

Serum Calcium Response Following Oral Zinc Oxide Administrations in Dairy Cows

By T. Thilsing-Hansen and R.J. Jørgensen

The Royal Veterinary and Agricultural University, Department of Clinical Studies, Cattle Production Medicine Research Group, Denmark.

Thilsing-Hansen T, Jørgensen RJ: Serum calcium response following oral zinc oxide administrations in dairy cows. Acta vet. scand. 2001, 42, 271-278. – Six non-pregnant cows were allocated into 3 groups. Group 1 comprised a pair of lactating cows, whereas groups 2 and 3 each comprised a pair of non-lactating cows. The cows in groups 1 and 2 were dosed intraruminally by stomach tube with zinc oxide at 120 mg Zn per kg of bodyweight at weekly intervals for a period of 33 days. Each cow received a total of 4 doses of zinc oxide. Group 3 served as non-treated control group. Blood samples were collected from all 6 cows daily. Serum was analysed for concentration of calcium. Within 12-24 h of each zinc oxide administration the serum calcium of the lactating cows dropped dramatically indicating the existence of an antagonistic effect between Zn and Ca. The first Zn induced hypocalcaemic episode in the lactating cows was followed by a rise in serum calcium to a level above the pre-dosing level and above the mean value of the control group. The depth of the hypocalcaemic response decreased with the number of zinc oxide dosings. This effect was explained as a response from the stimulation of the calcium homeostatic mechanisms. In the Zn dosed non-lactating cows responses were similar but less clear. The perspective of these findings is discussed in relation to resistance towards parturient hypocalcaemia.

hypocalcaemia.

Introduction

There are several published reports documenting interaction between zinc and calcium in pigs, chickens and rats. *Newland et al.* (1958) found a higher rate of zinc metabolism in pigs receiving high-calcium diets unsupplemented with zinc. *Hoekstra et al.* (1956) cured parakeratosis in swine by adding 50 ppm zinc to the diet, and *Stewart & Magee* (1964) alleviated the effects of zinc toxicity in rats by supplementing calcium and phosphorus. Also *Hsu et al.* (1975) claimed that high dietary calcium had a protective effect against the adverse effects of diet Zn. All these experiments point in the direction of the existence of an antagonistic effect between calcium and zinc in the above mentioned animal species.

Relatively few studies are concerned with the interaction between Ca and Zn in ruminants. Thompson and co-workers found that supplying lambs on a slightly Ca deficient diet with 0,5% or 1,0% zinc sulphate markedly reduced the net retention of calcium, as compared with control animals receiving no supplemental zinc (*Thompson et al.* 1959). A decreased intestinal absorption as well as an increased endogenous excretion mediated the loss of calcium. Thompson and co-workers suggested that calcium and zinc to some extent are antagonistic in ruminants. This hypothesis was supported by the findings of *Suttle & Field* (1969). In their study a change in dietary calcium from 1% to 2% increased faecal excretion of zinc in sheep. *Pond*

& Wallace (1986) obtained matching results, and suggested that the feeding of high dietary calcium (0,8%) to ewes decreased the absorption of dietary Zn.

During the years the prophylactic effect of dietary zinc on facial eczema in cattle has been well-documented (Smith *et al.* 1978). The occurrence of clinical cases of hypocalcaemia in conjunction with such prophylactic treatments led Smith and co-workers to investigate this area more closely.

By using zinc oxide in doses corresponding to 120 mg Zn/kg body weight Smith *et al.* (1984) produced a significant drop in serum-Ca from 109 mg Ca/l (2,72 mmol/l) prior to the zinc administration to 92 mg Ca/l (2,30 mmol/l) 24 h after the zinc dose in lactating dairy cows. The suggestion was made that the gut lumen was the site of the mineral interference. They based this assumption on the fact that a sudden increase in the daily zinc dose dramatically changes the relative concentrations of zinc and calcium in the gut lumen as the normal molar calcium concentration in the gut lumen is approximately 200 times those for zinc. Allen & Masters (1980) further claimed that the absorption of Zn under normal circumstances is relatively low, but when dietary Zn is high the homeostatic control mechanisms function less effectively and the absorption of Zn increases dramatically.

While the majority of the experiments performed pointed in the direction of the existence of an antagonistic effect between calcium and zinc in ruminants, like in other animal species, a few studies did not support this.

Bedi & Sawhney (1980) performed an experiment on growing Harijana calves showing that in the presence of a dietary Ca:P ratio of 1:1,53 the retention of Ca increased significantly when the diet was supplemented with 40-100 ppm Zn, and Leontowicz *et al.* (1995) found that loading sheep with Ca (45.28 g Ca/kg DM) did

not affect the absorption of Zn in the small intestine or in the segment between the mouth and ileum. Pond (1983) further stated that in contrast to swine a clinical Zn deficiency could not be induced in lambs by elevating the level of dietary calcium to 0,8% of DM. In support of this Kincaid (1979) found, that elevated amounts of dietary calcium had no effect on absorption of zinc in the lactating cow.

The aim of the present experiment was to examine further the antagonism between calcium and zinc in dairy cows by following the response in blood calcium after oral administration of zinc oxide. It was further the intention to investigate whether the expected hypocalcaemia would provoke a response from the mechanisms responsible for calcium homeostasis.

Materials and methods

Experimental animals

Six non-pregnant dairy cows were allocated into 3 treatment groups as shown in Table 1. Group 1 comprised 2 lactating cows, which were milked twice daily. Groups 2 and 3 each comprised a pair of non-lactating cows. From Table 1 it can be seen that the cows in group 3 had a lower parity than the cows in the other 2 groups. This is, however, considered to be without importance, as the cows in group 3 acted as untreated control cows.

Diets

All cows received a basic ration consisting of wrap grass silage. In addition to this the lactating cows received a lactation concentrate (NAG Kvaegfoder SL 12, DK 3200 Helsingør). Table 2 shows the mean daily intake of dry matter (DM) and calcium for the 6 cows during the experimental period. The composition of the diets was determined by atomic absorption spectrophotometry (Centrallaboratoriet, DJF, Forskningscenter Foulum, Tjele, Denmark).

Table 1. Characteristics of the experimental animals.

	Cow number	Age, (Years)	Parity	Weight, (kg)	Mean milk yield (litre/day)	Zinc oxide dosing (120 mg Zn/kg bw)
Group 1	0423	7	5	612	20	+
	0262	5	3	598	20	+
Group 2	0817	6	3	812	0	+
	0686	6	3	704	0	+
Group 3	0185	2	1	419	0	-
	0239	4	2	488	0	-

In addition to wrap grass silage and lactation concentrate the cows had free access to barley straw. The estimated daily intake of barley straw was 2 kg per cow per day, which contributed with 10 g of calcium/day.

Treatments

The cows in group 1 and 2 were given zinc oxide (ZINKOXID, Superfos Kemi A/S, DK-2950 Vedbaek) in doses of 120 mg Zn/kg body weight. Group 3 acted as a non-dosed control group.

Group 1 and 2 were dosed once a week at 11.00 a.m. The ZnO was mixed with tap water and given by intraruminal intubation.

Samples and analyses

Blood samples (Vacutainer[®], SST[®] Gel and Clot Activator) were collected each day at

10.00 a.m. (one hour after morning feeding) from the coccygeal vessels or from V. jugularis. On the day of zinc dosing and the day after additionally one daily blood sample was taken at 10.00 p.m.

The blood samples were centrifuged the following morning at 3400 rpm for 10 min. The serum was separated and stored at -37°C until analysis for serum calcium by atomic absorption spectrophotometry (Perkin-Elmer 5000, Analytical Instruments, Perkin-Elmer Corp., Norwalk, CT 06856 U.S.A.). Serum calcium was monitored twice on each blood sample.

The cows were observed every day for clinical signs of hypocalcaemia (cold extremities, subnormal rectal temperature, decreased feed intake).

The experiment was conducted over 33 days.

Table 2. Mean daily intake of DM and Ca in the 3 experimental groups. The contribution from barley straw is not included.

	DM intake, kg/day		Calcium intake, (g/day)	NRC recommendations (Anon. 1989) (Calcium, g/day)
	Wrap grass silage	Concentrate		
Group 1	4.1	6.8	51	84
Group 2	6.0	0	35	30
Group 3	5.5	0	32	18

Results

Clinical signs

Slightly cold extremities, decreased feed intake and lowered milk yield was recorded in the Zn-dosed lactating cows following the first Zn dose. Both cows recovered spontaneously without treatment. The Zn dosed non-lactating cows as well as the control group showed no clinical changes during the experimental period.

Serum calcium

The mean serum calcium concentration of the control cows during the entire experimental period was 2.26 mmol/l with minor day-to-day fluctuations as shown in Fig. 1.

From Fig. 1 it also appears that each zinc oxide treatment of the lactating cows was followed by an episode of hypocalcaemia. The drop in serum calcium among these lactating cows was more severe following the first ZnO treatment than following the succeeding ones (Fig. 2). In terms of numerical changes the mean serum

calcium concentration fell from 2.28 mmol/l to a minimum of 1.29 mmol/l 12-24 h after the first Zn-dose, from 2.39 mmol/l to 1.45 after the second dose, from 2.16 mmol/l to 1.50 mmol/l after the third dose and from 2.15 mmol/l to 1.58 mmol/l after the fourth dose.

Following the first hypocalcaemic episode serum calcium of the lactating cows rose to a level higher than the pre-dosing level and higher than the control group mean (Fig. 1). A similar trend, although less pronounced, was seen after the succeeding hypocalcaemic episodes.

The ZnO treated non-lactating cows also dropped in serum calcium following the zinc oxide dosings. The drops were however less extensive, and the difference in magnitude between the 4 hypocalcaemic episodes was apparently minor (Fig. 1 and 3).

Discussion

Our experiment clearly demonstrates the exis-

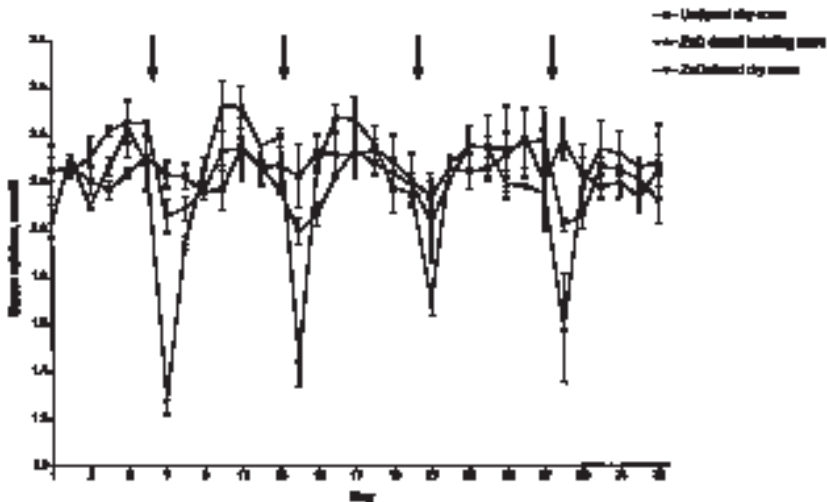


Figure 1. Mean serum calcium concentration (\pm SEM) of the ZnO (120 mg Zn pr. kg. bodyweight) treated lactating and non lactating cows as compared to the undosed non lactating control cows. The arrows indicate the 4 dosings.

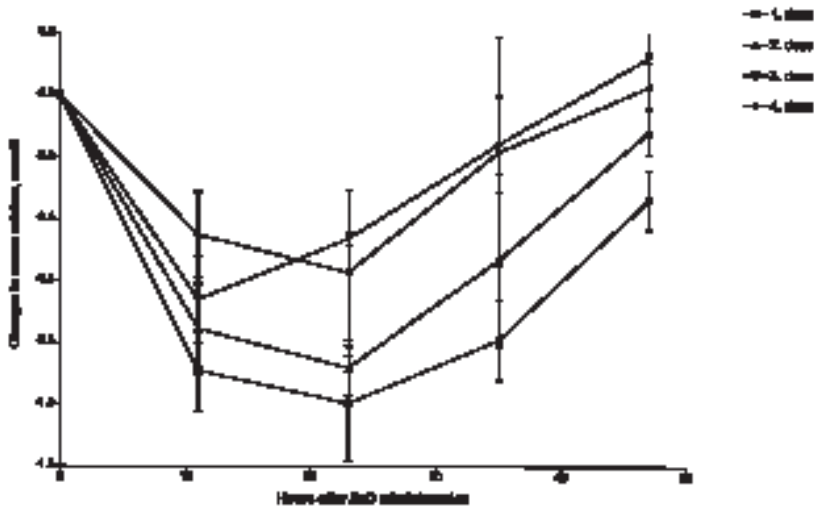


Figure 2. Detailed mean serum calcium course (\pm SEM) of the ZnO (120 mg Zn pr. kg. bodyweight) treated lactating cows during the interval 0-47 hours after each of the 4 ZnO-dosings. The starting points are reset/synchronized.

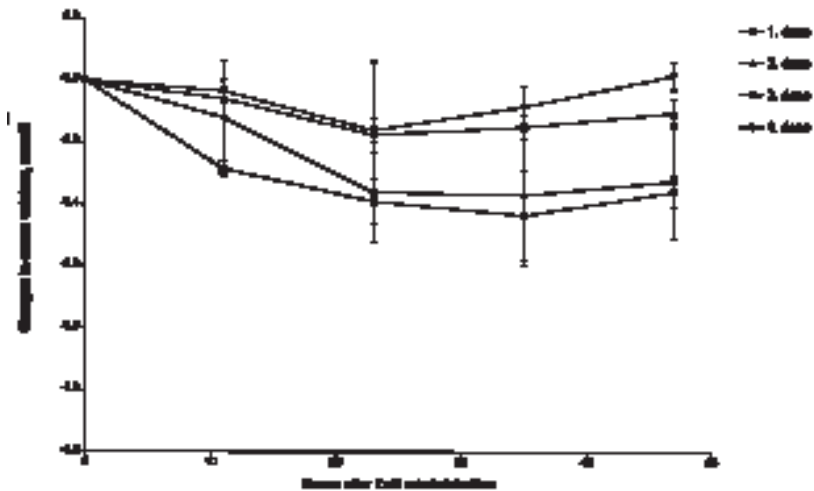


Figure 3. Detailed mean serum calcium course (\pm SEM) of the ZnO (120 mg Zn pr. kg. bodyweight) treated non-lactating cows during the interval 0-47 hours after each of the 4 dosings. The starting points are reset/synchronized.

tence of an antagonistic effect between calcium and zinc in dairy cows as evidenced by the drop in serum calcium following zinc oxide administration together with the clinical signs of hypocalcaemia displayed by the lactating cows after the first ZnO treatment. As such it confirms the observations made by *Smith et al.* (1984).

The exact mechanism of calcium/zinc-antagonism at the epithelial level is unknown. *Thompson et al.* (1959) did, however, find that the antagonistic effect between Ca and Zn included not only intestinal absorption but also endogenous faecal excretion indicating that more than one system is involved.

The results of the present experiment show that the response to oral zinc oxide administration differs much between the lactating and the non-lactating cows although the dose was the same in terms of mg zinc/kg body weight. The serum calcium drop was not as extensive, and the overcompensation tendency seen among the lactating cows following a hypocalcaemic episode was not as convincing in the non-lactating cows.

This difference in response may be explained by the continuous and comprehensive demand for dietary calcium to fulfil the needs for milk production in lactating cows (*Hove & Hilde* 1984), whereas the calcium requirement of non-lactating cows is limited. Therefore, compromising the dietary calcium supply more easily leaves lactating cows in extensive calcium deficiency as compared to dry cows. Earlier studies support this interpretation. When *Hove & Hilde* (1984) decreased the daily calcium intake of a lactating cow from 120 to 20 g/day serum calcium fell from 2.35 to 1.90 mmol/l within 5 h, whereas in pregnant dry cows *Goings et al.* (1974) recorded a less severe drop (2.35 to 2.07 mmol/l) following a shift in dietary calcium intake from 41-52 g/day to 8.2 g/day. This drop in serum calcium also oc-

curred more slowly reaching a minimum after 36 h. Interestingly, in both studies the lowered level of dietary calcium intake equals approximately 25% of NRC recommendations (*Anon.* 1989), and yet the responses in serum calcium in the lactating cows and in the non-lactating pregnant cows are different.

Besides lactation itself, another factor may have influenced the difference in response in the present study. According to Table 2 the daily calcium intake (from wrap grass silage and concentrate) was calculated to be below NRC recommendations (*Anon.* 1989) for the lactating cows and above NRC recommendations for the non-lactating animals. This may represent a common situation in dairy herds, but the extent to which it influenced the observed difference in response can not be extracted.

The observation of the occurrence of overcompensation in serum calcium following an episode of hypocalcaemia is in accordance with the results obtained by *Smith et al.* (1984). *Hove* (1987) described it as a reparative action, which overshoot and produce a period of hypercalcaemia. One might argue that a drop in milk production following ZnO dosing could contribute to this serum Ca overshooting. In this experiment the ZnO administration decreased the milk yield (2.25-3.5kg) for one or 2 days following administration, but on the day of maximum serum Ca overshooting the milk production was increasing although not fully re-established.

The mean drop in serum calcium concentration for the Zn-dosed lactating cows was more profound after the first ZnO dose than after the following doses, and the time required to reach the pre-dosing serum calcium level was longer following the first dose than following the succeeding ones (Figs. 1 and 2). These results indicate that such a single Zn-induced hypocalcaemic episode is sufficient to elicit a response from the cow's calcium homeostatic

mechanisms to succeeding similar challenges. Although the serum calcium response of the dry cows was less pronounced, the serum calcium level dropped following ZnO administration below the threshold level for subnormal plasma calcium of 2.18 mmol/l suggested by Hove (1986). The differences in serum calcium response following the succeeding ZnO dosings were however minor (Fig. 3), and apparently the Zn induced hypocalcaemia in terms of depth and duration was too small to trigger a post-hypocalcaemic response from the calcium homeostasis mechanisms.

The finding in this experiment, that a short-term induced hypocalcaemia in lactating cows apparently induced an increased resistance to similar succeeding challenges, is new. It may be seen in relation to the principle of feeding rations low in calcium before parturition for the prevention of milk fever (Boda & Cole 1953), because the supplementation of zinc oxide, as in this study, probably decreases the actual availability of the dietary calcium.

The perspective of reducing dietary calcium availability in late pregnancy cows by zinc administration is however questionable, as the zinc dose used in this experiment is around 6 times the dose recommended for facial eczema prevention (Smith *et al.* 1984), and therefore also seriously conflicts with feeding recommendations for zinc (Anon. 1989). Further more zinc toxicity has been recorded after long-term exposure of zinc in doses between 45 and 240 mg Zn/ kg bodyweight (Allen & Masters 1980, Smith 1980, Smith & Embling 1984).

Acknowledgement

This work was supported by the Danish Agricultural and Veterinary Research Council (grant no. 9801570). The authors wish to thank Barry L. Smith, Ruakura Research Centre, New Zealand, for his advise regarding zinc dosing and toxicity.

References

- Allen JG, Masters HG: Prevention of ovine lupinosis by oral administration of zinc sulphate and the effect of such therapy on liver and pancreas zinc and liver copper. *Austr. Vet. J.* 1980, *56*, 168-171.
- Anonymous: National research council. Nutrient requirements of dairy cattle. Sixth revised edition, Update 1989. National academy press. Washington, D.C. 1988.
- Bedi SPS, Sawhney PC: Influence of zinc on the metabolism of calcium, phosphorus, zinc, copper and iron in growing cow calves. *Indian J. Anim. Sci.* 1980, *50*, 25-29.
- Boda JM, Cole HH: The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. *J. Dairy Sci.* 1953, *37*, 360-372.
- Goings RL, Jacobsen NL, Beitz DC, Littledike ET, Wiggers KD: Prevention of parturient paresis by a prepartum, calcium-deficient diet. *J. Dairy Sci.* 1974, *57*, 1184-1188.
- Hoekstra WG, Lewis Jr PK, Phillips PH, Grummer RH: The relationship of parakeratosis, supplemental calcium and zinc to the zinc content of certain body components of swine. *J. Anim. Sci.* 1956, *15*, 752-764.
- Hove K: Cyclic changes in plasma calcium homeostatic endocrine system of the postparturient dairy cow. *J. Dairy Sci.* 1986, *69*, 2072-2082.
- Hove, K: Milk fever prevention and calcium homeostasis around calving in the dairy cow. *Irish veterinary news*, march 1987, 24-28.
- Hove K, Hilde BL: Plasma calcium in the lactating cow: Dependence on continuous intestinal absorption. *Can. J. Anim. Sci.* 1984, *64* (Suppl.), 227-228.
- Hsu FS, Krook L, Pond WG, Duncan JR: Interactions of dietary calcium with toxic levels of lead and zinc in pigs. *J. Nutr.* 1975, *105*, 112-118.
- Kincaid RL: Biological availability of zinc from inorganic sources with excess dietary calcium. *J. Dairy Sci.* 1979, *62*, 1081-1085.
- Leontowicz H, Gralak M, Leontowicz M, Kulsek G, Krzeminski R, Lesniewska V: The effect of calcium and sodium loading on organic matter digestibility and mineral absorption in sheep. 2. Absorption of minerals. *J. Animal and Feed Sci.* 1995, *4*, 311-319.
- Newland HW, Ullerey JA, Hoefer JA, Luecke RW: The relationship of dietary calcium to zinc metabolism in pigs. *J. Anim. Sci.* 1958, *17*, 886-892.
- Pond WG: Effect of dietary calcium and zinc levels

on weight gain and blood and tissue mineral concentrations of growing columbia- and suffolk-sired lambs. *J. Anim. Sci.* 1983, *56*, 952-958.

Pond WG, Wallace MH: Effect of gestation-lactation diet calcium and zinc levels and of parenteral vitamin A, D and E during gestation on ewe body weight and on lamb weight and survival. *J. Anim. Sci.* 1986, *63*, 1019-1025.

Smith BL: Effect of high concentration of zinc sulphate in the drinking water of grazing yearling dairy cattle. *N. Z. J. Agri. Res.* 1980, *23*, 175-178.

Smith BL, Coe BD, Embling PP: Protective effect of zinc sulphate in a natural facial eczema outbreak in dairy cows. *N. Z. Vet. J.* 1978, *26*, 314-315.

Smith BL, Collier AJ, Lawrence RJ, Towers NR: Hypocalcaemia associated with high dose rates of zinc oxide to lactating dairy cows. *N. Z. Vet. J.* 1984, *32*, 48-50.

Smith BL, Embling PP: The influence of chemical form of zinc on the effect of toxic intraruminal doses of zinc to sheep. *J. App. Toxicity.* 1984, *4*, 92-96.

Stewart AK, Magee AC: Effect of zinc toxicity on calcium, phosphorus and magnesium metabolism of young rats. *J. Nutr.* 1964, *82*, 287-295.

Suttle NF, Field AC: Effects of dietary calcium and phosphorus concentrations on the faecal excretion of copper, manganese and zinc in sheep. *Proc. Nutr. Soc.* 1969, *28*, A33-A34.

Thompson A, Hansard SL, Bell MC: The influence of aluminium and zinc upon the absorption and retention of calcium and phosphorus in lambs. *J. Anim. Sci.* 1959, *18*, 187-197.

Sammendrag

Serum calcium respons efter orale zinkoxid doseringer hos malkekøer.

Seks ikke drægtige køer inddeltes i 3 grupper. Gruppe 1 bestod af to lakterende køer, mens gruppe 2 og 3 hver bestod af to ikke lakterende køer. Køerne i gruppe 1 og 2 blev en gang om ugen over en periode på 33 dage tildelt 120 mg Zn pr. kg legemsvægt. Tildelingen skete intraruminalt via vomsonde. Hver ko modtog i alt 4 doser zinkoxid. Gruppe 3 fungerede som ubehandlet kontrolgruppe. Daglige blodprøver blev taget fra alle køer. Serum calcium blev målt. Et voldsomt fald i serum calcium indenfor 12-24 timer efter zinkoxid tildeling pegede i retning af en antagonistisk effekt mellem Zn og Ca. Blandt de lakterende køer efterfulgtes første Zn inducerede hypocalcæmiske episode af en stigning i serum calcium til et niveau over udgangsniveauet og over middelværdien for kontrolgruppen, mens dybden af det hypocalcæmiske respons mindskedes med antallet af zinkoxid doseringer. Dette forklarede som et respons via en aktivering af calcium homeostase mekanismerne. Lignende, men mindre udtalt respons, sås blandt de zink doserede goldkøer. Ovenstående fund diskuteres i relation til resistens mod parturient hypocalcæmi (mælkefeber).

(Received April 14, 2000; accepted January 25, 2001).

Reprints may be obtained from: Trine Thilsing-Hansen, The Royal Veterinary and Agricultural University, Department of Clinical Studies, Cattle Production Medicine Research Group, Dyrslægevej 88, DK-1870 Frederiksberg C, Denmark. E-mail: trh@kvl.dk, tel: +45 35 28 28 33, fax: +45 35 28 28 38.