

The effect of various forms of selenium supplied to pregnant goats on selected blood parameters and on the concentration of Se in urine and blood of kids at the time of weaning

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ABSTRACT: The aim of this trial was to compare the effect of supplementation of goats with different forms of selenium on the metabolism of their kids at the time of weaning. The experiment was performed with 45 kids of mothers supplemented with various forms of selenium. Group C was control while the other four groups were supplemented with selenium for six weeks before (0.3 mg/goat/day) and after parturition (0.9 mg/goat/day). Group Se-I received sodium selenite while the other groups received organic forms: Se-lactate-protein complex (Se-L), Se-proteinate (Se-P) and Se-yeast (Se-Y). The kids were weaned at three months of age and samples of blood and urine were taken. Parameters monitored in the blood included Se, glutathione peroxidase (GPx), Zn, Cu, thyroxine, triiodothyronine, protein, immunoglobulins, muscle enzymes, total antioxidant status, vitamin A and E. Se levels were determined in the urine. Selenium supplementation of goats from six weeks before delivery significantly influenced selenium concentrations in the blood of kids. Significant differences ($P < 0.0002$) were found between the control and all experimental groups and further between Se-Y and the other experimental groups (Se-Y: $243.0 \pm 20.3 \mu\text{g/l}$; Se-I: $156.3 \pm 34.3 \mu\text{g/l}$; Se-P: $152.6 \pm 41.5 \mu\text{g/l}$; Se-L: $146.7 \pm 20.0 \mu\text{g/l}$; C: $67.6 \pm 13.1 \mu\text{g/l}$). The highest concentration was found in the group supplemented with Se-yeast with a high content of selenomethionine. The other two organic forms of selenium (proteinate and lactate-protein complex) increased the concentration of Se in blood and the activity of GPx to the same extent as the inorganic form of selenium. Se supplementation did not have a negative effect on the concentration of copper and zinc in the blood serum of kids, but we found decreased concentrations of thyroxine in the experimental groups (Se-Y: $79.8 \pm 12.8 \text{ nmol/l}$; Se-I: $66.5 \pm 13.2 \text{ nmol/l}$; Se-P: $76.2 \pm 25.7 \text{ nmol/l}$; Se-L: $84.5 \pm 14.8 \text{ nmol/l}$; C: $92.7 \pm 13.4 \text{ nmol/l}$). Significant differences were found between the group C and groups Se-I and Se-P ($P < 0.05$). The supplementation of mothers with Se both in organic and inorganic forms was sufficient to prevent Se deficiency in kids at the time of weaning.

Keywords: ruminants; Se-yeast; sodium selenite; Se-lactate; Se-proteinate; Se-lactate-protein complex; zinc; copper; thyroxine; creatine kinase; glutathione peroxidase

Selenium is an essential trace element vital for the normal growth and health of animals. It is present in all cells and tissues and is necessary for maintaining the vital functions of humans and animals. The content of Se in the organism is naturally very low, the majority of Se being bound in tissues and blood in the form of selenoproteins. Selenium is a compo-

nent of at least 25 selenoproteins with antioxidant, anti-inflammatory and chemoprotective properties (Pappas et al. 2008). The most important are glutathione peroxidases (GPx1–GPx6), thioredoxin reductases (TrxR1–TrxR3), iodothyronine deiodinases (ID1–ID3), selenophosphate synthetase, selenoprotein P, and selenoprotein W. This element

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acts as a cofactor of the GPx family of enzymes which protect against oxidative stress. Specifically Se-dependent GPx enzyme recycles glutathione, reducing lipid peroxidation by catalysing the reduction of peroxides, including hydrogen peroxide. In general all these enzymes in their reduced state catalyse the breakdown of lipid hydroperoxides and hydrogen peroxides in cells (Navarro-Alarcon and Cabrera-Vique 2008). GPx and selenoprotein P are also involved in the regulation of the inflammatory response.

Selenium deficiency has been linked to many health problems in young animals such as increased neonatal mortality, decreased sucking reflex, weakness, higher occurrence of infectious diseases and white muscle disease (Enjalbert et al. 2006). Selenium deficiency may also result in immune and endocrine disorders, especially thyroid dysfunction, as Se is essential for thyroid function and thyroid homeostasis (Kohrle et al. 2005). In new-born animals and during sucking, the saturation of kids with selenium depends on the saturation of the mother (Ghany-Hefnawy et al. 2007; Misurova et al. 2009), as selenium permeates placental and mammary barriers. Placental transfer is more effective than the transmission of selenium to the calf through milk (Enjalbert et al. 1999). The concentration of selenium in milk depends on the amounts and the form of selenium in the ration (Givens et al. 2004; Heard et al. 2004; Juniper et al. 2006). The concentration of Se in milk is significantly increased mainly by products containing Se organically bound in the form of selenomethionine (Ortman and Pherson 1999; Muniz-Naveiro et al. 2006; Pechova et al. 2008). Selenium deficiency that affects animals living in areas with low natural concentrations of Se causes significant economic losses. Various methods of supplementation have been developed to increase intake of selenium. Recently, close attention has been paid to the development of new preparations containing organically bound selenium. However, the chemical form of selenium and quantity of selenium that is organically bound is often not precisely specified in these products. From the organic compounds, selenomethionine has been described most thoroughly. Selenomethionine fulfils the criteria of essential amino acids for humans and monogastric animals. The same can be said for ruminants, even though these carry bacteria in their rumen that produce Se-met (Schrauzer and Surai 2009). As speciation analysis of individual Se compounds is not available in many new products, the only way

to compare their supplementation efficiency is to use them experimentally in animals.

The aim of this work was to evaluate the effect of long-term supplementation of goats with one inorganic and three different organic forms of selenium on the metabolism of their kids at the time of weaning.

MATERIAL AND METHODS

The experiment was performed with 45 kids of mothers supplemented with various forms of selenium. The white shorthaired goats were divided into five groups. Diverse selenium supplementations were carried out from six weeks before the date of delivery until weaning. Group C was a control group where no selenium was added to the goats' diet. Goats from the remaining four groups were given various forms of selenium in granulated feed mixture with a concentration 0.9 mg/kg (according the producer's declaration). The composition of granulated feed mixture supplied by the producer is shown in Table 1. The Se-I group received

Table 1. The composition of granulated feed mixture according producer's declaration (nutrients are indicated per 1000 g of mixture)

Dry matter (g)	883.13
Net energy of lactation (MJ)	6.58
Crude protein (g)	116.11
Metabolizable energy (MJ)	10.77
Fiber (g)	65.38
Fat (g)	35.29
Ca (g)	7.9
P (g)	7.54
Mg (g)	1.77
Na (g)	3.11
K (g)	5.83
Fe (mg)	59.66
Co (mg)	0.59
J (mg)	1.50
Mn (mg)	93.64
Cu (mg)	24.36
Zn (mg)	99.41
Cl (mg)	4.08
Se (mg)	0.07 ^a

^ain the group C

Se in the form of sodium selenite, the other three groups received organic forms of selenium. Goats of Se-L group were given lactate-protein complex (0.17% Se, Selenium chelate, Agrobac Karel Gebauer, Czech Republic). The Se-P group received selenium proteinate (B-Traxim Se, Pancosma, Switzerland) and The Se-Y group received yeast enriched with selenium 0.5% Se (Sel-Plex, Alltech, USA). The exact chemical composition of the individual preparations of organic selenium is not known. Sel-Plex is constituted of organic selenium from yeast, whose chemical composition is characterised by the Official Journal of the European Union L 330/10 as selenomethionine (63%) and low-molecular compounds of selenium (34–36%). B-traxim Se is a selenised compound produced by the reaction of inorganic selenium on enzymatically hydrolysed protein and is registered according to the feed additive regulations in Canada as a selenium proteinate (No. 990637, Canadian Food Inspection Agency). Selene chelate is produced by the cultivation of *Lactobacillus acidophilus* on substrate containing sodium selenite and is described only as lactate-protein complex. The exact composition of the last two preparations is not known.

Until the date of delivery, goats received 300 g of granulated feed mixture per animal and day. The feed also included *ad libitum* hay, water and salt lick. The selenium concentration in hay was 0.065 ± 0.024 mg/kg. After the delivery, granulated feed ration was increased to 1 kg per animal and day. When sucking mother's milk, kids were given also hay *ad libitum*. As soon as they were weaned at the age of three months, the following numbers of male kids were sampled in each group: C: $n = 10$, Se-L: $n = 10$, Se-P: $n = 10$, Se-I: $n = 8$, Se-Y: $n = 7$. Blood was taken from *v. jugularis* and urine was sampled by cystocentesis after the kids were slaughtered.

We tested the following parameters in whole blood: selenium, glutathione peroxidase (GPx) and in serum: zinc (Zn), copper (Cu), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), triiodothyronine (T_3), thyroxine (T_4), total protein, immunoglobulins and total antioxidant status (TAS). All the biochemical variables were determined in the laboratory of clinical biochemistry in the Ruminant Clinic, University of Veterinary and Pharmaceutical Sciences, Brno. T_3 and T_4 were determined by chemiluminescence using IMMULITE device (DPC CZECH, Czech Republic). Total protein and enzyme activities were determined by the automatic analyser LIASYS

(AMS, Italy) using the tests listed in parentheses: total protein (L Protein total, Cat. No. 12751), AST (L AST, Cat. No. 10351), LDH (L LDH, Cat. No. 12352) – the tests were provided by BioVendor (Czech Republic); CK (CK NAC L 100, Cat No. 10004494) – Pliva Lachema (Czech Republic); TAS (Total antioxidant status, Cat. No. NX 2332) – Randox (United Kingdom). Immunoglobulins were determined using the zinc sulfate turbidity test (Slanina et al. 1976), the quantity of immunoglobulins is quoted in units of zinc sulphate turbidity (IU ZST). Vitamins were determined by fluorometry according to Bouda et al. (1980). GPx was assessed in whole heparinised blood according to the method described by Paglia and Valentine (1967) with the use of the Ransel-Randox set and the automatic biochemical analyser Cobas Mira (Roche, Switzerland). Copper and zinc concentrations in the blood serum were determined after deproteinisation using trichloroacetic acid with the F-AAS method and the AAS Solaar M6 (Unicam, Great Britain) device. Selenium was measured in the whole blood and in the urine using the HG-AAS method and the AAS Solaar M6 (Unicam, Great Britain) device, after microwave mineralisation of samples in the Milestone Ethos TC (Milestone Italy) unit using a method described in Pechova et al. (2005).

The results were statistically assessed using the *F*-test for the assessment of the variance of individual sets and using the dependent Student's *t*-test for sets with equality/non-equality of variance according to the results. The relation between the concentration of selenium in blood and GPx was tested by calculating the correlation coefficient (*r*). The results are quoted as a mean value with standard deviation. The relative increase of Se and GPx in the blood was calculated using the following formula: (the value in experimental group/the value in control) $\times 100$. Analyses were carried out using Microsoft Excel software.

RESULTS

Selenium concentrations in the whole blood of kids at the time of weaning were significantly influenced by the supplementation of their mothers (Figure 1). The highest concentration was seen in the group Se-Y: 243.0 ± 20.3 $\mu\text{g/l}$, while a slightly lower concentration was observed in the other experimental groups (Se-I: 156.3 ± 34.3 $\mu\text{g/l}$;

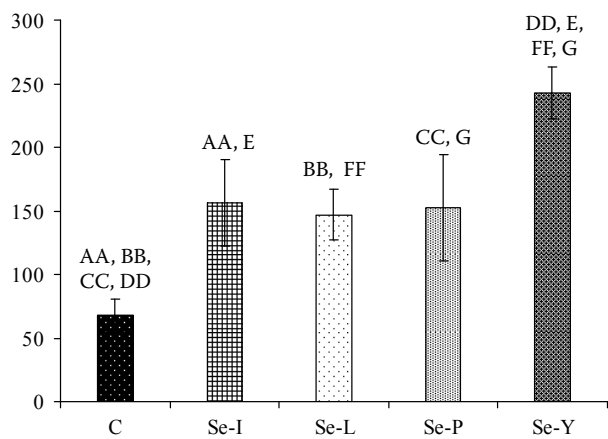


Figure 1. Selenium concentration in whole blood (µg/l) of weaning kids from mothers supplemented with different forms of selenium

C = control, Se-I = sodium selenite, Se-L = lactate-protein complex, Se-P = proteinate, Se-Y = yeast
 The same letters shows statistical significance of the difference between groups (AA = $P \leq 0.0001$, A = $P \leq 0.001$)

Se-P: 152.6 ± 41.5 µg/l; Se-L: 146.7 ± 20.0 µg/l), and the lowest concentration was found in the control group (C: 67.6 ± 13.1 µg/l). Significant differences were found between the control and all experimental groups ($P < 0.0001$) and between the group Se-Y and groups Se-L, Se-P, Se-I ($P < 0.001$). The average increase in selenium concentration in the blood was the following as compared with the control group (C = 100%): Se-Y, 359%; Se-I, 231%; Se-P, 226%; Se-L, 217%. Similar results were found in the activity of glutathione peroxidase in blood of kids (Figure 2) where the average increase was the following as compared with the control group (C = 100%): Se-Y, 327%; Se-I, 222%; Se-P, 253%; Se-

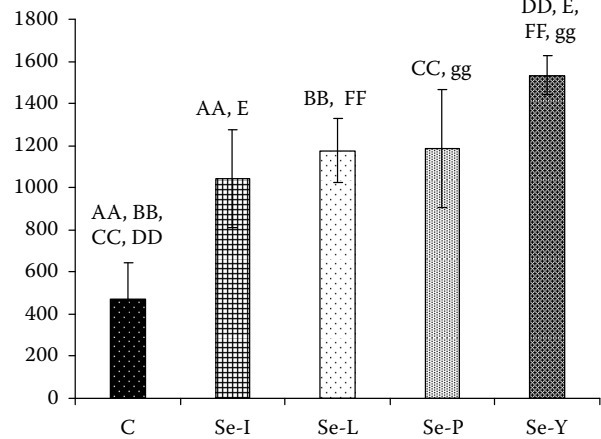


Figure 2. Glutathione peroxidase activity in whole blood (µkat/l) of weaning kids from mothers supplemented with different forms of selenium

C = control, Se-I = sodium selenite, Se-L = lactate-protein complex, Se-P = proteinate, Se-Y = yeast
 The same letters shows statistical significance of the difference between groups (AA = $P \leq 0.0001$, A = $P \leq 0.001$, aa = $P \leq 0.01$)

L, 251%. Significant differences were found between control and all experimental groups ($P < 0.0001$) and between the group Se-Y and the other experimental groups Se-L, Se-P, Se-I ($P < 0.01$). A highly significant correlation coefficient ($r = 0.943$; Figure 3) was calculated between the concentration of selenium in blood and activity of glutathione peroxidase.

The concentrations of Se in urine were relatively low, in individual animals the concentration fluctuated from 1 to 22 µg/l and showed high variability within groups as indicated by Figure 4. The variation coefficient in individual groups fluctuated from 43% to 80%. A higher excretion of Se via urine as compared with the control group was found in all

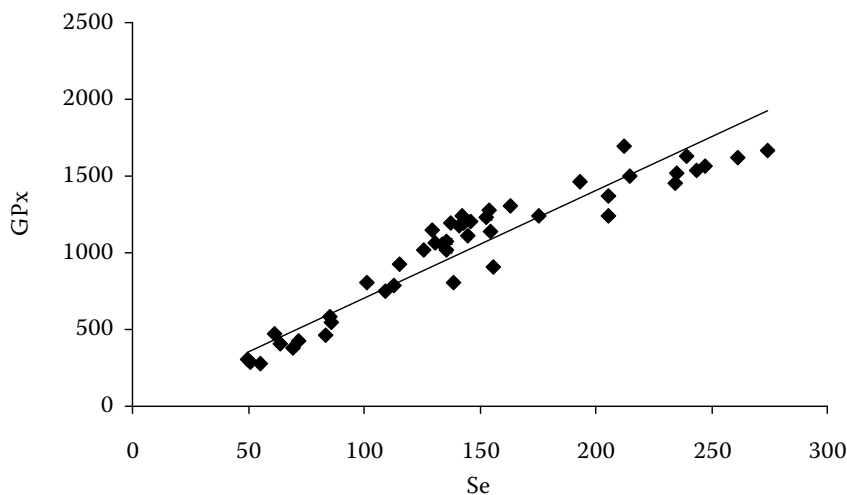


Figure 3. The correlation between the concentration of selenium (µg/l) and glutathione peroxidase activity (µkat/l) in whole blood of weaning kids ($n = 45$) from mothers supplemented with different forms of selenium

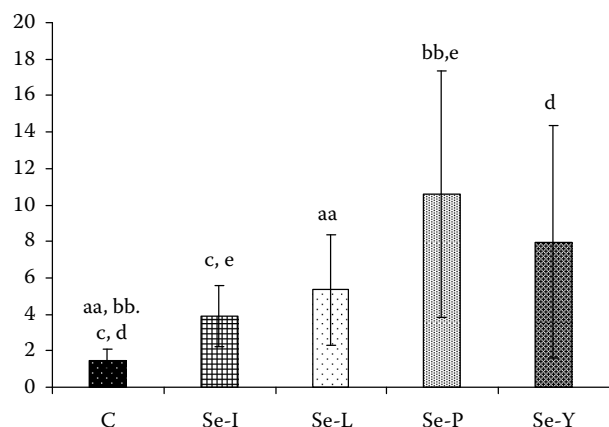


Figure 4. Selenium concentration in urine ($\mu\text{g/l}$) of weaning kids from mothers supplemented with different forms of selenium

C = control, Se-I = sodium selenite, Se-L = lactate-protein complex, Se-P = proteinate, Se-Y = yeast

The same letters show statistical significance of the difference between groups ($aa = P \leq 0.01$, $a = P \leq 0.05$)

experimental groups. The highest variability (80%) was in group Se-Y.

Among other biochemical parameters, the effect of selenium supplementation of mothers on the metabolism of muscles and proteins was monitored (Table 2). The concentration of total protein significantly differed only between individual organic forms (lower concentration was observed in group Se-L as compared with groups Se-P and Se-Y). More significant differences were found in immunoglobulins concentration where groups Se-P and Se-Y showed significantly higher levels as compared with the group Se-L and the control group. Muscle tissue state, determined on the basis of activity of selected enzymes, did not differ between the groups. Certain differences were seen in total

antioxidant status in serum that was higher in the group Se-Y as compared with the group Se-I and the control group. Antioxidant status was also significantly higher in the control group as compared with the group Se-I.

As for possible interactions with other trace elements, concentrations of Zn, Cu and thyroid hormones were monitored (Table 3). Significantly higher concentrations of Zn were observed in group Se-P as compared with the control group. Copper concentrations showed a positive trend in supplemented groups, while significantly higher Cu concentrations were determined in group Se-I as compared with the control. Certain differences between the groups were found in concentrations of thyroid hormones. The control group showed the highest concentration of thyroxine (significant differences were found against groups Se-I and Se-P). Triiodothyronine concentration was highest in group Se-L and lowest in group Se-I (significant difference was found in Se-I as compared with the control). There were no differences in the T_4/T_3 ratios between the groups.

DISCUSSION

The results indicate that the level of Se available to kids at the time of weaning is significantly influenced by the form of selenium administered to their mothers. Concentrations of selenium in individual experimental groups of kids show sufficient Se supply, whereas the animals from the control group showed Se deficiency. These results are consistent with Se concentrations in individual tissues (Sevcikova et al. 2011) where the unsupplemented group showed also selenium deficiency.

Table 2. Selected biochemical parameters in blood serum of weaning kids from mothers supplemented with different forms of selenium

	C	Se-I	Se-L	Se-P	Se-Y
Total protein (g/l)	66.62 \pm 3.31	66.28 \pm 4.22	64.70 \pm 0.99 ^{a,b}	67.81 \pm 3.94 ^a	69.69 \pm 3.53 ^b
Immunoglobulins (IU ZST)	25.30 \pm 1.59 ^{c,d}	25.26 \pm 3.49	24.43 \pm 1.98 ^{a,b}	27.13 \pm 2.47 ^{a,c}	27.54 \pm 2.51 ^{b,d}
AST ($\mu\text{kat/l}$)	1.67 \pm 0.25	1.85 \pm 0.36 ^a	1.49 \pm 0.20 ^a	1.55 \pm 0.26	1.71 \pm 0.22
CK ($\mu\text{kat/l}$)	2.16 \pm 0.62	2.10 \pm 0.46	1.87 \pm 0.51	1.98 \pm 0.54	1.97 \pm 0.35
LDH ($\mu\text{kat/l}$)	14.45 \pm 1.69	13.70 \pm 2.53	13.92 \pm 1.33	13.50 \pm 1.18	14.05 \pm 1.51
Total antioxidant status (mmol/l)	0.74 \pm 0.05 ^b	0.69 \pm 0.03 ^{b,cc}	0.71 \pm 0.03 ^a	0.73 \pm 0.22	0.77 \pm 0.05 ^{a,cc}

C = control, Se-I = sodium selenite, Se-L = lactate-protein complex, Se-P = proteinate, Se-Y = yeast

The same letters in one row show statistical significance of the difference between groups ($^{aa}P \leq 0.01$, $^aP \leq 0.05$)

Table 3. The concentration of selected biochemical parameters in blood serum of weaning kids from mothers supplemented with different forms of selenium

	C	Se-I	Se-L	Se-P	Se-Y
Zn ($\mu\text{mol/l}$)	10.30 \pm 0.89 ^c	10.23 \pm 1.77	9.97 \pm 0.75 ^{aa}	11.22 \pm 0.87 ^{aa,bb,c}	9.44 \pm 1.07 ^{bb}
Cu ($\mu\text{mol/l}$)	13.60 \pm 1.26 ^a	15.23 \pm 1.43 ^a	14.13 \pm 1.06	14.10 \pm 1.97	14.85 \pm 2.23
Vitamin A ($\mu\text{mol/l}$)	1.08 \pm 0.12	1.02 \pm 0.21	1.04 \pm 0.13	0.99 \pm 0.15	0.99 \pm 0.10
Vitamin E ($\mu\text{mol/l}$)	1.30 \pm 0.16	1.24 \pm 0.22	1.28 \pm 0.26	1.22 \pm 0.23	1.12 \pm 0.10
T ₃ (nmol/l)	3.03 \pm 0.58 ^c	2.30 \pm 0.52 ^{bb,c}	3.33 \pm 0.58 ^{a,bb}	2.64 \pm 0.75 ^a	2.87 \pm 0.63
T ₄ (nmol/l)	92.70 \pm 13.43 ^{b,cc}	66.51 \pm 13.17 ^{a,cc}	84.45 \pm 14.81 ^a	76.19 \pm 25.69 ^b	79.76 \pm 12.77
T ₄ /T ₃	31.29 \pm 6.14	29.93 \pm 6.96	25.66 \pm 3.58	29.19 \pm 6.89	28.57 \pm 4.64

C = control, Se-I = sodium selenite, Se-L = lactate-protein complex, Se-P = proteinate, Se-Y = yeast
The same letters in one row show statistical significance of the difference between groups (^{aa} $P \leq 0.01$, ^a $P \leq 0.05$)

Deficiency is defined as an animal with a blood Se concentration of below 80 $\mu\text{g/l}$ (Bickhardt et al. 1999). However, other authors report full blood Se concentrations ranging from 150 to 250 $\mu\text{g/l}$ as the reference values (Van Metre and Callan 2001). Views on toxic concentrations of Se in blood also differ: Underwood and Suttle (1999) stated that the critical toxic concentration of Se is 200 to 300 $\mu\text{g/l}$ in sheep, though no signs of toxicity were observed experimentally even at Se concentrations of 6000 $\mu\text{g/l}$ (Davis et al. 2008). Although Se concentrations in group Se-Y exceeded 200 $\mu\text{g/l}$, the animals showed no signs of toxicity. Similarly to our results, other authors did not observe toxicity in goats with Se concentrations of above 200 $\mu\text{g/l}$ (Hayashida et al. 2006; Pechova et al. 2008). The activity of glutathione peroxidase in blood strongly correlated with Se concentration in whole blood. The correlation coefficient (0.943) determined in the present study was higher than reported in goats (0.72) (Pavlata et al. 2011a). The graph indicates that GPx activity increases more slowly at blood Se concentrations of above 200 $\mu\text{g/l}$ with the highest values being around 1500 $\mu\text{kat/l}$ which can be considered as a plateau.

The highest concentrations of Se in whole blood were found in the group of kids whose mothers received Se-yeast. Similarly to our results, a higher Se concentration in the blood after Se-yeast supplementation as compared with an inorganic Se supplementation was observed also by other authors (Malbe et al. 1995; Ortman and Pherson, 1999; Ortman et al. 1999; Pavlata et al. 2001). In the other two groups supplemented with organically bound selenium (Se-P, Se-L), a similar blood concentration of Se was found as in the inorganic

Se group, though it was significantly higher than in the control group. Similar results were reported by Pavlata et al. (2011b). These results are inconsistent with Se concentrations in individual tissues (Sevcikova et al. 2011), where groups supplemented with lactate-protein complex and Se-proteinate showed higher concentrations than groups supplemented with inorganic selenium. This difference can be explained by the fact that selenium tissue concentrations of weaned kids are largely influenced by the intrauterine development whereas the concentration of selenium in the blood reflects the current Se supply (i.e., milk intake is critical in kids at the time of weaning). It has been found that Se concentration in the milk is significantly affected by the form of Se supplementation and that products containing organically bound Se in the form of selenomethionine markedly increase Se concentration in the milk (Ortman and Pherson, 1999; Muniz-Naveiro et al. 2006). Our previous study (Pechova et al. 2008) compared the effect of three forms of organic Se supplementation on Se levels in goat's milk. Increased Se concentration in the milk was found only when Se-yeast was supplemented (25.9 $\mu\text{g/l}$). Supplementation with a lactate-protein complex and Se-proteinate resulted in milk Se concentrations that were comparable with the control group (13.14 $\mu\text{g/l}$, 11.70 $\mu\text{g/l}$, 12.53 $\mu\text{g/l}$ respectively). However, selenium deficiency was not observed in experimental animals used during that study, which was also probably associated with the fact that control animals did not show lower concentrations of Se. Differing excretion of Se in milk was perhaps the cause of the higher Se concentrations in the blood of kids from the group Se-Y. The higher content of Se in the milk is due to

the fact that Se-methionine is directly incorporated into milk protein. Se-met is the only naturally occurring selenium compound that is significantly incorporated into body proteins (Schrauzer and Surai 2009). Supposedly, the presence of organic Se compounds other than selenomethionine led to the differences between the tested preparations. Exact information on the Se-met content in proteinate and the lactate-protein complex is not available. The published studies indicate that selenocysteine and Se-methylselenocysteine are produced during lactic fermentation by bacteria (*Lactobacillus*); and during yogurt fermentation yeast (*Saccharomyces*) produce mainly selenomethionine (Calomme et al. 1995; Alzate et al. 2008).

Although Se concentration in the blood was significantly different in individual groups (deficient, optimum and increased Se concentrations in kids' blood were observed), muscle metabolism was not substantially affected. Alteration in muscles was not observed even in the group with Se deficiency. Significantly higher concentrations of immunoglobulins as compared with the control were revealed in kids from groups Se-P and Se-Y. These differences could be associated with the positive effect of selenium on immunity. The effect of selenium on immune functions has been documented, though the results of experimental studies are equivocal. A higher production of antibodies after Se supplementation was observed by Rooke et al. (2004), but Hall et al. (2011) did not confirm the effect of various forms of Se supplementation on humoral immunity. A positive effect of Se supplementation on the level of antibodies in the colostrum was found by Pavlata et al. (2004).

The excretion of Se in the urine was significantly higher in all groups supplemented with Se as compared with the control group, which confirms the effect of Se intake on its excretion from the organism. Urine excretion has been reported to be the body's mechanism for maintaining Se homeostasis (Navarro-Alarcon and Cabrera-Vique 2008). Zachara et al. (2006) reported that losses in the urine represent 50–78% of the ingested element and they confirmed that the level of Se excretion in the urine was proportional to the level of Se intake. After absorption, various sources of Se are metabolised into selenide that represents the crossroads of selenium metabolism, from which it may be committed to specific selenoprotein synthesis or be removed from body by urinary excretion pathways that involve its detoxification by methylation to

methyl selenides. The effect of the form of the supplemented selenium on the amount of Se excreted in the urine cannot be unequivocally evaluated due to the high variability of our results. The amount of selenium excreted through kidneys represents the difference between absorbed and utilised selenium. It is therefore expected that selenomethionine, considering that it is incorporated into body proteins instead of methionine, is subject to higher retention. A study by Walker et al. (2010) indicates that when Se-yeast is administered to cows, 66% of received selenium is excreted in urine and 17% is lost through milk secretion, meaning that Se apparently retained in tissues accounted for 17% of the total Se intake of cows.

The present study monitored also other trace elements that are reported to interact with selenium (Schrauzer 2000; Moeini et al. 2011). Kantola and Vartiainen (2001) reported that after agricultural fertilizers started to be supplemented with selenium in Finland in 1984, concentrations of trace elements in human breast milk changed. After five to eight years, selenium concentrations increased, whereas copper and zinc levels decreased. As the composition of the mother's milk significantly influences the metabolism of offspring during the suckling stage, possible changes in the content of trace elements could be reflected by the health status of the offspring. In the present study, Se supplementation had no negative effect on the concentrations of copper and zinc in the blood serum of kids. As for copper, a positive trend was seen, which is consistent with the published positive correlation between Se and Cu in milk noted by Kantola and Vartiainen (2001) between 1993 and 1995. Pavlata et al. (2005) observed a negative effect of increased iodine supplementation on the concentration of Se and GPx in the blood of kids. Therefore, we monitored also thyroid hormones in order to determine whether iodine absorption is decreased and, subsequently, thyroid metabolism is impaired or not. We observed a trend of decreasing T_4 concentrations that reflects the amount of iodine in the organism. Similarly, Wichtel et al. (1996) observed decreased concentrations of thyroxine after Se supplementation in Angora goat kids. These results suggest that there probably exists a negative interaction, at the level of absorption, between iodine and selenium administered mainly in the inorganic form. Selenium is a significant part of iodothyronine deiodinase which is responsible for the conversion of T_4 to T_3 . Although kids

from the control group showed Se deficiency, the T_4/T_3 ratio was not significantly influenced. Awadeh et al. (1998) confirmed the influence of Se supplementation on the concentrations of T_3 and the ratio of T_3/T_4 in the plasma of cows, but there were no differences between supplementation with organic and inorganic forms.

The supplementation of mothers with Se both in organic and inorganic forms significantly influenced the Se supply of their kids at the time of weaning. The highest concentration of Se and GPx in blood was found in group Se-Y supplemented with selenium yeast, where selenium is in the form of selenomethionine. The other two organic forms of selenium (proteinate and lactate-protein complex) increased the concentration of Se in blood and the activity of GPx to the same extent as the inorganic form of selenium. All forms were sufficient to prevent Se deficiency in kids at the time of weaning.

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REFERENCES

- Alzate A, Fernandez-Fernandez A, Perez-Conde MC, Camara C (2008): Comparison of biotransformation of inorganic selenium by *Lactobacillus* and *Saccharomyces* in lactic fermentation process of yogurt and kefir. *Journal of Agricultural and Food Chemistry* 56, 8728–8736.
- Awadeh FT, Kincaid RL, Johnson KA (1998): Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *Journal of Animal Science* 76, 1201–1215.
- Bickhardt K, Ganter M, Sallmann P, Fuhrmann H (1999): Investigation on manifestation of vitamin E and selenium deficiency in sheep and goat. *Deutsche Tierärztliche Wochenschrift* 106, 242–247.
- Bouda J, Jagos P, Dvorak V (1980): Fluorometric determination of vitamins A and E in blood plasma, colostrum and the liver of cattle (in Czech). *Ceskoslovenska Fyziologie* 29, 351.
- Calomme MR, Vandenbranden K, Berghe DAV (1995): Selenium and *Lactobacillus* species. *Journal of Applied Bacteriology* 79, 331–340.
- Davis PA, McDowell LR, Wilkinson NS, Buergelt CD, Van Alstyne R, Weldon RN, Marshall TT, Matsuda-Fugisaki EY (2008): Comparative effects of various dietary levels of Se as sodium selenite or Se yeast on blood, wool, and tissue Se concentrations of wether sheep. *Small Ruminant Research* 74, 149–158.
- Enjalbert F, Lebreton P, Salat O, Schelcher F (1999): Effects of pre- or postpartum selenium supplementation on selenium status in beef cows and their calves. *Journal of Animal Science* 77, 223–229.
- Enjalbert F, Lebreton P, Salat O (2006): Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. *Journal of Animal Physiology and Animal Nutrition* 90, 11–12.
- Ghany-Hefnawy AL, Lopez-Arellano R, Revilla-Vaquez A, Ramirez-Bribiesca E, Tortora-Perez J (2007): The relationship between fetal and maternal selenium concentrations in sheep and goats. *Small Ruminant Research* 73, 174–180.
- Givens DI, Allison A, Cottrill B, Blake JS (2004): Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *Journal of the Science of Food and Agriculture* 84, 811–817.
- Hall JA, Harwell AM, Van Saun RJ, Vorachek WR, Stewart WC, Galbraith ML, Hooper KJ, Hunter JK, Mosher WD, Pirelli GJ (2011): Agronomic biofortification with selenium: Effects on whole blood selenium and humoral immunity in beef cattle. *Animal Feed Science and Technology* 164, 184–190.
- Hayashida M, Orden EA, Cruz EM, Cruz LC, Fujihara T (2006): Effect of soluble glass bolus administration on the blood selenium concentration in grazing Philippine goats under backyard condition. *Animal Science Journal* 77, 595–602.
- Heard JW, Walker GP, Royle PJ, McIntosh GH, Doyle PT (2004): Effects of short-term supplementation with selenium yeast on milk production and composition of lactating cows. *Australian Journal of Dairy Technology* 59, 199–203.
- Juniper DT, Phipps RH, Jones AK, Bertin G (2006): Selenium supplementation of lactating dairy cows: Effect on selenium concentration in blood, milk, urine, and faeces. *Journal of Dairy Science* 89, 3544–3551.
- Kantola M, Vartiainen T (2001): Changes in selenium, zinc, copper and cadmium contents in human milk

- during the time when selenium has been supplemented to fertilizers in Finland. *Journal of Trace Elements in Medicine and Biology* 15, 11–17.
- Kohrle J, Jakob F, Contempre B, Dumont JE (2005): Selenium, the thyroid, and the endocrine system. *Endocrine Reviews* 26, 944–984.
- Malbe M, Klaassen M, Fang W, Myllys V, Vikerpuur M, Nyholm K, Sankari S, Suoranta K, Sandholm M (1995): Comparison of selenite and selenium yeast feed supplements on Se-incorporation, mastitis and leucocyte function in Se-deficient dairy cows. *Journal of Veterinary Medicine Series A* 42, 111–121.
- Misurova L, Pavlata L, Pechova A, Dvorak R (2009): Selenium metabolism in goats – maternal transfer of selenium to newborn kids. *Veterinarni Medicina* 54, 125–130.
- Moeini MM, Kiani A, Karami H, Mikaeili E (2011): The effect of selenium administration on the selenium, copper, iron and zinc status of pregnant heifers and their newborn calves. *Journal of Agricultural Science and Technology* 13, 53–59.
- Muniz-Naveiro O, Dominguez-Gonzalez R, Bermejo-Barrera A, Bermejo-Barrera P, Cocho JA, Fraga JM (2006): Study of the bioavailability of selenium in cow's milk after a supplementation of cow feed with different forms of selenium. *Analytical and Bioanalytical Chemistry* 385, 189–196.
- Navarro-Alarcon M, Cabrera-Vique C (2008): Selenium in food and the human body: A review. *Science of the Total Environment* 400, 115–141.
- Ortman K, Pherson B (1999): Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. *Journal of Animal Science* 77, 3365–3370.
- Ortman K, Andersson R, Holst H (1999): The influence of supplements of selenite, selenate and selenium status of dairy heifers. *Acta Veterinaria Scandinavica* 40, 23–34.
- Paglia DE, Valentine WN (1967): Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* 70, 158–169.
- Pappas AC, Zoidis E, Surai PF, Zervas G (2008): Selenoproteins and maternal nutrition. *Comparative Biochemistry and Physiology B* 151, 361–372.
- Pavlata L, Illek J, Pechova A (2001): Blood and tissue selenium concentrations in calves treated with inorganic or organic selenium compounds – a comparison. *Acta Veterinaria Brno* 70, 19–26.
- Pavlata L, Prasek P, Filipek J, Pechova A (2004): Influence of parenteral administration of selenium and vitamin E during pregnancy on selected metabolic parameters and colostrum quality in dairy cows at parturition. *Veterinarni Medicina* 49, 149–155.
- Pavlata L, Slosarkova S, Fleischer P, Pechova A (2005): Effects of increased iodine supply on the selenium status of kids. *Veterinarni Medicina* 50, 186–194.
- Pavlata L, Chomat M, Pechova A, Misurova L, Dvorak R (2011a): Impact of long-term supplementation of zinc and selenium on their content in blood and hair in goats. *Veterinarni Medicina* 56, 63–74.
- Pavlata L, Misurova L, Pechova A, Dvorak R (2011b): The effect of inorganic and organically bound forms of selenium on glutathione peroxidase activity in the blood of goats. *Veterinarni Medicina* 56, 75–81.
- Pechova A, Pavlata L, Illek J (2005): Blood and tissue selenium determination by hydride generation atomic absorption spectrophotometry. *Acta Veterinaria Brno* 74, 483–490.
- Pechova A, Misurova L, Pavlata L, Dvorak R (2008): Monitoring of changes in selenium concentration in goat milk during short-term supplementation of various forms of selenium. *Biological Trace Element Research* 121, 180–191.
- Rooke JA, Robinson JJ, Arthur JR (2004): Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *Journal of Agricultural Science* 142, 253–262.
- Schrauzer GN (2000): Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *Journal of Nutrition* 130, 1653–1656.
- Schrauzer GN, Surai PF (2009): Selenium in human and animal nutrition: Resolved and unresolved issues. A partly historical treatise in commemoration on the fiftieth anniversary of the discovery of the biological essentiality of selenium, dedicated to the memory of Klaus Schwarz (1914–1978) on the occasion of the thirtieth anniversary of his death. *Critical Reviews in Biotechnology* 29, 2–9.
- Sevcikova L, Pechova A, Pavlata L, Antos D, Mala E, Palenik T, Panev A, Dvorak R (2011): The effect of various forms of selenium supplied to pregnant goats on the levels of selenium in the body of their kids at the time of weaning. *Biological Trace Element Research* 143, 882–892.
- Slanina L., Vajda V., Blazej J. (1976): Turbidimetric analysis of immunoglobulins in calves and their clinical evaluation (in Slovak). *Veterinarstvi* 9, 392–394.
- Underwood EJ, Suttle NF (1999): *The Mineral Nutrition of Livestock*. 3rd ed. CABI Publishing, Oxon, UK.
- Van Metre DC, Callan RJ (2001): Selenium and vitamin E. *Veterinary Clinics of North America: Food Animal Practice* 17, 373–402.
- Walker GP, Dunshea FR, Heard JW, Stockdale CR, Doyle PT (2010): Output of selenium in milk, urine, and feces is proportional to selenium intake in dairy cows fed a

- total mixed ration supplemented with selenium yeast. *Journal of Dairy Science* 93, 4644–4650.
- Wichtel JJ, Thompson KG, Craigie AL, Williamson NB (1996): Effects of selenium and kosine supplementation on the growth rate, mohair production, and thyroid status Angora goat kids. *New Zealand Journal of Agricultural Research* 38, 111–115.
- Zachara BA, Gromadzinska J, Wasowicz W, Zbrog Z (2006): Red blood cell and plasma peroxidase activities and selenium concentration in patients with chronic kidney disease: A review. *Acta Biochimica Polonica* 53, 663–677.

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