Effect of a long-term peroral supplementation with sodium selenite and selenium lactate-protein complex on selenium status in goats and their kids

L. Misurova, L. Pavlata, A. Pechova, R. Dvorak

Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The aim of this study was to evaluate the effect of a long-term peroral selenium supplementation in the form of sodium selenite and selenium lactate-protein complex by comparing selenium concentrations and glutathione peroxidase activity in blood of goats and their kids as well as comparing selenium concentrations in goat colostrums. For the study, a total of 27 clinically healthy pregnant white shorthair goats were used. They were divided to three groups, i.e., the control group (C) without any selenium supplementation, sodium selenite group (E1) and selenium lactate-protein complex group (E2). For four months, experimental goats received 0.43 mg of selenium per animal per day in diet; goats from the control group were given 0.15 mg of selenium per animal per day. At the beginning of the experiment, goats of all groups showed an average selenium concentration of 96 μg/l in whole blood. On the parturition day, samples of first colostrum from goats and heparinized blood from goats and kids were taken. In the control group (C), average blood selenium concentrations of 111.4 ± 33.5 μg/l were observed on the parturition day. In both experimental groups, selenium concentrations were significantly higher ($P < 0.05$). Average selenium concentration in the sodium selenite group (E1) was 177.2 ± 34.8 μg/l and in the group supplemented with selenium lactate-protein complex (E2) 159.0 ± 28.5 μg/l. Average glutathione peroxidase (GSH-Px) activity in blood of control goats (C) was 581.9 ± 99.2 μkat/l, in group E1 1 154.6 ± 156.2 μkat/l and in group E2 1 011.6 ± 153.6 μkat/l. GSH-Px activity in experimental groups was significantly higher ($P < 0.05$) as compared with the control group. Average selenium concentrations in colostrum was in the control group 40.1 ± 12.8 μg/l, in E1 99.0 ± 29.9 μg/l and in group E2 79.0 ± 17.7 μg/l. Colostral selenium concentrations in experimental groups were significantly higher ($P < 0.05$) as compared with the control group. No significant difference in the monitored parameters was found between experimental groups. In kids of control mothers (kC), average selenium concentrations in blood on the parturition day were 62.4 ± 22.9 μg/l; kids of mothers supplemented with sodium selenite (kE1) showed average selenium levels of 100.0 ± 31.2 μg/l, and the average selenium concentration in kids of mothers receiving lactate-protein complex was 83.4 ± 20.1 μg/l (kE2). Average GSH-Px activity in control kids (kC) was 402.1 ± 153.9 μkat/l. Kids from kE1 showed average activity of GSH-Px 806.1 ± 254.9 μkat/l and kids from group kE2 529.9 ± 119.8 μkat/l. Statistically significant difference ($P < 0.05$) was found only between kC and kE1 which showed significantly higher selenium concentration and GSH-Px activity. The results of this study confirm that both forms of selenium administered in experimental groups (i.e., sodium selenite and selenium lactate-protein complex) had similar biological effect in goats. However, results obtained in kids indicate a better effect of supplementation with sodium selenite.

Keywords: mother-kid relationship; trace element; glutathione peroxidase; colostrum; organic selenium; inorganic selenium

Selenium (Se) saturation in newborn kids fed on mother's milk depends on Se levels in the mother's organism. Although Se passes both placental and mammary barriers, placental transfer is more effective than transport of Se into milk (Enjalbert et al., 1999; Pavlata et al., 2003). Misurova et al. (2009) observed that newborn kids reach only 60–70% of the selenium concentration and glutathione per-

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oxidase (GSH-Px) activity in whole blood of their mothers.

In areas with low Se content in soil, to which belongs the Czech Republic, it is important to monitor Se levels in animals and, if necessary, consider Se supplementation (Pavlata et al., 2000, 2002; Ludvikova et al., 2005; Podhorsky et al., 2007). Selenium is supplemented either in inorganic forms such as sodium selenite, sodium selenate, barium selenate, or, more recently, in organic forms such as selenomethionine and selenium yeast (which contain Se bound primarily as selenomethionine), selenium chelates (where selenium is part of a chelate complex with proteins or amino acids), Se-methylselenocysteine contained in Se-enriched plants such as onion, garlic, broccoli, cabbage or radish (Wrobel et al., 2004; Pedrero et al., 2006; Kapolna and Fodor, 2006; Yamanoshita et al., 2007). In human nutrition, also other foodstuffs are increasingly used for their anticarcinogenic and antioxidative effects. They include Se-enriched green tea (Yu et al., 2007; Xu et al., 2007; Li et al., 2008), Se-enriched eggs and meat (Pan et al., 2007; Fisinin et al., 2008, 2009), Se-enriched mushrooms (Zhao et al., 2008) or Se-enriched *Lactobacillus* (Calomme et al., 1995; Xia et al., 2007).

As far as the impact of various Se forms in ruminants is concerned, the majority of studies focus on cattle or sheep. Studies on Se in goats are quite rare. Various Se forms in goat's milk were studied by Aspila (1991) and Pechova et al. (2008). Studies on health problems caused by selenium deficiency or a combined selenium and vitamin E deficiency in goats (Tontis, 1984; Bickhardt et al., 1999; Culjak et al., 1999; Ramirez-Bribiesca et al., 2001) resulted in efforts to understand the effect of Se supplementation on increased Se levels in blood and its impact on animal health (Hayashida et al., 2003; Ramirez-Bribiesca et al., 2005; Sanchez et al., 2007).

In ruminants, Se is supplemented either parenterally or perorally in feed, mainly in the form of licks,

Table 1. Nutrient content in diet of individual groups of goats with expected intake of 1.2 kg meadow hay and 350 g pelleted grain mixture diet

	Mixture diet per animal per day	
	Group C	Group E1 a E2
Dry matter (kg)	1.2	1.2
Crude protein (g)	152.8	152.8
Metabolizable protein (g)	97.2	97.2
Fiber (g)	305.0	305.0
Fat (g)	38.2	38.2
Net energy of lactation (MJ)	6.1	6.1
Metabolizable energy (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)	35.8	35.8
Cu (mg)	10.6	10.6
I (mg)	0.2	0.2
Se (mg)	**0.15**	**0.43**
Vitamin E (mg)	8.4	8.4
β-caroten (mg)	12.8	12.8

intraruminal boluses (Wichtel et al., 1994; Grunder and Auer, 1995; Krys et al., 2009), or in drinking water (Lokajova et al., 2004). Currently, the biological availability of individual Se forms is being investigated. This study will focus on a comparison between sodium selenite and the rarely administered selenium lactate-protein complex.

MATERIAL AND METHODS

A total of 27 clinically health pregnant white shorthair goats were used for the study. The goats were kept on the premises of the Ruminant Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno. They were divided into three groups (nine animals in each group). The first group was a control (C) without any selenium supplementation. Animals of the second group (E1) were given sodium selenite. In the third group (E2), goats received selenium lactate-protein complex (Selene Chelate Agrobac, Czech Republic). This form of selenium complex was produced by cultivation of *Lactobacillus acidophilus* on a substrate containing sodium selenite.

The experiment was started four months before expected parturitions. Goats of all groups showed average Se levels in whole blood of 96 µg/l. They were fed twice a day with granules (350 g per animal) and meadow hay. Granules intended for experimental goats were enriched with 0.9 Se per 1 kg of feeding mixture (Table 1). Goats received drinking water and salt lick was *ad libitum*. On the parturition day, heparinized blood was obtained by puncturing the *vena jugularis* of mothers and their kids in order to determine selenium concentration and glutathione peroxidase activity. Blood samples of kids were taken immediately after parturition before they sucked the first colostrum. In twins and triplets, mixed samples from all kids were used for analyses. First colostrum (150 ml) was taken by milking mothers; 10 ml of this volume was used to determine selenium concentrations. Blood and colostrum samples were stored frozen at -20°C until use.

For selenium concentration tests, all samples were mineralized in a closed system by microwave digestion equipment in the presence of HNO_3 and H_2O_2 (Milestone Ethos TC by Milestone, Italy). The mineralized sample was prepared for the determination of selenium by evaporation and dissolution in water to which 20% HCl was added. These samples were then tested for selenium concentrations by the AAS hydride technique using the AAS Solaar M6 (Unicam, Great Britain) (Pechova et al., 2005).

GSH-Px activity was established by the Paglia and Valentine method (1967) using the Ransel Set by Randox (Great Britain) and the Cobas Mira automatic analyzer (Roche, Switzerland).

Basic statistical parameters of results (means, standard deviations) in individual groups and a comparison between the results of groups (using the two-tailed Student *t*-test after *F*-test for equality of variations) were computed using Microsoft Excel XP.

RESULTS

Means, standard deviations and minimum and maximum of identified parameters are presented in Table 2 and Figures 1 to 5 where the statistical significance of differences between individual groups is also shown.

Table 2. Basic statistical characteristics (mean ± standard deviation, minimum, and maximum) of selenium (Se) concentration and glutathione peroxidase activity (GSH-Px) in whole blood of goats and kids and selenium concentration in colostrum of goats in individual groups on the parturition day

	Control group (C)			Sodium selenite (E1)			Lactate-protein selenium complex (E2)		
	$\bar{x}$ ± SD	min.	max.	$\bar{x}$ ± SD	min.	max.	$\bar{x}$ ± SD	min.	max.
Se (µg/l) – goats	111.4 ± 33.5	66.7	146.5	177.2 ± 34.8	122.0	235.9	159.0 ± 28.5	100.3	198.7
GSH-Px (µkat/l) – goats	581.9 ± 99.2	375.1	696.8	1154.6 ± 156.2	999.9	1439.0	1011.6 ± 153.6	820.7	1339.0
Se (µg/l) – colostrum	40.0 ± 12.8	15.1	54.1	99.0 ± 29.9	68.4	156.8	79.0 ± 17.7	54.2	108.9
Se (µg/l) – kids	62.4 ± 22.9	35.1	104.2	100.0 ± 31.2	72.8	166.3	83.4 ± 20.1	37.3	106.1
GSH-Px (µkat/l) – kids	402.1 ± 135.9	230.0	645.9	806.1 ± 255.0	545.0	1422.0	529.9 ± 119.8	238.1	673.2

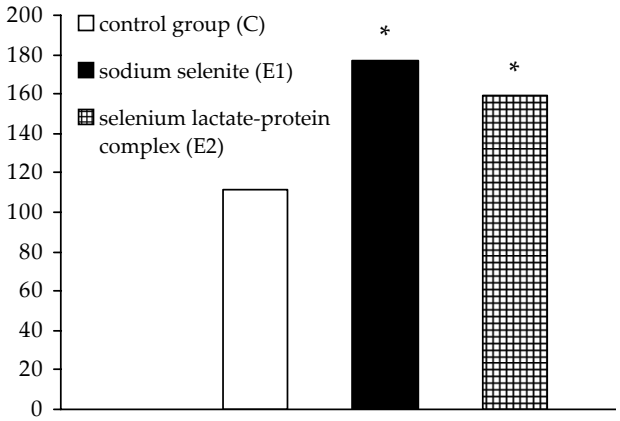

Figure 1. Selenium concentration (μg/l) in whole blood of goats from individual groups on the parturition day, and statistical significance of differences between the groups – *E1, E2 > C ($P < 0.05$)

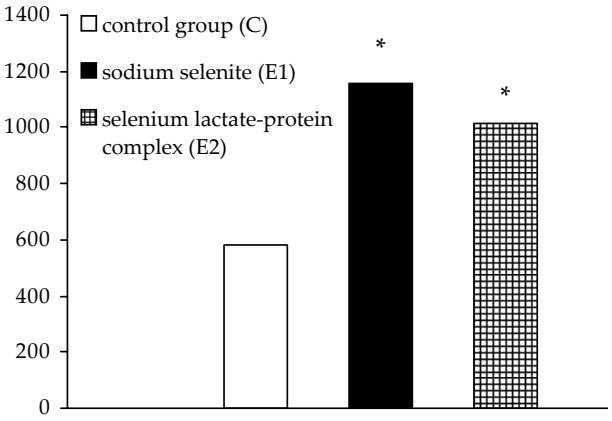

Figure 2. Glutathione peroxidase activity (μkat/l) in whole blood of goats from individual groups on the parturition day, and statistical significance of differences between the groups – *E1, E2 > C ($P < 0.05$)

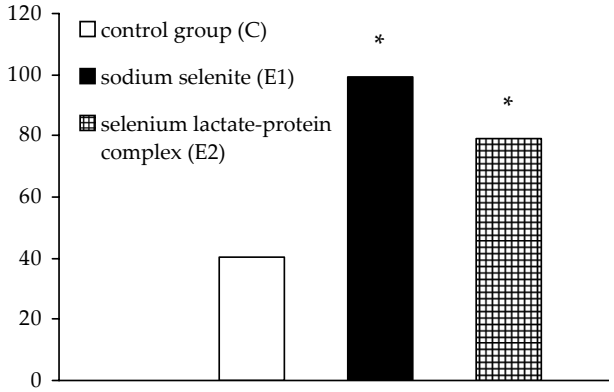

Figure 3. Selenium concentration (μg/l) in colostrum of goats from individual groups on the parturition day, and statistical significance of differences between the groups – *E1, E2 > C ($P < 0.05$)

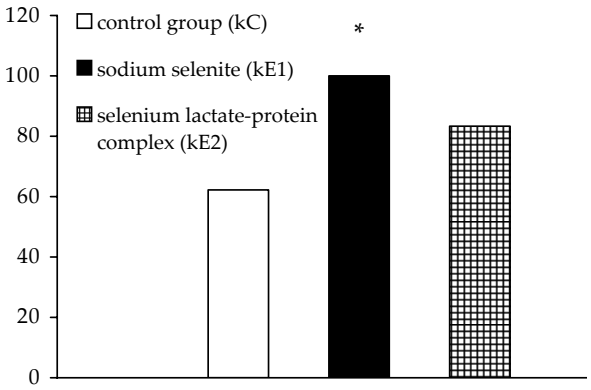

Figure 4. Selenium concentration (μg/l) in whole blood of kids from individual groups on the parturition day, and statistical significance of differences between the groups – *kE1 > kC ($P < 0.05$)

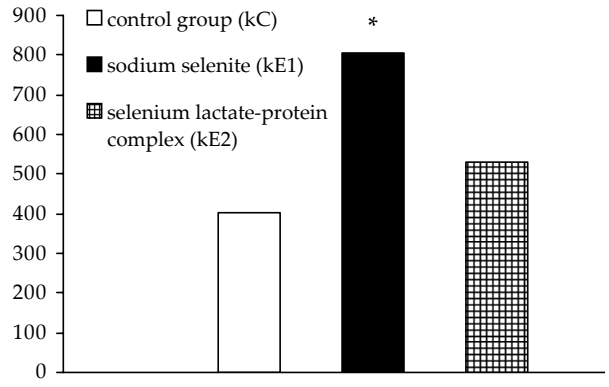

Figure 5. Glutathione peroxidase activity (μkat/l) in whole blood of kids from individual groups on the parturition day, and statistical significance of differences between the groups – * kE1 > kC ($P < 0.05$), kE1 > kE2 ($P < 0.05$)

Selenium levels and glutathione peroxidase activity in mother's blood was in both experimental groups (E1 and E2) statistically significantly ($P < 0.05$) higher than in the control group (C) (Figure 1 and 2). However, no statistically significant difference was found between the sodium selenite group (E1) and the group supplemented with selenium lactate-protein complex (E2), though the values tended to increase in the sodium selenite group. The same results were found in colostrum (Figure 3): both experimental groups showed significantly higher selenium levels as compared to the control group ($P < 0.05$). Again, no statistically significant difference was found between the sodium selenite group (E1) and the group supplemented with selenium lactate-protein complex (E2), though

the values tended to rise in the sodium selenite group.

In kids from the control group (kC), average selenium levels of 62.4 ± 22.9 μg/l were observed. Kids of sodium selenite mothers (kE1) had average selenium values of 100.0 ± 31.2 μg/l, and kids of lactate-protein mothers (kE2) had average selenium concentrations of 83.4 ± 20.1 μg/l (Figure 4). Compared to the control group, statistically significantly higher ($P < 0.05$) selenium levels were found only in kids of mothers that received sodium selenite.

The following average activity of glutathione peroxidase was identified in individual groups: 402.1 ± 153.9 μkat/l in the control group (kC), 806.1 ± 254.9 μkat/l in the sodium selenite group (kE1) and 529.9 ± 119.8 μkat/l in the lactate-protein group (kE2) (Figure 5). Compared to the control group, statistically significantly higher ($P < 0.05$) GSH-Px activity was observed only in kids supplemented with sodium selenite (kE1). Furthermore, a statistically significant difference in GSH-Px activity was found also between kE1 and kE2 groups. Therefore, the average glutathione peroxidase activity in the blood of kids of mothers supplemented with sodium selenite was higher than in those supplemented with the selenium lactate-protein complex.

DISCUSSION

The results of this study indicate that both selenium forms enhanced selenium levels in the blood of experimental goats as compared with the control group and that no significant differences were found between experimental groups. Similar results were reported by Travnicek et al. (2007) who compared the effect of supplementation with sodium selenite vs. selenium bound in the biomass of the alga *Chlorella* on selenium levels in the blood of ewes and their lambs. In their study, selenium levels in experimental groups were significantly higher than in the control group, but no significant difference between experimental groups was found in blood selenium levels. Baumgartner et al. (1998) reported similar results in dairy cows that received selenium in the form of selenomethionine and sodium selenite. In their study, no difference in blood selenium levels between experimental groups was observed, but statistically significantly higher selenium levels were found in the milk of cows that received selenium bound in selenomethionine. In the present study, no differences in colostral selenium levels were found between experimental groups. However, experimental groups showed statistically significantly higher colostral selenium levels as compared with the control group.

Selenium levels observed in newborn kids during this study indicate that supplementation with sodium selenite is more effective as compared with supplementation with a lactate-protein complex. Blood selenium levels and GSH-Px activity were statistically significantly higher in kids of mothers that received sodium selenite. Similar results that suggested a better effect of selenite on GSH-Px activity as compared with selenium yeast were reported by Knowles et al. (1999) who performed experiments on dairy cows. They found that GSH-Px activity was lower by 15% in the group supplemented with Se-enriched yeast as compared with the selenite group. In contrast, milk selenium levels were twice to three times as high as in dairy cows that received Se-enriched yeast.

However, the results of the present study differ from certain other studies that compared inorganic selenium forms and organically bound selenium, mainly in the form of Se-enriched yeast. Guyot et al. (2007) compared Se-enriched yeast and sodium selenite administered perorally to pregnant Belgian Blue cows two months before and after parturition. Plasmatic levels of selenium in dams and newborn calves as well as selenium levels in colostrum and milk were higher in the group supplemented with Se-enriched yeast. Pehrson et al. (1999) made similar findings in Hereford cows. Ortman and Pehrson (1999) compared the effect of selenate, selenite and selenium yeast in dairy cows. Blood and milk selenium levels significantly increased in dairy cows supplemented with Se-enriched yeast. No difference was found between selenate and selenite groups. Although GSH-Px activity significantly increased in all groups as compared with the control group, no statistically significant difference in GSH-Px activity was found among experimental groups. Nicholson et al. (1991) reported similar data in calves that were supplemented with sodium selenite and Se-enriched yeast for four months – organic selenium statistically significantly increased selenium levels and GSH-Px activity in the blood of animals receiving yeast.

It is clear from the abovementioned summary of publications that many studies have already compared various forms of inorganic and organically bound selenium. However, results that would un-

ambiguously confirm a better biological effect of one of these forms are still missing. There is comparatively little data in the literature on the selenium lactate-protein complex used in the present study. Pechova et al. (2008) monitored selenium concentration in milk after peroral supplementation of goats with Se-enriched yeast, selenium lactate-protein complex and selenium proteinate. They observed significantly higher selenium levels in milk of goats supplemented with Se-enriched yeast as compared with the control group, lactate-protein complex and selenium proteinate group. Pavlata et al. (2007) compared the effect of 5-month supplementation of goats with selenite and lactate-protein complex on Se levels and GSH-Px activity in blood. They observed a significant increase in selenium concentration and GSH-Px activity in the blood of all experimental goats as compared with the control group. However, they found no significant differences in Se levels and GSH-Px activity between the selenite group and lactate-protein group. Nevertheless, they noted a significant growth in the blood GSH-Px activity in the selenite group during the first month of the experiment, whereas GSH-Px activity in the lactate-protein group increased as late as three months after the beginning of supplementation.

Based on data supplied by the manufacturer and information obtained from various studies, the lactate-protein complex used in the present study was expected to contain organically bound selenium, as this form of selenium was produced by cultivation of *Lactobacillus acidophilus* on substrate containing sodium selenite. However, it is not completely clear how much and what form of organically bound selenium is contained in this preparation because no precise specification of the lactate-protein complex is available.

The assumption that the lactate-protein complex contains organically bound selenium is supported by, e.g., the study of Calomme et al. (1995) who found that various species of lactobacilli can concentrate selenium intracellularly in the form of selenocysteine in biomass. This could constitute a potential source of organically bound selenium. They did not confirm that the microorganisms incorporate Se into their proteins in the form of selenomethionine. Alzate et al. (2007) detected selenocysteine and Se-methylselenocysteine in their speciation studies of Se-enriched yogurt after enzymatic hydrolysis, the concentration of which increased with the amount of supplemented selenite. When they used selenate, biotransformation was very poor. Selenomethionine was found at the same level in both unenriched and enriched yogurt, indicating that *Lactobacillus* does not metabolize Se^4 to selenomethionine. Zhang et al. (2009) reported that *Bifidobacterium animalis* 01 could absorb 16.7–39.6% of inorganic selenium in the medium and transform most of it into organic selenium. Most of organic selenium (57–63%) was found in the protein fraction, 9.6–18.7% in the polysaccharide fraction, 0.3–0.8% in the nucleic fraction and 21–31% in other components. Based on chemical analysis, they determined selenomethionine as the main selenium component of the total protein. Alzate et al. (2008) compared selenium forms that are produced from lactic fermentation with two different types of microorganism, bacteria (*Lactobacillus*) and yeast (*Saccharomyces*). While *Lactobacillus* is responsible for yogurt fermentation, synergy of bacteria and the yeast *Saccharomyces* causes kefir fermentation. Lactic fermentation for yogurt elicited an incremental rise in selenocysteine and Se-methylselenocysteine, while fermentation to produce kefir also increased the selenomethionine concentration. Besides these studies that confirm the transformation of inorganic selenium to organically bound selenium, there are other studies that prove that, e.g., *Lactobacillus bulgaricus* is able to reduce selenite to insoluble elemental selenium, electro-dense Se^0 granules, thereby depositing in both the cytoplasm and the extracellular space (Xia et al., 2007). Alzate et al. (2008) and Zhang et al. (2009) reported as well that at higher selenite concentrations, Se^4 microorganisms are reduced to Se^0 as a result of detoxication mechanisms. Considering the abovementioned facts, it should be stated that further research into the lactate-protein complex must be better specified in terms of representation of various selenium forms that are the result of the culturing of *Lactobacillus acidophilus* in the presence of selenite. If it turns out that the greater part of selenium is transformed to elementary selenium Se^0, then using this preparation would practically mean the comparison of two forms of inorganic selenium. Podoll et al. (1992) compared two purely inorganic forms, i.e., sodium selenite and sodium selenate. They found no difference after peroral supplementation of lambs and horses with these two forms. However, in lactating dairy cows selenate increased serum Se levels more significantly. Both inorganic selenium forms at the dose of 0.3 mg/kg of dietary dry matter maintain sufficient

selenium levels and GSH-Px activity in the blood of these three animal species. Grace et al. (1995) compared selenium oxide and sodium selenate and came to the conclusion that both these forms increase blood Se level in dairy cows similarly.

CONCLUSION

Both selenium forms, i.e., sodium selenite and selenium lactate-protein complex, significantly increased Se levels in the whole blood and colostrum of adult goats as compared with the control group. In newborn kids, however, a significant increase in blood Se levels and GSH-Px activity was observed only in the kids of mothers supplemented with sodium selenite. This indicates that the biological efficiency of sodium selenite in goats in terms of selenium status of their newborn kids seems to be higher as compared with the selenium lactate-protein complex.

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Corresponding Author:

Doc. MVDr. Leos Pavlata, Ph.D., Dipl. ECBHM, University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Ruminant Clinic, Palackeho 1–3, 612 42 Brno, Czech Republic
Tel. +420 541 562 407, +420 602 501 562, Fax +420 541 562 407, E-mail:pavlatal@vfu.cz