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Research Article

Effect of Selenium supplementation on White Blood Cell Count in Awassi ewes

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Abstract | The aim of the current experiment was to evaluate the effects of selenium (Se) supplementation on white blood cells in non-pregnant, pregnant, and lactating Awassi ewes. Thirty ewes were divided into three groups, each containing 10 animals: the control group C (without Se supplementation), and two experimental groups, E1 (with sodium selenite supplementation) and E2 (with organic Se supplementation). All ewes became pregnant during the experimental period (July 2022 to March 2023), and the treatments were continued until two months after parturition. The total leukocyte count and differential white blood cells in blood smears were determined through microscopic examination, while the CD8+ and CD4+ subsets of T lymphocytes were assessed using flow cytometry. A significant (P≤0.05) reduction in leukocyte counts was observed during the gestation period in all experimental groups. In the lactation period, there was a significant (P≤0.05) elevation in the levels of total white blood cell count, percentage of lymphocytes, and CD4+ and CD8+ subsets of T lymphocytes in the E1 and E2 groups in comparison to the control group. The results of the present experiment revealed that the supplementation of selenium has effects on the leukocyte counts in lactating Awassi ewes.

Keywords | Awassi ewes, Selenium, WBC, $CD4^+$, $CD8^+$.

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INTRODUCTION

The mineral contents in the animal's diet depend on many factors, especially the mineral contents of soil used for cultivation of animal feed stuff. The deficiency of mineral elements is characterized by low productivity, poor growth, emaciation, and high mortality (Mazokopakis and Protopapadakis, 2007). Most areas in the world suffer from a deficiency of selenium in the plants; therefore, it must be provided in the diets of animals especially in the geographical areas that suffer from acute selenium deficiency (Flohe et al., 2000). A recent study proved that selenium plays a major role in decreasing the oxidative stress in the animal's body which results from the low concentration of the glutathione peroxidase enzyme in ruminants (Diaz-Sanchez et al., 2017). Many studies revealed that selenium influences the blood parameters in sheep (Boldizarova et al., 2005), goats (Diaz-Sanchez et al., 2017), and cows (Slavik et al., 2008).

Ghanem et al. (2016) reported that the addition of selenium in the diet of lambs suffering from a deficiency of selenium leads to an elevation in the respiratory and heart rates of the animals. While Qureshi et al. (2017) mentioned that the addition of selenium (0.3 mg/kg diet) exhibited a significant reduction in heartbeat, respiratory rate, malondialdehyde, cortisol and HSP-70 contents in the Balkhi and Damani sheep; while superoxide dismutase (SOD) and glutathione peroxidase contents and level of T3 and T4 hormones were improved by dietary selenium.

The addition of selenium (Se) as sodium selenite is the common practice applied to elevate dietary selenium in the rations of domestic animals. In natural conditions, the Awassi sheep are mostly saturated with selenium bound in vegetables and plants. The vegetables and plants contain organic selenium often in the form of selenocysteine, amino acids and selenomethionine (Wu et al.,1997; Whanger, 2002). Selenium addition can prevent the initiation of muscular dystrophy in sheep especially in lambs (Kursa 1969; Pavlata et al., 2001) and can enhance the antioxidant status in the animal's body (Behne and Kyriakopoulos, 2001; Fekete and Kellems, 2007). The most common clinical symptoms of selenium deficiency in sheep is muscular dystrophy, which occurs more commonly in young lambs whose mothers were suffering from a lack of selenium (Humann-Ziehank et al., 2013). Also, selenium deficiency leads to abortion, lack of fertility, retained placenta, several health problems in neonatal lambs, such as reduction of neonatal vitality, elevation of neonatal mortality, reduction of suckling reflex and reduction in the immune response in lambs (Rooke et al., 2008; Ross et al., 2014; Al-Moteoty, 2018; Milewski et al., 2021). In recent years, many studies were carried out that aimed to study the role of organic and / or inorganic forms of selenium in enhancing immunity comprising the functions of white blood cells (WBC) especially lymphocytes. Mammalian females immunity is enhanced significantly in the pregnancy period and in the perinatal period in association with the formation of the antibodies for their lambs. During the pregnancy period the role of lymphocytes is very important in the integration of whole immune responses (Trnka and Cahill, 1980; Mackay, 1993). The lymphocyte subsets cannot be known through its structures, but they can be known through their behavior and their cell surface molecules. There is a probability of T lymphocyte differentiation through $CD4^+$ and $CD8^+$ (Park et al., 2004) that can affect the immunity in the pregnancy period. Perinatal changes in the uterine tissues were studied by Abu Nasar et al. (2002), who mentioned a reduction in the WBC numbers in the uterine stroma. The WBC values in Awassi sheep is ranged between 5 and 11 × 10^9/L of blood (Scott et al., 2006). The normal levels of lymphocytes are ranged between 60% to 65% of the whole number of white blood cells (Thorp et al.,1991). The T lymphocytes comprise of two main subsets; $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. The normal levels of $CD8^+$ and $CD4^+$ T lymphocyte subsets are ranged from 4-22% and 8-22% respectively (Smyth et al., 1990).

The aims of the present study was to evaluate the effect of organic and inorganic selenium addition to the rations of pregnant, non-pregnant and lactating Awassi sheep on the levels of T lymphocyte subsets ($CD4^+$ and $CD8^+$), proportion of lymphocytes, and levels of white blood cells.

Ethical Approval

The study was performed after getting permission from the College of Veterinary Medicine, Al-Qasim Green University.

MATERIALS AND METHODS

This experiment was performed on thirty ewes of the Awassi breed sheep aged 15 months (according to farm record of age). The ewes were divided into three groups with 10 animals per each group: control group C (without Se supplementation) and two experimental groups comprising E1(with sodium selenite addition) and E2 (with addition of organically bound selenium found in *Chlorella* algae). The feed ration composition per head and day is shown in (Table 1). The average of selenium levels in the blood serum of ewes were as follows: E1: 110.4 ± 15.6; E2: 101.3 ± 17.3; C: 65.4 ± 12.4 µg/L (Travnicek et al., 2007). The experiment was performed in the period extending from July 2022 to March 2023. The ewes were in three stages including the non-pregnant, pregnant and lactation stages during the experiment. The all ewes became pregnant and parturitions occurred during the experimental period. The blood samples were collected every month, even after parturition from the ewes on Day 30 ± 3 and Day 60 ± 3. The differentiation of leukocytes in blood smears and the WBC were calculated using microscopic analysis according to Czech Standard No. 84 3206 and No. 84 3209 (Abdul–Rahman and Khrofa, 2018). While, the $CD8^+$ and $CD4^+$, T cell subsets were calculated through flow cytometry analysis. The lymphocyte percentage which is calculated microscopically through the differential counting of white blood cells were compatible with the normal levels reported by Thorp et al. (1991). The monoclonal antibodies used in the current study were bought from Cerotic GmbH (Düsseldorf, Germany). Anti-sheep CD8 antibody (MCA2216F) and Anti-sheep CD4 antibody (MCA2213F) were used for the assessment of $CD8^+$, and $CD4^+$ T lymphocytes, respectively.

Statistical Analysis

The data of the ewes were measured and represented as mean ± standard error. The data were statistically analyzed by ANOVA Tukey's test (Statistics 6). The significant values were accepted at ($P<0.05$).

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

Component	Groups								
	C			E1			E2		
	Amount (gm)	DM (gm)	Se (µgm)	Amount (gm)	DM (gm)	Se (µgm)	Amount (gm)	DM (gm)	Se (µgm)
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

C: control group, E1: sodium selenite, E2: organically bound Se in algae of genus *Chlorella*, DM: dry matter.

RESULTS

As shown in Table 2, the calculation of WBC count in the control group (C) was coincided with both experimental groups (E2 and E1 groups) during the whole period of experiment. Near to parturition, the values dropped to the lower limit of the normal values. The neutrophils and lymphocytes participated in reducing the levels of WBCs from the second month of pregnancy till their recurrent elevation during the post parturition period.

The low and stable levels of neutrophils prevents the probable inflammatory reactions for the total period of the experiment including the postnatal period. The percentage of lymphocytes (Table 3) and $CD4^+$ and $CD8^+$ subsets of T lymphocytes (Table 4 and 5) in pregnant and non-pregnant ewes was recorded without any significant differences ($P>0.05$) between the groups.

The different superscript lowercase letters found vertically on means indicate significant difference beween various stages of experiment at ($P<0.05$) . While, the different superscript uppercase letters found horizentally on means indicate significant difference beween treatment groups at ($P<0.05$).

In the postnatal period (post parturition) there was an significant elevation ($P\leq0.05$) in both the WBC count, percentage of lymphocytes and in the $CD8^+$ and $CD4^+$ subsets of T lymphocytes in E1 and E2 groups in comparison to the control group (Tables 2,3,4 and 5). This elevation was statistically significant ($P\leq0.05$) and the levels were higher than the normal level reached in the CD8+ and CD4+ subsets of T lymphocytes except group E2 the levels exceeded control group (Table 4 and 5).

In this study, there was a statistically significant increase ($P\leq0.05$) in the percentage of $CD4^+$ and $CD8^+$ during the 50-100 days of pregnancy, 100-150 days of pregnancy and lactation periods in comparison to non-pregnant period, but no significant differences between the groups were recorded (Table 5).

Table 2: The levels of white blood cells in the control and experimental groups.

Stages of the animals	No.of WBCs in the control group (without selenium) $\times 10^9$ / L	No. of WBCs in the E1 group (sodium selenite) $\times 10^9$ / L	No of WBCs in the E2 group (organic selenium) $\times 10^9$ / L
Pre experiment period	9.34 ± 1.2^{a}	9.47 ± 1.4^{a}	9.61 ± 1.6^{a}
Non-pregnant ewes	9.28 ± 0.84^{a}	9.92 ± 0.24^{a}	9.46 ± 0.72^{a}
0-50 days of pregnancy	8.51 ± 0.43^{b}	8.21 ± 0.32^{b}	8.62 ± 0.24^{b}
50-100 days of pregnancy	7.88 ± 0.35^{b}	7.62 ± 0.12^{b}	7.87 ± 0.34^{b}
100-150 days of pregnancy	6.64 ± 0.43^{c}	6.36 ± 0.72^{c}	5.12 ± 0.16^{c}
30 days of lactation	7.82 ± 0.35^{bB}	9.64 ± 0.22^{aA}	9.16 ± 0.65^{aA}
60 days of lactation	7.18 ± 0.47^{bB}	9.27 ± 0.28^{aA}	9.15 ± 0.34^{aA}

The different superscript lowercase letters found vertically on means indicate significant difference beween various stages of experiment at ($P<0.05$) . While, the different superscript uppercase letters found horizentally on means indicate significant

difference beween treatment groups at (P<0.05) .

Table 3: The percentage (%) of lymphocytes in control and experimental groups.

Stages of the experiment	Percentage of lym phocytes in the blood of control group (without selenium).	Percentage of lymphocytes in the blood of E1 group (sodium selenite)	Percentage of lymphocytes in blood of E2 group (organic selenium).
Pre experimental period	72.41 ± 4.4^{a}	71.38 ± 3.6^{a}	72.52 ± 4.1^{a}
Non-pregnant ewes	71.4 ± 3.4^{a}	70.6 ± 4.2^{a}	71.3 ± 5.2^{a}
0-50 days of pregnancy	68.2 ± 5.1^{a}	69.2 ± 3.8^{a}	69.6 ± 4.4^{a}
50-100 days of pregnancy	64.7 ± 4.6^{b}	63.6 ± 4.1^{b}	64.1 ± 3.2^{b}
100-150 days of pregnancy	64.6 ± 3.6^{b}	62.3 ± 2.6^{c}	62.4 ± 4.3^{c}
30 days of lactation	66.1 ± 2.4^{bB}	71.3 ± 3.1^{aA}	70.8 ± 2.5^{aA}
60 days of lactation	67.2 ± 4.5^{bB}	71.5 ± 2.4^{aA}	70.1 ± 3.6^{aA}

The different superscript lowercase letters found vertically on means indicate significant difference beween various stages of experiment at (P<0.05) . While, the different superscript uppercase letters found horizentally on means indicate significant difference beween treatment groups at (P<0.05) .

Table 4: The percentage (%) of CD4^{+} T lymphocytes in control and experimental groups.

Stages of the experiment	Percentage of CD4^{+} T lymphocytes in the blood of control group (without selenium)	Percentage of CD4^{+} T lymphocytes in the blood of E1 group (sodium selenite)	Percentage of CD4^{+} T lymphocytes in the blood of E2 group (organic selenium)
Pre experimental period	14.62 ± 2.4	14.35 ± 2.1^{a}	14.44 ± 2.2^{a}
Non pregnant ewes	14.53 ± 2.4	14.62 ± 1.7^{a}	14.42 ± 1.8^{a}
0-50 days of pregnancy	13.26 ± 2.1	13.55 ± 1.4^{a}	13.27 ± 1.4^{a}
50-100 days of pregnancy	14.37 ± 3.2	15.11 ± 1.2^{b}	15.26 ± 1.6^{b}
100-150 days of pregnancy	14.49 ± 2.5	15.72 ± 2.4^{b}	15.72 ± 2.2^{b}
30 days of lactation	14.64 ± 3.7^{B}	16.44 ± 4.8^{cA}	16.62 ± 3.6^{cA}
60 days of lactation	14.98 ± 3.3^{B}	17.36 ± 4.7^{cA}	17.56 ± 2.4^{cA}

The different superscript lowercase letters found vertically on means indicate significant difference beween various stages of experiment at (P<0.05) . While, the different superscript uppercase letters found horizentally on means indicate significant difference beween treatment groups at (P<0.05) .

Table 5: The percentage (%) of CD8^{+} T lymphocytes in control and experimental groups.

Stages of the experiment	Percentage of CD8^{+} T lymphocytes in the blood of control group (without selenium)	Percentage of CD8^{+} T lymphocytes in the blood of E1 group (sodium selenite)	Percentage of CD8^{+} T lymphocytes in the blood of E2 group (organic selenium)
Pre experimental period	10.62 ± 1.9	10.57 ± 1.7^{a}	10.38 ± 1.6^{a}
Non pregnant ewes	10.52 ± 2.5	10.73 ± 2.1^{a}	10.66 ± 1.7^{a}
0-50 days of pregnancy	10.25 ± 2.3	10.27 ± 2.2^{a}	10.42 ± 1.5^{a}
50-100 days of pregnancy	10.32 ± 2.7	11.86 ± 2.3^{b}	11.79 ± 1.2^{b}
100-150 days of pregnancy	10.73 ± 1.6	11.94 ± 1.4^{b}	11.96 ± 1.1^{b}
30 days of lactation	10.52 ± 3.6^{B}	12.62 ± 3.4^{cA}	12.57 ± 2.4^{cA}
60 days of lactation	10.55 ± 2.7^{B}	12.22 ± 3.4^{cA}	12.68 ± 2.8^{cA}

The different superscript lowercase letters found vertically on means indicate significant difference beween various stages of experiment at (P<0.05) . While, the different superscript uppercase letters found horizentally on means indicate significant difference beween treatment groups at (P<0.05) .

DISCUSSION

The neutrophils and lymphocytes participated in a reduction in the white blood cell count since the second month of pregnancy until their recurrent postnatal elevation. A reduction in the values of white blood cell count was the most prominent in Group E2 receiving organic selenium (5.12 ± 0.16 × 10^9/L) between 100-150 days of pregnancy. This observation was compatible with the value calculated in pregnant rhesus monkeys compared to non-pregnant animals (Rogers et al., 2005). The low level of neutrophils prevents the probable inflammatory process for the total period of the experiment, including the postnatal period. The percentage of lymphocytes, CD8+ and CD4+ subsets of T lymphocytes in pregnant and non-pregnant ewes remained within the normal physiological levels without significant differences between the groups. In the postnatal period there was a significant elevation in the white blood cell count, $CD4^+$ and $CD8^+$ subsets of T lymphocytes in the whole groups. The elevation in CD4+ and CD8+ subsets levels was twice higher than that reported by Thorp et al. (1991). Entrican et al. (2001) reported that the development of the fetus in the maternal uterus is associated with a huge stress on the maternal immunity system leading to the production of maternal immune-tolerance to the fetuses extraneous antigens. The histological study by Abu Nasar et al. (2002) revealed that the production of immune-tolerance in pregnant animals is highly concentrated in the tissues of uterus while the proportions of lymphocyte subsets are not affected in the peripheral blood of the pregnant ewes. Barrington and Parish,(2001) reported that the CD4+ and CD8+ subsets increases during the elevation of the antigen pressure, which is associated with the huge risk of infections during the puerperal period which needs the stimulation of the maternal immune system. In the puerperal period there was a significant elevation in the total leukocyte count comprising the neutrophils that participate in the mechanisms of the non-specific immunity in most species of animals. Reiterova et al., (2004) also reported a significant postnatal elevation in $CD4^+$ levels in *Toxocara canis* infected mice. The higher elevations in the levels of $CD8^+$ and $CD4^+$ subsets of lymphocytes were reported in E2 and E1 groups which were supplemented with organic selenium and sodium selenite coinciding with a high numbers of twins. The elevation in the percentage of $CD4^+$ between Day 30 and day 60 of lactation was statistically significant ($P< 0.05$) in E1 and E2 groups in comparison to the control group. Moodley et al. (2000) reported that the values of the $CD4^+$ / $CD8^+$ ratio which were higher than 1.0 as optimal in the animals free from diseases of specific cell-mediated immunity. In the current study the CD4+ / CD8+ ratio was 1.4 and this observation excludes presence of abnormal status in the specific cellular immunity. In the present study the statistical analysis of the average values during the total experimental period revealed presence of a high coefficient correlations between the following parameters: total proportion of lymphocytes and $CD8^+$ ($r_{xy} = 0.62$), total proportion of lymphocytes and $CD4^+$ ($r_{xy} = 0.78$), then $CD4^+$ and $CD8^+$ ($r_{xy} = 0.70$). Novoselec et al. (2022) reported that the addition of selenium to the rations of ewes in the late stages of pregnancy improves the oxidative stability, superoxide dismutase, and glutathione peroxidase activity, and decreases the lipid peroxidation in lambs and ewes.

The addition of organic selenium has a higher effect on the elevation of the activity of glutathione peroxidase and elevation of selenium concentration in the blood of lambs and ewes. Novoselec et al. (2022) also reported that the addition of (inorganic or organic) selenium to the rations of the ewes and their lambs have a positive influence on the metabolic activities and immune status of the lambs without any side effects. The selenium transfer through the placenta and milk, then it improves the vitality of the newborn lambs and increases the immunological responses in these lambs. So the postpartum and prepartum addition of selenium to the rations of pregnant ewes are very important to maintain normal selenium levels during the production stages of late gestation and early lactation in pregnant ewes and their lambs during the first six weeks of life (AL Mallah et al.,2017). The overall evaluation of the influence of selenium addition to the rations of sheep on the alterations in WBC count, percentages and levels of lymphocytes and their subsets ($CD8^+$ and $CD4^+$) in the non-pregnant period and during the gestation, puerperium and lactation period revealed a significant effect of either organic or inorganic selenium (Ahmed et al., 2019). There was a significant elevation in the percentage of CD8+ and CD4+ during pregnancy period and in the postnatal period and this suggests the importance of selenium supplementation to improve the immunological status of the animals especially at the pregnancy period and postnatal period (Alkass, 2017).

CONCLUSION

The results of the current study revealed that supplementation with both organic and inorganic selenium had positive effects on the blood parameters in the selenium-treated groups, especially in lactating animals, when compared to the control group.

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CONFLICT OF INTEREST

The author(s) participating in this scientific research declare that there is no conflict of interest regarding the publication of this paper.

NOVELTY STATEMENT

It was observed that giving organic and inorganic selenium which caused a significant increase ($P<0.05$) in levels of total of white blood cells, percentage of lymphocytes, and $CD4^+$ and $CD8^+$ subsets of lymphocytes of lactating ewes compared to control group.

AUTHORS CONTRIBUTION

All the author(s) contributed equally towards the completion of this paper.

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