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SERUM 25-HYDROXYVITAMIN-D CONCENTRATIONS IN BEEF CATTLE HERDS IN WHICH RUPTURES OF THE ACHILLES TENDON HAD OCCURRED*

By

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STURÉN, M.: *Serum 25-hydroxyvitamin-D concentrations in beef cattle herds in which ruptures of the Achilles tendon had occurred.* Acta vet. scand. 1985, *26*, 169—178. — The frequency of skeletal disorders including ruptures of the Achilles tendon has increased during the last years in Swedish beef cattle herds, especially in intensively raised calves. The serum concentrations of 25-hydroxyvitamin-D, calcium and inorganic phosphorus were analysed in 7 affected and 3 control herds. None of the animals in affected herds had detectable amounts of serum-25-OH-D_3 ($\leq$ 2 ng/ml) while the control animals had a concentration of 36.1 ng/ml (least squares mean). Calcium and inorganic phosphorus were also significantly lower in the affected herds ($P \leq 0.001$). In 1 herd the animals were randomly divided into 4 groups and treated in one of the following ways: Orally with vitamin AD_3E or D_3, intramuscularly with D_2 or D_3. The effect of treatment on serum Ca and P_i was measured 1 month later and was found to be equally high in the 4 groups.

skeletal disorders; calcium; inorganic phosphorus.

Skeletal disorders, especially in intensively raised calves, have become more common in the last decades in Sweden. The clinical picture is characterized by stiffness, unwillingness to move, and retarded growth. The growth zones in the long bones are abnormal. In some cases ruptures of the Achilles tendon and fractures occur (*Martin & Holzschuh* 1964, *Spratling et al.* 1970, *Jonsson et al.* 1972, *Johnsson et al.* 1977, among others).

Low calcium and/or low phosphorus (*Martin & Holzschuh* 1964, *Schulz* 1964) as well as low vitamin D supply in the diet

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(*Jonsson et al.* 1972, *Johnsson et al.* 1977) have been discussed as aetiological factors.

The purpose of the present investigation was to examine the vitamin-D status in beef cattle herds in which Achilles tendon ruptures had occurred and also to follow the effects of vitamin D_2 or D_3 treatments in some of the animals. The serum levels of 25-hydroxyvitamin-D were used as a measure of vitamin-D status.

MATERIAL AND METHODS

Affected animals

The study comprised 110 animals, 3.5—12 months old, from 7 herds in which Achilles tendon ruptures had recently occurred in 1 or more animals. Varying clinical signs of the above-mentioned skeletal disorders were seen in most of the other animals of the same age groups, i.a. stiffness, difficulty in moving and visibly enlarged growth zones of the long bones.

In 1 herd animals were tied up, in the others they were loose-housed on slatted floors. All animals were kept indoors the whole year.

The most common Swedish breeds were represented (Swedish Friesian, Swedish Red and White and crosses between them). One herd included a few limousine crosses.

Control animals

Sixty calves of the same age and breeds (except limousine crosses) as the affected animals were used as controls. They belonged to 3 herds in which no Achilles tendon ruptures or symptoms of skeletal disorders had been observed. The animals were loose-housed on slatted floors and kept indoors all the year.

Feeding

The characteristics of the diets are summarized in Table 1. Three of the affected herds were fed concentrates ad lib. and a restricted amount of roughage. The other 4 affected herds were given approximately equal proportions of energy from roughage and concentrates in the diets. The concentrates were based on oats and barley in different proportions. Two of the affected herds (nos. 1 and 6) were given neither protein nor mineral supplement, 4 of them (nos. 2, 3, 5, 7) were supplemented in both ways, while the remaining one (no. 4) was given only

Table 1. Characteristics of diets in 7 herds affected by ruptures of the Achilles tendon and in 3 control herds.

Herd	Roughage etc.	Concentrates		Roughage: concentrates ratio on energy basis	Mineral supplement[2]	Vitamin D_3 supplement IU/kg conc.
		Oats: barley ratio	Protein suppl. %			
(*Affected*)						
1	hay	75/25	0	10/90[1]	0	0
2	silage, straw	60/40	18	50/50	+	0
3	hay, straw, carrots	60/40	7.5	50/50	+	0
4	hay	70/30	0	50/50	+	0
5	hay, potatoes	100/0	25	50/50	+	0
6	hay	75/25	0	10/90[1]	0	0
7	hay	75/25	10	10/90[1]	+	0
(*Control*)						
8	straw	60/40	13	10/90[1]	+	1000
9	hay	55/45	20	10/90[1]	+	1000
10	straw	50/50	20	10/90[1]	+	900

[1] Ad lib. feeding of concentrates.
[2] In herds with + sign the mineral supply was judged to be adequate according to ARC 1965.

mineral supplement. No vitamin supplement was given to any of the affected herds. The 3 control herds were fed concentrates ad lib. with protein, mineral and vitamin supplements.

Analysis of samples

Blood samples were taken from all animals. The samples were centrifuged as soon as possible and serum was kept at —20°C until analysed. 25-OH-D_3 and 25-OH-D_2 were analysed by high performance liquid chromatography (HPLC) (*Holmberg et al.* 1984). The assay separates 25-OH-D_3 from 25-OH-D_2. Ca was determined by atomic absorption and inorganic phosphorus according to *Fiske & Subbarow* (1925).

After initial blood sampling the animals in herd 1 were randomly divided into 4 groups of 5 animals each and treated in one of the following ways:

— Orally with vitamin AD_3E granules in daily amounts corresponding to 15 000 IU D_3
— orally with 15 000 IU D_3 daily
— intramuscularly with a single dose of 375 000 IU vitamin D_2
— intramuscularly with a single dose of 375 000 IU vitamir D_3.

The effect of treatment was followed up in blood samples taken 1 month later.

Statistical analysis

The General Linear Model procedure from the Statistical Analysis System (*SAS Institute Inc.* 1982) was used.

Results from the initial sampling in the 10 herds were analysed with the following model:

$$y_{ijk} = \mu + d_i + h_{ij} + (dh)_{ij} + e_{ijk}$$

where

y_{ijk} = the ijth observation
μ = general mean
d_i = effect of the ith class of vitamin D supplementation (with or without) ($i = 1,2$)
h_{ij} = effect of the jth herd ($j = 1,2, \ldots 10$)
e_{ijk} = residual random term

The effect of class of vitamin D supplementation was regarded as fixed and the effect of herd as random.

Data from the treatment experiment were analysed with the following model:

$$y_{ijk} = \mu + d_i + a_j + e_{ijk}$$

where the elements have the same meaning as before and in addition:

d_i = effect of the ith class of vitamin D supplementation ($i = 1,2, \ldots 5$)
a_j = effect of the jth animal ($j = 1,2, \ldots 20$)

The 5 classes of vitamin D supplementation were: without vitamin D before first sampling and 4 different treatments after first sampling.

Values of 25-OH-D_2 and 25-OH-D_3 below the detection limit of the method (2 ng/ml) were given the arbitrary value of 1.8 ng/ml.

RESULTS

At the initial blood sampling all animals in the affected herds had undetectable serum levels of 25-OH-D_3, while the animals in the control herds had an average level of about 36 ng/ml (least squares mean). The 25-OH-D_2 were below or just above the de-

Table 2. Least squares means of 25-OH-D_2, 25-OH-D_3, Ca and P_i in serum of animals from affected and control herds.

	25-OH-D_2 ng/ml	25-OH-D_3 ng/ml	Ca mmol/l	P_i mmol/l	n
Affected herds	2.0 n.s.	1.8***	2.50***	2.26***	110
Control herds	1.8	36.1	2.80	3.03	60

*** Significantly different from control group: $P \leq 0.001$.
n.s. Not significantly different from control group: $P > 0.05$.

tection limit (2 ng/ml) in all animals in the affected as well as in the control herds.

Serum calcium and inorganic phosphorus (P_i) were significantly lower in the affected herds (Table 2). There were rather big differences between affected herds as regards serum Ca and P_i levels (Table 3). In 3 of the herds (1, 5, 6) serum Ca values were considerably lower than in the rest. The same 3 herds and

Table 3. Least squares means ± standard error for serum Ca and P_i in 7 affected (1—7) and 3 control herd (8—10).

Herd no.	Number of blood-sampled	Ca mmol/l	P_i mmol/l
1	20	2.12 ± 0.05	2.27 ± 0.09
2	18	2.72 ± 0.05	2.79 ± 0.09
3	15	2.65 ± 0.06	2.56 ± 0.11
4	14	2.63 ± 0.06	2.45 ± 0.11
5	9	2.35 ± 0.08	1.73 ± 0.13
6	15	2.40 ± 0.06	1.98 ± 0.10
7	19	2.63 ± 0.05	2.07 ± 0.09
8	20	2.83 ± 0.05	3.18 ± 0.09
9	20	2.76 ± 0.05	2.88 ± 0.09
10	20	2.82 ± 0.05	3.04 ± 0.09

a fourth (7) had distinctly lower P_i levels as well. The levels of significance of herd differences as regards Ca and P_i values are shown in Table 4.

The effect of different treatments in herd 1 on the 25-OH-D_2, 25-OH-D_3, Ca and P_i levels after 1 month are shown in Table 5. The 25-OH-D_3 values in animals treated with vitamin D_3 had increased to levels nearly as high as those in the control herds (Table 2). A corresponding increase in 25-OH-D_2 concentration occurred after vitamin D_2 treatment. The 25-OH-D_2 values measured after vitamin D_3 treatment were numerically higher than

Table 4. Levels of significance for herd differences as regards serum Ca (above the diagonal) and P_i levels (below the diagonal).

Herd	1	2	3	4	5	6	7	8	9	10
1		***	***	***	*	***	***	***	***	***
2	***		n.s.	n.s.	***	***	n.s.	n.s.	n.s.	n.s.
3	*	n.s.		n.s.	**	**	n.s.	*	n.s.	*
4	n.s.	*	n.s.		**	**	n.s.	*	n.s.	*
5	***	***	***	***		n.s.	**	***	***	***
6	*	***	***	**	n.s.		**	***	***	***
7	n.s.	***	***	*	*	n.s.		**	n.s.	*
8	***	**	***	***	***	***	***		n.s.	n.s.
9	***	n.s.	*	**	***	***	***	*		n.s.
10	***	*	***	***	***	***	***	n.s.	n.s.	

before treatment. The serum Ca and P_i values of all treatment groups increased to levels equal to or above those observed in the control herds. All treatments gave effects on Ca and P_i of equal magnitude.

Table 5. Serum levels (least squares means ± standard error) of 25-OH-D_2, 25-OH-D_3, Ca and P_i in herd 1 before and 1 month after start of 4 different vitamin D treatments.

Treatment	n	25-OH-D_2	25-OH-D_3	Ca	P_i
Before	20	1.8 ± 1.0^a	1.8 ± 1.0^a	2.12 ± 0.05^a	2.27 ± 0.06^a
Vit. AD_3E orally	5	5.3 ± 2.1^a	26.8 ± 2.4^b	2.80 ± 0.12^b	2.89 ± 0.12^b
Vit. D_3 orally	5	4.9 ± 2.2^a	28.1 ± 2.4^b	2.80 ± 0.12^b	2.96 ± 0.13^b
Vit. D_2 i.m.	5	21.9 ± 2.1^b	1.8 ± 2.4^a	3.03 ± 0.12^b	2.68 ± 0.12^b
Vit. D_3 i.m.	5	5.3 ± 2.2^a	27.6 ± 2.4^b	2.86 ± 0.12^b	2.66 ± 0.13^b

Values with different superscripts are significantly different from each other ($P \leq 0.01$).

DISCUSSION

The skeletal disorders in fattening bulls, sometimes clinically described as leg weakness, have been morphologically characterized as rickets, secondary nutritional hyperparathyroidism or osteochondrosis (*Spratling et al.* 1970, *Jonsson et al.* 1972, *Fichtner* 1978, *Reiland et al.* 1978, *Jensen et al.* 1981). The pathological changes show great variability, often with features of more than 1 of the above-mentioned diseases in the same animal. Leg weakness is regarded as a multifactorial disease. Dietary deficiencies of calcium and/or vitamin D, high energy and high protein feeding are the most commonly discussed aetiological factors.

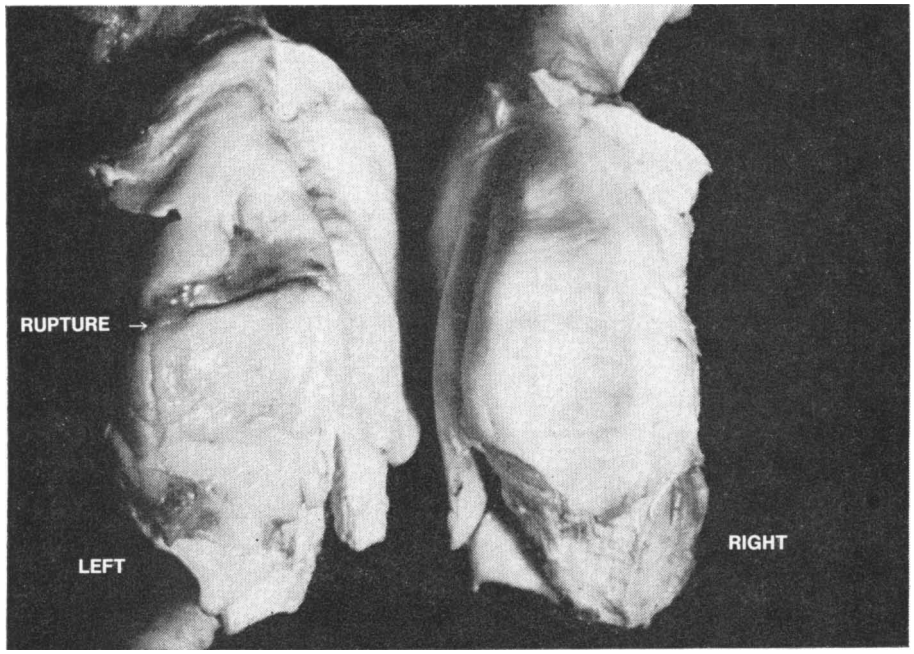

Figure 1. Rupture at the insertion of the Achilles tendon on the left calcaneus.

Leg weakness is not a well defined clinical entity and includes a variety of morphological changes. In the present study, in an attempt to make the clinical diagnosis more distinct, only herds in which ruptures of the Achilles tendon had occurred in 1 or more animals were included. Common features of these herds were the indoor feeding and a marked dietary vitamin D deficiency, detected by determination of 25-OH-D metabolites in serum.

The skeletal lesions in leg weakness have been thoroughly investigated morphologically and radiologically (i.e. *Jonsson et al.* 1972, *Reiland et al.* 1978, *Jensen et al.* 1981). The Achilles tendon lesions, however, are very sparsely described. They may be localized at the insertion on the calcaneus, showing partial ruptures at the junction between tendon tissue and bone tissue (*Johnsson et al.* 1978). Bone fragments from the calcaneus were found in the torn off fibrils of the tendon by *Martin & Holzschuh* (1964). Based on this observation they regarded the ruptures as a consequence of the skeletal lesions. In the present investigations the ruptures were seen at the insertion (Fig. 1), but also

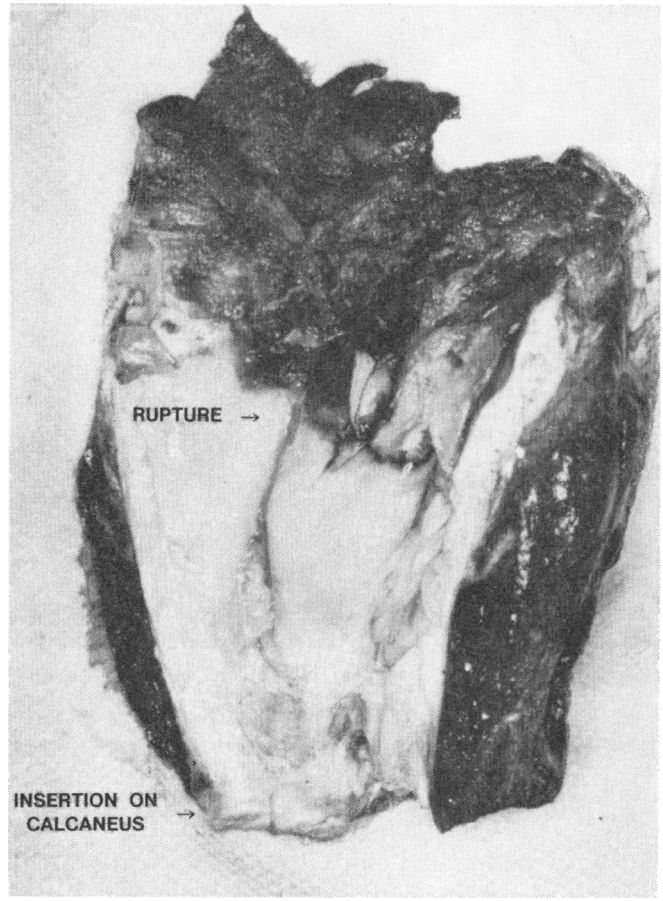

Figure 2. Rupture of the Achilles tendon approximately 5 cm proximal to the insertion on the calcaneus.

approximately 5 cm proximal to the calcaneus (Fig. 2), as was earlier reported by *Bach & Haase* (1966), pointing to a weakness not only of the calcified tissue but also of the tendinous tissue per se.

Vitamin D is of importance for the cross-linking of collagen fibrils in bone matrix (*Mechanic et al.* 1972). It was recently shown in rats that prolonged vitamin-D deficiency interferes with tendon collagen cross-link maturation (*Lee et al.* 1982), indicating an effect on the strength of the tendons as well. This point, however, needs further elucidation.

The observation that the decreased serum Ca and P_i values

in the affected herds were normalized after vitamin D treatment, irrespective of route of administration or type of vitamin D, gives a further indication of an aetiological importance of vitamin D deficiency for the described disease.

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SAMMANFATTNING

Serumkoncentrationen av 25-hydroxyvitamin-D hos köttdjur i besättningar där rupturer av achillessenan förekommit.

Frekvensen skelettskador, inkluderande achillessenerupturer, har under de senaste åren ökat i köttdjursbesättningar speciellt hos intensivt uppfödda kalvar. Serumkoncentrationen av 25-hydroxyvitamin-D, kalcium och oorganiskt fosfor mättes i 7 drabbade besättningar och i 3 besättningar utan kliniska symptom. Inte något av djuren i drabbade besättningar hade detekterbara nivåer av serum-25-OH-D_3 ($\leq$ 2 ng/ml) medan kontrolldjuren hade en koncentration på 36.1 ng/ml (least-squares mean). Även kalcium och oorganiskt fosfor var signifikant lägre i de drabbade besättningarna ($P \leq 0.001$). I 1 besättning gjordes försök med 4 olika former av D-vitamintillförsel (oral tillförsel av AD_3E eller D_3, intramuskulär tillförsel av D_2 eller D_3). Behandlingseffekten på Ca och Pi i serum mätt en månad senare, var densamma i de 4 grupperna, innebärande normaliserade nivåer.

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