BLOOD AND TISSUE SELENIUM CONCENTRATIONS IN CALVES TREATED WITH INORGANIC OR ORGANIC SELENIUM COMPOUNDS - A COMPARISON

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Received October 25, 2000
Accepted February 7, 2001

Abstract

Pavlata L., J. Illek, A. Pechová: *Blood and Tissue Selenium Concentrations in Calves Treated with Inorganic or Organic Selenium Compounds – a Comparison.* Acta Vet. Brno 2001, 70: 19–26.

Selenium concentrations were investigated in 5 calves treated parenterally with inorganic selenium (Group II), 5 calves treated orally with organic selenium (Group III), and 5 untreated control calves (Group I). Blood samples for analyses were collected at the beginning and at the end of the treatment period and tissue samples (liver, kidney, striated muscle, myocardium) after slaughter. Selenium was determined in the samples after microwave mineralisation using the hydride technique of AAS. Both treated groups showed highly significant increases in whole blood selenium concentrations (Group II from 53.4 ± 10.5 to 75.9 ± 4.0 $\mu g.l^{-1}$; Group III from 70.25 ± 12.07 to 127.5 ± 16.7 $\mu g.l^{-1}$). The comparison of tissue concentrations showed highly significant differences ($P < 0.01$) in the liver (213.3 ± 56.8, 206.5 ± 36.2 and 424.7 ± 88.4 $\mu g.kg^{-1}$ wet tissue for the Groups I, II, and III, respectively), striated muscles (92.4 ± 29.2, 81.4 ± 12.1, and 263.4 ± 47.4 $\mu g.kg^{-1}$ wet tissue for the Groups I, II and III, respectively), and myocardium (121.5 ± 31.8, 108.3 ± 9.6, and 251.4 ± 51.0 $\mu g.kg^{-1}$ wet tissue for the Groups I, II and III, respectively) not only between Groups I and II, but also between Groups II and III. The among-group differences in selenium concentrations in the kidney (991.9 ± 49.1, 960.6 ± 36.3, and 1050.5 ± 336.8 $\mu g.kg^{-1}$ wet tissue in the Groups I, II and III, respectively) were nonsignificant. It is apparent that oral administration of organic selenium resulted in higher tissue concentrations than parenteral administration of inorganic selenium. Highly significant ($P < 0.01$) correlations were found between selenium concentrations in the liver and striated muscles, in the liver and the myocardium, and in striated muscles and the myocardium (r = 0.78, 0.85, and 0.94, respectively). No relations were found between selenium concentrations in the kidney and other tissues. Highly significant ($P < 0.01$) correlations were also between the concentrations of selenium in the blood and in the liver, striated muscles, and the myocardium (r = 0.85, 0.80, and 0.77, respectively).

Cattle, liver, striated muscle, myocardium, kidney

Recent knowledge of biological effects of trace elements has prompted studies of their effects on animal health and performance. The importance of such studies is enhanced by the use of trace elements as feed additives as a replacement for antibiotic and hormonal growth stimulants. Considerable attention in this respect is paid to selenium. The principal source of this element for animals are plants. Groce et al. (1995), who studied correlations between the contents of essential trace elements in soil, forages, and blood, found a highly significant correlation coefficient (r = 0.96) for selenium. Availability of soil selenium to plants depends on soil acidity, structure and degree of aeration. Availability of oxidised forms is higher than that of the reduced form which remains undissolved in the soil. Selenium is incorporated into plant tissues mostly in the form of selenomethionine present in the protein component of grain. Hence, the content of selenium in grain closely correlates with the content of protein (Mahan 1999). Further factors influencing the content of selenium in plants include the vegetation phase,

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diluting effects of long-lasting rains, season, and fertilisation with sulphur (Ammerman and Miller 1975). Our earlier observations have shown that selenium deficiency in the Czech Republic is a serious and topical problem. Tests of blood samples collected from 326 cattle in 30 herds demonstrated insufficient or marginal saturation with selenium in 64 percent of the probands (Pavlata et al. 2000a). Tests carried out in slaughter cattle revealed selenium deficiency in more than 80 percent of the animals; the deficiency was observed more frequently in heifers and bulls than in cows (Pavlata et al. 2000b). Illek et al. (1999), who tested ten herds of high-producing Holstein cows, found selenium deficiency in dry cows in eight and in lactating cows in four of them. The results of the above studies emphasise the necessity of monitoring of the selenium status and of supplementation in animals showing deficiency. The major source of selenium used world-wide is sodium selenite. Most, but not all, problems resulting from insufficient supply of selenium can be solved by administration of selenite. Therefore increased attention of researchers is paid to organic selenium compounds. One of the supplementation forms used recently are yeast which incorporate selenium into amino acids. The organic form is more suitable for metabolic transformation (Mahan 1999). In the Czech Republic, animals showing selenium deficiency are most frequently treated with a local drug containing inorganic selenium and vitamins in a formula for parenteral administration. Therefore this drug was included into our experiment. Its objectives were a) to compare the effects of administration to calves of inorganic and organic selenium forms at recommended dosage in terms of blood and tissue selenium concentrations; b) to study the tissue distribution of selenium and to select tissues most suitable for the monitoring of the selenium status.

Materials and Methods

Fifteen one-month-old (± 4 days) Holstein calves, 55 to 65 kg in weight, born by cows of a single herd were selected for the experiment. The calves were housed in individual outdoor sheds, had free access to a selenium-free starter diet and received 2 l of milk per day up to the age of 2 months. The experimental period was 2 months. The calves were randomly divided into three groups of five. Group I were controls. Group II was treated (the 1st day of the experiment) intramuscularly with inorganic selenium and vitamin E (SELEVIT inj. ad us. vet., natrii selenis anhydricus 2.2 mg, tocoferoli acetas 25 mg per 1 ml) at the dose of 20 ml split to two injection sites in the neck region; the treatment was repeated one week later. The total amount of received net selenium was 40 mg; Group III was fed a starter diet supplemented with yeast-bound selenium (SEL-PLEX, Alltech, selenium 1000 $mg.kg^{-1}$) at the recommended concentration of 0.3 mg selenium per 1 kg throughout the experimental period. At the mean feed consumption of 2 kg per animal per day the calves of this group received a total of 36 mg of selenium per animal. Blood samples for the determination of selenium concentration were collected by puncture of v. jugularis into disposable heparinised tubes at the beginning and at the end of the experiment. The calves were slaughtered within two subsequent days and samples of liver, striated muscle, myocardium and renal cortex tissues were collected for analyses.

The blood and tissue samples were mineralised by microwave digestion in a closed system in the presence of nitric acid and hydrogen peroxide using the MILESTONE MLS-1200 apparatus. The dried mineralised product was dissolved in water and reduced by addition of hydrochloric acid. Selenium was then determined using the hydride technique of AAS in the UNICAM 939 AA spectrometer. The results are expressed in $\mu g.l^{-1}$ of blood, or $\mu g.kg^{-1}$ of wet tissue.

Correlation coefficients were calculated and results were processed by *t*-test after *F*-test for equality of variation using the EXCEL and STAT plus software.

Results

Blood selenium concentrations in the three groups at the beginning and after the end of selenium supplementation are shown in Fig. 1. No virtual difference between the initial and the final blood selenium concentrations was observed in Group I (69.21 ± 11.22 vs. 68.42 ± 10.7 $\mu g.l^{-1}$). The concentrations of selenium increased in Group II (inorg. Se) from 53.4 ± 10.5 $\mu g.l^{-1}$ to 75.9 ± 4.0 $\mu g.l^{-1}$ and in Group III (organic Se) from 70.25 ± 12.07 to 127.5 ± 16.7 $\mu g.l^{-1}$. The increases were highly significant in both the groups.

The results of analyses of tissue samples and significance of differences are given in Tables 1 and 2, respectively. The highest selenium concentrations were found in the kidney, followed by the liver and muscles. Highly significant differences were found for selenium concentrations in the liver, striated muscles, and the myocardium between Groups I and II and Groups II and III. No significant among-group differences were found in selenium concentrations in the kidney. Positive correlations were found among the concentrations of selenium in the blood, the liver, striated muscles and the myocardium. No correlations were found for the concentrations of selenium in the kidney and in any of the remaining tissues. The respective correlation coefficients are shown in Table 3.

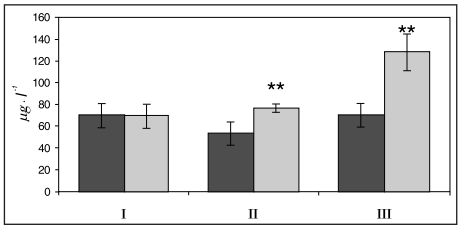

Fig. 1: Selenium concentrations (mean ± S.D.) in whole blood of the individual calf groups (I, II and III) at the beginning and after the experiment (** $P < 0.01$).

Table 1
Selenium concentrations ($\mu g.kg^{-1}$) in tissues of slaughtered calves of the individual groups

		Liver	Kidney	Muscle	Myocardium
Group I	mean	213.3	991.9	92.4	121.5
	S.D.	56.8	49.1	29.2	31.8
Group II	mean	206.5	960.6	81.4	108.3
	S.D.	36.2	36.3	12.1	9.6
Group III	mean	424.7	1050.5	263.4	251.4
	S.D.	88.4	336.8	47.4	51.0

Table 2
Significance of among-tissue differences in selenium concentrations (- non-significant; ** $P < 0.01$)

	Liver	Kidney	Muscle	Myocardium
Group I : Group II	-	-	-	-
Group I : Group III	**	-	**	**
Group III : Group II	**	-	**	**

Table 3
Correlation coefficients (r) for selenium concentrations in various samples including their significance (** $P < 0.01$)

	Blood	Liver	Kidney	Muscle	Myocardium
Blood	1	0.85**	0.16	0.80**	0.77**
Liver		1	0.38	0.78**	0.85**
Kidney			1	0.07	0.01
Muscle				1	0.94**
Myocardium					1

Discussion

The administration of inorganic or organic selenium to calves resulted in a highly significant increase in the concentration of blood selenium. In terms of absolute values, the concentration of blood selenium was markedly higher in Group III receiving organic

selenium. The mean blood selenium concentrations in Group II and Group III rose by 42% and 82%, respectively. It can be concluded that long term oral administration of recommended dose of organic selenium resulted in markedly higher blood selenium concentrations than parenteral administration of an inorganic selenium-containing drug used currently for the therapy of selenium deficiency. Apart from the irrelevant decrease by 1%, the blood selenium concentration in Group I remained constant throughout the experimental period. Considering the reference value of 100 $\mu g.l^{-1}$, corresponding to adequate selenium supply in cattle (Van Saun 1990; Fisher et al. 1995; Pavlata et al. 2000), the selenium status of all the calves at the beginning of the experiment (mean 64.3 ± 13.1, range 43 - 84 $\mu g.l^{-1}$), and in Groups I and II also at its end, must be assessed as deficient or marginal. The lower initial mean value in Group II was due to random allotment of two calves with the lowest blood selenium concentrations. The unchanging blood selenium concentration in Group I is indicative of a constant, but insufficient, supply of selenium to the animals fed the non-supplemented diet.

Increasing attention is paid world-wide to the comparison of effects of organic and inorganic selenium. Differences in the increase of blood selenium in animals treated for 8 weeks with sodium selenite or selenium yeast at equal doses of 0.2 ppm were reported by Malbe et al. (1995). Mean concentrations of blood selenium rose from 5.6 $\mu g.l^{-1}$ to 167 $\mu g.l^{-1}$ and 91 $\mu g.l^{-1}$ in the animals treated with selenium yeast and sodium selenite, respectively. The corresponding ratio of biological availability for organic and inorganic selenium was 1.9:1. Ortman et al. (1999) treated experimental groups with sodium selenite, sodium selenate, or selenium yeast at equal doses of 2 mg for 3 months. Whole blood and blood plasma concentrations in the animals treated with organic selenium were significantly higher than in any of the groups receiving inorganic selenium. The difference between the groups receiving inorganic selenium was non-significant. The same results were reported by Ortman and Pehrson (1999) who arranged a similar experiment and measured selenium concentrations in whole blood, blood plasma, and milk of dairy cows. Illek et al. (2000) compared whole blood selenium concentrations in pastured beef heifers receiving either selenium yeast or sodium selenite for two months. Their conclusions were consistent with those of the above authors.

The results of our experiment have extended the current knowledge on effects of long-term oral administration of yeast-bound selenium and double administration of approximately the same dose of inorganic selenium on the selenium status. The higher efficacy of organic selenium was confirmed also by results of analyses of selected tissues. The concentrations in Group II were almost identical with those in Group I and demonstrated that parenteral administration of inorganic selenium had only a minimum effect on tissue reserves. Occasional increases observed after the treatment were followed by rapid decreases and the concentrations at slaughter were low. It can therefore be concluded that, in terms of long-range effects, such treatment failed to improve the selenium status. This was evident also from the absence of differences in tissue selenium concentrations between selected calves of Groups I and II with approximately the same initial blood selenium concentrations. Although the parenteral treatment with inorganic selenium resulted in a significant increase in blood selenium concentrations, tissue concentrations and the overall selenium status were lower than in the calves treated orally with organic selenium. The highly significant differences in selenium concentrations in the liver, striated muscles and myocardium between the control group and the groups receiving organic selenium, as well as between the groups receiving organic and inorganic selenium demonstrate the higher efficacy of organic selenium also in terms of tissue concentrations. Similar results were published by Ortman and Pehrson (1997) as well as Knowles et al. (1999) who investigated blood, liver and milk selenium concentrations in cows receiving selenium yeast.

Any comparison of data on tissue concentrations as published by different authors is difficult, because their results are expressed in different units and their opinions on the suitability of various tissues for the assessment of selenium status are controversial. Van Vleet (1975) examined tissues of slaughtered normal weaned calves and found the following selenium wet tissue concentrations: liver - 0.12 ppm, kidney cortex - 0.63 ppm, muscles - 0.05 ppm. Salisbury et al. (1991) in their investigations of selenium concentrations in organs of slaughtered animals found 280 $\mu g.kg^{-1}$ and 920 $\mu g.kg^{-1}$ in bovine liver and kidney tissues, respectively. Stowe and Herdt (1992) studied dependence of selenium concentrations in blood serum on age in various animal species and reported the ranges 50 to 80 $ng.ml^{-1}$ for calves and lambs and 70 to 100 $ng.ml^{-1}$ for adult cattle, while the concentrations in liver tissue varied between 1200 and 2000 $ng.kg^{-1}$ dry matter irrespective of the age and species of animals. Zachara et al. (1993) in their studies of effects of various doses of selenium in feeds on tissue concentrations in lambs found for animals receiving the basic amount of selenium the highest concentrations in kidneys (1320 $\mu g.kg^{-1}$ wet tissue) and the lowest in striated muscles (30 $\mu g.kg^{-1}$ wet tissue). Liver, lung, and spleen concentrations ranged between 140 and 180 $\mu g.kg^{-1}$. In their study of selenium concentrations in foetal livers and kidneys, Abdelrahman and Kincaid (1993) demonstrated an increase in liver tissue in the period between pregnancy days 145 and 195 and a subsequent decrease in the period between pregnancy days 195 and 245, while the kidney concentrations remained constant. Mee et al. (1994), who studied effects of selenium and iodine status on the course of parturition and state of health of calves, considers kidney selenium concentrations lower than 5.06 $\mu mol.kg^{-1}$ as indicator of selenium deficiency. Zust et al. (1996) assessed the selenium status of calves on the basis of blood plasma and liver concentrations; in their view, marginal concentrations are 300 $\mu g.kg^{-1}$ of fresh liver tissue and 30 $\mu g.l^{-1}$ of blood plasma. Liver and kidney concentrations of selenium were used as indicators of selenium status within etiologic studies of heart malformations in calf foetuses by Orr and Blakley (1997).

This short survey shows that the liver and kidney are used most frequently in the assessment of selenium status. The distribution pattern found in our experiments was consistent with the data of other authors although absolute values are different. Our investigations, too, have confirmed that the highest concentrations are to be found in the kidney, followed by the liver and striated muscles. However, the kidney tissue is apparently less suitable for the monitoring of selenium status in supplementation experiments. Although the concentrations were high, they did not increase in the animals fed diets supplemented with either inorganic or organic selenium. This finding is consistent with the data published by Zachara et al. (1993) who fed lambs a diet supplemented with graded doses of selenium and found a linear increase of selenium concentrations in the liver and lung, but not in the kidney. The spleen, myocardium and striated muscles responded by an increase only in lambs receiving higher doses of selenium. Another reason for refusing the kidney as a matrix for the assessment of selenium status is the lack of significant correlations between the selenium concentrations in the kidney on the one hand, and in the blood, the liver, and striated muscles on the other hand.

The high correlation between blood and liver selenium concentrations ($r = 0.85$) indicates that the two matrices are suitable for the assessment of selenium status. Blood and selenium concentrations were determined also by Van Saun et al. (1989) in their study of the dam - foetus relation. Mean in foetal liver and blood serum concentrations were 2140 $\mu g.kg^{-1}$ dry matter and 21.4 $ng.ml^{-1}$, respectively. The corresponding values for dams were 950 $mg.kg^{1}$ and 44 $ng.ml^{-1}$, respectively. For bovine foetuses, the authors regard as appropriate liver and whole blood selenium concentrations of 2200 $\mu g.kg^{-1}$ dry matter and 120 $ng.ml^{-1}$, respectively. Similarly, Kirk et al. (1995) found the determination of selenium

concentrations in bovine foetal liver and in whole blood of dams suitable for the assessment of selenium status at the herd level. Moreover, their investigations demonstrated higher foetal liver selenium concentrations in dairy cattle than in beef cattle (777 ± 408 $\mu g.kg^{-1}$ vs. 443 ± 38 $\mu g.kg^{-1}$) and a closer correlation between selenium concentrations in the blood and calf liver in beef cattle than in dairy cattle. A highly significant correlation between blood and liver selenium concentrations was found also in our recent study in slaughtered bulls (Pavlata et al. 1999).

Another tissue which is apparently suitable for the monitoring of selenium status in cattle are muscles. The correlations between selenium concentrations in the blood and striated muscles (r = 0.80), in the blood and myocardium (r = 0.77), in the liver and striated muscles (r = 0.78), and in the liver and myocardium (r = 0.85) were highly significant. These results are consistent with our earlier finding of a highly significant correlation between the concentrations of selenium in the blood and diaphragmatic muscles (r = 0.91) (Pavlata et al. 1999). Moreover, supplementation with organic selenium resulted in an increase of selenium concentrations in the liver and myocardium by 100% and in striated muscles by more than 185%.

Conclusions

Long term oral administration of organic selenium in the form of selenium yeast resulted in higher blood and tissue concentrations than repeated parenteral administration of recommended therapeutic doses of inorganic selenium. The latter treatment was ineffective in terms of tissue concentrations and induced only a considerably smaller increase of blood concentrations. Taking in concentrations and correlation coefficients, the most suitable materials for the monitoring of selenium status in cattle are the blood, the liver, striated muscles, and the myocardium. On the other hand, the kidney was found unsuitable; although the concentrations were the highest, they remained practically constant even in the calves receiving organic selenium.

Porovnání vlivu anorganické a organické formy selenu na jeho koncentraci v krvi a tkáních telat

Na 15 telatech rozdělených do 3 skupin po pěti (kontrolní – I, pokusná – II a III) bylo provedeno sledování vlivu parenterálně aplikovaného anorganického selenu (u skupiny II) a perorálně podaného organicky vázaného selenu (skupina III) na jeho krevní a orgánové koncentrace. Obsah selenu v krvi byl stanoven na začátku a při ukončení dotačního pokusu a v orgánech (játra, ledviny, kosterní svalovina, myokard) po poražení telat. Stanovení selenu v krvi i orgánech bylo provedeno po mikrovlnné mineralizaci vzorků hydridovou technikou AAS. U obou pokusných skupin bylo zjištěno vysoce signifikantní zvýšení koncentrace selenu v plné krvi (u skupiny II z průměrné hodnoty 53,4 ± 10,5 $\mu g.l^{-1}$ na 75,9 ± 4,0 $\mu g.l^{-1}$, resp. z hodnoty 70,25 ± 12,07 $\mu g.l^{-1}$ na 127,5 ± 16,7 $\mu g.l^{-1}$ u skupiny III). Při porovnání orgánových hladin selenu byl zjištěn vysoce průkazný rozdíl ($P < 0,01$) v koncentraci selenu v játrech, kosterní svalovině a myokardu mezi skupinou číslo I a III, ale i II a III (při koncentraci selenu v játrech u skupiny I – 213,3 ± 56,8; II – 206,5 ± 36,2; III- 424,7 ± 88,4 $\mu g.kg^{-1}$ čerstvé tkáně, v kosterní svalovině u skupiny I – 92,4 ± 29,2; II – 81,4 ± 12,1; III – 263,4 ± 47,4 $\mu g.kg^{-1}$ čerstvé tkáně a v myokardu u skupiny I – 121,5 ± 31,8; II – 108,3 ± 9,6; III – 251,4 ± 51,0 $\mu g.kg^{-1}$ čerstvé tkáně. Obsah selenu v ledvinách nebyl mezi zvířaty jednotlivých skupin průkazně rozdílný (I – 991,9 ± 49,1; II – 960,6 ± 36,3; III– 1050,5 ± 336,8 $\mu g.kg^{-1}$ čerstvé tkáně). Efekt perorálně podaného organicky vázaného selenu tak byl z hlediska vzestupu orgánových hladin výrazně vyšší v porovnání s parenterální

aplikací anorganické formy. Dále bylo provedeno vyhodnocení těsnosti vztahu mezi jednotlivými zjištěnými hodnotami orgánových koncentrací pomocí korelačních koeficientů. Byl zjištěn vysoce průkazný vztah ($P < 0,01$) mezi obsahem Se v játrech a kosterní svalovině, játrech a myokardu a kosterní svalovině a myokardu ($r = 0,78$; $r = 0,85$; $r = 0,94$). Mezi hodnotami obsahu Se v ledvinách a ostatních tkáních nebyla závislost prokázána. Vysoce průkazná korelace ($P < 0,01$) byla zjištěna i mezi hladinou selenu v krvi a jeho obsahem v játrech, kosterní svalovině a myokardu ($r = 0,85$; $r = 0,80$; $r = 0,77$).

Acknowledgement

The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. 161700002/1999).

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