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Influence of Selenium on Innate Immune Response in Kids

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ABSTRACT. The effect is described of selenium supplemented in an inorganic and organic form on the innate immune response of goats. Though the phagocytic activity (as a marker of the immune function) was found to be lower in organic-Se-treated group than in control (54.5 ± 4.32 *vs*. 60.2 ± 9.15 %), it did not generally exhibit any significant differences; similarly, no differences were found in the phagocytic index. The production of reactive oxygen species (ROS) was determined using the luminol-enhanced chemiluminescence (CL) (estimated as peak CL, integral CL and a peak time after addition of calcium ionophore A23187, opsonised zymosan (OZP) and phorbol-12-myristate-13-acetate as effectors. A significant ROS increase reflected in integral CL and a peak time was found in the inorganic-Se-treated group when OZP was used as activator; other parameters did not exhibit significant changes. The supplementation of Se in inorganic form can thus be seen to influence positively the innate immune system of kids.

Abbreviations

Ca-I	calcium ionophore A23187	PMA	phorbol-12-myristate-13-acetate
CL	chemiluminescence	RLU	relative light unit(s)
GPx	glutathione peroxidase	ROS	reactive oxygen species
OZP	opsonized zymosan	WBC	white blood cells
PBS	phosphate-buffered saline		

Selenium, as an essential component necessary for maintaining the vital function of humans and animals (Řezanka and Sigler 2008), is present in all cells and tissues and cannot be replaced by any other trace element. The content of Se in the organisms is naturally very low, the majority of Se being bound in tissues and blood in the form of selenoproteins. Se can influence cell function of through antioxidant activities, thyroid hormone metabolism and regulation of activity of redox enzymes (Arthur *et al.* 2003); the main dietary form of Se is selenomethionine (Smith *et al.* 2004). The Se content is dependent on its amount in nutrition which reflects the Se amount in the soil. Some world regions contain <0.6 mg/kg (crops with <0.1 mg/kg) Se; this is considered as insufficient for animals and humans (Gupta and Gupta 2002). Generally, nutrients in the diet contribute to maintaining the optimum immune response and Se affects the function of neutrophils (Ferenčík *et al*. 2003). Dietary Se is essential not only for an optimum immune response but also for other activities on the immune system and Se excess as well as its long-term deficiency can damage human and animal organism. Se in higher concentrations (>900 μg/kg) is toxic and influences, among others, also the function of the immune system (Johnson *et al*. 2000). Clinical signs of selenosis in ruminants are alopecia, cracking of hooves, intradigital lesions and discoloration of the hard palate (Rampal *et al*. 2008).

Milad *et al.* (2001) reported that Se and vitamin E led to a significant rise of GPx activity in sheep blood and positively affected phagocytic activity of neutrophils (the GPx activity was also used for estimation of Se insufficiency in cattle; Pavlata *et al*. 2000). Musik *et al*. (1999) suggested that also inorganic Se compounds are biologically active and can modify neutrophile function.

Neutrophils have a pivotal function in innate immunity (Ahluwalia *et al*. 2004), providing this effect by phagocytizing, killing and digesting bacteria and fungi (Segal 2005). Neutrophils produce ROS during a process called the respiratory burst; the ROS overproduction can cause oxidative damage to membrane lipids, DNA, proteins and lipoproteins (Číž *et al.* 2008). An effective defense against the toxic influence of ROS in the organisms relies on a number of antioxidant systems, including also Se metabolism.

The aim of our experiments was to study the possible changes in innate immunity in kids and their mothers by addition of inorganic and organic form of Se to be feed.

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MATERIALS AND METHODS

Eighteen kids of the white short-haired goat with a strict clinical control of a healthy state were used. The animals were housed at the *Ruminant Clinic of University of Veterinary and Pharmaceutical Sciences*, Brno. The kids were divided into three groups: group *1* ($n = 6$) was not treated with Se (control), group *2* ($n = 6$) was treated with inorganic Se (disodium selenite) and *3* ($n = 6$) was treated with organic Se (Se lactate–protein complex, selene chelate; *Agrobac*, Czech Republic). (This form of Se complex is produced by cultivation of *Lactobacillus acidophilus* on a substrate containing disodium selenite.)

Content of Se was 1 mg per 1 kg concentrate. The animals were fed by the tested substances together with their rations. The mothers (age of 2–4 years) were divided into the same tested groups and received the same doses of Se as their kids. They started receiving the Se supplement 3 months before the parturition.

During the after-birth period the kids from each group were housed in pens with their mothers and they received mother's milk. The average content of Se in milk was determined on day 30 of lactation. Milk in the first group contained on average 25.0 μg/L Se, in the second group 21.2 μg/L and the third group 30.1 μg/L; no significant differences were observed among the tested groups. The kids were weaned at the age of 69 d of life and, after weaning, they kids were fed with a concentrate for goats (*Biokron*, Czech Republic) (Table I).

Table I. Composition of feed concentrate

Component or characteristic	Amount, g/kg
Dry matter	887
Crude protein	170
PDIE[a]	105
Starch	296
Sugar	46.2
Crude fibber	85.0
Fat	28.8
NEL[b]	6.29

[a]Metabolizable protein supply.
[b]Net energy for load (in MJ).

The consumption of the concentrate was 300 g per d per animal. Meadow hay and water were available *ad libitum*. The composition of concentrate in control group was the same but without Se. Other mineral supplements and vitamins were added in physiologically adequate quantity. No clinical signs of disease were observed during the experimental period. The average body mass of kids at the age of 120 d was in the first group 22.4 kg, in the second group 23.5 kg and in the third group 21.5 kg with no significant differences among the tested groups.

The blood samples were obtained from kids by punction of the jugular vein. Heparin (*Léčiva*, Czech Republic) in a concentration of 50 IU per mL of blood was used as anticoagulant in all cases. The blood samples were collected after 120 d of life. WBC were counted in Bürker chamber. Differential count of WBC was determined using May–Grünwald–Giemsa stained blood smear. In order to extrapolate the values of respiratory burst into the number of neutrophils these data were used to record differences in the numbers of WBC. Two hundred cells were evaluated on each slide.

Luminol-enhanced CL was used to measure the production of ROS by goat's blood neutrophils. OZP, PMA and Ca-I were used as activators; the concentrations used were optimal (*unpublished results*).

Luminol-dependent CL was performed to determine integral CL (RLU*s), peak CL (RLU) and peak-time (min). Luminol-enhanced CL-assay was done using a luminometer *Immunotech* (Czech Republic). For the measurements, the whole peripheral blood was diluted 1:100 in Hanks' balanced salt solution (HBSS; *Sigma-Aldrich*, USA) in order to block the effect of hemoglobin. Luminol (*Sigma-Aldrich*) was diluted in borate buffer (7.628 g $Na_2B_4O_7$, 1.237 g H_3BO_4 (both *Fluka*), 500 mL of deionized water, pH 9) in order to reach a working concentration of 10 mmol/L; all other chemicals used were diluted in HBSS. The reaction mixture contained diluted blood and 2.5 mmol/L of luminol. The following agents were used to stimulate the ROS production: OZP at a final concentration of 62.5 μg/L, PMA at a final concentration of 1.62 μmol/L, and Ca-I A23187 (all *Sigma-Aldrich*) at a final concentration of 4.8 μmol/L; no activator was added to controls. The level of spontaneous CL was compared with the background. The height of *y*-axis and the distance of *x*-axis from the peak was evaluated as integral, peak and peak-time, and the values of the curves were assessed. The obtained values were converted into the constant count of neutrophils using the known number of WBC in the whole blood and the differential count of WBC.

The ability of peripheral blood neutrophils to phagocyte was tested according to Větvička *et al.* (1982) by using the method of microspheric hydrophilic particles (*Artim*, Czech Republic). The results were given as phagocytic activity (percentage of phagocytizing cells out of the total count of observed cells) and phagocytic index (total count of engulfed particles divided by the number of cells that were counted). Blood from the kids (10 μL) was collected and mixed in a small plastic test tube with 10 μL of PBS-suspended microspheric hydrophilic particles (4×10^6 particles). The test tubes were incubated under intermittent shaking for 1 h at 37 °C. Two blood smears on slides were prepared from each test tube (in addition to blood smears

from original fresh blood) and stained with May–Grünwald–Giemsa. All applied methods were optimized on adult goats.

The results are presented as average ±SEM. Student's *t*-test was used to evaluate the effect of Se among tested groups. All calculations were performed with MS-Excel (*Microsoft*) software.

RESULTS

All characteristics obtained from tested blood (count of leukocytes, leukocyte differential count) were not significantly different and correspond to physiological values of goat blood.

When we used OZP as activator for assessment of CL activity (and thus ROS production) in the whole blood we observed a significant RLU*s increase, particularly in organic-Se-treated group (*3*). The group treated with inorganic Se (group *2*) exhibited a significant increase in the peak time (Table II *upper part*).

Table II. Luminol-enhanced chemiluminescence for all leukocytes and neutrophils

Group	Effector	Integral RLU*s	Peak, RLU	Peak time, min
Leukocytes				
1	Ca-I	131 212 ± 35 532	172 ± 62.5	862 ± 161.3
	OZP	253 317 ± 45 841	105 ± 18.4	1004 ± 189.4
	PMA	60 387 ± 14 181	36 ± 15.9	870 ± 343.3
2	Ca-I	126 655 ± 37 265	156 ± 70.6	851 ± 103
	OZP	290 442 ± 35 381	111 ± 15.5	1506 ± 62.1
	PMA	52 685 ± 3479	21 ± 1.6	698 ± 187
3	Ca-I	125 031 ± 26 426	133 ± 34.7	960 ± 77.2
	OZP	341 567 ± 41 084	131 ± 16.3	1311 ± 91.0
	PMA	57 205 ± 4893	9 ± 2.0	1173 ± 264
Neutrophils				
1	Ca-I	37 113 ± 12 417	50 ± 19.6	174 ± 42.5
	OZP	65 378 ± 16 076	29 ± 7.8	215 ± 62.5
	PMA	18 147 ± 8220	13 ± 7.8	187 ± 95.5
2	Ca-I	61 199 ± 23 398	78 ± 41.6	363 ± 64.5
	OZP	134 006 ± 29 365	52 ± 12.3	647 ± 79.6
	PMA	22 308 ± 2556	9 ± 1.2	295 ± 82.1
3	Ca-I	46 794 ± 5806	48 ± 7.8	410 ± 59.2
	OZP	133 930 ± 12 119	51 ± 4.9	556 ± 69.8
	PMA	23 710 ± 2828	9 ± 0.8	518 ± 130

After the data were recounted from leukocytes in the whole blood to neutrophil granulocytes, the results were more significant (Table II *lower part*). Groups treated by inorganic and organic Se showed a significant increase in RLU*s when OZP was used as an activator. In group *2* the value of peak-time was significantly higher ($p \leq 0.05$). A significant increase in peak time after the use Ca-I and PMA was shown in both groups receiving inorganic and organic Se.

Phagocytic activity was lower in the group treated by organic Se (54.5 ± 4.32 %) compared with controls (60.2 ± 9.15 %) and group *2* (60.7 ± 2.69 %). There were no significant differences among the tested groups in the phagocytic index.

DISCUSSION

Although ruminants generally display a good transfer of Se *via* placenta (Pavlata *et al*. 2005) the placenta of goats was shown not to provide immunoglobulin transfer.

Knowledge of phagocytic functions of neutrophils in ruminants is still rather poor. The percentage of phagocytizing cells in goat blood reached up to 67.8 ± 5.58 % (Benda and Hospes 1991); however, our values were lower (60.2 ± 9.15 %) with a phagocytic index of 21.7 ± 0.41. Although we did not find any

significant differences between the tested groups the results indicate that ruminants prefer the inorganic form of Se supplementation.

In contrast to the metabolism of some other trace elements (Zn, Fe, Cu), there is no homeostatic control of absorption during Se metabolism (Windisch *et al.* 1998), biological control being based mainly on renal excretion (Kirchgessner *et al*. 1997). The Se metabolism affects also alterations in renal function and/or failure (Berger *et al*. 2004). Organisms are more or less passively exposed to influx of dietary Se from small intestine and the exposure depends on the dietary concentration of this trace element. However, in ruminants a microbial processing in the forestomach can cause transformation of the inorganic form of dietary Se into organic one (Windisch 2002). Experiments with disodium selenite in ruminants showed that the majority of Se was present in biomass whereas the Se concentration in ruminal fluid was minimal (Falhar *et al*. 2009). Our data on the innate immune response indicate that inorganic form of Se can be beneficial for neutrophil function of kids.

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