19 Vitamins in Forages

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Vitamins are organic molecules with complex molecular structure and they are essential in minimum quantities for the health, growth and reproduction of ruminants (bovine, ovine and caprine) (Table 19.1). Vitamin deficiency results in a number of metabolic disorders of varying levels of gravity. Forage has the potential to play a significant role in the supply of vitamins to ruminants. However, as will be demonstrated, the vitamin content of forages is highly variable and unpredictable and the production of synthetic vitamins was essential to the advent of intensive livestock production.

Vitamin Requirements of Ruminants

Adult ruminants are different from monogastrics (pigs and poultry) with respect to their dependence on an exogenous supply of vitamins. Synthesis of B-group vitamins (thiamine, riboflavine, niacin …) and vitamin K occurs during the degradation and fermentation of feed ingredients by ruminal microorganisms. Vitamin D is synthesized by the action of ultraviolet radiation on the sterols present in the skin of ruminants, vitamin C is synthesized from C_6 sugars (glucose and galactose) and niacin from tryptophan (if the amino acid is present in excess). It is therefore mainly with respect to vitamins A and E that ruminants have specific dietary dependence.

However, very young ruminants, which do not possess a fully developed rumen and which lack a fully functional rumen microflora, cannot synthesize adequate B-group vitamins. Calves and lambs can therefore suffer from a B-group vitamin deficiency. In this instance, as in monogastrics, they are dependent on an exogenous supply of B-group vitamins.

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Table 19.1. Fat- and water-soluble vitamins with their major functions (adapted from McDowell, 1989).

Names	Functions
Fat-soluble vitamins	
A, retinol	Vision Maintenance of epithelial cells Reproduction Growth
E, α-tocopherol	Biological antioxidant Phospholipid membrane stability Immunomodulation
D, ergocalciferol D_2, cholecalciferol D_3	Phosphocalcium metabolism Growth Reproduction
K	Cofactor in coagulation
Water-soluble vitamins	
B_1, thiamine	Coenzyme in oxidative decarboxylation Role in neurophysiology
B_2, riboflavine	Intermediary in the transfer of electrons in biological oxidation–reduction reactions
B_3, PP factor, niacin	Constituent of coenzymes NAD and NADP in carbohydrate, lipid and protein metabolism
B_5, pantothenic acid	Coenzyme A precursor
B_6, pyridoxine, pyridoxamine	Amino acid metabolism Formation of biogenic amines
B_{12}, cobalamin	Integrity of nervous system Haematopoiesis Growth Gluconeogenesis from propionate
H, $B_{w,}$, biotin	Coenzyme in carbohydrate, lipid and protein metabolism
M, B_c, folacin	Growth Haematopoiesis Maintenance of immune system
Choline*	Synthesis of acetylcholine Component of phospholipids Methyl radical donor Lipotrophic factor
C, ascorbic acid	Collagen biosynthesis Transfer of electrons Oxidation reactions

*Choline is classified as one of the B-complex vitamins.
PP, pellagra-preventing; NAD, nicotinamide adenine dinucleotide; NADP, NAD phosphate.

Thus, in addition to the standard classification of vitamins according to their solubility in either water (C and B group) or in lipid solvent (A, D, E and K), vitamins for adult ruminants can be divided according to:

- self-supply (via either the rumen or endogenous supply) (K, C, D and B group);
- supply from the feed (A and E).

Different rearing methods and changes in ration formulation may alter the endogenous production of vitamins and result in a need to modify this classification. Animals reared indoors or fed rations rich in concentrates cannot produce sufficient vitamin D and thiamine. Furthermore, there is an increasing need for an exogenous supply of niacin, as, following genetic selection, the increase in productivity of ruminants begins to approach the quantitative limits of ruminal vitamin synthesis. The consumer demand for animal products of high quality is also imposing a need for an increased exogenous supply, particularly of niacin and vitamin E. The supply of niacin to high-producing cows at the beginning of lactation has a tendency to increase milk production and also the fat and protein content of milk (NRC, 1989; Hullár and Brand, 1993). In the nutrition of adult ruminants, the five most important vitamins with respect to an exogenous supply are therefore vitamins A, E, D and, in specific conditions, niacin and thiamine.

The supply of vitamins to ruminants other than from the synthetically produced material is totally dependent on a supply from fresh or conserved forage in the ration, since the concentrate portion of the feed is practically devoid of any naturally occurring vitamins A, E, D or their precursors (Brown, 1953; Machlin, 1984; McDowell, 1989).

Vitamin A or retinol exists only in animal products (McDowell, 1989). However, vegetable material can contain provitamins A (carotenoids), of which 80 forms are known, with β-carotene being the most important. β-Carotene is partially absorbed at the intestinal level, where it is transformed into vitamin A. It has been established that the conversion rate in the bovine is of the order of 400 international units (IU) of vitamin A mg^{-1} β-carotene (McDowell, 1989) (where 1 IU is defined as the biological activity of 0.300 μg of retinol). However, the efficiency of this conversion varies according to the isomer. The all-*trans* form of β-carotene has the highest activity, the neo-B and neo-U forms have only 53 and 38% of all-*trans*-form activity, respectively (Aitken and Hankin, 1970).

Vitamin E is found in eight known forms, four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) (McDowell, 1989). The α-tocopherol is the most active and is considered true vitamin E.

Vitamin D forms are derived from the ultraviolet irradiation of sterols in animals (D_3 = cholecalciferol) and in vegetables (D_2 = ergocalciferol), the two forms having the same biological potency in ruminants (McDowell, 1989). One international unit of vitamin D activity is defined as the activity of 0.025 μg of vitamin D_3.

The term niacin is used to cover nicotinic acid and nicotinamide, the two compounds possessing the same vitamin activity in ruminants (McDowell, 1989). Thiamine consists of a molecule of pyrimidine and a molecule of thiazole linked by a methylene bridge (McDowell, 1989).

The physiological requirements for these vitamins are difficult to define and can vary appreciably according to the measures used as reference, particularly

whether the criterion is the prevention of deficiency symptoms or the requirement for maximum animal performance. Standard tables of requirements tend to ignore the additional needs that have been identified as a result of different repeated stressors occurring during livestock production (unfavourable rearing conditions, infection, etc.). Present recommendations for ruminants concern vitamins A, E, D and niacin and are greater than the strict physiological needs, since they incorporate a margin of safety in order to ensure that the performance of the animals is not compromised (Table 19.2). The minimal recommendations are given for mean rearing conditions but are increased in accordance with a number of factors, such as the composition of the ration and the performance of the animal (growth, reproduction and milk production). Supply of highly fermentable diets increases the requirements for both thiamine and vitamin A (NRC, 1989). The continued increase in genetic potential of animals as a result of breeding results in a general increase in vitamin requirements.

Forage as a Source of Vitamins

The diet of ruminants is composed essentially of a combination of concentrates (cereals and protein supplements) and forages. Fresh or conserved forages are potential sources of vitamins A, E, D, niacin and thiamine (Aitken and Hankin, 1970; McDowell, 1989). However, although it is relatively easy to list vitamins present in forage, it is more difficult to give precise estimates of the mean values. Levels of vitamins found in forages are highly variable (Table 19.3). The factors that are responsible for this variability, and which include plant origin, climatic conditions, stage of maturity, conservation methods (drying, ensiling, dehydration …) and storage conditions, are described in a later section. It would appear that the minimal attention given to the vitamin content of forages, other than for the vitamins A, E and, to a lesser extent, D, is the result of the lack of literature data concerning the content of niacin and thiamine, since vitamin analysis is not routinely performed on forages.

At first sight, the majority of forages can make a significant contribution to the dietary supply of β-carotene and α-tocopherol to cover the daily recommendations of ruminants (RPAN, 1998; Fig. 19.1). Indeed, only approximately 15% of the forages sampled supplied less than 50 mg of β-carotene or α-tocopherol kg^{-1} dry matter

Table 19.2. Vitamin recommendations for ruminants (RPAN, 1998).

Ruminants	Vit. A (IU $head^{-1}$ day^{-1})	Vit. E (mg $head^{-1}$ day^{-1})	Vit. D (IU $head^{-1}$ day^{-1})	Niacin (g $head^{-1}$ day^{-1})
Dairy cow, lactating	80,000–120,000	100–1,000	15,000–50,000	1–2
Dairy cow, dry	75,000–125,000	500–900	10,000–20,000	0–1
Finishing cattle	40,000–70,000	200–1,500*	4,000–7,000	1–2

* Improvement of meat quality after slaughter.

Table 19.3. Vitamin content of fresh and conserved forages (adapted from Blaylock *et al.*, 1950; Brown, 1953; Keener, 1954; Wallis *et al.*, 1958; Albonico and Fabris, 1958; Hjarde *et al.*, 1963; Hoffmann and Nehring, 1967; Bunnel *et al.*, 1968; Aitken and Hankin, 1970; NRC, 1989).

	β-Carotene (mg kg^{-1} DM)			α-Tocopherol (mg kg^{-1} DM)			D (IU kg^{-1} DM)			Thiamine (mg kg^{-1} DM)			Niacin (mg kg^{-1} DM)		
Forages	*n*	M	SD	*n*	M	SD	*n*	M	SD	*n*	M	SD	*n*	M	SD
Green forages (1)	349	196 (15–606)*	108	86	161 (9–400)	91	25	365 (31–1800)	470	18	4.6 (1.9–8.3)	2.4	5	37 (13–56)	17
Dehydrated forages (2)	16	159 (66–271)	73	12	125 (28–238)	57	4	– (176–617)	–	7	4 (3.8–4.5)	0.5	7	53 (39–64)	9
Silages (3)	50	81 (2–276)	68	4	– (0–310)	–	10	440 (80–866)	311	1	0.1 –	–	2	– (1.1–34)	–
Hays (4)	68	36 (1–162)	34	10	61 (10–211)	62	40	1156 (90–5560)	1161	19	2.7 (0.2–4.5)	1.3	14	28 (6–52)	17

(1) Grasses (cocksfoot, timothy, ryegrass, fescue …) and legumes (lucerne, clover …); (2) lucerne; (3) legume silages (lucerne, clover), grass silages (cocksfoot, ryegrass …) and maize silage; (4) legume hays (lucerne, clover) and grass hays (timothy, ryegrass, fescue, cocksfoot …).
n, Number of samples; M, mean; SD, standard deviation.
* Range.

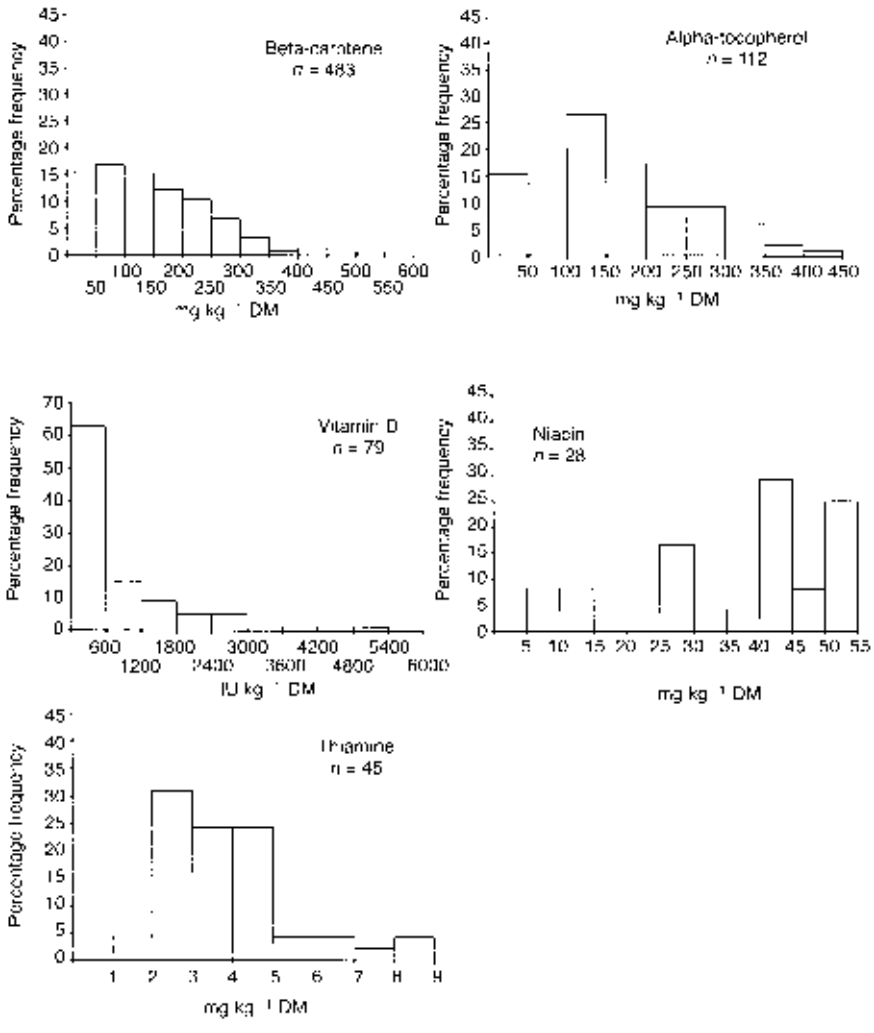

Fig. 19.1. Histograms representing frequency distribution of vitamin content of forages (adapted from Table 19.3). *n* = Number of samples.

(DM), the remaining 85% supplying in excess of 50 mg kg^{-1}. It would appear that forages, in general, are very good sources of the vitamins A and E. However, these data must be viewed with great prudence, due to lack of precision of most analytical methods for vitamins in forages and also to lack of estimates of the bioavailability of β-carotene and α-tocopherol in forages.

Methods of analysis used for feed materials have a tendency to overestimate concentrations of β-carotene and α-tocopherol in a wide range of raw materials (Aitken and Hankin, 1970; Ullrey, 1972; McDowell, 1989). The presence of geometric isomers of all-*trans*-β-carotene that have a lower biological activity results in

an overestimation of the biological activity of β-carotene (Table 19.4). This overestimation is dependent on the forage under consideration (+6–11% for fresh lucerne and *Cynadon dactylon*, +13–17% for the same material dried and +19–33% for dehydrated lucerne).

In most cases, even if the concentration of β-carotene and α-tocopherol in the forage is high, the requirements for vitamins A and E of ruminants cannot be adequately covered by their daily forage consumption (Nadai, 1968; Ferrando and Mainguy, 1970; McDowell, 1989). Between the ingestion of β-carotene and α-tocopherol present in forage and the metabolic use of vitamins A and E, two important steps are involved: the passage of the material through the rumen and intestinal absorption, with, in addition, for carotene its conversion into vitamin A (see later). A number of factors can therefore modify the nutritional supply of vitamins A and E from forage. The level of conversion of β-carotene varies (1 mg of β-carotene = 400 IU of vitamin A in a lactating dairy cow) according to the health status and the quantity consumed. The rate of conversion is reduced as the level consumed increases, and it is generally accepted that this conversion factor for ruminants is only valid at levels of intake corresponding to maintenance (Nadai, 1968; Bondi and Sklan, 1984).

Over 60% of the forages analysed (Table 19.3) had a vitamin D content of less than 600 IU kg^{-1} dry matter (Fig. 19.1), resulting in a severe risk of deficiency in housed animals. Niacin and thiamine, although found in forages, are present in quantities insufficient to cover recommendations (RPAN, 1998). The same caution as was given for vitamins A and E must be exercised in estimating the nutritional supply of vitamins D, niacin and thiamine from forages, in particular the

Table 19.4. Stereoisomers of β-carotene and biological efficiency of lucerne (fresh, dehydrated and sun-cured) and *Cynadon dactylon* (fresh and dried at 65°C).

Forages	Stereoisomers of β-carotene (%)			Biological efficiency of total carotene (% of all-*trans* β-carotene)	References
	Neo-B	All-*trans*	Neo-U		
Fresh lucerne	7	84	9	90	Livingston *et al.* (1966)
	4.6	90	5.4	94	Thompson *et al.* (1951)
	6	84	10	91	Kemmerer *et al.* (1944)
Dehydrated lucerne					
T = 104°C*	27	63	10	81	Livingston *et al.* (1966)
T = 160°C	46	36	18	67	
Sun-cured lucerne hay	12	77	11	87	Thompson *et al.* (1951)
Fresh *Cynadon dactylon*	8	81	11	89	Kemmerer *et al.* (1944)
	5	86	9	92	
Cynadon dactylon dried at 65°C	11	70	19	83	Kemmerer *et al.* (1944)

* Outlet temperature of drier.

sensitivity of different analytical techniques and effects on biological availability (McDowell, 1989).

In conclusion, therefore, although forages are potentially good sources of vitamins, the levels which they may contribute in the diet of ruminants are influenced by a number of factors inherent to both the plant and the animal. This range of influencing factors results in a major lack of precision in estimating the quantity of vitamins available to ruminants from forage.

Factors Affecting the Vitamin Content of Forages

The considerable variability found in the vitamin content of forages tends to suggest that a number of factors contribute to the variability encountered. These include:

- the origin of the plant (family, species and variety);
- climatic conditions;
- stage of maturity of the plant;
- conservation methods (drying, ensiling, dehydration …);
- storage conditions.

The origin of the plant

During the early stages of growth, the grasses and legumes have similar mean levels of β-carotene (approximately 300 mg kg^{-1} DM) (Table 19.5). However, at the stage of flowering and at maturity, legumes are richer in β-carotene than the grasses (Moon, 1939). Thus, the stage of growth does not affect the level of β-carotene of legumes to the same extent as in the grasses (Moon, 1939; Seshan and Sen, 1942a). In a comparison of grasses and legumes at the start of the flowering stage (Table 19.6), Livingston *et al.* (1968c) reported that the leaves had similar levels of carotene, but these levels were four to 11 times higher than the levels found in the stems (Seshan and Sen, 1942a; Olsson *et al.*, 1955; Ramanujan and Anantakrishnan, 1958; Park *et al.*, 1983). However, account must be taken of the fact that, at the start of flowering, the proportion of leaves in legumes (mean 40%) is twice that found in the grasses (mean 19%). Thus, at advanced stages of growth, the legumes are the better source of β-carotene, since the proportion of stem is less than in the grasses (Moon, 1939; Olsson *et al.*, 1955).

Results presented by Olsson *et al.* (1955) also indicate the existence of differences between plant species in terms of β-carotene (Moon, 1939; Seshan and Sen, 1942a; Park *et al.*, 1983). At an early growth stage (1st cycle) (Table 19.7) perennial ryegrass has the lowest level of β-carotene of the grasses (146 mg kg^{-1} DM)(Smith and Wang, 1941) and, of the legumes, white clover has the highest level (438 mg kg^{-1} DM). Thus, certain plant species possess an important capacity to synthesize carotene (Park *et al.*, 1983). During the course of a study, Olsson *et al.* (1955) noted that, at a stage of full bud, red clover had a concentration of β-carotene (206 mg kg^{-1} DM) close to that of lucerne (233 mg kg^{-1} DM) and that,

Table 19.5. Influence of plant origin and stage of maturity in β-carotene and α-tocopherol contents of fresh forages (mg kg^{-1} DM) during the first growth cycle (adapted from Brown, 1953; Hjarde *et al.*, 1963; Hoffmann and Nehring, 1967; Aitken and Hankin, 1970; NRC, 1989).

	β-carotene				α-tocopherol			
Forages and stages	*n*	M	Max.	Min.	*n*	M	Max.	Min.
Grasses								
Vegetative to ear	51	278	606	84	29	253	400	121
Early to end flowering	44	133	258	53	17	98	154	40
Mature	30	59	156	4	7	22	30	9
Legumes								
Vegetative to bud	62	309	552	140	19	129	202	79
Early to end flowering	34	192	488	97	6	116	127	109
Mature	7	130	252	80	–	–		

n, Number of samples; M, mean.

Table 19.6. Variation in carotene content of four grasses and three legumes harvested at early flowering during the first growth cycle (from Livingston *et al.*, 1968c).

Forages	Date harvested	Fraction	% (dry wt)	Carotene (mg kg^{-1} DM)
Grasses				
Fescue	18/6	Leaf	12	460
		Stem	88	101
Timothy cv. Verdant	2/7	Leaf	17	387
		Stem	83	95
Cocksfoot cv. Potomac	20/6	Leaf	21	521
		Stem	79	62
Reed canary grass	18/6	Leaf	26	640
		Stem	74	90
Legumes				
Red clover cv. Lakeland	14/6	Leaf	34	724
		Stem	66	66
Lucerne cv. Saranac	9/6	Leaf	39	629
		Stem	61	75
Birdfoot cv. Empire	1/6	Leaf	42	449
		Stem	58	62

at the stage of end ear, timothy (96 mg kg^{-1} DM) had a lower level than that of cocksfoot (175 mg kg^{-1} DM). Table 19.7 also shows that the growth cycle has little effect on the level of β-carotene in lucerne (Bruhn and Oliver, 1978) and in red clover, but strongly influences that in white clover and the grasses. According to these authors, the differences in level of β-carotene are mainly due to the ratio of leaf to stem in the plant and the regrowth.

Table 19.7. β-Carotene content (mg kg^{-1} DM) of legumes and grasses harvested at an early stage of development several times in the vegetation period, in years 1942–1946 (number of samples = 5) (from Olsson *et al.*,1955).

	First cut			Second cut			Third cut			Fourth cut		
Forages	M	Max.	Min.	M	Max.	Min.	M	Max.	Min.	M	Max.	Min.
Legumes												
Lucerne	263	315	235	239	365	172	272	380	225	293	424	244
Red clover	356	462	270	313	362	256	330	391	253	335	411	245
White clover	438	538	387	269	395	184	268	463	177	422	595	342
Grasses												
Timothy	238	369	133	128	222	63	192	233	119	248	356	142
Cocksfoot*	242	267	217	148	191	104	104	–	–	254	331	177
Perennial ryegrass	146	214	95	161	407	68	250	425	123	207	259	160

* Number of samples = 2 (1943 and 1944).
M, mean.

At early stages of growth, grasses contain nearly twice as much α-tocopherol as legumes (Table 19.5). However, at flowering, the legumes and grasses have approximately the same mean levels of α-tocopherol. Similar to the case with carotene, the ratio of leaf to stem is an important factor in the evolution of the level of vitamin E in forages (Brown, 1953), with the leaves being richer in α-tocopherol than the stems (Brown, 1953; Ramanujan and Anantakrishnan, 1958; Booth, 1964). Variability between species is also evident for α-tocopherol. Cocksfoot (313–362 mg kg^{-1} DM) is a better source of α-tocopherol than fescue and timothy (184–249 mg kg^{-1} DM) at the 20–25 cm stage (Brown, 1953). Furthermore, for thiamine, fescue is superior to brome grass and timothy (3.2 mg and 2.6 mg kg^{-1} DM, respectively) and lucerne is superior to clover (7.5 mg and 6.5 mg kg^{-1} DM, respectively) (Robinson *et al.*, 1948).

Brown (1953) reported approximately 22% variation in the level of α-tocopherol when comparing four varieties of lucerne. Thompson (1949) observed a variation of the order of 30% in the level of β-carotene in nine varieties of lucerne. However, comparing three varieties of red clover, Hjarde *et al.* (1963) found no significant difference in the levels of β-carotene and α-ζ-tocopherol. Vail *et al.* (1936), cited by Robinson *et al.* (1948), found no significant difference in the level of thiamine in four varieties of lucerne. Albonico and Fabris (1958) concluded that genetic factors should be considered as having only a weak influence on the vitamin content of forages.

In conclusion, plants that produce little leaf tend to contain low levels of β-carotene and α-tocopherol (Park *et al.*, 1983; McDowell, 1989). Other than the origin of the plant, the ratio of leaf to stem, which is the main factor influencing the levels of β-carotene and α-tocopherol in forage, is also influenced by both climatic conditions and the stage of maturity (Olsson *et al.*, 1955).

Climatic conditions

Bondi and Meyer (1946) measured the level of carotene in lucerne harvested at the budding and flowering stages at different times of the year in Palestine. The level of β-carotene was higher in April and May for the budding stage (266–273 mg kg^{-1} DM) and in November for the flowering stage (170 mg kg^{-1} DM) than in August (110 mg kg^{-1} DM for the budding stage and 72 mg kg^{-1} DM for the flowering stage). Albonico and Fabris (1958) demonstrated that the levels of β-carotene and α-tocopherol of 16 varieties of lucerne sampled in the south of Italy at the beginning of flowering were higher in March/April (349 and 282 mg kg^{-1} DM, respectively) than in July (251 and 110 mg kg^{-1} DM, respectively). Independently of the cycle of growth, the level of β-carotene of legumes and grasses was higher in the summer of 1942 – a fresh and humid summer – than in 1943 and 1944 – two summers that were hot and dry (Table 19.8; Olsson *et al.*, 1955). These results indicate that, for a given stage of growth and independently of the cycle of growth, a forage is richer in β-carotene and α-tocopherol when grown under mild, wet conditions. The beneficial effect of rainy conditions rests in the reduction in the quantity of sunlight received by the plant (Olsson *et al.*, 1955; Hjarde *et al.*, 1963; Park *et al.*, 1983). The ambient temperature and the light contribute more to the variability in the level of β-carotene than the supply of water (Olsson *et al.*, 1955; Repp and Walkins, 1958).

Beck and Redman (1940) showed that heat and intense light were detrimental to the production of carotene in the leaves of clover. However, the positive influence of the reduction in temperature and day length was also related to the increase in the leaf-to-stem ratio (Wilson, 1981). Thus the climatic conditions influence the levels of β-carotene and α-tocopherol of forages because they also influence the leaf-to-stem ratio.

The antirachitic value of forages is also dependent on levels of sunlight (Meissonier, 1981; McDowell, 1989). Green forages have low levels of vitamin D but, when the plant begins to die and the fading leaves are exposed to the ultraviolet light of the sun, high levels of vitamin D are synthesized (Thomas and Moore, 1948; McDowell, 1989).

Table 19.8. β-Carotene content (mg kg^{-1} DM) of grasses and legumes harvested at an early stage of growth during the summer, 1942–1944, in Sweden (from Olsson *et al.*, 1955).

	White clover		Red clover		Timothy		Perennial ryegrass	
Years	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
1942	538	395	462	362	369	222	214	147
1943	408	210	355	256	209	87	97	100
1944	387	225	270	298	133	63	95	68

The stage of maturity of the plant

The levels of β-carotene and α-tocopherol in the grasses and legumes are very high in the young stages and reduce as the plant matures (Smith and Wang, 1941; Brown, 1953; Clarke, 1953; Bondi and Sklan, 1984; Table 19.5). The levels of β-carotene and tocopherols of lucerne achieve maximum values between the end of the vegetative stage (Park *et al.*, 1983) and the budding stage (Burrows and King, 1968) and then decrease with maturity. The level of carotene drops at the end of the vegetative stage in clover (Olsson *et al.*, 1955) and cocksfoot (Clarke, 1953) and after the start of earing in timothy (Olsson *et al.*, 1955). According to Moon (1939) and Livingston *et al.* (1968c), the level of carotene in the leaves of grasses and legumes is maximal at the start of flowering and then diminishes with varying degrees of intensity (Thompson, 1949). Indeed, the green colour of their leaves is generally a good index of their carotene content (Maynard *et al.*, 1979). At maturity, plants may have 10% (in the case of grasses) to 40% (for legumes) of the value of carotene of immature plants.

As has already been indicated, the principal factor responsible for the variation in levels of β-carotene and α-tocopherol of forages in the course of their maturation is the change in the ratio of leaf to stem, because the leaves are considerably richer in these vitamins than the stems. The formation of stems is accompanied by an increase in the concentration of DM; there is thus a negative correlation between the DM content and the level of β-carotene (Olsson *et al.*, 1955).

Contrary to the situation when vitamin D is produced in fading leaves, the level of thiamine is positively correlated with the verdant nature of the forage leaf (Hunt *et al.*, 1935; Galgan *et al.*, 1950; McDowell, 1989). The vitamin D content of forages is also dependent on the stage of harvest. Indeed, vitamin D level increases even when the plant has reached maturity (Table 19.9; Keener, 1954), because of the increase in dead leaves (Thomas and Moore, 1951; McDowell, 1989). Thus, the levels of β-carotene and vitamin D are negatively correlated. Thomas and Moore (1948) demonstrated that the proportion of dead leaves in lucerne increases from 2.4% at budding to 6.5% at maturity. The dead leaves contain approximately 7064 IU vitamin D kg^{-1} DM, while the green leaves had levels close to zero. Thus, the quantity of dead leaves adhering to the plant during harvest can considerably modify the level of vitamin D, independently of the duration of exposure to sunlight during drying. During the same year, the level of vitamin D of forages at the same stage of development increases with the number of cuts (Keener, 1954; Table 19.9). It was suggested that the difference between a first and second cut was due to differences in the level of ergosterol and the relative importance of the sun's ultraviolet rays.

Methods of harvesting and conservation

The manner in which forage is treated between its harvest and being offered as feed to animals can influence its vitamin content.

Both β-carotene and α-tocopherol are destroyed by oxidation. This reaction is accelerated by ultraviolet light and heat (Seshan and Sen, 1942b; Bauernfeind,

Table 19.9. Influence of stage of maturity and number of cuts in vitamin D content of several forages (from Keener, 1954).

Cutting and date	Red clover (IU kg^{-1} DM)	Timothy (IU kg^{-1} DM)
First cutting		
25 June	31	40
2 August	750	571
15 September	1400	1100
Second cutting		
2 August	130	430
15 September	981	820
Third cutting		
15 September	500	540

1980). However, prolonged heating without oxygen has a minor effect (Seshan and Sen, 1942b; McDowell, 1989).

Before it becomes a photochemical process, the destruction of β-carotene in forages is first an enzymatic process and is due to a lipoxygenase system (Ferrando and Mainguy, 1970; Kalac and McDonald, 1981; Bondi and Sklan, 1984). The lipoxygenases, a group of isoenzymes, are present at varying levels in a wide number of species of plants (Waugh *et al.*, 1944; Larsen *et al.*, 1993). Lucerne is know to be an excellent source of lipoxygenases (Waugh *et al.*, 1944; McDowell, 1989). These enzymes are destroyed at around 80–100°C (Olsson *et al.*, 1955; Ferrando and Mainguy, 1970), wherein lies the interest of dehydration (Blaylock *et al.*, 1950; Bondi and Sklan, 1984).

According to Bauernfeind (1972), the enzymatic destruction of total pigments present in the leaves of lucerne is three to four times greater than photochemical destruction (Mitchell and Hauge, 1946). Enzymatic destruction of carotene begins when the forage is chopped and is more intense in the early stages of growth. Increasing the degree of chopping and grinding also accelerates enzymatic destruction, as does raising ambient temperature and humidity (Mitchell and Hauge, 1946; Ferrando and Mainguy, 1970; McDowell, 1989). During drying, the losses of β-carotene and α-tocopherol can equally be due to isomerization (Livingston *et al.*, 1966, 1970), but this isomerization represents only a small part of the total loss (Kalac and Kyzlink, 1979).

Vitamin D can be destroyed by excessive exposure to ultraviolet light (Ferrando and Mainguy, 1970; McDowell, 1989). When the crop is dry, thiamine is stable at 100°C for several hours, but the presence of humidity accelerates destruction (McDowell, 1989). Thus, thiamine is much less stable to heat in fresh material than in dried material. On the contrary, niacin is highly stable in air, to heat, to light and to the action of alkalis – hence its stability in feedstuffs (McDowell, 1989).

Haymaking

Drying crops either on the ground or in barns reduces the levels of β-carotene and α-tocopherol in forages (King *et al.*, 1967; Burrows and King, 1968; McDowell, 1989). Russell (1929) found that in excess of 80% of carotene from fresh lucerne was lost during the first 24 h of sun-drying. According to Akopyan (1958), cited by Kivimaë and Carpena (1973), the level of tocopherol in leaves of clover and maize was practically zero when the crop was dried for 4–5 days in the sun. With grasses, Ramanujan and Anantakrishnan (1958) obtained carotene and tocopherol losses in the region of 80% after 4 days of sun-drying. Park *et al.* (1983) showed that, as the exposure to the sun was increased, the level of destruction of β-carotene was increased (Table 19.10) and that this occurred irrespective of the stage of harvest of the lucerne. Forages exposed to rain and then dried in the sun have less β-carotene than sun-dried forage. Thus, if the forage rests exposed to the sun for an extended period of time and at the same time is exposed to several showers, the destruction of β-carotene is nearly complete.

According to Akopyan (1958) and Maynard *et al.* (1979), barn-drying has less of a destructive effect on carotene and tocopherol. Galgan *et al.* (1950) reported that barn-dried lucerne hay could contain three times more carotene than sun-dried lucerne hay. However, Ramanujan and Anantakrishnan (1958) reported that, when the drying time of grasses was considerably extended, irrespective of the method, the losses of carotene and tocopherol were practically the same.

During crop-drying on the ground, radiation from the sun can result in synthesis of vitamin D. However, again, it must be emphasized that the degree of radiation must not be excessive (Ferrando and Mainguy, 1970). Thomas and Moore (1948) showed that a sun-dried lucerne contained twice as much vitamin D (971 IU kg^{-1} DM) as a barn-dried lucerne (470 IU kg^{-1} DM).

The level of thiamine is much lower in sun-dried materials than in materials dried in an oven at 62°C (Galgan *et al.*, 1950). The lucerne dried in the sun had high losses of niacin when it was exposed to bad weather (Blaylock *et al.*, 1950; Scott, 1973).

Table 19.10. Effect of time of sun-drying (with or without rain) in β-carotene content of lucerne harvested at three stages of maturity during the second growth cycle (from Park *et al.,* 1983).

Duration of drying (h)	Vegetative (mg kg^{-1} DM)	Bud (mg kg^{-1} DM)	Flowering (mg kg^{-1} DM)
Sun-dry			
48 h	79.0	56.8	53.0
72 h	34.4	24.4	24.2
96 h	29.6	8.1	21.8
Rain damage, sun-dry			
48 h	22.2	22.0	22.6
72 h	13.9	12.0	23.6
96 h	12.7	5.3	10.8

Ensiling

In general, ensiling of grasses and legumes guarantees a better conservation of carotene and tocopherol than haymaking (Bauernfeind, 1980; Wolter, 1988; Hidiroglou *et al.*, 1994). As a result of a series of studies, Watson and Nash (1960) concluded that mean losses of carotene during ensilage were of the order of 30% (cited by Kalac and McDonald, 1981). It should be noted that the losses of β-carotene depend partly on the type of material ensiled, the quality of the ensiling process and the method of silage preparation. Kalac (1983) demonstrated that the losses of β-carotene during the aerobic phase (24 h) were of the order of 17 (cocksfoot) to 30% (lucerne and Italian ryegrass) (Table 19.11) and for maize approximately 19% after 48 h (Kalac and Kyzlink, 1980).

Either wilting prior to ensiling, which often precedes the ensiling of a forage, or the use of additives, such as organic acids (acetic, formic and propionic acids), tends to increase the loss of β-carotene during the preliminary aerobic phase (Kalac and Kyzlink, 1979, 1980; Kalac and McDonald, 1981). Acidification considerably increased the losses of β-carotene in lucerne, clover and maize, but did not have an effect on either Italian ryegrass or cocksfoot (Kalac and Kyzlink, 1980; Kalac, 1983). The destruction of β-carotene under acidic conditions is of enzymatic origin and results from an oxyreductase other than lipoxyreductases (Kalac and Kyzlink, 1980). The losses of β-carotene during fermentation differ according to the forage (Kalac, 1983), and are high for clover and lucerne (Patel *et al.*, 1966) and low for cocksfoot and Italian ryegrass (Table 19.11).

Ferrando and Mainguy (1970) and Kalac (1983) reported that there was no clear relationship between the quality of a silage and its carotene content.

Table 19.11. Changes in β-carotene content and pH of several types of silages (vegetative stage) (from Kalac, 1983).

Forages	Silage treatment	β-Carotene in fresh leaves (mg kg^{-1} fresh)	β-Carotene (% initial value), pH and quality of silage					
			Delayed sealing (h)			After fermentation and 180 days' storage		
			6 (%)	24 (%)	24 (pH)	(%)	(pH)	Quality
Cocksfoot	C	127	97.4	83.1	5.15	81.8	4.70	II
	F	–	92.7	80.8	4.30	76.3	4.40	II
Italian ryegrass	C	196	–	72.2	5.75	70.0	5.15	IV
	F	–	–	77.6	4.10	73.6	4.90	III
Lucerne	C	186	83.6	70.3	5.95	40.2	5.15	V
	F	–	47.0	11.3	4.35	8.4	5.25	IV
White clover	C	237	92.1	75.7	5.95	53.7	5.80	II
	F	–	69.7	48.6	5.10	28.6	5.30	III

C, control; F, formic acid.
Organoleptic assessment of quality: II, good; III, mean; IV, poor; V, very poor.

Bieber-Wlaschny (1988) indicated that grass silage could provide considerable levels of β-carotene (122 mg kg^{-1} DM), but that maize silage is a very poor source (11 mg kg^{-1} DM); lucerne silage (32 mg kg^{-1} DM) and clover silage (19 mg kg^{-1} DM) were intermediate.

Maize silage is practically devoid of vitamin E (Hidiroglou *et al.*, 1977; Bieber-Wlaschny, 1988). On the other hand, the ensiling of lucerne and brome preserves a major proportion of the vitamin E (King *et al.*, 1967).

The process of ensiling is therefore characterized by introducing great variability in the preservation of the β-carotene and α-tocopherol content of forages.

Dehydration

Rapid dehydration allows a major proportion of the β-carotene and α-tocopherol of forage plants to be conserved (Table 19.12). Dehydrated forages contain, on average, seven times the amount of carotene compared with when they are dried on the ground (Meissonier, 1974). The level of residual moisture of the treated material has a direct effect on the destruction of β-carotene and α-tocopherol (Ferrando and Mainguy, 1970; Burdick and Fletcher, 1985). Livingston *et al.* (1968a, b, 1970) demonstrated that the losses of β-carotene and α-tocopherol vary from 0 to 30% during the course of dehydration of lucerne. They observed that the highest losses occurred when the level of residual moisture was equal to or lower than 30 g kg^{-1}. The optimum moisture content appeared to be between 70 and 100 g kg^{-1} (Livingston *et al.*, 1970). Dehydration had no effect on losses of niacin (Blaylock *et al.*, 1950).

Storage conditions

The highest vitamin loss in absolute terms is observed in forages that are well preserved during their preparation, as in dehydrated materials (Park *et al.*, 1983). Bruhn and Oliver (1978) demonstrated that the losses of β-carotene and α-tocopherol in lucerne hay are significantly correlated with the duration of storage.

Table 19.12. β-Carotene and α-tocopherol losses of lucerne during dehydration.

Outlet temperature (°C)	Residual moisture of product (g kg^{-1})	Losses (%)		References
		Carotene	Tocopherol	
153	92	9	5	Knowles *et al.* (1968)
149	92	9	5	Livingston *et al.*
166	23	18	21	(1968a, b)
116–166	15–122	0–33	–	Livingston *et al.* (1970)
–	80	15	–	Ferrer (1972)

Kohler *et al.* (1955) demonstrated that losses in tocopherol during storage also depended on temperature; a ryegrass meal stored for 6 weeks at 3°C lost 8% of its tocopherol but the loss was of the order of 49% at 60°C (Maynard *et al.*, 1979). The conservation of dehydrated lucerne at ambient temperature (32°C) during 3 months results in considerable loss of carotene (42–72%) and tocopherol (35–73%) (Livingston *et al.*, 1968b, 1970). Thus, the storage at low temperature allows the maintenance of reasonable levels of β-carotene and α-tocopherol (Kivimäe and Carpena, 1973; Park *et al.*, 1983). The storage of dehydrated lucerne in a nitrogen atmosphere allows the duration of storage to be extended without any adverse effects on the quality of β-carotene (Melcion and Delort-Laval, 1973; Park *et al.*, 1983). Irrespective of the procedure of conservation, the carotene degrades slowly during storage; the loss is of the order of 50% in the first 6 months, but it can be greater (Fig. 19.2).

A second degradation of the carotene and tocopherol present after the period of conservation is known to occur during the time that forage is held in the feed bunker, prior to its distribution (McDowell, 1989).

Factors Affecting Vitamin Availability in Ruminants

The metabolic utilization of vitamins supplied by forages is not possible without transit through the digestive tract of the animal. The two most important factors limiting vitamin availability in ruminants are passage through the rumen and intestinal absorption. The act of digestion, principally at the level of the rumen,

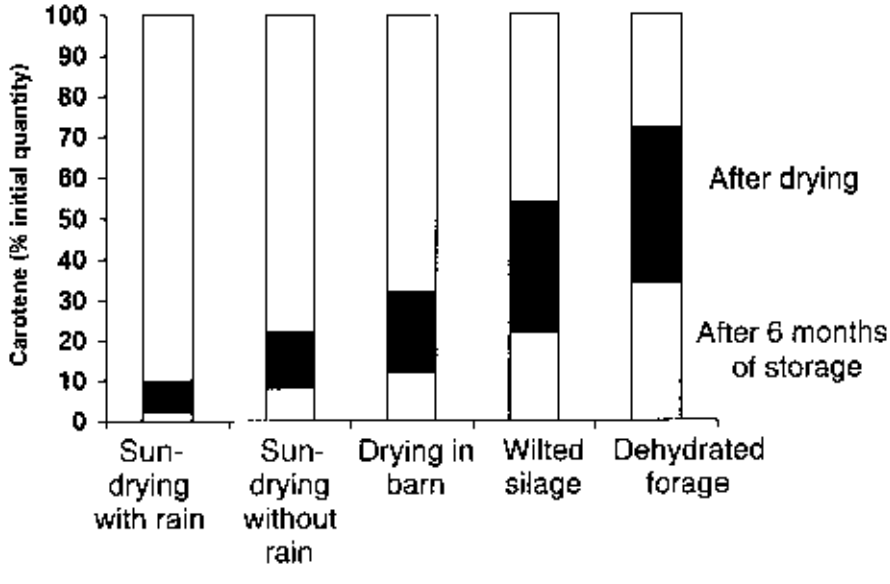

Fig. 19.2. Relative availability of carotene in comparison with its initial content in forages (from Wolter, 1988).

serves to release the vitamins from the combination in which they are found in plant tissue. Thus, the extent of degradation and liberation of vitamins from the forage in the rumen is an essential feature of the bioavailability of vitamins found in forage. According to Nadai (1968), the degree of liberation of carotene in the digestive tract varies according to the type of plant. This may explain why the carotene in green or dehydrated lucerne is more available than that from maize and, in particular, maize silage.

The variability of vitamin supply in forages identified in the foregoing section is one of the reasons why the ruminant-livestock industry has chosen the practice of meeting requirements from synthetic sources. In ration formulation, the supply of vitamins from raw materials is often ignored and the requirements for supplementation with synthetic vitamins are based on meeting 100% of the requirements. Most of the studies concerning the stability in the rumen and intestinal absorption of β-carotene and α-tocopherol have been carried out using synthetic sources. Certain comments on ruminal stability and intestinal absorption of vitamins A, E and D are therefore relevant to this discussion. Factors that need to be considered are the influence of rumen conditions on the availability of vitamins and also intestinal absorption. However, it is beyond the scope of this review to cover the ruminal stability and intestinal absorption of all vitamins and hence comments will be confined to vitamins A, E and D.

Vitamin A

Synthetic vitamin A is produced in the form of the ester, retinyl acetate, in the all-*trans*-isomer form, which has a biological activity of 100% (McDowell, 1989). Vitamin A is sensitive to the action of light, heat and oxidizing agents, as a result of the presence of the unsaturated side-chain. For this reason, synthetic vitamin A is found in encapsulated forms, in order to provide protection against the adverse effects of light and oxidation.

Degradation of vitamin A in the rumen and intestinal absorption

Many biochemical reactions that occur in the rumen are capable of destroying vitamin A, and a large number of studies have provided evidence of major losses of vitamin A in the rumen. These losses may be explained by different phenomena involving the rumen bacteria, including engulfment, oxidation and degradation. To date, no author has demonstrated absorption of vitamin A through the rumen wall, which would appear logical, given the vitamin's large molecular size.

Ruminal losses of β-carotene are, on average, 20%, with a range from 3 to 32% (Table 19.13). Potkanski *et al.* (1974) found that these losses were not affected by diet. Although Keating *et al.* (1964) showed that the addition of an antioxidant (ethoxyquin) or oxidizing agent (potassium nitrate) had no effect on the β-carotene disappearance, Cohen-Fernandez *et al.* (1976) suggested that the destruction of β-carotene could be due to partial oxidation. This contradicts the conclusions of Mitchell *et al.* (1967) and Lichtenwalner *et al.* (1973), who reported that degradation of vitamin A in the rumen is not significantly modified when oxidizing agents, including nitrates, were added to the rumen (Table 19.14). Given that the rumen is

Table 19.13. Ruminal disappearance of β-carotene.

Animal	Diet	Disappearance (%)	Method	References
–	–	31.9	*In vitro* 9 h	King *et al.* (1962)
Dairy heifer	High-hay	25.2 ± 5.6	*In vitro* 7 h	Davison and Seo (1963)
Steer	High-roughage	24.4	*In vitro* 16 h	Keating *et al.* (1964)
Mature wether	High-cellulose	23.1 ± 5.9	*In vivo*	Potkanski *et al.* (1974)
	High-starch	23.3 ± 6.9		
Cow	High-concentrate	4.7 ± 1.7	*In vitro* 24 h	Cohen-Fernandez w*et al.* (1976)*

* β-Carotene from different sources, pure β-carotene or commercial lucerne meal.

Table 19.14. Vitamin A recovery from abomasal fluid of beef cattle (%)* (from Mitchell *et al.,* 1967).

Steers no.	Observation no.	Supplement		Mean
		Control	Nitrate†	
1	1	43.1‡	62.2	
	2	32.4	46.2	
	3	44.6	55.8	
	4	69.1	32.2	48.2
3	1	41.8	45.1	
	2	35.2	39.5	
	3	42.1	66.4	
	4	35.5	55.7	45.7
Mean		43.0	50.4	

* Calculated from the ratio of vitamin A to chromic oxide in abomasal fluid compared with the ratio administered 24 h later.
† 90 mg of potassium nitrate steer^{-1} day^{-1}.
‡ Each observation is the average result of triplicate determinations.

a highly reducing environment, it is unlikely that oxidation plays a major role. It was therefore concluded that β-carotene disappearance in the rumen was due mainly to the action of bacterial hydrogenases (Ferrando, 1980) and it is probable that vitamin A undergoes reductive degradation. In *in vitro* studies, the highest rate of degradation of vitamin A incubated for 4 h in either rumen fluid or autoclaved rumen fluid was in the presence of an active rumen microflora (Table 19.15; Klatte *et al.*, 1964). It was concluded that the degradation of vitamin A was due to the action of the microflora, although it was accepted that autoclaving could have limited other biological functions. Rode *et al.* (1990) showed that nine different types

Table 19.15. Disappearance of vitamin A after 4 h in distilled water or ruminal fluid (from Klatte *et al.,* 1964).

Incubation fluid	Number of assays	Disappearance (%)
Distilled water	11	16
Rumen liquor	11	36
Autoclaved rumen liquor	2	13

of bacteria were capable of transforming retinol propionate without inducing an accumulation of retinol, which is the only form of vitamin A that can be absorbed (Table 19.16).

Rode *et al.* (1990) measured the disappearance of vitamin A from the rumen of steers given diets based on concentrates, hay or straw and found levels of degradation of 67, 16 and 19% respectively. Weiss *et al.* (1995) confirmed *in vitro* that degradation of retinyl acetate was higher (72%) with rumen fluid obtained from a concentrate-type fermentation, in comparison with fluid obtained when the diet was based on forage (20%).

Using measurements of rumen flow in steers (Mitchell *et al.*, 1967) and sheep (Brongniart, 1987), the degradation of vitamin A *in vivo* was shown to be of the order of 60%. From a review of the literature, a mean rate of degradation of vitamin A in the rumen of 66% was found (Table 19.17). Similar losses of vitamin A from the rumen after intraruminal administration of vitamin A were also reported by Granseigne and Robert (1985) (Fig. 19.3). The rate of degradation of the two esters (acetate and palmitate) was highest in the first 2 h and then tended towards a maximum of 60%. Retinyl palmitate appeared to be less degradable than the acetate form.

Intestinal absorption of fat-soluble vitamins is dependent on the digestion and absorption of fat (Ullrey, 1972; McDowell, 1989). Vitamin A is absorbed in the form of retinol via the same mechanism as lipids, requiring the activity of bile salts,

Table 19.16. Degradation of vitamin A by rumen bacteria (from Rode *et al.,* 1990).

	Retinyl propionate degradation (%)			Retinol accumulation (%)		
Strains	18 h	42 h	66 h	18 h	42 h	66 h
Ruminobacter amylophilus WP109	13	17	30	0	0	0
Ruminobacter amylophilus WP91	67	76	85	0	5	0
Eubacterium ruminantium G1–1(1)	37	45	37	2	3	5
Fusobacterium necrophorum 1A	18	50	69	0	0	0
Fibrobacter succinogenes D	9	26	27	0	0	0
Ruminococcus flavefaciens 4	0	69	63	0	10	5
Propionibacterium acnes ATC6919	48	48	73	0	0	0
Butyrivibrio fibrisolvens XBF	48	95	96	0	0	0
Megasphaera elsdenii AW106	46	59	46	0	0	0

Table 19.17. Measurements of ruminal degradation of vitamin A *in vivo.*

Animal	Diet	Degradation (%)	References
Mature steer	High-roughage	57 (31–67)	Mitchell *et al.* (1967)
Steer	High-hay	73.9 (70–80)	Mitchell *et al.* (1968)
Mature steer	20% concentrate	52	Warner *et al.* (1970)
	40% concentrate	56	
	60% concentrate	70	
	80% concentrate	62	
Sheep	High-concentrate	64 ± 9	Brongniart (1987)

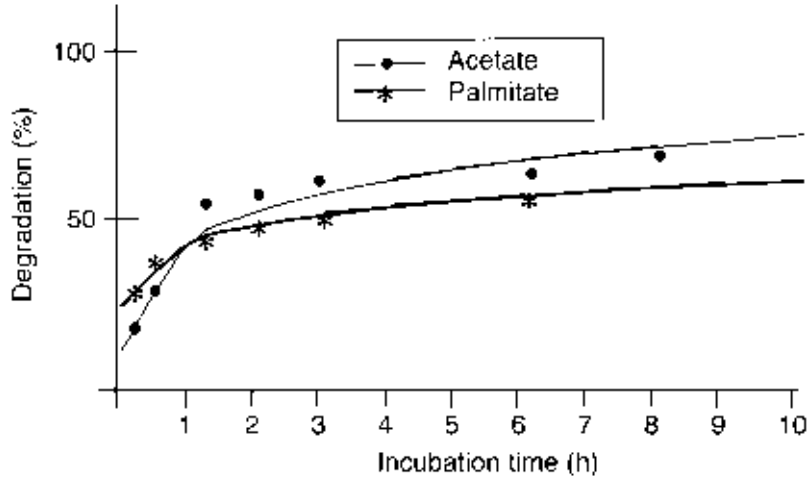

Fig. 19.3. Degradation curve of two esters of vitamin A in the rumen of sheep offered a concentrate ration (from Granseigne and Robert, 1985).

and thus any perturbation of lipid absorption results in a similar perturbation in vitamin A absorption (Bondi and Sklan, 1984; McDowell, 1989). Vitamin A transport across the brush border is not dependent on a membrane transport system but is dependent on the physicochemical characteristics of the surface contact of the molecule with the surface of the enterocyte. Absorption of vitamin A at the level of the intestine was found to be 30–50% of that ingested (Olson, 1984). This figure is in agreement with the level of degradation of 60% in the rumen, on condition that there is no further loss during intestinal absorption.

Ferrando (1980) reported that only 10% of β-carotene from forages would be used by ruminants. The low utilization of β-carotene from forages was due to its low solubilization and hence limited absorption (Bondi and Sklan, 1984), rather than its ruminal degradation. Indeed, Potkanski (1974), cited by Kalac and McDonald (1981), found that postabomasal absorption of carotene was only 5–13% of total carotene intake. Furthermore, intestinal absorption of carotene was reduced as the

level consumed increased (Olson, 1984). Following absorption, β-carotene is totally converted into vitamin A in the enterocytes of sheep and goats, but only partially converted in the enterocytes of the bovine. Eaton *et al.* (1959) found that differences existed between breeds in ability to convert carotene into vitamin A. The Holstein breed converted carotene into vitamin A 1.4 times more efficiently than the Guernsey breed.

A particular characteristic of the metabolism of vitamin A is that it is stored in the liver in the A1-all-*trans* esterified form by fatty acids, essentially palmitic acid. The liver may contain 90% of the total vitamin A in the organism and 30–60% of the quantity absorbed each day may be stored in the liver.

Studies in sheep on liver storage of vitamin A as a function of elevated dietary supply showed that the coefficient of liver storage, (vitamin A in the liver/vitamin A ingested) × 100, was of the order of 30% for supplementation with 20 IU g^{-1} of feed (Robert, 1987; Table 19.18). This value is relatively low in comparison with the coefficient of storage of 56% observed in monogastrics (Uzu, 1988), but is in accord with a substantial rumen degradation of vitamin A. In this study, it was also reported that coefficients of liver storage were reduced when levels of supplementation were increased. This could be explained by saturation of either the absorption pathway or the capacity of the liver to store vitamin A or a combination of the two.

During manufacture, synthetic vitamin A is normally encapsulated to guarantee stability against oxidation during fabrication of premixes and compound feed. It has been found that the type of encapsulation can play an important role in the level of ruminal degradation of the vitamin. Robert (1995a) measured the degradation of vitamin A in the rumen of lactating cows, using the nylon-bag technique. The results demonstrated that the encapsulation process could significantly reduce the rate of vitamin degradation in the rumen. It was further demonstrated that the solubilization of the encapsulation was significantly greater with a hay-based ration compared with a ration based on maize silage, probably as a result of the higher pH in the rumen with the hay-based diet compared with maize silage, producing conditions more favourable for the solubilization of the encapsulation material. The observations indicate that, dependent on the diet and the encapsulation process, different levels of vitamin A may bypass the rumen and be absorbed in the intestine.

Table 19.18. Measurements of hepatic storage of vitamin A (from Robert, 1987).

Diet	Number of animals	Planned vitamin A supplementation (IU g^{-1} feed)	Vitamin A level in feed (IU g^{-1} feed)	Total vitamin A hepatic storage (IU × 10^3)	Coefficient of hepatic storage (%)
1	15	0	2.4	11	–
2	15	20	19.8	523	34.1
3	15	40	45.6	961	25.0
4	15	60	61.3	928	18.9
5	15	80	83.1	898	12.1
6	15	100	109.2	989	10.1

Vitamin E

The biological role of tocopherols is related to the antioxidant activity of vitamin E, in particular as a means of protecting polyunsaturated fats in tissue membranes from peroxidation. Tocopherols have a low stability to heat, light and basic pH. Taking into account their antioxidant properties, in the presence of oxygen they themselves are oxidized, with the formation of quinones in the dimer and trimer forms. Acylation of the hydroxyl group present on the ring improves vitamin E stability and, for the most part, synthetic forms of vitamin E are present in the form of esters (acetate, succinate, …). These synthetic forms have no antioxidant properties *per se* but this property is recovered at the metabolic level, given the fact that absorption at the intestinal level occurs in the form of the alcohol.

Degradation of vitamin E in the rumen and intestinal absorption

Effective supplementation with vitamin E requires that the dietary vitamin supplement arrives intact at the site of absorption in the small intestine, where it can be absorbed and participate in metabolism.

The question of apparent low plasma appearance has been addressed by several authors. Using a preparation in which mature ewes had the pylorus ligatured, Alderson *et al.* (1971) were unable to obtain increased blood levels of vitamin E when 5000 IU of α-tocopherol (1 mg = 1.36 IU) was introduced into the rumen. It was concluded that vitamin E is not absorbed through the rumen wall. The addition of either an oxidizing agent (sodium nitrate) or an antioxidant (ethoxyquin) into the rumen of steers had no influence on the rate of disappearance of supplementary vitamin E added to the rumen at the level of 4190 IU (Tucker *et al.*, 1971; Table 19.19). It is notable that, in these two experiments, quite disparate and unexplainable rates of degradation of vitamin E were reported (27% and 62%). Hidiroglou *et al.* (1970) reported that there was a higher retention of radiolabelled tocopherol in sheep when it was administered intramuscularly compared with oral administration, which could be explained by pre-intestinal destruction of vitamin E. The fact that the rumen microflora contains vitamin E strengthens the hypothesis of a direct action of the rumen bacteria (Hidiroglou and Jenkins, 1974).

The destruction of vitamin E by the rumen microorganisms was studied in rumen-fistulated steers, each supplemented with 20,000 IU of vitamin E day^{-1} and given four diets containing different levels of lucerne hay and maize (Alderson *et al.*, 1971; Table 19.20). Degradation of vitamin E ranged from 8 to 42% as the level of maize incorporation in the ration increased. These results tend to suggest that

Table 19.19. Influence of oxidant or antioxidant agents on the degradation of vitamin E *in vivo* (from Tucker *et al.,* 1971).

	Trial 1		Trial 2	
	Control	Ethoxyquin	Control	Sodium nitrate
% of vitamin E degraded	26.8	24.4	61.5	64.3

Table 19.20. Disappearance of α-tocopherol in the rumen.

Animal	Diet	Disappearance (%)	Method	References
Steer	20% concentrate 40% concentrate 60% concentrate 80% concentrate	8.4 ± 1.2 22.2 ± 3.5 25.0 ± 5.3 42.4 ± 7.7	*In vivo*	Alderson *et al.* (1971)
Adult steer	High-concentrate	39–52	*In vivo*	Shin and Owens (1990)
Sheep	Forage/concentrate 50/50	6 ± 4	*In vitro* 24 h	Astrup *et al.* (1974a)
Beef steer	High-concentrate	4	*In vitro* 24 h	Leedle *et al.* (1993)
Jersey steer	High-forage	7.7 ± 3.5	*In vitro* 24 h	McDiarmid *et al.* (1994)
Dairy cow	High-silage	18	*In vivo*	Robert (1995b)
Dry cow Dairy cow lactating	High-forage Forage/concentrate 50/50	0 0	*In vitro* 24 h	Weiss *et al.* (1995)

vitamin E was partly degraded by the rumen microflora and particularly the amylolytic bacteria. Shin and Owens (1990) also demonstrated that between 39 and 52% of dietary supplemental vitamin E disappeared before reaching the duodenum of steers.

These results are contrary to those obtained by Leedle *et al.* (1993), who, based on assumptions made by Alderson *et al.* (1971), examined the stability of vitamin E in an *in vitro* system, using rumen liquor obtained from steers which had been offered a diet rich in concentrates. After periods of incubation of up to 24 h at 39°C, there was no loss of 1000 IU of DL-α-tocopherol acetate (1 mg = 1 IU). These authors concluded that there was no destruction of vitamin E by the rumen microorganisms and they suggested that the contradictory results obtained by other authors were due to the methods of vitamin E extraction and analysis. Using a diet based on 50% oats and 50% hay and with an *in vitro* rumen system, Astrup *et al.* (1974a) observed only slight degradation of vitamin E (Table 19.20). Similarly, with diets rich in either forage or concentrates, McDiarmid *et al.* (1994) and Weiss *et al.* (1995) observed only slight degradation of DL-α-tocopherol acetate.

Leedle *et al.* (1993) considered that structural differences between vitamins E and A were responsible for the relative differences in stability. Vitamin A possesses four double bonds in the side-chain, which are susceptible to reduction in the anaerobic rumen environment (biohydrogenation), whereas vitamin E possesses only one double bond on the inside of the aromatic ring, conferring greater stability.

It is quite possible that values obtained using *in vitro* fermentation systems may underestimate ruminal degradation, compared with values measured *in vivo*. In an *in vivo* trial with cows cannulated at the duodenum and given diets based on maize silage (ratio of forage to concentrate 80/20), Robert (1995b) reported only moder-

ate degradation of vitamin E (approximately 20%) (Table 19.20). The level of supplementation was 1500 IU animal^{-1} day^{-1}, which was an exceptionally high level but which was used for reasons of experimental methodology. Indeed, it is possible that this very high dose caused a saturation of the microbial degradation of vitamin E.

In ruminants, absorption of vitamin E is slower than in other species, the peak level in the plasma occurring approximately 24–48 h after oral administration. Intestinal absorption is linked to the absorption of lipids and requires the presence of bile salts and pancreatic lipases. Only the alcohol form is directly absorbed from the jejunum. This absorption process can be saturated. Using radiolabelled L-α-tocopherol, Tikriti (1969) obtained levels of absorption of the order of 40–50% of that escaping degradation in the rumen of dairy cows and, in goats, Astrup *et al.* (1974b) obtained levels of absorption of approximately 58% of the vitamin E that was introduced into the abomasum. Similarly to the case with β-carotene, intestinal absorption reduces as the level consumed increases (Machlin, 1984).

Vitamin E is stored both in adipose tissue and in the liver, but all tissues contain detectable amounts. Adipose tissue would appear to be capable of storing an unlimited quantity, whereas storage of vitamin E in the liver occurs for only a limited period. Furthermore, high-level supplementation of vitamin E for a short period does not increase the level of reserves in a similar manner to that following similar supplementation with vitamin A.

A low level of degradation of vitamin E in the rumen, which as yet remains to be confirmed, would be in accordance with responses in terms of animal health and performance obtained in practice. It is also in accordance with the more recent practice of using high-level supplementation with vitamin E to improve carcass quality. Bozzolo *et al.* (1993) (Table 19.21) were able to improve the quality of external carcass fat in sheep by supplementing with 4 g of DL-α-tocopherol acetate for 4 days prior to slaughter. The improvement resulted from the antioxidant properties of tocopherols. It is important to note that these authors obtained a significant increase in the plasma concentration of vitamin E, which was similar to that reported by Hidiroglou and Karpinski (1988). However, the high variability recorded in the concentrations of vitamin E in the treated sheep demonstrates a lack of homogeneity in the response. The authors considered that the variability was a result of the oral administration.

For improvement in meat quality, different authors have recommended supplementation with vitamin E at elevated dose levels (500–1000 IU animal^{-1} day^{-1}) during a period of 90–100 days before slaughter of cattle. Such levels of supplementation have proved beneficial in increasing tissue levels of α-tocopherol and reducing the percentage of metmyoglobin in muscle and the concentration of free radicals in the meat. Mitsumoto *et al.* (1993) orally supplemented steers with 1500 IU vitamin E animal^{-1} day^{-1} for 250 days before slaughter. Proportions of metmyoglobin, which is a brown oxidation product, were, in muscle of the control and treated animals, 19 and 7% on the day of slaughter and 87 and 40% after 9 days of storage, respectively. This reduced oxidation has the potential to dramatically improve the quality and storage life of meat obtained from beef animals and available on supermarket shelves (Faustman *et al.*, 1989a, b; Arnold *et al.*, 1992).

Table 19.21. Vitamin E and external tissue colour of sheep (from Bozzolo *et al.,* 1993).

	Control	Supplemented
Number of animals	120	120
Supplementary supply of DL-α-tocopherol acetate	0	4 g per animal (4 days before slaughter)
Colour of fat (%)		
Creamy white	19	38
Creamy brown	46	27
Yellow	34	34
Plasma level of vitamin E (μg ml^{-1})	0.7 ± 0.18	1.93 ± 0.83

Vitamin D

In high-producing, lactating, dairy cows at the start of lactation and under normal conditions, the presence of vitamin D prevents hypocalcaemia and the occurrence of milk fever. The presence of 1,25-dihydroxycholecalciferol also stimulates mineral uptake and particularly calcium uptake by bone, which explains the occurrence of rickets and bone dystrophy in the absence of vitamin D (rickets found in young animals and osteomalacia in lactating females).

Theoretically, the requirements for supplementary vitamin D in ruminants are low, because animals exposed to sunlight are capable of synthesizing sufficient vitamin D and, as indicated earlier, a supply can be obtained from sun-dried forages. Regular supplementation with vitamin D allows establishment of adequate reserves, which cover requirements during periods when the climate is unfavourable and when diets based on ensiled materials are offered. It is for these reasons that synthetic vitamin D has been developed, which confers the additional advantage of providing a controlled supply.

Studies of degradation of vitamin D incubated for 24 h in rumen liquor obtained from steers fed a diet based on forage showed a loss of approximately 75% of vitamin D (Sommerfeldt *et al.*, 1979b). However, incubation of vitamin D in sterilized rumen fluid resulted in no degradation, indicating that degradation is due to the activity of the rumen microorganisms (Sommerfeldt *et al.*, 1979b). Such activity produces three metabolites of vitamin D (stereoisomers of 10-ceto-19-norvitamin D_3) (Horst and Reinhardt, 1983), which have anti-vitamin D activity but which have the advantage of protecting ruminants from vitamin D toxicity in the event of consumption of high quantities. Although D_2 is comparable to D_3 in antirachitic function (McDowell, 1989), Sommerfeldt *et al.* (1979a, 1983) showed that the two forms of vitamin D (vitamin D_2 and D_3) do not pass through identical metabolic pathways in the rumen, resulting in a reduction of vitamin D_2 activity in comparison with vitamin D_3. Vitamin D is absorbed from the intestine in association with lipids and thus requires the presence of bile salts. Only approximately 50% of the vitamin D ingested is absorbed (Miller and Norman, 1984; McDowell, 1989).

It is probable that a proportion of supplementary vitamin D in the diet is destroyed by the rumen and does not reach the small intestine. This is justification for retaining the use of supplementary vitamin D in the diet as a means of covering requirements during periods of need. However, account must be taken of the ease with which hypervitaminosis and vitamin D toxicity can occur. Severe bone demineralization (osteofibrosis) in conjunction with hypercalcaemia and the occurrence of urinary calculi plus calcification of soft tissues has been observed following chronic doses of between 50 and 100 times requirement. In Europe, legislation limits dietary supplementation for ruminants to a maximum of 4000 IU vitamin D kg^{-1} of diet.

Conclusion

This review has demonstrated that, whilst forages may be considered as a potential source of vitamins, there is great variability in the quantity of key vitamins that remain bioactive in the feed and, perhaps of more importance, it is almost impossible, without costly feed evaluation, to accurately predict the quantity that will be supplied to ruminants. The review has also identified the major influence that rumen fermentation has on the quantity of vitamins reaching the small intestine. The value of correct vitamin supply to ruminants cannot be overstated and there is increasing evidence of the benefits of hypersupplementation with certain vitamins.

In view of the tremendous importance of correct vitamin supply, plus the uncertainty of supply from feed components, it is totally realistic to rely on the precision and reliability that is achieved by supply from synthetic vitamins. Accuracy of vitamin supply to ruminants is assisted by the use of formulation technology, which protects vitamins during feed processing and also helps ensure the delivery of the target dose to the absorption site in the intestine. In this manner, optimum benefit can be gained from these highly valued but relatively inexpensive feed additives. Although forage has the potential to contribute to the vitamin supply of ruminants, it is very difficult to envision how this supply can be reliably incorporated into ration formulation systems.

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