Impact of long-term supplementation of zinc and selenium on their content in blood and hair in goats

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ABSTRACT: This paper evaluates the impact of long-term supplementation of different forms of zinc (Zn) and selenium (Se) on the content of these substances in the blood and hair of goats. Two analogous supplementation experiments were performed. 37 goats divided into four groups were used in the first trial with the Zn supplementation. Group A (n = 10) was a control group (with no Zn administered). A further three groups (B, C, D) were supplemented with Zn in various forms. Group B (n = 9) with zinc oxide, Group C (n = 9) with zinc lactate and Group D (n = 9) with zinc chelate. The second trial with Se supplementation was carried out on 20 goats divided into four groups. Group E (n = 5) was a control group. The other three groups were administered Se. Group F (n = 5) was supplied with a selenium lactate-protein complex, Group G (n = 5) with sodium selenite and Group H (n = 5) with selenium yeast. Three months later blood and hair samples were taken from all animals and Zn and Se concentrations were determined in whole blood, plasma, and hair. Glutathione peroxidase (GSH-Px) activity was determined in the Se supplementation trial group. At the end of the trial the Zn concentrations in plasma and whole blood were without major differences between the groups. The plasma concentration of Zn did not increase from the initial value at the start of the trial. In hair the average concentration of Zn was 95.2–100.0 mg/kg in all groups. No conclusive relation was confirmed between the values of Zn in hair and its concentration in blood. The Se concentration in whole blood (μg/l) at the end of trial in supplemented groups (F – 188.8 ± 24.6; G – 197.2 ± 10.9; H – 190.1 ± 26.3) was significantly higher (P < 0.01) than in the control group (E – 103.1 ± 23.5). Similarly, the activity of GSH-Px (μkat/l) was significantly higher in all supplemented groups (F – 872.3 ± 94.8; G – 659.5 ± 176.4; H – 839.8 ± 150.8) than in the control group (E – 379.1 ± 63.5). Se content in hair (μg/kg) was higher also in all trial groups (F – 242.3 ± 41.5; G – 200.5 ± 46.9; H – 270.0 ± 106.8) than in the control group (E – 174.7 ± 38.0). However, it was significantly (P < 0.05) higher only in Group F. A conclusive correlation was identified between the Se concentration in whole blood and its content in hair (r = 0.54; P < 0.05; n = 20). Based on the results it can be concluded that none of the supplemented forms of Zn increased its concentration in blood, plasma and hair. On the other hand, the administration of Se led to an increase in the Se concentration in blood, increased the activity of GSH-Px in whole blood and the Se content in hair. Based on the proven correlation and regression relation between the Se concentration in blood and its content in hair, hair can be considered as a suitable material for the diagnosis of long-term Se status in goats. Goats with sufficient Se status are those that have more than 160 μg/kg of Se in hair dry weight.

Keywords: trace element; glutathione peroxidase; organic selenium; inorganic selenium; organic zinc; inorganic zinc; ruminants; metabolism

Selenium (Se) and zinc (Zn) rank among the essential microelements that influence metabolism, the immune system and overall health condition. Deficits in any of these trace elements can result in a number of health disorders (Underwood and Suttle, 1999a,b; Pavlata et al., 2009). Animals in the Czech Republic frequently suffer from a lack of essential microelements (Ludvikova et al., 2005;

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Pavlata et al., 2005a; Podhorsky et al., 2007) and for this reason they are added to feeds in various organic as well as inorganic forms (Pechova et al., 2008, 2009; Krys et al. 2009; Mala et al., 2009; Misurova et al., 2009a).

The diagnosis of microelement status is based on the direct determination of the element's concentration in blood or other tissues and organs (liver, muscle tissue, kidneys). Alternatively, other indirect diagnostic methods can be used, such as for example an identification of the activity of an enzyme dependent on the particular microelement, such as glutathione peroxidase in the case of Se (Pavlata et al., 2000; Ludvikova et al., 2005; Misurova et al., 2009b). Milk and hair are other materials used for diagnostics (Gabryszuk et al., 2008). A growing hair is considered to be a metabolically active tissue and as such it can reflect the concentration of minerals present in the hair follicle at the time of the hair formation. Hair analysis, however, can be negatively influenced by secondary contamination with mineral elements from urine, faeces and feed (Combs et al., 1982). It is believed that trace elements are contained in hairs at concentrations much higher than those in blood plasma, urine or other tissues (Perrone et al., 1996). The taking and storage of hair samples is relatively simple compared to the handling of other tissues and liquids. This is why hair has potentially great importance in identifying the mineral status in individual animals (Combs, 1987; Perrone et al., 1996). A potential problem in hair analysis is insufficient standardisation of results but with the application of good modern laboratory practice and validated methodology it is possible to obtain reliable results (Bass et al., 2001; Klevay et al., 2004).

The level of a mineral in the blood often does not correspond to the content of minerals in the whole body because the composition of plasma is buffered against deficiencies by different homeostatic mechanisms. Moreover, the blood concentration of microelements is relatively low and depends on the current diet; therefore, the diagnostic value of such analytical results may be fairly small. Studies have shown that the analysis of hair and nails are appropriate alternatives to blood and urine tests or to biopsies (Gabryszuk et al., 2010). It has been reported, for example, that in ruminants the values of hair Zn reflect the amount of Zn supplied in the diet in cattle and goats better than values measured in other tissues (Miller et al., 1966). On the contrary, Beeson et al. (1977) reported that the content of Zn measured in hair increased significantly only in some cases even with a significant increase in Zn supplementation in feed. The testing of hypotheses regarding the detection of Se in cattle hair (Kursa and Kroupova, 1975) proved that hair could be a suitable indicator for the diagnosis of clinical and sub-clinical Se deficiency.

A number of studies focusing on the suitability of using hair as diagnostic material have been carried out in humans (Teresa et al., 1997; Bass et al., 2001; Ilhan et al., 2003; Wu, 2007). For example, in patients with stomach cancer the values of hair Se are remarkably lower than those of healthy individuals (Wu, 2007). Hac et al. (2002) describe a significant correlation between the measured values of plasma and hair Se. Similarly, Klevay et al. (2004) show a conclusive ratio between Se in hair and in the tissues of kidneys, liver and lungs. It is also described in humans that the content of Zn in hair correlates remarkably well with its concentration in other tissues, such as bones and testicles (Klevay et al., 2004). Lowe et al. (2009) summarised 49 recent studies in humans and compared the suitability of 32 biomarkers of Zn status. According to their findings the concentration in human and animal hair is the best indicator of Zn status immediately following the values measured in urine and plasma. Also, Gellein et al. (2008) point out the suitability of using human hair and the potential suitability of using the hair of ruminants for this purpose.

This paper's objective was to evaluate the impact of long-term supplementation of different forms of Zn and Se on their content in blood and hair in goats and to assess whether hair is a suitable material for the diagnosis of Se and Zn status.

MATERIAL AND METHODS

Animals

Over two years two separate experiments were carried out on breeding white shorthaired goats stalled at the Ruminant Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno. Goats included in the experiments were gravid, apparently in good health and without clinical signs of illness. The first trial with Zn supplementation was carried out on 37 individuals. The animals were divided into four groups depending on the current measured level of overall Zn in blood plasma in such a way that the initial Zn concentration in blood was bal-

Table 1. Number of individuals in the group (*n*) and measured concentrations of plasma Zn (mean ± standard deviation) in μmol/l in the individual groups of animals at the beginning of trial

Group	A	B	C	D
Zn	10.0 ± 0.7	10.0 ± 1.3	9.9 ± 1.5	10.0 ± 2.1
n	10	9	9	9

anced in all groups. Individual groups are described in Table 1.

The second trial with Se supplementation was carried out on 20 goats. The animals were divided into four groups depending on the currently measured concentration of Se and glutathione peroxidase activity (GSH-Px) in whole blood of goats. The groups are described in Table 2.

The Zn supplementation trial included goats that were about two months before expected parturition. The Se supplementation trial began approximately one month before the expected parturition. Since the trials took three months each, the gravid goats gave birth during the trial and the kids were stalled along with their mothers. In both cases the animals in groups were stalled in shared boxes with straw litter and had access to troughs with drinking water.

Feeding

In both trials the goats were fed identical feed rations consisting of meadow hay (1.2 kg of hey per animal per day), supplementary diet (0.6 kg per animal per day), water and salt blocks *ad libitum*. Goats after parturition were fed an additional 0.15 to 0.5 kg of oat. The supplementary diet composition was as follows: barley 30%, wheat 20%, lucerne meal 18%, unpeeled sunflower extracted meal 10%, wheat bran 10%, corn 5%, malt sprouts 5%, dicalcium phosphate 1.1%, sodium chloride 0.7%, and calcium carbonate 0.2%. The basic composition was identical in all groups while the contents of supplemented Zn and Se differed between individual trial groups. The nutrient composition of the feed is provided in Table 3 (not including the oat added in the period after parturition).

The supplementary diet for Group B included inorganic Zn in the form of zinc oxide (ZnO), for Group C there was Zn lactate (zinc lactate trihydrate, Zinc Chelate, Agrobac, Czech Republic) and for Group D Zn chelate (Bioplex Zn, Alltech, USA). The supplementary diet for goats in Group A was not enriched with Zn and contained 21mg Zn/kg dry matter. The Zinc concentration in the diet for Groups B, C, and D was 60 mg Zn/kg dry matter. The diet of Group F contained Se in the form of a lactate-protein complex (Selene chelate, Agrobac Karel Gebauer, Czech Republic), Group G were given sodium selenite and Group H selenium yeast with the declared content of selenomethionine (Sel-Plex, Alltech, USA). Group E was not fed any additional Se. The Se concentration in the diet in the trial groups was 0.9 mg/kg dry matter while the Se content in the diet for Group E was 0.15 mg/kg dry matter. The experimental feed rations were used over the course of three months in both trials.

Sampling and laboratory tests

Following three months of feeding the diet specified above blood and hair samples were taken from all animals. In the long-term supplementation Zn trial samples were taken to determine the Zn concentration in blood plasma, whole blood and hair. In the Se trial the Se concentration was determined in whole blood, plasma and hair. GSH-Px activity in whole blood was also measured.

Blood was obtained from *vena jugularis* and collected in disposable heparinized plastic test tubes. Within two hours of sampling the blood plasma was separated from whole blood by centrifugation.

In individual plasma or whole blood samples the Se content was established using HG-AAS (Hydride

Table 2. Number of individuals in the group (*n*) and measured concentrations of Se (mean ± standard deviation) in μg/l and GSH-Px activity in μkat/l in the whole blood of individual groups of animals at the beginning of trial

Group	E	F	G	H
Se	99.4 ± 15.2	109.1 ± 14.0	113.0 ± 17.9	104.5 ± 21.3
GSH-Px	662.6 ± 110.8	656.9 ± 86.6	667.8 ± 104.7	640.8 ± 145.9
n	5	5	5	5

Table 3. Nutrient composition of hey and supplementary feed for all groups of goats

	Meadow hey	Supplementary feed
Weight (kg)	1	1
Dry matter (kg)	0.85	0.9
Crude protein (g)	88	150
Fiber (g)	270	111
Fat (g)	20	25
Net energy of lactation (MJ)	3.8	5.9
Metabolizable energy (MJ)	6.8	9.9
Ca (g)	5.4	6.7
P (g)	2.6	6.9
Mg (g)	1.7	2.4
Na (g)	0.4	3.4
K (g)	14.8	9.6
Mn (mg)	20	40
Cu (mg)	6	11
J (mg)	0.1	0.4
vitamin E (mg)	0	20
β caroten (mg)	7	12

Generation Atomic Absorption Spectrometry) according to the method of Pechova et al. (2005) at AAS Solaar M6 (Unicam, Great Britain) following microwave mineralisation at Milestone Ethos TC (Milestone, Italy). The Zn concentration in whole blood was measured with flame AAS following mineralisation at Milestone Ethos TC (Milestone, Italy). Samples of blood and blood plasma (2 ml) were mineralized in the presence of 1 ml H_2O_2 and 2 ml HNO_3. The zinc concentration in blood plasma was determined by the method of flame AAS at Solaar M6 (Unicam, Great Britain) following the deproteination of the sample by the addition of trichloracetic acid at the 1 : 1 ratio. The concentration of zinc was determined in the supernatant after centrifugation.

The activity of GSH-Px was measured in whole heparized blood according to the method of Paglia and Valentine (1967), using the Ransel – Randox test set at the Cobas Mira automatic biochemistry analyser.

Hair samples were cut with stainless steel scissors on the right side of the animals, behind the shoulder blade. Using vinyl sampling gloves the samples were placed in individual plastic bags. The material was subsequently processed in the laboratory using the modified method of Bires (1986). Hair was placed in flasks and embedded in a detergent aqueous solution (10 ml/l H_2O) of 250 ml per 2 g of hair at 25 °C. Following this the solution was placed in an electric shaker for 10 min. The procedure was repeated once after screening. Subsequently, 300 ml of distilled water at 25°C was used for washing the hair on the electric shaker for 5 min. The procedure was repeated until the water discharged from the flask had characteristics equal to those of the water poured in initially (approx. 10 times). After draining the hair samples were dried at 110 °C to obtain constant weight. A hair sample of 0.25–0.5g was weighed from the prepared material with a precision to four decimal places. A combustion mixture of 6 ml of concentrated HNO_3 and 3 ml HF was added to this sample.

Hair sample mineralisation was carried out using a microwave mineralisation method at Milestone Ethos TC (Milestone, Italy). Zn concentration was subsequently determined in the obtained pure mineralisate using the flame AAS at AAS Solaar M6 (Unicvam, Great Britain). The mineralized hair samples for determining Se concentration were first evaporized and following reduction Se was determined by HG-AAS following the method of

Pechova et al. (2005). The resulting concentrations of elements were converted depending on the charge to the contents of Se and Zn in a kilogram of dry hair.

All tests were carried out at the laboratories of the Ruminant Clinic.

Statistical methods

The basic statistical parameters of the set of results (mean, standard deviation) obtained from individual groups and their statistical comparisons were processed in Microsoft Excel XP. The results were statistically assessed using an *F*-test to evaluate the distribution of values of individual sets and according to the results of the two-tailed Student's *t*-test for sets with even/uneven distribution. The paired *t*-test was used to determine whether the supplementation form had a conclusive impact on the element content in the biological material. The correlation and regression analysis of the values relationship in various biological materials was also performed in Microsoft Excel XP.

RESULTS AND DISCUSSION

Zinc supplementation experiment

The basic statistical characteristics of Zn concentration in various biological materials (blood plasma, whole blood, hair) in individual goat groups are provided in Table 4.

The mean concentration of Zn in blood plasma at the end of the experiment varied between 9.1 and 11.3 µmol/l in individual groups whereas before Zn supplementation the mean values were around 10 µmol/l (Table 1). Zinc concentration in whole blood at the end of the trial reached average values of 34.1 to 36.3 µmol/l in individual groups, i.e., approximately three times higher than that in blood plasma. In the control group the Zn concentration in blood plasma represented 31.6% of the whole blood value. In the group supplemented with zinc oxide it was 30%, in the group supplemented with zinc lactate it was 29% and in the group supplemented with chelate it was 25.1%. Similarly, the Zn concentration in hair was well balanced between the groups reaching average values of 95.2 to 100 mg/kg of hair dry matter.

Tables 4 and 1 clearly indicate that long-term supplementation with Zn did not have a conclusive impact on Zn concentrations in the tested biological materials ($P > 0.05$). No significant difference was found in the Zn concentration in the tested materials. Similarly, a comparison of Zn concentration results at the beginning of the trial and at the end demonstrates that for all groups Zn supplementation did not lead to an increase in its plasma concentration.

This result is rather surprising as following from reports from the literature and from our own recent experience we expected that Zn supplementation would increase its concentration in the biological materials and lead to differences between individual trial groups. For example, Pechova at al. (2009) describe significant differences in Zn concentrations

Table 4. Basic statistical characteristics of Zn concentration values (mean ($\bar{x}$) ± standard deviation (s), minimum (min), maximum (max)) in various biological materials after three months of the experiment in individual groups of goats supplemented with various Zn forms and control group (without Zn supplementation)

		A – control $n = 10$	B – Zn oxide $n = 9$	C – Zn lactate $n = 9$	D – Zn chelate $n = 9$
Plasma (µmol/l)	$\bar{x}$ ± s	11.3 ± 1.4	10.3 ± 1.1	9.9 ± 1.6	9.1 ± 1.6
	min	8.6	7.7	8.0	6.1
	max	13.0	12.0	12.9	11.3
Whole blood (µmol/l)	$\bar{x}$ ± s	35.6 ± 6.2	34.4 ± 7.4	34.1 ± 9.1	36.3 ± 5.3
	min	27.1	27.8	26.9	27.8
	max	46.6	48.1	55.2	47.1
Hair (mg/kg)	$\bar{x}$ ± s	97.9 ± 10.1	97.9 ± 7.0	95.2 ± 5.1	100.0 ± 8.9
	min	85.5	87.0	87.7	88.0
	max	120.9	108.6	104.6	113.5

in the blood plasma of goats after a 14-day supplementation with its various forms. In their short trial they daily applied 500 mg/Zn/animal/day in the form of zinc lactate, zinc chelate, amino-acid-polypeptide and zinc oxide orally. The plasma Zn concentration in the trial animals clearly increased from the initial mean values 10.4–10.7 μmol/l to 13.5–16.6 μmol/l in the supplemented groups. A similar result was obtained by Puchala et al. (1999) in goats supplemented with zinc oxide and Zn-Met at various doses. The 120-day supplementation resulted in increases in Zn concentration in blood plasma without regard to the form. Ryan et al. (2002) reported significant differences in sheep supplemented with organic forms (Bioplex) compared to inorganic forms (sulphate zinc). The Zn concentration in blood reached a higher value after Bioplex. In contrast to this, Spears and Kegley (2002) reported similar results to those of our experiments. During their trial with cattle in the growing stage they administered Zn oxide and then determined the values in plasma and found no statistical differences in comparison with the control unsupplemented group. Similarly, Pechova et al. (2006) reported no differences in the concentrations measured in the blood plasma of 500 cows in various stages of lactation after supplementation with Zn chelate (Bioplex).

The results of our experiments document that supplementation with various forms of Zn does not always result in an anticipated increase of its concentration in blood. This is likely to be connected with the fact that the Zn concentration in blood is greatly influenced by the overall saturation of an organism with this element. If the animals enter the trial without a significant deficit of the supplemented element, the probability of increasing its concentration in blood is much lower than in deficient animals. This can be supported by the results of the above-mentioned study (Pechova et al., 2009). Following a rapid, significant increase in Zn concentration in blood plasma in goats after 14 days of supplementation a further 14 days of supplementing the same amount of Zn there resulted in a rather significant decrease in Zn concentrations and the values among supplemented groups became more balanced. The average Zn concentration after 28 days of supplementation was around 12.15–12.86 μmol/l. We can assume that organisms utilise the Zn supplemented in their diet quickly, which is connected with the increase in blood concentration, but when the tissues are sufficiently saturated with Zn the Zn blood concentration stabilises and drops. We cannot rule out that after a short period of Zn supplementation during our trial the Zn concentration rose but then over the course of the experiment dropped to the initial level. Our measured average Zn concentration in blood plasma was under 12 μmol/l, a level generally recommended for ruminants (Pavlata et al., 2005a), but it remains a question whether this value is adequate for diagnostics in goats. Since even long-term supplementation with Zn did not have an impact on the Zn concentration in blood, we can speculate regarding whether the blood Zn concentration is sufficient.

Zn concentration in whole blood and hair did not show significant differences neither between supplemented groups nor between supplemented groups and the control group indicating that the animals had been probably sufficiently saturated with zinc at the beginning of the trial. Hair Zn concentration values measured in groups ranged from 97.8–100 mg/kg of dry matter and are well balanced and in absolute values very similar to the results described by Miller et al. (1966) who determined a value of 100 mg/kg dry matter of hair in healthy goats. Philips et al. (2004) reported normal Zn values in sheep wool as 127 mg/kg dry matter of hair. Even though some older studies (Miller et al. 1966; Miller, 1970) indicate that the Zn values measured in hair reflect the Zn content in the diet of cattle and goats much better than values measured in other tissues, according to more recent results it appears more likely that the diagnostic value of Zn in goat hair is rather debatable. This is in spite of the fact that in humans the determination of microelements in hair is considered as promising (Lowe et al., 2009). Klevay et al. (2004), for example, state that the Zn content in hair correlates remarkably with its concentration in other tissues. The determination of concentration in blood elements has a lower informative value. Our study also documents that the Zn concentration in blood elements was not greatly influenced by supplementation with various forms of zinc. The absolute Zn concentrations in whole blood were well balanced across all groups (supplemented and unsupplemented). The results only indicate a certain tendency toward a higher proportion of Zn tied to blood elements in the supplemented groups – most pronounced in goats supplemented with organic Zn. The Zn concentration in blood plasma in the group supplemented with zinc lactate and chelate reached only 29 and 25%, respectively, whereas in

the control group and group supplemented with zinc oxide it was 31.6 and 30% of the whole blood value, respectively.

Correlation analysis of the relationship between Zn concentration values in various biological materials demonstrates that the Zn concentrations in blood plasma, whole blood and hair are not mutually dependent ($P > 0.05$). The determination of zinc concentrations in hair and its statistical evaluation in relation to other tested parameters do not support the use of hair as a marker of Zn status.

Selenium supplementation experiment

The basic statistical characteristics of Se concentrations in various biological materials (blood plasma, whole blood, hair) and GSH-Px activity in whole blood in individual goat groups after three months of supplementation with various forms of Se are provided in Table 5.

Comparison of Se concentrations in whole blood before the trial and at the trial end is shown in Figure 1. Comparison of GSH-Px activity in whole blood before the trial and at the end of the trial is shown in Figure 2.

Table 5 documents higher Se plasma concentrations in all supplemented groups compared to Group E. However, statistical significance of the difference in concentration was not confirmed. It is apparent that the measured Se concentrations in whole blood following three-month supplementation with Se of all forms were remarkably higher ($P \leq 0.01$) in all trial groups compared to the control group. Equally, the activity of GSH-Px in all supplemented groups was evidently higher than in the control group. Supplemented groups had an even higher content of Se in hair although a statistically significant difference ($P < 0.05$) was recorded only in Group F as compared to the unsupplemented control Group E.

The results shown in Figure 1 describe a significant impact of all forms of Se supplementation on increasing its concentration in whole blood. However, there are no obvious differences with regard to the form of Se administered ($P > 0.05$). With regard to the evaluation of the impact of different forms of Se on GSH-Px activity in whole blood, Figure 2 indicates that while in control group (E) the activity definitely dropped over the course of the trial ($P < 0.01$), in groups supplemented with organic Se (F and H) there was either an increase ($P < 0.01$) or the activity remained at the same level as at the beginning of the trial (Group G supplemented with inorganic Se form). However, if we compare the activities of GSH-Px between the groups at the end of the trial, in Group G the GSH-Px activity was higher than in the control group.

Table 5. Basic statistical characteristics of Se concentration values and GSH-Px activity (mean ($\bar{x}$) ± standard deviation (s), minimum (min), maximum (max)) in various biological materials after three months of the experiment in individual groups of goats supplemented with various Se forms and control group (without selenium supplementation) showing a significant difference between the control group and supplemented groups (*$P < 0.05$, **$P < 0.01$)

		E – control $n = 5$	F – lactate-protein complex $n = 5$	G – sodium selenite $n = 5$	H – Sel-Plex $n = 5$
Plasma (µg/l)	$\bar{x}$ ± s	99.1 ± 32.6	112.2 ± 12.7	118.6 ± 13.1	117.6 ± 22.4
	min	67.7	90.3	102.8	90.1
	max	146.0	104.5	136.8	154.1
Whole blood (µg/l)	$\bar{x}$ ± s	103.2 ± 23.5	188.9 ± 24.7**	197.2 ± 10.9**	190.1 ± 26.3**
	min	65.4	143.5	182.0	146.7
	max	134.7	215.5	211.5	223.8
GSH-Px (µkat/l)	$\bar{x}$ ± s	379.1 ± 63.5	872.3 ± 94.8**	659.5 ± 176.4*	839.8 ± 150.8**
	min	313.2	740.5	343.0	640.5
	max	464.9	1028.8	841.7	1086.1
Hair (µg/kg)	$\bar{x}$ ± s	174.7 ± 38.0	242.3 ± 41.5*	200.5 ± 46.9	270.0 ± 106.8
	min	125.8	195.4	131.2	137.8
	max	232.7	310.1	256.1	431.8

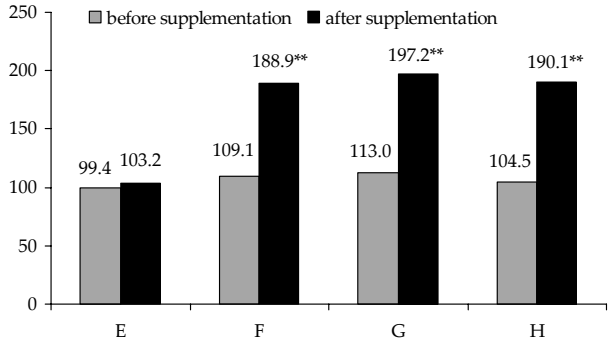

Figure 1. Comparison of Se concentration in whole blood (μg/l) before and after three-month supplementation with its various forms in the diet

E – control, F – Se lactate-protein complex, G – selenite, H – Sel-Plex
*$P < 0.01$

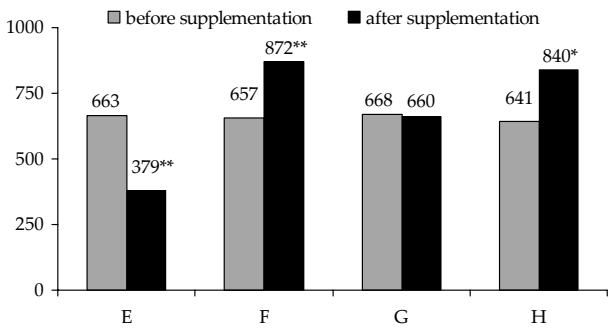

Figure 2. Comparison of GSH-Px activities in whole blood (μkat/l) before and after three-month supplementation with its various forms in the diet

E – control, F – Se lactate-protein complex, G – selenite, H – Sel-Plex)
*$P < 0.05$, ** $P < 0.01$

Se concentrations in all groups (Table 5) clearly indicate a trend toward a higher mean Se content in the hair of goats administered Se. However, a significantly higher Se content in hair ($P < 0.05$) was only observed in Group F compared to the control group. A even higher mean hair Se content was determined in Group H but with because of the rather low number of animals included in the experiment and the greater distribution of values within the groups this difference in Se content is not significant.

This comparison shows that supplementation with all Se forms had a very similar impact on increases in Se concentration in the whole blood (and blood plasma) of the animals. From the results of hair Se and GSH-Px activity it can be seen that long-term supplementation with organic Se forms (yeast bound, lactate-protein complex) resulted in a trend towards higher Se concentration or GSH-Px activity compared to supplementation with inorganic Se (sodium selenite). The impact of supplementation with various forms of Se on concentrations in blood (or other biological materials) in ruminants and other animals is the subject of numerous studies by other authors and the results provided therein are not always consistent.

It is possible to conclude, however, that the majority of studies document an improved biological effect or higher concentration of Se in blood following the application of organic forms of selenium. For example, Malbe et al. (1995) reported differences in the increase in blood Se observed when using sodium selenite and selenium yeast in cows with extremely low Se levels. According to the blood Se level they estimated that organic Se had a higher biological availability (1.9 : 1.0) compared to inorganic. Also, Ortman et al. (1999) supplemented with inorganic and organic Se in the yeast form over the course of three months and found a significantly higher Se concentration in blood and plasma in the group supplemented with organic Se compared to the group administered the inorganic Se. A higher Se increase in blood, plasma and milk following the application of Se yeast compared to selenite was also reported by Ortman and Pehrson (1999) in dairy cows. Pavlata et al. (2001) administered various forms of Se to calves and found increases in blood Se concentration when using both inorganic and organic forms of the element but when comparing the absolute values the increase in blood Se concentration was remarkably higher in the group supplemented with organic Se. Not all studies on administration of different forms of Se to goats clearly document a better biological effect of organic Se. For example, Pavlata et al. (2011) reported that when comparing supplementation of selenite and a selenium lactate-protein complex there was a similar increase in the blood concentration in goats but in goats supplemented with inorganic Se there was a faster increase in the GSH-Px activity compared to the group supplemented with the lactate-protein complex. This can be explained by the higher and faster biological availability of Se supplied in selenite (for synthesis of selenium-dependent GSH-Px) compared to the Se which is part of the lactate-protein complex. Se in this form

is probably metabolized as Se-amino acid and can be used for GSH-Px synthesis only after degradation to selenide (Windisch, 2002).

Shiobara et al. (1998) describe the influence of the form of Se on its concentration in the hair of rats. In their view the amount of Se reaches a constant value in hair after two weeks of diet change and the type of Se used influences the concentration. In our experiment, we found lower values using selenite than with Se-Met. Similar results were obtained by Salbe and Levander (1990). Similarly Pechova et al. (2008) describe increased values of Se in the milk and whole blood of goats after supplementation in the form of yeast (Sel-Plex) where most of the element is present as Se-Met even if the animals are well saturated with this element. Selenium in this form is absorbed with higher efficiency by way of active transportation. The lactate-protein complex of Se administered in their study did not serve to increase its concentration in milk or whole blood at the end of the trial. In contrast, in our study there was a significant increase in all parameters even in the group supplemented with the lactate-protein complex. The lactate-protein complex was also the only form that increased the Se concentration in hair significantly ($P < 0.05$). These differences in results for the lactate-protein complex form could be down to the duration of the trial. Pechova et al. (2008) carried out short experiments and the results were evaluated after only 20-day supplementation of Se to trial animals. Another factor that could have influenced the results in various experiments is the fact that animals included in the experiment had Se values around 99.4–109.1 μg/l, i.e., marginal Se saturation, whereas in the above mentioned experiment were included animals with sufficient initial Se status. The limit value of deficiency in whole blood is 80 μg/l (Bickhardt et al., 1999), or 100 μg/l according to Pavlata et al. (2005a), for example. Also, the GSH-Px activity values in our experiment indicate that the animals were marginally saturated at the trial beginning. Pavlata et al. (2000) describe limit values of 600 μkat/l in cattle and above 525 μkat/l in kids (Pavlata et al., 2005b). Based on correlation and regression analysis between the Se concentration and GSH-Px activity in the whole blood of goats in other experiments the limit value for GSH-Px activity was defined at 700 μkat/l whole blood (Misurova et al., 2009b). Our GSH-Px activity results at the trial beginning were around 641–663 μkat/l. The defined GSH-Px activities corresponded with our expectations when the activity of this enzyme dropped only in the control unsupplemented Group E at the end of the trial (to 379.1 μkat/l) while in the supplemented groups it increased or remained at a value similar to that at the trial beginning. Also, in this case the group supplemented with the lactate-protein complex form reached the highest mean values (872.3 μkat/l) and the group supplemented with the selenite exhibited the lowest activity of this enzyme of all supplemented groups (659.5 μkat/l). This result is different from the previous experiment where the GSH-Px activity increased faster and more significantly after selenite supplementation (Pavlata et al., 2011).

As well as evaluating the absolute values of individual parameters and comparing them among the groups we performed an evaluation of correlations between the parameters monitored in the animals at the end of the experiment (Table 6). The analysis showed a statistically important correlation and dependence ($P < 0.05$) between the Se concentration in whole blood and plasma, whole blood and hair, and a highly significant dependence ($P < 0.01$) between GSH-Px activity and Se concentration in whole blood.

The correlation between Se concentration in whole blood and GSH-Px activity can be described as the closest even though in our experiment this dependence was somewhat lower than the results published by Pavlata et al. (2005b) who defined

Table 6. Coefficients of correlation (r) between Se content ($n = 20$) in various biological materials ($^*P < 0.05$, $^{**}P < 0.01$)

	r	Regression line equation
Whole blood – plasma	0.53*	$y = 0.276x + 64.9$
Whole blood – hair	0.54*	$y = 0.906x + 68.0$
Whole blood – GSH-Px	0.72**	$y = 3.803x + 41.8$
Plasma – hair	0.42	–
GSH-Px – hair	0.35	–
GSH-Px – plasma	0.33	–

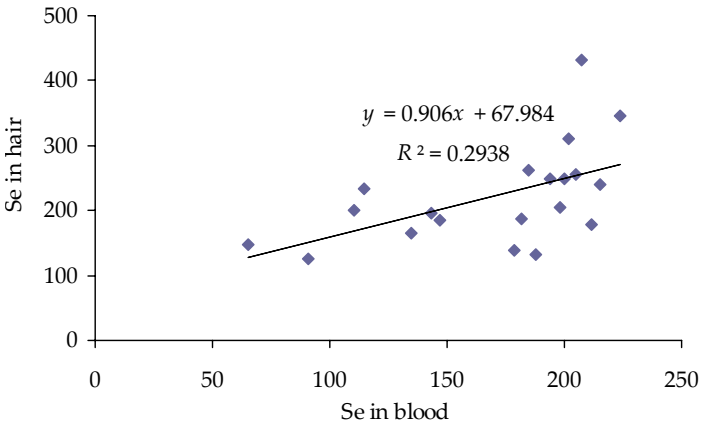

Figure 3. Regression analysis of correlation between selenium concentration in whole blood (μg/l) and hair (μg/kg) including the regression line equation. Values for all animals at the end of the trial are compared (n = 20)

a correlation coefficient (r = 0.93) for 56 pairs of values in growing kids. Nonetheless, the values in goats defined by Misurova et al. (2009b) r = 0.64 evaluating the correlation in 25 animals are very close to our results.

Further, we succeeded in proving a significant (albeit not too close) correlation (r = 0.54) between Se concentration in whole blood and its content in hair (Figure 3) but not between other indicators and hair. If we substitute the Se concentration value of 100 μg/l of whole blood, we calculate the corresponding content of Se in hair to be 158.5 μg/kg of dry hair matter. An impact on hair selenium was also demonstrated by Kursa and Kroupova (1975) who measured the hair Se values in cattle in the South Bohemian region, in an area of enzootic incidence of nutritional muscular dystrophy. Very low Se concentrations (90 μg/kg) were found in young cattle, calves and lambs, in herds with previous incidence of this disease. When Se (Selevit inj., Spofa) was injected into these animals 10 days before hair sampling, the measured Se values were remarkably higher (290 μg/kg).

The use of hair for the diagnostics of Se deficiency has also been described in humans. For example, Gellein et al. (2008) point out the suitability of using human hair as a biomarker. Hac et al. (2002) confirmed correlation dependence between Se concentration in blood plasma and in hair. Wu (2007) also suggests the possibility of using hair for this purpose. In light of our results hair can be considered as a suitable material for the indicative diagnostics of Se status even if the determination of Se in whole blood is more accurate, especially if combined with determination of GSH-Px activity or in combination with other tests that assess current (Se concentration in plasma and whole blood) and long-term selenium status (GSH-Px activity, Se in hair). Ashton et al. (2009) summarised the results of the existing 18 studies in humans which sought to determine the most appropriate markers of selenium status. They concluded that the most suitable biomarkers are blood plasma, erythrocytes and whole blood. As additional suitable markers they suggested selenoprotein P and GSH-Px activity. In their opinion other parameters, including hair tissue, lack convincing evidence for their suitability.

In our study the value of Se content in the hair exceeded 160 μg/kg of dry matter; this can be considered as evidence of sufficient long-term selenium status in goats.

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