Comparison of organic and inorganic forms of selenium in the mother and kid relationship in goats

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ABSTRACT: The goal of the experiment was to compare the effect of four different forms of selenium (Se) – sodium selenite (SS), lactate-protein selenium complex (SL), selenium enriched yeast (SY), and selenium-proteinate (SP) supplemented to pregnant goats on Se concentration and glutathione peroxidase (GSH-Px) activity in the blood of goats on the day of delivery and also on Se concentration and GSH-Px activity in the blood of newborn kids. The experiment involved 33 pregnant goats of White Short-haired breed. The supplementation started 6 weeks before the parturition. The goats were divided into 5 groups: control group C, not supplemented, and 4 trial groups (SL, SP, SS, SY), which received Se in the above stated forms by the means of supplemented pellets (300 g per animal per day) at a rate 900 μg Se/kg of dry matter. The average Se concentrations in the blood of the goats were 79.6 μg/l in group C, 152.6 μg/l in group SL, 167.1 μg/l in group SP, 144.9 μg/l in group SS, and 152.9 μg/l in group SY. Selenium concentrations in all 4 trial groups were significantly higher ($P < 0.01$) than in control group, however no significant difference was found between individual trial groups. Likewise, the activity of GSH-Px in goat blood increased significantly in all supplemented groups compared to the controls; however we did not discover any significant differences in activity of GSH-Px between the individual selenium-supplemented groups. The Se concentrations in the blood of kids were significantly ($P < 0.01$) higher in the selenium-supplemented groups (SL – 94.9 μg/l, SP – 87.5 μg/l, SS – 87.6 μg/l, SY – 92.5 μg/l) than in the control group (C – 49.4 μg/l), but we did not discover any differences between the individual experimental groups. The activity of GSH-Px in the blood of the kids tended towards higher values in the supplemented groups than in the control group, but the values were significantly higher ($P < 0.05$) only in groups SY and SL. We have found significant correlation between GSH-Px activity and Se concentration in the blood of goats ($r = 0.86$) and newborn kids ($r = 0.95$). Likewise, there was significant correlation between Se concentration in the blood of goats and their kids ($r = 0.74$). We discovered that the kids are reaching physiologically only about 60% of Se status in whole blood in comparison with their mothers. Our results are suggesting that all the above forms of Se were similarly utilised and transferred into the foetus in the goats.

Keywords: selenium-proteinate; selenium lactate-protein complex; sodium selenite; selenium-yeast; glutathione peroxidase; organic selenium; inorganic selenium; selenomethionine

During pregnancy and lactation the mother's demands for selenium increase because selenium is necessary for the foetus and the newborn kids (Smith and Picciano, 1986; Anan et al., 2009). A decrease in the concentration of selenium in the blood was confirmed during parturition and beginning of lactation (Prosbová et al., 1982; Vrzgula et al., 1982). Selenium concentrations also decreased in the liver of pregnant animals as compared to the non-pregnant ones; it was discovered that selenium

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concentrations in the liver decreased further in lactating animals (Anan et al., 2009).

Supplementing selenium during pregnancy can affect not only the mother's organism but can also supply the organism of the newborn kid. In selenium-supplemented mothers the selenium concentration increased in the blood, colostrum, and milk; it was also proved that the selenium level increased in the allantoic sac (Hefnawy et al., 2008). Selenium penetrates the placenta and the mammary barrier and the transfer via the placenta is more efficient than the transfer of selenium into the calf's organism via milk uptake (Enjalbert et al., 1999). Placental transfer is bi-directional and this may affect the net retention of selenium in maternal, foetal, and neonatal tissues. It is still elusive whether selenium rapidly passes the placenta or is actually concentrated in placental tissues. It seems that transport process may be a more complex action than a simple passive transfer mechanism (Pappas et al., 2008).

The newborn kid can be supplied by administration of selenium to mothers (Misurova et al., 2009a, b). Selenium is usually added to feed in inorganically or organically bound forms – sodium selenite and selenate vs. selenomethionine, selenium proteinate, lactate-protein selenium complex, selenium-enriched yeast, selenium-enriched alga *Chlorella*, selenium-enriched alga *Scenedesmus quadricauda*, dimethylselenonium propionate etc. (Trávníček et al., 2008, 2010; Heindl et al., 2010; Skřivan et al., 2010a, b; Wang et al., 2011). In some experiments it was documented that the organic form of selenium (especially selenomethionine) is more available than inorganic forms (Pavlata et al., 2001; Gunter et al., 2003; Kuricová et al., 2003; Weiss and Hogan, 2005; Guyot et al., 2007; Pechova et al., 2008; Steen et al., 2008; Sevcikova et al., 2011; Pavlata et al., 2011a). However, several papers comparing various forms of Se supplementation have recently been published and some results not confirming better biological effect of organic-bound forms of selenium have occurred (Leeson et al., 2008; Heindl et al., 2010; Pavlata et al., 2011b). Therefore further research in this field is necessary.

The objective of our study was to compare various forms of selenium (inorganic and organically bound) supplemented orally to pregnant goats and their effect on selenium concentration and activity of glutathione peroxidase (GSH-Px) in goat and newborn kid blood before the first intake of colostrum.

MATERIAL AND METHODS

The experiment was conducted with 33 pregnant goats of the White Short-haired breed at the Ruminant Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno. Goats included in the experiment were gravid, apparently in good health and without clinical signs of illness. Few months before the trial started the goats were fed with identical feed rations consisting of hay, oats, and granulated grain mixture (300 g per animal and day) where nutritional content corresponded with recommended standards for the given species and category of animals. Selenium content in grain mixture was in long term 300 μg/kg of dry matter (DM) (Se was supplemented in the form of sodium selenite).

For the experiment the goats were divided into 5 groups (depending on the currently measured concentration of selenium and glutathione peroxidase activity in whole blood of goats – Table 1): control group (C) with no selenium supplementa-

Table 1. Concentration of selenium (μg/l) and glutathione peroxidase activity (μkat/l) in whole blood of goats before start of experiment (insignificant differences between all groups)

Group	Se (mean ± SD)	GSH-Px (mean ± SD)
Control (C)	105.4 ± 16.0	703.0 ± 113.7
Lactate-protein Se complex (SL)	100.8 ± 19.6	622.6 ± 103.7
Selenium-proteinate (SP)	101.8 ± 24.2	630.8 ± 180.8
Sodium selenite (SS)	101.8 ± 23.8	605.9 ± 137.6
Se-enriched yeast (SY)	106.6 ± 19.3	648.0 ± 134.2

Se = selenium, GSH-Px = glutathione peroxidase, SD = standard deviation

tion (n = 7) and 4 groups fed selenium-enriched pellets at a rate of 900 µg Se/kg of DM. The first experimental group (SS) received selenium in the form of sodium selenite (n = 7), the second experimental group (SP) received it in the form of proteinate (B-Traxim Se, Pancosma, Geneva, Switzerland) (n = 6), the third experimental group (SL) was supplemented with a lactate-protein selenium complex (0.17% Se, Selene chelate, Karel Gebauer AGROBAC, Třemešné, Czech Republic) (n = 7), and the fourth group (SY) received yeast enriched with selenium (Sel-Plex, Alltech, USA) (n = 6).

Sel-Plex is organic selenium from yeast the chemical composition of which is characterized 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 manufactured from the reaction of inorganic selenium on enzymatically hydrolysed protein and is registered in the feed additive regulation in Canada as a selenium proteinate (#990637, Canadian Food Inspection Agency). Selene chelate is produced by cultivation of *Lactobacillus acidophilus* on substrate containing sodium selenite and is described only as lactate-protein selenium complex without any exact chemical composition.

Supplementation began 6 weeks before parturition. The animals in groups were stalled in shared boxes with straw litter and had access to troughs with drinking water.

The goats were fed twice a day with pellets (Table 2) at a rate of 300 g per animal and day and they had *ad libitum* access to hay, drinking water, and to salt lick (NaCl). The pellets were fed in appropriate amount (n-times 150 g per one feeding) to the individual groups into the common feeders of the size that enabled all animals to eat at the same time.

The selenium concentration in hay was 65 ± 24 µg/kg DM. The basic composition of feed rations was identical in all groups while the contents of Se differed between control and experimental groups.

Blood was sampled immediately after parturition from *vena jugularis*. If the kids were twins or triplets we used a mixed blood sample.

Selenium levels were assessed in the laboratory of the Ruminant Clinic of the University of Veterinary and Pharmaceutical Sciences Brno. The blood samples were mineralised in a closed system using a microwave digestion technique in the presence of HNO_3 and H_2O_2 in the Milestone Ethos TC equipment (Milestone, Bergamo, Italy). After evaporation and transfer of the mineralisate to an aqueous solution selenium was reduced by adding 20% HCl. This sample was used to assess selenium on the AAS Solaar M6 apparatus (Unicam, Leeds, UK) by a hydride technique of atomic absorption spectophotometry (Pechová et al., 2005). The resulting values of selenium concentrations are given in µg/l of whole blood.

Table 2. Nutrient composition of the pellets

	Control group	Experimental groups
Dry matter (g)	883.13	883.13
Net energy of lactation (MJ)	6.58	6.58
Crude protein (g)	116.11	116.11
Fiber (g)	65.38	65.38
Fat (g)	35.29	35.29
Sugar (g)	25.82	25.82
Starch (g)	423.72	423.72
Ca (g)	7.90	7.90
Fe (mg)	59.66	59.66
Co (mg)	0.59	0.59
P (g)	7.54	7.54
I (mg)	1.50	1.50
Mg (g)	1.77	1.77
Mn (mg)	93.64	93.64
Na (g)	3.11	3.11
Cu (mg)	24.36	24.36
K (g)	5.83	5.83
Zn (mg)	99.41	99.41
Cl (mg)	4.08	4.08
Se (mg)	0.07	0.97

The activity of glutathione peroxidase was assessed according to the method of Paglia and Valentine (1967) using the commercial test Ransel GPX Cat. No. RS 506 (Randox Laboratories, Ltd., Crumlin, UK) photometrically on the Cobas Mira apparatus (Roche, Basel, Switzerland). The activity of glutathione peroxidase is given in µkat/l of whole blood.

The data were statistically analysed by ANOVA using general linear model and using the F-test

Table 3. Selenium concentration (μg/l) in whole blood of goats (G) and kids (K) on the day of parturition

Group		Mean	SD	Minimum	Maximum
Control (C)	G	79.6	12.2	65.1	105.5
	K	49.4	12.6	36.8	68.4
Lactate-protein Se complex (SL)	G	152.6^{aa}	28.4	104.5	195.1
	K	94.9^{bb}	23.3	59.4	139.4
Selenium-proteinate (SP)	G	167.2^{aa}	34.5	115.5	209.1
	K	87.5^{bb}	25.4	67.6	140.2
Sodium selenite (SS)	G	144.9^{aa}	30.4	94.7	190.7
	K	87.6^{bb}	26.3	60.6	144.8
Se-enriched yeast (SY)	G	152.9^{aa}	18.6	124.0	186.2
	K	92.5^{bb}	26.3	67.3	144.3

$^{aa}P < 0.01$ C : SL, C : SP, C : SS, C : SY in goats
$^{bb}P < 0.01$ C : SL, C : SP, C : SS, C : SY in kids
SD = standard deviation

to evaluate the variances of the individual sets and according to results of the double Student's *t*-test for sets with equal/non equal variances. The correlations were tested using correlation and regression analysis determining the correlation coefficient (r), and regression line equation with documented value of reliability (r^2). Microsoft Excel 2010 programme was used for the evaluations.

RESULTS

Selenium concentrations of all four experimental groups of goats on the day of parturition (Table 3) were significantly ($P < 0.01$) higher than in the control group, but no significant differences were detected between the individual experimental groups. Likewise, the activity of glutathione peroxidase in goat blood (Table 4) was higher in all supplemented

Table 4. Glutathione peroxidase activity (μkat/l) in whole blood of goats (G) and kids (K) on the day of parturition

Group		Mean	SD	Minimum	Maximum
Control (C)	G	442.5	129.6	197.2	602.1
	K	338.3	141.5	157.8	537.3
Lactate-protein Se complex (SL)	G	888.8^{aa}	173.9	656.9	1149.1
	K	695.9^{b}	262.4	370.4	1198.5
Selenium-proteinate (SP)	G	904.2^{aa}	291.1	464.0	1363.9
	K	524.3	286.8	329.9	1091.4
Sodium selenite (SS)	G	802.3^{aa}	170.5	555.2	1074.1
	K	592.1	278.2	313.8	1134.9
Se-enriched yeast (SY)	G	782.2^{aa}	156.8	593.9	1089.6
	K	643.9^{b}	234.5	396.2	1070.4

$^{aa}P < 0.01$ C : SL, C : SP, C : SS, C : SY in goats
$^{b}P < 0.05$ C : SL, C : SY in kids
SD = standard deviation

Table 5. Coefficients of correlation (r, r^2) and regression line equations between selenium content (Se) and glutathione peroxidase activity (GSH-Px) in whole blood of goats and their kids (n = 33)

Correlation	r	r^2	P	Regression line equation
Se – GSH-Px in goats	0.86	0.75	*	$y = 5.49x - 8.48$
Se – GSH-Px in kids	0.95	0.91	*	$y = 9.52x - 230.95$
Se goats – Se kids	0.74	0.55	*	$y = 0.53x + 9.35$
GSH-Px goats – GSH-Px kids	0.77	0.59	*	$y = 0.88x - 113.07$

*$P < 0.01$

groups compared to the control; however we did not discover any significant differences in activity of glutathione peroxidase between the individual selenium-supplemented groups.

When comparing concentration of selenium and activity of GSH-Px in whole blood of goats before the trial started (Table 1) and on the day of delivery (Tables 3 and 4) it is obvious that while the values of both examined parameters increased in experimental groups ($P \leq 0.05$) – average Se concentration in whole blood increased from 100.8–106.6 to 144.9–167.2 µg/l and activity of GSH-Px increased from 605.9–648.0 to 782.2–904.2 µkat/l, there was significant decline of both parameters in control group (Se from 105.4 ± 16.0 to 79.6 ± 12.2 µg/l and GSH-Px from 703.0 ± 113.7 to 442.5 ± 129.6 µkat/l).

Tables 3 and 4 give the selenium concentrations and the activity of glutathione peroxidase in the blood of kids of the individual groups prior to the first colostrum feeding.

Selenium concentrations in the blood of kids were significantly ($P < 0.01$) higher in the selenium-supplemented groups than in the control but we did not discover any differences between the individual experimental groups. The activity of glutathione peroxidase in the blood of kids tended towards higher values in the supplemented groups than in the control, but the values were significantly ($P < 0.05$) higher only in groups SY and SL; the values in the individual experimental groups did not differ significantly.

Irrespective of whether they received selenium supplements or not the correlations between selenium concentrations and activity of glutathione peroxidase were calculated in all goats and their kids. Equations of the regression line and correlation coefficients were obtained between the concentration of selenium and activity of glutathione peroxidase in whole blood of goats and kids which proved a close significant correlation (Table 5).

For the correlation between selenium concentration and activity of glutathione peroxidase in whole blood of goats $y = 5.49x - 8.48$ and $y = 9.52x - 230.95$ for kids. Using this equation, the selenium concentration of 100 µg/l is equivalent to the activity of glutathione peroxidase in whole blood of 541 µkat/l of adult goats and 721 µkat/l in whole blood of newborn kids. Regression analyses gave the regression line equation for concentrations of selenium ($y = 0.53x + 9.35$) implying that a 100 µg/l concentration of selenium in goat blood is equivalent to 62 µg/l in the whole blood of their newborn kids. For the correlation between selenium concentration and activity of glutathione peroxidase in whole blood of kids the selenium concentration of 62 µg/l is equivalent to the activity of glutathione peroxidase of 359 µkat/l. Similarly, for the correlation between the glutathione peroxidase activity in the whole blood of goats and kids the activity of 541 µkat/l in goats is equivalent to 365 µkat/l in kids.

DISCUSSION

We compared the inorganic form of selenium (sodium selenite) and newer forms of organically bound selenium in the form of yeast and selenium-proteinate from two different producers. Selenium-enriched yeasts are produced under growth of a selected species of *Saccharomyces cerevisiae* in a Se enriched medium. It ranks among organic forms of selenium but when we explore the exact composition with an accent on the form of selenium, the results are contradictory. By means of speciation analysis it was discovered that it contains approximately 90% of selenomethionine (Schrauzer, 2006).

The lactate-protein selenium complex which we used is produced by cultivation of *Lactobacillus acidophilus* on a substrate containing sodium selenite but it has not been yet specified how much of the organically bound selenium does the lactate-protein complex contain and in which form of selenium. Calomme et al. (1995) support the assumption that the lactate-protein complex contains organically bound selenium; they discovered that the concentration of selenium by various lactobacilli species can be intracellular, as selenocysteine in the biomass and in this way a potential source of organically bound selenium. These authors did not discover the microorganisms to incorporate selenium in the form of selenomethionine into protein. Alzate et al. (2008) compared selenium forms that are produced during lactic fermentation with two different types of microorganisms, bacteria – *Lactobacillus* and yeast – *Saccharomyces*. *Lactobacillus* is responsible for yogurt fermentation and produced an increment in selenocysteine and Se-methylselenocysteine, but the *Saccharomyces* causes kefir fermentation with increased selenomethionine concentration. It is assumable that the difference between the lactate-protein complex and Se-yeast lies in the bond to various amino acids. B-Traxim Se is a newly developed organic Se product using soybean peptides as the ligand (Leeson et al., 2008) but it is not declared which form of selenium the product contains and in what amount.

On the basis of our results we may conclude that all forms of selenium applied increased the level of selenium in the blood of mothers and in the blood of kids, but we did not discover significant differences between the individual forms of selenium. Other authors also came to the conclusion that both the inorganic and organic form of selenium increased the selenium concentrations in the blood of lambs (Boldižárová et al., 2005), dairy cows (Juniper et al., 2006), and goats (Misurova et al., 2009a) in contrast to the control group. Leeson et al. (2008) compared selenite, Se-enriched yeast, and B-Traxim Se in broilers and discovered that plasma concentrations of selenium did not differ significantly when using various forms of selenium. However the activity of glutathione peroxidase in the blood of broilers was significantly higher in the selenite-supplemented group than in the group supplemented with Se-enriched yeast or B-Traxim Se. Likewise, Pavlata et al. (2011b) described that the effects of supplementation with selenite and lactate-protein selenium complex in goats are similar with regard to selenium status, but that the increase in glutathione peroxidase activity occurred much faster with selenite, which therefore appears to be a more biologically available form of selenium for creation of biologically active selenoproteins.

Many authors compared, in the first place, inorganic forms of selenium such as selenite and selenate and Se-enriched yeast and discovered higher concentrations of selenium in the blood (Pehrson et al., 1999; Pavlata et al., 2001; Guyot et al., 2007; Phipps et al., 2008), body (Sevcikova et al., 2011), and in milk (Ortman and Pehrson, 1999; Givens et al., 2004; Muñiz-Naveiro et al., 2005) of animals supplemented with Se-enriched yeast. Pechova et al. (2008) compared selenium concentrations in goat milk after per oral administration of selenium in the form of Se-enriched yeast, lactate-protein complex, and selenium proteinate and they discovered that the levels of selenium in milk were significantly higher only in the group supplemented with Se-enriched yeast than in the controls as well as in both groups supplemented with other forms of selenium. Sevcikova et al. (2011) compared the effect of long-term supplementation with some different forms of selenium (Se-yeast, lactate-protein Se complex, Se-proteinate, and sodium selenite) on body reserves of selenium in kids at the time of weaning. The supplementation influenced selenium concentration in all examined tissues of kids, but the highest efficiency was found in kids supplemented with Se-yeast, also the other two organically bound forms of Se (proteinate and lactate-protein) were more efficient than the sodium selenite.

The different results reached by some authors in terms of how ruminants use up the various forms of selenium may be influenced by the availability of selenium from the feed. The availability of selenium for ruminants is affected by the type of the diet, by the sulphur, iodine, and calcium content, and content of cyanogenic glycosides (Spears, 2003; Pavlata et al., 2005). Microbial processes in the forestomachs may also change the chemical form of selenium by changing the degree of oxidation or transformation of inorganic selenium into organic and vice versa (Windisch, 2002). Selenium metabolism is also influenced by the fact if selenium is received in an organically or inorganically bound form. In the organism the absorbed inorganic selenium (selenate, selenite) is rapidly transformed into metabolically available selenide which is transformed through selenophosphates into func-

tional selenoproteins containing selenocysteine. Organically bound selenium (selenomethionine, selenocysteine) is absorbed via the absorption system of amino acids. In this way selenomethionine and selenocysteine become accessories to the amino acid pool and in the course of proteosynthesis they either become part of tissue proteins and are thus eliminated from functional selenium metabolism, or these amino acids are oxidised and release selenide which is then exploited in the same way as inorganic selenium (Windisch, 2002; Zeng, 2009).

Data comparison of Se concentration and GSH-Px activity in whole blood of the goats before the trial started and on the day of delivery is showing that while the long-term supplementation of grain mixture with Se content of 0.3 mg/kg DM assures adequate supply of the goats with selenium, elimination of the Se supplement and leaving only natural sources of Se in the feed of control group (that means Se supply of 0.07 mg/kg DM in the grain feed and 0.065 mg/kg DM in the hay) causes decline of blood Se concentration and GSH-Px activity. On the other hand, the grain feed with Se content of 0.97 mg/kg DM assured increased supply of selenium in the goats and their kids in all experimental groups.

The values of selenium concentration in the whole blood of goats from control group oscillating around 80 µg/l can be classified as marginal to insufficient selenium supply because the concentration of selenium in blood should fluctuate around 100 µg/l (Pugh, 2002).

We discovered significant correlations between selenium concentration and activity of glutathione peroxidase in goats and their kids. Selenium concentration of 100 µg/l is equivalent to glutathione peroxidase activity of 541 µkat/l in whole blood of goats and to 721 µkat/l in whole blood of newborn kids. Concerning the blood of newborn kids, Misurova et al. (2009b) came to the same conclusions; they discovered that 100 µg/l is equivalent to the activity of glutathione peroxidase of 720.3 µkat/l, but they detected a higher equivalent value of glutathione peroxidase in the blood of adult goats – 699.5 µkat/l. In one-month old kids Pavlata et al. (2005) discovered that 100 µg/l is equivalent to 523.1 µkat/l activity of glutathione peroxidase in whole blood. The correlation between the selenium concentration in the blood of goats and their kids was also significant. We discovered that the concentration of 100 µg/l of selenium in goat blood is equivalent to the concentration of about 60 µg/l of selenium in the whole blood of their newborn kids; this finding corresponds with the findings of Misurova et al. (2009b) that selenium concentration in the blood of newborn kids is by 40% lower than in their mothers. This finding should be taken into account for interpretation of selenium concentration and GSH-Px activity in whole blood of newborn kids. On the basis of our results the limits for adequate selenium status in kids are concentration of Se higher than 60 µg/l of whole blood or GSH-Px activity higher than 350 µkat/l.

We compared four different forms of selenium (sodium-selenite, selenium enriched yeast, lactate-protein selenium complex, and selenium-proteinate) supplemented per orally to the pregnant goats six weeks before parturition and their effect on Se concentration and GSH-Px activity in the blood of goats and their kids. All the above forms increased Se levels in the blood of supplemented animals when compared with control group but there was no significant difference found between the individual forms. The kids from supplemented mothers had higher Se levels in the blood than control group kids. Selenium concentrations in the blood of newborn kids were reaching about 60% of Se concentrations in the blood of their mothers regardless on supplementation status.

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