Effect of Selenium Fertilization on Selenium in Feedstuffs and Selenium, Vitamin E, and β-Carotene Concentrations in Blood of Cattle

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ABSTRACT

Selenium (n = 56), total vitamin E, and homologues of natural vitamin E in feedstuffs (n = 52) and the concentrations of Se (n = 241), vitamin E (n = 244), and β-carotene (n = 227) in blood were measured. The mean (±SD) Se content in hay, grass silage, oats, and barley produced using fertilizers enriched with Se was 0.13 (±0.169), 0.17(±0.704), 0.23 (±0.107) and 0.21 (±0.119) mg/kg of DM, respectively, and the mean (±SD) vitamin E contents, calculated as dl-α-tocopherol acetate equivalents, were 39.7 (±13.0), 120.0 (±40.27), 24.4 (±3.83) and 34.5 (±3.57) IU/kg of DM, respectively. The mean Se concentrations in whole blood of cows, heifers, bulls and calves fed hay (n = 62), silage (n = 111), or pasture (n = 68) varied from 183 to 244 μg/l. The mean concentrations of total vitamin E in serum of lactating cows fed hay (n = 21), silage (n = 29) or pasture (n = 26) were 2.8 (±1.43), 6.5 (±3.03) and 8.2 (±2.64) mg/l, respectively. For calves, concentrations of vitamin E in serum were as low as 0.25 mg/L. The mean concentration of β-carotene in serum of lactating cows fed grass silage (n = 26) or pasture (n = 28) was 13.7 (±6.61) and 15.4 (±6.15) mg/L, respectively, but, in lactating cows fed hay (n = 20), concentrations were 2.5 (±1.07) mg/L.
(**Key words**: selenium, vitamin E, homologues, β-carotene)

INTRODUCTION

Nordic countries have soils that are low in Se (24), and the acidity of the soil impairs utilization of Se by plants (7), resulting in low selenium content in Finnish forage plants. The mean Se (milligrams per kilogram DM) for hay samples was 0.014 (range 0.002 to 0.048), and, for grain samples, the mean value was 0.007 (range 0.002 to 0.085) (24). Therefore, sodium selenite has been added to animal feed in Finland since 1969. Based on studies conducted in the 1980s (7, 30), the Ministry of Agriculture and Forestry (Helsinki, Finland) decided, beginning July 1, 1984, to increase Se content of feed by the addition of 6 mg/kg of Se as sodium selenite to grass fertilizers and 16 mg/kg of Se to other fertilizers. The Se content allowed in multinutrient general fertilizers was changed to 6 mg/kg of fertilizer in 1990. The supplementation of other fertilizers with Se was prohibited. However, supplementation of dietary minerals with inorganic Se is still legal in Finland.

Results on the content of vitamin E in feed have been reviewed (15). The introduction of high performance liquid chromatography (HPLC) has increased the accuracy of measuring vitamin E and its homologues. Bieri and McKenna (5) defined the biopotence of other vitamin E homologues in relation to d-α-tocopherol, 40% β-tocopherol, 10% γ-tocopherol, 1% δ-tocopherol, and 25% α-tocotrienol; 1 mg of d-α-tocopherol is equivalent to 1.49 IU, and 1 mg of dl-α-tocopherol is equivalent to 1.1 IU.

The adequacy of vitamin E intake by animals can only be evaluated reliably from measurements on the animal itself. Despite the variety of different vitamin E homologues and their presence in feed, the only significant component of vitamin E in cow blood is α-tocopherol. However, the distribution of feed homologues is reflected in serum concentrations (25).

Carotenoids are present in animal systems, but animals cannot synthesize them de novo and depend on intake from their feed (22). Green plants are an exemplary source of β-carotene (22) as is good grass silage (21).

This study provides further information about the antioxidant status of Finnish cattle, including changes in Se status of cattle since Se fertilization was initiated.

MATERIALS AND METHODS

The 22 herds studied were thought to represent well-managed Finnish herds of average size. The

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TABLE 1. Selenium content of feed samples produced on research farms in 1988. Fertilizers containing sodium selenite were used.

	n	$\bar{X}$	SD	Median	Minimum	Maximum
		(mg/kg of DM)				
Hay	15	0.13	0.169	0.07	0.024	0.719
Silage	12	0.17	0.704	0.15	0.059	0.330
Barley	12	0.21	0.107	0.17	0.056	0.435
Oats	17	0.23	0.119	0.28	0.050	0.320

main roughage source was sun-cured, timothy hay cut in the late vegetative stage for 10 of the herds and timothy silage from hay cut at the early bloom stage for 12 of the herds. Feeding methods were examined during farm visits. All minerals used contained inorganic Se at 10 mg/kg of DM, and the mean daily supply of sodium selenite from minerals was 2 mg. The mean daily supply of vitamin E from commercial solution, when used, was 20 to 200 IU. The cows from all farms on which hay was fed were given minerals and, except on one farm, additional vitamins (administered as commercial per os solutions or injections).

Most farms that produced silage permitted the cows free access to silage, the dry cows on 2 farms and the lactating cows on 4 farms were an exception. On 2 farms, dry cows did not receive minerals, and, on 3 farms, dry cows did not receive supplementary vitamins. During lactation, cows on 3 farms were not given minerals and cows on 2 farms were not given vitamins.

Heifers on all hay farms that produced hay were fed hay and grain. Minerals were used on all farms, and supplementary vitamins (administered as commercial per os solutions or injections) were given on half of the farms. Half of the farms producing silage offered silage to the heifers for ad libitum consumption; others received <5 kg of DM/d. Minerals were used on all but 2 farms, but only 3 farms administered supplementary vitamins. Growing bulls were largely fed silage, home-grown grain, and concentrate. Two of the farms did not use minerals, and 3 farms did not administer vitamins. Calves <1 wk of age were fed whole milk only; older calves were fed a milk powder substitute. Half of the older calves received minerals. On farms where hay was fed, calves were managed without commercial vitamin solutions, but on farms where silage was fed, half of the calves received vitamins.

Samples of feed produced on the farm were taken during farm visits. At the same time, the use fertilizers containing Se was confirmed. Blood samples with and without anticoagulant (EDTA) were taken during the late indoor (April to May) and pasturing (August to September) seasons in 1989.

The feed samples were frozen and kept at −18°C until analysis. Blood samples with EDTA, which were taken from the cattle for Se analyses, were frozen immediately after sampling and kept at −18°C until analysis. Samples without anticoagulant were centrifuged at 2000 rpm for 10 min to separate the serum, which was stored frozen before being analyzed for Se, vitamin E, and β-carotene. The Se of the feed and whole blood samples was determined using the fluorometric method described by Lindberg (19).

Preparation of serum samples for HPLC analysis of vitamin E involved precipitation of proteins with ethanol and hexane extraction of lipid material. After evaporation of the hexane phase, the samples were redissolved in a small volume of hexane for vitamin E analysis. Sample preparation before HPLC analysis of vitamin E in the feeds started with grinding, followed by Soxhlet extraction with ethanol, and further extraction with hexane. Ascorbic acid or butylated hydroxytoluene was present in all extraction procedures to avoid spontaneous oxidation of the substances to be analyzed. Total lipids in serum were determined according to the method of Epstein et al. (6). Vitamin E in the serum samples and in the feeds was determined with a normal phase HPLC system, including a mobile phase based on heptane and fluorescence detection; the excitation wavelength was set at 295 nm, and the emission wavelength was set at 325 nm. Pure dl-α-tocopherol was used as the external standard. This normal phase HPLC system allowed all eight natural vitamin E homologues to be separated and quantified (8, 25).

For the determination of β-carotene, the serum sample (0.1 ml) was mixed with 0.9 ml of ethanolic KOH (0.5*N* KOH in 94% alcohol) and incubated in the dark at room temperature (20°C) for 4 h. Carotenes were extracted from the supernatant (0.9 ml) with 1.0 ml of hexane. After centrifugation the absorbance of the hexane phase was measured against the control (β-carotene) at 436 nm. The control was prepared using saline instead of serum. The standard was prepared from β-carotene (E. Merck, Darmstadt, Germany).

TABLE 2. Concentration of Se in blood of cattle fed silage or hay during the indoor season and in blood of cattle during the pasture season.[1]

	Silage			Hay			Pasture		
	— (μg/L) —		(n)	— (μg/L) —		(n)	— (μg/L) —		(n)
	$\bar{X}$	SD		$\bar{X}$	SD		$\bar{X}$	SD	
Dry cows	223	34.1	16	244	72.0	14	200	36.0	17
Lactating cows	214	24.6	29	218	49.2	22	205	43.0	25
Heifers	213	29.2	23	218	64.0	12	. . .	. . .	. . .
Calves	203	48.6	18	231	101.8	14	183	36.1	21
Growing bulls	206	35.5	25	. . .	. . .	. . .	194	30.7	5

[1]Dry cows were sampled during the dry and periparturient periods. The mean whole blood Se for all calves fed milk (from 2 to 7 d of age) and not receiving supplementary inorganic Se was 310 μg/L, and that for all calves not receiving supplemental Se was 202 μg/L (from 2 to 90 d of age).

The Student's *t* test was used for comparison of concentrations of Se in whole blood and of vitamin E and β-carotene concentrations in serum among cattle fed hay, silage, or pasture grass. Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Se

The range of Se content in feed was large (Table 1). Under field conditions, it is difficult to narrow the range because many factors affect the Se content of plants (7).

The Se status all groups of cattle was adequate (Table 2). The mean whole blood Se concentration in cows exceeded the nominal concentration of 200 μg/L set for optimal health (28). Selenium concentration also exceeded 100 μg/L, which is considered to be sufficient (16). No signs of a decrease in the concentration of Se in serum of dry cows were detected that might predispose cows to postcalving disorders, such as retained placenta, which were a concern in earlier studies (11).

Based on the Se concentrations that were determined in this study, heifers and growing bulls were at no risk of suffering nutritional muscular degeneration caused by poor nutrition. Growing cattle were found

TABLE 3. Total vitamin E and proportions of vitamin E homologues in feed.

		Vitamin E homologues[1]							
	Vitamin E	α-T	α-T3	β-T	β-T3	δ-T	δ-T3	γ-T	γ-T3
	(mg/kg of DM)								
Hay (n = 15)									
$\bar{X}$	30.7	25.9	0.1	1.0	0.2	2.3	. . .	0.6	. . .
SD	8.89	8.69	0.27	0.53	0.39	1.33	. . .	0.79	. . .
Silage (n = 15)									
$\bar{X}$	96.7	76.5	0.1	7.9	2.6	8.1	0.5	0.9	. . .
SD	31.68	26.20	0.55	4.07	2.81	3.86	0.73	1.20	. . .
Barley (n = 12)									
$\bar{X}$	76.9	10.8	47.0	0.8	5.9	3.1	8.0	0.4	1.0
SD	7.55	1.67	6.39	0.17	3.31	2.16	1.58	0.17	0.45
Oats (n = 7)									
$\bar{X}$	37.5	10.4	22.0	1.2	2.3	0.3	0.5	. . .	0.6
SD	5.99	1.77	4.17	0.35	0.58	0.25	0.56	. . .	0.28
Barley treated with propionic acid (n = 3)									
$\bar{X}$	7.2	1.5	2.6	0.3	0.9	0.8	0.4	0.2	0.6
SD	2.80	0.78	1.14	0.25	0.05	0.60	0.31	0.11	0.33

[1]α-T = α-Tocopherol, α-T3 = α-tocotrienol, β-T = β-tocopherol, β-T3 = β-tocotrienol, δ-T = δ-tocopherol, δ-T3 = δ-tocotrienol, γ-T = γ-tocopherol, and γ-T3 = γ-tocotrienol.

to be healthy even with markedly lower Se concentrations in the blood (26).

The transfer of Se through the placenta from the dam to the fetus is efficacious when the dam is fed organic Se compounds and exceeds that when fed sodium selenite (14). In addition, fetal calves are able to concentrate Se (17). High concentrations of Se in the blood of calves are partially due to the effective transfer of Se from organic Se in feed to the milk of the dam (1, 17). The decrease in whole blood Se of cows during the pasturing season was thus reflected in the Se status of the calf. The highest concentration of Se measured in the whole blood of calves was 495 μg/L, a concentration potentially high enough to cause immunosuppression (18). Selenium concentrations of 400 μg/L in cows, however, have been considered normal (29).

Vitamin E

Total vitamin E in hay, silage, barley, oats, and grain preserved with propionic acid were ($\bar{X}$ ± SD) 30.9 ± 8.89, 96.7 ± 31.68, 76.9 ± 7.55, 37.5± 5.99, and 7.2 ± 2.80 mg/kg DM, respectively. The proportions of different vitamin E homologues are presented in Table 3.

Total biological activity, expressed as dl-α-tocopherol acetate equivalents, was calculated using the coefficients proposed by Bieri and McKenna (5): 100 for dl-α-tocopherol acetate, 1.49 for α-tocopherol, 0.60 for β-tocopherol, 0.15 for γ-tocopherol, and 0.37 for α-tocotrienol. The equivalent contents of dl-α-tocopherol acetate in hay, silage, barley, oats, and grain that had been preserved with propionic acid were 39.7 ± 13.0, 120 ± 40.27, 34.5 ± 3.83, 24.4 ± 3.57, and 3.5 ± 1.78 IU/kg DM, respectively, and the vitamin E biopotences in the different feeds were 128, 124, 45, 65, and 47, respectively. In the calculation of biopotence, the amount of dl-β-tocopherol acetate equivalents was compared with the total vitamin E content. The main part of the total biological activity of vitamin E of the roughages was due to α-tocopherol, but a considerable part of activity of the grains was due to α-tocotrienol. On a weight basis, barley contained nearly twice as much vitamin E as oats, but, when the activity was expressed as dl-α-tocopherol acetate equivalents, the apparent activity of barley was severely reduced because barley contained relatively large amounts of the less biologically active homologue α-tocotrienol (Table 4). Therefore, the total biological activity of vitamin E in a diet depends not only on the proportions of individual

TABLE 4. Serum vitamin E concentration in cattle fed silage or hay during the indoor season and in cattle during the pasturing season.

	Total vitamin E	Vitamin E/lipid	Vitamin E homologues[1]			
			α-T	β-T	γ-T	α-T3
	(mg/L)	(mg/g)	(%)			
Lactating cows						
Fed silage (n = 29)						
$\bar{X}$	6.5[a]	1.23	97.46	0.49	1.50	0.52
SD	3.03	0.36	1.04	0.36	0.63	0.56
Fed hay (n = 21)						
$\bar{X}$	2.8[b]	0.61	94.91	0.61	2.72	1.75
SD	1.43	0.12	1.99	0.70	1.48	1.56
Fed pasture (n = 21)						
$\bar{X}$	8.2[c]	2.08	98.89	0.28	0.66	0.43
SD	2.64	0.37	0.42	0.07	0.23	0.18
Dry cows						
Fed silage (n = 16)						
$\bar{X}$	4.5[d]	1.39	98.80	0.18	0.78	0.16
SD	2.24	0.64	0.69	0.25	0.43	0.31
Fed hay (n = 14)						
$\bar{X}$	1.5[e]	0.57	96.91	0.36	1.66	106
SD	0.35	0.10	2.86	0.96	1.74	1.31
Fed pasture (n = 27)						
$\bar{X}$	6.3[df]	2.20	99.15	0.28	0.59	. . .
SD	2.08	0.50	0.42	0.11	0.33	. . .

[a,b,c]Means of lactating cows with different superscripts differ ($P < 0.05$).

[d,e,f]Means of dry cows with different superscripts differ ($P < 0.05$).

[1]α-T = α-Tocopherol, β-T = β-tocopherol, γ-T = γ-tocopherol, and α-T3 = α-tocotrienol.

TABLE 5. Serum vitamin E concentration in calves of herds fed silage or hay during the indoor season and in calves of herds during the pasturing season.

Calves	Total vitamin E	Vitamin E/lipid	Vitamin E homologues[1]			
			α-T	β-T	γ-T	α-T3
	(mg/L)	(mg/g)	(%)			
Fed silage (n = 17)						
$\bar{X}$	2.8[a]	0.61	96.79	0.45	2.37	0.38
SD	0.90	0.29	2.08	0.90	1.32	0.73
Fed hay (n = 14)						
$\bar{X}$	1.26[b]	0.42	95.19	0.14	3.88	0.79
SD	0.70	0.24	4.86	0.38	3.76	1.40
Fed pasture (n = 21)						
$\bar{X}$	2.5[a]	0.88	95.89	0.57	2.58	1.86
SD	2.13	0.51	2.41	0.27	1.30	2.11

[a,b]Means with different superscript differ ($P < 0.05$).

[1]α-T = α-Tocopherol, β-T = β-tocopherol, γ-T = γ-tocopherol, and α-T3 = α-tocotrienol.

homologues present but also on their biological potencies.

The total vitamin E content of feed (Table 3) was similar to the content reported previously in a Swedish study (8). The vitamin E content of hay was higher than that reported by Hakkarainen and Pehrson (8), but lower than that reported by Hidiroglou et al. (13). The vitamin E content of silage was lower than that reported in the Swedish study (8). The low vitamin E content of grain preserved with propionic acid was in agreement with earlier observations (3). Preservation with propionic acid destroys approximately 90% of the vitamin E of grain under conditions of high humidity. Moisture alone may also destroy vitamin E (9).

The high biopotence and content of vitamin E in silage increases the importance of high quality silage as a source of vitamin E. The NRC (23) recommendation for vitamin E for dairy cows can be met at all stages of lactation at 6 kg DMI/d of high quality silage.

The concentrations of vitamin E measured in the serum of lactating cows (Table 4) reflected the vitamin E content of roughages. In addition, reduced grain in the diet increased the probability of low concentrations of vitamin E in dry cows fed hay instead of silage. The vitamin E content in the serum of cows fed silage was higher than that reported in earlier studies (13, 25). The distribution of different vitamin E homologues in the silage group was in accordance with observations (25). The high vitamin E content of silage and the high α-tocopherol proportion in homologues was reflected in serum concentrations. The high percentage of α-tocopherol in the total vitamin E content of pasture grass (8) emphasized the role of α-tocopherol in the serum homologue distribution of cattle in the pasturing group. The proportion of γ-tocopherol was large for cows and calves fed dry hay and barley. If the diet contains large amounts of grain, the other homologues, and particularly α-tocotrienol, should also be considered (8). In addition, dietary tocotrienols in the body can be reduced to the corresponding concentrations of tocopherols (10).

The total vitamin E concentrations in calves in herds fed silage, hay, or pasture were 2.8 ± 0.90, 17, 1.2 ± 0.70, and 2.5 ± 2.13 mg/L, respectively, for 17, 14, and 21 calves, respectively.

The low vitamin E content in milk (2) was reflected in the vitamin E status of calves fed whole milk only (Table 5) (13). The dispersion of the vitamin E status of cows in this study was not reflected to the same extent in the concentrations of vitamin E in serum of the calves in the different feeding groups. The low vitamin E status of calves was in agreement with the findings of earlier studies (13, 25, 27), and the minimum value (0.25 mg/L) was of the same magnitude as that measured previously in newborn calves (12, 13). To ensure that the vitamin E status of calves exceeds the nominal value of 2 mg/L, a daily dose of 125 IU is recommended for calves fed whole milk or milk powder (27). This value is higher than that recommended by the NRC (23). Other studies (25) have also recommended that intake be increased.

β-Carotene

Mean concentrations of β-carotene were not different ($P > 0.05$) between cows fed silage and those fed pasture grass, but dry and lactating cows and heifers in the group fed silage had higher β-carotene concentrations in serum than did cattle in the group fed hay ($P < 0.001$; Table 6).

TABLE 6. Concentration of β-carotene in serum of cows.

Cows	Season		
	Pasture	Indoor	
		Silage	Hay
	(mg/L)		
Dry			
n	17	15	13
$\bar{X}$	13.3[a]	11.8[a]	3.2[b]
SD	3.26	5.41	1.14
Minimum	7.1	6.1	1.4
Lactating			
n	26	28	20
$\bar{X}$	15.4[c]	13.7[c]	2.5[d]
SD	5.15	6.61	1.07
Minimum	6.6	2.9	1.1

[a,b,c,d]Means within a row with different superscripts differ ($P < 0.05$).

β-Carotene status in the serum of cattle is a good reflection of the value of feed as a β-carotene source. Pasture grass, silage, and hay contain, on average, 380, 62, and 45 mg of β-carotene/kg of DM, respectively (20). Cows receiving grass silage or pasture grass have equally high β-carotene concentrations, and no deficiencies have arisen (21).

CONCLUSIONS

Because of the large variation in Se content in feed, Se intake of cattle should be secured by Se supplementation. If the current fertilizer usage practice in Finland is continued, however, Se supplementation in minerals needs to be retained because of the large variation in SE content in feed. Based on the Se concentrations in blood presented here, Se supplementation is not needed for calves, heifers, and growing bulls.

To ensure a sufficient vitamin E intake, calves need to receive a supplemental daily dose of 100 to 150 mg of dl-α-tocopherol acetate until they can consume 1 kg of silage DM/d. The vitamin E concentration in serum of heifers and growing bulls fed grass silage can be considered adequate (25). The NRC (23) recommendation for vitamin E intake for dairy cows is met at all stages of lactation with a normal diet. The high vitamin E content of high quality grass silage can compensate for the vitamin E destroyed in the wet storage of grain. The commercial vitamin E recommendations for supplementation given by Bieber-Wlashny (4), which are about three times as high as those of the NRC (23), are met with a ration containing good grass silage as main roughage.

The concentration of β-carotene in the blood of cows fed grass silage is comparable with that of cows on pasture.

REFERENCES

1 Aspila, P. 1991. Metabolism of selenite, selenomethionine and feed-incorporated selenium in lactating goats and dairy cows. Ph.D. Diss., Univ. Helsinki, Helsinki, Finland.
2 Batra, T. R., K. Singh, S. K. Ho, and M. Hidiroglou. 1992. Concentration of plasma and milk vitamin E and plasma β-carotene of mastitic and healthy cows. Int. J. Vitam. Nutr. Res. 62:233.
3 Bauernfeind, J. 1980. Tocopherols in foods. Page 99 *in* Vitamin E, A Comprehensive Treatise. L. J. Machlin, ed. Marcel Dekker, Inc., New York, NY.
4 Bieber-Wlashny, M. 1988. The use of vitamin E in the dairy cow. Page 1 *in* Roche Symp. on Anim. Nutr. F. Sissel, ed. Hoffman-LaRoche, Basel, Switzerland.
5 Bieri, J. G., and M. C. McKenna. 1981. Expressing dietary values for fat-soluble vitamins: changes in concepts and terminology. Am. J. Clin. Nutr. 34:289.
6 Epstein, E., E. S. Baginski, and B. Zak. 1972. Extraction of lipids from serum and measurement of total serum lipids. Ann. Clin. Lab. Sci. 2:244.
7 Gissel-Nielsen, G., U. C. Gupta, M. Lamand, and T. Westermark. 1984. Selenium in soils and plants and its importance in livestock and human nutrition. Page 398 *in* Advances in Agronomy. Vol 37. N. C. Brady, ed. Academic Press, Orlando, FL.
8 Hakkarainen, J., and B. Pehrson. 1987. Vitamin E and polyunsaturated fatty acids in Swedish feedstuffs for cattle. Acta Agric. Scand. 37:341.
9 Hakkarainen, R.V.J., J. T. Työppönen, and S. G. Bengtsson. 1983. Relative and quantitative changes in total vitamin E and isomer content of barley during conventional and airtight storage with special reference to annual variation. Acta Agric. Scand. 33:395.
10 Hakkarainen, R.V.J., J. T. Työppönen, S. Hassan, S. G. Bengtsson, S.R.L. Jönsson, and P. Lindberg. 1984. Biopotency of vitamin E in barley. Br. J. Nutr. 52:335-349.
11 Harrison, J. H., D. D. Hancock, and H. R. Conrad. 1984. Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci. 67:123.
12 Hidiroglou, M., T. R. Batra, L. F. Laflamme, and F. Markham. 1992. Possible roles of vitamin E in immune response of calves. Int. J. Vitam. Nutr. Res. 62:308.
13 Hidiroglou, M., T. R. Batra, and G. L. Roy. 1994. Changes in plasma α-tocopherol and selenium of gestating cows fed hay or silage. J. Dairy Sci. 77:190.
14 Jacobsson, S. O., and H. E. Oksanen. 1966. The placental transmission of selenium in sheep. Acta Vet. Scand. 7:66.
15 Kivimäe, A., and C. Carpena. 1973. The level of vitamin E content in some conventional feeding stuffs and the effects of genetic variety, harvesting, processing and storage. Acta Agric. Scand. Suppl. 19:161.
16 Koller, L. D., P. J. South, J. H. Exon, and G. A. Whitbeck. 1983. Selenium deficiency of beef cattle in Idaho and Washington and a practical means of prevention. Cornell Vet. 73:323.
17 Koller, L. D., G. A. Whitbeck, and P. J. South. 1984. Transplacental transfer and colostral concentrations of selenium in beef cattle. Am. J. Vet. Res. 45:2507.
18 Larsen, H. J., G. Övernes, and K. Moksnes. 1988. Effect of selenium on sheep lymphocyte responses to mitogens. Res. Vet. Sci. 45:11.
19 Lindberg, P. 1968. Selenium determination in plant and animal material, and in water. Acta Vet. Scand. Suppl. 9:5.
20 Lindner, H., and M. Gadient. 1982. β-Carotene in cattle reproduction. European β-carotene field studies 1976-1981. Literature Review. F. Hoffmann-La Roche, Basel, Switzerland.
21 Lotthammer, K. H. 1979. Importance of β-carotene for the fertility of dairy cattle. Feedstuffs 51:16.

22 McDonald, P., R. A. Edwards, and J.F.D. Greenhalgh. 1981. Animal Nutrition. 3rd ed. Longman Group Ltd., New York, NY.
23 National Research Council. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Nat. Acad. Sci., Washington, DC.
24 Oksanen, H. E., and M. Sandholm. 1970. The selenium content of Finnish forage crops. J. Sci. Agric. Soc. Finl. 42:251.
25 Pehrson, B., and J. Hakkarainen. 1986. Vitamin E status of healthy Swedish cattle. Acta Vet. Scand. 27:351.
26 Pehrson, B., J. Hakkarainen, and J. Työppönen. 1986. Nutritional muscular degeneration in young heifers. Nord. Vet. Med. 38:26.
27 Reddy, P. G., J. L. Morril, and R. A. Frey. 1987. Vitamin E requirements of dairy calves. J. Dairy Sci. 70:123.
28 Smith, K. L., J. S. Hogan, and H. R. Conrad. 1988. Selenium in dairy cattle: its role in disease resistance. Vet. Med. 83:72.
29 Stevens, J. B., W. G. Olson, R. Kraemer, and J. Archambeau. 1985. Serum selenium concentrations and glutathione peroxidase activities in cattle grazing forages of various selenium concentrations. Am. J. Vet. Res. 46:1556.
30 Yläranta, T. 1985. Increasing the selenium content of cereal and grass crops in Finland. M.S. Thesis, Univ. Helsinki, Helsinki, Finland.