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Review

Carotenoids for ruminants: From forages to dairy products

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Abstract

Carotenoids are involved in the nutritional and sensory characteristics of dairy products, and are potential biomarkers for traceability of cows' feeding management. We review general and recent knowledge on carotenoids from forage to milk and dairy products in ruminants. Nearly 10 carotenoids (*i.e.*, xanthophylls and carotene) have been quantified in forages, and their concentrations vary highly according to development stage and length of conservation. Sensitivity of β-carotene to ruminal degradation varies among studies, depending on its dietary source. Data suggest that carotenoid digestion would be linked to dietary lipids for transit, and to specific transporters of lipophilic molecules for absorption. Among ruminants, only bovines accumulate high concentrations of carotenoids, mainly β-carotene, possibly due to lower Vitamin A synthesis efficiencies in enterocytes. Carotenoid flows in plasma and tissues in dairy cows remain to be investigated, especially the ability of adipose tissue to release β-carotene in depleted or underfed animals. Carotenoids in cows' milk mainly consist of all-*trans*-β-carotene and, to a lesser extent, lutein. In milk, concentration is more variable for β-carotene than for retinol, for which the plasma concentration is well regulated. Milk concentration of β-carotene depends on its dietary supply. Both animal and feeding factors that affect milk yield (*i.e.*, breed, parity, physiological stage, level of intake) generally also control milk β-carotene concentration by concentration/dilution mechanisms, and by efficiency of extraction from plasma. The β-carotene concentration in cheese is highly linked to milk concentration, whereas high losses of retinol occur during cheese-making. The color of dairy products highly depends on their carotenoid concentration,

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suggesting that color may be a promising rapid measurement tool for traceability of feeding conditions. Feeding management of dairy cows allows efficient control of carotenoid concentration and color in dairy products.

Keywords: Carotenoids; Retinol; Color; Forage; Milk; Dairy product; Ruminant

Contents

1. Introduction

Carotenoids are a family of more than 600 molecules that are synthesized by higher plants and algae and are involved in photosynthetic processes. They are characterized by a linear polyisoprene structure with conjugated double bonds, either *per se* (lycopene, $C_{40}H_{56}$), or as derived by cyclisation of the two extremities, with oxidation (xanthophylls such as lutein and zeaxanthin, $C_{40}H_{56}O_2$) or without oxidation (carotenes, $C_{40}H_{56}$). Carotenoid molecules, especially those without hydroxyl groups, are lipophilic.

Carotenoids form the main group of natural pigments. Xanthophylls, carotene and lycopene are responsible for yellow, orange and red coloring, respectively. Plant carotenoids are transferred into animal products, sometimes to a large extent (egg yolk) or to a lesser extent, as in ruminant products where they modify the color of milk and dairy products and body fat. Consumers are sensitive to product color, although preferences differ among countries and regions within countries (Sheath et al., 2001). A yellow color in milk is associated with pasture, which in many European countries carries connotations of "natural" feeding. Thus, carotenoids could be indicators of grazing production systems (Prache et al., 2003a,b).

In ruminants, as in other animals, carotenes (mainly β-carotene) are precursors of retinol (*i.e.*, Vitamin A) through cleavage (Yang and Tume, 1993). Among the roles of retinol in ruminants, as in other mammals, its influence on reproduction has been extensively studied. A deficiency in retinol may reduce reproductive efficiency in dairy cows, especially through impaired ovarian function and increased incidence of abortion (Hurley and Doane, 1989). Retinol is also involved in various functions, such as vision, growth and male fertility.

Together with Vitamin E and polyphenols, carotenoids are natural antioxidants in ruminant diets. They play a role in cell communication and immune function by protecting cells against free radical attack (van den Berg et al., 2000). It has been demonstrated that carotenoids and retinol are able to reduce mastitis in dairy cows (Chew, 1995), although the effect of β-carotene was not systematic (Folman et al., 1987; Oldham et al., 1991). Carotenoids also have a positive role in fertility independent of the role of retinol (Hurley and Doane, 1989). In addition to their role in cow health, higher carotenoid concentrations in milk contribute to an improvement in the nutritional value of dairy products. Even if it is obvious that in many countries carotenoids in human diets derive mainly from carrots and other fruits and vegetables, a higher concentration in dairy products could contribute to an increased supply, giving milk a better image in the consumers' mind. In addition, carotenoids could play a role in stabilising oxidizable compounds in milk. Also, higher

retinol in milk may improve human Vitamin A status in areas where Vitamin A deficiency is common.

Despite these various roles of carotenoids, little attention has been paid to carotenoid transfer from diet to milk in cows. This review summarises current knowledge on variations in carotenoid and retinol concentration in milk caused by animal and nutritional factors, on their transfer to dairy products, and on the relationships between carotenoid concentration and color of dairy products.

2. Carotenoids in feedstuffs

Despite the large variety of carotenoids in plants, no more than 10 are found in ruminant feeds (Fig. 1), and the most quantitatively important are β-carotene and lutein. Due to the limited interest paid to carotenoids in ruminant diets to date, chemical analyses are often non-specific and concern "carotene", which is a mixture of several molecules and isomers. Lutein concentration, or total xanthophylls, have also been determined in some experiments. Other molecules have generally not been determined, due to non-optimised methods of analysis that have generally been developed for another matrix, such as blood or milk, or for human foods. Indeed, it is difficult to avoid sample contamination by chlorophylls. To date, the methods adapted for forages have included time-consuming procedures using TLC (Livingston et al., 1968b), but more rapid methods using HPLC have been reported recently (Cardinault et al., 2006). However, it is important to emphasize that most methods of sample storage (*i.e.*, freeze-drying, freezing–thawing, refrigerator storage) together with long storage before analysis lead to degradation of these molecules (Park et al., 1983).

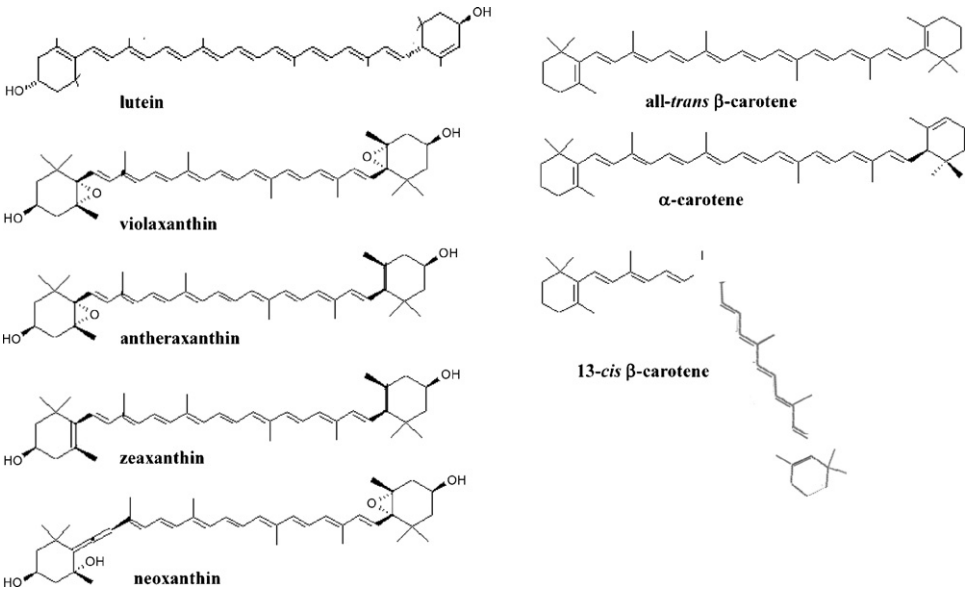

Fig. 1. Structure of the main carotenoids found in forages.

2.1. Diversity of carotenoids

Carotenoid concentration in forages depends on synthesis and degradation. Synthesis is achieved from isoprene units (*e.g.*, Armstrong and Hearst, 1996) in plastids and occurs mainly in leaves. According to the species, leaves contain 5–10 times more carotenoids than do stems (Morrison, 1954; Livingston et al., 1968b). Degradation occurs rapidly by oxidation, mainly due to light exposure and solar radiation. With accurate methods of analysis, it has been shown that cultivated forages contain four major carotenoids being: lutein, zeaxanthin, another xanthophyll identified as epilutein, and all-*trans*-β-carotene. In cocksfoot (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*) and red clover (*Trifolium pratense*), the average proportions of lutein, zeaxanthin, epilutein and β-carotene are 630, 120, 80 and 170 g/kg, respectively, and these proportions vary to a low extent with forage species (Chauveau-Duriot et al., 2005). Neoxanthin and violaxanthin were detected in lucerne (*Medicago sativa*) by Livingston et al. (1968b), and violaxanthin, antheraxanthin and 13-*cis*-β-carotene were detected in a natural grassland pasture by Calderon et al. (in press). In this experiment, lutein, violaxanthin, zeaxanthin, epilutein, antheraxanthin, all-*trans*-β-carotene and 13-*cis*-β-carotene represented 490, 140, 100, 90, 30, 110 and 40 g/kg of total carotenoids, respectively. Prache et al. (2003a) reported very small amounts of α-carotene in natural grassland pasture. Differences in the numbers of carotenoids described in forages may arise either from small differences in determination method, or from a wider diversity of molecules in natural grasslands. Until now, no glycosylated forms of xanthophylls have been reported in forages.

2.2. Carotenoids in fresh forages

Among species differences are less important than within species differences due to drying. A few experiments have compared several species. Park et al. (1983) found 50% more carotene in bromegrass (*Bromus inermis*) and reed canarygrass (*Phalaris arundinacea*) than in wheat grass (*Agropyron repens*) hybrids, while Chauveau-Duriot et al. (2005) found that red clover contained 25% more carotenoids than cocksfoot or perennial ryegrass. In tropical grasses, Reynoso et al. (2004) found no difference in β-carotene and lutein concentrations between bermudagrass (*Cynodon dactylon*) and Pangola (*Digitaria decumbens*). However, based on available literature and unpublished data, it is not possible to draw conclusions on systematic differences between cultivated species and natural grasslands, or between grasses and legumes. Moreover, N fertilization increases the carotene concentration of forages (Park et al., 1983). According to these authors, this could be due to activation of carotenoid biosynthesis by proteins.

The effect of maturity stage and/or season remains controversial. On pasture in France, Prache et al. (2003a) reported that total carotenoid concentrations varied only slightly between May and the end of June (620–700 mg/kg DM), then decreased to 430 mg/kg DM at the beginning of August. This decrease was due to both carotene and xanthophylls. In France during a very dry summer, Calderon et al. (in press) did not observe an effect of maturity stage on the carotenoid concentration of natural grassland during June. In New Zealand, McDowall and McGillivray (1963) showed that differences in carotene concentration of ryegrass throughout the grazing season were much less than differences in maturity

stage. This was confirmed by Williams et al. (1998) who summarised available values for β-carotene. Similarly, lucerne carotenoid concentration decreases between vegetative, bud and bloom stages, only partly due to the decrease in leaf-to-stem ratio (Livingston et al., 1968b; Park et al., 1983, review by Williams et al., 1998). Taken together, these results show that carotenoid concentration decreases with forage age, whatever the season or number of cuts or regrowth.

Lutein and β-carotene concentrations in green forages are two- to three-fold higher for the same species in humid *versus* dry tropics (Reynoso et al., 2004). These authors explained this result by the higher proportion of leaves in humid tropics, but it is likely that other factors, such as temperature or solar radiation, also modify the extent of carotenoid synthesis.

Maize silage is low in carotenoids, but very few data are available. While old data suggested very low concentrations, three samples in our laboratory gave concentrations as follows: epilutein 3–10, lutein 25–37, zeaxanthin 6–10, β-carotene 24–35, and total carotenoids 70–80 mg/kg DM. These initial results, which are not representative of the variability in maize silages, remain to be confirmed.

2.3. Carotenoids in preserved forages

Sun-drying forages strongly decreases carotenoid concentration, especially when rain occurs during haymaking (Park et al., 1983). Chauveau-Duriot et al. (2005) showed an average 83% loss in carotenoids between direct-cut silage and hay (Fig. 2). Lutein and epilutein losses occur less rapidly than for zeaxanthin and carotene. This carotenoid loss is mainly due to solar radiation, because exposure to UV rays in the dark is capable of destroying all carotenoids (Cardinault et al., 2004). However, moderate losses of carotenoids

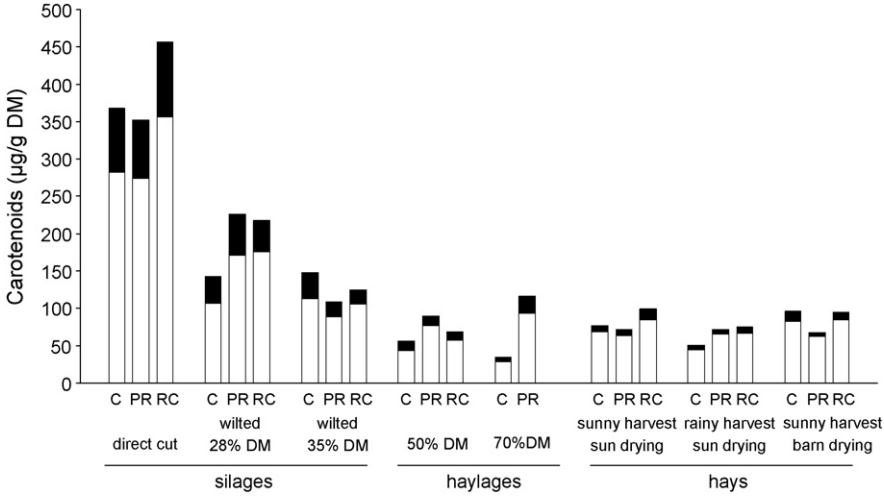

Fig. 2. Effects of modes of preservation of cultivated forages on their concentrations in xanthophylls (□) and β-carotene (■) [C = cocksfoot (*Dactylis glomerata*); PR = perennial ryegrass (*Lolium perenne*); RC = red clover (*Trifolium pratense*)]. After Chauveau-Duriot et al. (2005).

occur during in-barn hay storage (Bruhn and Oliver, 1978), probably because of the presence of oxygen.

Independent of wilting, the ensiling process decreases carotenoid concentration to various degrees. Carotenoid losses are pH-dependent and favoured by aerobic conditions. Carotenoid losses are higher for legumes than for grasses when pH is high (*i.e.*, about 5) and when sealing of the silo is delayed, and these losses increase with time of storage in the silo (Kalac and McDonald, 1981; Kalac, 1983). Maximum losses can reach 80% of the initial concentration but, in well-made silages, the concentration loss is generally less than 20%.

Lucerne dehydration results in loss of both carotene and xanthophylls, and the extent of the loss depends on the process. This loss is higher for high processing temperatures, especially for xanthophylls, and when the final product has a high moisture concentration. With a temperature of 120 °C, and an end product with 8% moisture, dehydration has a limited effect on carotenoids, whereas more drastic conditions (more than 150 °C, less than 3% final moisture) may result in up to 33% carotene and 73% xanthophylls losses (Livingston et al., 1968a; Burdick and Fletcher, 1985). Either way, dehydration has a less negative effect on carotenoids than silage or haymaking: Williams et al. (1998) reviewed available literature and determined mean β-carotene values of 196, 159, 81 and 36 mg/kg DM for green forages, dehydrated forages, silages and hays, respectively.

2.4. Carotenoids in concentrates

Most concentrate feeds eaten by cows are very low in carotenoids. It is difficult to find reliable composition data for animal feeds, but some information is available (Holden et al., 1999; Nys, 2000). Maize contains lutein and zeaxanthin, and lower amounts of other xanthophylls, such as cryptoxanthin, zeinoxanthin, which are concentrated in corn gluten meal. Processing feeds for concentrates often involves heating, which probably destroys many carotenoids. By-products of tomato, which is available in some areas as tomato pulp silage, and red grapefruit, which is fed as fresh or ensiled citrus pulp, contain lycopene, whereas its concentration is probably reduced in dried citrus pulp by processing. In these feedstuffs, β-carotene concentrations are low.

2.5. Relationship between carotenoids and other chemical components of forages

Because carotenoids are lipophilic molecules related to lipids, there is a likely relationship between ether extract and carotenoid concentration. Analysis of 27 samples combining 3 forages and 9 types of conservation showed a correlation of 0.60 between these parameters (Chauveau-Duriot et al., 2005). Fifteen analyses of grass and/or red clover silages gave a correlation of 0.82 (unpublished data from IGER (UK) and INRA) for both carotenes and xanthophylls. Lipids in forages are generally positively related to crude protein and negatively related to cell walls. This general trend occurs among stages of vegetation and types of conservation within species. A group of 20 observations from natural grassland gave correlations between carotenoids and crude protein ($r = 0.71$), and between carotenoids and crude fibre ($r = -0.73$) (Calderon et al., in press). It would be useful to study these relationships further, by additional data obtained with a wider variety of forages (*e.g.*, forage

species, silage or haymaking, storage) to improve knowledge on quantitative relationships between carotenoids and other well characterized biochemical plant components.

3. Digestion, absorption and metabolism of carotenoids

The carotenoid concentration of cows' milk is determined by the nature and amount of dietary supply through forage intake as well as by their transfer from the vegetable matrix delivered to the mammary gland. As could be deduced from the low carotenoid recovery in milk, the efficiency of this transfer seems to be strongly limited. It is likely that the different steps of carotenoid transfer from diet to milk (*i.e.*, rumen digestion, intestinal absorption and tissue metabolism) could influence carotenoid availability to the mammary gland.

3.1. Ruminal digestion

The first event in the digestive process of carotenoids is degradation of the vegetable matrix that releases carotenoids into the rumen liquid phase (Mora et al., 1999). The extent of carotenoid degradation by microorganisms in the rumen remains uncertain because of the wide range of results, most on β-carotene, from *in vitro* and *in vivo* studies. Whereas some authors reported no degradation (Dawson and Hemington, 1974; Cohen Fernandez et al., 1976a), others found moderate (10–25%; Davison and Seo, 1963; Potkanski et al., 1974; Cohen Fernandez et al., 1976b; Mora et al., 1999) or higher β-carotene disappearance (40–55%; King et al., 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 (N. Cardinault et al., unpublished (INRA, Theix, France)). The few studies on factors influencing carotenoid degradation in the rumen have shown small differences in lutein degradation between goats and steers fed the same diet (46 g/kg *versus* 99 g/kg, respectively) but not for β-carotene (Mora et al., 1999) suggesting low species and molecular particularities. No effects of diet composition (Potkanski et al., 1974; Davison and Seo, 1963) have been reported, although rations containing soy, cereal and cotton seeds that are enriched in lipoxygenases (*E.C.* 1.13.11.12) could stimulate rumen degradation of carotenoids (Larsen et al., 1993).

3.2. Transport and absorption in the intestine

Data on passage of carotenoids through the gut are exclusively based on non-ruminant animals. In these species the amount and nature of dietary lipids influence carotenoid solubilisation and subsequent absorption in the intestine because carotenoids are transported with the lipid phase. In the digestive tract, xanthophylls, as polar molecules, are exposed at the outer surface of emulsions and micelles and consequently the efficiency of their transfer from emulsion to micelles is higher (25–40%) than for carotenes (α or β, 12–18%) that are less polar molecules integrated in the core of these particles (Furr and Clark, 1997; Garrett

et al., 1999). Transfer of carotenoids from emulsion to micelles could be a limiting step for intestinal carotenoid absorption, especially in the case of high carotenoid, or very low lipid, intake. In ruminants, this mechanism remains unknown but could likely present some specificities, as a consequence of the ruminal metabolism of dietary lipids which induces a modification of the composition of the lipids entering in the duodenum. Biliary secretions may also influence carotenoid composition in the digestive tract through carotenoid enterohepatic recycling. Indeed, as evidenced in sheep, 180 g/kg of an intravenous β-carotene dose was recovered in bile per day, part of it probably as metabolites, including Vitamin A (Boling et al., 1969).

A general assumption derived from experiments from the late 1970s was that carotenoid absorption is passive. However, differences among animal species, molecules (van Vliet, 1996), or parts of the intestine involved in carotenoid absorption suggest an active mechanism that was recently evidenced by studies in CaCo-2 cells. First, β-carotene absorbability was demonstrated to be time- and dose-dependent, as well as saturable (During et al., 2002). Second, the implication of the facilitative transporter scavenger receptor B1 (SR-B1) in the transport of carotenoids through the membrane of the mucosal side of the enterocytes has been demonstrated (Reboul et al., 2005; During et al., 2005; van Bennekum et al., 2005).

In humans, mean values of intestinal digestibility of β-carotene range between 90 and 520 g/kg (van Vliet, 1996), depending on factors such as nature and amount (de Pee and West, 1996). At this time, data on apparent intestinal digestibility of carotenoids in ruminants are scarce. In sheep, lutein from fresh red clover appears to be absorbed more efficiently than all-*trans*-, or 13-*cis*-, β-carotene (557, 217, and 233 g/kg, respectively; Cardinault et al., 2006), which is consistent with faster appearance of dietary xanthophylls (*i.e.*, lutein and canthaxanthin) than carotenes (α or β) in the plasma of pre-ruminant calves (Bierer et al., 1995). The effect of the amount of carotenoid ingested does not lead to a clear conclusion because faecal recoveries of β-carotene in sheep after increasing abomasal spot doses of purified molecule from 1.38 to 6.15 mg did not vary (Cohen Fernandez et al., 1976a) whereas, in growing bovines, total intestinal digestibilities increased from 660 to 880 g/kg with increasing β-carotene intake from 42 to 2679 mg/day (Mora et al., 2001). Among the dietary components, lipids may affect carotenoid absorption in ruminants. Indeed, total intestinal apparent digestibility of β-carotene supplied to bovines in abomasal spot doses was higher when dissolved in lipid solutions (360 g/kg in fatty acids or monoacylglycerols; 540 g/kg in triacylglycerol solutions) than in water (150 g/kg). Triacylglycerols likely favour incorporation of carotenoids into digestive micelles, bile salt secretion and carotenoid flow across enterocyte carriers. However, triacylglycerols could also stimulate microbial degradation of carotenoids in the hindgut (Cohen Fernandez et al., 1976a). As in non-ruminants, other effectors such as dietary fibre (Cohen Fernandez et al., 1976a), sterols (Traber, 2004) or other liposoluble micronutrients of the diet (Vitamin E, Furr and Clark, 1997) could impair absorption of any carotenoid, but no data are available in ruminants, although Wing (1969) reported low variability among individuals in total tract carotenoid digestibility.

3.3. Metabolism

Carotenoid availability for secretion in milk is governed by their transport into lymph and plasma, their metabolism within tissues (especially conversion into Vitamin A and

by utilization as pigments or antioxidants), as well as their storage in adipose tissues or secretion into bile by the liver.

3.3.1. Conversion into Vitamin A

Each provitamin carotenoid can be processed more or less efficiently in numerous cell types (mainly in enterocytes and hepatocytes; Borel et al., 2005) to yield two molecules of retinal by oxidative cleavage *via* the β-carotene 15,15′-monooxygenase (*E.C.* 1.14.99.36). In non-ruminants, this process is more efficient with all-*trans*-β-carotene, and xanthophylls (zeaxanthin) have an inhibitory effect on β-carotene conversion (Grolier et al., 1997). In ruminants, Yang and Tume (1993) demonstrated higher activity of the enzyme from sheep *versus* cow or goat intestine. Expression of the cleavage enzyme was not regulated by β-carotene dietary supply in bovine enterocytes (Morales et al., 2004). Thus, β-carotene transfer across enterocytes would be enough to induce the yellow coloration of fat in carcass and dairy products of grazing bovines (Yang and Tume, 1993; Mora et al., 2001). β-Carotene conversion may also occur in the mammary gland of lactating dairy cows, as suggested by increased Vitamin A concentrations in milk, but not in plasma, after parenteral administration of β-carotene (Schweigert and Eisele, 1990).

3.3.2. Transport in plasma

In spite of a wide diversity of xanthophylls and carotenes in forages (see Section 1), β-carotene (especially the all-*trans* isoform) is the main circulating carotenoid in bovines (Yang et al., 1992). The 9-*cis* and 13-*cis* isoforms have been also observed, as well as α-carotene and lutein (Nozière et al., 2006). In sheep and goats, the main plasma carotenoid is lutein (Yang et al., 1992), but zeaxanthin, and more rarely β-carotene and three unidentified polar components have been reported in sheep (Prache et al., 2003a; Cardinault et al., 2006). To our knowledge, violaxanthin and antheraxanthin have never been detected in ruminant plasma, perhaps due to microbial degradation in the rumen (Cardinault et al., 2004), to conversion into zeaxanthin (van den Berg et al., 2000) or to rapid transfer to tissues from intestinal chylomicrons. In bovines, plasma carotenoids are essentially associated with HDL (more than 800 g/kg) in pre-ruminant calves (Bierer et al., 1995), steers (Yang et al., 1992) and dairy cows (Schweigert et al., 1987), which is consistent with plasma lipoprotein composition. However, in sheep and goats, plasma carotenoids (essentially lutein) are mainly (over 570 g/kg) associated with the LDL + VLDL fraction (Yang et al., 1992). Thus, the relative participation of carotenoids from dietary origin through CM in total plasma carotenoids remains negligible (due to the chylomicron half-life less than 5 min) but this does not preclude an important quantitative contribution of chylomicrons for carotenoid transport, because chylomicrons represent the primary mode of carotenoid transport in plasma and the first step of carotenoid delivery to tissues. Thus their quick and easy uptake by tissues could explain lower plasma concentrations of xanthophylls, especially lutein, compared to β-carotene in spite of a higher concentration in the diets and higher digestibility values.

3.3.3. Body pools

In bovines, carotenoids have been quantified in liver and adipose tissues (Yang et al., 1992, 2002; Knight et al., 1996; Mora et al., 2001), and the latter may constitute the main

body pool of carotenoids (mainly β-carotene), depending on growth stage of the animal (Nozière et al., 2006). The liver may play a central role in carotenoid availability to the mammary gland by regulating their distribution between biliary recycling, conversion into Vitamin A, or mobilisation of carotenoid stores and secretion as part of liver VLDL. Elsewhere, adipose tissues have mainly a storage function from which β-carotene could be mobilized independently of lipids when dietary supply of carotene is reduced, as suggested both in bovine (Knight et al., 2001; Reynoso et al., 2004; Nozière et al., 2006) and ovine (Prache et al., 2003b) or when the animals are mobilising lipid. Patterson (1965) reported a linear relationship between plasma carotene and free fatty acid concentrations in parturient dairy cows in negative energy balance. Consistent with this latter hypothesis, epinephrine-stimulated lipid mobilisation induced a concomitant decrease in β-carotene concentrations in bovine adipose explants, while the reverse occurred with insulin (Arias et al., 2005).

Some key steps could control carotenoid transfer from diet to the milk in dairy cows. Specificities occur in the digestive tract of ruminants due to mechanisms of rumen outflow which cause carotenoid flow to the duodenum to be spread out over time, to the composition of duodenal lipophilic nutrients (except when protected sources are fed, dietary lipids are hydrolysed to fatty acids in the rumen) and to enterohepatic recycling. Elsewhere, characteristics of expression and activity of the SR-B1 transporter and of the cleavage enzyme in the tissues of the dairy cows are not well known. Data on carotenoid flux, transport and metabolism within and among tissues such as intestinal wall, liver, adipose tissues and mammary gland are insufficient to fully understand regulation of milk carotenoid concentration and composition.

4. Animal and nutritional factor effects on milk retinol and carotenoid concentrations

Carotenoids in cows' milk mainly consist of all-*trans*-β-carotene and, to a lesser extent, lutein, zeaxanthin, β-cryptoxanthin (Havemose et al., 2004; Martin et al., 2004; Hulshof et al., 2006; Calderon et al., in press). Traces of 13-*cis*-β-carotene have been detected in cows' milk (A. Lucas et al., unpublished (INRA, Theix, France)), but their origin (native or from isomerisation during conservation or extraction) remains unclear. The β-carotene proportion of total carotenoids in cow's milk ranges from 750 g/kg to the more commonly found value of 850 g/kg. Retinol in milk mainly exists in the ester form, arising mainly from esterification of the alcohol form derived from the liver by the mammary gland (Tomlinson et al., 1974), but also arising from uptake of the retinol ester derived from β-carotene and retinol dietary intake. The *cis* isomer of retinol is present in very low amounts in raw cows' milk (Panfili et al., 1998), but it accounts for 15–35 g/kg of total retinol in goats' milk (Fedele et al., 2004).

The carotenoid and retinol concentrations in cows' milk reported in the literature vary greatly among studies, from 1 to 17 and from 1 to 12 μg/g fat for carotene and retinol, respectively (Tables 1 and 2; Figs. 3 and 4). Large variations also occur for β-carotene in plasma (from 1 to 16 μg/ml), whereas retinol concentrations in plasma are less variable (from 0.1 to 0.6 μg/ml). Several non-dietary and dietary factors have been identified to account for this variability, and these studies used a wide range of techniques for extraction

Table 1
Effect of breed on carotene[1,2,3,4,5] and retinol in milk and plasma of dairy cows

Reference	Breed	No. of cows	Carotene		Retinol	
			Milk (μg/g fat)	Plasma (μg/ml)	Milk (μg/g fat)	Plasma (μg/ml)
Baumann et al. (1934)[1]	Guernsey	12	11.7		6.8	
	Jersey	12	7.8		8.0	
	Brown Swiss	10	7.1		9.5	
	Holstein	11	5.4		11.8	
	Ayrshire	11	5.1 (0.81)[6]		9.2 (0.90)[6]	
Krukovsky et al. (1950)[2]	Guernsey	28	17.7		4.9	
	Jersey	16	10.7		5.3	
	Brown Swiss	33	7.6		6.8	
	Holstein–Friesian	51	4.9 (0.44)[6]		5.8 (0.22)[6]	
Thompson et al. (1964)[3]	Guernsey	7	12.0		5.1	
	Shorthorn	30	5.5		7.7	
	Friesian	10	7.0		10.0	
	Shorthorn	10	6.8		9.1	
	Ayrshire	60	6.4		9.1	
	Friesian	60	5.8		10.0	
	Jersey	50	8.3		9.6	
Graves-Hoagland et al. (1989)[4]	Jersey	9		7.1 a		0.42 a
	Holstein	30		3.9 b		0.38 a
Keen and Wilson (1992)[1]	Jersey	Herd milk	13.0			
	Jersey 60%	Herd milk	13.0			
	Jersey 39%	Herd milk	10.5			
	Jersey 29%	Herd milk	9.0			
	Friesian	Herd milk	6.0			
Winkelman et al. (1999)[4,5]	Jersey	2566	11.3			
	Friesian	5105	6.8			
Morris et al. (2002)[2]	100% Jersey	720 (milk)	7.5	16.1		
	62% Jersey		8.1	14.5		
	62% Friesian	3501 (plasma)	7.3	13.0		
	100% Friesian		5.2	12.4		
Martin et al. (2004, unpublished)[4,7,8]	Montbeliarde	28	2.9 a	2.4 a	3.5 a	0.49 a
	Tarentaise	28	3.1 a	2.7 b	3.3 a	0.46 a
Nozière et al. (2006)[4]	Holstein	12	2.8 a	3.6 a	4.2 a	0.68 a
	Montbeliarde	20	2.6 a	3.4 a	4.2 a	0.46 b

Table 1 (*Continued*)

Reference	Breed	No. of cows	Carotene		Retinol	
			Milk (μg/g fat)	Plasma (μg/ml)	Milk (μg/g fat)	Plasma (μg/ml)
Coulon and Grolier (unpublished)[4,7,8]	Holstein	14	2.3 a	2.0 a		
	Montbeliarde	24	2.3 a	1.9 a		
	Tarentaise	4	2.8 a	1.7 a		
Coulon and Grolier (unpublished)[4,7,8]	Montbeliarde	20	2.8 a	3.8 a		
	Tarentaise	12	2.5 a	3.0 b		

Means in the same column and the same experiment with different letters (a and b) differ ($P < 0.05$).

[1] Carotene.
[2] Carotenoids.
[3] Active carotenes.
[4] β-Carotene.
[5] β-Carotene estimated by modelling from milk color and fat percentage measurements.
[6] Pooled standard error for all breeds within the reference.
[7] Butterfat estimated to 40 g/kg.
[8] Unpublished data from INRA, Theix, France.

or quantification by spectrophotometry or chromatography. In addition, particularly in less recent studies, the results are expressed differently (*i.e.*, as carotin, carotenoids, carotene or β-carotene), whereas the biochemical importance for each term has generally not been specified. This probably accounts for the high variability among publications in the absolute values reported. This section reviews non-dietary and dietary factors affecting carotenoid

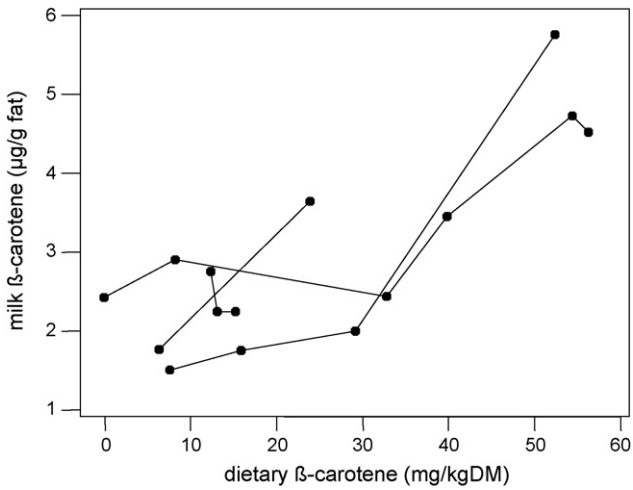

Fig. 3. Within study relationships between dietary β-carotene and milk fat β-carotene concentrations in mid-lactation cows. After J.B. Coulon and P. Grolier (unpublished (INRA, Theix, France)), Martin et al. (2004) and Nozière et al. (2006).

Table 2
Effect of nature of forage on carotene[1,2,3] and retinol in milk and plasma of dairy cows

Reference	Nature of forage	No. of cows	Carotene		Retinol	
			Milk (μg/g fat)	Plasma (μg/ml)	Milk (μg/g fat)	Plasma (μg/ml)
Baumann et al. (1934)[1]	Winter rations	46	5.8		7.0	
	Pastures	10	8.7 (0.68)[4]		11.3 (0.51)[4]	
Krukovsky et al. (1950)[2]	Late pasture	43	8.9		5.6	
	Barn	40	3.9		3.7	
	Early pasture	45	13.9 (0.38)[4]		7.9 (0.21)[4]	
Havemose et al. (2004)[3]	Grass silage	12	14.2			
	Maize silage	12	4.7 (0.32)[4]			
Martin et al. (2004, unpublished)[3,5]	Concentrate based	8	2.90 b	1.74 a	3.75 ab	0.52 a
	Maize silage	8	2.43 ab	1.26 a	2.84 a	0.50 a
	Ryegrass silage	8	4.72 cd	4.39 a	5.18 ab	0.48 a
	Ryegrass hay	8	3.45 b	2.89 b	4.47 ab	0.44 a
	Natural grass hay	8	2.44 a	1.41 a	3.21 a	0.46 a
	Early pasture	8	5.39 d		5.67 b	
	Late pasture	8	4.52 c	4.67 c	4.07 ab	0.48 a
Nozière et al. (2006)[3]	Natural grass hay	16	1.77 a	2.3 a	3.57 a	0.51 a
	Ryegrass silage	16	3.64 b	4.72 b	4.78 b	0.59 a
Coulon and Grolier (unpublished)[3,5,6]	Cocksfoot hay	42	2.25 a	1.7 a		
	Natural grass hay	42	2.75 b	2.3 b		
	Ryegrass hay	42	2.25 a	1.6 a		
Coulon and Grolier (unpublished)[3,5,6]	Natural grass hay (Alpes)	6	2.00 a	2.2 b		
	Cocksfoot hay	14	1.75 a	1.6 ab		
	Natural grass hay (Auvergne)	6	1.50 a	1.4 a		
	Pasture	6	5.75 b	8.4 c		
Martin et al. (unpublished)[3,5]	Concentrate based	16	0.98 a		5.19 a	
	Grass based	16	3.16 a		6.05 b	

Means in the same column and the same experiment with different letters (a–d) differ ($P < 0.05$).
[1] Carotene.
[2] Carotenoids.
[3] β-Carotene.
[4] Pooled standard error for all diets within the reference.
[5] Unpublished data from INRA, Theix, France.
[6] Butterfat estimated to 40 g/kg.

and retinol concentrations in milk and plasma. The data apply to cows, unless specified otherwise.

4.1. Variations related to non-dietary factors

4.1.1. Species

Milk of goats and ewes, in contrast to that of cows, contains only retinol and xanthophylls and generally no β-carotene, thus explaining differences in color between bovine and small

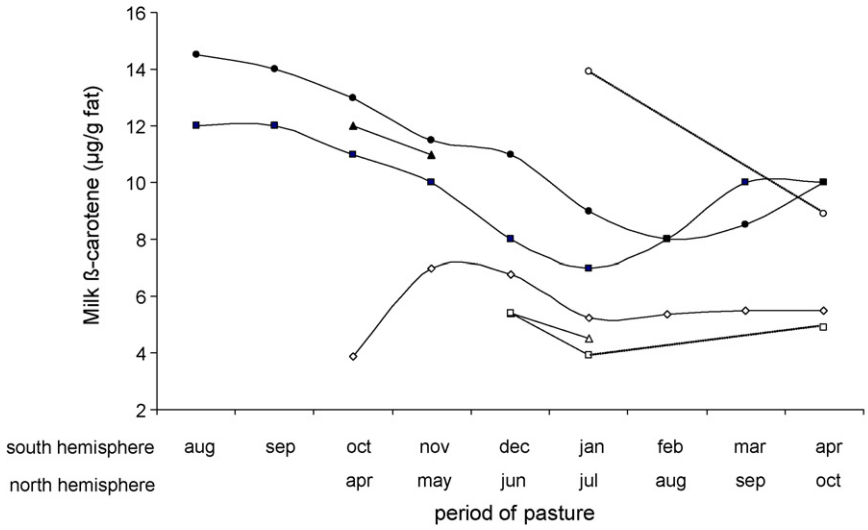

Fig. 4. Effect of season on β-carotene in milk of cows at pasture, in south hemisphere (solid symbols) and north hemisphere (open symbols). After Krukovsky et al. (1950) (◯), McDowall and McGillivray (1963) (▲), Thompson et al. (1964) (◊), Keen and Wilson (1992) (■), Waghorn and Knight (1992) (●), Martin et al. (2004) (△) and Calderon et al. (in press) (□).

ruminant dairy products. As described in Section 3, differences among species are partly related to a higher efficiency of conversion of carotenoids, particularly β-carotene, into retinal in the enterocytes in ovines than bovines. Serum concentrations, as well as liver and subcutaneous fat concentrations, of both β-carotene and lutein are much lower in sheep and goats than in cattle, whereas serum concentrations of retinol are slightly higher in sheep and goats than cattle (Yang et al., 1992). Retinol concentrations in milk and cheese are about two-fold higher in goats than cows (Martin et al., 2004; Lucas et al., in press).

4.1.2. Breed

β-Carotene concentrations in cow milk vary widely among dairy breeds (Table 1). Carotene concentrations in milk fat are nearly three-fold higher in Guernsey than in Holstein–Friesian, Ayrshire and Shorthorn cows, whereas they are intermediate in Jersey and Brown Swiss. Similar differences among these breeds occurred in local butter in India (Dubey et al., 1991). Morris et al. (2002) reported on large sample populations that carotenoid concentrations in both milk and plasma are positively linearly related to the Jersey-to-Friesian gene ratio. The effect of breed on carotene concentration or color in butterfat, as well as on β-carotene concentration in plasma, is maximal under high carotene diets (Baumann et al., 1934; Graves-Hoagland et al., 1989; Keen and Wilson, 1992). Slight differences in plasma and milk β-carotene concentrations, and color of milk between Holstein, Montbeliarde and Tarentaise cows were also reported. Lastly, higher β-carotene and xanthophyll concentrations in the fat of farmhouse cheeses produced with Montbeliarde than with Abondance herds were reported, with Holstein

herds having intermediate concentrations (Lucas et al., 2006a). It should be noted that, in these studies, both plasma and milk concentrations of β-carotene were lower than those obtained with Guernsey, Jersey or Brown Swiss cows, but comparable to those obtained with Holstein–Friesian cows in studies performed in New Zealand.

Retinol concentrations in butterfat also vary among breeds, but the differences are less marked and the ranking of breeds is different than for carotenoids. Retinol concentrations are 1.6-fold higher in Holstein–Friesian than in Guernsey cows, whereas Jersey cows have intermediate values. Dubey et al. (1991) reported no effect of genetic group (Holstein–Friesian, Brown Swiss, Jersey) on retinol concentrations in local butter in India. Milk and plasma retinol concentrations appear comparable between Holstein, Montbeliarde and Tarentaise cows. Unlike for β-carotene, Jersey and Holstein cows exhibit similar plasma retinol concentrations (Graves-Hoagland et al., 1989).

4.1.3. Stage of lactation

β-Carotene and retinol concentrations are much higher in colostrum than in milk, and these concentrations decrease rapidly during the first week post-partum (Johnston and Chew, 1984). This is associated with a rapid decrease in plasma concentrations peripartum, mainly 1 week pre-partum, and minimal concentrations are reached at calving for retinol, or 1 week post-partum for β-carotene (Johnston and Chew, 1984; Michal et al., 1994). It is likely that this decrease is not only due to the moderately decreased intake, but to an increased uptake by the mammary gland. The decrease in β-carotene and retinol plasma concentrations can be limited when cows receive dietary supplements during this period (Michal et al., 1994; Oldham et al., 1991), but the impact on colostrum composition remains unknown.

Changes in milk β-carotene and retinol concentrations during early and mid lactation are poorly documented. Jensen et al. (1999) reported that β-carotene concentration in milk fat increased from 0.7 to 2.0 μg/g during the first 24 weeks of lactation, whereas retinol concentration only varied slightly. At the same time, both β-carotene and retinol concentrations increased in plasma, from 0.5 to 6.5 and from 0.15 to 0.35 μg/ml, respectively, as also previously reported (Johnston and Chew, 1984; Michal et al., 1994). This could be related to the concomitant increase in dry matter (and presumably β-carotene) intake. It is noticeable that these experimental results clearly diverge from models of β-carotene secretion in which β-carotene yield follows fat yield or color (Waghorn and Knight, 1992; Winkelman et al., 1999). In mid lactation, there is a more marked increase in β-carotene concentration with time in milk than in plasma (Nozière et al., 2006), partly due to a concentration effect related to the decrease in milk yield over time, although it could also reflect an increase in the extraction of β-carotene from plasma to milk, but this has not yet been reported. However, due to seasonal variations in dietary β-carotene concentration, stage of lactation and nature of the diet are often partly confounded. Thus, a specific effect of stage of lactation has not been clearly established, and this could be an important factor affecting concentrations of β-carotene, and other micronutrients, in milk.

4.1.4. Parity

Variability due to parity appears very low compared to variation related to other non-dietary factors. In early lactation, Larson et al. (1983) observed that β-carotene

concentrations in milk decreased from 1.4 to 1.2 μg/g fat between lactations 1 and 2, then increased with lactation number, to 1.6 μg/g. These variations were inversely related to milk yield, and positively related to milk fat content. No effect of lactation number on plasma β-carotene and retinol concentrations from 4 weeks pre- to 13 weeks post-calving occurred in multiparous cows (Ascarelli et al., 1985).

4.1.5. Health status

Low plasma concentrations of β-carotene or retinol are associated with an increased incidence of udder infection (Chew et al., 1982). Cows suffering from severe mastitis tend to produce milk containing less β-carotene and more retinol than non-infected cows (47 μg/l *versus* 54 μg/l β-carotene and 215 μg/l *versus* 185 μg/l retinol, respectively), but differences are slight in both mid and early lactation (Chew et al., 1982; Johnston and Chew, 1984).

4.1.6. Milk yield and milk fat

The effect of milk production level on milk carotenoid and retinol concentrations has been indirectly assessed through factors affecting milk yield, such as breed, stage of lactation, parity and level of intake. More directly, once daily milking, which induces a decrease in milk yield and an increase in milk fat content compared to twice daily milking, causes production of more yellow cheese (Martin et al., unpublished (INRA, Theix, France)). Changes in milk β-carotene and retinol concentrations induced by changes in milk yield do not only respond to concentration/dilution effects. This is consistent with Jensen et al. (1999), who suggested active transport of β-carotene from blood to milk leading to a quantitatively limited, and genetically determined, maximum daily secretion of β-carotene.

4.1.7. Individual variability and heritability

Plasma and milk concentrations of carotenoids are highly variable among individuals. Genetic studies on 2321 dairy cattle in New Zealand concluded that the coefficient of variation is much higher for plasma and milk carotenoid concentrations (30% and 26%, respectively) than for milk yield and fat concentrations (14% and 10%, respectively, Morris et al., 2002). A high coefficient of variation of 45% was also reported for milk color in a population of 9516 cows (Winkelman et al., 1999). In Holstein dairy cows with similar milk yield and milk fat concentration, retinol and β-carotene concentrations in both plasma and milk varied according to sire (Jensen et al., 1999). The heritability of carotenoid concentrations in plasma was reported to be much higher than in milk fat (0.46 *versus* 0.11, respectively, Morris et al., 2002). The heritability of milk fat color is also breed-dependent (*e.g.*, it is higher for Friesians than for Jerseys (Winkelman et al., 1999)). Both genetic and phenotypic correlations between plasma and milk fat carotenoid concentrations are high, indicating that selecting bulls in terms of plasma carotenoids could control carotenoid concentration in the milk of their daughters (Morris et al., 2002). Milk retinol concentrations vary less than milk β-carotene concentrations. For 36 cows receiving a grass-silage diet, the coefficient of variation was 18% for retinol *versus* 46% for β-carotene (Nozière et al., 2006).

4.2. Carotenoids and retinol in milk: variations related to dietary factors

4.2.1. Nature of forage

The concentration of β-carotene in milk is highly dependent on the concentration of β-carotene in the diet (Table 2; Fig. 3). As noted above, grass concentrations of β-carotene vary according to vegetation stage and decrease during drying and preservation proportional to the degree of light exposure, since β-carotene is highly UV-sensitive. A comparison of seven diets revealed that milk concentrations and yield of β-carotene were directly related to β-carotene in plasma and to the amount of β-carotene ingested by the cows (Martin et al., 2004). They were higher with mountain natural grassland (especially with young, but also aged, swards) and ryegrass silage than with ryegrass and natural grassland hays, concentrate-rich diets and corn silage diets (Martin et al., 2004). Similar results have been obtained in other studies comparing pastures *versus* hays (J.B. Coulon and P. Grolier, unpublished (INRA, Theix, France); B. Martin et al., unpublished (INRA, Theix, France)), grass silage *versus* hay (Nozière et al., 2006) and grass silage *versus* maize silage (Havemose et al., 2004). Within a given period of pasture grazing, the carotene and Vitamin A concentrations in milk fat and plasma may also be influenced by the botanical composition of the pasture (McDowall and McGillivray, 1963), but differences between white clover and ryegrass observed by these authors were slight.

4.2.2. Maturity stage and grazing management

Pasture feeding has been associated with high β-carotene concentrations in milk for a long time (Baumann et al., 1934), with high variations related to season (Fig. 4) when both maturity stage and lactation stage are confounded. Grazing cows in mid lactation have a decreased β-carotene level in milk throughout the first cycle of vegetation. With diversified mountain grassland, the extent of the decrease appears moderate (averaging −20%), from 5.4 to 4.5 μg/g fat (Martin et al., 2004) or from 4.6 to 3.6 μg/g fat (Calderon et al., in press). This may be attributed to the balance between the decrease in β-carotene concentration and intake of grass and the decrease in milk yield, inducing a concentration effect. It cannot be excluded that β-carotene digestibility varies with grass maturity, but this has not yet been demonstrated. The increase in β-carotene concentration and intake of grass from the late first cycle to early regrowth is accompanied by an increase in β-carotene concentration in milk from 3.8 to 4.8 μg/g fat (Calderon et al., in press). Similarly, McDowall and McGillivray (1963) observed higher carotene and retinol concentrations in milk fat, and higher plasma concentrations of carotene with cows grazing immature *versus* mature ryegrass. According to these authors, this could not be explained by differences in carotene intake, but rather by variations in the quantity and nature of the lipids present in the ryegrass at different stages of maturity that may interact with carotene bioavailability. However, this hypothesis remains unproven.

Because grazing management affects both the amount and nature of grass ingested by animals, it is a potential factor for variation in milk composition however, data on effect of grazing management on milk concentrations of carotenoids remain scarce. On diversified mountain grassland where strip grazing and rotational grazing were compared with 75 m^2/cow/day *versus* 113 m^2/cow/day, respectively, Calderon et al. (in press) observed no difference in milk β-carotene concentrations, but the milk contained 25% more lutein under

strip *versus* rotational grazing. This may be related to differences between the two groups in feeding choices at pasture, as supported by results on terpenes in the same study (Tornambé et al., 2006).

Compared to β-carotene, variations in milk lutein concentrations among forages or according to stage of development have been reported as lower (Martin et al., 2004; Nozière et al., 2006; Calderon et al., in press), higher (Havemose et al., 2004), or similar (B. Martin et al., unpublished (INRA, Theix, France)), and are related to variations in plasma concentrations (B. Martin et al., unpublished (INRA, Theix, France); Nozière et al., 2006). Between forages, milk concentrations of retinol varied to a lower extent than for β-carotene, and plasma retinol concentrations remained unaffected (Martin et al., 2004; Nozière et al., 2006; B. Martin et al., unpublished (INRA, Theix, France)).

In goat milk, the retinol concentration (*cis* + *trans* isomers) is higher in pasture-fed than in indoor-fed animals (650 μg/100 g DM *versus* 499 μg/100 g DM, repectively). This difference mainly concerns all-*trans* isomer (from 633 to 482 μg/100 g DM) whereas the 13-*cis* isomer is unaffected, averaging 17 μg/100 g DM (Fedele et al., 2004).

4.2.3. β-Carotene and vitamin supplementation

Several studies have reported that β-carotene has a specific role in reproductive efficiency of cattle besides its Vitamin A activity. Supplementation of β-carotene up to 600 mg/day has increased plasma concentrations of both β-carotene (Bindas et al., 1984; Oldham et al., 1991; Michal et al., 1994) and retinol (Michal et al., 1994) before calving, both in early and mid lactation. However, the potential positive effect on milk concentrations of β-carotene and retinol in cows remains unproven due to a lack of results (Chawla et al., 2003; Chawla and Kaur, 2004), and may depend on both the initial retinol and the β-carotene status of the animals. A single parenteral administration of β-carotene resulted in a marked increase in plasma concentrations of β-carotene, but not of retinol, and also increases milk concentrations of both β-carotene and retinol, which suggests local conversion of β-carotene into retinol in the mammary gland (Schweigert and Eisele, 1990).

In cows, dietary Vitamin A supplementation increases plasma, colostrum and milk concentrations of retinol, but reduces carotenoid concentrations in both milk and plasma (Deuel et al., 1941, 1942). This concentration decrease occurs with supplementation of 0.7×10^6 IU/day, but only a small additional reduction is observed with two- to six-fold higher supplementation. Similar results occurred for plasma and liver in steers (Knight et al., 1996). The change in plasma carotenoid concentration was −5.3 and −0.9 μg/ml (*i.e.*, −43% and −36% of initial level for pasture-fed and feedlot-fed animals, respectively). In goats, oral administration of provitamin A carotenoids either as a single massive dose or the same dose split into four equal doses markedly increased the retinol concentration in milk (Goyal et al., 1984). Similarly, oral administration of a single dose of retinyl acetate to lactating goats greatly increased both plasma and milk retinol concentrations (Sharma et al., 1983).

Lastly, Vitamin E supplementation (2500 IU/day for 132 days) as an antioxidant to improve lipid stability in meat, leads to a decrease in plasma and tissue concentrations of β-carotene in pasture-fed cattle (Yang et al., 2002) that may be related to a decrease in absorption, or to competition between α-tocopherol and β-carotene for transport in lipoproteins.

4.2.4. Persistence and latency delays

In mid lactation, a change from grass silage to a hay diet induces rapid decreases in concentrations of β-carotene and color of both plasma and milk. Changes occur the first day, and peak differences in β-carotene concentrations of milk between grass silage and hay diets occur 10–15 days after the shift in forage type in both milk and plasma (Nozière et al., 2006). This duration was irrespective of the initial plasma concentration, although it did vary strongly among individuals before the shift in diet. The average decrease in plasma β-carotene concentrations after the change from a grass silage to a hay diet was very fast, reaching −0.21 μg/ml/day during the first 10 days (Nozière et al., 2006). Comparable rates, but with a longer duration of the decrease in plasma carotenoid concentrations (−0.13 to −0.22 μg/ml/day over 30–50 days) have been reported for beef cattle changed from high carotene to low carotene pellets (Knight et al., 1994) and from pasture to feedlot (Knight et al., 1996). Factors affecting rate or duration of the plasma pool decrease in β-carotene during depletion remain unclear.

Data on latency for β-carotene appearance in milk remain scarce. In mid lactation, a change from a hay diet to diets with different hay-to-grass silage ratios (from 0:100 to 100:0) induces a rapid increase in plasma color that stabilises only after 2 or 4 weeks depending on the amount of β-carotene in the diet (F. Calderon et al., unpublished (INRA, Theix, France)). Latency is likely due to carotenoid metabolism (*i.e.*, storage and mobilisation by tissues) and to HDL dynamics and metabolism. Also, a change from a hay-concentrate (35:65) diet to pasture induces an increase in milk color that does not stabilise after 36 days of pasture (Prache et al., 2002).

4.2.5. Energy balance

With a fixed amount of carotenoids and retinol ingested, restricted energy intake during mid lactation (from 6.2 to 4.5 MJ NEl/day) induces an increase in β-carotene (from 3.21 to 4.06 μg/g fat) and retinol (4.08–5.48 μg/g fat) concentrations in milk, whereas plasma concentrations remain unaffected, averaging 4.72 and 0.58 μg/ml, respectively (Nozière et al., 2006). For β-carotene, the increase in milk concentration is mainly related to the decrease in milk yield, whereas the amount of β-carotene secreted in milk remains unaffected. In contrast, retinol concentration in milk increases slightly more than accounted for by decreased milk yield (from 3.60 to 4.26 mg/day). A lower ruminal destruction of retinol due to reduced concentrate level in the diet may not be excluded (Rode et al., 1990; Weiss et al., 1995). However, because plasma concentrations remained unchanged, the increase in retinol secretion in milk may rather be related to a release of β-carotene from adipose stores.

5. Relative influence of production factors on carotenoid and retinol concentrations in milk and dairy products under practical herd management conditions

The variation range of β-carotene and retinol concentrations in cows' milk fat observed with various milk production factors detailed in Section 4 indicates that the nature of the forage is the main variable that explains variation of β-carotene and retinol concentrations

in cows' milk fat. Breed is also an important factor in variation in these micronutrients, particularly the β-carotene concentration. Conversely, the influence of parity and energy balance on the β-carotene and retinol concentrations in milk fat seem to be relatively small. This theoretical approach does not account for interactions between milk production factors that occur in production systems. In order to address these limits, we will simultaneously consider effects of the production factors directly under real conditions of herd management.

5.1. Herd milk

Lucas et al. (2006a) reported the influence of production factors under practical conditions of herd management. In dairy products derived from herd milks, the micronutrient concentrations in cheese fat varied by a factor of 6.3 for retinol, 16.8 for β-carotene, and 9.4 for xanthophylls. The main factor influencing carotenoid and retinol concentrations was the nature of the basal forage in the ration and, more particularly, the level of green grass fed. Herd breed (*i.e.*, Montbeliarde, Holstein and Abondance), also appeared to influence carotenoid, but not retinol concentrations in cheese fat. Level of Vitamin A supplementation had no effect when herds were fed fresh grass, but was the main milk production factor influencing the retinol concentration of cheese when herds were fed preserved forage-based rations. Stage of lactation appeared to have no influence on carotenoid and retinol concentrations in cheese fat, but calvings were dispersed throughout the year, which probably weakened potential effects of lactation stage on herd milk.

5.2. Bulk milk

In practical conditions where herd milks are pooled in transport tankers, the composition variability is reduced and the influence of production factors are partly cancelled. Consequently, variations in the carotenoid and retinol concentrations in bulk milk products can only be linked to factors that are indirectly responsible for variations, such as the region or the season of production, due to the regional or seasonal dependence of some management practices. Seasonal variations in carotenoid and retinol composition of bulk milk products are mainly due to the nature of the forage fed, which is the production factor that is most closely related to season. Except in countries where cows graze throughout the year (*e.g.*, New Zealand), milk fat is generally richer in carotenoids and retinol when it is produced during the summer months from grazing animals than during the winter months when animals are fed preserved forages (Hartman and Dryden, 1965). In France, Agabriel et al. (2004) noted that average milk fat concentrations of retinol, β-carotene and xanthophyll were 1.2-, 1.6- and 1.8-fold higher, respectively, when milk was produced during the grazing *versus* the indoor feeding period. However, these differences were not statistically significant for retinol. Stage of lactation may also play a role in the season effect, in particular in production systems characterized by seasonal calvings. In countries where cows are on pasture all year, carotenoid and retinol concentrations are higher in winter *versus* summer milk, unlike in most other countries. For example, McDowell and McDowall (1953), working in New Zealand, found the highest Vitamin A potency values (45.0–50.2 IU/g) in winter (July–August) butter and the lowest potency values (28.3–33.4 IU/g) in summer/early autumn (January–March) butter. According to these authors, the seasonal variations cannot

be attributed to a lack of carotene in the summer diet, but, at least in part, to a lower bioavailability of the carotenoids from the grass during this period.

The strong regional effects sometimes noted (*e.g.*, Smit et al., 2000) may be attributed to differences in feeding, breed or calving period depending on the production region. Moreover, these regional effects may interact with season effects. For instance, in South Africa, Smit et al. (2000), compared the composition of bulk milks produced in fives locations either in summer or winter, and showed that variations in micronutrient concentrations according to locations were higher in summer (factors of 3.8 and 3.0 for β-carotene and retinol, respectively), than in winter (factor of 3.0 and 1.7, respectively).

6. Transfer of carotenoids and retinol from raw milk into milk products

Carotenoids and retinol are sensitive to different physico-chemical factors including air, oxidizing agents and ultraviolet light. Their degradation is accelerated by increasing temperature and is catalyzed by mineral ions. Retinol can also be quite unstable at pH 4.5 or lower. A low pH causes partial isomerisation of retinol from the all-*trans* form to the less potent *cis* forms, and hydrolysis of retinol esters to more labile retinol (Erdman et al., 1988). Consequently, technological treatments such as heating and acidification applied when processing milk to produce dairy products, as well as the immediate processing and storage environment (*i.e.*, light, temperature) are likely to degrade these micronutrients and influence the vitamin potency of the resulting dairy products. Furthermore, processing of some milk products (*e.g.*, cheese, butter) involves selective transfer of constituents from milk to milk products. Given that carotenoids and retinol are fat-soluble, they mainly behave like milk fat. However, a small proportion of the retinol and carotenoids are associated with whey proteins (McGillivray, 1957; Puyol et al., 1991) and/or concentrated in the milk fat globule membrane (Zahar et al., 1995). As a result, a certain amount of these micronutrients could be lost to whey during cheese-making and into buttermilk during butter-making.

6.1. Effect of thermal processing

Thermal processing of milk appears to cause little or no loss of retinol and carotenoids (Hartman and Dryden, 1965). B. Martin et al. (unpublished (INRA, Theix, France)) recently observed that milk pasteurisation (at 72 °C for 30 s) causes a 6% loss of retinol in milk fat, but no loss of β-carotene and lutein. In contrast, pasteurisation results in isomerisation of retinol from the all-*trans* form to *cis* forms that directly depends on the intensity of the heat treatment. Panfili et al. (1998) detected no 13-*cis* retinol in raw cows milks, whereas it represented 25 g/kg of total retinol in pasteurized milks with mild heat and 57 g/kg in pasteurized milks treated for 15 s at temperatures ranging from 72 to 76 °C. Milk subjected to more severe heat treatments had a higher degree of isomerisation, as shown by the proportion of 13-*cis* retinol ranging from 137 to 250 g/kg of total retinol in UHT milks and sterilised milks, respectively. Similarly, 13-*cis* retinol had, on average, 29 and 121 g/kg of total retinol in pasteurized cream and UHT cream, respectively. To our knowledge, there are no data on level of carotenoid isomerisation in relation to thermal processing. Relevant data from other foodstuffs indicates that heat treatment does not substantively affect total

β-carotene, but results in isomerisation from the all-*trans* form to *cis* forms, especially the 13-*cis* form (Marx et al., 2003). β-Carotene seems to be less sensitive to heat-inducing isomerisation than retinol. Following a 10-min heat processing at 80 or 90 °C, 13-*cis*-β-carotene is either below detectable quantities or represents only 10 g/kg of β-carotene. With higher temperatures and/or length of heating, the proportion of 13-*cis*-β-carotene progressively increases, but still represents only 120 g/kg of total β-carotene following a 40-min treatment at 130 °C (Marx et al., 2003). In this latter case, 9-*cis*-β-carotene is also detected, but represents only 27 g/kg of total β-carotene.

6.2. *Effect of the cheese-making process*

During cheese manufacturing, between 800 and 950 g/kg of the retinol and carotenoids in the original milk are recovered in the curd (Hartman and Dryden, 1965). In many studies, little or no change in the concentration of these components has been observed during ripening or storage of cheese for up to a year (Hartman and Dryden, 1965). In contrast, Randoin and Causeret (1958) observed a large loss of retinol during milk cooking (from 360 to 580 g/kg) and ripening (310 and 590 g/kg after 1 and 5 months of ripening, respectively) in the making of Gruyère cheese. In a recent study (Lucas et al., 2006b), the rate of transfer of retinol, β-carotene and xanthophylls from milk fat into cheese fat, considering four cheese-making technologies and original milks covering a large range of concentrations of these micronutrients in milk fat was examined (Fig. 5). On average, 950 g/kg of β-carotene, but only 660 g/kg of retinol and 640 g/kg, of xanthophylls originally present in milk fat were recovered in cheese fat. In addition, despite the varying heating temperatures, acidification levels and ripening times among the cheese-making technologies studied, the rate of retinol and carotenoid loss did not vary by the cheese-making technology. These results suggest that β-carotene is very stable, whereas retinol and xanthophylls are partially damaged and/or lost into whey during cheese-making. However, Kon et al. (1944) have reported that whey fat was richer in carotenoids than the original milk fat (+50%), which could be explained by the lower size of the fat globules in whey *versus* milk and the higher proportion of carotenoids in the milk fat globule membrane *versus* in the globule core. In contrast, these authors observed that retinol concentration was similar between milk fat and whey fat,

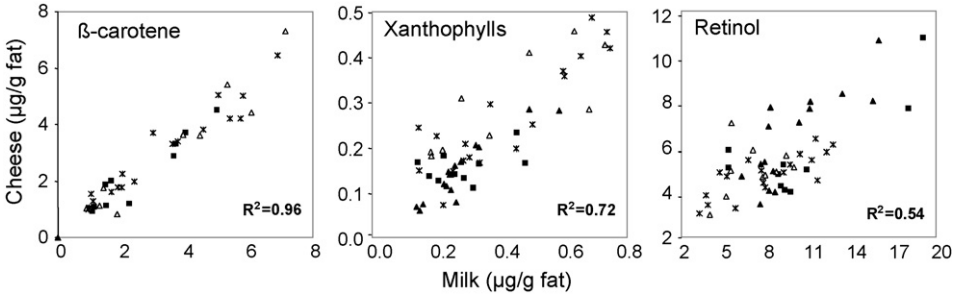

Fig. 5. Relationships between milk and cheese compositions in β-carotene, xanthophylls and retinol. Abondance (■), Tomme de Savoie (△), and Cantal-type (×) cow cheeses, and Rocamadour (▲) goat cheese. After Lucas et al. (2006b).

which suggests that loss of retinol in cheese-making is mainly due to its destruction. In particular, the high sensitivity of retinol to photoisomerisation following exposure of milk to light (De Man, 1981) could explain loss of this vitamin during cheese-making. That the 13-*cis* isomer of all-*trans*-retinyl palmitate, which is known to result from light exposure, is present in large amounts in cheese (140–260 g/kg of total retinol), but not in milk (Panfili et al., 1998), confirms the instability of retinol during cheese-making. In addition, according to Marsh et al. (1994), exposure of cheese to light causes a marked destruction of retinol throughout the cheese, not just in the surface layers. However, only a certain portion of the milk-native retinol appears to be lost rapidly on light exposure, with the remaining portion being resistant to further destruction (De Man, 1981). This may explain why retinol loss is similar regardless of the cheese-making technology. In contrast, the cheese-making process did not lead to increased proportions of 13-*cis*-β-carotene, which averaged 150 g/kg in both milk and cheese fat (Lucas et al., 2006b).

6.3. *Effect of the butter-making process*

Berl and Peterson (1945) studied the rate of transfer of carotenoids and retinol from milk into cream, skimmed milk, buttermilk and butter. The recovery rate of the milk-native retinol ranged from 920 to 994 g/kg in cream, from 23 to 40 g/kg in skimmed milk, from 4 to 11 g/kg in buttermilk and from 929 to 1020 g/kg in butter. Similarly, the distribution of carotene following butter-making was about 825–941 g/kg in cream, 95–143 g/kg in skimmed milk, 8–20 g/kg in buttermilk and 895–941 g/kg in butter. These proportions may add to more than 1000 g/kg which, according to these authors, is because of the method of calculation and analytical methods. Furthermore, Kon et al. (1944) studied the rate of transfer of retinol and carotenoids from milk fat into the fat of cream, butter and buttermilk, and reported that the retinol concentration was similar in milk fat, cream fat, buttermilk fat and butterfat. Milk fat, cream fat and butterfat have similar carotenoid concentrations, but buttermilk fat was richer in carotenoids than the original milk fat (+24%). The authors suggested that was due to the lower size of the fat globules in buttermilk *versus* milk and the higher proportion of carotenoids in the fat globule membrane *versus* the globule core. In addition, the degree of isomerisation of all-*trans* retinol during the manufacture of butter and yogurt appears to be low. Indeed, Panfili et al. (1998) measured 13-*cis* retinol proportions of only 76 and 71 g/kg of total retinol in butter and in yogurt, respectively.

7. Links between milk carotenoids and the sensory properties of dairy products

Milk carotenoids influence sensory properties of dairy products either indirectly through their antioxidant properties or directly through their yellowing properties.

7.1. *Antioxidant properties*

Dairy lipids can undergo oxidation leading to changes in the quality of the final dairy products, sometimes impacting negatively on consumer acceptability. Lipid auto-oxidation, as well as light-induced oxidation, is affected by a complex interplay of pro- and

antioxidants. The main antioxidant compounds in milk are enzymes (*i.e.*, superoxide dismutase, catalase, glutathione peroxidase), lactoferrin, Vitamins C and E, and carotenoids. Vitamin E and carotenoids act as fat-soluble antioxidants in, for example, the milk fat globule membrane which is regarded as a major site of auto-oxidation (Lindmark-Mansson and Akesson, 2000). β-Carotene is also particularly involved in prevention of photo-oxidation as it absorbs light in a concentration-dependent manner that would otherwise be absorbed by riboflavin, thereby inducing quality changes (Mortensen et al., 2004). Carotenoids function as singlet oxygen scavengers and may also react with other reactive oxygen species (Lindmark-Mansson and Akesson, 2000). In terms of the oxidative stability of milk, several reports have highlighted the beneficial action of α-tocopherol supplementation in cows on spontaneous oxidized flavour-related problems in milk (St-Laurent et al., 1990), but the influence of carotenoids is not as clearly defined. For instance, Barrefors et al. (1995) reported β-carotene concentrations of 3.5 and 4.9 mg/g fat in milk in different herds with and without off-flavour, respectively. However, it is particularly difficult to identify direct relationships between milk β-carotene and its vulnerability to oxidation because β-carotene-rich milks (*e.g.*, from pasture-fed cows) are also rich in α-tocopherols, which makes it difficult to accurately individualise the specific role of β-carotene, which may only be a coincidental factor, as suggested by Krukovsky et al. (1950), and β-carotene-rich milks are also PUFA-rich and consequently more vulnerable to oxidation.

7.2. Color of dairy products

Assessment of color in dairy products is achieved in most studies by tristimulus color measurement instruments (Kneifel et al., 1992; Chatelain et al., 2003) giving results expressed in the L, a, b Hunter system, where "L" defines the position of the sample on the dark-light axis, "a" on the green-red axis and "b" on the blue-yellow axis. The absence of standardised sample preparation procedures and the illumination conditions (*i.e.*, incident light) raise major problems in comparison of literature.

7.2.1. Milk

The white appearance of milk results from its physical structure (*i.e.*, the dispersion of both casein micelle and fat globules responsible for the diffusion of incident light and consequently of the high L value of milk). All the measurement conditions such as temperature and composition parameters such as fat, protein, Ca and P, as well as technological treatments that influence the physical structure of milk also modify the L component of the milk color measurement. Milk color assessment is mainly applied to identify technological parameters such as homogenisation, thermal treatment (including Maillard reactions), fat concentration, photo-degradation, storage conditions or additives (for review, see Chatelain et al., 2003). The 'a' and 'b' components of the milk color are also influenced by a number of factors linked to milk's natural pigment concentration. The main pigments are riboflavin, a green compound present in the aqueous phase which is a strong photosensitizer, and β-carotene and to a lesser extent lutein which have maximal absorbance at wavelengths 497–466 and 481–453 nm, respectively. Milk carotenoids are responsible for the yellow coloration (higher 'b' value) of cattle milks in comparison to sheep and goat milks which are devoid of β-carotene, and they are also responsible for the yellow coloration of cattle

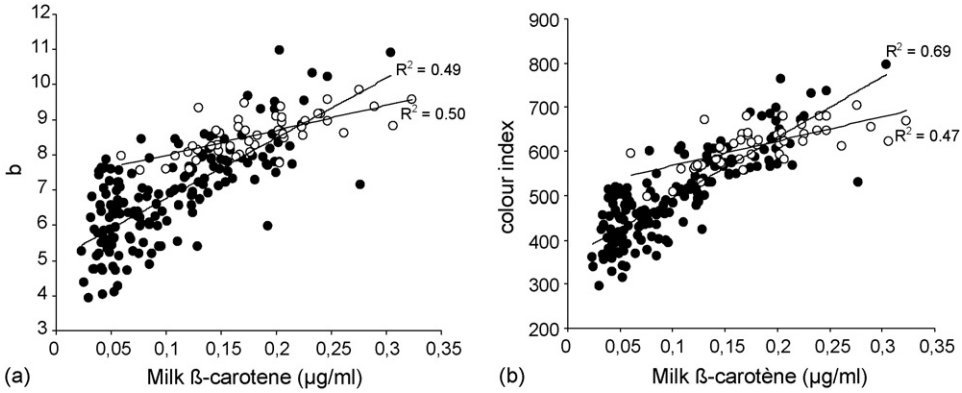

Fig. 6. Relationships between milk yellow coloration (b component) or "color index" (see text) and milk β-carotene concentration (color measurements achieved on individual milks immediately after milking (●) or on bulk milks stored 2–3 days (◯)). After Nozière et al. (2006) and Martin et al. (2004).

milks derived from breeds or diet regimens that have a high milk carotenoid concentration (see Section 5). Nevertheless, an overview of recent results obtained in our laboratory highlighted only a weak relationship between milk carotenoids and its yellow milk coloration (the "b" component; Fig. 6a). In a study using individual milk samples analysed immediately after milking, milk carotenoids were responsible for 49% of the variability in the blue-yellow axis ('b'). This relationship is similar ($R^2 = 0.50$) in the case of color measurements 2–3 days after the sampling of bulk milks collected over a 1 year period in industrial dairy plants in the Massif Central of France. This weak relationship also demonstrates that a simple color measurement cannot be accurately used to determine milk carotenoid concentration. Conversely, on untreated full-fat raw milks, we recently adapted a method proposed by Prache and Theriez (1999) based on the spectrum interpretation of light reflected by carcass adipose tissue in the 450–510 nm range which can distinguish carcasses of lambs raised on grass from those raised indoors and fed concentrates. This color index, which was adapted to milks by using the 450–530 nm range, varies according to the milk β-carotene concentration, and was able to explain 47–69% of the variability of β-carotene concentration, depending on the study (Fig. 6b). It clearly discriminated milks produced by grazing cattle from milks produced with cattle fed concentrate and hay based diets (Martin et al., 2005a). By directly applying this color index to individual milk samples produced by cows fed different diets corresponding to a wide array of nutritional situations, it was possible to discriminate milk produced by cows receiving the high-concentrate (*i.e.*, 650 g/kg of DM intake) or maize-silage-based diets from milk produced by cows fed grass silage or diversified pasture. Nevertheless, due to variability, the color measurement index was unable to distinguish milks from hay based diets from other milks. To accurately discriminate carotene-rich diets from carotene poor diets, this measurement must be completed 4–5 weeks after the diet change due to carotenoid latency and persistency. For example, after a change from a hay and concentrate based diet (*i.e.*, carotenoid poor) to pasture (*i.e.*, carotenoid rich), the color index perfectly discriminated milks produced from these diets only after a 36-day treatment period (Fig. 7). After a change from a carotene-rich diet

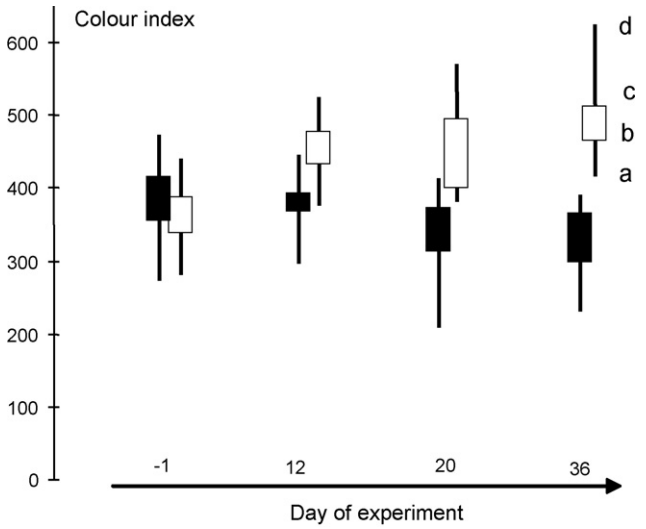

Fig. 7. Variations in the color index of milk from cows maintained on pasture (open symbol) or after shift from pasture to hay and concentrate diets (solid symbols). (a) Minimum and (d) maximum, 50% of the values comprised between (b) and (c). After Prache et al. (2002).

(*i.e.*, grass silage) to a carotene poor diet (*i.e.*, hay), both the "b" component and the color index for the resulting milk could not be conclusively discriminated, even after 50 days, but simultaneous use of plasma and milk indexes proved effective in discriminating 100% of the cows according to their diets from day 15 onwards (Nozière et al., 2006).

7.2.2. Butter and cheeses

As stated above, milk carotenoids are transferred into butter and cheeses with minimal losses and thus contribute to their yellow coloration. Depending on the specific target market, the yellow color may be perceived as a positive or negative attribute. For instance, it is considered as negative for the color-sensitive markets of the Middle-East (Keen and Wilson, 1992). The marked yellow color of New Zealand milk fat resulting from the use of Jersey cows fed diets consisting predominantly of fresh grass raises problems for exportation. In contrast, in Europe, the yellow color of dairy products is generally seen as a positive trait that contributes to consumers' preference for dairy products produced in summer (*e.g.*, derived from fresh grass based diets; Casalis et al., 1972). The hedonic preference of consumers for summer butter and cheese produced from pasture-fed cows is more marked when the sensory assessment is made under daylight in comparison to red light which masks the natural color differences (Houssin et al., 2002).

The yellow coloration of dairy products is generally a more important issue in high fat dairy products such as butter and full-fat cheeses. Because carotenoids are fat-soluble, the yellow coloration is a function of both fat color and concentration, and fat color is a function of the carotenoid concentration in the fat (Kneifel et al., 1992). The color of butter and cheese is also largely influenced by other factors. For example, it varies during ripening and storage according to duration, temperature and light exposure (Kristensen et al., 2001; Kneifel et

al., 1992) or according to contamination by pigment-producing microorganisms such as *Brevibacterium linens*. When these process related factors are controlled, the "b" value of butter or cheeses will not accurately evaluate their β-carotene concentrations (Kneifel et al., 1992), even if milk production conditions generate large variations. Factors linked to animal characteristics and feed systems that affect milk fat carotenoid concentration (see Section 4) also modify the "b" component of butter and cheese color. In particular, the yellow coloration is higher in dairy products from cows than from ewes or goats, and higher for cattle when breeds such as Jersey cows are used instead of Holstein or Montbeliarde. The yellow coloration is also higher when cows are fed pasture or grass silage than hay based, grain or maize silage diets (see review by Martin et al., 2005b).

8. Conclusions

Carotenoid transfer from diet to milk is relatively low. However, the milk concentration of carotenoids, mainly β-carotene and to a lesser extent retinol, varies substantively while contributing to the nutritional and sensory properties of dairy products. The variability in milk explains a high proportion of the variability in dairy products. Carotenoid and retinol concentrations in milk are governed by numerous dietary and non-dietary factors including animal breed and feeding management. However, the nature and amount of forage consumed appears to be the main factor controlling carotenoid and retinol concentrations in dairy products. Feeding management at pasture, and techniques leading to a reduced degradation of carotenoids in preserved forages increase carotenoid concentrations in milk. Moreover, control of carotenoid levels in milk may also be improved by a better knowledge of digestive and metabolic processes, including storage/release events involved in transfer of ingested carotenoids to the mammary gland. Carotenoids play a major role in color of dairy products. Thus, rapid measurement of color in milk or dairy products appears to be a promising tool in feed system traceability. Overall, it seems clear that carotenoid concentration and color in dairy products can be rapidly and efficiently controlled by dairy cow feeding management.

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