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SERUM ENZYMES IN PREGNANT EWES AND THEIR NEWBORN LAMBS ON A VITAMIN E-DEFICIENT RATION

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Nutritional muscular dystrophy (NMD) in young lambs is a rather common disease in many parts of Norway. In some years the morbidity may be 30—40 % in the flocks, and as the mortality is relatively high, the disease causes great losses.

The present investigations were designed to see how far a winter feeding extremely low in vitamin E would cause NMD in pregnant ewes and their newborn lambs. Two experiments were performed during two successive years.

In many parts of Norway sheep are kept indoors during the winter for 6—7 months. The main food consists of hay, supplemented with certain concentrates, in the mating and lambing season. In the years after the second world war alkali-treated straw has become a very usual feed for ruminants, including sheep, in this country. The treated straw is a valuable foodstuff, rich in carbohydrates and with very good dietetic properties. It has, however, negative protein value and a total lack of all fat soluble vitamins.

Hay, even of good quality at harvesting, may also be a rather poor source of vitamins in the late winter and early spring, as the tocopherol and carotene contents show a heavy decrease during storage.

The experiments described are based upon the hypothesis that the main etiological factor of NMD in lambs in Norway is a vitamin E-deficiency. A survey of the literature on NMD in lambs is given by *Ribe* (1963). This report will deal mainly with the serum enzyme investigations, and the conclusions drawn are based upon the serum enzyme values.

Serum enzymes for diagnostic purposes

Since La Due et al. (1954) observed an elevation of a transaminase enzyme in serum by myocardial infarction in human beings, the value of serum enzyme determinations for diagnostic purpose has received considerable attention. Both in human and veterinary medicine some of the enzymes studied have proved to be of real diagnostic value for some groups of diseases. For cases of muscular dystrophy the deviation of the serum enzyme aspartate aminotransferase (AspAT)¹) is a good indicator of cell destruction.

An elevation of the serum AspAT level is, however, not specific for a muscle degeneration. A necrosis in other organs rich in this enzyme, e.g. the liver, will cause an increase in the serum as well. A differentiation between a muscular and a hepatic degeneration is possible in ruminants by means of determinations of a second serum enzyme, AlAT. In muscular dystrophy an elevation in both enzymes is observed, whereas a liver degeneration will not cause any increase in serum AlAT activity in ruminants (Cornelius et al. 1959, Setchell 1961). This is contrary to the findings in human beings, cats and dogs, where a liver complaint causes a considerable rise in the serum AlAT level.

A third serum enzyme, lactate dehydrogenase (LDH) has been determined in these experiments. A strong correlation is shown to exist between the serum levels of AspAT and LDH. Blincoe & Marble (1960) found a correlation coefficient of +0.97 in serum from 28 lambs on widely different transferase levels. An estimation of LDH will thus support the AspAT findings.

¹⁾ The nomenclature for enzymes suggested in the Report of the Commission on Enzymes (1961) is used in this paper. The report did not include suggestions for abbreviations, but new abbreviations are introduced elsewhere in accordance with the systematic and common names. Thus for two enzymes included in this study:

a) Aspartate aminotransferase, hitherto known as glutamate-oxalacetate transaminase (GOT), is now abbreviated AspAT.

b) Alanine aminotransferase, hitherto known as glutamate-pyryvate transaminase (GPT) is now abbreviated AlAT.

The close correlation between these two enzyme systems indicates that they have a common origin and that they will be released in the same proportion by destruction of tissue cells.

METHODS AND MATERIAL

Enzyme determinations

Blood samples were taken from the pregnant ewes every week during the winter in Experiment 1 and every second week in Experiment 2. From the lambs blood was collected at shorter intervals, mostly twice a week. The serum samples were analyzed on the same and following day.

AspAT and AlAT were determined by the method of *Reitman & Frankel* (1957) as described in Sigma Technical Bulletin No. 505. LDH was determined by the method of Berger and Broida according to Sigma Technical Bulletin No. 500.

Animals and feeding

In Experiment 1 6 adult Oxford Down ewes were placed indoors on October 9th. Until November 25th, when the mating season was over, the animals were given a balanced ration of hay, concentrates and alkali-treated straw. In the following period, which lasted till June 12th, the animals were given the experimental diet. Five of the 6 ewes became pregnant, and 9 lambs were born in the middle of April, one of which died immediately after birth. The average birth weight was 4.5 kg. In Experiment 2 12 ewes, 6 of Oxford Down and 6 of the Dala breed were used. The experimental period and the time of lambing were similar to those of the previous year. The animals were turned out to pasture on June 1st. The number of lambs born and birth weights are given in Table 1.

In Experiment 1 all the ewes received the same experimental diet. The daily ration consisted of 3 kg alkali-treated straw,

Table 1. Number of lambs born and birth weight in Experiment 2.

	No. of ewes	No. of lambs born	Total birth weight, kg	Mean birth weight, kg
Group 1	4	8	33.9	4.2
Group 2	4	5	25.3	5.1
Group 3	4	7	29.7	4.2

270 g dried skim milk, 1.5 g granulated cod liver oil, 12 g dicalciumphosphate and admission to licking stones with trace elements ad lib. The granulated cod liver oil had a content of 3.000 i.u. vitamin A, 600 i.u. vitamin D_3 per g, and was free from α -tocopherol.

Towards the time for lambing the ration of dried skim milk was increased, to meet the protein requirement for ewes in lactation.

In Experiment 2 the animals were divided into 3 groups. Group 1 was fed as described for Experiment 1. In group 2 the animals were given 10 g cod liver oil in addition to the same ration. Cod liver oil is widely used as a vitamin A- and D-supplement to a winter feeding. In this experiment it was of great interest to study the effects of its content of polyunsaturated fatty acids in a vitamin E-deficient ration. All the vitamin A was removed before use.

In group 3 the alkali-treated straw was substituted with hay. Hay and dried skim milk were given in amounts equal to the nutritional value of the ration in group 1. Group 3 was meant to serve as a control group. Analyses of the hay with respect to the α -tocopherol content in the spring showed, however, a value of 5 mg α -tocopherol per kg. This is a very low value, as analyses of Scandinavian timothy hay have shown a content of up to 60 mg α -tocopherol per kg (Nordfeldt & Ruudvere 1961).

RESULTS AND DISCUSSION

Experiment 1

Ewes. All the ewes behaved normally during the winter and gained weight, from an average live weight of 63.3 kg at mating time to 70.7 kg at time of lambing. In the late spring there was a tendency to wool napping.

The mean serum enzyme values of the ewes in different periods of the indoor feeding are presented in Table 2.

	From Oct. 9th to Nov. 25th	From Nov. 25th to April 15th	From April 15th to June 12th	The whole experimental period	No. of samples
AspAT AlAT LDH	110 ± 51 13.5 ± 3.6 1042 ± 201	74 ± 21 12.5 ± 4.7 729 ± 253	$\begin{array}{ccc} 95 & \pm & 46 \\ 12.3 \pm & 2.8 \\ 746 & \pm 212 \end{array}$	80 ± 31 12.4 ± 4.3 734 ± 242	150 144 150

Table 2. Mean serum enzyme values \pm s in ewes.

The serum enzyme values found in this experiment are in agreement with values of normal sheep found in other experiments at this institute. Aas Hansen (1964) found in 93 normal sheep a mean AspAT value of 74.7 ± 13.6 and an AlAT value in 64 animals of 17.4 ± 5.1 Sigma-Frankel units.

In our experiment there is an insignificant increase in the AspAT values in the periods before mating and after lambing. The elevations are, however, not so marked that they indicate any cell destruction. The cause of the increased serum enzyme levels in these periods might be the higher level of protein given. Schimke (1962) and Waldorf et al. (1963) have found in rats that the activity of enzymes of central importance in the catabolism of amino nitrogen increases with higher protein intake. Aas Hansen could not, however, find any augmented serum enzyme values in sheep fed with high amounts of a protein-rich concentrate mixture of equal parts of dried skim milk and soya bean meal.

In the experimental period, which lasted for 22 weeks, no clinical symptoms or enzymatic criteria of muscular dystrophy could be found in the maternal sheep. This is in agreement with the results of Wright & Bell (1964). They did not find any changes in the serum transferase levels in adult ewes given an α -tocopherol free diet for 18 weeks. In younger ewes (9 months) they found that the same diet produced elevated serum enzyme values within 9 weeks. The mean age of the ewes in our experiment was $2\frac{1}{2}$ years at the beginning of the experiment, no animal being younger than 18 months.

Lambs. In order to find the normal serum enzyme levels in young lambs, 11 control lambs from normally-fed ewes were tested and gave the following results:

AspAT 64 ± 15 ; AlAT 8.4 ± 3.0 ; LDH 738 ± 109 .

Blincoe & Marble (1960) found in 31 control lambs values of AspAT 56 ± 31 and LDH 1152 ± 297 (21 lambs) respectively.

As the lambs in the experiment became 3 to 4 weeks old the serum enzyme values increased rapidly and reached in some cases very high levels. Blood samples were collected twice a week, and if the AspAT values exceeded 1000 Reitman-Frankel units, the lambs were treated with an intramuscular injection of 45 mg α-tocopherol. After treatment the serum enzyme values rapidly decreased to normal levels.

Blincoe & Marble found in 17 lambs with muscular dystrophy mean values of AspAT and LDH 1290±1310 and 22310±10000 units respectively. The highest serum enzyme values in a single lamb observed in the present experiment were AspAT 6400, AlAT 480 and LDH 17600 units. Even with these greatly increased values the lamb showed no clear symptoms of muscular dystrophy.

Experiment 2

Ewes. Each of the 3 groups in this experiment included 2 Oxford Down ewes and 2 of the Dala breed. The ewes of the latter breed are of smaller size and did not gain weight to the same extent during the winter as the Oxford Down ewes. In the early spring the animals in group 2, which were fed with cod liver oil, lost their appetite, and 2 of the ewes showed increasing serum enzyme values. The cod liver oil supplement was abolished, and the 2 ewes treated with injections of 150 mg α -tocopherol.

Table 3.	Mean serum enzyme values \pm s in ewes in the experimental
	period from mating to turning out to pasture.

	No. of samples	AspAT	AlAT	LDH
Group 1	56	95± 75	14.5±4.5	802±129
Group 2	60	139 ± 122	15.6 ± 6.2	1015 ± 252
Group 3	56	76 ± 29	13.0 ± 4.1	757 ± 166

As shown in Table 3, the ewes in the cod liver oil supplemented groups had the highest mean serum enzyme values.

The animals in group 1 which received an α -tocopherol free diet showed slightly increased values in comparison with the hay-fed group.

Lambs. In this experiment too most of the lambs developed very high serum enzyme levels after 3 to 4 weeks. Cases were found in all 3 groups. Two of the lambs which first showed increased values (in groups 1 and 3) were treated with α -tocopherol. The remaining lambs were not treated, as it became obvious that the high serum enzyme levels culminated without treatment.

The lambs in group 2, whose mothers had received supplements of cod liver oil, showed the lowest increase in the serum enzyme levels. This was contrary to what might have been

carring out to Present.				
	No. of samples	AspAT	AlAT	LDH
Group 1	56	$427{\pm}375$	$52{\pm}55$	1890±1316
Group 2	39	159 ± 56	18 ± 16	1312 ± 355
Group 3	66	268 ± 235	37 ± 47	1664 ± 1204

Table 4. Mean serum enzyme values ± s in lambs from birth to turning out to pasture.

expected on this auto-oxidizable ration. Two ewes in this group, however, had been treated with a single injection of α -tocopherol about 1 month prior to lambing. Nevertheless, 2 of the 3 lambs born of these ewes showed distinct elevated enzyme values. A more reliable explanation is found in the relation between single and twin lambs. Group 2 had a greater percentage of single lambs than the other groups. Whether this fact is correlated to the cod liver oil supplement it is difficult to say, with such a limited number of animals, but in ewes given 4 % fish liver oil without additional vitamin E prenatal deaths of lambs have previously been found (Welch et al. 1958).

Table 5 shows the mean serum enzyme values in single and twin lambs in all groups in the period from birth to being turned out to pasture.

T a ble 5. Serum enzyme values \pm s in single and twin lambs.

-	No. of samples	AspAT	AlAT	LDH
Single lambs Twin lambs	42	92± 77	14±11	1235± 346
	119	370±513	46±29	1810±1236

From these data it is relevant to suggest that twin lambs more easily develop high serum enzyme levels than single lambs under conditions where the mother ewes have small reserves of vitamin E. Wright & Bell found that single lamb foetuses contained twice as high concentrations of Se^{75} in the organism as twin lambs, when the ewes received supplements of Se^{75} .

These experiments have, further, confirmed earlier observations of *Blincoe & Marble* that twin lambs are no more alike with respect to serum enzyme activity than single lambs selected at random.

Serum enzyme values in lambs put out to pasture

In order to see how the change from indoor feeding to pasturing would influence the serum enzyme activity in lambs, blood samples were taken from all lambs on the day when they were turned out to pasture, and on the 5th and 12th day thereafter. Two lambs, 1 from group 1 and 1 from group 2, were at this time killed for histological examination.

Table 6. Serum enzyme values \pm s in lambs before and after being put out to pasture.

Group	AspAT	AlAT	LDH
On day of tu	rning out to pasture		
1	217 ± 187	26 ± 26	1427 ± 304
2	$53\pm~28$	8.0 ± 5.7	1270 ± 226
3	118 ± 75	23 ± 17	1284 ± 232
After 5 days	at pasture		
1	849± 911	137 ± 178	3459 ± 489
2	632 ± 1046	72 ± 119	3349 ± 3911
3	449 ± 478	68 ± 81	2419 ± 2460
After 12 day	rs at pasture		
1	153 ± 102	28 ± 14	1219 ± 436
2	93 ± 29	27 ± 18	1375 ± 356
3	104 ± 37	$19\pm~8.0$	1263 ± 368

As will be seen from Table 6, a great increase has occurred in the activity of all the three enzymes investigated, and in all groups during the first 5 days at pasture. In the following 7 days, however, the values became almost normal without any treatment and without clinical signs of disease at any time.

There is reason to believe that the mean cause of the serum enzyme elevation at pasture is the physical activity and greater exertion during the first days.

A similar increase in the serum enzyme after physical exercise without any observable histological changes has been found in human beings by Schlang (1961) and by Halonen & Konttinen (1962) and in rats by Altland & Highman (1961) and Critz (1966). A possible explanation of the increase in enzyme activity may be an augmented permeability of the cell or mitochondrial membrane. This may be caused by catechol amines released from

the adrenal cortex into the circulation during exercise. In dogs, Highman et al. (1959) found raised transferase levels in serum after injections of epinephrine and norepinephrine. No histologic changes were seen, and the authors conclude that the cellular damage responsible for the rise in serum enzyme levels need not be sufficiently great to be demonstrable by the usual histological methods. Aas Hansen has observed higher serum AspAT values in cattle and sheep at pasture than when stall-fed. Young et al. (1965) found significantly higher concentrations of serum AspAT in sheep at pasture than in the same sheep when penned. For LDH the reverse results were found.

Since the increased serum enzyme values in most of the lambs fell to normal levels after 12 days at pasture, it is natural to suppose that the lambs had then become adapted to the existing pasture environment and that they were now adequately supplied with α -tocopherol through their dams' milk.

Experience in this country shows that clinical cases of muscular dystrophy in lambs are seldom found later than 14 days at pasture (*Ribe* 1964).

Post-mortem examinations

Two lambs with very high serum enzyme values, 2500—3000 RF units of AspAT and about 6000 units of LDH were used for histological examination. The lambs were killed about 14 days after the culmination of the serum enzyme values. No great pathological changes were found in the skeletal muscles. In both lambs there was observed a cell infiltration between the muscle fibres, but no demonstrable cell degenerations.

CONCLUSIONS

As far as elevated serum enzyme activity may be taken as an expression of tissue degeneration, the following conclusions may be drawn from these experiments:

- 1. A winter feed free from α -tocopherol does not necessarily give rise to nutritional muscular dystrophy in adult pregnant ewes. It does not seem to influence the birth weight or vitality of the lambs born.
- 2. Addition of cod liver oil to a vitamin E-deficient ration aggravates the deficiency and causes loss of appetite and increased serum enzyme values.

- 3. Lambs born from ewes fed on an α-tocopherol-deficient diet will develop considerable increases in serum enzyme activity after 2 to 4 weeks of age.
- 4. Treatment with 45 mg α-tocopherol i.m. will cause a very rapid decrease in the serum enzyme activity in lambs.
- 5. Even very high serum enzyme values in lambs may culminate and subside to normal levels without clear clinical symptoms and without treatment.
- 6. Twin lambs are possibly more susceptible to nutritional muscular dystrophy than single lambs.
- 7. A heavy increase in serum enzyme values occurs when lambs are turned out to pasture. This elevation is greatly diminished after 12 days at pasture.
- 8. Timothy hay may be very low in α-tocopherol in the spring. Given to pregnant ewes without vitamin E-supplements from other sources, it may cause increased serum enzyme values in young lambs.
- 9. Histological examination of muscle tissue from lambs with high serum enzyme values, may show only minor signs of degeneration.

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SUMMARY

A two-years experiment has been performed to investigate the effect of a vitamin E-deficient diet to pregnant adult ewes and their newborn lambs. 18 ewes and 28 lambs have been tested with serum enzyme determinations, and the conclusions drawn are based upon the serum enzyme values. Adult pregnant ewes do not develop increased serum enzyme values on a vitamin E-deficient winter diet, unless a supplement of cod liver oil is given. The lambs from ewes given a vitamin E-deficient ration, show a heavy increase of the serum enzyme values after 2 to 4 weeks of age. This seems to be more pronounced in twins than in single lambs. Even with very high serum enzyme values in lambs, clinical symptoms of muscular dystrophy may be scarce, and the enzyme levels may decline without treatment. Turning out to pasture causes a rise in serum enzyme values in lambs during the first 5 days. Within 12 days at pasture the values become almost normal. Histological examination of skeletal muscle tissue from lambs very high in serum enzymes, may show only small pathological changes.

ZUSAMMENFASSUNG

Serumenzymen bei tragenden Mutterschafen und deren neugeborenen Lämmern auf einem E-Vitamin-armen Futter.

In zwei Wintern wurden Versuche durchgeführt um den dystrophie-hervorrufenden Effekt eines E-Vitamin-armen Futters, verabreicht an tragende Mutterschafe und deren Lämmer, zu untersuchen. 18 Schafe und 28 Lämmer wurden mit Hilfe von Serumenzym-Bestimmungen getestet, und die hieraus gezogenen Konklusionen sind hauptsächlich auf die Serumaktivität von AspAT (GOT), AlAT (GPT) und LDH basiert.

Ausgewachsene trächtige Schafe zeigen keine Steigerung in den Serumenzym-Werten nachdem sie den Winter hindurch ein E-Vitaminarmes Futter bekommen haben — falls ihnen kein Zuschuss von flüssigem Tran gegeben wird. Die Lämmer, die geboren werden, sind von normaler Grösse und Vitalität. In dem Alter von 2 bis 4 Wochen tritt jedoch eine erhebliche Steigerung in den untersuchten Serumenzymen auf. Diese Steigerung ist mehr ausgesprochen bei Zwillingen als bei Einzellämmern. Deutliche klinische Symptome einer Muskeldystrophie können ebenfalls ausbleiben. Nach einer Behandlung mit 45 mg α -Tocopherol i.m. fallen die Serumenzym-Werte schnell zu dem normalen Niveau. Auch ohne Behandlung kann die Serumenzymaktivität sich normalisieren, es bedarf dann jedoch etwas längerer Zeit.

Beim Ausbinden auf die Weide tritt bei den Lämmern im Laufe der ersten Tage eine markante Steigerung in den Serumenzym-Werten auf. Das Niveau ist ohne Behandlung nach 1 bis 2 Wochen stark reduziert.

Bei einer Mikroskopie der Skelettmuskulatur von Lämmern mit sehr hohen Transferase-Werten wurden auffallend kleine pathologische Veränderungen festgestellt.

SAMMENDRAG

Serumenzymer hos drektige söyer og deres nyfödte lam på en E-vitaminfattig fóring.

Forsök er utfört i to vintrer for å undersöke den dystrofifremkallende effekt av en E-vitaminfattig diett til drektige söyer og deres nyfödte lam. 18 söyer og 28 lam er blitt testet ved hjelp av serumenzymbestemmelser, og de konklusjoner som er dratt, er vesentlig basert på serumaktiviteten av AspAT (GOT), AlAT (GPT) og LDH.

Voksne, drektige söyer viser ingen stigning av serumenzymverdiene om de står på en E-vitaminfri diett vinteren igjennom, såfremt de ikke blir gitt tilskudd av flytende tran. Lammene som födes, er av normal störrelse og vitalitet. Ved 2—4 ukers alder vil det imidlertid opptre en kraftig stigning av de undersökte serumenzymer, tilsynelatende mer uttalt hos tvillinger enn hos enkeltlam. Tydelige kliniske symptomer på muskeldystrofi kan likevel utebli. Serumenzymverdiene vil raskt gå ned til normalt nivå etter behandling med 45 mg α-tocopherol i.m. Også uten behandling kan serumenzymaktiviteten nomalisere seg, men da over noe lengere tidsrom.

Ved slipping på beite vil det i löpet av de förste dögn opptre en markant stigning av de undersökte serumenzymverdier hos lammene. Nivået vil uten behandling være sterkt redusert etter 1—2 uker.

Ved mikroskopi av skjelettmuskulatur fra lam med meget höye transferaseverdier, kan det finnes påfallende små patologiske forandringer.

(Received August 3, 1966).