SEASONAL FLUCTUATIONS IN SERUM TOCOPHEROL CONCENTRATIONS IN BOVINE AND OVINE SAMPLES SUBMITTED TO VETERINARY INVESTIGATION CENTRES

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Introduction

The measurement of -tocopherol concentration in serum is considered to be a satisfactory method for assessing the vitamin E status of sheep (Fry and others 1993; Njeru and others 1995) and cattle (Njeru and others 1995). Values less than a marginal band of 1.0-1.5 mg/l (2.4-3.2 mol/l) are regarded as being deficient for ruminants (McMurray and Rice 1982; Pehrson and others 1986). Evidence is accumulating that recommended dietary concentrations of vitamin E (25iu/kg DM - Agricultural Research Council 1980) do not always maintain normal plasma -tocopherol concentrations in sheep (Suttle - personal communication) and that proprietary injectable preparations containing vitamin E can give very short lived responses in initially deficient lambs (L.Stubbings, personal communication). Vitamin E is required in far larger quantities than other vitamins and there is concern that supplements used during winter feeding may be inadequate for cattle as well as sheep. Seasonal fluctuations in vitamin E status were therefore investigated retrospectively by collating the data for samples from cattle and sheep submitted for analysis to the Veterinary Investigation Services (VIS) in the United Kingdom.

Materials and Methods

The serum samples were submitted for analysis by veterinary practitioners via their VIC and analysed on the day of receipt. Samples collected in England & Wales were analysed at Shrewsbury VIC (SVIC) and samples in Scotland by the Clinical Biochemistry Unit of the Moredun Research Institute (MRI), Edinburgh.

Samples were also taken from a flock in the Eastern Counties where vitamin E responsive disease had been seen in previous years. A group of ewes were serially sampled at 2 monthly intervals and the vitamin E concentration in serum measured. Serum was treated with ethanol and the vitamin E extracted into hexane. The mixture was The hexane layer removed for High Performance Liquid Chromatography (HPLC). Vitamin E was detected in eluent by fluorescence with excitation at 296nm and measurement at 330nm in both laboratories. Inter laboratory comparisons showed good agreement between methods.

Animals were considered to be deficient in vitamin E if the serum contained less than 2.4 mol/l and comparisons between month of sampling, species and laboratories were assessed by the Chi-square test.

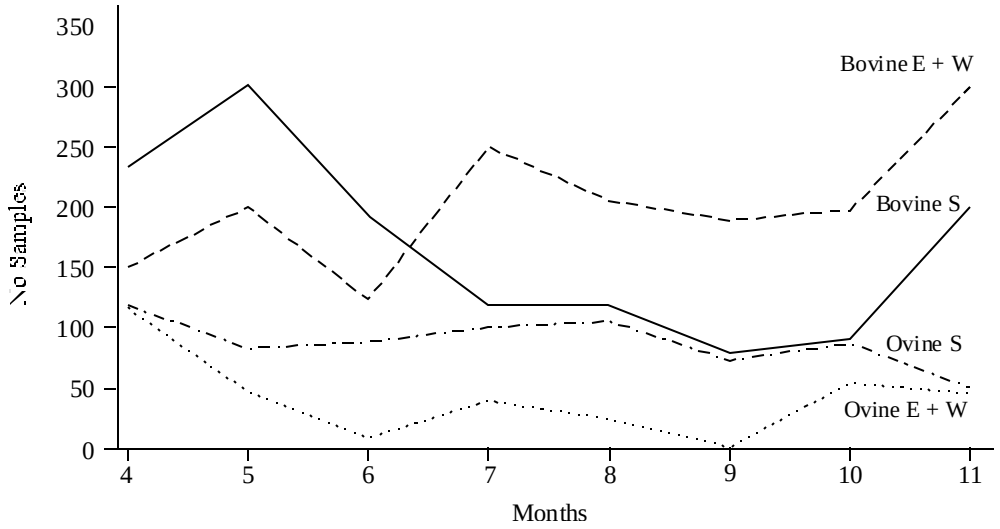

Figure 1. Number of samples submitted during 1995 from cattle and sheep in Scotland (S) and England and Wales (E+W).

Results

The number of samples submitted for analysis each month (Figure 1) showed a slight seasonal variation particularly in the sheep samples in England and Wales (E+W). Over 600 analyses / month were requested in April, May & November and around 400 in September & October. Bovine samples outnumbered Ovine by 4:1 in E+W and 2:1 in Scotland. In total 3022 bovine and 1084 ovine samples were examined.

The population distributions in cattle and sheep were skewed towards low values in the earlier months but normal in summer. The seasonal fluctuation in average values is therefore better indicated by median values as shown in Figure 2. The median values for cattle and sheep in E+W showed marked increases to peak values in August followed by a decline, the cattle attaining peak values twofold greater

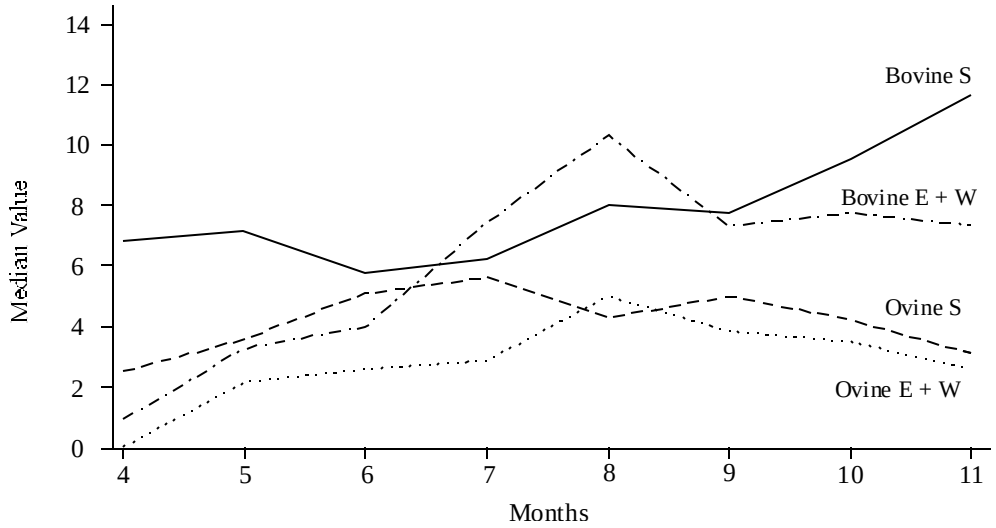

Figure 2. Medians of Vitamin E levels in cattle and sheep from Scotland (S) and England and Wales (E+W) during 1995

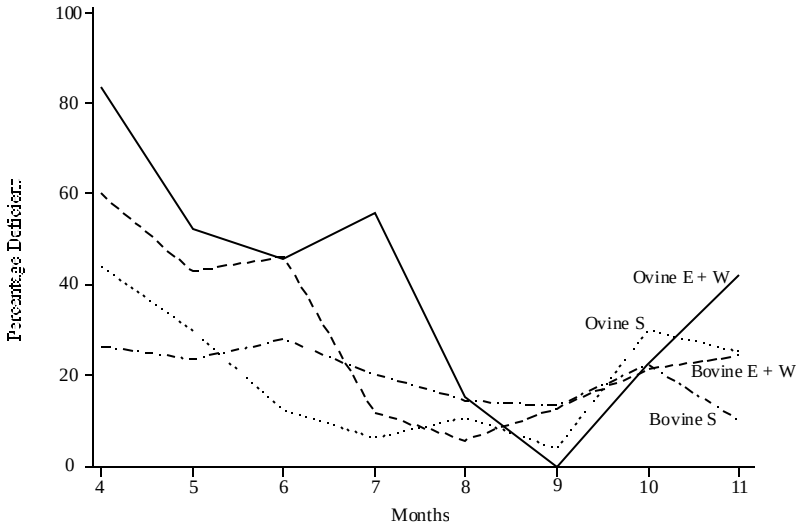

Figure 3. Proportion of Vitamin E "deficient" values (< 2.4 μmol/l) by species and source in 1995.

than those in sheep. In Scotland the seasonal fluctuations in sheep was less marked with an earlier peak in July while cattle showed an Autumn rise.

The proportion of "deficient" values recorded for each species and centre is shown in Figure 3. Vitamin E deficiency was most prevalent in April and more prevalent in E+W than in Scotland. There were marked seasonal declines in serum levels in cattle in E+W and in sheep in both areas until September but it was July-August before the prevalence fell below 20% in E+W. Bovine samples showed a different trend, one of slow steady decline from April to November.

In the serially sampled ewes (Figure 4) the vitamin E levels were adequate in November but had fallen to deficient levels by January and even further by March.

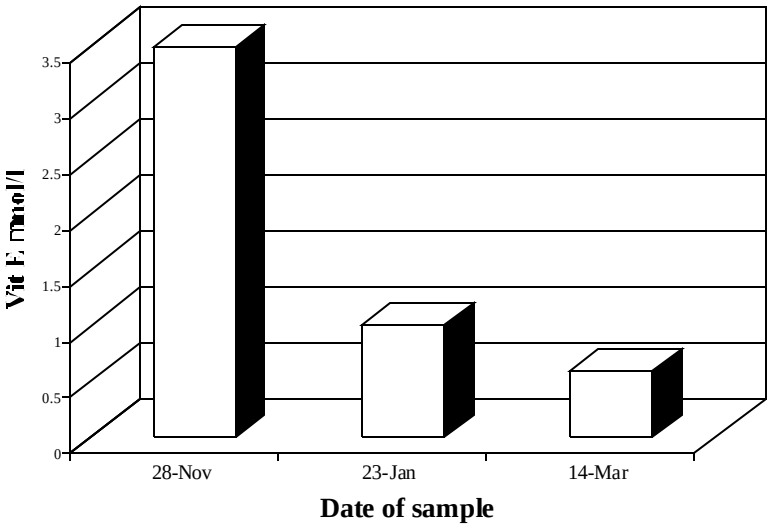

Figure 4. Mean Vitamin E levels in sheep sampled each two months between November and March.

Discussion

There are obvious limitations to the interpretation that can be placed on data that is prone to bias. The samples assayed were taken from animals with present or anticipated health problems in which low vitamin E status was believed to be a contributory factor. The seasonal fluctuation in the number of samples submitted did not run parallel to the prevalence of deficiency (Figures 1 v Figure 3) indicating that the practitioners' accuracy of diagnosis or prognosis was not constant. The seasonal fluctuations in the proportion of subnormal levels will reflect the vitamin E status of the wider healthy populations of cattle and sheep if the accuracy of diagnosis and prognosis is low and vitamin E status is not influenced by other disease states. Many of the requests were for glutathione peroxidase and copper as well as vitamin E indicating uncertainty as to the cause of the disorder. With serum copper, seasonal fluctuations in the proportion of subnormal values in a previous survey were the same in samples submitted for analysis other than copper, as for those where copper assays were specifically requested (Suttle and Small, unpublished data). This was also seen in pooled herd samples (Bain and others 1986).

There is no doubt that large numbers of sheep and cattle have a subnormal vitamin E status over the winter/spring period throughout the country and that this will leave the young prone to white muscle disease and older cattle to acute myoglobinuria at turnout. Both diseases may involve selenium deficiency and susceptibility to oxidant stress from polyunsaturated fatty acids in spring pasture (McMurray and Rice 1982). The slow improvement in vitamin E status of cattle and sheep in England and Wales long after the onset of grass growth may reflect a high turnover of the vitamin in a situation of oxidant stress.

The presence of such high proportions of sheep and cattle in 'at risk' populations with low serum tocopherol values probably reflects the inadequate levels of vitamin E in supplements meant to avoid deficiency. Assuming that ruminants sometimes require >30iu/kg DM in the diet (i.e. >300mg tocopherol/d for an adult dry cow), the injectable preparations on the market providing 1.4- 2.7 mg/kg liveweight (about 0.7 - 1.4g tocopherol for an adult cow at recommended doses), are clearly inadequate for long term prevention. A mineral/vitamin supplement must contain 1200 iu E/kg DM when ingested as 2.5% of total food intake to meet this daily requirement but most supplements contain far less. Wider use of large oral doses of vitamin E - measured in grams (say 5g/100kg LW) not milligrams, is recommended.

Contrasts between species and centres are difficult to interpret because of the lack of information concerning the animal sampled. The proportion of beef cattle in the bovine populations sampled may vary between centres. In the sampled sheep population there are likely to be changes in age distribution of the samples submitted in the spring months and a later onset of grazing in Scotland. The distinctive pattern shown by Scottish cattle may reflect a common husbandry system in which outwintered cattle are fed largely on vitamin E rich silage. It is suggested that more detailed information is transmitted to the central analytical laboratories with such samples (e.g. age, sex, housed or grazing) to facilitate interpretation. By making fuller use of diagnostic samples, animal experimentation can be minimised and research and diagnostic priorities set. Routine requests for profiles including vitamin E analysis in samples taken from July to October should be discouraged.

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