Acta vet. scand. 1986, 27, 351—360.

From the Experimental Station, Veterinary Institute, Skara and the Department of Clinical Nutrition, College of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

VITAMIN E STATUS OF HEALTHY SWEDISH CATTLE *

By

Bo Pehrson and *Juhani Hakkarainen*

PEHRSON, B. & J. HAKKARAINEN: *Vitamin E status of healthy Swedish cattle.* Acta vet. scand. 1986, 27, 351—360. — Using high pressure liquid chromatography the serum concentration of vitamin E was measured in dairy cows fed either hay or silage as their main roughage, in calves fed milk-replacer, and in young intensively fed bulls. The concentrates fed to the cows, calves and bulls were supplemented with 5—10, 25 and 5—10 mg DL-α-tocopheryl acetate per kg, respectively, and the milk-replacer for the calves was supplemented with 50 mg DL-α-tocopheryl acetate per kg powder. Cows fed silage as their main roughage had higher serum vitamin E concentrations ($\bar{x}$: 3.8—5.2 mg/l) than cows fed only hay ($\bar{x}$: 2.5—4.1 mg/l). Lactating cows had higher vitamin E concentrations than dry cows ($\bar{x}$: 4.1—5.2 and 2.5—3.8 mg/l, respectively) and calves and bulls had much lower vitamin E concentrations ($\bar{x}$: 1.4 and 1.2 mg/l, respectively) than cows. Thirty per cent of the calves and 41 % of the bulls had serum vitamin E concentrations less than 1.0 mg/l, suggesting that in these animals the conventional level of supplementation of feeds with DL-α-tocopheryl acetate in Sweden is probably inadequate for the prevention of nutritional muscular degeneration and other negative effects.

nutritional muscular degeneration; feed supplementation; α-tocopherol; DL-α-tocopheryl acetate; dairy cattle; milk-replacers; intensively fed young bulls; milk-fed calves; meat production.

Since the introduction of high performance liquid chromatography (HPLC) it has been possible to determine accurately vitamin E and its isomers. This measurement on blood plasma or serum can be used to give an indication of the vitamin E status of cattle. However, several dietary and environmental factors

* The investigation was supported by the Swedish Council for Forestry and Agricultural Research.

may affect the requirements of cattle for vitamin E and make it difficult to establish what constitutes an "adequate" serum vitamin E concentration. Nevertheless it is claimed that it is possible to establish whether cattle are severely vitamin E deficient (*McMurray et al.* 1983). These authors state that concentrations of α-tocopherol in plasma below 0.2 mg/l indicate a severe deficiency. Moreover, serum concentrations less than 1.0—1.5 mg/l are often found in young cattle with nutritional muscular degeneration (*Arthur* 1982, *McMurray & Rice* 1982, *Pehrson et al.* 1986) and such values must therefore be considered to be suboptimal.

McMurray et al. (1983) reported that concentrations of 3.0—5.0 mg/l of α-tocopherol were normal in the plasma of young cattle fed silage or grass. *Lynch* (1983) found mean values of 1.4 and 3.1 mg α-tocopherol/l in the serum of dairy cows which were receiving no vitamin E supplementation, when they were fed hay and silage, respectively; milk-fed calves had much lower values (0.5—0.8 mg/l). *Lundin & Palmquist* (1983) found 6.2—7.1 mg α-tocopherol/l in the plasma of lactating cows fed corn silage, alfalfa hay and concentrates without vitamin E supplementation.

The aim of the present study was to measure the concentration of vitamin E in the serum of different categories of healthy Swedish cattle and to evaluate whether the amounts of supplementary vitamin E added to commercial feeds are adequate.

MATERIALS AND METHODS

In January and March 1983 blood samples were taken from 81 cows at different stages of lactation and from 20 calves belonging to 18 dairy herds. Seven of these herds were fed hay as the only roughage and the 11 others were fed more than 60 % of their roughage dry matter as silage. Concentrates (commercial protein feed plus grain) were fed to the cows in quantities related to their milk yields. The silages had been conserved with formic acid and were of good quality with a pH below 4.5.

The calves were from 1—8 weeks old and fed milk-replacer; they also had free access to hay and concentrates.

Blood samples were also taken from 29 intensively fed young bulls, aged from 6—10 months, in 8 different herds. They were fed restricted amounts of hay (< 1 kg/day) but had free access to concentrates (protein feed plus grain).

The protein feed in the concentrates was supplemented with minerals, trace elements and vitamins, but no further supplements were provided to the animals. The concentrates fed to the cows, calves and bulls contained on average 5—10, 25 and 5—10 mg DL-α-tocopheryl acetate per kg, respectively.

The feeding systems were different in different herds. In most of the herds a commercial protein feed and home-produced grain were fed separately, but in some herds a commercial mixture was used. The total amounts fed and the proportions of protein and grain varied with the nutritional value of the roughage, but with few exceptions the proportions fed to the dairy herds were between 25—35 % protein feed and 75—65 % grain; the corresponding figures for the concentrates fed to the bulls were 10—15 % protein feed and 90—85 % grain.

The milk-replacers fed to the calves were supplemented with 50 mg DL-α-tocopheryl acetate per kg powder.

Blood samples were taken from a jugular vein into plain tubes and stored at 4°C for 3 h before they were centrifuged at 2000 rpm for 10 min. The serum was removed, immediately frozen and stored at —20°C until analysed for vitamin E.

The isomers of vitamin E in 0.8 ml serum were analysed as follows: the serum proteins were precipitated with 0.8 ml absolute ethanol and the lipid material extracted with 1.6 ml n-hexane. A portion (1.4 ml) of the hexane phase was evaporated to dryness and the residue dissolved in 70 µl hexane. Ten µl of this solution was injected onto a Merck Hibar RT 125-4 5 µm silica column held thermostatically at 25°C. The mobile phase consisted of n-hexane containing 1.5 % tetrahydrofuran. The flow rate during isocratic elution was 1.5 ml/min.

The Perkin-Elmer HPLC system consisted of a model 3 B pump module, fitted with a PE ISS-100 Auto Sampler. A Perkin-Elmer 204 S fluorescence spectrophotometer equipped with a microflow-cell unit was used for detection. The wavelength settings were 295 nm and 330 nm for excitation and emission, respectively. The peaks were identified as described previously (*Hakkarainen* et al. 1983), and the peak areas were calculated with a Sigma 10B Chromatographic Data Station; they were quantified by using an external α-tocopherol standard as described by *Thompson & Hatina* (1979).

The coefficient of variation (CV) for the determination of α-tocopherol in serum was 1.9 %, and 98 % of α-tocopherol added to samples was recovered.

The serum lipids were analysed by the method of *Epstein et al.* (1972) and the determination had a mean CV of 3.1 %.

RESULTS

Dairy cows which were fed silage as their main roughage had higher serum vitamin E concentrations than cows which were fed only hay as roughage (Table 1). The differences were significant ($P < 0.05$, 0.01 or 0.001) in both the lactating and the dry cows, and remained so whether the vitamin E concentrations were expressed as mg/l or as mg/g lipid. Irrespective of the type of roughage being fed, the lactating cows had significantly ($P < 0.01$ or 0.001) higher vitamin E concentrations than the dry cows, when the results were expressed as mg/l. However, the lactating cows had much higher levels of total lipids in their sera (4.6 g/l compared with 2.7 g/l), and as a result there was no significant difference between the vitamin E concentrations in the herds which fed hay as the only roughage, when the values were expressed as mg/g lipid. In the herds feeding silage the dry cows had a significantly ($P < 0.01$) higher concentration of vitamin E, expressed as mg/g lipid, than the lactating cows.

The calves fed milk-replacer had significantly lower vitamin E concentrations than dairy cows, but there was no significant influence from the type of roughage given to the cows.

The intensively fed young bulls had slightly lower vitamin E concentrations than the calves fed milk-replacer.

α-tocopherol was the dominant component of the total vitamin E content of the serum of all the groups, constituting 95 % in the dairy cows, about 92 % in the calves and 88 % in the bulls. Of the non-α-tocopherol isomers, γ-tocopherol formed the largest component in the cows and calves and α-tocotrienol in the bulls.

DISCUSSION

Vitamin E concentrations in serum are usually expressed in mg/l. However, it has been proposed that they should be expressed in mg/g serum lipid because the vitamin E levels appear to be correlated with serum total lipids (*Horwitt et al.* 1972). On the other hand it is evident that the serum vitamin E level is also highly dependent on the vitamin E content of the diet, and that serum vitamin E levels can be increased independently

Table 1. Vitamin E concentrations in serum from different categories of cattle. The results of statistical calculations are given in the text.

Herds	Animals		Total vit. E mg/l		Total vit. E mg/g lipid		Isomers (percentage distribution) α-tocopherol		β-tocopherol		γ-tocopherol		α-tocotrienol	
			$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Farms feeding silage as main roughage	Lactating cows,	n=21	5.20	1.67	1.09	0.28	97.02	0.85	0.26	0.05	1.89	0.60	0.82	0.20
	dry cows,	n=19	3.75	1.45	1.50	0.59	97.18	1.93	0.05	0.17	2.32	1.88	0.45	0.43
	milk-fed calves,	n=14	1.42	0.60	0.62	0.23	91.62	3.61	0.18	0.26	5.71	3.33	2.49	2.22
Farms feeding hay as sole roughage	Lactating cows,	n=20	4.11	1.23	0.95	0.21	95.41	1.36	0.54	0.16	2.68	0.92	1.38	0.34
	dry cows,	n=21	2.52	1.02	0.97	0.22	96.33	1.41	0.43	0.43	2.26	0.86	0.98	0.28
	milk-fed calves,	n= 6	1.48	0.37	0.55	0.17	92.02	2.81	1.37	0.61	4.83	1.60	1.78	1.01
Farms with restricted roughage and concentrates ad lib.	Growing young bulls,	n=29	1.22	0.54	0.50	0.14	88.32	4.84	0.25	0.18	2.04	1.94	9.39	4.32

of the serum lipids (*Hakkarainen et al.* 1984). A comparison of the results observed in lactating and dry cows in the present investigation (Table 1) shows that conclusions may differ, depending on the way in which the results are expressed. Thus, serum vitamin E concentrations in the lactating cows were significantly higher than in the dry cows when they were expressed as mg/l, but the difference disappeared in herds fed hay as the only roughage when the values were expressed as mg/g lipid.

In order to establish a connection between the serum vitamin E concentration and the risk of cattle suffering from clinical disorders it would probably be better to relate the serum vitamin E values to the concentrations of polyunsaturated fatty acids (PUFA) in the serum, particularly after taking the peroxidisability of the individual PUFA into consideration. Most of the PUFA are normally hydrogenated in the rumen, thereby decreasing the net absorption of the high peroxidisable linoleic (C 18:2) and linolenic (C 18:3) acids. However, under certain circumstances, the process of hydrogenation seems to cease and the risk of cell damage by PUFA is increased (*McMurray & Rice* 1982), unless there is a parallel increase in the concentration of the protective vitamin E isomers.

In the present investigation the concentrates fed to the cows were supplemented with 5—10 mg DL-α-tocopheryl acetate per kg. The significantly higher levels of serum vitamin E, when expressed as mg/l, in the lactating cows than in the dry cows were certainly the result of their higher intake of concentrates and, thereby, of vitamin E, because equal amounts of roughages were offered to all the cows in each herd. It is well known that lactating cows have higher levels of total lipids than dry cows (*Maynard et al.* 1931, *Riis* 1964). When comparing the lactating and dry cows it seems probable that the supplementary DL-α-tocopheryl acetate contributed at least half the total serum vitamin E in the lactating cows.

By comparing the cows fed either hay alone or predominantly silage, and assuming an equal intake of roughage by both groups, it is evident that feeding good quality silage increased the vitamin E concentration in serum by about 1 mg/l. This increase is slightly lower than that reported by *Lynch* (1983). Depending on the stage of lactation it can be calculated approximately that dairy cows not receiving any supplementary vitamin E should

have a serum vitamin E concentration of 1—2 mg/l when hay is used as the only roughage and 2—3 mg/l when significant amounts of silage are fed. This conclusion is in good agreement with the results reported by *Lynch* (1983) from the USA. Other researchers (*McMurray et al.* 1983, *Lundin & Palmquist* 1983) have shown that feeding silage or grass can increase serum vitamin E values above 2—3 mg/l. In cows fed exclusively on grass, serum vitamin E values above 4—5 mg/l can be expected from 3—4 weeks after turning out to the end of the grazing season (*Pehrson & Hakkarainen*, unpublished).

The calves fed milk-replacer and the young growing bulls had significantly lower serum vitamin E concentrations than the cows, whether the levels were expressed as mg/l or mg/g lipid. Similarly low vitamin E levels in milk-fed calves have earlier been reported by *Lynch* (1983). Our calves had free access to hay and to vitamin E supplemented concentrates, but between 1—8 weeks of age calves would eat little of these feedstuffs. The serum levels of vitamin E were therefore certainly derived mostly from the milk-replacer. Six of the 20 calves had serum vitamin E concentrations below 1.0 mg/l. It can therefore be questioned whether the vitamin E content of the milk-replacers used in Sweden should be supplemented with more vitamin E than the 50 mg/kg powder DL-α-tocopheryl acetate added at present.

The bulls received a diet based on restricted amounts of hay (< 1.0 kg per day), and free access to concentrates which were supplemented with DL-α-tocopheryl acetate at the same level as the concentrates for the cows. The bulls consumed about 5—7 kg of concentrates daily, giving them a higher intake of supplementary vitamin E than the dry cows, which ate only 2—3 kg kg of the concentrates daily. However, the cows consumed much more hay than the bulls and therefore their total intake of vitamin E was greater, which probably was the main reason why the vitamin E values of the bulls were only about half those of the dry cows in the group fed hay as the only roughage. Twelve of the 29 bulls had serum vitamin E concentrations less than 1.0 mg/l, and it is possible that a destruction of vitamin E in the rumen may have contributed to the very low values. *Alderson et al.* (1971) have thus observed that such a destruction can occur when the diet contains high amounts of grain; our bulls received 85—90 % of their concentrates as grain.

It has been suggested that serum vitamin E levels less than 1.0—1.5 mg/l can be potentially hazardous for ruminants (*Arthur* 1982, *McMurray & Rice* 1982, *Øvernes et al.* 1985, *Pehrson et al.* 1986). If this is true, the present investigation shows that the serum vitamin E levels of calves fed milk-replacer, and of rapidly growing young bulls, are not satisfactory under conventional Swedish conditions. It must be remembered, however, that an animal's requirement for vitamin E depends on a variety of dietary and environmental factors, among which are its intake of e.g. PUFA, selenium and sulphur-containing amino-acids (*Scott* 1978). Until the quantitative relationships between these factors and adequate serum vitamin E concentrations in cattle are understood it will not be possible to know whether a given vitamin E concentration is satisfactory or not. However, from a practical point of view and bearing these reservations in mind, it is reasonable to consider serum vitamin E values of 2—3 mg/l as satisfactory when cows are fed hay as their main roughage, and 3—4 mg/l when they are fed silage as the major roughage.

The fact that many Swedish milk-fed calves and young bulls apparently have low serum vitamin E levels suggests the need for an increase in the level of supplementary vitamin E in the feeds for these animals. The relationships which have been established between vitamin E and the resistance of animals to infections (*Nockels* 1979, *Sheffy & Schultz* 1979) and the high infection rates observed in practice, particularly in systems for intensive meat production, suggest the importance of controlled experiments with different levels of vitamin E in the feed. *Adams & Zimmerman* (1984) have reported improved growth rate in young cattle treated with vitamin E.

It has previously been shown that the major isomer of vitamin E in blood is α-tocopherol, irrespective of both the species of animal and the distribution of isomers in the feed (*Rice & McMurray* 1982, *Hakkarainen et al.* 1984, *Työppönen et al.* 1984, *Hakkarainen et al.* 1986, *Ronéus et al.* 1986) and this finding was confirmed by the results of the present investigation. In the cows, calves and bulls respectively, more than 95 %, 92 % and 88 % of the total serum vitamin E content was α-tocopherol. In the bulls α-tocotrienol was the principal other isomer, presumably because α-tocotrienol is the principal isomer in barley and oats (*Hakkarainen et al.* 1983, *Hakkarainen & Pehrson* 1986) and these were the main feedstuffs for the bulls.

REFERENCES

Adams, C. R. & C. R. Zimmerman: Update on supplemental vitamin E in beef cattle rations. Feedstuffs 1984, *56*, 21—22.

Alderson, N. E., G. E. Mitchell Jr., C. O. Little, R. E. Warner & R. E. Tucker: Preintestinal disappearance of vitamin E in ruminants. J. Nutr. 1971, *101*, 655—660.

Arthur, J. R.: Nutritional inter-relationships between selenium and vitamin E. Rep. Rowett Inst. 1982, *38*, 124—135.

Epstein, E., E. S. Baginski & B. Zak: Extraction of lipids from serum and measurement of total serum lipids. Ann. Clin. Lab. Sci. 1972, *2*, 244—254.

Hakkarainen, R. V. J., J. T. Työppönen & S. G. Bengtsson: Changes in the content and composition of vitamin E in damp barley stored in airtight bins. J. Sci. Food Agric. 1983, *34*, 1029—1038.

Hakkarainen, R. V. J., J. T. Työppönen, S. Hassan, S. G. Bengtsson, L. Jönsson & P. Lindberg: Biopotency of vitamin E in barley. Brit. J. Nutr. 1984, *52*, 335—349.

Hakkarainen, J. & B. Pehrson: Vitamin E and polyunsaturated fatty acids in Swedish feedstuffs. Acta agric. scand. 1986, *36*, in press.

Hakkarainen, J., J. Työppönen & L. Jönsson: Vitamin E requirement of the growing rat during selenium deficiency with special reference to selenium dependent and selenium independent glutathione peroxidase. Zbl. Vet. Med. A, 1986, *33*, 247—258.

Horwitt, M. K., C. C. Harvey, C. H. Dahm & M. T. Steacy: Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann. N. Y. Acad. Sci. 1972, *203*, 223—236.

Lundin, P. K. & D. L. Palmquist: Vitamin E supplementation of high fat diets for dairy cows. J. Dairy Sci. 1983, *66*, 1909—1916.

Lynch, G. P.: Changes of tocopherols in blood serum of cows fed hay or silage. J. Dairy Sci. 1983, *66*, 1461—1465.

Maynard, L. A., E. S. Harrison & C. M. McCay: The changes in the total fatty acids, phospholipid fatty acids and cholesterol of the blood during the lactation cycle. J. biol. Chem. 1931, *92*, 263—272.

McMurray, C. H. & D. A. Rice: Vitamin E and selenium deficiency diseases. Irish Vet. J. 1982, *36*, 57—65.

McMurray, C. H., D. A. Rice & S. Kennedy: Nutritional myopathy in cattle; from a clinical problem to experimental models for studying selenium, vitamin E and polyunsaturated fatty acids interactions. In: Trace Elements in Animal Production and Veterinary Practice. Eds. Suttle, N. F., R. G. Gunn, W. M. Allen, K. A. Linklater & G. Wiener. Brit. Soc. Anim. Prod. 1983, p. 61—73.

Nockels, C. F.: Protective effects of supplemental vitamin E against infection. Fed. Proc. 1979, *38*, 2134—2138.

Øvernes, G., U. Nymoen & A. Frøslie: E-vitamin hjå sau og lam. (Vitamin E in ewes and lambs). Norsk Vet. tidskr. 1985, *97*, 173—176.

Pehrson, B., J. Hakkarainen & J. Työppönen: Nutritional muscular degeneration in young heifers. Nord. Vet.-Med. 1986, *38*, 26—30.

Rice, D. A. & C. H. McMurray: Recent informations on vitamin E and selenium problems in ruminants. Proceedings Roche Vitamin Symposium, London 1982, p. 1—19.

Riis, P. M.: Investigations on lipid metabolism in cattle. Thesis. The Royal Veterinary and Agricultural College, Copenhagen 1964.

Ronéus, B., R. V. J. Hakkarainen, C. A. Lindholm & J. Työppönen: Vitamin E requirements of adult standardbred horses evaluated by tissue depletion and repletion. Equine vet. J. 1986, *18*, 50—58.

Scott, M. L.: Vitamin E. In: The Fat Soluble Vitamins. Handbook of Lipid Research 2. Ed. DeLuca, H. E., Plenum Press. New York and London 1978, p. 133—210.

Sheffy, B. E. & R. D. Schultz: Influence of vitamin E and selenium on immune response mechanisms. Fed. Proc. 1979, *38*, 2139—2143.

Thompson, J. N. & G. Hatina: Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography. J. Liq. Chromatogr. 1979, *2*, 327—344.

Työppönen, J., J. Hakkarainen, T. Juokslahti & P. Lindberg: Vitamin E requirement of mink with special reference to tocopherol composition in plasma, liver, and adipose tissue. Amer. J. vet. Res. 1984, *45*, 1790—1794.

SAMMANFATTNING

E-vitaminstatus hos svenska nötkreatur.

Med HPLC-analys av blodprov kartlades E-vitaminstatus hos mjölkkor som fick hö resp. ensilage som grovfoder, hos ej avvanda kalvar från samma besättningar och från intensivt uppfödda slakttjurar. Kraftfodret till korna, kalvarna och tjurarna hade berikats med 5—10, 25 resp. 5—10 mg DL-α-tokoferylacetat per kg och kalvarnas mjölkersättningspulver med 50 mg per kg. Kor från besättningar med ensilage som huvudsakligt grovfoder hade högre E-vitaminvärden i serum ($\bar{x}$: 3,8—5,2 mg/l) än kor som fick enbart hö som grovfoder ($\bar{x}$: 2,5—4,1 mg/l). Lakterande kor hade högre E-vitaminvärden än sinkor ($\bar{x}$: 4,1—5,2 resp. 2,5—3,8 mg/l). Både kalvar och tjurar hade betydligt lägre värden ($\bar{x}$: 1,4 resp. 1,2 mg/l) än korna. Trettio procent av kalvarna och 41 procent av tjurarna hade serumvärden understigande 1,0 mg/l. Detta tolkas som att de tilsatser av DL-α-tokoferylacetat till foder för dessa djurkategorier som f n är regel i Sverige sannolikt är otillräckliga för att eliminera risker för muskeldegeneration och andra negativa effekter.

(Received May 12, 1986).

Reprints may be requested from: Bo Pehrson, the Experimentel Station, P. O. Box 234, Veterinary Institute, S-532 00 Skara, Sweden.