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THE EFFECT OF INTRAMUSCULARLY ADMINISTERED VITAMIN D_3 ON SERUM VITAMIN D METABOLITES AND ELECTROLYTES IN VITAMIN D_3 DEFICIENT DAIRY COWS*

By

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STURÉN, M.: *The effect of intramuscularly administered vitamin D_3 on serum vitamin D metabolites and electrolytes in vitamin D_3 deficient dairy cows.* Acta vet. scand. 1985, *26*, 179—190. — The effect of intramuscular administration of vitamin D_3 (1×10^6 IU D_3/100 kg bodyweight) to 3 different dairy breeds on the serum levels of vitamin D_3, 25-OH-D_2, 25-OH-D_3, Ca, inorganic P and Mg was studied.

The vitamin metabolites and the electrolytes were analysed on 9 occasions during a 36-day period. Vitamin D_3 was analysed on 6 occasions during the same period. No significant breed differences were observed except for 25-OH-D_3 ($P \leq 0.05$). The D_3 level rose in 1 day from < 2 ng/ml to 906 ng/ml and decreased to below 50 % of the peak level after 6 days. At the end of the experiment (day 36) vitamin D_3 was < 2 ng/ml. 25-OH-D_3 rose from < 2 ng/ml to 106 ng/ml in 6 days and stayed at this level during the whole experiment. 25-OH-D_2 decreased from 16 ng/ml to 5 ng/ml during the observation period.

25-OH-D_2; 25-OH-D_3; serum electrolytes response curve; breed differences.

Parenterally administered vitamin D_3 in a dose of 10 million IU has in the last decades been widely used for prevention of parturient paresis in dairy cows (e.g. *Seekles et al.* 1958, *Gregorovic* et al. 1967). A good preventive effect without side effects has been reported in most studies. However, in two independent Swedish field trials (*Jönsson & Pehrson* 1970, *Gustafsson et al.* 1971), a poor effect and serious side effects were reported in

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Swedish Red and White cattle. When a corresponding trial was made in Swedish Friesian cattle good effects and no side effects were noticed (*Jönsson & Pehrson* 1971). A breed difference in vitamin D metabolism was proposed as an explanation of the discrepancy in the results.

The purpose of the present investigation was to follow the effect of a single intramuscular injection of vitamin D_3 on 25-hydroxycholecalciferol (25-OH-D_3), 25-hydroxyergocalciferol (25-OH-D_2), calcium, inorganic phosphorus, and magnesium in serum from cows of different dairy breeds.

MATERIAL AND METHODS

Animals

Eight Swedish Friesian cows (SLB), 8 Swedish Red and White cows (SRB) and 6 Jersey cows (SJB) in an experimental herd of the Swedish University of Agricultural Sciences, Uppsala, were used. Their ages varied from 41 to 68 months. The cows had calved 2 or 3 times. They were kept in individual stalls indoors all the year round with the exception of visits to outside paddocks for 2×6 h per week during April-November. The average bodyweight was 605 kg for SLB, 505 kg for SRB and 375 kg for SJB cows.

Diet

The cows were given 1.7 kg dry matter of roughage (hay and grass silage) per 100 kg bodyweight. Concentrates (36 % crushed barley, 36 % crushed oats, 13 % soybean meal, 6 % rapeseed meal, 7 % molasses and 2 % dicalciumphosphate) were given according to milk production.

Experimental design

The cows were given vitamin D_3* intramuscularly in a dose of 1.0 million IU D_3 per 100 kg of bodyweight. Blood samples from

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* Duphafral® D_3 1000.
"Formula: Vit. D_3 Cryst. 1 000 000 I.U. (= 25 mg Cholecalficerol) — Alc. Benzyl. — Dinatr. Phosphas — Acid Citric. — Ol. Arachid. — Ol. Ricin. Polyxyaethylenat. — Propylenglycol. — Aqua Bidestill. q.s. ad 1 ml."
The generous gift of Duphafral ® D_3 from Ferrosan AB, Malmö, is gratefully acknowledged.

the jugular vein were drawn into vacutainer tubes (Becton- Dickinson) immediately before treatment and on 8 occasions during the following 35 days. The blood samples were sent immediately by mail to the laboratory and were centrifuged within 24 h. Serum was kept at —20°C until analysed.

Chemistry

25-OH-D_3 and 25-OH-D_2 were analysed by a high performance liquid chromatographic method (HPLC) (*Holmberg et al.* 1984) with detection limits of 2 ng/ml for each.

Vitamin D_3 was analysed by a modified HPLC method (*Aksnes* 1980) with vitamin D_2 as an internal standard. Serum samples were extracted with diethylether in a horizontal shaker for 1 h at room temperature. The water phase was frozen and the diethylether decanted. The ether phase was evaporated to dryness and th residue dissolved in 1 ml of hexane ether (4:1). This phase was fractionated on a silica column in a Pasteur pipette. The vitamin D fraction was further cleaned on a SEP-PAK C18 mini column with acetonitril/water as a mobile phase. The quantification was performed on a HPLC C18 column 8 mm ID×100 mm (Waters RCM system). This assay separates D_2 from D_3. The overall recovery was approximately 50 %. The intra-assay coefficient of variation was 11 % ($n = 13$) for a sample with 20 ng vitamin D_3 per ml. The interassay coefficient of variation of the same sample was 17 % ($n = 8$). The practical detection limit was set at 2 ng/ml.

In blood samples taken before vitamin D_3 administration, vitamin D_2 was analysed as described but without internal standard. No vitamin D_2 was found and the vitamin D_2 supply was not changed during the experiment, which was the prerequisite for using vitamin D_2 as a standard in vitamin D_3 analyses.

Standard solutions were made from crystalline vitamin D_3 and D_2 (Sigma), from crystalline 25-OH-D_3 (Philips Duphar B.V.) and from 25-OH-D_2 (a generous gift from Lage Aksnes, University of Bergen, Norway). They were all stored at —25°C in 95 % ethanol.

Calcium and magnesium were determined by atomic absorption spectrophotometry and inorganic phosphorus according to *Fiske & Subbarow* (1925).

The effect of a 24-hour delay between blood sampling and

Table 1. The effect of a 24-hour delay between blood sampling and centrifugation on the analytical results of serum 25-OH-D_3, calcium (Ca), magnesium (Mg) and inorganic phosphorous (P_i) in 12 blood samples.

	Centrifuged immediately $\bar{x}$	Stored 24 h before centrifugation $\bar{y}$	r	Regression line
25-OH-D_3 ng/ml	20.8	22.0	0.96***	$y = -0.88 + 1.10x$
Ca mmol/l	2.51	2.53	0.99***	$y = -0.02 + 1.01x$
Mg mmol/l	0.94	0.96	0.99***	$y = 0.1 + 0.91x$
P_i mmol/l	1.63	1.67	0.96***	$y = 0.07 + 0.97x$

centrifugation on the analytical results was studied in samples from 12 cows. Table 1 shows that the delay had only a minor influence on the results.

Statistical analysis

The General Linear Model procedure from the Statistical Analysis System (*SAS Institute Inc.* 1982) was used. The following model after omission of non-significant two-way interactions was found appropriate:

$$y_{ijklm} = \mu + b_i + l_j + s_k + c_{ijkl} + d_m + (bd)_{im} + (ld)_{jm} + (sd)_{km} + e_{ijklmn};$$

where

y_{ijklmn} = the ijklmnth observation
μ = general mean
b_i = effect of the ith breed (i = 1,2,3,)
l_j = effect of the jth number of lactation (j = 1,2)
s_k = effect of the kth stage of lactation (k = 1,2,3)
c_{ijkl} = effect of the lth cow (l = 1,2 .... 22)
d_m = effect of the mth day of sampling (m = 1,2, ... 9)
$(bd)_{im}$ = effect of the interaction between the ith breed and the mth day of sampling
$(ld)_{jm}$ = effect of the interaction between the jth number of lactation and the mth day of sampling
$(sd)_{km}$ = effect of the interaction between the kth stage of lactation and the mth day of sampling
e_{ijklmn} = residual random term with variance σ_e^2

The effect of cow was regarded as random and all other effects as fixed. The stage of lactation was grouped in three classes: 0—60, 61—150, and more than 150 days of lactation.

RESULTS

As shown in Table 2 there were breed differences for 25-OH-D_3 while the effect of breed was non-significant for all other variables. The effects of number of lactation and stage of lactation were non-significant for all variables. The effect of cow was significant for vitamin D_3, 25-OH-D_2, 25-OH-D_3, Mg, Ca and P_i. The effect of sampling day, too, was significant for all variables measured.

Table 2. Levels of significance for the effects of breed, number of lactation, stage of lactation, animal, day of sampling and the effects of interaction between breed and day of sampling, between number of lactation and day of sampling and between stage of lactation and day of sampling on vitamin D_3, 25-OH-D_2, 25-OH-D_3, magnesium (Mg), calcium (Ca) and inorganic phosphorus (P_i).

Source of variation		Vitamin D_3	25 OHD_2	25 OHD_3	Mg	Ca	P_i
Breed	[B]	NS	NS	*	NS	NS	NS
Number of lactation	[L]	NS	NS	NS	NS	NS	NS
Stage of lactation	[S]	NS	NS	NS	NS	NS	NS
Cow	[C]	*	***	***	***	***	***
Day of sampling	[D]	***	***	***	***	***	***
B * D		**	NS	*	NS	NS	NS
L * D		NS	*	**	NS	NS	NS
S * D		NS	*	NS	NS	NS	NS

Levels of significance: NS (non significant) = $P > 0.05$;
* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Fig. 1 shows that the vitamin D_3 level in serum increased rapidly after the vitamin D_3 administration. The mean peak value of 905 ng/ml occurred on day 1, followed by a decrease to less than 50 % of this value on day 6. At the end of the experiment the values were below the detection limit in 50 % of the animals.

The 25-OH-D_3 level increased from below 2 ng/ml in all animals at start to 48 ng/ml (least squares mean) on day 1 and to 106 ng/ml on day 6, a level which was almost constant for the rest of the observation period (Fig. 2). 25-OH-D_2 showed a peak

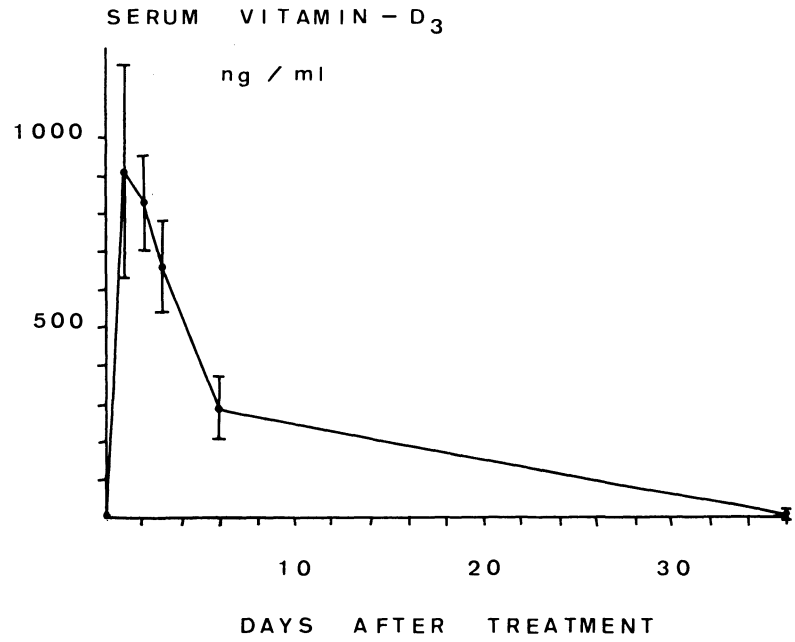

Figure 1. The effect of i.m. administration of 1×10^6 IU vitamin D_3/100 kg bodyweight on serum concentration of vitamin D_3 in 22 cows (means ± s.d.).

value on day 1, insignificantly higher than the initial level of 16 ng/ml (least squares mean), from which it successively decreased to 5 ng/ml on day 36 (Fig. 3).

The electrolytes studied showed rather small but significant variations (Fig. 4). Serum Ca increased during the first 2 days, decreased until day 8 and increased again until day 14. Inorganic phosphorus increased during the first 3—6 days. During that time the Mg values showed a decrease.

DISCUSSION

The present study was primarily aimed at a comparison between 3 breeds as regards their reaction to a bodyweight-related dose of parenterally administered vitamin D_3. The response curve of vitamin D_3 in serum was almost identical in the 3 breeds, while there were breed differences for 25-OH-D_3, with the lowest response to the treatment in the SRB breed and the highest in the SJB.

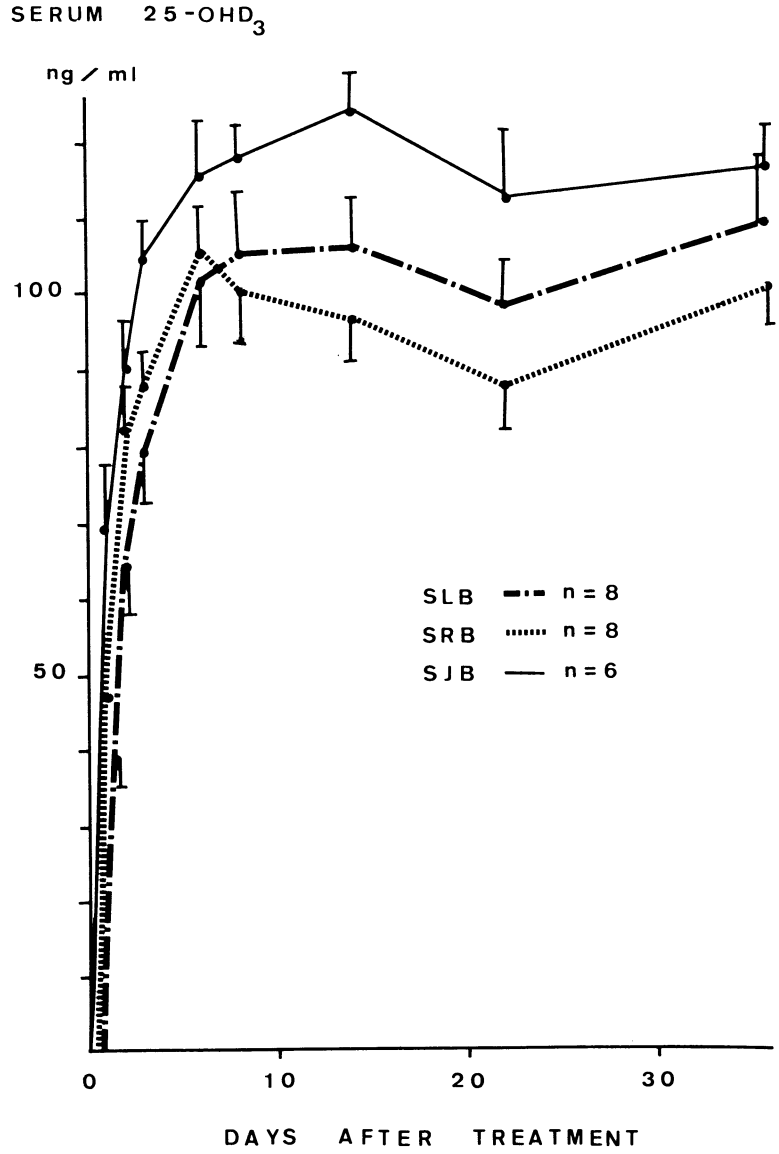

Figure 2. The effect of i.m. administration of 1×10^6 IU vitamin D_3 on serum concentration of 25-OH-D_3 in cows of 3 different breeds (means ± s.e.m.).

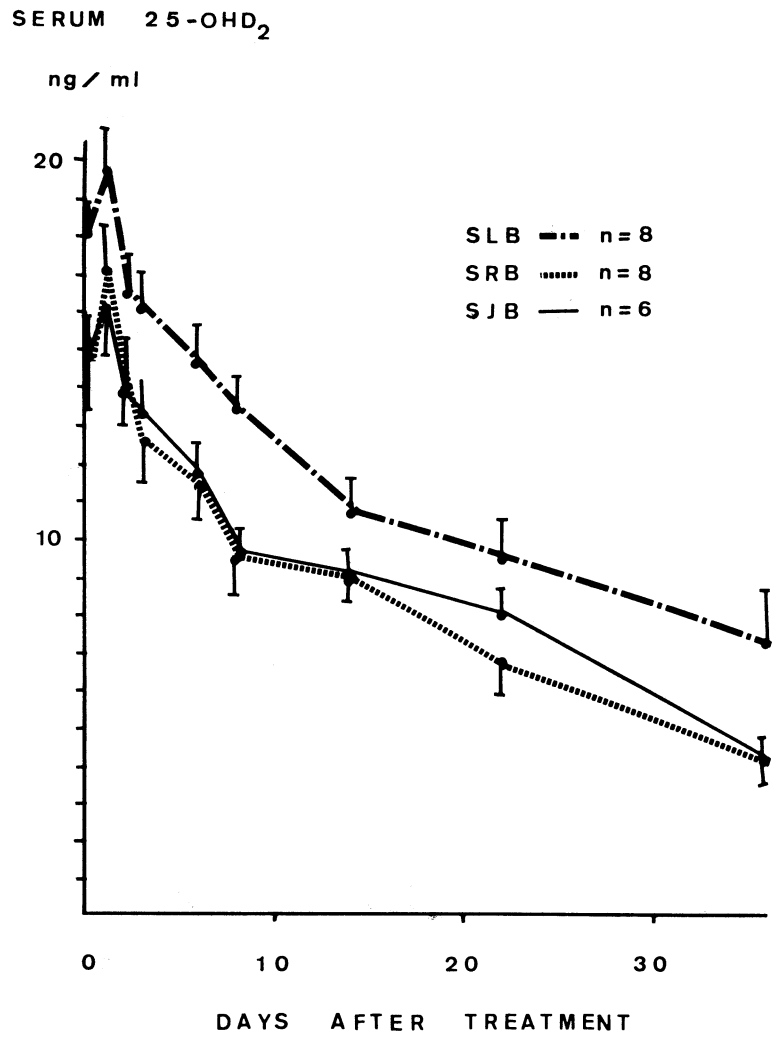

Figure 3. The effect of i.m. administration of 1×10^6 IU vitamin D_3 on serum concentration of 25-OH-D_2 in cows of 3 different breeds (means ± s.e.m.).

Thus the hypothesis of a breed difference in the response to vitamin D_3 administration as an explanation of the high incidence of side-effects observed after using it as prophylaxis of parturient paresis in SRB cows (*Jönsson & Pehrson* 1970) has not been confirmed in the present study.

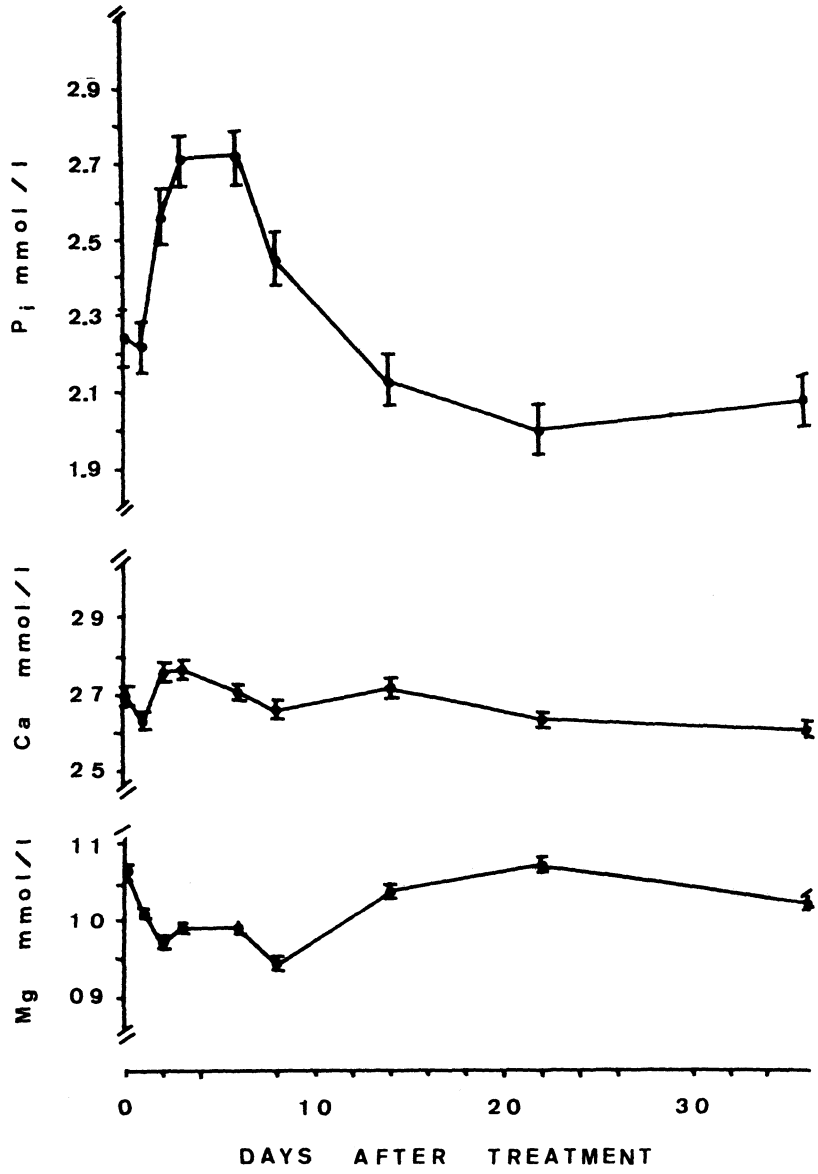

Figure 4. The effect of i.m. administration of 1×10^6 IU vitamin D_3 on serum concentration of inorganic phosphorus (P_i), calcium (Ca) and magnesium (Mg) in 22 cows (means ± s.e.m.).

The very fast increase of the serum vitamin D_3 level after injection is markedly different from the results reported by *Horst & Littledike* (1979) and *Littledike & Horst* (1982), showing an increase of plasma vitamin D_3 to only 150 ng/ml after repeated injections of vitamin D_3 in doses considerably higher than those used in the present study. Their peak value was not reached until 10—12 days and the pre-injection level 90 days after the first injection. The discrepancy between the results may partly be explained by different resorption rates from injection sites caused by different vehicles of the vitamin. *Littledike & Horst* used vitamin D_3 in ethanol, while D_3 in solubilized form (see Material and Methods) was used in the present study. Differences in diet, especially the deficient vitamin D supply in the present study, may also have contributed to the discrepancy, since it has been shown in man that the half-life of parenterally administered vitamin D_3 is much shorter in a deficiency state (*Mawer* 1980).

Initially no animal in the present study had detectable amounts of 25-OH-D_3 in serum because of their limited access to solar radiation and absence of dietary vitamin D_3 supplement. In Swedish dairy cows on a vitamin D_3 supplemented diet and pastured during the summer the 25-OH-D_3 level is 20—50 ng/ml (*Sturén* to be published). The peak value of 25-OH-D_3 in the present study was at the same level (106 ng/ml) as that reported by *Littledike & Horst* (1982) despite their use of a considerably higher dose 10×10^6 IU). Their peak value was not reached until about 30 days after the first of 2 injections, whereas the corresponding time in the present study was 6 days. The initial values of 25-OH-D_3 in their animals were 25—60 ng/ml. The different results may be explained by the fact that the animals in the present study were vitamin D deficient before treatment. It has been shown in humans that the increase of serum 25-OH-D_3 after intravenous administration of vitamin D_3 is much faster in vitamin D deficient than in normal subjects (*Mawer* 1980).

After a short and insignificant increase the level of 25-OH-D_2 in serum decreased successively during the observation period despite a constant dietary supply of vitamin D_2. A decrease was also observed by *Littledike & Horst* (1982), who suggested a competition between D_2 and D_3 for 25-hydroxylation in the liver as an explanation.

The toxicity of vitamin D may be related to the available

amount of vitamin D binding protein, as the toxic effect is created by the non-protein-bound part of the vitamin (*Bouillon et al.* 1982). Normally vitamin D binding protein is present in large surplus in man (*Haddad & Walgate* 1976) and laboratory animals (*Bouillon et al.* 1978). Whether this is valid also for cattle remains to be shown. If not, quantitative differences in vitamin D binding proteins between breeds or individuals might be an explanation of the different susceptibility to large doses of vitamin D.

Gahne & Juneja (1978) showed that the so-called GC factor in cattle is identical to the vitamin D binding protein and has a genetic polymorphism. In man no correlation between the genetic GC pattern and the vitamin D metabolism has been found (*Bouillon et al.* 1980). No corresponding study has so far been made in cattle.

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SAMMANFATTNING

Effekten av intramuskulärt tillfört vitamin D_3 på serumkoncentrationen av D-vitaminmetaboliter och elektkrolyter hos D-vitamindeficienta kor.

Effekten av intramuskulär tillförsel av D-vitamin (1×10^6 IU D_3/100 kg kroppsvikt) till 3 mjölkkoraser studerades.

Ca, oorganiskt P, Mg, 25-hydroxyvitamin-D_2 och 25-hydroxyvitamin-D_3 i serum analyserades vid nio tillfällen under en 36-dagars period. Vitamin D_3 analyserades vid 6 tillfällen under samma period. Inga rasskillnader kunde konstateras utom för 25-OH-D_3 ($P \leq 0.05$). D_3-nivåen steg inom ett dygn från < 2 ng/ml till 905 ng/ml för att sedan sjunka till under halva toppvärdet på 6 dagar. På dag 36 var halten åter < 2 ng/ml. 25-OH-D_3 steg från < 2 ng/ml till 106 ng/ml på 6 dagar och låg sedan kvar på denna nivå under hela observationstiden. 25-OH-D_2-nivån minskade från 16 ng/ml til 5 ng/ml under försökets gång.

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