81	5.48	0.75	4.56	5.88
Gamma tocoferol	0.06	0.63	0.65	0.05	0.38	0.46	0.05	0.39	0.41
Vitamin D3	0.11	0.90	nd	0.12	0.84	nd	0.12	0.90	nd
Retinol	0.47	6.96	1.88	1.47	14.28	3.84	1.47	13.85	2.76
TBARS (ppb) a	214.79	1073.54	ND	198.37	1132.32	ND	198.37	968.23	ND
Hexanal (RU/1000) b	102.45	629	ND	25.95	341	ND	27.45	305	ND
FRAP (microM Fe+2) c	308.36	166.43	ND	481.75	204.52	ND	481.75	163.67	ND

A0, A35 and A70: Diet with 0, 35 and 70% Lucerne pasture respectively.
M: Raw Milk; WMP: Whole Milk Powder; CH: Cheese (regianitto).
a: Thiobarbituric Reactive Substances
b: Relative Area Units
c: Ferric Reducing antioxidant activity
d: A0, A35 and A70, feed treatments with 0, 35 and 70% Lucerne pasture
ND: not done; nd: not determined
L*, a*, b*: CIELab Sysytem, D65 Illuminant and 10º geometry.

Table 9. Mean values of antioxidant vitamins, color parameters and oxidative stability indicators in two different seasons.

As indicated for feed components, numerous aldehydes are produced during oxidation, including octanal, nonanal, pentanal, and hexanal. Hexanal is the dominant aldehyde produced during oxidation (Dupuy and others 1987; Ajuyah and others 1993). It arises from both the 9 and 13 hydroperoxides of linoleate, and from other unsaturated aldehydes formed during the oxidation of linoleate (Shahidi and Pegg, 1994). It is a useful tool to assess lipid secondary oxidation products in milk (Erickson, 1999; Smet, 2009). This compound was higher in silage milk and could reflect a lower oxidative stability in milk and dairy products derived from silage-fed cows. Whole milk powder from pasture showed the same fatty acids profile as raw milk (table 9), and could constitute a natural source for n-3 fatty acids. In a similar way, antioxidants profile reproduced the composition of raw milk with estimated losses around 20 and 40% being higher for retinol and vitamin D3.

Higher natural antioxidants in whole milk powder lowered TBARS values in pasture (A70) samples compared to silage samples (A0) after 18-months shelf life assay (20ºC in sealed bi-laminated plastic pouches under nitrogen atmosphere).

The total antioxidant activity was measured using the Ferric reduction assay (FRAP). Antioxidant compounds such as α-tocopherol, trolox, vitamin C, uric acid and billirubin, among others, are able to reduce ferric- to ferrous-tripyridyltriazine which develops a blue colour (Benzie and Strain 1996) with an adsorption maximum at 593 nm. Smet and others. (2009), demonstrated that FRAP and DPPH assays provide useful information about the oxidation process, particularly about the very early changes in the oxidative stability of milk. FRAP values for A0, A35 and A70 milk, were 332, 428 and 448 micromolar equivalents of Fe+2 respectively ($P<0.05$) and served as an indicator of the antioxidant capacity of milk. In milk powder, no differences were detected, thus indicated that some thermo labile antioxidants could be destroyed due to the processing of milk.

It can be concluded that there is not a unique method to describe the antioxidant capacity of biological samples. The complexity and diversity of mechanisms that contribute to the onset of oxidation and the mechanisms that counteract oxidative reactions involve multiple pathways. Also α-tocopherol, β-carotene and retinol contents were higher in pasture samples throughout the shelf life experiment.

4.2. Instrumental sensory odour

The electronic nose (E-nose) has been successfully applied to distinguish seasonal variations in whole milk powder (Biolatto and others., 2005). Also its application permitted the differentiation of virgin olive oil that showed different volatile production and vitamin E degradation patterns during the frying process (Messina and others., 2009).

The application of e-nose measurement to milk samples permitted to demonstrate that a dietary shift from silage to Lucern pasture, induced changes in the odour profile of the raw milk.

As shown in figure 4, at the beginning of the experiment (time 0), all samples were grouped differently than after turn into pasture. Also changes in the profile were detected at time 60 days, thus indicated an interaction between diet and time in the experiment, probably due to changes in the nature of the volatile compounds produced by Lucern plants as the experiment proceeded.

Data were analyzed using a Discriminant Function Analysis (DFA) and were discriminated with 86% of recognition of each sample within each group for the original cases.

A positive correlation was found for e-nose measurements and the content of different antioxidant vitamins in milk. Rossetti and others., 2010, showed than individual e-nose sensors correlated with the concentration of antioxidant vitamins in milk. The sensor called LY2/gCTI correlated positively with α-tocopherol, β-carotene and retinol, and negatively with γ-tocopherol ($p<0.05$). The sensor P30/1 correlated positively with retinol and the sensor P10/1 showed a positive correlation with γ-tocopherol ($p<0.05$).

Odour components have shown also to be transferred from raw milk to whole milk powder from cows fed 0, 35 and 70% Lucern pasture. When the value of each sensor was compared in both products, a linear relationship was found with a correlation coefficient of 0.9851 ($P<0.05$). In reggianito cheese, and after six months maturation, this relation was different (figure 5b), probably due to the development of odour compounds during the fermentation and maturation processes.

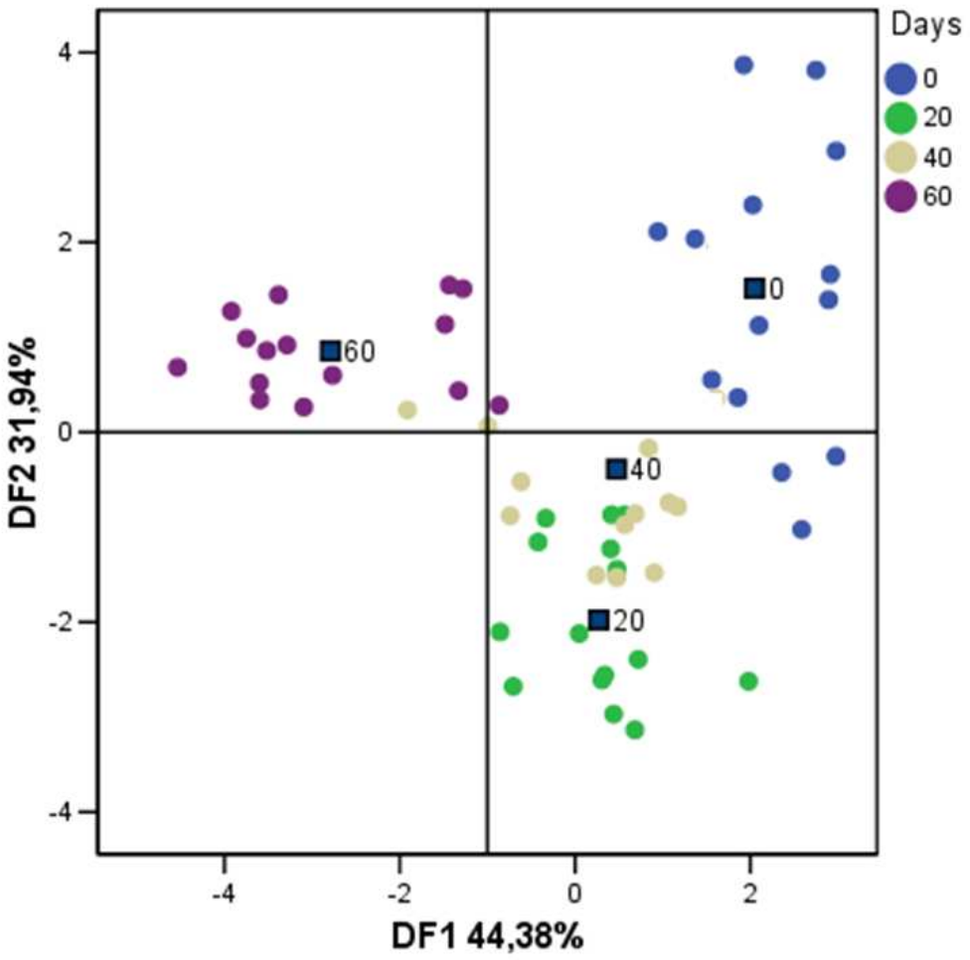

Figure 4. Odor profile of ALF (70% Lucerne pasture diet) milk at 0 (time 0), 20 (time 20), 40 (time 40) and 60 (time 60) days after the shift from silage to pasture diet. Odor profile was determined in the head space of milk samples (3 samples per cow per time) with 3 mL of sample in a vial, incubated at 50ºC for 10 min with an agitation speed of 500 rpm (Autosampler HS100, Alpha MOS). For electronic nose analysis (Alpha Fox 4000, Alpha MOS), 1 mL of headspace sample was injected and the acquisition was obtained either with 18- semi-conductor oxide metallic sensors (MOS) or 4 mL of headspace was injected in a quadrupole mass spectrometer (Alpha Kronos, Alpha MOS) with an electronic impact (70 eV) ion source (Rossetti et al., 2010).

Further research will allow explaining the biochemical changes that occur during the transformation of milk from different productive origin; in this concern the use of an e-nose approach represents an alternative powerful tool to traditional methods of odour measurements.

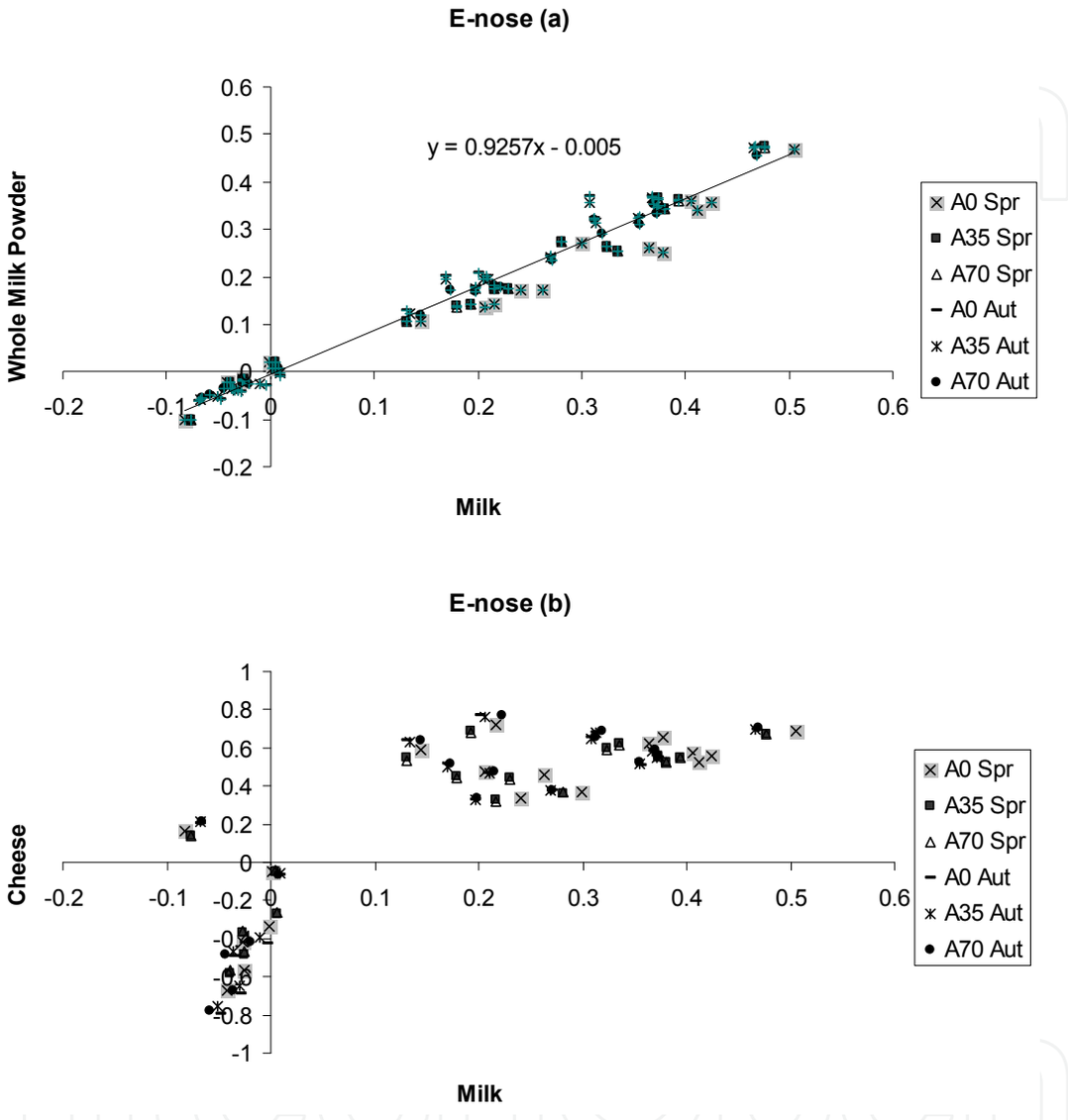

Figure 5. Relationship between e-nose sensors values in raw milk and its derived whole milk powder or reggianito cheese sample. Each point represents the value for each individual sensor applied to raw milk, and the whole milk powder or reggianito cheese that derived from the same alaboration batch. 0, 35 and 70 indicates Lucerne pasture percentage in the diet. Aut: autumn; Spr: spring.

4.3. Natural pigments in milk and cheese

Consumer's perception for natural and functional dairy products is an actual concern. In a survey over 179 consulted consumers, 87 % answered that would associate functional and natural concepts. In addition, 47% associated cheese with functional dairy products (other choices were yogurt, milk powder, ice-cream and dietary supplements).

In this concern, the concentration of carotenoids, especially β-carotene and lutein, could serve as natural pigment in milk fat and cheese.

Changes in beta carotene content were accompanied with changes in the yellow color in milk. As indicated in Calderon and others, (2007), the color index was a valuable tool to discriminate pasture from silage milk. The reflectance spectrum of raw milk samples was measured in the spectral region associated to light absorption by carotenoids, involving wavelengths from 450nm to 530nm (with a resolution of 10nm). In this range spectral data was translated to have reflectance values equal to zero at 530nm. On the translated spectrum, the integral value (IV) was calculated (Prache and others, 1999) as follows:

$$IV = \left(\frac{T450}{2} + T460 + T470 + T480 + T490 + T500 + T510 + T520 + \frac{T530}{2} \right) * 10$$

where T is the translated value corresponding to the reflectance intensity at each wavelength. Results in figure 6 indicate a linear relationship between IV and β-carotene. The β-carotene content could be predicted in function of IV by the following expression:

$$Z_{\beta-carotene} = -0.11 + 0.895\, Z_{IV}$$

Where Z is the standardized value of the variables β-carotene and IV ($Z_i = \frac{x_i - \overline{x}}{\sigma^2}$).

This result indicates that the transformation of spectral data in the range of carotenoids, may be used as a representative index of the feeding system.

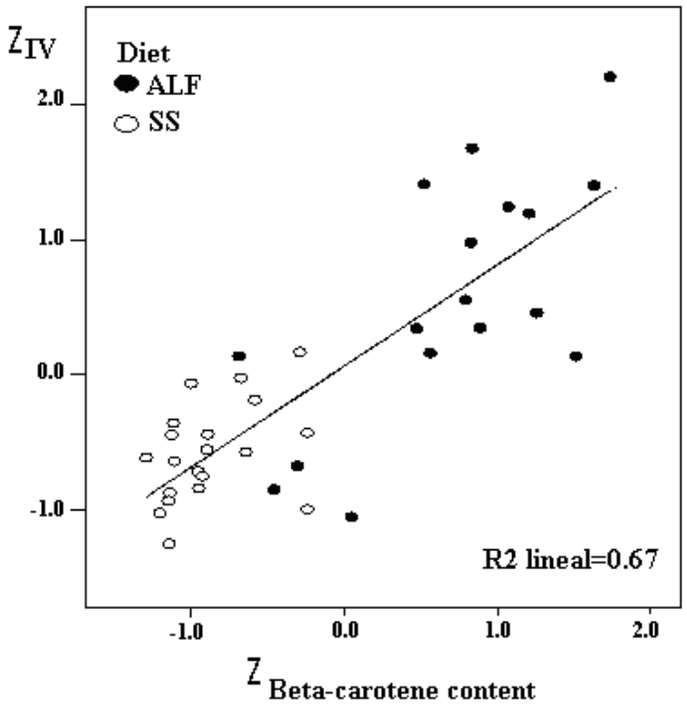

Figure 6. Correlation between IV and β-carotene content. ALF: 70% grazed Lucerne (●) values in the region of SS: Sorghum Silage diet (○) samples corresponded to milk at the beginning of the assay (time 0)

Experimental data obtained with reggianito cheese, which was elaborated within the experimental pilot plant of INTA are shown in table 5. Colour parameters indicate differences in cheese attributable to feeding management. Pasture cheese from A35 and A70 treatments presented higher b* values, which indicates a more yellow color, than A0. Differences were also distinguished by visual appreciation (figure 7). No significant differences were observed for L* parameter.

Figure 7. Aspect of reggianito cheese elaborated with milk from A0, A35 and A70 (feeding treatments containing 0, 35 and 70% Lucern pasture respectively) after six-month maturation. Left to right: A0, A35 and A70 treatments.

5. Conclusions

Productive strategies can be used to develop naturally added value dairy products. These strategies may be considered together with the consumer's perception about the role of functional and natural dairy products on human health. Preliminary studies on "pasture milk", clearly demonstrated the important changes in total lipids and fatty acid composition, especially in the contribution of C18:2 and C18:3 due to cultivar, cutting date and season. The importance of these fatty acids as substrate for CLA and n-3 PUFA concentration in milk lipids need to be considered.

The disadvantage of milk enriched with n-3 and CLA fatty acids is the possibility to suffer oxidation due to its high content of double-bonded molecules, which are prone to oxidation onset. This will not probably affect raw milk, but can bring inconvenient for the processing and commercialization of dairy product.

Therefore, pasture enhanced antioxidant status on raw milk, could be a strategy to solve part of the problem and consequently to avoid the development of rancidity and other off flavors and off odors in dairy products.

In addition, naturally pigmented cheese and milk products are positively accepted by consumers.

Pasture value added milk has an aspect which covers two purposes: preventing food spoilage and giving a fresh and healthier look to dairy products.

Author details

A.M. Descalzo, L.Rossetti, R. Páez, G. Grigioni, P.T. García, L. Costabel, L. Negri, L.Antonacci, E. Salado, G. Bretschneider, G. Gagliostro, E. Comerón and M.A. Taverna
Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina

6. References

Ajuyah AO, Fenton TW, Hardin RT, Sim JS. 1993. Measuring lipid oxidation volatiles in meats. J Food Sci 58:270–3.

Al-Mabruk, R.M., Beck, N.F.G., Dewhurst, R.J.(2004). Effects of Silage Species and Supplemental Vitamin E on the Oxidative Stability of Milk. Journal of Dairy Science, 87(2): 406-412.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. J. Dairy Sci. 85:2948–2963.

Benzie, I.F.F. and Strain, J.J. (1996), The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem. 239, 70-76.

Besle, J.M., Viala, D., Martin, B., Pradel, P., Meunier, B., Berdague, J.L., Fraisse, D. Lamaison, J.L. Coulon, J.B. (2010). Ultraviolet-absorbing compounds in milk are related to forage polyphenols, Journal of Dairy Science, 93(7): 2846-2856.

Butler, G., Nielsen, J.H., Slots, T., Seal, C., Eyre, M.D., Sanderson, R., Leifert, C. (2008). Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. J. Sci. Food Agric. 88:1431–1441.

Calderón, F., Chauveau-Duriot, B., Martin, B., Graulet, B., Doreau, M. and Nozière, P. (2007). Variations in Carotenoids, Vitamins A and E, and Color in Cow's Plasma and Milk During Late Pregnancy and the First Three Months of Lactation, Journal of Dairy Science, 90(5): 2335-2346.

Calderón, F., Tornambé, G., Martin, B., Pradel, P., Chauveau-Duriot, B., Nozière, P. (2006). Effects of mountain grassland maturity stage and grazing management on carotenoids in sward and cow's milk.Anim. Res. 55 (6) 533-544.

Castillo A ; Taverna M; Paez R. ; Cuatrin A ; Colombatto D; Bargo F ; Garcia M. ; Garcia P ; Chavez M ; Beaulieu A.; Drackley J (2006). Fatty acid composition of milk from dairy cows fed fresh lucerne based diets. Animal feed science and technology, 131: 241-254.

Chilliard Y., Ferlay A., Doreau M., (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids, Livest. Prod. Sci. 70 : 31–48.

Cohen Fernandez, S., Budowski, P., Ascarelli, I., Neumark, H., Bondi, A., 1976b. Pre-intestinal stability of betacarotene in ruminants. Int. J. Vit. Nutr. Res. 46, 439–445.

Cohen-Fernandez, S., Budowski, P., Ascarelli, I., Neumark, H., Bondi, A., 1976a. Low utilization of carotene by sheep. Int. J. Vit. Nutr. Res. 46, 446–453.

Comeron, E.A., Salado, E., Cuatrin, A., Paez, R., Riba, N., Molinelli, P., 2007. Modificación de la calidad de leche por la dieta ofrecida. 1.- Consumo, producción y composición de la leche. Rev. Arg. Prod. Animal, 27(1), 387.

Davison, K.L., Seo, J., 1963. Influence of nitrate upon carotene destruction during in vitro fermentation with rumen liquor. J. Dairy Sci. 46, 862–864.

Dawson, R.M., Hemington, N., (1974). Digestion of grass lipids and pigments in the sheep rumen. Br. J. Nutr. 32, 327–340.

Delagarde , R., Peyraud, J.L., Delaby, L. 2004. High grazing pressure in early-spring increases herbage intake of grazing dairy cows in late-spring. En : Land Use Systems in Grassland Dominated Regions. Proc. 20th General Meeting of EGF, 21-24 June, Luzerna, Suiza, 647-649.

Dewhurst, R.J., Scollan, N.D., Youell, J., Tweed, J.K.S. and Humpherys, M.O. (2001) Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. Grass and Forage Science, 56:68-74

Dhiman, T. R., G. R. Anand, L. D. Satter, and M. W. Pariza. 1999. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82: 2146 – 2156.

Dillon, P., 2006. Chapter 1: Achieving high dry-matter intake from pasture with grazing dairy cows. In: Fresh herbage for dairy cattle, the key to a sustainable food chain. Eds. Elgersma, A., Dijkstra, J. & Tammingna, S.,Wageningen UR Frontis Series Volume 18, Springer, Dordrecht, Netherlands. pp 1-26.

Dillon, P., J. R. Roche, L. Shalloo, and B. Horan. 2005. Optimising financial return from grazing in temperate pastures. Pages 131–147 in XXth Int. Grassl. Congr., Cork, Ireland. Wageningen Academic Publishers, Wageningen, the Netherlands.

Dillon, P., T. Hennessy, L. Shalloo, F. Thorne, and B. Horan. 2008. Future outlook for the Irish dairy industry: A study of international competitiveness, influence of international trade reform and requirement for change: Review. Int. J. Dairy Technol. 61:16 - 29.

Dufour, E., and T. Haertlé. 1991. Binding of retinoids and β-carotene to β-lactoglobulin. Influence of protein modifications. Biochim. Biophys. Acta 1079:316–320.

Dupuy HP, Bailey ME, St. Angelo AJ, Vercellotti JR, Legendre MG. 1987. Instrumental analysis of volatiles related to warmed-over flavor of cooked meats. In: St. Angelo AJ,

Bailey ME, editors. Flavor quality of fresh meats: warmed over flavor. Orlando, Fla.: Academic Press. p 165–90.

Elsgerma, A. Ellen, G., Horst, H., Mouse, B.G., Boer, H and Tamminga, S. (2003) Influence of cultivar and cutting date on the fatty acid composition of perennial rygrass. Grass and Forage Science, 58:323-331.

Erickson, M. (1999). Chemical and microbial stability of fluid milk in response to packaging and dispensing. Intl. J. Dairy Technology, 50(3): 107-111.

Garcia, PT , Pordomingo,A, Perez CD, Rios,MD, Sancho, AM, Casal, JJ. (2007) Influence of cutting and season on the fatty acid composition of forage crops for forage crops beef production. In Proceedings of the 54 th ICoMST, pp:101-102.

Garcia, S. C and W. J. Fulkerson. 2005. Opportunities for future Australian dairy systems: a review. Aust. J. Exp. Agr. 45: 1041-1055.

Graulet B. (2010). Vitamins naturally occuring in bovine milk. In : Improving the safety and quality of milk, Volume 2 : Improving quality in milk products. Ed. M. Griffiths, Woodhead Publishing Ltd, Cambridge, UK.

Griinari J.M., Bauman D.E. (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants, in: Yurawecz, M.P., Mossoba M.M., Kramer J.K.G., Pariza, M.W., Nelson G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, Vol. 1, Champaign, Illinois: AOCS Press, pp. 180–200.

Guimont, C., E. Marchall, J.M. Girardet , G. Linden. 1997. CRC in Food Science and Nutrition, 37:393-410.

Hartman A.M., Dryden L.P., Vitamins in milk and milk products, American Dairy Science Association, USA, 1965.

Hidiroglou, M., Batra, T.R., Roy, G.L. (1996). Changes in plasma [alpha]-tocopherol and selenium of gestating cows fed hay or silage. Journal of Dairy Science, 77(1): 190-195.

Igene JO, Pearson AM, Merkel RA, Coleman TH. (1979). Effect of frozen storage time, cooking and holding temperature upon extractable lipids and TBA values of beef and chicken. J Animal Sci 49(3):701–7.

Ip MM, Masso-welch PA, Shoemaker SF, Shea-eaton WK. (1999). Conjugated linoleic acid inhibits proliferation and induces apoptosis of normal rat mammary epithelial cells in primary culture. Exp Cell Res. 1999; 250:22-34.

Jahreis, G., Fritsche, J., Steinhart, H. (1997). Conjugated linoleic acid in milk fat: High variation depending on production system. Nutrition Research, 17(9):1479-1484.

Jiang, Q., Christen, S., Shigenaga, M. K., & Ames, B. N. (2001). Gamma tocopherol, the major form of vitamin E in the US diet, deserves more attention. American Journal of Clinical Nutrition, 74(6), 714–722.

Juárez, M, Dugan, M E.R., Aalhus, J L., Aldai, N., Basarab, J.A., Baron, V. S., McAllister, T.A. (2010). Dietary vitamin E inhibits the trans 10-18:1 shift in beef backfat. Canadian Journal of Animal Science. 90(1):9-12.

Keenan, T. W., Morre, D.J., Olson, D.E., Yunghans, W.N., Patton, S. (1970). Biochemical and morphological comparison of plasma membrane and milk fat globule membrane from bovine mammary gland. The journal of cell biology, 44: 80-93.

King, T.B., Lohman, T.G., Smith, G.S., 1962. Evidence of rumeno-reticular losses of Vitamin A and carotene. J. Anim. Sci. 21 (Suppl.), 1002 (abstr.).

Kolver, E. S., and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403–1411.

La Terra, S., Marino, V.M., Manenti, M., Licitra, G., Carpino, S. (2010). Increasing pasture intakes enhances polyunsaturated fatty acids and lipophilic antioxidants in plasma and milk of dairy cows fed total mix ration. Dairy Sci. Technol. 90: 687–698.

Ladikos D, Lougovois V. (1990). Lipid oxidation in muscle foods: a review. Food Chem 35:295–314.

Lucas, A., Rock, E., Chamba, J-F., Verdier-Metz, I, Brachet, P., Coulon, J-B (2006) Respective effects of milk composition and the cheese-making process on cheese compositional variability in components of nutritional interest Lait 86 (1) 21-41.

Macdonald, K. A., J. W. Penno, J. A. S. Lancaster, and J. R. Roche. 2008. Effect of Stocking Rate on Pasture Production, Milk Production, and Reproduction of Dairy Cows in Pasture-Based Systems. J. Dairy Sci. 91:2151-2163.

McEvoy, M., E. Kennedy, J. P. Murphy, T. M. Boland, L. Delaby, and M. O'Donovan. 2008. The Effect of Herbage Allowance and Concentrate Supplementation on Milk Production Performance and Dry Matter Intake of Spring-Calving Dairy Cows in Early Lactation. J. Dairy Sci. 91:1258-1269.

Messina, V., Biolatto, A., Descalzo, A., Sancho, A., Baby, R., Walsöe de Reca, N. (2009). Effect of pan-frying in extra-virgin olive oil on odour profile, volatile compounds and vitamins International Journal of Food Science and Technology, 44:552–559.

Mora, O., Romano, J.L., Gonzalez, E., Ruiz, F.J., Shimada, A., 1999. In vitro and in situ disappearance of betacarotene and lutein from lucerne (Medicago sativa) hay in bovine and caprine ruminal fluids. J. Sci. Food Agric. 79, 273–276.

Müller, C.E.; Möller, J.; Krogh Jensen S.; Udén, P. (2007) Tocopherol and carotenoid levels in baled silage and haylage in relation to horse requirements. Animal Feed Science and Technology 137: 182–197.

Negri, L, Sancho, A., Biolatto, A., Taverna, M.A. ; Descalzo, AM. (2008). HS-SPME-GC/FID: Application To The Study Of Volatile Components In Bovine Milk. Res Nº 456. COLACRO XII - Latin-American Congress of Chromatography and Related Techniques, October, 28-30, Florianopolis, Brasil.

Páez, R., Descalzo, A., Cuatrin, A, Comerón, E.A., Salado, E.E., Campos, S., Robledo, M. and Audero, G. (2007), Modificación de la calidad de leche por la dieta ofrecida. 2. Composición de ácidos grasos y estabilidad oxidativa. TPP 28. Revista Argentina de Producción Animal 27(1), 389-390.

Pecetti, L., Tava, A., Felicioli, A., Pinzauti, M. and Piano,E. (2002), Effect of three volatile compounds from lucerne flowers on their attractiveness towards pollinators. Bulletin of Insectology 55 (1-2), 21-27.

Potkanski, A.A., Tucker, R.E., Mitchell Jr., G.E., 1974. Pre-intestinal carotene losses in sheep fed lucerne or isolated carotene beadlets. Int. J. Vitam. Nutr. Res. 44, 3–7.

Prache, S., Cornu, A., Berdagué, J.L. and Priolo, A. (2005), Traceability of animal feeding diet in the meat and milk of small rumiants. Small Rumiant Research 59, 157-168.

Prache,S., Kondjoyan N., Delfosse,O., Chauveau-Duriot, B., Andueza, D., Cornu, A. (2009). Discrimination of pasture-fed lambs from lambs fed dehydrated lucerne indoors using different compounds measured in the fat, meat and plasma. Animal, 3:4; 598–605

Reis, R. B., and D. K. Combs. 2000. Effects of increasing levels of grain supplementation on rumen environment and lactation performance of dairy cows grazing grass–legume pasture. J. Dairy Sci. 83:2888–2898.

Rossetti, L., Langman, L., Grigioni, G.M., Biolatto, A., Sancho, A.M., Comeron, E. Descalzo. (2010) Antioxidant status and odor profile in milk from silage or lucerne-fed cows. Australian Journal of Dairy Technology. 65 (1):3-9.

Salado, E.E., Bretschneider, G., Cuatrin, A., Carassai, A., Espiñeira, M. y Haedo, E. 2010a. Suplementación de vacas lecheras en pastoreo con niveles crecientes de concentrado energético: 1. Producción y composición de leche. Rev. Arg. Prod. Anim. 30 (supl. 1): 520-521.

Salado, E.E., Bretschneider, G., Gaggiotti, M. y Cuatrin, A. 2010b. Suplementación de vacas lecheras en pastoreo con niveles crecientes de concentrado energético: 3. Ambiente y digestión ruminal. Rev. Arg. Prod. Anim. 30 (supl. 1): 523-525.

Sardar, S., Chakraborty, A., Chatterjee, M. (1996). Comparative effectiveness of vitamin D[3] and dietary vitamin E on peroxidation of lipids and enzymes of the hepatic antioxidant system in Sprague-Dawley rats. International Journal for Vitamin and Nutrition Research, 66(1): 39-45.

Schingoethe, D.J., Parsons, J.G., Ludens, F.C., Tucker, W.L., Shave, H.J. (1978). Vitamin E status of dairy cows fed stored feeds continuously or pastured during summer. J Dairy Sci 61:1582-1589.

Schroeder, G.F. , Delahoy, J.E. , Vidaurreta, I., Bargo, F., Gagliostro, G.A. and Muller, L.D. (2003). Milk Fatty Acid Composition of Cows Fed a Total Mixed Ration or Pasture Plus Concentrates Replacing Corn with Fat. Journal of Dairy Science, 86 (10), 3237-3248.

Shahidi F, Pegg RB. 1994. Hexanal as an indicator of the flavor deterioration of meat and meat products. In: St. Angelo AJ, editors. Lipid oxidation in food. ACS Symposium Series 500. Washington, D.C.: American Chemical Society. p 105–19.

Smet, K., De Block, J., De Campeneere, S., De Brabander, D., Herman, L., Raes, K., Dewettinck K., Coudijzer, K.(2009). Oxidative stability of UHT milk as influenced by fatty acid composition and packaging. International Dairy Journal 19: 372–379.

Søren Krogh Jensen, Anna Kirstin Bjørnbak Johannsen And John E. Hermansen (1999). Quantitative secretion and maximal secretion capacity of retinol, β-carotene and α-tocopherol into cows' milk. Journal of Dairy Research, 66, pp 511-522

Tamminga, S. 1993. Influence of feeding management on ruminant fiber digestibility. Page 571–602 in Forage Cell Wall Structure and Digestibility. D. R. Buxton, H.G. Jung, R. D. Hatfield, and J. Ralph, ed. ASA-CSSA-SSSA, Madison, WI.

Tava, A. and Pecetti, L. (1997), Volatiles from Medicago sativa complex flowers Phytochemistry 45 (6), 1145-1148.

Tesoriere, L., D'Arpa, D., Re, R., Livrea, M.A. (1997). Antioxidant reactions of all-trans retinol in phospholipid bilayers: effect of oxygen partial pressure, radical fluxes, and retinol concentration. Archives of Biochemistry and Biophysics, 343(1): 13-18.

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J.-P., Coulon, J.-B., & Berdagué, J.-L. (2000). Transfer of monoterpenes and sesquiterpenes from forages into milk fat. Lait, 80, 635–641.

White, S. L., G. A. Benson, S. P. Washburn, and J. T. Green Jr. 2002. Milk production and economic measures in confinement or pasture systems using seasonally calved Holstein and Jersey cows. J. Dairy Sci. 85:95–104.

White, S. L., J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295 –2301.