The Nutrition of the Young Ayrshire Calf

11. The Toxicity of Cod-liver Oil

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(*Received 22 December* 1951)

Previous observations have shown that the addition of small quantities of cod-liver oil to a ration for calves that by itself produces muscular dystrophy, results in a considerable increase in the severity of the disease, and that the further addition of 50 mg α-tocopherol is not sufficient to reverse the effect (Blaxter, Watts & Wood, 1951, 1952). Cod-liver oil can be toxic when included in the rations of goats and sheep, but in cattle recent attempts to show that it is toxic have failed. The early results of Agduhr (1927), Agduhr & Stenström (1929) and of Slagsvold (1925) suggested that cod-liver oil causes muscular dystrophy in calves, but numerous experiments in the United States (Gullickson & Fitch, 1944; Davis & Maynard, 1936, 1938; Barnes, Davis & McCay, 1938) and also in Europe (Horn, 1934; Paloheimo, 1937; Schmalfuss, Werner & Esskuchen, 1938) failed to confirm them. In Britain, muscular dystrophy of calves has not been reported, as far as we are aware, and recommendations are given by various authorities (Fream, 1932; Mackintosh, 1937), regarding the use of cod-liver oil as a supplement to skim-milk or dried skim-milk rations (Woodman, 1948). Thus, in *Bulletin* no. 10 of the Ministry of Agriculture and Fisheries (Mackintosh, 1937) the following recommendation is made: 'Dried whole milk can be introduced, replacing a quarter of the new milk allowance, when the calf is 10–14 days old, and the change-over may be completed when the calf is 3–4 weeks old. When dried separated-milk powder is used, 1 oz. of good quality cod-liver oil should be added to each gallon of remade milk. These dried milks can be used as long as is considered desirable, but after 8 weeks the allowance can be gradually reduced and its place taken by a dry meal and cake mixture, hay and water.' Similarly in *Bulletin* no. 48 of the Ministry of Agriculture and Fisheries (Woodman, 1948) the recommendation is to give '1–2 oz. (cod-liver oil) per day up to a maximum of 3–4 oz. at 6 weeks of age'. These quantities are considerably larger than the ½ oz. which led to symptoms in our early experiments. It was therefore thought desirable to test whether, under practical conditions, dystrophy would follow the inclusion of cod-liver oil in the diet. For this purpose practical recommendations were followed—calves were initially reared on whole milk and then transferred to dried skim milk and cod-liver oil.

EXPERIMENTAL

Calves

Twenty-eight Ayrshire bull calves were used as experimental animals. They were bought in the open market when a few days old, and each was then confined in an individual pen, in a calf house thermostatically maintained at 60° F. On arrival each was given 20 ml. 'anti-coliform' serum by subcutaneous injection, four capsules of halibut-liver oil each containing 4500 i.u. vitamin A and 450 i.u. vitamin D, and cow's whole milk diluted with water. For the first week they were given 3, increasing to 4, l. of whole milk as the sole diet. Analyses of the milk from the cows in the Hannah herd showed the content of tocopherols to be relatively high (Blaxter, Brown & MacDonald, 1952).

Treatments

Subsequently, the calves were allocated by lot to one of the following treatments:

Positive controls. Dried skim milk 750 g, arachis oil containing 2000 i.u. vitamin A and 200 i.u. vitamin D 20 ml. and a gelatin capsule containing 50 mg DL α-tocopheryl acetate daily.

Low level cod-liver oil. Dried skim milk 750 g and cod-liver oil 1 oz. daily.

Medium level cod-liver oil. Dried skim milk 750 g and cod-liver oil 2 oz. daily.

High level cod-liver oil. Dried skim milk 750 g and cod-liver oil 4 oz. daily.

Negative controls. Dried skim-milk powder 750 g and the arachis oil solution of vitamins A and D 20 ml. daily.

Feeding arrangements

During the 2nd week after arrival of the calves, the arachis oil or cod-liver oil was added to the whole milk. The dried skim milk dissolved in tap water was gradually substituted for the whole milk and the calves were consuming the experimental diets 12–15 days after purchase. The oils were stirred into the warmed milks immediately before feeding. No emulsion was formed since the oil separated completely after standing for a few minutes. Feeding was normally twice daily but during the 1st week, and with any weakly animals, it was thrice daily. The allowance of dried skim milk was increased to 1000 g after 50 days.

Plan of experiment

In each of groups 1–4 inclusive there were six calves, in group 5 there were four. It was impossible to carry out the whole experiment at once, and so 'replications' of the experiment were carried out at different times. The first replication was begun early in September 1950 and the second in November 1950. Two further replications were begun in January 1951 and the remaining two in March 1951. For reasons given later the negative controls were all included in the replications of March 1951.

The calves were not given any roughage or solid food, but they consumed bedding on occasions. Straw was used as bedding for the first 30–40 days when it was replaced by peat moss.

Methods

Detailed observations of the posture and gait as well as of appetite, 'condition' and general wellbeing of each calf were made daily. The calves were weighed at weekly intervals, on which occasions they were allowed to run about for a few minutes under unrestricted conditions outside the calf house. When abnormalities occurred that were thought to be indicative of serious dystrophy the calf was killed. Unaffected calves and control calves were killed some 50–60 days after purchase. Three calves for which the ante-mortem evidence suggested that the cause of death was not entirely the result of muscular dystrophy were subjected to veterinary pathological examination by Mr A. L. Wilson and the staff of the Veterinary Department, West of Scotland Agricultural College.

At all post-mortem examinations the entire musculature was closely examined, sketches and notes were made of affected muscles and photographs taken. Where it was impossible to decide whether a muscle was normal or dystrophic, sections were taken for histological study. Chemical analyses were limited to the determination of dry matter, ash and creatine content of eight skeletal muscles and the heart. The methods have already been described (Blaxter & Wood, 1952). The tocopherol content of blood and tissues will be reported in a later paper (Blaxter, Brown & MacDonald, 1953).

RESULTS

General

The design of the experiment makes possible a comparison of the incidence of the disease in calves given the same diet at widely differing times of the year. In September the calves were born from cows at grass, whereas each subsequent replication was composed of calves from cows that had received winter rations for increasing periods of time. The last replication was composed of calves from cows that had been housed throughout a long and moderately severe winter. Where possible, visits or telephonic inquiries were made on the farms on which these calves had been born. In no case was any abnormality of the feeding regime of the herd cows noticed. It would seem that the calves were representative of those produced under average conditions of farm management in the west of Scotland.

The design also permits an accurate and unbiased comparison of differences between the first four treatments. Comparison of these with the negative controls is only valid for the March replication. As there is considerable evidence that the incidence of dystrophy under constant dietary and environmental conditions increases with advance of the winter (see below), this comparison is a highly critical one, since in March all animals were at their maximal sensitivity.

Observations made before death

Only one of the calves that received cod-liver oil failed to show any of those signs which previously have been ascribed to dystrophic involvement of part or parts of the skeletal or cardiac musculature. Two of the calves that received arachis oil and vitamin E were noted to be slightly splay-footed or flat-footed, but these signs could

not be categorically interpreted as indicative of any dystrophic lesions. Two of the four calves that received no additional vitamin E in the March replication showed similar slight flat-footed gaits. One animal in this group (replication no. 6*a*), however, showed quite definite signs of dystrophy of the muscles of the back. The signs when first noticed were mild and never appeared to become more pronounced, in fact, they slowly regressed as the experiment continued. Before slaughter, this calf was classified as normal.

The clinical signs observed in the most severely affected calves were broadly the same as in previous experiments—abnormal gait, flexion of the knees, winged scapulas, outward rotation of the hocks, and inability to rise. Three signs that may have occurred but had not previously been noted in dystrophic calves were also seen. These were, firstly, acute abdominal respiration, secondly, severe heart attacks with consequent prostration, and, lastly, presumed dystrophy of the lumbar muscles. Abdominal respiration occurred in calves in which post-mortem examination showed an almost complete dystrophy of both the internal and external intercostal muscles and the diaphragm. Affected calves stood in a characteristic attitude with their heads down and outstretched. No movement of the thoracic wall occurred and the abdominal wall took over the entire function of those muscles normally employed in breathing. Such respiration was accompanied by expiratory grunts. One animal thus affected died, but all the others were slaughtered to avoid unnecessary suffering. Dystrophy of the muscles of the back resulted in the extension of both forelegs and hind-legs to minimize strain on the affected muscles. The application of the hand to the back of such calves resulted in a voluntary depression of the back, whereas in normal calves this action generally results in a humping of the back. Heart attacks were in most cases precipitated by exercise. The calf would collapse after being walked outside or even after having become excited in its pen. Heavy breathing and tachycardia were predominant signs and soon the heart became irregular in its beat. For several hours after recovery the pulse was erratic and more than one attack sometimes occurred in one day.

Further clinical signs were also apparent, those of bloat. Two calves, both in the group that received 1 oz. cod-liver oil (replication nos. 1 and 5) suffered very severely from bloat. The bloating occurred every time food was given and was associated with pain. The more usual remedies had no effect, and both animals were killed, although they had shown only slight signs of dystrophy. A further calf that was given 4 oz. cod-liver oil (replication no. 4) died very suddenly when a blood sample was being taken, and post-mortem examination showed a twist of the ileum at the level of the ileo-caecal valve. Slight bloating also occurred in one calf that received 2 oz. cod-liver oil. No bloating ever occurred in the ten calves that did not receive cod-liver oil. Whether bloating and abnormality of the intestinal tract were related to dystrophy of the smooth muscular coat of the intestine could not be determined. It was, however, very suggestive that at post-mortem examination of the five remaining calves of the group that received 4 oz. cod-liver oil two showed a complete lack of the normal elasticity and tonus of the walls of the rumen.

Table 1 summarizes the signs shown by each calf, its fate, and the number of days

Table 1. *Summary of the clinical history of each calf*

Treatment	Replication no.	Fate	Signs and observations	No. of days of treatment
Positive controls:				
Arachis oil supplying vitamins A and D, 50 mg vitamin E daily	1	Killed	Very slight signs of muscle weakness	53
	2	Killed	No signs	42
	3	Killed	No signs	41
	4	Killed	No signs	40
	5	Killed	Very slightly flat-footed	37
	6	Killed	No signs (killed early for comparative purposes)	25
Low-level cod-liver oil, 1 oz. daily	1*	Killed *in extremis*	Continuous bloating necessitating constant surgical interference	40
	2	Killed *in extremis*	*Dystrophic*; respiratory muscles involved	43
	3	Killed	Only slight signs of dystrophy shown	41
	4	Died	*Dystrophic*; diaphragm and heart involved	35
	5*	Killed *in extremis*	Continuous bloating and poor thriving	25
	6	Killed	Only very slight signs of dystrophy shown	38
Medium-level cod-liver oil, 2 oz. daily	1	Killed	No signs observed	53
	2	Died	*Dystrophic*, heart signs, carcass haemorrhagic	35
	3	Killed	Slight signs of muscular involvement	40
	4	Killed *in extremis*	*Dystrophic*; diaphragm and chest involved	39
	5	Killed *in extremis*	*Dystrophic*; diaphragm and chest involved	22
	6	Killed *in extremis*	*Dystrophic*; severe heart attacks	25
High-level cod-liver oil, 4 oz. daily	1	Killed	*Dystrophic*; limb muscles severely involved	53
	2	Killed	Slight signs of muscular dystrophy	42
	3	Died	*Dystrophic*; heart signs and severe muscular involvement	25
	4*	Died	Only slight signs of dystrophy. A twist of the large bowel found at post-mortem examination	20
	5	Died	*Dystrophic*; diaphragm and chest involved	17
	6	Killed *in extremis*	*Dystrophic*; diaphragm and chest involved	17
Negative controls:				
Arachis oil alone. No vitamin E	5*a*	Killed	No signs	41
	5*b*	Killed	Very slight signs of muscle weakness	41
	6*a*	Killed	Very slight signs of dystrophy which regressed	71
	6*b*	Killed	No signs	71

* It is not known whether the dietary cod-liver oil was concerned in the death of these animals (see p. 37).

elapsing between the introduction of cod-liver oil into the ration and death. The calves that were killed *in extremis* and those that died are indicated. From the table one conclusion is quite clear; those groups that received no cod-liver oil showed no serious signs of dystrophy whereas the feeding of cod-liver oil resulted in very severe dystrophy. There was no very great difference in the severity of the disease between the three groups that received cod-liver oil. The mean numbers of days between commencement of cod-liver oil feeding and death of those calves that died or were killed when *in extremis*, however, were, with 1 oz. cod-liver oil, 36 days; with 2 oz., 30 days; and with 4 oz., 20 days. If the calves suffering from bloat, previously referred to, are excluded, the numbers of days were: 34, 30 and 21 respectively. Similarly, scoring the calves on the basis of the severity of signs, 0 representing no signs and 4 severe signs, gave the following scores out of a possible total of 36: group receiving no cod-liver oil, 2; group receiving 1 oz., 15; 2 oz., 13; and 4 oz., 19. These results do not indicate very large differences between the various doses of cod-liver oil as far as the severity of signs was concerned, but show that those calves that received the largest doses succumbed most quickly.

Table 2. *Mean gain in weight of the calves, time taken to consume their rations and loss of hair from their limbs*

Treatment	No. of calves	Gain in weight (g/day)	Time taken to consume ration (sec)	No. of calves losing hair from face and limbs
Negative controls:				
Arachis oil alone	4	308	78	2
Positive controls:				
Arachis oil with vitamin E	6	344	47	2
Low-level cod-liver oil, 1 oz. daily	6	271	109	4
Medium-level cod-liver oil, 2 oz. daily	6	365	84	5
High-level cod-liver oil, 4 oz. daily	6	267	172	5

Table 2 summarizes the mean gains in body-weight of the calves and the mean length of time they took to drink their rations. These gains and times refer only to the period before an increase was made in the quantity of diet offered. There were no statistically significant differences between the gains in body-weight of the calves. This conclusion may also be drawn from Fig. 1, in which the body-weights of the calves in the last replication of the experiment are shown. The calves that received either 2 oz. or 4 oz. cod-liver oil were both dystrophic and were killed to prevent suffering. It is remarkable that such a severe disease does not affect gain of body-weight.

Previously it had been shown (Blaxter, Watts & Wood, 1952) that muscular dystrophy was associated with a decline in the nitrogen balance and an increase in heat production. The observation that body-weight gain is not appreciably affected would thus appear at variance with these results. The explanation is to be found in the presence of slight but unmistakable oedema of the musculature (see Table 5). Protein

is lost, but water is gained. The differences between the groups in time taken to consume their rations were large. The values in Table 2 refer to those recorded at least 7 days before death or slaughter. The longer time taken by dystrophic calves to consume food is in agreement with previous results (Blaxter, Watts & Wood, 1952). Hair loss refers to depilation of the face and limbs. Bate, Espe & Cannon (1946) have

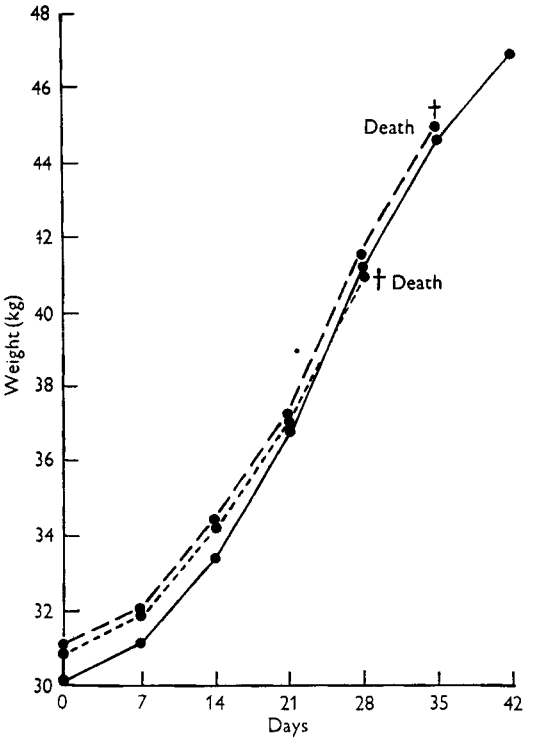

Fig. 1. Effect of dystrophy induced by cod-liver oil on the body-weight of calves. ———, positive control (arachis-oil solution of vitamins A and D with α-tocopherol); - - - - -, high-level cod-liver oil, 4 oz. daily; – – –, medium-level cod-liver oil, 2 oz. daily.

associated similar loss of hair in young calves with ingestion of non-homogenized fat. This may be the reason for the hair loss of the present calves. It was more severe with cod-liver oil than with arachis oil, and was not stopped by the addition of vitamin E.

Post-mortem examination

The appearance of the affected musculature was comparable to that already reported in our previous experiments (Blaxter, Watts & Wood, 1951, 1952) and to descriptions of the naturally occurring disease *weisses Fleisch* of cattle. The results are summarized in Table 3. No attempt has been made to classify them minutely into smaller groups than 'limbs' and 'other skeletal muscles', since dystrophy may be present to the same general extent in a carcass and yet one particular group of muscles remain apparently normal. None of the animals that were given arachis oil with or without vitamin E had abnormal heart or diaphragmatic muscles. In the

group of animals receiving α-tocopherol only part of one single muscle in one calf—the gluteus accessorius of the calf in replication no. 3—was thought dystrophic. When the same ration without vitamin E was given, a patchy appearance of the supra- and infraspinatus was noted in two calves and in a third a definite dystrophic lesion of the longissimus dorsi was observed. A dystrophy involving the back muscles had been diagnosed earlier in this calf, but the signs had regressed. This lesion appeared to be in the process of involution, and is remarkable in that it was strikingly asymmetrical.

Pronounced dystrophy was present in animals of all groups that received cod-liver oil. All skeletal muscles seemed to be affected, but suspensory muscles to the limbs appeared to be slightly more affected than others. On the basis of the post-mortem reports the severity of the lesions in the groups was summarized as shown in Table 4. Scoring of the severity of dystrophy showed that there was an increase with increasing dosage of cod-liver oil. There were nevertheless animals in all these groups that showed only slight signs, and in each of the groups that received 1 oz. cod-liver oil, and 2 oz. cod-liver oil, there was one animal with apparently normal muscles. Such variation has already been noted in our previous experiments.

Dry-matter, ash and creatine contents of skeletal muscles

The mean dry-matter and ash contents of the skeletal muscles are given in Table 5.

It will be seen from the results that in every muscle group the muscles of animals that received cod-liver oil contained less dry-matter than those of comparable calves that received none, whether they had received additional α-tocopherol or not. The muscles of calves given cod-liver oil also contained more ash than did the muscles of calves given an arachis oil solution of vitamins A and D. These results are in complete agreement with our previous ones (Blaxter & Wood, 1952). Statistical analysis of these results was not made owing to the obvious non-homogeneity of the variances of the values as between control calves and those given cod-liver oil. Non-homogeneity may be inferred from Fig. 2, where it is clear that the range of variation of creatine content is far greater in the calves given cod-liver oil than it is in the control calves. This was also true of the results of determinations of dry matter and ash content. The marked asymmetry of distribution and resultant shifting of the mean is quite apparent. The graphical representation, however, shows the marked effect of even 1 oz. cod-liver oil in shifting the mean of the distribution.

The creatine content of skeletal muscles is shown further in Table 6. The muscles of animals given cod-liver oil contained less creatine than those given arachis oil and a supplement of α-tocopherol, and with three exceptions less than those given arachis oil alone. From the results it would appear that the muscles of the shoulder—the infraspinatus and the supraspinatus—tended to be the most affected, as had been inferred by inspection. In the individual calf, however, this was not always so.

From the chemical analyses it appeared that 4 oz. cod-liver oil did not cause a greater disturbance of muscle composition than either 2 oz. or 1 oz.; this at first appears anomalous, but is probably to be accounted for by the fact that animals were killed when they showed signs. Thus calves that died with severe dystrophy after

Table 3. *Summary of presence of dystrophic muscles in calves*

Treatment	Replication no.	Incidence of dystrophy (visual assessment only) in the Heart	Diaphragm	Limbs	Other muscles
Positive controls: Arachis oil supplying vitamins A and D, 50 mg vitamin E daily	1	Normal	Normal	Normal	Normal
	2	Normal	Normal	Normal	Normal
	3	Normal	—	Normal	Part of gluteus accessorius white
	4	Normal	Normal	Normal	Normal
	5	Normal	Normal	Normal	Superficial muscles pale
	6	Normal	Normal	Normal	Normal
Low-level cod-liver oil, 1 oz. daily	1	Dystrophic	Normal	All limbs partly dystrophic	Peritonitis present—muscles partly dystrophic
	2	Pale	—	Forelimbs completely dystrophic	All skeletal muscles wet and oedematous
	3	Dystrophic and ventricle partly hardened	Pale	Dystrophic in parts only	Slight dystrophy of superficial musculature
	4	Dystrophic and ventricle partly hardened	Completely dystrophic	Dystrophic and haemorrhagic	Shoulder suspension and back muscles dystrophic (very severe)
	5	Flabby	Normal	Normal	Normal
	6	Flabby and pale	Pale	Very slightly affected	Normal
Medium-level cod-liver oil, 2 oz. daily	1	Very pale and patchy	—	Pale, otherwise normal	
	2	One pillar and wall dystrophic	Normal	Partly dystrophic	Shoulder suspensory muscles partly dystrophic
	3	Enlarged and dystrophic	Pale	Forelimb pale, hind-limb partly dystrophic	Not affected to any great extent
	4	Enlarged and dystrophic	Radially dystrophic	Severely dystrophic	All muscles inluding intercostals slightly affected, subcutaneous muscles affected
	5	Severely dystrophic	Completely dystrophic	Completely dystrophic	All muscles very severely dystrophic, external intercostals worse than internal
	6	Very severely dystrophic	Radially dystrophic	Completely dystrophic	Back muscles dystrophic, shoulder and neck muscles normal
High-level cod-liver oil, 4 oz. daily	1	Normal	—	Partly dystrophic	Partly dystrophic
	2	Very large and flabby but no lesions	Normal	Partly dystrophic	Apparently normal
	3	Very large and possibly dystrophic, very difficult to tell*	—	Shoulder muscles dystrophic, rest pale	Some obvious areas dystrophic with considerable haemorrhage
	4	Dystrophic pillars	Normal	Partly dystrophic	Scalenae and deltoids dystrophic (twist at ileo-caecal valve)
	5	Very severely dystrophic and haemorrhagic	Partly radially dystrophic	Severely dystrophic	Intercostals completely dystrophic, hind-limb suspensory completely dystrophic; back muscles normal
	6	Normal size, small pin-point lesions	Completely dystrophic	Forelimb muscles oedematous and pale; hind-limb muscles dystrophic	Intercostals partly dystrophic, back muscles dystrophic; neck and shoulder suspensory muscles completely dystrophic
Negative controls: Arachis oil alone	5*a*	Normal	Normal	Shoulder muscles very slight dystrophic	Back muscles slightly pale
	5*b*	Normal	Normal	Shoulder muscles (spinatus group) patchy in colour	Normal
	6*a*	Normal	Normal	Normal	Unilateral severe dystrophy of longissimus dorsi
	6*b*	Normal	Normal	Normal	Neck slightly pale

* Owing to unequal distribution of blood

Table 4. *Classification of the calves according to severity of muscular dystrophy*

Replication no.	Positive controls: Arachis oil with vitamin E	Negative controls: Arachis oil alone	Low level cod-liver oil 1 oz. daily	Medium-level cod-liver oil 2 oz. daily	High-level cod-liver oil 4 oz. daily
1	Normal	—	Slight	Normal	Severe
2	Normal	—	Severe	Severe	Slight
3	Very slight	—	Slight	Slight	Very severe
4	Normal	—	Very severe	Severe	Severe
5	Normal	Very slight (?)	Normal	Very severe	Very severe
6	Normal	Very slight (?)	Slight	Very severe	Very severe
5*a*	—	Very slight	—	—	—
6*a*	—	Normal	—	—	—
Total score (percentage of possible)	1·7	7·5	41·7	58·3	75·0

The classification is based on an assessment of the severity of muscular dystrophy as normal, very slight, slight, severe or very severe. For numerical comparison these classes are given the values 0, 1, 3, 6, and 10 respectively.

Table 5. *Mean dry-matter content and mean ash content of the dry matter of the skeletal muscles of the calves*

Treatment	Mean dry-matter content (%)				Mean ash content of the dry matter (%)			
	Shoulder (two muscles)	Arm (three muscles)	Forearm (two muscles)	Biceps femoris	Shoulder (two muscles)	Arm (three muscles)	Forearm (two muscles)	Biceps femoris
Positive control, no cod-liver oil, 50 mg tocopherol daily (six calves)	21·4	21·7	20·9	21·8	4·9	4·9	5·1	5·3
Negative control, no cod-liver oil, no tocopherol (four calves)*	21·5	21·7	21·6	21·9	5·4	5·3	5·0	5·6
Low-level cod-liver oil, 1 oz. daily (six calves)	19·0	20·1	20·0	20·7	7·7	5·6	5·7	6·4
Medium-level cod-liver oil, 2 oz. daily (six calves)	20·0	20·5	20·1	19·4	7·5	6·4	6·1	6·6
High-level cod-liver oil, 4 oz. daily (six calves)	19·9	20·9	20·0	21·0	6·0	5·4	5·4	5·9

* Four calves only in March replications. The values do not differ significantly from those obtained with two animals given α-tocopherol in concomitant replications.

having received 4 oz. cod-liver oil for 17–20 days had muscles closely resembling in composition those of calves that took twice as long to succumb when given 2 oz. A further explanation may be that an acute toxicity of cod-liver oil may result in a greater disturbance of one functionally indispensable muscle and a lesser disturbance of the remainder, whereas chronic toxicity may result in a more generalized effect. The results of Pappenheimer (1940) showing that disuse of a muscle renders it immune to muscular dystrophy, and the earlier results with calves showing a voluntary redistribution of the load on affected muscles (Blaxter, Watts & Wood, 1952) would suggest that gait and stance adaptations in chronic dystrophy, which result in partial disuse of particular muscles, possibly play a part in the total severity of the disease at death.

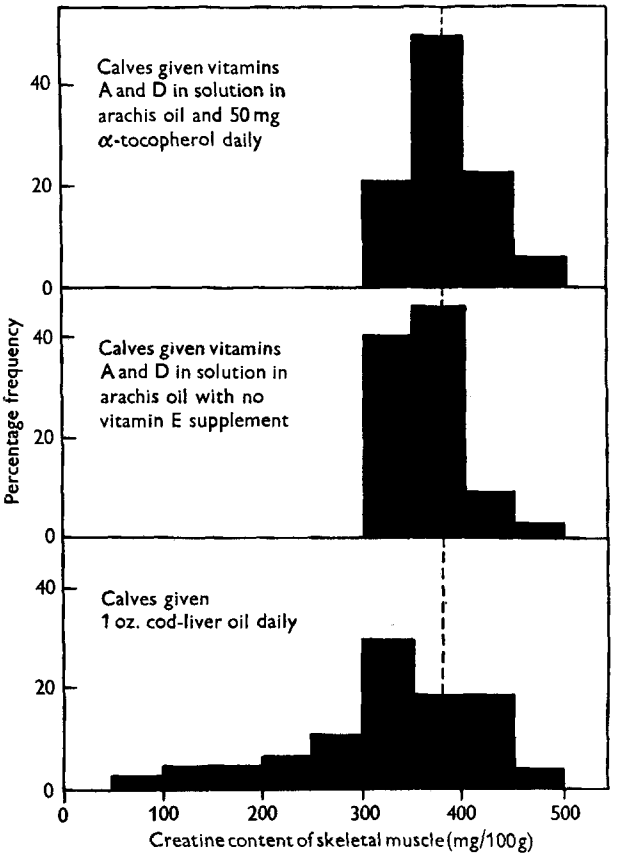

Fig. 2. Effect of 1 oz. cod-liver oil daily in the ration of the calves on the creatine content of their muscles.

Table 6. *Mean creatine content of skeletal muscles of the calves*

(Mean values for six calves in each experimental group and for four negative control calves)

Muscle	Positive controls: No cod-liver oil + 50 mg α-tocopherol daily (mg/100 g)	Low-level cod-liver oil 1 oz. daily (mg/100 g)	Medium-level cod-liver oil 2 oz. daily (mg/100 g)	High-level cod-liver oil 4 oz. daily (mg/100 g)	Negative controls: No cod-liver oil and no α-tocopherol (mg/100 g)
Supraspinatus	380	272	282	323	346
Infraspinatus	365	272	304	303	335
Long head of triceps	401	327	340	372	417
Lateral head of triceps	348	313	296	332	356
Anterior brachial	374	369	333	362	350
Anterior extensor of metacarpus	379	345	331	376	363
Coracoradialis	363	363	351	362	354
Rectus femoris	429	400	378	413	399
Mean of all muscles	380	333	327	355	365

The muscles of the calves that received the arachis oil solution as the sole supplement to their diet were slightly lower in creatine content than those of the calves that received vitamin E. The differences were small and variation within groups excludes their significance. The apparently dystrophic muscle of the back of the calf in replication no. 6*a* had normal creatine, ash and dry-matter contents, suggesting that the hypothesis of recovery was in fact correct.

Composition of the heart muscle

The results of analyses of the hearts of individual calves are given in Table 7. Again no statistical analysis has been made since the distributions within the groups that were dystrophic were clearly asymmetrical. An indication of normality or otherwise of the hearts of the individual calves may, however, be obtained by computation of the mean values and their standard deviations of the control group, for dry matter $20{\cdot}38 \pm 0{\cdot}18\%$ and for creatine $258{\cdot}5 \pm 26{\cdot}4$ mg/100 g. Using twice the standard deviation as a criterion of the range above which the ash, or below which the dry-matter or creatine content, is unlikely to fall, the values of 5·8% for ash, 19·4% for dry-matter and 205 mg/100 g for creatine are obtained. These limits do not represent exact fiducial limits, for the numbers in the control group are small. They nevertheless allow a more critical appraisal of the results of Table 7. Values that may be judged abnormal have been marked with an asterisk. These results show that two cardiac muscles of abnormal ash content were encountered in the group that received arachis oil without additional vitamin E. Of the eighteen calves in the groups that received cod-liver oil, three were judged to have oedematous muscles, seven to have muscles with an increased ash content and twelve to have muscles with a low creatine content. It was evident that the cardiac muscle could be low in creatine content and yet be comparatively normal in ash and dry matter.

The correlation between a visual assessment of dystrophy and the assessment of dystrophy from the creatine content alone was fair. In the twenty-eight calves, only two showed disagreement. The heart of the calf that received 2 oz. cod-liver oil daily (replication no. 4), although judged to be dystrophic at post-mortem, had a normal creatine content, and the heart of the calf that received 4 oz. cod-liver oil in the same replication was normal chemically, though at post-mortem examination the pillars of the left ventricle were classified as partly dystrophic. Sampling errors may be involved in these discrepancies, though the agreement between duplicate chemical analyses was within $\pm 5\%$.

Histological examination

Broadly speaking, the lesions observed in samples of heart and skeletal muscle were the same as previously found (MacDonald, Blaxter, Watts & Wood, 1952). Agreement between the results of the histological examination and chemical analysis of muscles was good for the skeletal muscles, but only fair for the heart muscle. In no instance was cardiac muscle classified as abnormal histologically when the creatine content was normal, but the reverse was often true. This might have been due to

Table 7. *Mean content of dry matter, ash and creatine in the walls of the ventricles of the hearts of the calves*

Replication no.	Positive controls: Arachis oil with 50 mg α-tocopherol daily			Negative controls: Arachis oil alone			Low-level cod-liver oil 1 oz. daily			Medium-level cod-liver oil 2 oz. daily			High-level cod-liver oil 4 oz. daily		
	Dry matter (%)	Ash (percentage of dry weight)	Creatine (mg/100 g)	Dry matter (%)	Ash (percentage of dry weight)	Creatine (mg/100 g)	Dry matter (%)	Ash (percentage of dry weight)	Creatine (mg/100 g)	Dry matter (%)	Ash (percentage of dry weight)	Creatine (mg/100 g)	Dry matter (%)	Ash (percentage of dry weight)	Creatine (mg/100 g)
1	20·0	5·4	260	—	—	—	20·1	6·0*	188*	19·9	5·1	213	19·4	5·0	204
2	21·1	5·2	258	—	—	—	—	8·2*	189*	19·6	5·2	158*	19·9	5·5	223
3	20·1	5·5	251	—	—	—	19·6	5·8	183*	19·8	5·6	195*	19·2*	7·1*	196*
4	19·9	5·5	280	—	—	—	19·0*	5·7	194*	Lost	6·5*	214	20·1	5·8	237
5	20·6	Lost	289	19·7	5·7	231	19·7	5·5	232	19·4	6·2*	191*	18·6*	6·3*	104*
6	20·6	5·6	213	20·4	6·5*	234	20·6	5·4	196*	21·1	5·8	152*	20·4	5·9*	195*
5*a*	—	—	—	20·7	6·2*	275	—	—	—	—	—	—	—	—	—
6*a*	—	—	—	21·1	5·6	262	—	—	—	—	—	—	—	—	—
Mean	20·4	5·4	258·5	20·5	6·0	250·5	19·8	6·1	197	20·0	5·7	187	19·6	5·9	193·1

* Values that may be regarded as abnormal (see text, p. 45).

absence of the focal lesion from the tissue blocks examined, and thus represent a sampling error.

In addition to the changes in muscles previously reported, two changes were observed in early dystrophy. First, the sequence of coagulation and clumping of the sarcoplasm appeared to be preceded by a granulation leading to a total disappearance of cytoplasm, but without coagulation and necrosis. Breaking of fibres and fraying of the ends of the myofibrils were also common. The loss of staining power which was previously reported appears very characteristic of these early changes. Secondly, in several muscle sections the sarcolemmal reaction was not observed. A degeneration of the cytoplasm, generally without segmentation and necrosis, was followed by vacuole formation, but a collapse of the sarcolemma did not occur nor did the nuclei of the sarcolemma proliferate. The reason or reasons for this failure of response or, indeed, for the more usual response of the sarcolemmal nuclei is not known. It appears, however, that sarcolemmal nuclear proliferation only follows the collapse of the sarcolemmal tube.

The opportunity was taken to examine the livers, kidneys and spinal cords of affected and normal animals. Liver damage was detected in only one animal in which a fatty degeneration had taken place. No significance is attached to this observation for it may have been the result of terminal failure of appetite. No lesions other than minute changes in widely scattered glomeruli were found in the kidneys. This was unexpected since, in view of the breakdown of muscle, the presence of tubular casts was thought probable. The spinal cords were examined by sectioning at six levels, two cervical, two thoracic and two lumbar. There was no demyelination or abnormality of the pyramidal tracts, and the anterior horn cells were perfectly normal.

From these observations it is apparent that neither central nor peripheral nerves are involved in the disease, and they emphasize once again that the primary changes are in the sarcoplasm of the muscle cells.

DISCUSSION

The results of the experiment are quite clear. Administration of cod-liver oil to calves results in a high incidence of muscular dystrophy and a large number of deaths, either sudden or after a short period of prostration. The design of the experiment did not clearly distinguish between the toxicity of 1 oz. and 4 oz. of cod-liver oil as far as muscle composition was concerned, since those calves given the highest level died at an earlier age. The rations given were those recommended by a variety of authorities on calf rearing. The dried skim milk and cod-liver oil were both of high quality, and the results must be viewed with some concern. They confirm the earlier reports of Agduhr (1927), Agduhr & Stenström (1929), and Slagsvold (1925), as briefly reviewed by Blaxter, Watts & Wood (1952). These early reports were not confirmed by American or German workers. Similarly, no deaths of calves in this country as the result of muscular dystrophy have been reported from the field, though it is safe to assume either that cod-liver oil has been added to skim-milk rations or that it has been incorporated in milk substitutes which contain very high quantities of dried skim milk. If our experiments and the earlier Scandinavian experiments are unimpeachable,

dystrophy should have occurred, and the absence of reports to that effect needs explanation. One obvious explanation is that dystrophy, or death as the result of dystrophy, may have occurred without correct interpretation of the cause. Diagnosis is difficult. A dystrophic calf is a calf that is muscularly weak and lethargic, and so in fact is a calf with diarrhoea or the more common infections. Many calves have signs very easily mistaken by the farmer—and indeed by us, for calf pneumonia; still others die suddenly in convulsions that might be thought due to lead poisoning (Allcroft & Blaxter, 1950), magnesium deficiency (Duncan, Huffman & Robinson, 1935), or

Table 8. *Seasonal incidence of muscular dystrophy as judged by the performance of calves receiving cod-liver oil at different times of the year*

Month in which the experiment began	No. of calves	Deaths due to dystrophy including calves killed *in extremis* (%)	Score of all animals (see Table 4) (%)	Mean muscle* creatine of all animals (mg/100 g)
September	4	25	30	359
November	4	50	50	354
January	8	50	63	329
March†	8	62	72	318

* Mean value of seven forelimb muscles for each calf.

† Not including the calves that received vitamins A and D in arachis oil without a supplement of vitamin E.

'colic'. A further explanation is afforded by calf no. 85 which received dried skim-milk powder and an arachis-oil solution of vitamins A and D without any additional vitamin E. This animal was judged to be slightly dystrophic when 22 days of age, but the signs regressed with time and only a small but nevertheless quite definite lesion was recorded at post-mortem examination. Regression of signs in calves on farms is a possibility which needs consideration. Finally, the incidence of the disease is not 100% even at the highest level of treatment. The cause of this is not known, but further information on this aspect may supply evidence regarding the apparent absence of field cases of cod-liver oil injury in this country.

The heart failure of some of the calves is of considerable interest since a comparable 'sudden heart death' has been observed in pigs and sheep in Germany and Scandinavian countries (Karsten, 1931; Nieberle & Cohrs, 1949*a*; Seifried, 1943; Nieberle & Cohrs, 1949*b*). These diseases are characterized by sudden death upon the slightest exertion. A grey heart muscle, sometimes with a patchy appearance, is seen at post-mortem examination and on histological study a grainy vacuolar degeneration or a hyaline, scaly ('Schollig') degeneration is observed. Both appear to be followed by a nuclear proliferation of the sarcolemma. A variety of causes has been suggested for these diseases, from infection to a reflex-conditioned coronary insufficiency. Seifried (1943), however, has suggested that a vitamin deficiency may be involved in their aetiology and the present results suggest that this may be so. A similar type of disease in calves called *Transportnekrose* or transit-disease has also been described (Nieberle & Cohrs, 1949*b*). Descriptions of the heart in this disease agree well with present findings and our previous observations.

In the naturally-occurring diseases that are comparable to *weisses Fleisch* a seasonal incidence has been recorded (Hjärre & Lilleengen, 1936*a*, *b*; Nieberle & Cohrs, 1949*b*; Mahr, 1936). From the present results a similar seasonal incidence; may be inferred, as shown in Table 8. A higher number of calves died or were killed *in extremis* in March than at any other time of the year, and the scores based on the severity of the disease confirm this, as indeed does the creatine content of the muscles. This suggests a depletion of the dams of these calves of vitamin E or perhaps other protective factors, during the late winter months.

SUMMARY

1. An experiment involving twenty-eight Ayrshire bull calves was carried out to provide information on the toxicity of cod-liver oil at levels recommended for use in farm practice in Great Britain.

2. The daily addition of 1, 2 or 4 oz. cod-liver oil to a dried skim-milk ration resulted in death or severe dystrophy of a high proportion of the animals.

3. Besides severe dystrophy of limb muscles, dystrophy of diaphragmatic and intercostal muscles was observed. A high incidence of 'bloating' and a lack of tonus of the rumen walls at post-mortem were encountered. Heart lesions were particularly severe. Gain in body-weight was not affected.

4. Additional observations on the histological development of the lesions are presented. No involvement of either the central or peripheral nervous system could be detected nor was any gross abnormality present in the livers or kidneys of dystrophic calves.

5. The incidence of the disease was affected by the season of the year, a higher number of calves dying in late winter than in early autumn.

6. Four calves given the basal ration with vitamins A and D showed very mild signs of dystrophy which regressed. At post-mortem only very slight, but nevertheless definite, lesions were observed.

7. In view of the use of cod-liver oil in the rations of young calves as supplements or as components of calf starters and milk substitutes, the results are discussed in relation to the absence of reports of deaths of such a nature under field conditions in Great Britain.

Our thanks are due to Mr D. Paterson and Miss M. Hutcheson for the great care they took in looking after the experimental animals, and to Mr A. L. Wilson, M.R.C.V.S. and his staff of the Veterinary Department, West of Scotland Agricultural College, for the examination of the carcasses of three of the calves. We are also most grateful to Miss Breckenridge for technical assistance.

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