Incidence of Hypovitaminosis E in Calves and Therapeutic Remedy by Selenium-Vitamin Supplementation

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Abstract

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The objective of the research project was to assess incidence of hypovitaminosis E in calves of dairy cows during milk nutrition from different farms in the Czech Republic and to verify the effect of hypovitaminosis E therapy consisting in oral and parenteral administration of a selenium-vitamin supplement.

Vitamin E concentration in blood serum of 350 calves from 39 different dairy farms was determined. The average vitamin E concentration being 3.55 ± 2.24 μmol·l^{-1}, hypovitaminosis (defined as vitamin E concentration below 4.64 μmol·l^{-1}) was identified in 77.7% of the calves included in the study. There were only 9 farms where the average concentration of vitamin E in blood of calves was sufficient.

The effect of hypovitaminosis E therapy based on selenium-vitamin supplement administration was assessed in a group of 10 calves divided into 2 groups (PO and SC) of five. The calves included in the PO group were administered recommended doses of the selenium-vitamin supplement (420 mg Tocopheroli alpha acetas) in two oral doses (Day 0 and Day 7) while the calves from the SC group were giwen the same supplement using the same administration pattern but subcutaneously (350 mg Tocopheroli alpha acetas). Concentration of vitamin E in blood serum of the calves was determined on Days 0, 1, 2, 3, 6, 8, 9, and 12 of the trial. It was found in both groups that supplements given to calves with hypovitaminosis E (PO – 1.65 ± 0.50 μmol·l^{-1}; SC – 1.38 ± 0.38 μmol·l^{-1}) led to a significant increase in serum concentration of vitamin E (PO – 3.41 ± 1.58 μmol·l^{-1}, $p < 0.05$; SC – 5.63 ± 0.82 μmol·l^{-1}, $p < 0.01$) as soon as on Day 1. The concentration of vitamin E, however, decreased again from post-administration Day 2 or Day 3 on (Day 6: PO – 1.50 ± 0.42 μmol·l^{-1}; SC – 3.05 ± 0.88 μmol·l^{-1}). Repeated supplementation led to a significant increase in concentration of vitamin E in both groups again, which was substantially higher in the SC group (8.8 ± 1.55 μmol·l^{-1}) compared to PO group (2.93 ± 1.29 μmol·l^{-1}). After the second application, too, the concentration of vitamin E dropped fast in both groups (Day 12: PO – 2.05 ± 0.90 μmol·l^{-1}; SC – 3.53 ± 0.90 μmol·l^{-1}).

It may be concluded that the incidence of hypovitaminosis E among calves of dairy cows is very high and the effect of selenium-vitamin supplements to calves with this diagnose is insufficient as far as long-term achievement of the concentrations of vitamin E is concerned.

Cattle, tocopherol, vitamin E, preventive diagnostics, Czech Republic

Along with selenium (Se), vitamin E ranks among very important antioxidant agents protecting the organism from the effect of reactive oxygen forms. As an extinguisher of peroxidation reactions in membranes, vitamin E is probably the most important antioxidant in cell membranes (Putman and Comben 1987; Rice and Kennedy 1988). The antioxidant effect of Se depends mainly on glutathione peroxidase (GSH-Px), in which selenium is contained. Vitamin E and GSH-Px operate at different sites in the cell. The function-site for GSH-Px is cell cytosol and vitamin E operates within lipid membranes. One important function of both systems is protection of polyunsaturated fatty acids (PUFA) in membranes, which are very sensitive to the effect of reactive oxygen forms.

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Hypovitaminosis E in animals is associated with PUFA metabolism alteration, which may subsequently lead to impaired function of cells such as polymorphonuclear neutrophils, which provide the main mechanism of protection against infection (Smith et al. 1997). Vitamin E supplementation to cows around parturition has been reported to prevent suppression of blood neutrophil and macrophage function during early postparturition period (Politis et al. 1995). Vitamin E supplementation also raises titres of specific antibodies after vaccination, increases *in vitro* T and B cell mitogenesis, interleukin production and phagocyte activity (Mudroň et al. 1992; Chew 1995). Clinical signs associated with incidence of hypovitaminosis E include nutritional muscle dystrophy and other health deficits that have to do with a drop in activity and immune function deficit (Weis 1998; Allison and Laven 2000).

Calves are born with a very low blood serum concentration of vitamin E as its transplacental transmission is limited. Vitamin E concentrations increase as the calf ages. The main sources of vitamin E for neonates are colostrum and milk. Vitamin E concentrations in milk are 6-7 times higher than in later-produced milk (Hidiriglou 1989; Van Saun et al. 1989; Herdt and Stowe 1991; Tomkins and Jaster 1991; Quigley and Drewry 1998)

Incidence of hypovitaminosis E in calves is associated with a significant increase in risk of illness, mainly due to immune function suppression. Therefore determining the concentration of this vitamin should be paid due attention as part of preventive diagnostics the search for causes of increased calve morbidity. Where hypovitaminosis has been diagnosed, efficient therapy should be started and prevention applied. However data on the current rates of incidence of hypovitaminosis E in calves in the Czech Republic are not available. Incidence of selenium deficit has nevertheless been reported (Pavlata et al. 2001b; Pavlata et al. 2002a). Its therapy and prevention is often based on combination preparations containing selenium and vitamin E (Pavlata et al. 2001a; Pavlata et al. 2002b; Pavlata et al. 2003; Pavlata et al. 2004a). Since our objective was to determine the incidence of hypovitaminosis E in calves during first month after birth and find out whether potential administration of the used doses of selenium-vitamin supplements is sufficient to deal with hypovitaminosis E in calves, too.

Materials and Methods

a) As part of preventive diagnostics of metabolic diseases and the search for the causes of increased morbidity among calves of dairy cows during milk nutrition a population of 350 calves aged 2 - 30 days was included in the trial. The calves were from 39 farms in different regions in the Czech Republic; at least 5 calves per farm were sampled for blood from *v. jugularis* to determine vitamin E (α-tocopherol) concentrations in blood serum. Vitamin E blood serum concentrations were determined by fluorometry as described by Thompson et al. (1973) and Bouda et al. (1980), using a 204 Perkin-Elmer fluorescence spectrophotometer. The testing was performed in the biochemical laboratory of the Department of Laboratory Diagnostics of the Clinic of Ruminant Diseases of the University of Veterinary and Pharmaceutical Sciences. Basic statistical parameters of the obtained set of values were computed with the help of Microsoft Excel 2003. Based on the results of vitamin E concentration determination, incidence of hypovitaminosis E in calves was assessed. The inclusion criterion for the group of calves with hypovitaminosis was vitamin E serum concentration lower than 4.64 $\mu mol \cdot l^{-1}$ (Illek et al. 1990).

b) The trial designed to verify the effect of hypovitaminosis E therapy in calves by application of selenium-vitamin supplements included 10 calves of dairy cows (with predominance of Holstein blood) with hypovitaminosis, aged 15 - 29 days, with live weight of 48 - 60 kg, stabled at the Clinic of Ruminant Diseases of the University of Veterinary and Pharmaceutical Sciences. The calves were fed with up to 6 litres a day of native mixed cow milk 3 times daily and had access to prairie hay. The calves were divided into 2 groups of five (PO and SC). The five calves in the PO group were individually orally administered Combinal-Selevit sol. ad. us. vet. (Galena, Opava, Czech Republic). The calves in the SC group (n = 5) were individually applied subcutaneously Selevit inj. ad. us. vet. (Biotika, Slovenská Ľupča, Slovak Republic). The calves were given the same supplements in the same way and at the same dosage again on Day 7 of the trial as recommended by the manufacturer. The doses and the content of active substances in the supplements used and the total amount given are shown in Table 1.

Blood for vitamin E determination was collected from all calves prior to supplement administration (Day 0) and after therapy on Day 1, 2, 3, 6, 8, 9 and 12 again. Vitamin E concentration was determined along the above-described

methodology. The calculation of the basic statistical parameters (mean, standard deviation), the comparison of results between the groups and evaluation of change of E vitamin concentration in blood serum of the calves were performed using Microsoft Excel 2003. The comparison of mean vitamin E concentrations between PO and SC groups was performed, after testing the distributions with the F-test, using the Student's *t*-test for groups with equal distribution. The dynamics of vitamin E change within each group was evaluated by the paired Student's *t*-test.

Table 1. Supplemented amount of selenium-vitamin

Supplement	Administration mode	Supplement formula (in 1 ml)	Recommended therapeutic dose per calf	Amount given	Total amount of vitamin E given in a single dose
Combinal -Selevit sol. ad us. vet. (Galena, Opava, ČR)	p.o.	Tocoferoli alfa acetas 60 mg, Natrii selenis pentahydricus 4 mg	10 ml·100 kg^{-1} body weight	7 ml	420 mg
Selevit inj. ad us. vet. (Biotika, Slovenská Ľupča, Slovenská republika)	s.c.	Tocoferoli alfa acetas 25 mg, Natrii selenis anhydricus 2.2 mg	20 ml·100 kg^{-1} body weight	14 ml	350 mg

Results

a) By testing a group of 350 calves a mean vitamin E serum concentration of 3.55 ± 2.24 μmol·l^{-1} was found. Further statistical parameters of the obtained set of values are presented in Table 2.

Table 2. Basic statistical parameters of the set of vitamin E values (μmol·l^{-1}) in blood serum of the calves included in the trial

	Vitamin E
Mean	3.55
S D	2.24
Minimum	0.25
Maximum	12.25
Median	3.00
Mode	2.50
Variance	5.03
Number	350

Table 3. Incidence of hypovitaminosis E among the tested calves (n = 350)

Vitamin E (μmol·l^{-1})	Number	%	Hypovitaminosis (cumulative %)
< 1.00	13	3.7	3.7
< 2.00	67	19.1	22.9
< 3.00	94	26.9	49.7
< 4.00	59	16.9	66.6
< 4.64	39	11.1	77.7
≥ 4.64	78	22.3	

A very high incidence of hypovitaminosis E was identified among the calves (Table 3). Most calves tested (77.7%) had vitamin E blood serum concentrations lower than the recommended value of 4.64 μmol·l^{-1}.

An evaluation of results based on the mean vitamin E concentrations in calves from individual farms revealed that only 9 farms out of the total of 39 farms had a mean concentration higher than 4.64 μmol·l^{-1}. The recorded mean vitamin E concentrations ranged from 1.35 to 8.63 μmol·l^{-1}. At individual farms, vitamin E concentrations of individual calves were relatively stable. The variation coefficient ranged between 11 and 70%. 24 farms had a variation coefficient lower than 40% and the coefficient was over 60% at 4 farms only.

b) Both oral and parenteral administration of the selenium-vitamin supplements in calves with significant hypovitaminosis E was observed to lead to a statistically significant increase in vitamin E blood serum concentration of the calves (Table 4). This increase was significantly higher in the SC group compared with the PO group. Vitamin E blood serum concentrations of the calves nevertheless decreased again from post-application Day 2 or 3. Repeated supplementation led to a significant increase in vitamin E concentrations in both groups, but the increase was significantly higher for the SC group again. After the second application, a very fast decrease in vitamin E serum concentration occurred again in both groups. Towards the end of the trial (Day 5 after the second application of the supplements), mean vitamin E concentrations did not exceed 4.00 $\mu mol \cdot l^{-1}$.

Table 4. Dynamics of change in vitamin E serum concentrations ($\mu mol \cdot l^{-1}$) of calves after oral (PO group) and parenteral (SC group) administration of a selenium-vitamin supplement (given on Days 0 and 7); statistical significance of the change in vitamin E concentration values compared with the preceding and the initial sampling is indicated (* $p < 0.05$; ** $p < 0.01$)

		Day 0	Day 1	Day 2	Day 3	Day 6	Day 8	Day 9	Day 12
PO	Mean	1.65	3.41	3.00	2.07	1.50	2.93	2.44	2.05
	S.D.	0.50	1.58	1.32	0.76	0.42	1.29	0.80	0.90
	% of previous value		207*	88	69	72	196*	83	84**
	% of initial value	100	207*	182	125	91	178	148	124
SC	Mean	1.38	5.63	6.65	5.70	3.05	8.80	5.25	3.53
	S.D.	0.38	0.82	1.05	0.64	0.88	1.55	1.28	0.90
	% of previous value		408**	118*	86	56**	289**	60*	67*
	% of initial value	100	408**	482**	413**	221**	638**	380**	256**

Comparing the results between the two groups (Fig. 1) it may be concluded that although the change of vitamin E blood concentrations had very similar dynamics in both groups of calves, the absolute values of the concentration increase were significantly higher for the SC group of calves, i.e. after parenteral supplement administration. The initial mean vitamin E concentrations being 1.65 ± 0.50 and 1.38 ± 0.38 $\mu mol \cdot l^{-1}$ for the PO and SC groups respectively, the highest vitamin E concentration was observed in calves in the SC group – 8.8 ± 1.55 $\mu mol \cdot l^{-1}$ (Day 8 of the trial), compared with the peak value of 3.41 ± 1.58 $\mu mol \cdot l^{-1}$ in the PO group (post-application Day 1).

The results thus show that although injection of the supplement has led to the necessary short-time increase in vitamin E serum concentration in calves with hypovitaminosis E, this positive therapeutic effect lasted for a very short time only, despite repeated therapy with the supplement. Oral administration of the supplement did increase vitamin E concentration, too, but the increase has not led to the required vitamin E concentration.

Discussion

The generally very high incidence of hypovitaminosis E in the calves of dairy cows included in the experiment during the first month of their lives must be perceived as very negative. Hypovitaminosis E in calves may have a whole array of negative consequences for their health status and development. Besides clinical forms of nutritional muscle dystrophy,

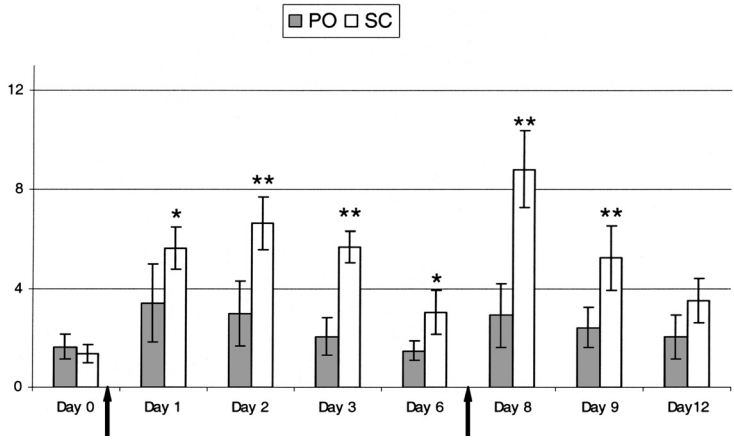

Fig. 1. Vitamin E serum concentrations (μmol·l^{-1}) of the calves after oral (the PO group) and parenteral (the SC group) administration of the selenium-vitamin supplement; statistical significance of the difference in vitamin E concentration between the groups for individual collections is indicated (* $p < 0.05$; ** $p < 0.01$). The black arrows on the x axis indicate the days the supplements were given (Day 0 and Day 7).

reported in calves whose vitamin E serum concentrations are lower than 3 μmol·l^{-1} (Kováč 1991), hypovitaminosis E has a major negative effect on the immune function, predisposing calves to infectious diseases.

The very high incidence of hypovitaminosis E in calves may be due to numerous factors. Although calves are born with very low vitamin E serum concentrations, the concentration increases in physiological conditions over the period of colostral and subsequently milk nutrition (Hidiroglou 1989; Van Saun et al. 1989; Pavlata et al. 2004b). It is therefore essential that calves are fed a sufficient amount of quality colostrum and milk rich in vitamin E. Vitamin E status of the organism of calves in early postparturition period is thus significantly determined by vitamin E status of the gestating cows along with other factors such as the overall quality of nutrition and metabolism and potential incidence of any diseases which can affect the composition of the colostrum and milk the cows produce as well as resorption of vitamin E from the digestive system in calves. The low levels of vitamin E concentration in blood may be e.g. due to supplementation of high doses of vitamin A (Eichler et al. 1997).

With vitamin E binding to fats, it is desirable that colostrum and milk are sufficiently rich in natural fats. Normal colostrum and milk are characterized by 8-13 times higher α-tocopherol levels compared with skimmed colostrum and milk. Research shows that while a progressive increase in α-tocopherol serum concentration occurs in calves fed natural colostrum and milk, this increase has not been observed in calves fed skimmed colostrum and milk (Rajaraman et al. 1997).

In the light of the great importance of quality colostrum intake for reaching the necessary concentrations of vitamin E in blood (Njeru et al 1994), the observed high incidence of hypovitaminosis E at Czech farms with calves of dairy cows may be ascribed especially to the poor colostral nutrition of calves that is so frequent at farms in the Czech Republic. For example, within an analysis of the causes of increased morbidity among calves at 27 cattle farms, protein metabolism testing revealed colostral nutrition deficits in approximately 80% of cases. The main cause underlying this situation seems to be insufficient nursing care and insufficient colostrum intake by the calves (Pavlata et al. 2003). Tocopherol concentrations may also decrease as a result of stress (Sconberg et al. 1993; Nockels et al. 1996), which, in its turn, may be induced by insufficient nursing care.

High incidence of hypovitaminosis E in calves thus points to a need for improved nursing care throughout the early postparturition period, with special focus on colostral nutrition quality. It also proves the need for preventive diagnostics of metabolic diseases in this cattle category, too, since early identification of subclinical forms of disease and implementation of therapeutic and preventive measures may help eliminate subsequent negative consequences on development and growth of future breeding animals.

Our results elucidate the effect of selenium-vitamin supplements in hypovitaminosis E therapy and show that the used doses of the supplements were not sufficient to ensure long-time adjustment of vitamin E serum concentration in the calves. Administration of the supplements did lead to a significant increase in vitamin E serum concentrations in the calves, but for a short period only, despite repeated administration. The significant increase in vitamin E levels after its administration to calves has been observed as early as within 14 - 32 h from administration (Hidiroglou et al. 1989; Eichler et al. 1997).

A raise in vitamin E concentration in calves above 4 $\mu mol \cdot l^{-1}$ was achieved after subcutaneous administration of the supplement only. This shows that as to inducing an increase in vitamin E serum concentrations, parenteral α-tocopherol administration was significantly more efficient compared with the oral administration. This is the more noteworthy that the overall dose of applied α-tocopherol was higher for calves to which the supplement was administered orally. Although it has been known that vitamin E concentration remains stable throughout fermentation in the rumen (Leedle et al. 1993; Weiss et al. 1995) and higher tocopherol plasma concentrations were recorded after intraruminal administration of the vitamin compared with intraduodenal administration (Roquet et al. 1992), it is evident that the increase in vitamin E concentrations is faster and greater after injection administration of tocopherol. In dairy cows, too, injection administration of vitamin E is regarded as a suitable strategy e.g. in those cases when oral supplementation of vitamin E fails to eliminate depression of plasma α-tocopherol in cows in the postparturition period (Weiss et al. 1990). Weiss et al. (1992) reported that even long-term oral administration of vitamin E to cows in the dry period has a lower effect on increasing α-tocopherol plasma concentration compared with injections. The duration of higher vitamin E concentrations is relatively short even after injections of the supplement.

The insufficient effect of administration of the supplements used in our trial to adjust vitamin E serum concentrations has probably to do also with the fact that the initial vitamin E concentrations were very low and the supplemented vitamin was employed to create a body reserve for the calf. Although it is generally true that α-tocopherol plasma concentrations correlate with vitamin E intake (Weiss et al. 1992; Njeru et al. 1995), the current state of body reserve needs not be closely tied to its serum concentration. There is no close correlation between vitamin E concentrations in blood and tissues and the serum concentration therefore need not be a reliable indicator of the current reserve in tissues (Rajaraman et al. 1997).

Our results show that the selenium-vitamin supplements we used are capable of bringing vitamin E concentration up for a short time, but their supplementation is insufficient for its adjustment in the longer run. Considering the fact that the supplements contain selenium, increasing the dosages cannot be recommended. Therefore if calves are diagnosed with hypovitaminosis E along with selenium deficit and the recommended dosage of selenium-vitamin supplements has been exhausted, the recommendation may be to go on with further hypovitaminosis E therapy using supplements containing vitamin E alone and/or combining vitamin E with other vitamins containing no potentially toxic selenium.

Výskyt hypovitaminózy E u telat a její terapie aplikací selenovitaminových preparátů

Cílem práce bylo vyhodnotit výskyt hypovitaminózy E u telat dojného skotu v období mléčné výživy v různých chovech České republiky a ověřit efekt terapie hypovitaminózy E perorální a parenterální aplikací selenovitaminového preparátu.

Koncentrace vitaminu E byla stanovena v krevním séru 350 telat v období mléčné výživy pocházejících z 39 různých chovů dojnic. Při průměrné zjištěné koncentraci vitaminu E 3,55 ± 2,24 μmol·l^{-1}, byla hypovitaminóza (koncentrace vitaminu E nižší než 4,64 μmol·l^{-1}) zjištěna u 77,7% vyšetřených telat. Pouze v 9 chovech byla průměrná koncentrace vitaminu E v krvi telat dostatečná.

Hodnocení efektu terapie hypovitaminózy E selenovitaminovými preparáty bylo realizováno na deseti telatech rozdělených do 2 skupin (PO a SC) po pěti. Telatům skupiny PO byl selenovitaminový preparát (420 mg Tocoferoli alfa acetas) aplikován dvakrát (den 0 a 7) perorálně a telatům skupiny SC ve stejném schématu subkutánně (350 mg Tocoferoli alfa acetas). Koncentrace vitaminu E v krevním séru telat byla stanovena 0., 1., 2., 3., 6., 8., 9. a 12. den trvání pokusu. U obou skupin bylo zjištěno, že po aplikaci preparátů telatům s hypovitaminózou E (PO - 1,65 ± 0,50; SC - 1,38 ± 0,38 μmol·l^{-1}) došlo již 1. den k průkaznému zvýšení sérové koncentrace vitaminu E (PO - 3,41 ± 1,58 μmol·l^{-1}, $p < 0,05$; SC – 5,63 ± 0,82 μmol·l^{-1}, $p < 0,01$). Od 2., resp. 3. dne po aplikaci, však docházelo k opětovnému poklesu koncentrace vitaminu E (6. den pokusu: PO – 1,50 ± 0,42 μmol·l^{-1}; SC – 3,05± 0,88 μmol·l^{-1}). Po opakované aplikaci preparátů došlo opět k průkaznému vzestupu koncentrace vitaminu E u obou skupin, který byl podstatně vyšší u skupiny SC (8,8 ± 1,55 μmol·l^{-1}) v porovnání s PO (2,93 ± 1,29 μmol·l^{-1}). I po druhé aplikaci preparátů došlo u obou skupin k rychlému poklesu koncentrace vitaminu E (12. den pokusu: PO - 2,05 ± 0,90 μmol·l^{-1}; SC - 3,53 ± 0,90 μmol·l^{-1}).

Lze konstatovat, že výskyt hypovitaminózy E v chovech telat mléčného skotu je velmi vysoký a efekt aplikace selenovitaminových preparátů telatům s touto diagnózou je z hlediska dlouhodobějšího dosažení potřebných koncentrací vitaminu E nedostatečný.

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