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REVIEW

Carotenoids, ergosterol and tocopherols in fresh and preserved herbage and their transfer to bovine milk fat and adipose tissues: A review

Pavel Kalač

University of South Bohemia, Faculty of Agriculture, Department of Applied Chemistry, České Budějovice, Czech Republic

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Abstract
The requirements of cattle for the fat-soluble vitamins A, D and E, their provitamins and some carotenoids have increased under conditions of intensive husbandry with insufficient provision of fresh forage, their cheapest natural source. Moreover, bovine milk fat and adipose tissues participate in the intake of these micronutrients by humans. Four major carotenoids occurring in forage crops are lutein, all-*trans*-ß-carotene, zeaxanthin and epilutein. Fresh forage is their richest source. Losses are significantly higher in hay as compared with silage, particularly if prepared from unwilted herbage. Maize silage is a poor source of carotenoids as compared with ensiled grasses and legumes. Ergosterol contents in forages increase under environmental conditions favourable for the growing of moulds, particularly at higher humidity and lower temperatures. Credible data on changes of ergosterol during herbage preservation, particularly drying and ensiling, have been lacking. Alpha-tocopherol is the most important among eight related compounds marked as vitamin E. It is vulnerable to oxidation and herbage ensiling is thus a safer preservation method than haymaking. As with carotenoids, maize silage is a poor source of α-tocopherol. Overall, information on factors affecting the content of ergosterol and tocopherols in fresh herbage, on changes during forage preservation and on transfer to bovine fats has been much more limited than data for ß-carotene.

Key words: forage; silage; fat-soluble vitamins; carotenoids; ergosterol; tocopherols; bovine milk fat; bovine adipose tissue

Abbreviations: DM, dry matter; **DMI**, dry matter intake; **ERG**, ergosterol; **HPLC**, high performance liquid chromatography; **IU**, international unit

✉ Pavel Kalač, University of South Bohemia, Faculty of Agriculture, Department of Applied Chemistry, Studentská 13, 370 05 České Budějovice, Czech Republic

kalac@zf.jcu.cz

INTRODUCTION

The health of livestock is the main factor affecting the quality, safety and health effects of foods of animal origin in human nutrition. Feed quality is a very important factor determining both animal health and transfer of essential micronutrients

(particularly vitamins, provitamins and trace elements) into animal products.

Cattle requirements for the fat-soluble vitamins A, D and E in intensive husbandry with insufficient provision of fresh forage and with low exposure to sunlight have been revised during the last decade. These requirements can be met by appropriate vitamin supplements. Nevertheless, fresh and preserved forages are the safest and cheapest natural sources of these vitamins and their provitamins. Moreover, provitamin carotenoids and tocopherols possess antioxidative effects protecting unsaturated fatty acids in milk and body fats from oxidation (for a review see Krzyzewski et al. 2012).

The aim of the review is to collate and evaluate recently available data on the content of carotenoids, ergosterol and tocopherols in fresh and preserved forages for cattle nutrition and their transfer to bovine milk and adipose tissues.

CAROTENOIDS

Carotenoids are a family of over 800 known natural pigments produced by higher plants and algae. Some of them participate in photosynthetic processes, others occur in flowers and fruits as attractants giving them yellow, orange or red colouring.

Carotenoids are linear or partially cycled polyisoprenes (tetraterpenoids) with 40 carbon atoms and numerous conjugated double bonds. Some of them are hydrocarbons (e.g. carotenes or lycopene), others contain oxygen atoms (particularly xanthophylls such as lutein or zeaxanthin). Both the groups are fat-soluble.

Plant carotenoids are partially transferred into lipidic fraction of animal products. In ruminants they affect the colour of milk and dairy products, particularly of butter, some cheeses, and also body fat. Consumers often associate the yellowish colour of milk and dairy products with "natural" feeding and the good health status of dairy cows. Carotenoid contents in these products could be thus an indicator of various production systems (Martin et al. 2005, Röhrle et al. 2011).

Several carotenoids, preferably carotenes and among them particularly all-*trans*-ß-carotene, are precursors of retinol (vitamin A_1) produced within animal organisms. Retinol is known mainly for its role in vision; however, it participates in reproduction processes and growth of ruminants. It is therefore commonly supplemented. Moreover, carotenoids are the important antioxidants (Kotíková et al. 2011).

Thus, carotenoids and retinol are nutritionally desirable constituents of milk. In addition, carotenoids may stabilise oxidisable compounds in milk. The recommended daily intake of retinol is 0.4–0.6 and 0.8–1.0 mg for children and adults, respectively. The total content of vitamin A in foods is expressed in International Units (IU, and one IU is equal to 0.3, 0.6 and 1.2 µg of retinol, ß-carotene and other provitamins A (e.g., α-carotene or γ-carotene), respectively. Milk and dairy products fulfil some 15% of the vitamin A requirements in the population of the Czech Republic.

Information on factors affecting the level of carotenoids in fresh herbage, hay and silage and their transfer from diet to cow's milk is thus needed. A thorough review on carotenoids from forages to dairy products was published by Nozière et al. (2006a). Only more recent and selected older data, either weighty or not included in the mentioned review, will be therefore collated in this chapter.

Carotenoids in fresh forages

Earlier data on the content of carotenoids in feeds may be somewhat misleading, as the measurement of carotenes was preferred. Traditional chemical analyses often determined "carotene" as a non-specified mixture of carotene isomers, including sometimes even other carotenoids. Recent analytical methods, preferably high-performance liquid chromatography (HPLC), enable the separation of individual carotenoids, isomeric carotenes and even various stereoisomers (*cis-trans*) differing in their biological value.

Less than 10 carotenoids have been reported in ruminant feeds. The four major carotenoids occurring in forage crops are lutein, all-*trans*-ß-carotene, zeaxanthin and epilutein (in order of descending quantity) and the minor carotenoids are neoxanthin, violaxanthin, antheraxanthin and 11-*cis*-ß-carotene. The actual content depends on synthesis and degradation. Synthesis proceeds in plastids, mainly in leaves. Highly unsaturated carotenoids undergo oxidative and isomerisation processes after plant harvest. Cautious handling with samples prior to analyses of fresh forage is therefore necessary.

Informative data for some herbages are given in Table 1. Nevertheless, the actual content of carotenoids is affected by several factors. There exist inter-species differences; however, it is not yet possible to draw conclusions on the differences among grasses, legumes or herbs. Carotenoid content increases most probably with increasing

nitrogen fertilisation. The effects of season or stages of maturity remain partly controversial, but generally speaking, the contents of carotenoids decrease with forage age (Nozière et al. 2006a, Graulet et al. 2012). Diurnal variations occur with the highest levels in the morning. The contents in leaves are considerably higher than in stems. Thus, the contents in forage increase with an elevated leaf-to-stem ratio during plant development. These differences have to be respected during herbage sampling for the determination of carotenoids.

Table 1. Content of carotenoids (mg kg^{-1} dry matter) in fresh forages

Forage (weight proportion)	Total β-carotene	All-*trans*-β-carotene	Lutein	Epilutein	Zeaxanthin	Reference
Natural mountain grassland (on mid-June and rotational grazing)	63.8	47.3	193	36.0	49.8	Calderón et al. 2006[1]
Trifolium pratense – first cut	29.0	16.0	136	40.0	–	Cardinault et al. 2006
Permanent grassland (0.45 *Phleum pratense* + 0.45 *Festuca pratensis*) – first cut	44.7	–	167	–	–	Müller et al. 2007
Herbs mixture (0.43 *Cichorium intybus* + 0.21 *Plantago lanceolata*) – mid-August	63.1	–	262	–	–	Petersen et al. 2011
Clover-grass mixture (0.78 *Trifolium repens* + 0.21 *Lolium perenne*) – mid-August	32.2	–	232	–	–	Petersen et al. 2011
Forage mixtures – first regrowth:						
Lotus corniculatus + *Phleum pratense*	56.2	–	–	–	–	Lindqvist et al. 2011
T. pratense + *P. pratense*	39.1	–	–	–	–	
T. pratense + *Festuca pratensis*	35.6	–	–	–	–	

[1] = total xanthophylls content was 328 mg kg^{-1} dry matter. The proportions of plants are expressed on dry matter basis.

Carotenoids in preserved forages

High losses of carotenoids have been reported during field sun-drying of herbage and the losses strongly increase under prolonged haymaking during rainy weather. Carotenoid destruction is mainly due to solar radiation, particularly its UV rays. For instance, in a report of Pickworth et al. (2012), ß-carotene contents expressed as vitamin A equivalents were 39,865 and 2,923 IU per kg of DM in fresh fescue pasture and fescue hay, respectively. Generally, hay is thus a limited source of available carotenoids in ruminant feeding.

Data on carotene changes during forage ensiling up to1980 have been reviewed (Kalač and McDonald 1981). Several results from that period should be mentioned here. In a study of 11 different silages, Zarend and Steger (1971) reported that all-*trans*-ß-carotene, biologically the most effective provitamin A, constituted about 70% of all ß-carotene isomers. The proportion of all-*trans*-ß-carotene decreased during grass ensiling with respect to the less available 9-*cis*- ß-carotene and 9,15-di-*cis*-ß-carotene. The decrease in biological activity was about 15%. Isomerisation changes, however, represented lower losses than the total decreases in ß-carotene during ensiling.

An illustrative case of carotenoid content in silages and hay is given in Table 2. A further recent report (Höjer et al. 2012) gives mean contents of 24.9–49.4 and 175–237 mg kg^{-1} DM for ß-carotene and lutein, respectively, in slightly wilted silages of various grasses combined with red clover (*Trifolium pratense*), preserved with a mixture of formic and propionic acids.

Volatile fatty acids, particularly formic and propionic acids, have been steadily used as efficient silage preservatives. Their application has been proved to increase ß-carotene losses considerably, especially in combination with oxygen access such as during delayed sealing of the silo or during the silage feed out period. High losses were observed in red clover, white clover (*Trifolium repens*), lucerne (*Medicago sativa*) and sunflower (*Helianthus annuus*), but not in grasses, rye, oats or kale (*Brassica oleracea* var. *acephala*) (Kalač and Kyzlink 1981, Kalač 1983). Mean ß-carotene losses were 3.9, 8.0 and 22.5% of the initial content in ensiled wilted legume-grass mixtures (see Table 1) in silages prepared

without an additive, inoculated with a mixture of lactic acid bacteria combined with structural polysaccharide-hydrolysing enzymes and with a mixture of formic and propionic acids, respectively (Lindqvist et al. 2011). However, Shingfield et al. (2005) observed minimum differences in ß-carotene contents in silages prepared from a wilted mixture of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) under a very similar experimental design as in the previous report. The differences can be explained by a proportion of legumes sensitive to acids in the former article.

Table 2. Content of carotenoids (mg kg^{-1} dry matter) in silages and hay (adapted from Calderón et al. 2007a)

Carotenoids	Grass silage[1]	Maize silage	Hay
All-*trans*-ß-carotene	106	24.0	0
Lutein	250	25.0	18.0
Antheraxanthin	21	0	0
Violaxanthin	59	0	0
Zeaxanthin	53	9.8	1.7
Unidentified xanthophyll (probably epilutein)	28	9.6	1.4
Total carotenoids	517	68.0	21.0
Dry matter (g kg^{-1})	227	272.0	854.0

[1] = Grass silage of an unwilted mixture of first cut *Lolium perenne* with natural grassland.

Also forage wilting prior to ensiling can be a critical period for carotenoid losses, particularly if prolonged under unfavourable weather conditions (Park et al. 1983). However, if legume-grass mixtures were wilted under optimum conditions for only 3 h, mean ß-carotene losses were just about 12% of the level in fresh herbage (Lindqvist et al. 2011).

Maize silage has been known as a limited source of available ß-carotene and "maize (corn) silage vitamin A deficiency syndrome" has been described in cattle. As the data in Tables 1 and 2 show, maize silage is low in ß-carotene. According to Pilipavičius and Mikulionienė (2010), ß-carotene content steadily decreased from some 80 to 10 mg kg^{-1} DM in maize silages prepared at five stages, starting from very young plants up to maize at dough grain stage, respectively.

Overall, losses of ß-carotene in forages under the main preservation methods can be characterised by the mean data cited by Nozière et al. (2006a). The losses were 19, 59 and 82% of initial level in green forages in dehydrated forages, silages and hays, respectively.

Transfer of carotenoids to bovine fats

Both carotenoids and retinol transformed from provitamins A in the cow's body are valuable components of bovine milk fat and adipose tissues. Their contents in fats are affected by several factors, such as nutrition, season, dairy cow management, genetics and stage of lactation. Cattle differ from most farm animals because of the considerable level of carotenoids circulating in blood. ß-Carotene in milk comes from the blood after the uptake by the mammary gland. Variability of its content depends mainly on its dietary supply.

A commonly known effect of season has been demonstrated by the data of Agabriel et al. (2007) in French tank (bulk) milk. Milks produced during the grazing period were yellower than during winter feeding with preserved forages. ß-Carotene, lutein and retinol contents varied between 2.5–3.5, 0.27–0.32 and 5.2–7.7 mg per kg of milk fat, respectively, between February and March. The respective values were 4.9–5.3, 0.48–0.59 and 7.2–7.6 mg kg^{-1} milk fat between May and September. Grass silage was a better source of carotenoids than maize silage.

Shingfield et al. (2005) reported a significantly higher content of ß-carotene in milks from dairy cows fed grass silage than from their counterparts fed hay from the same swards. The respective contents were 0.19–0.22 and 0.14 mg kg^{-1} of milk. The secretion of ß-carotene in milk was related to

the intake, but at a very low mean efficiency of 0.07%. The differences in retinol contents in the milk of dairy cows fed silage and hay were of low significance. Havemose et al. (2006) observed a higher transfer of ß-carotene to milk from grass-clover silage than from hay.

The contents of ß-carotene and retinol were 0.17±0.02 and 0.41±0.06 mg l^{-1}, respectively, in milk from five Danish organic farms (Mogensen et al. 2012). The values of 0.30 and 0.40 mg kg^{-1} of ß-carotene and retinol, respectively, were reported by Höjer et al. (2012) in milk of Swedish red dairy cows fed with silages of grasses and red clover.

The effects of winter feeding of dairy cows with grass silage and maize silage on carotenoids content in milk are demonstrated in Table 3. The results prove that maize silage feeding causes a lower carotenoid level in milk than dairy cows feeding with silage of various grasses. Overall, maize silage has been considered as a poorer source of carotenoids than silage of other crops, especially if produced from whole-crop maize damaged by frost.

Table 3. The effects of winter feeding of dairy cows with grass and maize silages on carotenoids content in milk (mg kg^{-1} of milk fat). Adapted from Larsen et al. (2010).

Carotenoid	Grass silage + concentrate	Grass silage + maize silage + concentrate
β-Carotene	3.8±1.6	2.4±1.0
Lutein	0.28±0.07	0.18±0.05
Zeaxanthin	0.07±0.03	0.05±0.04

There are results available on the changes in milk carotenoids following switches in the feeding of preserved forages. The change from grass silage to a hay diet induced a rapid decrease in the contents of all-*trans*-ß-carotene both in milk and in adipose tissues (Nozière et al. 2006b). The contents of retinol were 4.08 and 2.97 mg kg^{-1} of milk fat in milks from silage and hay fed dairy cows, respectively. The reverse change was studied by Calderón et al. (2007b). ß-Carotene content in milk increased rapidly, while the level of lutein and zeaxanthin was affected only slightly. The authors suggest that in diets high in carotenoids, the secretion of ß-carotene into milk is not limited by the amount of ß-carotene arriving to the mammary gland but by mechanisms involved in the ß-carotene transfer from plasma to milk.

Comparing milk composition from conventional and organic farms in the United Kingdom, Ellis et al. (2007) reported insignificant differences both in ß-carotene content, 4.99±2.10 *vs.* 5.35±1.35 mg kg^{-1} of milk fat, and in retinol content, 16.3±3.7 *vs.* 14.1±2.6 mg kg^{-1} of milk fat. A somewhat higher retinol level in the conventional farms was probably because of an increased vitamin A supplementation in concentrates. Similar results were observed in Sweden (Fall and Emanuelson 2011): the median content of ß-carotene was 0.18 and 0.19 mg l^{-1} in conventional and organic milks, respectively, and that of retinol 0.32 mg l^{-1} in both the groups during the indoor season.

The carotenoids participate in the oxidative stability of milk. Havemose et al. (2004) determined a more intensive lipid oxidation in milks from dairy cows fed grass silage than from cows fed maize silage, despite a higher antioxidative capacity of the former milks. Such a disproportion was probably caused by a higher proportion of unsaturated fatty acids vulnerable to oxidation in milk fat of dairy cows fed grass silage.

In general, transfer of carotenoids from various forages to bovine adipose tissue follows a similar course as was described for cow´s milk. Available data were recently reviewed by Daley et al. (2010).

Overall, fresh forage is the richest source of carotenoids. Losses are significantly higher in hay as compared with silage, particularly if prepared from unwilted herbage. Maize silage is a poor source of carotenoids as compared with ensiled grasses and legumes.

ERGOSTEROL

Vitamin D is an essential nutrient for mammals. Its main role is the maintenance and regulation of calcium homeostasis. The deficiency causes rickets

in children and growing animals and osteomalacy in the elderly humans and in adult animals. Moreover, further implications of its deficiency in man have been identified, particularly an increased risk of cancer, cardiovascular diseases, diabetes and reduced immune response. Low vitamin D intake and status have been reported worldwide.

The term vitamin D includes vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol). Vitamin D_2 is produced from its provitamin ergosterol (ERG), occurring in fungi (yeasts, e.g. leaven, moulds and mushrooms) if exposed to UV-B radiation (wavelength below 315 nm). Vitamin D_3 is produced in mammals from its provitamin 7-dehydrocholesterol by the action of sun UV-B radiation. It is therefore often referred to as animal vitamin D although this is not quite correct as it has been found in several plant species, preferably in the *Solanaceae* family.

The parent vitamins D_2 and D_3, occurring mainly in milk, are hydroxylated in mammal liver to 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3, which are further hydroxylated in the kidneys preferably to the metabolically active 1,25-dihydroxyforms of both the vitamins. The biological activity of vitamins D_2 and D_3 is generally considered equal.

The US National Research Council (2001) recommends providing lactating cows with 0.75 µg vitamin D (30 IU; 1 IU = 0.025 µg vitamin D) per kg of bodyweight daily.

The recommended vitamin D daily intake for man varies between 2.5–10 µg, the highest being for children, pregnant and nursing women. The usual vitamin D content, including all provitamins, vitamins and their hydroxy metabolites, is 1, 4 and 10–20 µg kg^{-1} in milk, cream and butter, respectively.

Mean vitamin D_2 content of 0.034±0.012 and 0.61±0.05 µg kg^{-1} was recently reported for milk (1.5% fat) and butter in Denmark. The content in cow's milk was highest during the period of May–July and lowest in February–April, while in butter the range was not too wide (Jakobsen and Saxholt 2009).

Ergosterol in herbage

Available data on ERG and vitamin D_2 content in fresh and preserved forages have been scarce. Most publications on vitamin D_2 in grass and hay date back 50–80 years (Jäpelt et al. 2011a and literature cited therein). Nevertheless, these data should be perceived with caution. Vitamin D activity was formerly determined by biological assays based on the ability to cure rickets in vitamin D-deficient rats. These methods cannot distinguish the individual vitamin D constituents, and traditional chemical methods using HPLC are problematic in complex matrices such as plant materials. These limitations were recently overcome using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Jäpelt et al. 2011b).

The contents of ERG and vitamin D_2 in herbage have been generally evaluated as a marker of the level of fungal biomass, while the standpoint of animal requirements is scarce.

The former viewpoint was reported in a series of studies dealing with fungal contamination of grasses saved for late autumn and winter grazing in low-input cattle management under the environmental conditions of several countries in Central Europe. The overall data are given in Table 4. ERG contents, determined by an HPLC method, varied widely between 20–400 mg kg^{-1} DM due to numerous factors. The grasses were analysed between October and January usually as second or third cuts. The previous cuts were harvested between June and August.

The date of the delayed harvest was generally found to be the main factor affecting the increased ERG content. Moreover, the longer lag between summer and winter cuts, the higher ERG content, as was reported by Opitz von Boberfeld and Banzhaf (2006) in *Festulolium* spp. and tall fescue (*Festuca arundinacea*), by Opitz von Boberfeld et al. (2006) in tall fescue or by Skládanka et al. (2008) in two *Festulolium* spp., cocksfoot (*Dactylis glomerata*) and *Arrhenatherum elatius*. Lower ERG levels in *Festulolium pabulare* suggests its higher resistance to mildew infestation than that of cocksfoot or *Arrhenatherum elatius* (Skládanka et al. 2008). ERG content increased in tall fescue with increasing N-fertilisation (Wolf and Opitz von Boberfeld 2003).

ERG content increases with increasing humidity and decreasing temperatures during autumn and winter months, the conditions favourable for mould growth (Golinski et al. 2006). Nevertheless, the correlation between ERG and mycotoxins, particularly zearalenone and deoxynivalenol content, was not commonly observed (Skládanka et al. 2011). This can be explained however by the occurrence of both toxinogenic and non-toxinogenic mould species, all producing ERG. Moreover, a decreasing ambient temperature decreases mycotoxin formation while enhancing ERG production.

Table 4. Mean ergosterol contents (mg kg^{-1} dry matter) in herbage saved for autumn and winter grazing in Central Europe

Herbage	Time of harvest	Ergosterol	Reference
Festuca arundinacea	January	70–250	Wolf and Opitz von Boberfeld (2003)
4 *Festulolium* spp.	December or January	100–200	Opitz von Boberfeld and Banzhaf (2006)
Festuca arundinacea *Lolium perenne* *Festuco-* or *Lolio-Cynosuretum* pastures	November–January	100–400	Opitz von Boberfeld et al. (2006)
Festulolium pabulare	October–December	95–110	Skládanka et al. (2008, 2009)
Dactylis glomerata		137–146	
Arrhenatherum elatius		140	
Festuca arundinacea	October–December	40–220	Skládanka et al. (2011)
Festulolium pabulare		20–100	
Festulolium braunii		35–240	

Jäpelt et al. (2011a) took into consideration animal requirements for ERG. They determined, using LC-MS/MS method, changes in ERG and vitamin D_2 content in four cuts between June and November under Danish conditions in six varieties of perennial ryegrass (*Lolium perenne*) during the first year after sowing. Vitamin D_2 levels were marginal, only up to 0.2% of ERG content. Generally, ERG content increased considerably after the first cut on 4 June (<1 mg kg^{-1} fresh weight) to the third cut on 2 September (mean level about 10.5 mg kg^{-1} fresh weight) and then decreased to mean content of about 6.1 mg kg^{-1} fresh weight at the fourth cut on 10 November. The mean dry matter was 19.2% in the study. Thus, values would be about five times higher if calculated per dry matter. These values are comparable with mean ERG content of 3.8 and 13.1 mg kg^{-1} DM reported for several grasses cut in June and July, respectively (Skládanka et al. 2011) but considerably lower than those in Table 4 determined between October and January by the HPLC method. Also in the Danish report (Jäpelt et al. 2011a), ERG contents increased under environmental conditions favourable for the growth of moulds, namely at higher humidity and lower temperature. Overall, perennial ryegrass and probably other grasses seem to be a considerable source of ERG for ruminants.

Unfortunately, credible data on changes of ERG and vitamin D_2 during herbage preservation, particularly drying and ensiling, have been lacking as has been an information on the relationship between ERG in fed herbage and bovine milk.

TOCOPHEROLS

Vitamin E is a generic name used for a group of four isomeric tocopherols (α- up to δ-) and four tocotrienols (designed similarly α- up to δ-). All the compounds are fat-soluble. α-Tocopherol is the most potent form of vitamin E, accounting for almost all vitamin E activity in living tissues. An antioxidation effect based on the quenching of free radicals, and thus the prevention of biomembranes damage, is the major biological function of vitamin E. Moreover, vitamin E improves immune responses. The IU is defined as the activity of 1 mg of synthetic *d,l*-α-tocopheryl acetate.

In cattle, vitamin E improves reproductive performance, reduces clinical mastitis and enhances macrophage function. Its metabolism and roles have been reviewed by Debier and Larondelle (2005). The reduced use of fresh forage as a source of vitamin E has led to a substantial increase in recommended intake for dairy cows. Recent recommendations are for about 80 IU kg^{-1} of dry matter intake (DMI) in the dry period and immediately post-partum, and about 20 IU kg^{-1} DMI during lactation. Vitamin E intake is generally considered adequate when α-tocopherol content in blood plasma is above 3–3.5 μg ml^{-1} or above 2 when expressed as a ratio to plasma cholesterol content. No further benefits from vitamin E supplementation are observed above these levels (Baldi 2005).

In human nutrition, the requirement greatly depends on the intake of polyunsaturated fatty acids. Due to their increasing intake, recent

vitamin E daily need is about 20–30 mg. The main sources are foods of plant origin, particularly oils. The content ranges from between 0.2–1.2, 2–5, 10–50 and up to 20 mg kg^{-1} in bovine mature milk, colostrum, butter and bovine tissue fat, respectively.

Tocopherols in fresh and wilted forages

Information on tocopherols in fresh forage and forage wilted prior to ensiling is limited. The available data for α-isomer are collated in Table 5. Values for γ-isomer are very scarce. Among the data in Table 5, the content reported by Lynch et al. (2001) from Ireland is considerably lower than that from other European countries. Overall, α-tocopherol content of about 50–75 mg kg^{-1} DM seems to be believable for legumes, while the values reported for perennial ryegrass by Beeckman et al. (2010) were at least double.

As results in Table 5 indicate, losses of α-tocopherol during wilting prior to ensiling varied between 20 and 35% of initial content and increased with the prolonged wilting. Thus, it may be expected that the losses during haymaking are even higher.

Table 5. Content of α-tocopherol (mg kg^{-1} dry matter) in fresh and wilted forages

Forage		Mean ± SD	Range	Country	Reference
Pasture grass, 2nd cut, (F)		14.5±0.36	13.9–15.2	Ireland	Lynch et al. (2001)
Meadow grass, 2nd cut, (F)		8.0±0.25	7.6–8.4	Ireland	Lynch et al. (2001)
Phleum pratense + *Festuca pratensis*	(F)	74	–	Sweden	Müller et al. (2007)
	(HW)	53	–		
Lolium perenne	(F)	156±11.3	–	Belgium	Beeckman et al. (2010)
	(SW)	133±13.0	–		
	(MW)	126±36.7	–		
	(HW)	100±53.8	–		
Pasture grasses between Nov. 15 and Jan. 31		33.9	–	Spain	Tejerina et al. (2011)
Trifolium pratense, 2nd cut		1.01±0.21	0.59–1.76	Ireland	Lynch et al. (2001)
T. pratense	(F)	74±5.7	–	Belgium	Beeckman et al. (2010)
	(SW)	55±5.1	–		
	(MW)	48±3.2	–		
	(HW)	51±1.6	–		
T. pratense + *Phleum pratense*	(F)	51.1	–	Sweden	Lindqvist et al. (2011)
	(SW)	40.1	–		
T. pratense + *Festuca pratensis*	(F)	59.7	–	Sweden	Lindqvist et al. (2011)
	(SW)	48.6	–		
Trifolium repens, 2nd cut		1.40±0.39	0.44–2.29	Ireland	Lynch et al. (2001)
T. repens	(F)	49±0.7	–	Belgium	Beeckman et al. (2010)
	(SW)	40±1.1	–		
	(MW)	43±8.1	–		
	(HW)	39±0.6	–		
Lotus corniculatus + *P. pratense*	(F)	58.8	–	Sweden	Lindqvist et al. (2011)
	(SW)	41.1	–		

SD, standard deviation. Forage: **F**, fresh; **SW**, slightly wilted; **MW**, medium-wilted; **HW**, heavily wilted

Tocopherols in preserved forages

Available information on α- and γ-tocopherols in silage and hay are collated in Table 6. As in fresh herbage, the data of Lynch et al. (2001) are considerably lower than the content reported from other laboratories. The overall values are lower than the content in fresh herbage given in Table 5. Data for hay have been scarce. Nevertheless, it may be supposed that tocopherols content is lower than in ensiled forages.

Table 6. Content of tocopherols (mg kg^{-1} dry matter) in silages and hay

Forage	α-Tocopherol		γ-Tocopherol		Country	Reference
	Mean ± SD	Range	Mean ± SD	Range		
Silage						
Grass, 1st cut	9.75±0.94	2.0–31.4	–	–	Ireland	Lynch et al. (2001)
2nd cut	9.8±1.07	1.53–16.3	–	–		
3rd cut	2.32±0.50	0.33–5.19	–	–		
Phleum pratense + *Festuca pratensis* (SW)	60.7	53.8–67.8	16.0	10.8–19.6	Finland	Shingfield et al. (2005)
Phleum pratense + *Festuca pratensis* (F)	34.0	–	5.3	–	Sweden	Müller et al. (2007)
(HW)	23.8	–	3.5	–		
Grass-clover	11.1±2.3	–	–	–	Denmark	Havemose et al. (2006)
Grasses + *Trifolium repens* + *T. pratense*	29.7	–	–	–	Denmark	Mogensen et al. (2012)
Grasses + *T. pratense*	20–37	–	–	–	Nordic countries	Höjer et al. (2012)
Lolium perenne (SW)	165±21.8	–	–	–	Belgium	Beeckman et al. (2010)
(MW)	72±31.5	–	–	–		
(HW)	57±12.6	–	–	–		
Trifolium pratense (SW)	39±2.9	–	–	–		
(MW)	40±4.2	–	–	–		
(HW)	39±2.2					
T. pratense + *Phleum pratense*	32.3	20.7–46.2	–	–	Sweden	Lindqvist et al. (2011)
T. pratense + *Festuca pratensis*	42.5	35.2–54.0	–	–		
Lotus corniculatus + *Phleum pratense*	59.6	56.8–65.2	–	–		
Trifolium repens (SW)	41±6.2				Belgium	Beeckman et al. (2010)
Maize	12.7	–	–	–	Denmark	Mogensen et al. (2012)
Hay						
Mixture of grasses	22.3	–	9.4	–	Finland	Shingfield et al. (2005)
Grass-clover	13.8	–	–	–	Denmark	Havemose et al. (2006)

SD, standard deviation. Silage prepared from forage: **F**, fresh; **SW**, slightly wilted; **MW**, medium-wilted; **HW**, heavily wilted

Information on the effects of ensiling is fractional. Lynch et al. (2001) observed a significantly lower α-tocopherol content in silages prepared from third-cut grasses as compared with previous cuts. Müller et al. (2007) reported α-tocopherol losses between 40 and 60% of the initial content during grass ensiling. Different mean losses were determined by Lindqvist et al. (2011) during ensiling of mildly wilted legume-grass mixtures. Low α-tocopherol shrinkage of 3.5 and 13% of the content in ensiled forage was observed in control silage and in silage preserved with a mixture of formic acid and propionic acid, respectively. In contrast, an increase of 27% was found in silages prepared with an additive containing lactic acid bacteria, cellulase and hemicellulase.

Unfortunately, data for whole-crop maize silage are lacking. Beeckman et al. (2010) reported a level of about 5 mg of α-tocopherol per kg DM in ensiled meal of maize cobs (CCM). This content was comparable with that in hay.

Transfer of tocopherols to bovine fats

Vitamin E content in milk is affected by several factors, such as nutrition, season, dairy cow management, genetics and stage of lactation.

In a report of Shingfield et al. (2005), α-tocopherol content was 1.10–1.15 and 0.54 mg kg^{-1} in milk from cows fed grass silages and hay, respectively. The secretion of α-tocopherol in milk was related to the dietary intake. Nevertheless, the mean efficiency of the transfer was only 2.8%. In contrast, Havemose et al. (2006) did not find a significant difference: 0.47 or 0.50 mg l^{-1} in milk from cows fed grass-white clover silage or meadow hay, respectively. In a previous work of Havemose et al. (2004), grass-red clover silage was shown to be a richer source of available tocopherols than maize silage. The content of α-tocopherol was 0.85 and 0.38 mg l^{-1} and that of γ-tocopherol 0.03 and 0.01 mg l^{-1} in milk of dairy cows fed grass-clover silage or maize silage, respectively. Mogensen et al. (2012) reported comparable α-tocopherol content of 0.82±0.23 mg l^{-1} in milk from five Danish organic herds. Twofold level of about 1.60 mg kg^{-1} was determined in milk of Swedish Red dairy cows fed with grass-red clover silages. Apparent recovery of α-tocopherol from feed to milk was approximately 6% (Höjer et al. 2012).

A shift from grass silage to hay diet caused a rapid decrease of α-tocopherol content in milk during the initial 2 weeks. Then the content slightly increased and remained stable for further 6 weeks. At the end of the experiment, the content was 21.4 and 14.2 mg kg^{-1} of milk fat following grass silage and hay feeding, respectively, under conditions of high energy intake, while the respective values were 26.2 and 17.4 mg kg^{-1} of milk fat under the energetic underfeeding (Nozière et al. 2006b).

An inverse shift from hay diet to diets with an increasing proportion of grass silage and lucerne protein concentrate as sources of α-tocopherol was investigated by Calderón et al. (2007b). Unfortunately, α-tocopherol content in hay and grass silage was not determined; its level in the lucerne concentrate was 500 mg kg^{-1} dry matter. A rapid increase of α-tocopherol in milk was observed during the first week following the change and then a plateau was reached. At the end of 6-week experimental period the content of α-tocopherol in milk fat was linearly related to the proportion of grass silage and lucerne protein concentrate in the diet. Alpha-tocopherol concentrations at that time were 11.3 and 7.8 mg kg^{-1} of fat in milk of cows fed the experimental diet and of those fed hay, respectively.

Thus, silage is a richer source of α-tocopherol than hay due to higher losses of this compound during grass drying and hay storage.

Agabriel et al. (2007) demonstrated the effect of season in French tank milks. Mean α-tocopherol contents were 18.8–21.7 and 10.5 mg kg^{-1} of milk fat between May and September, and in March, respectively. The difference was attributed to the proportion of grazed grass or grass silage in the forage. Similarly, the lowest α-tocopherol content was observed during the winter period both in British conventional and organic farms (Ellis et al. 2007).

As data reviewed by Debier et al. (2005) show, colostrum contains considerably higher levels of vitamin E as compared with mature cow's milk. The usual reported contents were 1.9–5.3 and 0.28–0.92 mg l^{-1} for colostrum and mature milk, respectively.

α-Tocopherol is an important antioxidant in milk preventing oxidative changes of unsaturated fatty acids. The processes result in spontaneous oxidised off-flavour (Juhlin et al. 2010). Havemose et al. (2004) determined a higher antioxidative capacity (including also carotenoids) in milk of cows fed grass silage as compared with milk from cows fed maize silage. Despite this, lipid oxidation was higher in former milk and higher content of linolenic acid, C18:3, in the milk fat of cows fed grass silage was the cause.

Available data on transfer of α-tocopherol from herbage to bovine tissues have recently been

reviewed (Daley et al. 2010, Kalač 2011). Overall, beef from animals finished on green fodder and silages (except for maize silage) has a lower content of intramuscular fat, but a higher vitamin E content than meat from animals fed on grain-based rations. The α-tocopherol contents ranged between 2.0 and 4.6 mg kg^{-1} in former tissues, while between 0.8 and 2.2 mg kg^{-1} in latter beef.

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