DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

CSG 15

Research and Development

Final Project Report

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Project title	Review of Vitamin E Deficiency Survey (Project LS3401)
DEFRA project code	LS3635
Contractor organisation and location	British Society Of Animal Science PO Box 3 Penicuik Midlothian EH26 0RZ
Total DEFRA project costs	£ 2555.32
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Executive summary (maximum 2 sides A4)

This project reviewed the Vitamin E Deficiency survey of UK farmers run by Reading University though the National Sheep Association and funded by DEFRA in 2000 (project code LS3401). The chief reviewer discussed the survey with Prof. John Arthur of the Rowett Institute, Aberdeen and consulted current and in-press literature and with colleagues world-wide to comment on the survey criteria and its results.

The six recommendations cover:

A) information that can be provided as guidelines to the UK Sheep industry
B) areas of research which could be considered for funding by DEFRA and other funding bodies that may lead to a better understanding of Vit E /Selenium problems and likely management strategies that could overcome this seroius threat to the welfare and production of lambs in the UK sheep industry.

Project title	Review of Vitamin E Deficiency Survey (Project LS3401)	DEFRA project code	LS3635

Scientific report (maximum 20 sides A4)

Reports attached
Please press enter

Report by J J Robinson, SAC, Animal Biology Division, Aberdeen on

An Epidemiological Survey of Vitamin E Deficiency in the UK Sheep Flock

(MAFF project code LS3401)

Background to the approach taken to evaluate the survey

The survey has been evaluated against the following background information:

- In its role as an essential structural component of lipid biomembranes, Vitamin E protects cells against the damaging effects of lipid peroxides and free radicals that are produced during oxidative metabolism.

- The 'free radical' protective qualities of Vitamin E are complemented by those of selenium in the form of the selenoenzymes, including 4 glutathione peroxidases and 3 thioredoxin reductases (Underwood & Suttle, 1999).

- In the case of white muscle disease (WMD) concurrent deficiencies of Vitamin E and selenium are required to produce the disease (Combs, 1998).

- Selenium-dependent deiodinase enzymes convert thyroxine (T_4) into the active thyroid hormone, tri-iodothyronine (T_3) in extrathyroidal tissues, including the brown adipose tissue (BAT) of the newborn lamb (Arthur & Beckett, 1994). Low T_3 results in a failure of transcription of the uncoupling protein gene required to induce the heat-generating capacity of BAT mitochondria immediately following the birth of the lamb.

- Overt signs of Vitamin E/selenium deficiency are often non-specific (eg lack of vigour at birth, ill-thrift and poor growth). Even in the case of WMD, visual symptoms are often similar to those for joint-ill, spinal abcesses and pneumonia. Biochemical measurements involving the status of both nutrients are therefore essential in order to confirm diagnosis (Suttle & Jones, 2000).

Critical assessment of the survey

- The accuracy with which Vitamin E deficiency was defined is critical to the value of the survey. In section 4.8 (page 11) it is stated that 'the typical signs reported by Stubbings (1999) were adopted and adapted for use in the questionnaire'. This reference is not, however, listed in the report and no information is presented to link typical signs of

Vitamin E deficiency with the biochemical measurements considered by Suttle & Jones (2000) to be essential in confirming diagnosis.

- In Case Study 1 (page 12) reference is made to a 'recent case' in which 25% of 8 – 12 week old lambs developed mild lameness/stiffness in their left fore leg which responded within 48 hours to 'vitesel' injection. The question is asked 'Why always left fore?' The observation is obviously interesting but should perhaps be viewed in the context that mild fore leg lameness can occur following neck injections. Due to the nature of sheep handling facilities on farms, a within-farm 'same side' injection routine could easily occur. If so, could it generate a localised muscular dystrophy and radial paralysis, via tissue damage at the injection site, in animals of marginal Vitamin E/selenium status? Although not yet proven for sheep (Suttle & Jones, 2000) there is the notion that Vitamin E/selenium deficiency may impair immune cell function and antibody production following vaccination, (see references cited by Suttle & Jones, 2000), thus implying a demand for these nutrients in the immune response. Could there be a vaccination-induced increase in the demand for Vitamin E/selenium that is of sufficient magnitude to trigger a muscular dystrophy?; 8 to 12 weeks of age coincides with the recommended time for active immunisation against the *Colostridial* and *Pasteurella* diseases. Up to this age protection from disease is afforded by colostral-derived passive immunity (Lewis, 2000).

- The inherent limitations of the methods used to assess Vitamin E deficiency means that caution must be exercised in the interpretation of information on its incidence in relation to farm type (lowland, upland and hill), flock management (entirely outdoors *vs* part-time indoors) and feeding strategy (roughage type and concentrate ingredients). In this regard there are interesting associations between the incidence of what are regarded, within the survey, as 'typical symptoms' of Vitamin E deficiency and the preceding factors. These merit comment and are:-

  (a) A higher incidence in hill and upland farms than lowland farms.

  (b) Greater prevalence of 'typical symptoms' following the introduction of a period of in-door housing, with lambs from ewes housed for more than 4 weeks pre-lambing being at greater risk than those from ewes housed for less than 4 weeks.

  (c) An increased incidence on straw-based diets.

  (d) In out-door flocks a doubling in the incidence in silage-fed as opposed to non-silage-fed ewes.

The higher incidence of 'typical symptoms' in hill and upland as opposed to lowland farms is understandable in that a higher proportion of hill and upland farms are in known selenium deficient areas; this in turn would be expected to accentuate the expression of Vitamin E deficiency.

The authors of the survey suggest that the greater incidence of typical symptoms of Vitamin E deficiency in housed flocks may be due to the likelihood that they are 'pedigree animals of a fast-growing breed (potentially a pre-disposing factor), ewe-lambs or multiple lamb-bearing ewes' (bottom of page 21). The assumption presumably is that ewe-lambs and multiple lamb-

bearing ewes are more susceptible to deficiency yet, within the survey, there was no clear evidence for a litter size effect (section 5.5, page 26).

The observation of a link to housing in the expression of 'typical symptoms' of Vitamin E deficiency could be explained by an increase with housing in the incidence of other diseases (joint-ill, spinal abcesses, pneumonia) with similar symptoms to those for Vitamin E deficiency. Alternatively, it could reflect a causal relationship linked to the stress of in-door housing or inadequacy of specific dietary nutrients. In this regard it is of interest that in addition to their increased reliance on straw and silage as opposed to hay as forage, a higher percentage of housed than outdoor flocks (18.2 *vs* 8.5; Table 34, page 23) used home-mixed cereals. If the use of home-mixed cereals to improve the late-pregnant ewe's energy status was not accompanied by adequate Vitamin E supplementation, it would reduce maternal tissue catabolism (an energy deficit-driven process) and thus decrease the release of Vitamin E from the mobilised adipose tissue where it is stored. The net effect would be a reduction in colostral Vitamin E from which the newborn lamb is dependent on its supply as Vitamin E does not cross the sheep placenta in significant amounts (reviewed by Robinson *et al*, 2002).

The increased incidence of 'typical symptoms' of Vitamin E deficiency in lambs from ewes given straw-based diets is clearly important in view of the extent to which barley straw is now used as the sole roughage in the diet of late pregnant ewes kept indoors. However, in the absence of information, in the survey, as to whether the straw-based diets were adequately supplemented in order to correct the low Vitamin E concentrations it is not possible to separate a direct dietary-induced deficiency of Vitamin E from an indirect one, caused perhaps by increased stress associated with inadequate supplementation of a poor quality forage (barley straw) with energy, protein and other essential nutrients.

Where silage is the sole forage, Vitamin E status is likely to be highly variable depending on the maturity of the crop at harvesting, the extent of wilting and the nature of the ensiling process. While these factors decrease the Vitamin E content of silages and therefore may contribute to the increased incidence of the 'typical' Vitamin E deficiency symptoms observed in outdoor ewes given silage in the survey, wilting and storage also reduce the Vitamin E content of hay (McDowell *et al*, 1996). It is therefore unclear why the incidence of symptoms of Vitamin E deficiency was much lower in lambs from ewes receiving hay as opposed to silage as their sole forage in late pregnancy. Although the survey results imply that silage is a poorer source of Vitamin E than hay they do not exclude the possibility that forage type (hay *vs* silage) may affect the availability and utilisation of Vitamin E. Additionally, some treatments of silages may cause destruction of vitamin E in an analagous fashion to hydroxide treatment of barley. Such processes can decrease tocopherol levels in feeds very rapidly, i.e. within 7-14 days. Thus when silage is stored for months there is a clear danger that the vitamin E will gradually disappear. Results could even be interpreted as indicating that the type of forage may affect the amount of Vitamin E needed at tissue and cellular levels. These are speculations that can only be resolved by combining direct measurements of forage Vitamin E contents with biochemical measurements of Vitamin E and selenium status of the ewe and the content of the vitamin in her colostrum.

- The comments (pages 24 and 25) from veterinary surgeons are interesting and informative with the majority of those involving forage quality, conservation methods (forages and cereals) and the use of home-grown cereals and home mixing of concentrates, bearing out the known scientific findings on factors influencing the Vitamin

E status of feeds. The comment that the problem has been accentuated since the implementation of a reduction in fishmeal-based protein in ewe diets is not surprising since fishmeal is rich in both Vitamin E and selenium. Furthermore when dietary supplements of fishmeal are given to ewes that are in energy deficit (the commercial norm) during late pregnancy they enhance the yield of colostrum and therefore the amount of colostral Vitamin E available to the newborn lamb.

- The observation in the survey that lambs born to Suffolk and Texel sires tended to be more susceptible to Vitamin E deficiency is supported by anecdotal information claiming greatly improved vigour in pure-bred Suffolks following Vitamin E/selenium supplementation above the accepted norm. Such claims, however, require scientific verification.

- The comment on page 28, under climate factors, that 'wet weather exacerbates the problem' presumably is a direct result of increased stress on the animal. To my knowledge the Vitamin E requirement to combat stress has not been defined. The claim that the problem also occurs 'on good grass in the summer' may reflect a cancelling out of the high Vitamin E content if fresh grass with its accompanying high anti-oxidant demands arising from high polyunsaturated fatty acid (PUFA) concentrations. In contrast to the stress situation, here there is a well-recognised need to increase Vitamin E intake above the recommended 1mg/day/kg ewe weight for maintenance and 5 mg/kg of milk, by a further 3 mg of Vitamin E per g of PUFA in the diet (Putman & Comben, 1987).

- In the section on 'Use of fertilizers' (5.10, page 28) reference is made to the success of 'pasture applications of selenium in deficient areas'. This approach is used in New Zealand where the major advance has been the development of selenium encapsulation techniques (prilling) that make the selenium safe to handle (Sykes, A R, 2002, personal communication). An unreferenced comment is also made in this section to the beneficial effect of seaweed dressings to soil but here caution must be exercised as recent studies show that seaweed also contains high concentrations of arsenic (Feldmann *et al*, 2000).

- Although the greater evidence of stillbirths in flocks producing lambs showing typical signs of WMD is as expected, the associated increase in abortion observed in the survey is more difficult to explain. Information on the incidence of retained placentas following normal lambing would have been useful in identifying Vitamin E/selenium deficiency as the cause; a high incidence would point to a direct involvement of the deficiency.

- The reference on page 30 to a 'decreased resistance to infection as a consequence of Vitamin E deficiency', although appearing to be supported within the survey by the associated increase of diarrhoea in lambs, should be interpreted with caution. While it is often assumed that Vitamin E deficiency reduces disease resistance convincing evidence in sheep is still lacking (Suttle & Jones, 2000).

Recommendations

- Sound biochemical measurements are available for the diagnosis of Vitamin E/selenium status. Ranges for diet, serum and tissue concentrations of Vitamin E and selenium in which expected benefits from supplementation can be expected have been tabulated (Suttle & Jones, 2000; Table 51.1, page 332). These should be adopted, in the first instance, as diagnostic criteria. In addition, wherever possible vitamin E status should be determined as alpha tocopherol concentrations in sheep particularly since isomers such as gamma tocopherol occur in feedstuffs.

- Vitamin E/selenium deficiency occurs widely in major sheep-producing countries such as Australia and New Zealand and methods of combating it under extensive conditions have been developed (eg Whelan *et al*, 1994; Sykes, A R, 2002, personal communication). These should be studied for their application under UK conditions.

- There are sufficient examples from experimental studies that stress is an important predisposing factor in Vitamin E/selenium deficiency. These examples not only include stress at the level of the whole animal (McDowell *et al*, 1996; Suttle & Jones, 2000) but also stress imposed at the cellular level, as occurs for example when semen is cryopreserved (Anderson *et al*, 1996). There is therefore a need for quantitative information on the effects of stress-inducing factors such as climate and flock management procedures (indoor housing, turn out to pasture, gathering, handling etc) on the additional anti-oxidant demands for Vitamin E and selenium at cellular level. Even if this information could only be obtained with a crude level of accuracy that would enable general recommendations to be made for the likely additional requirements of Vitamin E and selenium for each of the main sheep production systems it would be a major advance on the existing situation.

- Within pedigree flocks, in which there is general uniformity between animals in nutrition and management, there is often a significant proportion of ewes in the flock with an unexpectedly low Vitamin E/selenium status. This prompts the question 'Is there genetic polymorphism for Vitamin E and/or selenium requirements'? Although the survey did not identify breed differences in the incidence of 'typical' Vitamin E deficiency symptoms, this does not rule out the possibility of genetic polymorphism. Such polymorphisms occur in selenoenzymes in other species (Forsberg *et al* 2000)

- Although there are positive correlations between the concentrations of Vitamin E in plasma and in key body tissues (Hoppe *et al*, 1993; pig data), it is not known whether the composition of, for example, skeletal muscle (lipid amount and its fatty acid composition) influences either the availability of, or requirement for, Vitamin E at the cellular level. Also, observations such as those in the present survey which indicate effects of forage type on the incidence of deficiency symptoms raise questions regarding possible effects of diet type on Vitamin E requirements.

- An absolute requirement for any future surveys or research which addresses vitamin E deficiency in the UK sheep flock is that there is an element of confirmation of the actual vitamin E intake and either plasma or tissue concentration in the animals. As has been mentioned already several symptoms that point to vitamin E deficiency can result from other ailments in the animals. Misidentification of vitamin E related disorders could lead to association with management conditions and diets that actually have nothing to do with the disease.

Acknowledgement

This report was drafted by J J Robinson following discussions with Professor John Arthur, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB.

References

Anderson, J M L, Ap Dewi, I & Axford, R F E (1996). The effect of selenium supplementation on fresh and frozen ram semen. Animal Science 62: 672.

Arthur, J R and Beckett, G J (1994). Roles of selenium in Type 1 iodothyronine 5'-deiodinase and in thyroid hormone and iodine metabolism. In Selenium in Biology and Human Health. [Burk, R F editor, pp 95-115] Springer, New York]

Combs, G F Jr (1998). In The Vitamins. Fundamental Aspects in Nutrition and Health. 2nd Edition, Chapter 7, page 205. Academic Press, San Diego.

Feldman, J, John, K & Pengprecha, P (2000). Arsenic metabolism in seaweed-eating sheep from Northern Scotland. Fresenius J Anal Chem, 368: 116-121.

Forsberg, L. de Faire, U, Marklund,S L, Andersson, P M, Stegmayr, B & Morgenstern, R. (2000) Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. *Blood Cells Molecules And Diseases* 26:423-426,

Hoppe, P P, Schöner, F-J, Wiesche, H, Stahler-Geyer, A, Krammer, J & Hochadel, H (1993). Effects of graded dietary α-tocophenol supplementation on concentrations in plasma and selected tissues of pigs from weaning to slaughter. J Vet Med A 40: 219-228.

Lewis, C J (2000). Clostridial diseases. In Diseases of Sheep. 3rd Edition. [Martin, W B & Aitken, I D, Editors; pp 131-143] Blackwell Science, Oxford.

McDowell, L R, Williams, S N, Hidiroglou, N, Njeru, C A, Hill, G M, Ochoa, L & Wilkinson, N S (1996). Vitamin E supplementation for the ruminant. Animal Feed Science and Technology 60: 273-296.

Putman, M E & Comben, N (1987). Vitamin E. Veterinary Record 121: 541-545.

Robinson, J J, Rooke, J A & McEvoy, T G (2002). Nutrition for conception and pregnancy. In Sheep Nutrition, pp 189-211 (Freer, M & Dove, H, Editors). CABI, Wallingford, UK.

Suttle, N F and Jones, D G (2000). Micronutrient imbalance. In Diseases of Sheep. 3rd Edition. [Martin, W B and Aitken, I D, Editors; pp 332-344] Blackwell Science, Oxford.

Underwood, E J and Suttle, N F (1999). The Mineral Nutrition of Livestock. 3rd Edition, page 426. CABI International.

Whelan, B R, Peter, D W & Barrow, N J (1994). Selenium fertilizers for pastures grazed by sheep. 1. Selenium concentrations in whole blood and plasma. Australian Journal of Agricultural Research 45: 863-875.