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FEED ADDITIVES

Implications of Selenium in Large Animal Nutrition

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White muscle disease has been produced in lambs by feeding their dams prenatally and through lactation on an alfalfa hay–oats diet containing less than 0.02 p.p.m. Se, and has been prevented by raising the dietary Se level to 0.06 p.p.m. or more. Diagnoses of muscle damage were made by histological examination and by demonstration of elevated, though extremely variable, serum glutamate–oxalacetate transaminase levels. Provision of Se in a single dose to protect throughout pregnancy was effective via the parenteral route when a slow-absorption vehicle was used, but less effective when a similar amount was given orally, as sodium selenite, in aqueous solution. Levels of Se in the blood of lambs closely approximated that of their dams in the respective treatment groups. Whole blood levels of 0.11 p.p.m. Se in the ewes and 0.12 p.p.m. Se in the lambs were found compatible with WMD prevention in this study.

For many years, selenium was viewed by nutritionists solely as a toxic material from the point of view of potential hazards to livestock (*32*). It is, therefore, particularly interesting that recent work has attributed beneficial functions to this element. For example, minute amounts of selenium added to the diet will, under varying circumstances, prevent necrosis of the liver of rats (*23*), exudative diathesis of chicks (*25*), multiple necrotic degeneration in mice (*5*), liver necrosis in pigs (*8*), and white muscle disease in lambs (*15, 20*). In addition, there has been evidence presented indicating a positive growth response in lambs to selenium supplementation of the ewe (*6, 14, 18*), although other data show that such responses are not consistently obtained (*12, 26, 34*). Also, in Canadian experiments, administration of subtoxic levels of selenium to sheep produced an increase in wool fiber thickness and total fleece weight (*29*). Reviews on the subject of selenium in nutrition have been prepared by Hashem (*11*), Sharman (*27*), Schwarz (*22*), Cousins and Cairney (*4*), and Scott (*24*), among others.

Experiments in this general area of interest carried out at Oregon State University have been oriented toward the control of white muscle disease (WMD) in young ruminants, and have involved diets composed of natural feedstuffs. The procedures followed have been described in some detail by Schubert *et al.* (*21*). While it has been tacitly accepted by the investigators that the disease is of dietary origin, the precise nature of the nutrient interrelationships has not been described, due partly to the complexity of dealing with diets composed of crude feedstuffs, and more particularly to what Schwarz has termed "(the) elusive quality about the element" which has made selenium analysis most difficult (*22*). This paper describes an experiment designed to demonstrate relationships between selenium content of forage

given pregnant ewes, the blood of their lambs, and incidence of white muscle disease in the latter.

Experimental

Sixty crossbred range ewes secured from ranches where WMD was not a problem, were allocated to five groups of 12 each, and after breeding were placed on daily ration treatments as shown in Table I.

The criterion for selection of the hays used in the Control and Basal rations was documented evidence of absence or presence of WMD in animals fed hays produced on the same ranches over a number of years. The purpose of the oats was to serve as a convenient carrier for selenium supplementation in Lot 3, and they were fed unsupplemented throughout all other groups. Previous experimentation had shown local oats to be innocuous as far as WMD was concerned (*19*), and chemical analyses of a representative sample showed Se content to be only 0.01 p.p.m. Feeding of the test rations began approximately 60 days after breeding, and continued throughout pregnancy and at least 6 weeks postpartum. The rations were fed twice daily in equal portions, and water and iodized salt were available *ad libitum*. Selenium analyses were obtained on the two types of alfalfa hay (Control and Basal) using neutron activation analysis with radiochemical separations.

Weights were taken on all lambs at birth and at 2 and 6 weeks postpartum. At 6 weeks, the lambs were slaughtered and examined both grossly and histopathologically for evidence of WMD. Sections of heart and skeletal muscle from constant sites were fixed in neutral formalin and subjected to hematoxylin-eosin and von Kossa's silver nitrate stains. At this time also, blood samples were drawn and subjected to analyses for serum glutamate–oxalacetate transaminase (SG-OT) levels as related to muscle damage (*2*), and for Se.

Results

The selenium relationships between the forage consumed by the ewes, the blood of the ewes, and the blood of their lambs at 6 weeks of age are presented in Table II. Selenium analyses on whole blood were obtained by the method of Cousins and Cairney (*4*) and were performed on composite samples including equal volumes from all ewes or all lambs in each treatment lot.

Further insight into the effectiveness of the various treatments is given by consideration of WMD incidence in the lambs. Two criteria were used to evaluate myopathy—histopathological examination of samples of heart and skeletal muscle, as described, and SG-OT levels, expressed in Sigma-Frankel units (*28*). Data for both techniques are presented in Table III.

The SG-OT data are particularly useful in defining the extent and severity of muscle damage. That there was considerable variation in this regard is evident from the large standard error figures listed following the means. While no lesions were detectable in any of the treated groups (Lots 3, 4, 5), there was an apparent elevation of SG-OT levels in Lot 4 lambs, whose mothers received the single oral dose of 30 mg. Se, over the Control group lambs. This elevation was not nearly so great as that which occurred in the lambs of the unsupplemented Basal group, however.

A further criterion of the nutritional effect of Se may be the rate of growth of the young in the various treatment groups as shown in Table IV.

There was no significant improvement (as determined by analysis of variance) in this trial of growth rate of lambs from ewes which received additional Se, either through the forage or by oral or parenteral supplementation. This differs with previous observations at this Station (*18*) but may have been affected both by individual genetic differences in the small test groups and by incidence of multiple births, which was lowest in the Basal lot.

Discussion

The fact that neutron activation analysis did not establish the exact Se content of the Basal hay makes estimation of complete Se intakes impossible. However, one can calculate from the limits set on the data that the level of Se was not more than 0.02 p.p.m. If a level of 0.02 p.p.m. of Se is arbitrarily assigned to this hay, the total amounts of the element given per ewe in the various treatment groups approximates the following: Lot 1, 17.2 mg.; Lot 2, 5.0 mg.; Lot 3, 30.9 mg.; Lot 4, 35.0 mg.; and Lot 5, 35.0 mg. (These figures are computed for a feeding period of 92 days prepartum and 42 days postpartum, at the levels of Se listed in Table I.) It is thus possible to establish an approximate level of Se requirement for protection against WMD of 0.13 mg. daily, or 0.06 p.p.m. Se in the diet, based on the lowest protective level, achieved in Lot 1 on the Control hay. This latter figure is in reasonable agreement with the minimum amount required for consistent prevention of exudative diathesis in chicks (*16, 30*).

Close agreement in blood levels of Se between ewes and their lambs suggests an efficient system of maternal transport for the element. It is notable that the single administration of aqueous sodium selenite was insufficient to maintain either the blood levels of ewes or lambs

Table I. Descriptions of Treatment

Lot No.	Treatment
1	Control ration: 4 lb. alfalfa hay (nonconducive to WMD) plus 1/4 lb. oats/head/day
2	Basal ration: 4 lb. alfalfa hay (conducive to WMD) + 1/4 lb. oats/head/day
3	Oral selenium: Basal ration + 0.1 p.p.m. Se as Na_2SeO_3 fed continuously
4	Oral selenium: Basal ration + 30 mg. Se as Na_2SeO_3 in one dose
5	Injected selenium: Basal ration + 30 mg. Se as Na_2SeO_3[a] in one subcutaneous injection

[a] The parenteral treatment was prepared in a peanut oil base with 2% beeswax to delay absorption.

Table II. Se Levels in Forage and Blood of Ewes and Lambs

Lot No.	Forage Se, P.P.M.	Whole Blood Se, P.P.M	
		Ewes	Lambs
1	0.07	0.11	0.18
2	<0.02	0.02	0.02
3	<0.02	0.22	0.20
4	<0.02	0.06	0.06
5	<0.02	0.13	0.12

Table III. Incidence of WMD by Treatment

Lot No.	Total Lambs	WMD Lesions[a]		SG-OT
		Gross	Micro	
1	22	0	0	76 ± 4
2	17	7	1	478 ± 538
3	21	0	0	89 ± 47
4	21	0	0	184 ± 337
5	14	0	0	80 ± 4

[a] Data for micro lesions indicate those found on histological examination over and above those visible grossly.

Table IV. Lamb Growth Data

Lot No.	Mean Lamb Weights, Lb.		
	Birth	2 Weeks	6 Weeks
1	8.3 ± 2.0[a]	15.8 ± 2.5	26.4 ± 4.6
2	8.6 ± 2.2	15.5 ± 3.6	25.9 ± 6.9
3	8.3 ± 1.9	16.6 ± 3.6	27.6 ± 8.5
4	9.1 ± 1.9	15.8 ± 3.4	26.3 ± 7.4
5	9.2 ± 1.9	18.6 ± 3.3	31.4 ± 6.1

[a] Standard deviation.

in Lot 4 as high as those in Lot 1 on the Control hay. Lot 5 lambs, although their blood Se was lower than that of the Control group, showed no evidence of WMD and therefore may be considered to represent a satisfactory Se status.

The varying responses to different means of administration of Se are noteworthy. It would appear desirable, from the results of this experiment, to provide a continuous supply of this low level of Se. This was achieved by either continuous feeding (Lots 1 and 3) or injection in a slow absorption vehicle (Lot 5). Use of a single aqueous dose containing 30 mg. of Se (Lot 4) was not completely effective in preventing muscle damage, as evidenced by SG-OT data and by some evidence at necropsy. These findings are not surprising in the light of demonstrated losses of radioactive Se^{75} via exhalation [40% of injected dose in 10 hours (*9*)]; and by fecal excretion [51% of rumen infusion over 72 hours (*3*)]. McConnell and Portman (*13*) observed significant selenium exhalation as dimethyl selenide, and later the Wisconsin workers showed that exhalation losses of injected Se were almost doubled on a crude diet (as was fed in this study) over those when a purified diet was fed. On the other hand, Sharman (*27*) has stated that retention of Se has been greater when the element was provided in a "naturally occurring" organic form than when given as selenite.

Wide variability in SG-OT levels in lambs from WMD-affected groups as noted herein has also been experienced by other workers (*2, 33*). This variation on similar dietary regimes would appear to reflect other influences, including individual genetic differences and environmental factors having a bearing on the enzyme activity.

Comment should be made regarding possible involvement of Se in the reproductive processes. Data in Table III do not suggest consistently higher numbers of lambs born to those groups of ewes which received adequate Se supplementation. It would be unwise to draw conclusions from the lambing performances of these small groups of ewes during a single session; however, it may be noted that other investigators have made more extensive observations (*10*) indicating improved reproduction in sheep given Se supplementation.

While this experiment was not designed to investigate the mode of action of Se in the animal body, some speculation may be permissible. A considerable body of evidence has been accumulated implying that Se acts as an antioxidant. It has been recognized for years, for example, that minute amounts of Se will impart remarkable nondrying properties to linseed and tung oils (*32*). More recently, Bieri (*7*) has shown that low levels of dietary Se significantly reduce autoxidation of tissue lipides, while Olcott *et al.* (*17*) have suggested that selenomethionine may be modified in vivo to a derivative having antioxidant properties. On the other hand, English workers have ascribed to Se a specific, nonantioxidant function mediated through ubiquinone (*7*), although evidence exists which refutes the involvement of ubiquinone in muscular dystrophy of chicks (*31*).

In the present study, as in previous Oregon experiments with WMD, crude diets were used which presumably contained significant amounts of natural tocopherols. The efficacy of Se in preventing WMD in the young, as contrasted to ineffectiveness of α-tocopherol (*15*), may be interpreted as a demonstration of separate, specific functions for these two materials, or else as a reflection of different rates of transfer of them across the placental barrier from mother to fetus. Further accumulation of data concerning antioxidant levels of the natural feeds used and resulting animal tissues will be necessary to elucidate this point. It would appear from this work that muscle damage may be precipitated in young ruminants when the maternal diet contains less than 0.02 p.p.m. of Se even in the presence of (unspecified) amounts of natural antioxidant activity, and may be prevented when the Se content is raised to 0.06 p.p.m. or more.

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