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A SERUM ENZYME INCREASING EFFECT OF NON-TOXIC HERRING MEAL FED TO SHEEP

By
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A disease of cattle and sheep, often terminating fatally, occurred in this country under farm conditions in the early sixties. The disease was characterized by extensive necroses and degenerations of the liver. In a series of experiments at the Veterinary College of Norway, the disease was found to be due to the presence of a toxic agent in certain batches of herring meal (*Koppang* 1964, *Aas Hansen* 1964, *Koppang et al.* 1964).

The toxic principle, identified as dimethylnitrosamine (DMNA) by *Ender et al.* (1964) and *Sakshaug et al.* (1965) has been shown to be formed during the processing of the meal when nitrite is used as a preservative of the raw material.

In the experiments referred to, performed mostly on sheep, serum enzyme determinations were carried out at frequent intervals in an attempt to provide indices of the degree of liver damage (*Aas Hansen*).

The author of the present article had the opportunity to take part in and to duplicate some of the enzyme determinations. His attention to the actual problem was caught by the finding of highly elevated aspartate aminotransferase levels in some of the control animals receiving apparently non-toxic herring meal as well as in sheep fed meals causing severe liver injuries and death.

Koppang et al. suggested tentatively that the elevated enzyme activities in control animals were of muscular origin since lesions of skeletal muscles were seen in some instances. The possibility of environmental influences was mentioned: the fact that the animals were kept in rather narrow stalls with little or no exer-

cise. Calling attention to the rather great variations in serum aspartate aminotransferase levels of normal sheep, they concluded that their own findings provided no proof of toxic influences in the control groups.

The experiments described in the following were performed in order to provide further information about the effects of different qualities and quantities of feed proteins on serum or tissue enzyme activities in sheep:

1. Effects of high and normal dietary protein levels, and of different stalling systems.
2. Effects of different protein sources.
3. Effects of different levels of herring meal.
4. Effects of herring meals high and very low in dimethylnitrosamine.
5. Effects of an abrupted herring meal feeding.
6. Effects of herring meal feeding on serum lactate dehydrogenase isoenzyme distribution.
7. Effects of herring meal feeding on tissue enzyme activity.

Results of other experiments performed in this department on the same subject are briefly mentioned.

Activity of the following enzymes was determined:
Aspartate aminotransferase (EC 2.6.1.1) AspAT = GOT,
alanine aminotransferase (EC 2.6.1.2) AlAT = GPT,
total lactate dehydrogenase (EC 1.1.1.27) LDH, the five isoenzymes of LDH, and α-hydroxybutyrate dehydrogenase (HBD).

MATERIAL AND METHODS

Animals and feeding

Number and type of sheep, description of the diets, and duration of the feeding trials are presented under each experiment. All animals were kept indoors and individually fed at 7 a.m. and at 3 p.m. Blood was collected from the jugular vein at 9 a.m.

Analytical procedures

The enzymes were determined in serum obtained by centrifugation for 15 min. at 3,000 r.p.m. As far as possible analyses were performed within two days, the dehydrogenases preferentially on the day of sampling.

Analytical procedures were according to Sigma Technical Bulletins. For aspartate aminotransferase and alanine aminotransferase: Sigma Technical Bulletin No. 505 (1964). Sigma Frankel (S-F) units. For total lactate dehydrogenase: Sigma Technical Bulletin No. 500 (1960). Berger-Broida (B-B) units. LDH isoenzymes were determined electrophoretically according to the procedure described by *Baustad & Tollersrud* (1969) with the modifications reported by *Tollersrud* (1970). The isoenzymes are termed LDH_1 to LDH_5 in order from the anode.

α-hydroxybutyrate dehydrogenase was analysed according to Sigma Technical Bulletin No. 495 (1964), and the activity is expressed as Sigma units of HBD.

Supernatants of tissue homogenates were provided as previously described by *Tollersrud & Nafstad* (1970).

EXPERIMENTAL AND RESULTS

1. Effects of high and normal dietary protein levels and of different stalling systems

The experiment was carried out to investigate if the increased serum enzyme values which were previously observed in control animals fed non-toxic herring meal could be due to a relatively high daily intake of protein or, as suggested, be a consequence of the stalling system used.

Sixteen adult non-pregnant ewes at a live weight of about 60 kg were placed in spacious pens or on narrow stalls and grouped as follows:

	Penned	Stalled
High protein level	4	4
Normal protein level	4	4

The high protein level was provided by a diet consisting of 270 g herring meal, 100 g ground barley, and 800 g hay. According to chemical analyses of the foodstuffs the daily intake of digestible crude protein per animal was 205 g. In the normal level group herring meal was substituted by 300 g of a standard concentrate mixture containing 13 % protein, the daily ration providing 77 g of digestible crude protein. The energy value of the two diets was very near the same, 0.78 fattening feed units (f.f.u.).

The herring meal used was a commercial type of good quality which in earlier experiments had caused no toxic symptoms. The experiment lasted for 14 weeks, and blood samples were collected once weekly.

Results

As demonstrated in Figs. 1 and 2, the groups receiving the highest levels of dietary protein, irrespective of the stalling systems, developed highly increased serum activities of aspartate

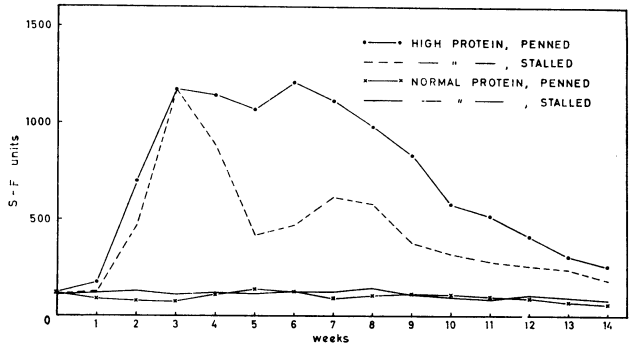


Figure 1. Mean serum aspartate aminotransferase activity of groups fed high and normal protein levels at different stalling systems.

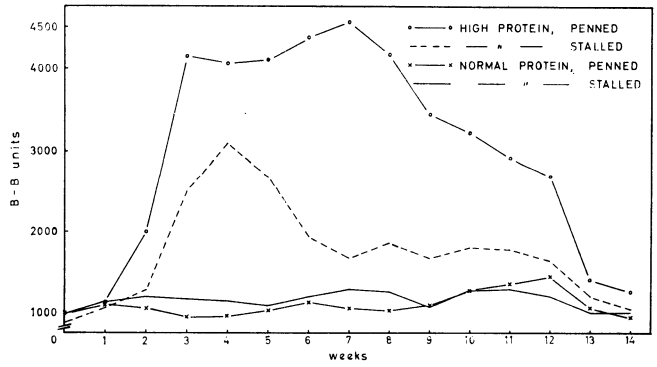


Figure 2. Mean serum lactate dehydrogenase activity of groups fed high and normal protein levels at different stalling systems.

aminotransferase and total lactate dehydrogenase. Alanine aminotransferase was, however, in all animals kept on a normal level*).

The sheep were clinically healthy during the whole experiment and gained weight at an average of 5.7 kg per animal on

*) Since alanine aminotransferase proved to be within normal limits also in all the subsequent experiments, and since high correlations consistently were found between the three other enzymes ($r = 0.8$—0.9), curves only for aspartate aminotransferase are regularly shown.

the high protein diet and 8.2 kg on the normal protein level. No difference was found in weight gain between the groups with respect to stalling systems.

The results indicate that the increased serum enzyme values were mainly due to the diet and not so much to the environmental conditions. The sheep which were kept loose in pens maintained the increased levels for a longer period than those kept restricted.

2. Effects of different protein sources

To investigate if protein-rich concentrates other than herring meal would yield a similar increase of serum enzyme activity, rations containing soybean meal, ground nut meal, and whale meat meal were compared to a herring meal diet equivalent in digestible crude protein.

Sixteen female lambs, homogenous in age and size, of about 40 kg live weight, were divided into four groups.

The daily rations were composed to give 0.64 f.f.u. and 160—170 g digestible crude protein.

The herring meal used in this experiment was specially manufactured for the purpose to ensure that no preservatives causing toxic effects had been added to the raw material.

The experiment lasted for 14 weeks for the herring meal group. As no changes had occurred in the other groups within 10 weeks, the experimental feeding of these animals was interrupted.

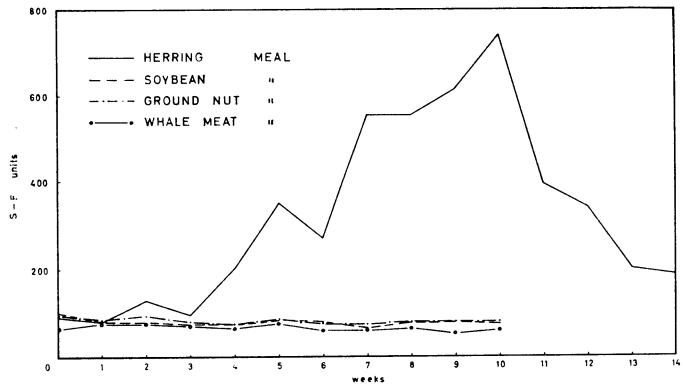


Figure 3. Mean serum aspartate aminotransferase activity of groups fed protein-rich concentrates of different origin.

Results

The aspartate aminotransferase mean levels for the different groups are illustrated in Fig. 3. This enzyme was closely correlated to lactate dehydrogenase and to α-hydroxybutyrate dehydrogenase, whereas no increase was observed in alanine aminotransferase.

The experiment strongly indicated that herring meal had a special effect on certain enzyme systems compared to other protein sources.

All animals behaved clinically normal during and after the experiment.

3. Effects of different levels of herring meal

To test the influence on serum enzyme activity of different quantities of herring meal in the daily ration, four pairs of sheep were each given 200 g, 100 g, 50 g, and 0 g of herring meal, respectively. The herring meal was of the same quality as used in Experiment 2. In addition was given hay and ground barley in amounts providing a daily intake of 156 g, 102 g, 77 g, and 50 g digestible crude protein. Mineral licks were available to all animals and water was given ad lib.

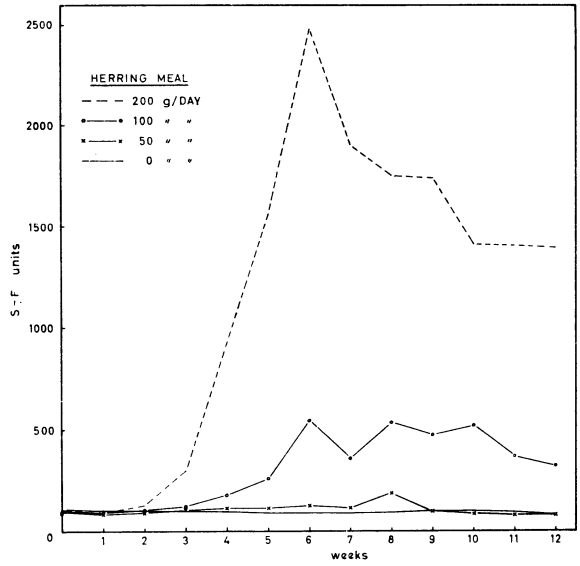
The experimental animals belonged to the same flock which had been used in Experiment 2, after having been kept at pasture for eight weeks.

The experiment lasted for 12 weeks, and blood was collected once weekly.

Results

In Fig. 4 is demonstrated that increasing daily intakes of herring meal were reflected in the serum aspartate aminotransferase activity. After six weeks the mean enzyme level on the highest intake was about 25-fold increased. Even quantities as low as 50 g of herring meal daily per animal were sufficient for the attainment of a response in serum aspartate aminotransferase activity. The same tendency was obtained in LDH and HBD.

One of the sheep receiving the highest level of herring meal was shortly after the peak of the enzyme curve killed for pathologic examination. Macroscopic and histological examinations performed at the Department of Pathology, revealed no injury



F i g u r e 4. Mean serum aspartate aminotransferase activity of groups fed different levels of herring meal.

of the liver or of other organs which could be supposed to cause the high serum enzyme levels.

4. Effects of herring meals high and very low in dimethylnitrosamine

In a series of experiments, primarily performed to establish the maximum non-fatal dose of DMNA in sheep, the effects of toxic and non-toxic herring meals on serum enzymes were studied.

Two batches of herring meal were obtained from the Norwegian Herring Oil and Meal Industry Research Institute, Bergen, where chemical analyses of the meals had been undertaken. According to a polarographic method worked out at The Central Institute for Industrial Research, Oslo, batch I initially contained 70 mg DMNA per kg and batch II less than 0.1 mg per kg. (Repeated analyses of the meals during the experimental period gave values up to 120 mg per kg for batch I and up to 1.5 mg per kg for batch II). No difference of practical consequence existed in the nutritional contents of the meals.

Lambs of the old Norwegian breed, Spelsau, weighing between 20 and 30 kg, were used as experimental animals. Fifteen ani-

mals, divided into three groups were fed meals from batch I (called DMNA groups) in quantities corresponding to 0.5, 0.25, and 0.15 mg of DMNA per kg body weight. Daily consumption of meal per kg body weight was 7.2 g, 3.6 g, and 2.2 g, respectively. Twelve animals divided into three control groups received corresponding amounts of meal from batch II. The animals were weighed every second week and the rations adjusted accordingly. Ground barley was added to equal the individual daily ration of concentrates to 300 g. In addition 0.5 kg of hay was given.

The experiment lasted for 16 weeks. Blood was collected once a week, and more frequently in cases of apparent toxic symptoms.

Results

Clinical observations

In each of the DMNA groups four animals died, as indicated in Figs. 5 and 6. The death occurred first on the highest level, between days 47 and 62 of the experiment. On the medium level the animals died between days 63 and 85, and on the lowest level between days 103 and 118. One sheep on each level survived. After 180 days these animals were killed, and hepatic disorders of a chronic character were found at necropsy. All sheep that died showed liver injuries similar to those earlier described by *Koppang* (1964). More acute histological changes were predominating on the highest DMNA level.

Clinical symptoms of DMNA poisoning were relatively small. Reduced appetite was sometimes observed a few days before death occurred. In many cases, however, the sheep were found dead in the morning without having shown symptoms in the evening before.

Among the control animals no deaths or clinical abnormalities occurred. At the end of the experiment six sheep, two from each subgroup, were killed for post-mortem examination at the Department of Pathology. No macroscopic or histologic lesions were detected in liver, heart, kidneys, or skeletal muscle.

Serum enzyme activity

Mean aspartate aminotransferase and total lactate dehydrogenase values for the DMNA groups and control groups are shown in Figs. 5 to 8. It appears that a higher aspartate amino-

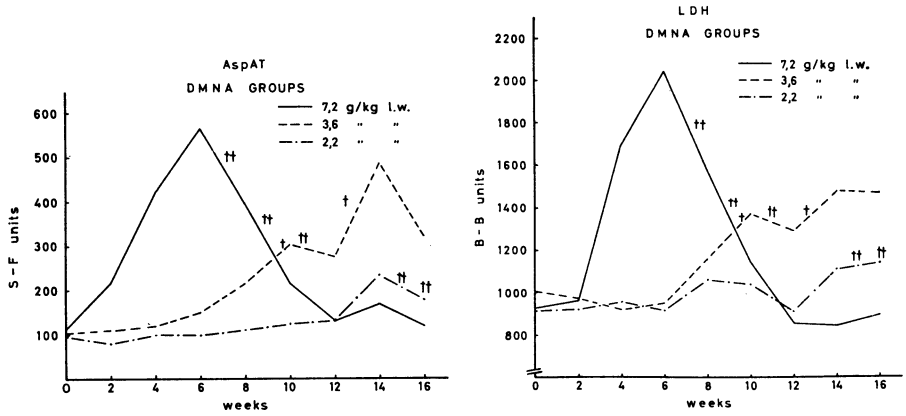


Figure 5. Mean serum aspartate aminotransferase activity of groups fed different levels of herring meal with a high content of dimethylnitrosamine. (Each cross represents the death of one animal).

Figure 6. Mean serum lactate dehydrogenase activity of groups fed different levels of herring meal with a high content of dimethylnitrosamine. (Each cross represents the death of one animal).

transferase mean level was reached in the control group where no pathologic organ changes were observed than in the DMNA groups with extensive liver injuries and deaths. Comparing the serum enzyme curves of the two main groups, great similarities are found. The peak enzyme levels were reached after six to eight weeks on the highest meal consumption. Lower intake caused less pronounced enzyme increases with peak levels obtained after 12—14 weeks.

Serum alanine aminotransferase activity was in no case found elevated.

The results of the present experiment confirm earlier suggestions that the very marked serum enzyme increase following feeding of normal, non-toxic herring meal, were not due to organ lesions detectable by ordinary light microscopy.

It is noticeable that sheep fed the low levels of toxic meal died without having shown highly increased serum enzyme activities. This may possibly be due to a decreased enzyme synthesis in the more chronic stages of liver injuries. It must, however, be realized that the very pronounced serum enzyme elevations recorded in the high DMNA group with liver changes of

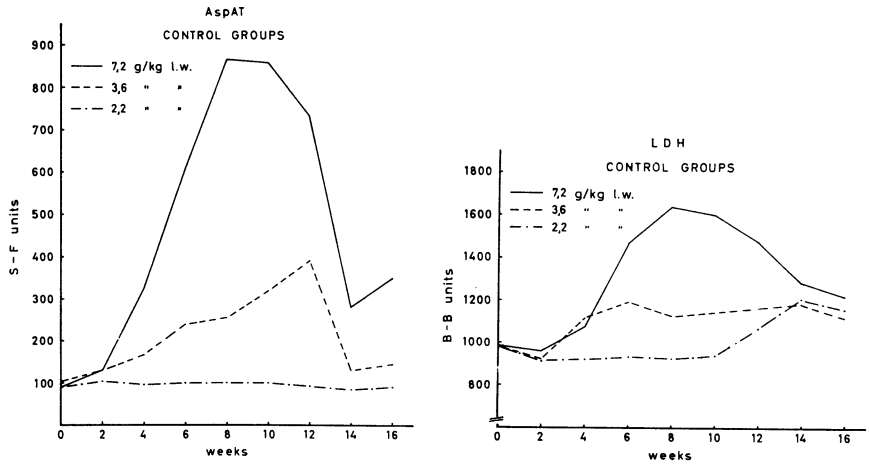


Figure 7. Mean serum aspartate aminotransferase activity of groups fed different levels of herring meal with a very low content of dimethylnitrosamine.

Figure 8. Mean serum activity of lactate dehydrogenase of groups fed different levels of herring meal with a very low content of dimethylnitrosamine.

an acute character, may be due, at least partly, to the herring meal per se.

This suggestion was tested in a further trial where each of two adult sheep received orally a gelatine capsule containing 1 mg of DMNA daily.

The diet consisted of 250 g whale meat meal, 100 g ground barley, and 0.5 kg of hay. This amount of DMNA corresponded to the level present in 300 g of herring meal with a DMNA content of 3—4 mg per kg. During the experimental period of 14 weeks no elevations of the serum enzymes could be observed. The animals showed no clinical symptoms, and were put out to pasture. After three and four weeks, respectively, they died, apparently from toxic hepatosis.

Another two sheep on the same diet were given a gelatine capsule containing 50 mg of DMNA daily. Both of them died after 21 days. Clinical symptoms were not observed until the day of death, and changes of serum enzyme levels were first recorded within the last week. Pathologic examinations performed at the Department of Pathology showed an acute toxic hepatosis.

5. Effects of an abrupted herring meal feeding

An experiment was carried out to see how herring meal thoroughly moistened with water and redried would influence serum enzyme activity. According to *Ender & Havre* (1966) the DMNA contents of herring meal can be greatly reduced by this procedure. Herring meal of a commercial quality, containing 3—4 mg DMNA per kg, had in an earlier experiment, when fed untreated, given highly increased serum enzyme values without clinical symptoms. The same meal, after moistening and redrying at about 50°C during two days, was given in quantities of 300 g + 100 g ground barley and 0.5 kg hay to two adult sheep of about 70 kg.

As seen from Fig. 9 a heavy increase occurred in aspartate aminotransferase, lactate dehydrogenase, and α-hydroxybutyrate

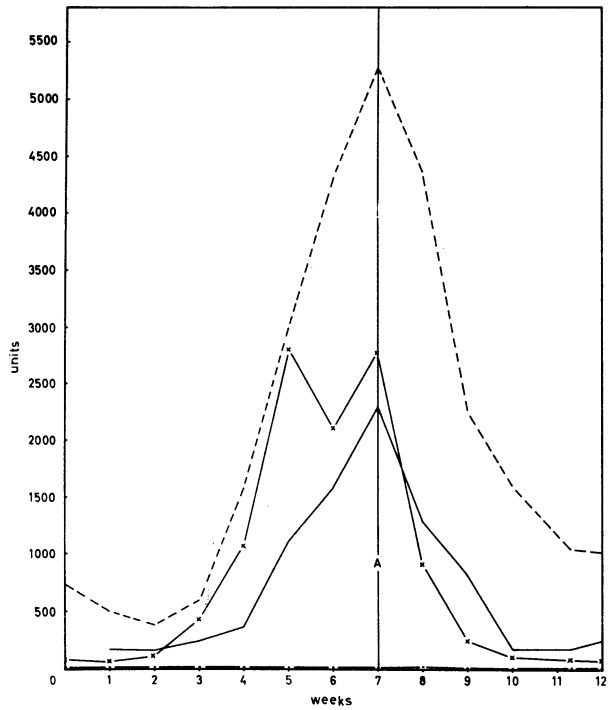


Figure 9. Mean serum enzyme activities in sheep fed moistened and redried herring meal, and after abrupt changing of the diet (A). LDH - - - - -; AspAT x ——— x; HBD ———; AlAT —.—.—. For units, see text.

dehydrogenase whereas alanine aminotransferase was not changed.

After seven weeks the diet was abruptly changed to the diet usually fed. It consisted of hay, grass silage, and a concentrate mixture, providing an equal energy intake, but a considerable lower protein level. Blood samples were analysed twice weekly to record the enzyme values after changing of the diet. Fig. 9 illustrates how aspartate aminotransferase and α-hydroxybutyrate dehydrogenase were returned to normal levels within three weeks and lactate dehydrogenase after about four weeks.

The results indicate that the increased enzyme release from tissue to blood in herring meal-fed sheep is a reversible process, not depending on tissue damage, and apparently not related to DMNA.

As shown in previous experiments, sheep fed continuously with herring meal reveal a drop in the serum enzyme activity after a peak level has been reached. In most cases, however, the enzyme values are remained elevated, compared with the initial levels, as long as herring meal is fed.

6. Effects of herring meal feeding on serum lactate dehydrogenase isoenzyme distribution

Since the LDH isoenzyme distribution in serum to some degree might indicate the origin of enzyme release, the electrophoretic pattern of six sera with highly increased levels of total LDH due to non-toxic herring meal feeding was compared to the distribution earlier obtained in six sera of normal, adult sheep (*Tollersrud* 1970). The results are given in Table 1.

Table 1. Mean percentage distribution of LDH isoenzymes in serum of control sheep, and in serum of sheep fed herring meal.

Serum from	n	Total LDH	% LDH_1	% LDH_2	% LDH_3	% LDH_4	% LDH_5
Control sheep	6	758	48.4	10.6	31.1	8.9	1.0
Herring meal-fed sheep	6	3383	66.0	9.0	14.1	9.9	1.0

It appears from the table that there was a considerable difference between the relative distribution of LDH isoenzymes. The LDH_1 fraction was greater and LDH_3 smaller in sheep fed herring meal than in the controls. An increase of the heat-stable

anodic fraction was confirmed by a relative heat stability test. In normal serum of adult sheep 25—30 % of total LDH is inactivated by heating to 50°C for 30 min. (*Tollersrud*), whereas the loss in sera of herring meal-fed sheep was generally less than 10 %.

As shown previously (*Tollersrud*) both heart, liver, and kidney of sheep are relatively rich in LDH_1, whereas for skeletal muscle the cathodic fractions predominate. A skeletal muscular origin of the serum enzyme increase must therefore be considered unlikely.

To differentiate between the other main organs as the site of release on the basis of the LDH isoenzyme distribution in serum seems hardly possible. Zymograms of sera from sheep suffering from hepatosis due to feeding of DMNA-containing herring meal, however, showed a similar pattern.

7. *Effects of herring meal on tissue enzyme activity*

It has previously been shown in vitamin E-deficient pigs that an inverse relationship existed between serum and tissue enzyme levels (*Tollersrud & Nafstad* 1970). The decreased tissue activity might be the result of a continuous enzyme leakage to the blood due to an increased cellular permeability or be a consequence of a decreased enzyme synthesis in the injured organs.

In the present experiment the activities of aspartate aminotransferase and total lactate dehydrogenase were determined in organs of four experimental sheep. Two of the animals had been fed herring meal, resulting in highly increased serum values of these enzymes at the time of killing: AspAT — 2040, LDH —

Table 2. Tissue activity of aspartate aminotransferase and total lactate dehydrogenase in casein-fed sheep and in sheep fed non-toxic herring meal. Units $\times 10^4$ per g fresh tissue.

Tissue	On casein diet		On herring meal diet	
	AspAT	LDH	AspAT	LDH
Heart	7.56	19.00	8.00	24.81
Liver	4.68	6.63	8.84	18.80
Skeletal muscle	5.68	31.00	6.84	29.10
Spleen	1.04	4.50	1.06	4.30
Kidney cortex	2.02	21.50	2.60	19.00
Kidney medulla	1.06	10.60	0.96	10.10

5235. The two other sheep were fed a casein diet at an equal protein level, but maintained normal serum values: AspAT — 77, LDH — 815. Results of the tissue enzyme analyses are shown in Table 2.

The most striking differences between diets were for both enzymes found in the liver. The LDH activity of heart muscle was also somewhat higher on the herring meal diet, whereas no distinct differences were obtained for other tissues. The results indicate an increased enzyme synthesis in sheep fed herring meal.

DISCUSSION

It has been demonstrated by several authors that adaptation to varying levels of dietary protein in rats is associated with changes of the activity of a number of hepatic enzymes. Liver activities of aspartate and alanine aminotransferases are generally found to be increased with increasing protein levels, whereas lactate dehydrogenase has shown an inverse relationship to dietary protein in rats (*Schimke* 1962; *Hurwitz & Freedland* 1968).

Increased serum enzyme levels as a consequence of high liver activity has, as far as known, never been reported in animals. In children recovering from severe malnutrition (kwashiorkor and marasmus), *McLean* (1962) found greatly increased serum alanine aminotransferase and isocitrate dehydrogenase levels without acute cell injury. He suggested that the increased serum enzyme levels were produced when enzyme synthesis proceded more rapidly than synthesis of the structural components of the cells.

In the experiments described only herring meal has given rise to increased serum enzyme activities. Feeding with meal produced from the salt-water fish capelin (Mallotus villosus), preserved with a benzoate-formate mixture, gave a similar strong and rapid enzyme elevation. Protein sources of other origin, vegetable as well as animal, at the same dietary level have caused no serum enzyme changes. Own experiments (not published) have shown that the effect of herring meal is not due to the fat fraction, since ether- and benzene-extracted herring meals have given the same results as non-extracted meals. Nor is it due to different drying methods used during the processing, since the effect is found on steam-dried herring meal as well as on meals

dried by the more common method of using hot flue gases from fuel oil combustion.

The results indicate that one or more factors in fish meal has a special effect on the enzyme synthesis. This product is more rich in certain amino acids than the other diets used, especially in the sulphur-containing amino acids methionine and cystine, and also in lysine and threonine. A strong evidence exists, as recently published by *Sanchez & Swendseid* (1969) that methionine or a metabolic product of methionine, such as cystine, acts as an inducer for some hepatic enzymes. Methionine has been shown to increase blood corticosterone levels, and may thus act through the adrenal cortex to promote the induction of enzymes or reduce their rate of degradation.

Feeding experiments performed in this department on sheep receiving 1 % and 2 % of methionine and lysine in addition to a hay-barley ration gave, however, no response in serum enzyme activity.

Herring meal is also more rich in certain mineral elements than other feedstuffs, such as iodine, zinc, and organic bound selenium. Traces of nitrite are found even in unpreserved raw materials, and formation of minute amounts of dimethylnitrosamine occurs in all batches of herring meal. A primary or secondary inducer effect of one of these factors is not impossible.

If the hypothesis of an increased de novo synthesis of enzymes in herring meal-fed sheep were right, the increased activities in serum must have been due to an increased diffusion rate.

The rate of diffusion is limited by the permeability of the cell membrane. Under normal conditions it is minimal. A variety of findings indicate, however, that the membrane can be varied physiologically and is, therefore, under metabolic control (*Hess* 1963). The concentration gradients of different enzymes between liver and serum in domestic animals do not seem to be known. They possibly vary between species. It is noticeable that herring meal fed to pigs in this department has caused no elevation of the serum enzyme levels such as constantly found in sheep and to a lesser extent in cattle.

That no increase of serum alanine aminotransferase has been found in herring meal-fed sheep, may be connected with the low liver content of this enzyme in ruminants (*Cornelius et al.* 1959).

A practical consequence of the results is that serum enzyme

determinations for diagnostic purposes will give a false picture of the clinical condition in sheep fed herring meal, even in moderate quantities. In this country herring meal has been recommended in liberal amounts as a prophylactic diet against muscular dystrophy due to its abundant contents of selenium. As a paradoxical result serum enzyme assays of healthy sheep fed the prophylactic diet may thus give analytical data which can easily be mistaken for indications of an advanced stage of the disease.

Further work as to the mechanism of these dietary effects may provide more information and allow a better understanding of the nature of the phenomenon. The increased emphasis on the production of fish protein for human consumption should justify similar studies also in man.

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SUMMARY

In a series of experiments it was demonstrated that highly increased activities of aspartate aminotransferase (= GOT), total lactate dehydrogenase (LDH), and α-hydroxybutyrate dehydrogenase (HBD) occurred in serum of sheep on herring meal feeding. The alanine aminotransferase (AlAT = GPT) level remained unchanged. The enzyme increase was apparently not related to the liver toxic agent dimethylnitrosamine (DMNA) occasionally occurring in lethal doses in meals produced from raw materials preserved with excesses of nitrite. Histological changes of the liver or other tissues were never detected in experimental animals killed at a stage with very high serum enzyme values. Diets equivalent in digestible crude protein consisting of vegetable as well as of animal protein sources other than fish meal, did not give rise to elevated serum enzyme values.

Electrophoretic separation of the LDH isoenzymes in serum of herring meal-fed sheep showed an increased percentage of the LDH_1 fraction, which is predominant in liver, heart, and kidney. Determination of enzyme activities in various tissues resulted in a markedly higher concentration in livers from herring meal-fed animals than in sheep fed casein at an equal protein level. It is suggested that herring meal may have a special promoting effect on the de novo synthesis of the enzymes concerned.

As a practical consequence of the experiments it must be emphasized that serum determinations of these enzymes for diagnostic purposes will give a false picture of the clinical condition of sheep fed even moderate amounts of herring meal.

SAMMENDRAG

Virkningen av fôring med atoksisk sildemel på enkelte serumenzymer hos sau.

I en lengre forsøksserie ble det vist at fôring med sildemel til sau forårsaket en sterk stigning i serumaktiviteten av aspartataminotransferase (AspAT = GOT), total laktatdehydrogenase (LDH) og α-hydroksybutyratdehydrogenase (HBD), mens alaninaminotransferase-nivået (AlAT = GPT) var uforandret. Enzymforhøyelsen hadde tilsynelatende ingen relasjon til det lever-toksiske agens dimethylnitrosamin (DMNA) som har forekommet i letale doser i sildemel av sterkt nitrittkonservert råmateriale. Patologiske forandringer som kunne forklare enzymstigningen, ble ikke påvist, hverken i lever eller andre organer på forsøksdyr som ble avlivet på et stadium med meget høye serumverdier.

Andre proteinrike fôrmidler som jordnøtt, soya, hvalkjøttmel og kasein gitt i ekvivalente proteinmengder ga ikke samme utslag som sildemel og loddemel.

Elektroforetisk separasjon av LDH isoenzymene i serum av sildemelfôrete dyr viste en sterk økning av fraksjonen LDH_1 som er dominerende i lever, hjerte og nyrer hos sau. Bestemmelse av enzymaktiviteten i forskjellige vev viste en markant høyere verdi i lever hos sauer som var fôret med sildemel enn hos dyr som var fôret med annet animalsk protein i tilsvarende mengder. Observasjonene kan tyde på at sildemel har en spesiell induserende effekt på syntesen av visse enzymer.

En praktisk konsekvens av resultatene er at serumenzymbestemmelser i diagnostisk øyemed hos sauer som blir fôret med sildemel, f. eks. profylaktisk overfor muskeldystrofi, vil kunne gi et sterkt forfalsket bilde av den kliniske tilstand.

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