Changes in white blood cells in sheep blood during selenium supplementation

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ABSTRACT: The aim of the experiment was to evaluate the impact of selenium supplementation on white blood cell parameters in the blood of ewes. The total white blood cell (WBC) and differentiation of leukocytes in blood smear were detected by a microscopic analysis, and the $CD4^+$ and $CD8^+$ subsets were detected by flow cytometry. A decrease in the count of WBC was recorded during pregnancy; it was statistically significant only in the group supplemented with organic selenium. In the postpartal period there was a statistically significant increase in the percentages of $CD4^+$ and $CD8^+$ subsets but differences between the groups were not statistically significant. The results of the experiment documented that the supplementation of different forms of selenium did not markedly influence the dynamics of blood parameters in non-pregnant, pregnant and lactating ewes if the intake of vitamins and other essential microelements was adequate.

Keywords: ewes; immunity; T lymphocytes; $CD4^+$; $CD8^+$

Selenium (Se) supplementation as sodium selenite is the most common source used to increase dietary Se in diets of farm animals. In natural conditions sheep are mainly saturated with Se bound in plants. Plants contain organic selenium primarily in the form of amino acids, selenomethionine and selenocysteine (Wu et al., 1997; Whanger, 2002). The actual state in the system of supplementation can eliminate the occurrence of muscular dystrophy (Kursa, 1969; Pavlata et al., 2001) and can support body antioxidant statues (Behne and Kyriakopoulos, 2001; Fekete and Kellems, 2007).

In recent years research on the physiological role of selenium has been aimed at organic and/or inorganic forms of Se in the processes of immunity including the functions of white blood cells (WBC), mainly lymphocytes. The immunity of females of mammals is activated significantly in the period of pregnancy and in the peripartal period in connection with the formation of antibodies for the offspring and with a higher risk of infection of reproductive organs.

During pregnancy the process of the lymphocyte recirculation is a critical element in the integration of systematic immune responses (Trnka and Cahill, 1980; Mackay, 1993). The subsets cannot be identified by their structure, but they can be identified by their characteristic cell surface molecules and by their behaviour. A possibility of T lymphocyte differentiation by receptors $CD4^+$ and $CD8^+$ (Park et al., 2004) improves the information on a potential impact of selenium supplementation on immunity processes in the period of pregnancy. Peripartal changes in intraepithelial lymphocytes in ewes were studied in the uterine tissue by Nasar et al. (2002), who determined a decrease in the white blood cell count in the uterine stroma.

In sheep blood the WBC count is normally between 5 and 11 × 10^9/l blood (Scott et al., 2006). The normal value of lymphocytes is from 60% to

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65% from the total number of WBC (Thorp et al., 1991). T lymphocytes consist of two main subsets, $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells. Normal physiological values of $CD4^+$ and $CD8^+$ T lymphocyte subsets are from 8% to 22% and from 4% to 22%, respectively (Smyth et al., 1990).

The aim of the experiment was to evaluate the impact of inorganic and organic selenium supplementation in non-pregnant, pregnant and lactating sheep on the state of white blood cells, proportion of lymphocytes and T lymphocyte subsets ($CD4^+$ and $CD8^+$).

MATERIAL AND METHODS

An experiment was conducted on fifteen ewes of the Sumava sheep breed at 18 months of age. The ewes were divided into three groups of five animals: control C (no Se supplementation) and experimental ones E1 (sodium selenite) and E2 (organically bound Se in algae of the genus *Chlorella*). The feed ration formulation per head and day is shown in Table 1. Average contents of Se in the blood serum of ewes were as follows: E1: 114.2 ± 23.6; E2: 103.1 ± 20.3; C: 68.6 ± 16.8 μg/l (Travnicek et al., 2007).

The experiment was conducted from July 2005 to March 2006. The ewes were in the stage of non-pregnancy, pregnancy and lactation during the experiment. All ewes became pregnant and parturitions took place in the course of six weeks. Blood for analyses was collected in monthly intervals. After parturition blood samples were taken from lambing ewes on Day 30 ± 5 and Day 60 ± 5. The WBC count and differentiation of leukocytes in blood smear were detected by a microscopic analysis in accordance with Czech Standard No. 84 3206 and No. 84 3209, and the $CD4^+$ and $CD8^+$ T cell subsets were detected by flow cytometry. Monoclonal antibodies used in the present study were purchased from Serotec GmbH (Düsseldorf, Germany). MCA2213F and MCA2216F were used for the detection of $CD4^+$ and $CD8^+$ T lymphocytes, respectively. Isotype-matched secondary antibody, (FITC)-labelled antimouse IgG2a (Exbio s.r.o., Jilove, Czech Republic, Catalogue No. 1F-458-C100). Data of ewes were calculated, and described as mean ± standard deviation. Data were statistically analyzed by ANOVA Tukey's test (Statistics 6). The significance was accepted at $P < 0.05$. The correlations were analyzed by MS Excel 2003.

RESULTS AND DISCUSSION

The dynamics of WBC count (Figure 1) in the control group (C) coincided with both experimental groups (E1, E2) during the whole experiment. As parturitions were approaching, the values dropped to the lower limit of the normal values given by Scott et al. (2006). Both lymphocytes and neutrophil granulocytes participated in a decrease in the count of WBC since the second month of pregnancy and in their recurrent postpartal increase. A decrease in the values of WBC count was the most marked and statistically significant ($P < 0.05$) in Group E2 receiving organic selenium: to $5.0 \pm 0.5 \times 10^9$/l of blood between Day 50 and Day 100 of pregnancy. This finding corresponds to the value measured in pregnant rhesus monkeys (*Macaca mulatta*) compared to non-pregnant animals (Rogers et al., 2005). Figure 2 documents that the above-mentioned decrease was not caused by lymphocytes because their proportions were characterised by heterogeneous

Table 1. Average composition of the daily ration per ewe and selenium intake

Component	Group								
	C			E1			E2		
	amount (g)	DM (g)	Se (μg)	amount (g)	DM (g)	Se (μg)	amount (g)	DM (g)	Se (μg)
Hay	1 180	1 010	40	1 180	1 010	40	1 180	1 010	40
Lucerne	240	218	6	240	218	6	240	218	6
Scraped oat	270	236	9	270	236	9	270	236	9
Mineral mixture	6	6	0	6	6	180	6	6	180
Total	1 696	1 470	55	1 696	1 470	235	1 696	1 470	235
Selenium content (μg/kg DM)			37			160			160

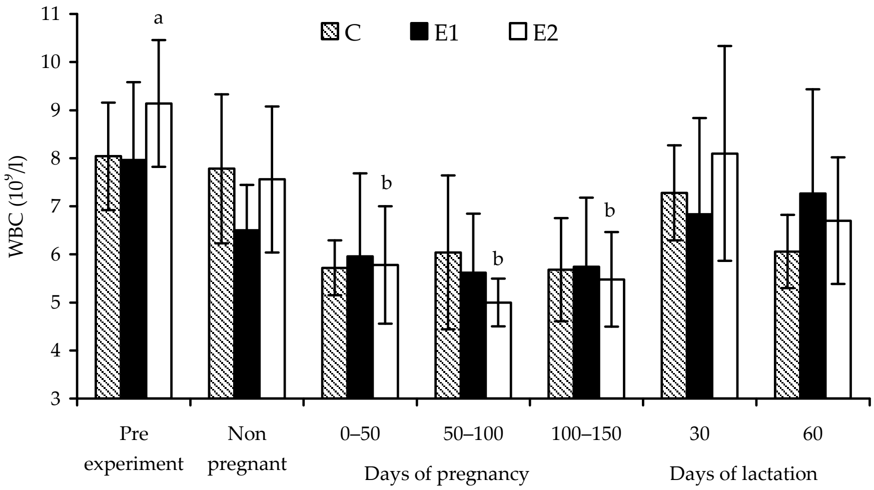

Figure 1. White blood cell count in the blood of ewes (10^9/l)

$^{a:b}P < 0.05$

variations (approximately 10%) without statistically significant differences for the experiment duration. The percentage of lymphocytes determined microscopically from the differential counting of WBC corresponded to the normal values (Thorp et al., 1991). The stable and low count of neutrophil granulocytes prevents potential inflammatory process (Naskalski et al., 2007) for the whole period of observation including the postpartal period. Contrary to the total count of WBC the percentages of lymphocytes (Figure 2) and $CD4^+$ and $CD8^+$ subsets of T lymphocytes (Figures 3 and 4) in non-pregnant and pregnant ewes remained on a very stable level within the normal physiological values (Smyth et al., 1990) without statistically significant differences between the groups.

After parturition there was an increase both in the WBC and in the $CD4^+$ and $CD8^+$ subsets of T lymphocytes in all groups. This increase was statistically significant while the values twice higher than the normal values reported by Thorp et al. (1991) were reached in the $CD4^+$ subset, and in the $CD8^+$ subset except Group E2 the values exceeded the upper limit of the normal ones given by Smyth et al. (1990). According to Entrican et al. (2002) the development of an embryo in the maternal organism is accompanied by a higher stress on the immunity system consisting in the establishment of mother's immunotolerance to the embryo's extraneous antigens. Based on histological examinations of Nasar et al. (2002) it is to assume that the onset of immunotolerance in pregnant ewes is concentrated in the uterine tissue whereas the proportions of lymphocyte subsets in the peripheral blood are not influenced.

According to Barrington and Parish (2001) $CD4^+$ (Figure 3) and $CD8^+$ (Figure 4) subsets are increasing when the antigen pressure increases, which corresponds to a higher risk of infections in the puerperal period requiring the enhancement of mother's immunity system. In this situation there was an increase in the total count of WBC (Figure 1) comprising also neutrophil granulocytes that par-

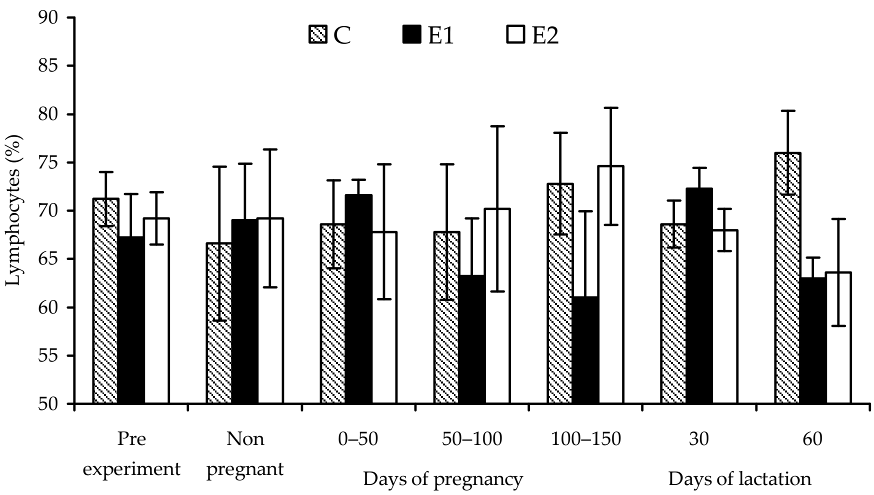

Figure 2. Percentage of lymphocytes in the blood of ewes (%)

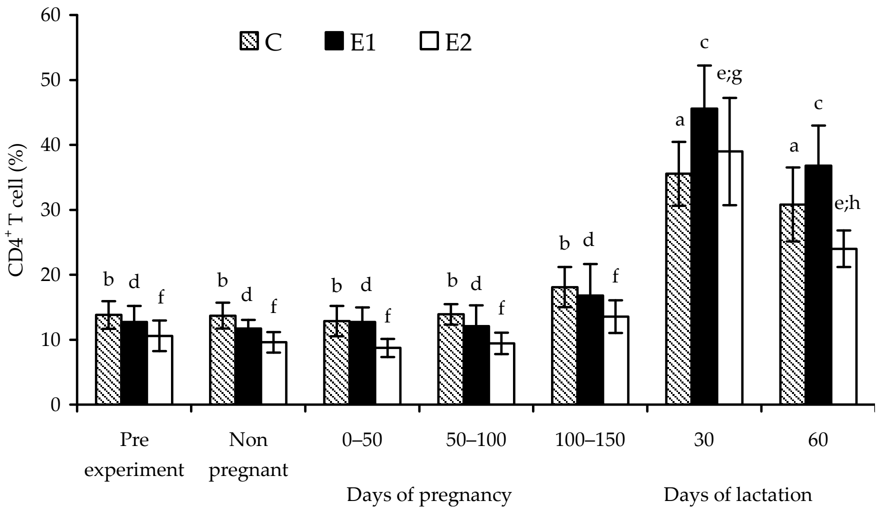

Figure 3. Percentage of $CD4^+$ T lymphocytes in the blood of ewes (%)

$^{a:b,\ c:d,\ e:f}P < 0.001$; $^{g:h}P < 0.05$

ticipate in processes of non-specific immunity. A marked postpartal increase in $CD4^+$ was also reported by Reiterova et al. (2004) in mice infected with *Toxocara canis*. The fastest normalisation of the extreme values of $CD4^+$ and $CD8^+$ subsets was recorded in Group E2, which was supplemented with organic selenium and had 100% twins. A decrease in the percentage of $CD4^+$ between Day 30 and Day 60 of lactation was statistically significant ($P < 0.05$) only in Group E2.

The $CD4^+/CD8^+$ ratio was on average 1.5 before parturition and 1.8 in the postpartal period, which excludes a disorder of specific cell-mediated immunity. It follows from the paper of Yuben et al. (2000), who considered the values of the $CD4^+/CD8^+$ ratio higher than 1.0 as optimal.

The statistical analysis of average values obtained for the whole experimental period provided significant coefficients of correlation between these parameters: total proportion of lymphocytes and $CD4^+$ (r_{xy} = 0.81), total proportion of lymphocytes and $CD8^+$ (r_{xy} = 0.65), and $CD4^+$ and $CD8^+$ (r_{xy} = 0.71).

The overall evaluation of the effect of selenium supplementation in sheep on changes in WBC count, percentages of lymphocytes and their subsets ($CD4^+$ and $CD8^+$) in the period of non-pregnancy and during pregnancy, puerperium and lactation did not show a statistically significant effect of either inorganic or organic selenium. The decrease in the WBC count during pregnancy and its postpartal recurrent increase until Day 60 of lactation was not statistically significant. In this period there was a statistically significant increase in the percentage of $CD4^+$ and $CD8^+$ but no significant differences between the groups were recorded. The experimental results document that the supplementation of inorganic and organic selenium form did not markedly influence the dynamics of blood parameters in non-pregnant, pregnant and

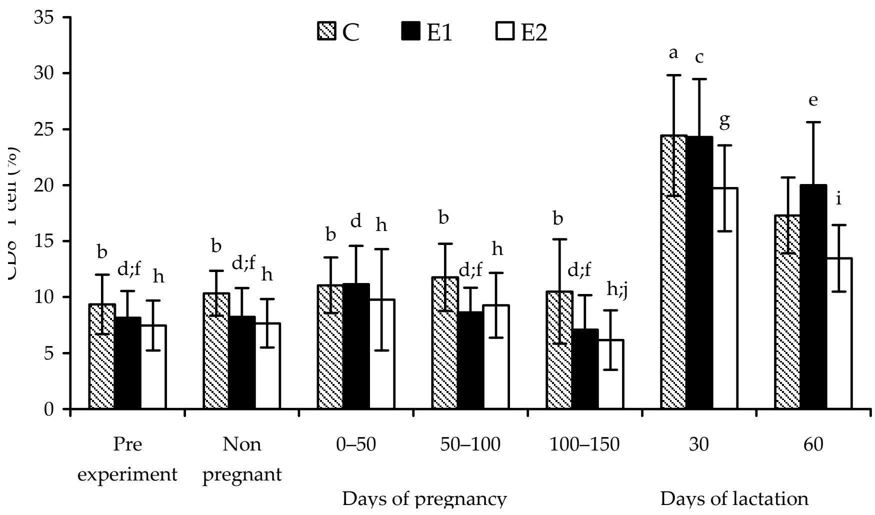

Figure 4. Percentage of $CD8^+$ T lymphocytes in the blood of ewes (%)

$^{a:b,\ c:d,\ g:h}P < 0.001$; $^{e:f}P < 0.01$; $^{i:j}P < 0.05$

lactating ewes in comparison with control group if there was provided a sufficient supply of vitamins and other essential microelements.

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