The effect of inorganic and organically bound forms of selenium on glutathione peroxidase activity in the blood of goats

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ABSTRACT: The goal of the experiment was to compare the effect of supplementation of inorganic and the new organically bound (lactate-protein selenium complex) form of selenium (Se) in feed for goats. The 31 goats were split into three groups: control (C) without Se supplementation, AN group administered sodium selenite, ORG group administered lactate-protein selenium complex (Selene Chelate, Agrobac, Czech Republic) produced by cultivation of *Lactobacillus acidophilus* on a substrate containing natrium selenite. The total Se intake in goats was 0.15 mg in group C, and 0.43 mg in the groups AN and ORG. The effect of Se supplementation was assessed based on the determination of Se concentration and the activity of glutathione peroxidase (GSH-Px) in whole blood. Samples were taken before the beginning of Se supplementation, 14 and 30 days after the start of supplementation, and then two and three months after the beginning of supplementation. Average Se concentrations in the blood of goats in individual groups (C, AN, ORG) before the start of supplementation were 109.6 ± 34.3, 117.5 ± 34.7, and 105.4 ± 43.6 μg/l respectively, and the activity of GSH-Px in whole blood was 745.3 ± 289.2, 810.7 ± 280.4, and 791.0 ± 398.1 μkat/l respectively. While in group C goats neither the Se concentration nor the GSH-Px activity changed substantially during the experiment, in the goats in the experimental groups there was a statistically significant increase ($P < 0.01$) in both Se concentrations and the GSH-Px activities. At the end of the experiment Se concentrations in the blood of AN and ORG groups amounted to 168.5 ± 12.2 and 168.8 ± 26.8 μg/l. The GSH-Px activities in goats supplemented with Se also increased significantly over the course of the experiment (at the end of the experiment it was 1178.0 ± 127.3 in the AN group and 1030.1 ± 152.3 μkat/l in the ORG group), and the difference between the groups was significant ($P = 0.038$). Regarding the dynamics of GSH-Px activity changes during the monitored period, a markedly quicker increase in GSH-Px activity was recorded in the AN group – one month after the beginning of Se supplementation, compared to three months after the beginning of Se supplementation in the ORG group. The results thus show that the effects of supplementation with selenite and the lactate-protein selenium complex are similar with regard to Se status, but that the increase in GSH-Px activity occurred much faster with selenite, which therefore appears to be a more biologically available form of selenium for creation of biologically active selenoproteins.

Keywords: selenium metabolism; ruminants; selenoproteins; organic selenium; blood selenium

The biological functions of selenium in organisms are mediated through various selenoproteins. Some selenoproteins have enzymatic functions (glutathione peroxidases, iodothyronine deiodinases, etc.) and are very important for key biological functions (antioxidant activity, thyroid function, immunity, cancer prevention, health of mammary gland, reproduction, etc.) (Schrauzer, 2000a, 2003; Birringer et al., 2002; Pavlata et al., 2004; Rowntree et al., 2004; Mala et al., 2009).

The selenium status of young ruminants is affected by the supplementation status of the mother during gravidity, since selenium passes through the placenta to the foetus (Abdelrahman and Kincaid, 1993; Pavlata et al. 2003; Misurova et al., 2009a,b).

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Adult animals are dependent on feed for their selenium. Its biological availability is influenced by numerous factors, including selenium status, amount of the element in the diet, element form (inorganically or organically bound), development of rumen fermentation, type of diet, antagonism to other elements or other dietary components, and other factors. The main pathways for Se loss from the organism include urine, excrement, milk, and, potentially, exhaled air (Underwood and Suttle, 1999; Leng et al., 2000; Pavlata et al., 2001a, 2005a; Boldizarova et al., 2003; Spears, 2003).

Since the Czech Republic numbers among those areas with a high occurrence of selenium deficiencies in animals and humans (Pavlata et al., 2000, 2001b, 2002, 2005b; Kvicala et al., 2003; Ludvikova et al., 2005a,b; Podhorsky et al., 2007) a lot of attention has been paid on Czech farms to the issue of selenium supplementation for ruminants and other animal species. Animals are parenterally supplemented by individual application of selenium-vitamin preparations (Pavlata et al., 2004, 2005c), and orally through mineral feed or water additives, or through mineral licks (Pavlata et al., 2001a; Travnicek et al., 2007; Krys et al., 2009).

Selenium is usually added to ruminant feed in inorganically or organically bound forms. Common inorganic forms of selenium include sodium selenite and selenate, while organically bound forms are represented mainly by selenomethionine, which occurs naturally in plants or preparations based on selenised yeast, selenium proteinate, or selenium-enriched unicellular alga Chlorella, that also contain other selenium compounds, such as dimethylselenonium propionate and Se-allylselenocysteine (Larsen et al., 2001; Davis et al., 2005, 2006; Travnicek et al., 2007). To this list has recently been added organically bound selenium in the form of a lactate-protein complex. However, the results of experimental studies comparing this form of selenium with other available sources are rare in the literature (Pechova et al., 2008a,b; Misurova et al., 2009b). Thus, the aim of our study was to compare the effects of supplementation with traditional inorganic and this new organically bound form of selenium in feed for goats.

MATERIAL AND METHODS

The experiment was performed on 31 white short-haired, one-year-old pregnant goats with an average weight of around 40 kg split into three groups. The experiment was carried out in the stables of the Ruminant Clinic of the Faculty of Veterinary Medicine at the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. Goats in individual groups were placed in independent, straw padded boxes providing free movement and drinkers. One group (C, n = 11) was a control – without Se supplementation in the feed, while the other two groups (AN and ORG) were experimental, and their feed contained Se added in pellets of meal to the amount of 0.9 mg Se/kg. The AN group (n = 10) was administered sodium selenite, while the ORG group (n = 10) was administered selenium in the form of a lactate-protein complex (Selene Chelate, Agrobac, Czech Republic), which contains 0.8 % Se. This form of Se was produced by

Table 1. Nutritional composition of the diet for goats at the calculated average consumption of 1 kg of meadow hay and 0.35 kg of granulated additive feed mix for goats in all groups

	Group	
	C	AN, ORG
Dry matter (kg)	1.2	1.2
Crude protein (g)	152.8	152.8
Digestible crude protein (g)	97.2	97.2
Fiber (g)	305.0	305.0
Fat (g)	38.2	38.2
NEL (MJ)	6.1	6.1
ME (MJ)	10.5	10.5
Ca (g)	9.3	9.3
P (g)	5.3	5.3
Mg (g)	2.5	2.5
Na (g)	1.3	1.3
K (g)	18.1	18.1
Mn (mg)	33.3	33.3
Zn (mg)	35.7	35.7
Cu (mg)	10.5	10.5
J (mg)	0.2	0.2
Se (mg)	0.1	0.4
Vitamin E (mg)	8.4	8.4
β-carotene (mg)	12.7	12.7

C = control; AN, ORG = experimental groups supplied with inorganic and organically bound selenium

cultivation of *Lactobacillus acidophilus* on a substrate containing natrium selenite. It is supposed that this complex contains Se-methionine and Se-cystein, but there is no definitive data regarding the amounts of these Se-containing amino acids.

Apart from the content and form of Se, the feed was identical for all 31 goats (hay, water, salt lick ad libitum, pellets – 0.35 kg/animal/day). The nutritional composition of the diet is displayed in Table 1. The total Se intake in goats was 0.1 mg in group C, and 0.4 mg in the groups AN and ORG.

The effect of Se supplementation was assessed based on determination of Se concentration in whole blood and the activity of glutathione peroxidase (GSH-Px) in whole blood. Blood sample were taken from the *v. jugularis* into disposable testing tubes using lithium heparinate as an anti-coagulant. Samples were taken before the beginning of Se supplementation, 14 and 30 days after the start of supplementation, and then two and three months after the beginning of supplementation. Selenium was measured in individual whole blood samples using the HG-AAS method and the AAS Solaar M6 (Unicvam, Great Britain) device, after microwave mineralization of samples in the Milestone Ethos TC (Milestone, Italy) unit using the method of Pechova et al. (2005). In some samples glutathione peroxidase activity was assessed in whole heparinized blood according to the method described by Paglia and Valentine (1967) with the use of the Ransel-Randox set and Cobas Mira automatic biochemical analyzer. All the tests were performed in the biochemical laboratory of our institute.

The results are quoted as a mean values with standard deviation. Comparison of the results between groups was achieved using an *F*-test to assess the variance of individual sets, and using the dependent Student's *t*-test for sets with equality/non-equality of variances according to the results. The dynamics of changes in selenium concentration and GSH-Px activity in individual animal groups were evaluated using the paired Student's *t*-test. All data was analysed using Microsoft Excel XP software.

RESULTS

The average selenium concentrations in the blood of goats in the individual groups (C, AN, ORG) before the start of supplementation were 109.6 ± 34.3, 117.5 ± 34.7, and 105.4 ± 43.6 μg/l, respectively, and the activity of GSH-Px in whole blood was 745.3 ± 289.2, 810.7 ± 280.4, and 791.0 ± 398.1 μkat/l, respectively (Figures 1 and 2). While in the goats in group C neither the Se concentration nor the GSH-Px activity changed substantially over the course of the experiment (120.4 ± 28.9 μg/l, and 763.0 ± 169.8 μkat/l at the end of the experiment), in the goats in the experimental groups there was a statistically significant increase ($P < 0.01$) in the Se concentration and GSH-Px activity. Already at the first sampling 14 days after the beginning of supplementation with Se the concentration of Se in group AN increased to 152.3 ± 19.8 μg/l and in the ORG group to 147.4 ± 35.4 μg/l. At the end of the experiment the Se

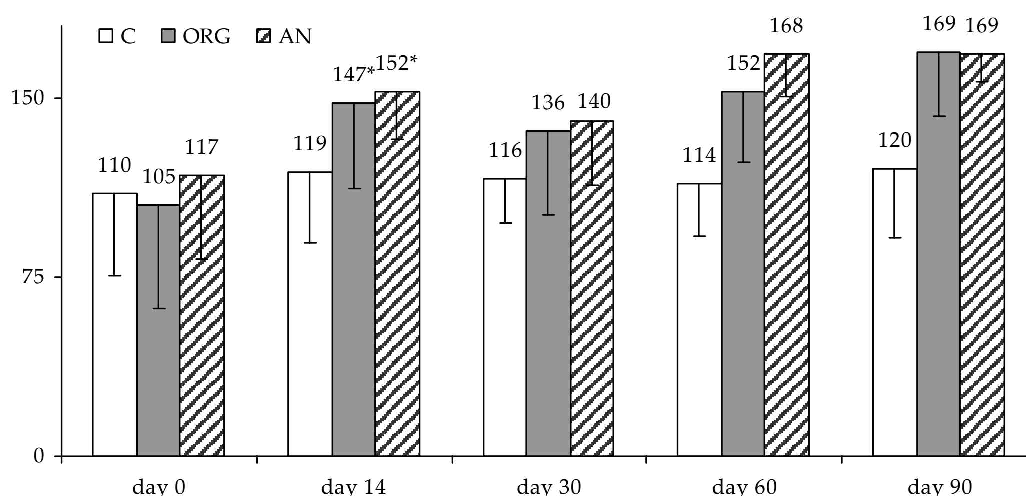

Figure 1. The concentration of Se in whole blood (μg/l) before the start of supplementation (day 0), 14 days, 30 days, 60 days, and 90 days after the beginning of supplementation

*indicates samples which for the first time gave a statistically significant increase in values ($P < 0.01$), compared to samples before selenium supplementation

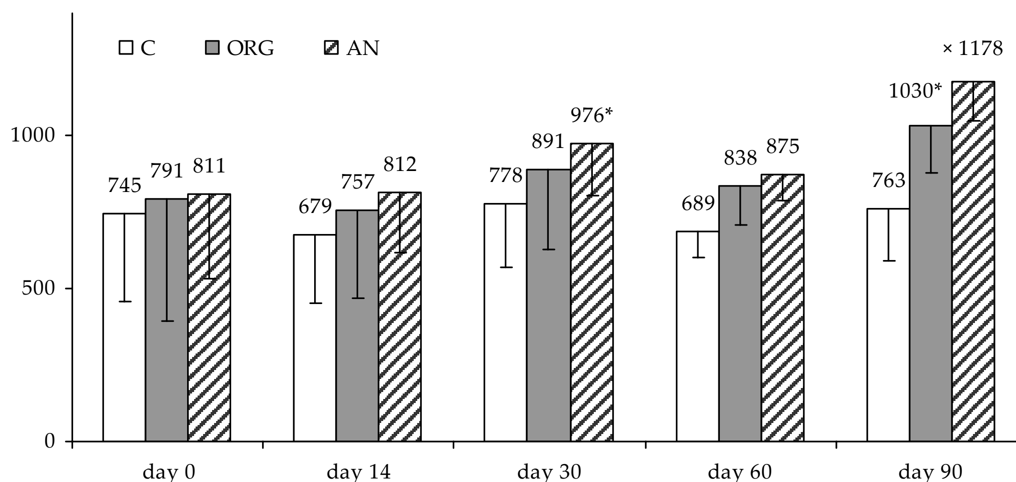

Figure 2. The activity of GSH-Px in whole blood (µkat/l) before the start of supplementation (day 0), 14 days, 30 days, 60 days, and 90 days after the beginning of supplementation

*indicates samples which for the first time gave a statistically significant increase in values ($P < 0.01$), compared to samples before selenium supplementation

×denotes statistically a significant difference ($P < 0.05$) between the ORG and AN groups in a single sampling

concentrations in the blood of AN and ORG groups were calculated to be 168.5 ± 12.2 and 168.8 ± 26.8 µg/l. There was no significant difference in the Se concentration between the supplemented groups. The GSH-Px activity in goats supplemented with Se also increased significantly over the time course of the experiment (at the end of the experiment it was 1178.0 ± 127.3 in the AN group and 1030.1 ± 152.3 µkat/l in the ORG group), and the difference between the groups was significant ($P = 0.038$).

Regarding the dynamics of changes in GSH-Px activity during the monitored period, a significantly quicker increase in GSH-Px activity was recorded in the AN group – one month after the beginning of Se supplementation, compared to three months after the beginning of Se supplementation in the ORG group (Figure 2).

DISCUSSION

Before the start of selenium administration, the concentrations of selenium in the whole blood of goats in all groups were very similar and were around 110 mg/l of whole blood. These values can be taken to indicate sufficient to marginal supply with selenium as the concentration of selenium in blood should be around 100 mg/l (Pugh, 2002). The experimental groups received an approximately threefold higher amount of selenium via their feed rations compared to the control group over the course of the experimental period. The recommended approximate selenium requirement for pregnant goats is reported as 0.2–0.5 mg per 1 kg of dry matter (Sommer et al., 1994; Dercksen et al., 2007). The selenium status of the control group C can be assessed as insufficient, while the experimental group goats received selenium at an amount close to the higher recommended limit.

After the commencement of selenium supplementation there was a quick, significant increase in selenium concentration in blood, which subsequently held at a concentration of around 150 µg/l during the experimental period or increased slightly to values of around 170 µg/l. No significant differences were found between the two supplemented groups during the experiment. Supplementation of both selenium forms (sodium selenite and the lactate-protein selenium complex) can be assessed as successful and equally effective from the standpoint of rate and absolute increase in selenium concentrations in whole blood. The results thus strongly indicate that there are probably no significant differences in absorption and subsequent metabolism following supplementation with the two different forms of selenium. It is also apparent that selenium concentration in whole blood is a good indicator of the current Se status of animals, since after increased supplementation, or after application of selenium, its concentration in blood increases quickly (Kim and Mahan, 2001; Cristaldi et al., 2005). This experiment did not reveal significant differences between selenium sup-

plementation in the form of selenite and in the form of a lactate-protein complex with regard to selenium blood concentration, although numerous reports claim that application of organically bound selenium provides a better effect (Ortman and Pehrson, 1999; Pehrson et al., 1999; Falkowska et al., 2000; Pavlata et al., 2001a; Gunter et al., 2003; Davis et al. 2008). The higher concentration of selenium in blood and in tissues after application of organically bound forms of selenium results from the fact that the most frequently used organically bound form of selenium is selenomethionine, directly incorporated into tissue proteins instead of methionine (Schrauzer, 2003). Since information concerning lactate-protein complex metabolism is scarce, an explanation of the results is not easy. Due to the nature of its chemical bond, it can be expected that the post-absorption metabolism of the selenium lactate-protein complex will differ from the metabolism of only selenomethionine, resp. selenomethionine in selenised yeast, or that the proportion of proteinate-bound selenium in the used preparation is small. This claim can be supported by our previous results, where we recorded, after application of a lactate-protein selenium complex, much lower concentrations of selenium in goat milk compared to application of selenium yeast which contains a high proportion of selenomethionine (Pechova et al., 2008a).

Differences have been described between the metabolism of inorganically and organically bound selenium with regard to absorption and post-absorption metabolism. It is claimed that the absorption of inorganic selenium and iodine, as opposed to zinc, iron, copper and manganese, in intestines, is regulated only minimally, and that during passage through the intestines, these elements do not significantly interact with other components of the diet. It can thus be stated that the organism is more or less passively exposed to selenium supplementation from the intestine depending on the amount of the element in the diet (Windisch, 2002). The principal homeostatic regulation of selenium metabolism in the organism occurs through renal excretion. Post-absorption selenium metabolism is influenced by its intake in either inorganic or organically bound form. Absorbed inorganic selenium (selenate, selenite) is quickly transformed in the organism into metabolically available selenide (H_2Se) and then converted by selenophosphates into functional selenoproteins containing selenocysteine (Windisch, 2002). Organically bound selenium (selenomethionine, selenocysteine) is absorbed through the absorption system of amino acids. Ingested selenomethionine is either metabolized directly to give reactive forms of selenium or stored in place of methionine in body proteins. Selenomethionine metabolism is closely linked to protein turnover (Schrauzer, 2000b, 2003). Selenomethionine and selenocysteine thus become parts of the amino acid pool, and either become constituents of tissue proteins during protein synthesis, which temporarily eliminates them from functional selenium metabolism, or are oxidized to release selenide which is further used as inorganic selenium (Windisch, 2002).

Comparing the results of GSH-Px activity in whole blood, it is apparent that after selenium supplementation in both experimental groups, there was a significant increase in the activity of this selenium-dependent enzyme. However, in the AN group supplied with selenite, the significant increase occurred faster (in a month), and the GSH-Px activity in whole blood was increased in absolute terms. Three months after the beginning of selenium supplementation in the form of selenite, we found a conclusively higher activity of GSH-Px compared to the group supplemented with the lactate-protein selenium complex. These results indicate that supplementation with selenite provided selenium that was more quickly biologically available for creation of GSH-Px, which is a biologically significant selenoprotein. Given the fact that selenium is incorporated into the erythrocytic GSH-Px already during erythropoesis, erythrocytic GSH-Px is considered as a suitable indicator of biologically active selenium (Hoffmen et al., 1978; Gerlof, 1992). It is to be expected that selenide required for creation of active selenoproteins, probably originates later, compared to selenite, due to the more complex pre- and post-absorption metabolism of lactate-protein complex. Naturally, the recorded difference in the onset of GSH-Px activity can be caused by numerous possible differences in the metabolism of the used forms of selenium. The differences could relate to, e.g., differences in metabolism at the level of the gizzard, resorption at the level of intestine, or the influence of other factors. It is a well-known fact that the metabolism of selenomethionine is dependent on vitamin B_6 status, because B_6-dependent enzymes are involved in the metabolic activation of selenomethionine. Tissue deposition of selenomethionine and its utilization for GSH-Px synthesis also depend on methionine status. In methionine-deficient rats supplemented with selenomethionine GSH-Px activity was lower

than in selenomethionine-supplemented methionine-adequate rats (Schrauzer, 2000b).

The results thus show that the effect of supplementation with selenite and a lactate-protein selenium complex is similar with regard Se status, but that GSH-Px activity increase much faster with selenite, which therefore appears to be a biologically better available form of selenium for creation of functional biologically active selenium proteins.

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