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NUTRITIONAL MUSCULAR DEGENERATION IN BEEF CALVES

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Introduction

Since the introduction of milk-substituting diets — so-called milk replacers — to fatten calves, severe economic losses have been caused by the appearance of muscular degeneration (M.D.) in the Netherlands.

With the exception of those calves which died or were slaughtered in cases of emergency because of M.D., several animals show changes due to M.D. after slaughter without any clinical symptoms of the disaese having been present during life or during ante mortem examination. There is another problem confronting meat inspectors as the outer layer of the skeletal muscles often is normal.

Though the losses are not more than a few per cent, the total production of 700.000 fattened calves a year made those losses important enough to pay attention to this disease. Normally, the gain in weight shown by the calves varies from 90 to 100 kg. within twelve weeks; the veal is highly appreciated in the Netherlands as well as in other neighbouring countries to which a fair amount of the produce is exported. When animals are affected with M.D., the gain in weight declines rapidly and the disease frequently terminates in death.

Therefore there was sufficcnt reason to study various aspects of M.D. in calves, to increase our knowledge of the aetiology and, if possible, of methods of treatment and prevention. It also was necessary to study methods of clinical diagnosis to be able to detect symptoms of M.D. in an aerly stage, to make it possible to discontinue production or treat the animals if this was likely to be succesful.

The literature includes a large number of data on M.D. in many animals, but not much on calves (Hjärre et al., 1936; Blaxter et al., 1952; Safford et al., 1954, Oldfield et al., 1958; Maplesden et al., 1960; Swart, 1963).

Selenium deficiency as well as vitamin E and C deficiency are believed to cause M.D. As regards the point of onset of M.D., it is not known whether this is to be found in the muscle fibres or in the nervous system; opinions differ on the repair of degenerated muscle fibres.

To study clinical symptoms and methods of treatment, an experiment with new-born Friesian calves was designed, to which an α-tocopherol-deficient milk replacer was administered, the controls being placed on a similar diet

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including sufficient α-tocopherol. The experiments and the results of the experiments and investigations are briefly reported; a more detailed report will follow later on.

Material and methods of inducing M.D. in calves

Material abtained from cases of spontaneous M.D. in about forty calves was received from slaughter-houses, from which several samples of muscle, heart, kidney, brain and spinal cord, all as fresh as possible, were taken for histopathological investigation.

Experiment A, ten calves.

Instead of the normal milk-replacing diet given to two controls (Table 1), a basal diet recommended as being deficient in vitamin E (Blaxter et al.) was modified to our special purpose (Table 2) and administered to eight new-born calves, starting on the second day of life. On the first day of life, the calves received two colostrum meals from their mothers. Analysis of the basal diet employed showed that the α-tocopherol content was 45 mg/kg., which was eventually found to contain sufficient vitamin E to prevent M.D. but did not permit a normal gain in weight of the calves.

Per kg. of liquid milk replacer 125 grams of dry mixture were dissolved in preheated water. The rate of feeding eventually was approximately equivalent to the weekly body weight. The daily gain in weight of the controls was 0,809 kg., that of the experimental animals being 0,755 kg. There was no evidence of M.D. during life and after slaughter. Specimens of muscles, of the nervous system and of various organs were studied as control material.

The experiment was carried out in a modern, air-conditioned stable which was disinfected at regular intervals. The calves were kept separate, clinically examined and weighed once a week and exercised twice weekly.

Experiment B, eight calves.

This experiment was carried out in the same stable and under similar conditions. The basal diet was altered (Table 3) and examined for its α-tocopherol content, which now was less than 5 mg./kg. On the first day of the experiment, all calves were given 3 ml. of Duphafral A.D_3, containing 25.000 I.U. of vitamin A and 12.500 I.U. of vitamin D_3/ml. and arranged in three groups, all receiving the basal diet.

The first group of three calves was given 2 ml. of Duphafral A.D_3 daily.

The second group of three calves was given 30 ml. of cod liver oil (600 I.U. of vitamin A and 75 I.U. of vitamin D_3/ml.) daily instead of the 2 ml. Duphafral A.D_3. to check the view advanced by some authors that unsaturated fats in the diet will precipitate or increase myodegeneration. This daily dose also contained 5 mg. of α-tocopherol. The third group of two calves were given 2 ml. of Duphafral A.D_3 and 4 ml. of Duphafral E (50 mg. of α-tocopherol-acetate/ml.) daily and served as controls in this experiment. The additives were given with the morning feed.

To determine the onset of M.D. as accurately as possible, biopsy specimens were taken at the time of onset of the first clinical symptoms of M.D.; the specimens were cut from certain muscles (biceps femoris muscle, semitendineus muscle and semimembranaceus muscle) and studied histopathologically. Some of the calves had indigestion; one died from enteritis within ten weeks and another from very severe M.D. within twelve weeks; the daily gain in weight of the other calves was 0,652 kg.

Results of the experiments

Some of the results of experiment B are summarised in Table 4. The composition of the diet was indeed deficient in vitamin E, although it took a long time until a diagnosis of M.D. could be established.

The positive results of experiment B gave us an opportunity to study the clinical symptoms.

Therapeutic trials were made in two of the calves positive for M.D. In each case 1.000 mg. of α-tocopherol-acetate were administered subcutaneously for three days, followed by a daily dose of 600 mg. for two and a half weeks administered by the same route; 300 mg. were then added to the daily morning ration.

The carcass of the treated calf of the first group was in good condition and gross lesions due to M.D. were not observed by the present authors or by the veterinary-surgeon inspector who was informed of the history of these experimental calves. The meat was released for human consumption after the large muscles had been cut for inspection and samples taken for histopathological examination.

The treated calf which had received cod-liver oil was also released after a cooking test for abnormal flavour.

The fact that M.D. dit not occur in the third group showed that an

adequate dose of α-tocopherol in the diet will prevent M.D.; they were passed as fit for human consumption.

Clinical symptoms

The experiments showed that calves kept in the normal way will rarely develop marked early symptoms of M.D. They are never allowed to leave their place in the stable. The initial symptoms of this condition are loss of appetite and difficulty in rising. Loss of appetite, however, is not specific for M.D., nor is slowness in rising. In this case, lesions will be advanced and when selective treatment is not carried out, death is very near.

Weighing at regular intervals, for instance, once weekly and exercising the animals on the way to the scales may supply important data on the onset of M.D. During exercise, calves with M.D. soon become fatigued and show shortness of breath as well as abnormal postures and movements. They want to lie down too soon and are unwilling to rise. In some moder te cases of M.D., however, these sympotons were not always apparent. In the present experiment, a number of calves became increasingly trained in exercise; the clinical symptoms and fatigue were less marked than were those in the untrained animals.

To obtain more exact data on the clinical condition of calves affected with M.D., a number of biochemical tests were performed to determine the activity of certain enzymes in the bloodserum, viz., glutamic oxalacetic transaminase (G.O.T.), glutamic pyruvic transaminase (G.P.T.), lactic dehydrogenase (L.D.H.), sorbit dehydrogenase (S.D.H.) and creatine phosphokinase (C.P.K.). The three first named enzymes are indicative of M.D. as well as degeneration of the liver; S.D.H. activity is specific of liver disease, C.P.K. activity of muscular disease.

Testing the C.P.K. activity at regular intervals was very useful in these experiments as it showed the onset of the damage to muscular tissues. In more chronic states of M.D., C.P.K. activity decreased; but then in addition L.D.H. activity can give a good indication.

The creatine and creatinecontent of the urine were also studied, but this did not provide any exact data on the state of M.D.

Pathological changes in skeletal muscles

Macroscopical examination

The changes due to M.D. observed in the biopsy specimens and carcasses in spontaneous and experimental M.D. are common knowledge. All gradiations of discolouration and pallor alternating with normal tracts of tissue were observed. In some cases, practically all the skeletal muscles as well as the diaphragm were more or less involved. The latter accounts for the laboured breathing and the abdominal type of respiration.

The disease was symmetrical in every case but the severity of the lesions was not alway identical on both sides.

Calcium granules were discernible in some cases when the cut surface was touched with tip of the finter.

There was no macroscopical evidence of M.D. in the two controls and the two treated calves.

Microscopical examination

As could be expected, all degrees of degenerative changes in the muscle fibres were observed, ranging from initial lesions such as slight swelling, incipient vacuolation and hyaline degeneration to total damage, calcification and proliferation of connective tissue. Myolysis was reflected in loss of striation of the fibrils; the sarcolemmic sheaths often were partially or completely empty. In chronic stages, substitution by fine connective tissue fibres deriving of the muscle fibres were frequently restored by proliferation of swollen vesicular sarcolemmic nuclei.

Various forms of regeneration were found to occur in the more extensively damaged tissues. A kind of cellbuds of a healthy fibre entered a necrotic muscle fibre in some cases, marked proliferation of sarcolemmic nuclei being observed in others. When endomysial capsules were damaged, growth was precarious.

The muscular coat of the medium-sized and smaller arteries was often swollen as if hyaline degeneration had set in. A number of slight haemorrhages were found to be present, indicating increased permeability of the capillaries.

Several specimens of various muscles of the two successfully treated calves were examined and serial sections were frequently prepared for de-

tailed study.

Although there was no macroscopical evidence of M.D., there were some foci which probably had been the seat of extensive lesions due to M.D. before treatment was initiated. Nearly all muscle fibres were normal, often showing thin striations and containing large numbers of swollen sarcolemmic nuclei, and small foci of necrosis, still containing a few macrophages among sarcolemmic nuclei in process of repair, were observed. This shows that the removal of necrotic material, present in large quantities in severe cases of M.D., in fact takes more time than four weeks of treatment. The experiment also showed that complete repair even of totally degenerated muscular tissue is possible.

Pathological changes in the heart muscle

Degenerative lesions which were smaller in number and milder than those in the skeletal muscles, were observed in the heart muscle, mainly in the wall of the left centricle, sometimes also in the wall of the right centricle and most often beneath the endocardium and epicardium, though only in cases of spontaneous M.D. Purkinje's fibres usually were not affected; in a few cases, they showed hyalinisation and pycnosis of the nuclei. Types of regeneration accompanying the necrotic changes were also observed in the heart muscle.

No other obvious changes were observed.

Glycogen content of degenerated muscles

The litarture doet not include any data on the glycogen content of degenerated muscles. The glycogen content was assumed to decrease in severe cases. To verify whether this was actually the case, sections of normal and degenerated muscle were stained using Schiff's periodic acid technique (P.A.S.). Three sections cut from the specimens were stained with P.A.S., one of these sections having been previously treated with saliva and another with 0,5 per cent diastase in a sodium acetate buffer solution with pH = 7,-. These two sections were incubated in the saliva or diastase at 37° C. for thirty minutes. Saliva was more effective in removing glycogen than was diastase. Glycogen was present as fine and coarse granules, especially in the striations. Comparison between these slides made it possible to determine the amount of glycogen present and its localisation. Comparison between

normal and degenerated muscle tissue showed that there was a marked decrease in degenerated fibres and complete absence of necrotic fibres. P.A.S.-positive material was observed in the walls of medium-sized and small arteries and in thickened walls of capillaries, in which is was amorphous. It may be that there is a muco-polysaccharide structure which is responsible for the diathesis which is a common feature in these cases.

Pathological changes in the central nervous system

Degenerative changes in animals affected with M.D. are reported by some authors. The brains and spinal cords of nine calves with M.D. and twelve controls were studied and compared with each other.

Microscopical examination of the cases of M.D. revealed congestion of the capillaries and several petechial haemorrhages througout the brain and spinal cord. The brain and spinal cord of the controls did not show any deviations.

These findings were verified by microscopical examination.

Besides severe hyperaemia of the blood vessels, particularly the capillaries, and multiple small haemorrhages, perivascular oedema was observed in the Virchow-Robin spaces. The haemorrhages which varied in number and size, were mainly localized about the small blood vessels. In some sections stained with P.A.S., no changes in the walls of the blood vessels were observed.

The neurons, nerve fibres and glial tissues were completely normal in all cases studied.

Pathological changes in the kidney

In a number of papers on spontaneous and experimental cases of M.D. in various animals, vitamin E deficiency is reported as having caused lesions in the kidneys. To verify this statement, the kidneys of twenty-two calves with spontaneous and experimental M.D. and those of a number of controls were studied.

In some of the kidneys of the calves with M.D. and the controls, lesions due to subacute and chronic focal interstitial nephritis were observed. With the exception of some dilated tubules with increased albumin content and flattened epithelium, which had occasionally flattened nuclei too, in the cases of M.D., no significant differences were found between the groups.

Conclusions

1. Muscular degeneration may be experimentally induced in calves by feeding them an α-tocopherol-deficient milk substituting diet.
2. Supplementing the deficient diet with cod-liver oil in a dose of thirty ml. daily did not affect the severity of the lesions or the veal qualities.
3. The lesions appearing in cases of experimentally induced M.D. are identical to those in spontaneous cases occurring in the Netherlands.
4. Clinical symptoms may be produced by exercising the calves for a longer period.
5. The diagnosis should be supported by biochemical tests and biopsy.
6. Treatment of very severe cases of M.D. with large doses of α-tocopherol-acetate is effective; the gross lesions disappear within a month.

Table 1

Diet 1 (normal)

Powdered skim-milk	75,575 %
Mill-rendered maize-starch	3,000 %
Maize glucose	3,000 %
Palm-kernel fat	8,000 %
Lard	8,000 %
Lecithin	1,000 %
Synthetic emulsifyer	0,300 %
Iodinized salt	0,200 %
Calciumhydrophosphate ($CaHPO_4.2H_2O$)	0,200 %
Copperoxide (CuO)	0,010 %
Magnesiumoxide (MgO)	0,150 %
Aureomycinprep. (22 g./kg.)	0,300 %
Carbomethylcellulose	0,250 %
Nicotinamid	0,005 %
Vitamin C	0,008 %
Vitamin E (pure)	0,002 %

Per kg. added fat 3 g. dodecylgallate used as antioxydant.
Per 100 kg. mixture 1,5 S.U. of vitamin A and 0,2 S.U. of vitamin D3 are added.

Table 2

Basal diet 2 (experiment A)

Powdered skim-milk	75,882 %
Mill-rendered maize-starch	3,000 %
Maize glucose	3,000 %
Palm-kernel fat	8,000 %
Sunflower oil	8,000 %
Glycerine monostearate	1,000 %
Iodinized salt	0,200 %
Calcium hydrophoshate	0,200 %
Copperoxide	0,005 %
Magnesiumoxide	0,150 %
Aureomycinprep. (22 g./kg.)	0,300 %
Carbomethylcellulose	0,250 %
Nicotinamid	0,005 %
Vitamin C	0,008 %

Per 100 kg. mixture 1,5 S.U. of vitamin A and 0,2 S.U. of vitamin D3 are added.

Table 3

Basal diet 3 (experiment B)

Powdered skim-milk	70,882 %
Lard	20,000 %
Glucose	7,000 %
Glycerine monostearate	1,000 %
Iodinized salt	0,200 %
Calciumhydrophosphate	0,200 %
Copperoxide	0,005 %
Magnesiumoxide	0,150 %
Aureomycinprep. (22 g./kg.)	0,300 %
Carbomethylcellulose	0,250 %
Nicotinamid	0,005 %
Vitamin C	0,008 %

Table 4

Grouping, biopsy an autopsy findings, gain in weight and period for which the individual anmials were subjected to the experiment

Calf no	Diet sub-group	First positive biopsy after	Biopsy findings	Treatment from ... to ...	Days of testing	Autopsy findings	Gain in weight kg	Daily gain in weight kg
I 1	Basal diet + AD3	100 days	very severe lesions	132nd-161nd day	161	limited foci of very mild lesions with regeneration	93	0,608
I 2	"	81 "	" " "	-	87	very severe lesions	41,5	0,482
I 3	"	111 "	mild lesions	-	157	mild lesions	106	0,679
II 4	Basal diet + cod-liver oil	-	-	-	74	no lesions	69	0,403
II 5	"	129 days	moderate lesions	135th-163th day	163	no lesions	111	0,689
II 6	"	119 "	" "	-	132	moderate lesions	91	0,689
III 7	Basal diet + AD3 + α-tocopherol	-	-	-	145	no lesions	108	0,620
III 8	"	-	-	-	142	no lesions	107	0,625

Calf No. I 2 was sacrificed on the 87th day and used for autopsy in view of its poor condition; during the last three weeks, it had shown clinical symptoms of M.D.; severe changes due to M.D. were present in all muscles.

Calf No. II 4 died spontaneously after some efforts to get it to rise; it had been affected with severe enteritis; there was no evidence of myodegeneration in specimens of nearly all muscles.

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Literature

Blaxter, K.L., Watts, P.S. and Wood, W.A.; The nutrition of the young Ayrshire calf: Muscular dystrophy in the growing calf. Brit.J.Nutr., 6, 125, 1952.

Hjärre, A. and Lillengen, K.; Ein Beitrag zur Aetiologie und Pathogenese des sogenannten "Weissen Fleisches" beim Kalbe. Virchows Archiv, 297, 565, 1936.

Maplesden, D.C., Harvey, J.D. and Branion, H.D.; Nutritional muscular dystrophy in calves, I: Addition of dried brewer's yeast, phosphorus and tocopherol to a dystrophic diet. Canad.Vet.J., 1, 10, 1960.

Maplesden, D.C. and Loosli, J.K.; Nutritional muscular dystrophy in calves, II: Addition of selenium and tocopherol to a basal dystrophic diet, containing cod-liver oil. J.of Dairy Sc., 43, 645, 1960.

Oldfield, J.E., Ellis, W.W. and Muth, O.H.: White muscle disease (Myopathy) in lambs and calves, III: Experimental production in calves from cows fed Alfafa hay. J.A.V.M.A., 132, 211, 1958.

Safford, J.W., Swingle, K.F. and Marsh, H.: Experimental tocopherol deficiency in young calves. Am.J.Vet.Res., 15, 373, 1954.

Swart, F.W.J.; Spier(muscular) dystrophy. Tijdschr.v.Diergen., 88, 809, 1963.

Summary

Muscular degeneration appearing in veal calves when milk-substituting diets were administered, was studied in spontaneous and experimentally induced cases.

The experimental studies in which muscular degeneration was induced in calves by a diet deficient in vitamin E, the clinical symptoms, a number of biochemical tests performed in establishing the diagnosis, methods of treatment used and the histopathological investigation of muscular and other tissues are reviewed.

Résumé

La dégénération musculaire de veaux gras qui se produit après l'administration de préparations remplaçant le lait, a été étudiée sur des cas spontanés et sur des cas provoqués en guise d'expérience.

Un aperçu est présenté des expériences qu'on veut obtenir de veaux souffrant de dégénération musculaire en les mettant au régime vitamine-E-déficient, des symptômes cliniques et de quelques méthodes biochimiques diagnostiques des thérapies et des investigations histopathologiques des muscles et des autres organes.

Zusammenfassung

Es wurden spontane und experimentell erzeugte Fälle von Muskeldegeneration bei fetten Kälbern untersucht, die auftraten bei Anwendung einer Vollmilchersatzdiät.

Es wird eine Übersicht gegeben von den Versuchen zur Erzeugung einer Vitamin E-armen Diät, von den klinischen Symptomen und einigen biochemischen Diagnosetests, von der angewandten Therapie und von den histopathologischen Untersuchungen der Muskeln und anderer Gewebe.