



Effect of Slow-Release Pellets of Selenium and Iodine on Performance and Some Blood Metabolites of Pregnant Moghani Ewes and Their Lambs

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Abstract

The need for minerals during pregnancy in mammals increases by progress of gestation due to embryo development. Therefore, the supply of trace minerals for pregnant ewes in late pregnancy may be of vital importance. The present study was carried out to evaluate the effect of selenium and iodine release slowly on the performance and some blood parameters of pregnant ewes and their lambs. Experiments were performed on 40 pregnant ewes from 3 to 4 years old in the last month of pregnancy with a weighing average of 52 ± 5 kg and a factorial experiment (2×2) in a completely randomized design with 4 treatments and 10 replicates used for 120 days (30 days before and 90 days after parturition of ewes). Experimental treatments were containing the following: (1) control, (2) ewe receiving bolus containing iodine, (3) ewe receiving bolus containing iodine and selenium, and (4) ewe receiving bolus containing selenium. One month before the expected birth date, the pellets will be fed to each of the livestock by bolus gun. During the test period, ewes will not receive any other mineral supplements and will be kept in a group in a herd. Ewes were blood sampled at 10 days prepartum and 30 and 60 days postpartum. The results showed that body weight on birth and weaning of lambs and average daily gain of lambs were highest in bloused animals that were related to the slow-release selenium tablet lambs ($P < 0.05$). Lambs born to mothers receiving 0.3 mg day^{-1} of selenium per day had a keratin phosphokinase concentration of T_4 concentration and T_4 to T_3 lower than that of zero selenium ($P < 0.05$). Daily gain, glutathione peroxidase activity, and T_4 concentration at iodine 0.4 mg day^{-1} were significantly higher than iodine level ($P < 0.05$). The adding of slow- release pellets of iodine and selenium improved the immune system.

Keywords Iodine · Pellet · Selenium · Slow-release

Implications Nutritional status during pregnancy and the proper growth rate of lambs in early life are two essential factors in good sheep production systems. Traditionally, farmers do not use any mineral supplements for their animals. The need for minerals during pregnancy in ewe increases for embryo development and reaches its peak during the late pregnancy, including the supply of trace minerals for pregnant ewes to protect the fetus and improve the growth of lambs born. This study aimed to investigate the effect of slow-release pellets of iodine and selenium on the performance of pregnant ewes and their lambs. The adding of slow-release pellets of iodine and selenium improved the immune system.

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Introduction

The nutritional status during pregnancy and the proper growth rate of lambs in early life and before puberty are two essential categories in breeding sheep. Live weight at the onset of mating is a vital determinant in the reproductive performance of lambs [1]. Inappropriate feeding, especially dietary constraints in late pregnancy, can delay intrauterine growth. The energy needs of the mother increase in the third full pregnancy period; hence, the energy constraints have adverse effects on breast milk production, colostrum immunoglobulin G (Ig G) levels, and lamb's growth. Oxidative stresses intensify in late pregnancy with energy constraints. Environmental conditions and malnutrition associated with physiological changes in late pregnancy and near birth, such as a change in the secretion of progesterone, lactoferrin, and estradiol-10 beta, induce insulin resistance in the mother [2].

One of the ways to optimize livestock production and reproductive conditions is through the use of metabolic enhancers by improving nutrient metabolism and eliminating or reducing stress conditions. Metabolic enhancers, such as anabolic steroids, somatotropin, beta-agonists, vitamins, and minerals, which are fed to supranatural levels, increase the growth rate, improve feed efficiency, increase meat production, reduce carcass fat, and improve reproductive performance. Mineral substances are one of the most vital metabolic enhancers such as selenium and iodine as essential micronutrients for some body functions of livestock [3], so that iodine and selenium have significant effects on the thyroid gland and its hormones. Researchers have reported that excessive iodine supplementation in lactating ewes increases the concentration of iodine in milk, but on the other hand, such high iodine intake will disrupt the oxidative and non-oxidative equilibrium in the thyroid gland. To compensate for this imbalance, an appropriate amount of glutathione peroxidase selenium is required [4]. Researchers reported that in rations with equal amounts of selenium, with increasing iodine, the T_3 concentration dropped several days after lambing. A lower quantity of T_3 in plasma with high iodine amount was probably due to higher iodine concentrations in plasma [5]. Selenium is one of the elements with a wide range of functionalities in the body due to the role of antioxidants and the entry of important compounds such as several seleno-proteins [3]. Different methods have been used, such as the use of mineral licking blocks, the feeding of common trace micronutrients, the injection of elements, and the use of slow-release pellets or bolus [6]. Free access to livelier blocks of minerals usually results in variable intake in animals, so that around 50% of herds may not receive any supplementation. Feeding the usual supplements of the ingredients also shows short-term responses to these supplements [7]. The injection also requires the sequential injections of these elements [7]. Slow-release tablets or pellets are stored in ruminant animals

based on density and release the desired elements at a controlled rate for a specified period of about 6 months and permanently. Previous studies have shown that slow-release trace pills can release their own rumen and improve the status of trace minerals in sheep [8]. The use of slow-release pellets of copper, cobalt, and selenium has been reported to improve the performance and status of the above elements in comparison to the control group in sheep [7]. In pregnant ewes, severe iodine deficiency can reduce fetal brain weight and reduce birth weight [9]. For example, the most commonly reported goat's need for iodine is higher than other ruminants. The proper concentration of iodine in dairy cows is 0.3 mg kg^{-1} dry matter, which is not sufficient to prevent goiter in suckling calves fed breast milk [10].

Sevcikova et al. [11] studied the effects of both organic and inorganic significantly affected selenium on selenium levels in pregnant goats and their kids at time of weaning. Pechova et al. [12] examined the effect of both organic and inorganic of selenium fed on pregnant goats on blood parameters and the concentration of selenium in urine and blood, which was sufficient to prevent selenium deficiency in the kids at time of weaning. Kachuee et al. [13] investigated a positive correlation between serum selenium concentration and copper and iron levels, as well as a negative correlation between selenium and zinc in the treated doses during late gestation in Merghoz goats and their kids. Kendall et al. [7] reported that using slow-release pellets containing copper, cobalt, and selenium, as well as the use of zinc, cobalt, and selenium slow release pellets, improved the performance and status of the above elements compared to the control group in sheep. Aliarabi and Fadayefar [6] reported that with the use of slow-release pellets containing zinc, selenium, and cobalt, the selenium deficiency of the ewes and their lambs was overcome and also a significant reduction in signs of white blood cell disease has been reported. The activity of alkaline phosphatase and glutathione peroxidase enzymes and serum triiodothyronine concentrations in the pill intake group was significantly higher than the control group. The small livestock farming system in Iran is mainly traditional, and in this system, mineral additions are not normally used. Therefore, this study aimed to investigate the effect of slow-release pellets of iodine and selenium on performance and some blood parameters of Moghani pregnant ewes and their lambs.

Materials and Methods

Animal and Diets

In order to conduct this experiment, 40 pregnant ewes (Synchronized for parturition) of 3 to 4 years old in the last month of pregnancy with an average weight of $52.5 \pm 5 \text{ kg}$ were selected and tested in a factorial (2×2) completely

randomized design with 4 treatments and 10 replicates for each treatment was used for 120 days (30 days before and 90 days after parturition). Factors include two levels of 0 and 0.3 mg selenium per day and two levels of 0 and 0.4 mg iodine per day. The treatments were (1) pregnant ewes without a pellet as control (zero iodine and selenium levels), (2) pregnant ewes receiving pellet of selenium (with a release rate of 0.3 mg selenium per day), (3) pregnant ewes receiving pellet iodine (with a release rate of 0.4 mg iodine per day), and (4) pregnant ewes receiving pellets containing selenium and iodine (with a release rate of 0.3 mg selenium per day and 0.4 mg iodine per day).

The composition and components of the ewe's diets are presented in Table 1 for 30 days before and 90 days after parturition. Within 30 days of the expected date of parturition, the pellets will be fed to each ewe by bolus pill gun special sheep. To ensure the use of the pellets, the ewes will be kept individually for half an hour. During this time, they tagged for identification at the next stages of the experiment, and their horns and backs marked by spray paint. The trial period was 120 days (from 1 month before parturition to 90 days after lamb birth). During the test period, ewes did not receive any other mineral supplements and were kept in a group in a herd.

Analytical Procedures

Blood samples were taken from all the ewes at 8:00 am to prepare blood samples 10 days before and 30 and 60 days after parturition. Blood samples per ewe were collected in 2 separate tubes per day, containing heparin for preparation of a

Table 1 Chemical composition and components of basic diets

Ingredients	Close-up diet % of DM	Lactation diet % of DM
Alfalfa hay	30.3	55.2
Barley straw	38.6	–
Corn grain	13.5	20.2
Soybean meal	2.48	6.1
Barley grain	13.77	12.1
Wheat bran	–	4.5
Calcium-carbonate	1.1	1.5
Calcium-phosphate (mono)	0.25	0.4
Chemical compositions		
Metabolizable energy (Mcal kg ⁻¹)	2.131	2.45
Crude protein	12	16
Neutral detergent fiber	46.3	32
Ether extract	2.43	2.8
Ash	7.94	8.5
Calcium	1.05	1.5
Phosphorus	0.27	0.4

*No mineral supplement was added to the daily ration

complete blood sample and an anticoagulant tube for serum extraction. Blood samples were kept at -80°C until analysis to measure glutathione peroxidase activity. Non-heparin blood samples were centrifuged at 3000 rpm for 15 min, and their serum was stored to determine the concentration of selenium, zinc, copper, triiodothyronine, and thyroxin hormones and phosphocreatine kinase enzyme activity at a temperature of 20°C until analysis.

As mentioned, complete blood samples were sent to the laboratory at all sampling days for determination of blood parameters and immediately analyzed on the same day. Blood parameters including white blood cell count, lymphocyte count, red blood cells, and hemoglobin were determined using an automatic cell counting machine (Diatron Abacus C Model 2.8, manufactured by Austria).

The activity of the glutathione peroxidase (GPX) enzyme was determined by Paglia and Valentine [14] using RANSEL kit of RANDOX, UK. The basis of this experiment is as follows: glutathione peroxidase catalyzes the reaction of glutathione oxidation by the use of cumene hydroperoxide (ROOH). Then, in the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is converted into a reduction form. The decrease in absorption was measured at 340 nm.

According to the plan, the manufacturer of the kit first diluted 50 μL of Heparin blood sample with 3 mL of diluent solution, and then mixed 20 μL of diluted sample with 1 mL of R1 and 40 μL of the R2 reagent. After 1 min, it was absorbed against blank containing distilled water, and was read by Varian's Cary® range of UV-Vis spectrophotometry (Australia made, Cary 100 model). Then they were read after 1 and 2 min, respectively. Absorption of blank from sample absorption was reduced and the mean absorbance of the sample was calculated in a minute, and using the following formula (1), the activity of glutathione peroxidase enzyme was determined:

8412

\times Mean changes in sample absorbance during 1 min

= activity of glutathione peroxidase ($\mu\text{kat l}^{-1}$) (1)

Finally, the results were expressed in microkatal in liter. The following procedure was used to convert units in liters to microkatal in liters from the formula (2).

$0.0167 \times \text{unit in liters} = \text{microkatal per liter}$ (2)

The activity of the enzyme keratin phosphokinase was determined using the Pars test company's kit and based on the International Federation of Clinical Chemistry International Federation (IFCC). According to the order of the company,

the manufacturer of the solution kit of reagent no. 1 was added to the ratio 4 to 1 with the reagent no. 2. Forty microliters of the sample with 1000 μL of mixed solution 1 and 2 was added, and the amount of light absorption after 1 min was read. The timer was used, and after just 1, 2, and 3 min, the optical difference was determined from the previous minute. The number of light absorption differences after minutes 1, 2, and 3 is combined and divided by number 3 and the mean of multiplied by 4127 multiplied. The activity of creatine phosphokinase was calculated from the formula (3).

4127

$$\begin{aligned} & \times \text{Mean changes in sample absorption during 1 min} \\ & = \text{Activity of keratin phosphokinase (unit } \Gamma^{-1} \text{)} \end{aligned} \quad (3)$$

This experiment was performed using an ELISA Kit manufactured by the Anti-Gotstor Co. and based on an immune-enzyme immunoassay. The triiodothyronine in the specimens competes to attach to the polyclonal antibody of the anti- T_3 rabbit coated on the wells to the T_3 attached to the horseradish peroxidase (HRP- T_3) enzyme. After the incubation time, the wells are drained and rinsed. The enzyme substrate was then added to each well, and the enzyme activity was inversely proportional to the concentration of T_3 in the samples. The T_3 standards with a specific concentration were tested in conjunction with the unknown samples. The number of catalyzed wells for standards and samples was selected in binary and placed the remaining wells along with the absorbent material in a special bag and closed it. Then, add 20 μL of standards and samples to each well. Also, for all wells was added 100 μL of conjugated enzyme conjugate (HRP- T_3). The plates were then shaken slowly for 15 s until the contents of the wells were well mixed. Then, the wells were covered with distinctive plates and the wells were incubated for 1 h at room temperature (22–28 $^{\circ}\text{C}$) and in the dark. Empty the contents of the wells, and the wells were washed five times with 300 μL of the buffer. At the end of the wash, all the liquid contained in the wells was depleted with mild blows on the absorbent paper. Also, 100 μL of the prepared substrate was added to all wells and incubated for 20 min at room temperature and in the dark. Then, the amount of 100 μL of stopping solution was added to all wells and the light absorption of each well was read at 450 nm with ELISA reader. Finally, using the mean light absorption of the standards and their specific concentration, the standard curve was plotted. Then, based on this, the concentration of the triiodothyronine hormone was obtained. The T_4 concentration measurement method was similar to that of T_3 . Zinc, selenium, and copper concentrations were measured by atomic absorption spectrometry (Varian SpectraAA220, made Australia). Plasma selenium

concentration was measured by atomic absorption and hydride ion production method.

Statistical Analysis

All data collected during the experiment were prepared using Excel software and were prepared for statistical analysis. Data analysis was performed using SAS statistical software [15]. The statistical model of the design for birth weight parameters, daily weight gain, and weight from milking was a simple factorial experiment in a completely randomized design. The statistical model was as follows (formula (4)):

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk} \quad (4)$$

where Y_{ijk} = the amount of each observation; μ = mean of the total of the studied variable; A_i = effect of selenium level; B_j = Effect of iodine level; AB_{ij} = Interaction between selenium and iodine levels; and e_{ijk} = effect of test error.

Mean comparison was done by Duncan's test method, and assumption of error 0.05 and $P < 0.05$ was considered significant.

Since the interaction of treatment at the time was not significant in all blood factors, these factors were mixed with mixed procedures in the form of repeated measures per unit time in a factorial experiment in a completely randomized design, the statistical model of which is shown below formula 5.

$$\begin{aligned} Y_{ijkl} = \mu + A_i + B_j + AB_{ij} + T_l + AT_{il} + BT_{jl} + ABT_{ijl} \\ + e_a + e_b \end{aligned} \quad (5)$$

where Y_{ijkl} = the amount of each observation; μ = mean of the total studied variables; A_i = the effect of the level of selenium; B_j = the effect of iodine level; AB_{ij} = interaction between the surface of selenium and iodine; T_l = time effect; AT_{il} = interaction between the selenium level and time; BT_{jl} = interaction of iodine and time levels; ABT_{ijl} = interaction of selenium levels and iodine and time levels; e_a = Main error effect; and e_b = minor error effect.

Results

Performance of Lambs

The results of the mean birth weight, mean weaning weight (90 days), and the mean daily weight gain of lambs born to mothers receiving slow-release pellets containing iodine and selenium are shown in Table 2. The results indicated lambs born to mothers receiving 0.3 mg day^{-1} of selenium, had mean weaning weight, and had the average daily gain higher than zero selenium ($P < 0.05$). However, the birth weight of lambs

Table 2 Average birth weight, weaning weight (90 days), and daily weight gain of lambs born to mothers receiving slow-release pellets containing iodine and selenium

treatments	Average birth weight (kg)	Weaning weight (kg)	Average daily gain (kg day ⁻¹)
Selenium (0) iodine (0)	3.07	23.11 ^b	0.230 ^b
Selenium (0) iodine (0.4)	3.10	24.18 ^a	0.285 ^a
Selenium (0.3) iodine (0)	3.19	24.30 ^a	0.292 ^a
Selenium (0.3) iodine (0.4)	3.13	24.34 ^a	0.294 ^a
SEM	0.026	0.14	0.006
<i>P</i> value			
Treatment	0.63	0.002	0.001
Selenium levels	0.58	0.001	0.001
Iodine levels	0.34	0.001	0.001
Selenium × iodine	0.57	0.10	0.06

Means without a common letter within the same column differ ($P < 0.05$)

SEM standard error of the mean

was not affected by selenium levels ($P > 0.05$). Also, there was no significant difference between the level of 0.4 mg iodine per day and the level of zero in terms of birth weight of lambs and the mean weaning weight ($P > 0.05$) but the average daily gain in lambs born to mothers receiving 0.4 mg day⁻¹ iodine was significantly higher than zero ($P < 0.05$). On the other hand, there was no interaction between iodine and selenium in relation to birth weight, mean weaning weight, and the average daily gain of lambs ($P > 0.05$).

Blood Minerals

The results of serum selenium concentrations ($\mu\text{g L}^{-1}$) of lambs born to mothers receiving slow release iodine and selenium pellets at age 10, 60, and 90 days are shown in Table 3. As it is seen, plasma selenium concentrations of pellet receiving ewes were significantly higher in postpartum than control ewes. The results showed that lambs born to mothers receiving 0.3 mg day⁻¹ of selenium had higher serum selenium amount than zero selenium ($P < 0.05$). The serum concentration of copper at 60 years of age was significantly higher than the 10 and 60 days of the kids.

Blood Enzymes

Based on Table 4, the activity of glutathione peroxidase enzyme was greater in slow-release pellet receiving ewes than the control ewes, but the activity of creatine phosphokinase was lower in the control group than the control group ($P < 0.05$). There was no significant difference in creatine phosphokinase concentration between 60 and 90 days postpartum ($P > 0.05$).

Thyroid Hormones

According to the results of this study as shown in Table 5, lambs born to mothers receiving 0.3 mg day⁻¹ of selenium had a T₃ higher concentration than zero selenium ($P < 0.05$). Lambs born to mothers receiving 0.3 mg day⁻¹ of selenium had a concentration of T₄ and a T₄ to T₃ lower than that of zero selenium ($P < 0.05$).

Moreover, there was no significant difference between the level of 0.4 mg iodine day⁻¹ with zero levels for T₃

Table 3 Selenium, zinc, and copper concentrations (mg L⁻¹) of lambs born to mothers receiving slow-release pellets containing iodine and selenium at 10, 60, and 90 days of age

Treatments	Selenium	Zinc	Copper
Selenium (0) iodine (0)	0.045b	0.422	0.368
Selenium (0) iodine (0.4)	0.043b	0.435	0.380
Selenium (0.3) iodine (0)	0.078a	0.395	0.385
Selenium (0.3) iodine (0.4)	0.088a	0.410	0.378
SEM	0.005	0.011	0.010
10 days of age	0.058b	0.366a	0.360b
60 days of age	0.062a	0.354b	0.390a
90 days of age	0.062a	0.362a	0.348b
SEM	0.004	0.003	0.005
<i>P</i> value			
Treatment	0.001	0.528	0.265
Selenium levels	0.001	0.224	0.186
Iodine levels	0.42	0.471	0.499
Selenium × iodine	0.62	0.807	0.192
Age	0.001	0.001	0.001
Treatment × age	0.69	0.49	0.869

Means without a common letter within the same column differ ($P < 0.05$)

SEM standard error of the mean

Table 4 Glutathione peroxidase enzyme activity of the whole blood and serum creatine phosphokinase enzyme activity in lambs born to mothers receiving slow-release pellets containing iodine and selenium at the age of 10, 60, and 90 days

Treatments	Glutathione peroxidase (U L ⁻¹)	Creatine phosphokinase (U L ⁻¹)
Selenium (0) iodine (0)	70.50b	208.12a
Selenium (0) iodine (0.4)	78.35b	198.40a
Selenium (0.3) iodine (0)	255.42a	166.25b
Selenium (0.3) iodine (0.4)	260.40a	170.83b
SEM	1.58	7.03
10 days prepartum	158.42c	145.20b
30 days postpartum	167.92b	207.30a
60 days postpartum	190.25a	202.12a
SEM	1.75	5.26
<i>P</i> value		
Treatment	0.001	0.001
Selenium levels	0.001	0.001
Iodine levels	0.31	0.81
Selenium × iodine	0.63	0.587
Age	0.001	0.001
Treatment × age	0.72	0.07

Means without a common letter within the same column differ ($P < 0.05$)

SEM standard error of the mean

concentration and T₄ to T₃ ratio ($P > 0.05$). However, T₄ concentration at iodine 0.4 mg day⁻¹ was significantly higher than the iodine level ($P < 0.05$). Concentrations of T₃ and T₄ to T₃ ratios were significantly different at different ages, and T₄ concentration was not significant. The concentration of serum T₃ at 60 days of age was significantly lower than that of 10 and

90 days of age ($P < 0.05$), and there was no significant difference between 10 and 60 days of age. The ratio of T₄ to T₃ at 60 years of age was significantly lower than the 10 and 90 days of lambs ($P < 0.05$), and there was no significant difference between 10 and 90 days of age. Also, the interaction between selenium and iodine levels was significant for T₄

Table 5 Concentrations of T₃ and T₄ hormones and T₄ to T₃ serum levels in lambs born to mothers receiving slow-release pellets containing iodine and selenium at 10, 60 and 90 days of age

Treatments	T ₃ hormone (ng mL ⁻¹)	T ₄ hormone (ng mL ⁻¹)	T ₄ to T ₃ serum levels
Selenium (0) iodine (0)	1.90a	52.50	27.64
Selenium (0) iodine (0.4)	2.10ab	65.80	31.34
Selenium (0.3) iodine (0)	1.85a	45.30	24.49
Selenium (0.3) iodine (0.4)	2.20a	42.40	19.28
SEM	0.16	1.15	1.25
10 days of age	1.80	58.50	25.10a
60 days of age	1.65	50.35	20.39ab
90 days of age	2.10	56.40	23.14b
SEM	0.23	1.25	1.85
<i>P</i> value			
Treatment	0.01	0.001	0.001
Selenium levels	0.001	0.001	0.001
Iodine levels	0.56	0.001	0.71
Selenium × iodine	0.35	0.001	0.98
Age	0.002	0.001	0.06
Treatment × age	0.09	0.19	0.39

Means without a common letter within the same column differ ($P < 0.05$)

SEM standard error of the mean

concentrations but there was no interaction between treatment and age in relation to the concentration of T_3 and T_4 hormones and the ratio between T_4 and T_3 lambs ($P > 0.05$).

Blood Hematological Parameters

In this study, the effect of selenium level was significant only on the concentration of red blood cells ($P < 0.05$), and no significant effect was observed on hemoglobin, white blood cell and lymphocyte concentrations ($P > 0.05$). There was no significant difference between the levels of 0.4 mg iodine day⁻¹ with zero levels on the concentration of any of the evaluated traits in the field of hematology ($P > 0.05$). Only the concentration of blood lymphocytes at different ages was significant so that at 90 days of age, a lower concentration was observed compared to 10 and 60 days of age. The concentration of lymphocytes at 10 and 60 days of age was not significantly different. There was no interaction between the levels of selenium and iodine and between treatment and age in relation to red blood cell, hemoglobin, white blood cell, and blood lymphocyte in lambs ($P > 0.05$) (Table 6).

Discussion

Performance of Lambs

In the present study, the significant effect of mother's supplementation with selenium and its effect on the performance of lambs were observed. Similarly, Gabryszuk and Kelewik [16] reported that selenium injections into pregnant ewes increased the mean daily weight gain from birth to age 28. Similar results were also obtained by Ziaei et al. [17], which showed a higher increase in the weight gain of lambs born from ewes fed with selenium-fed diets compared to lambs born to ewes. Soliman et al. [18] showed that vitamin E injections with selenium into ewes during the last 4 weeks of pregnancy and the period of infancy significantly increased the mean body weight and daily gestational weight of the offspring from birth to time. There was a significant increase in weight of birth and mean body weight in ewes. It has also been reported that injection of vitamin E and selenium into ewes before mating and lambing caused a significant increase in daily weight and average body weight from birth to 60 days [19]. The significant daily weight gain and total body weight gain may be due to higher concentrations of thyroxin in them [20], which affects their metabolic process. However, the results of this study are contrary to the results of previous studies where selenium, whether organic or inorganic, was received alone during pregnancy and lactation had no significant effect of selenium on birth weight and the growth of their lambs [21]. Also, Kachuee et al. [13] showed that the supplementation of

maternal selenium to Merghoz goats in the 4 weeks of pregnancy and the infancy period for 4 weeks did not have any significant effect on birth weight and not on lamb's growth during 4 weeks old. In this study, significant effects of iodine supplementation on the growth performance of the kids were observed. In agreement with the findings of this study, daily weight gain and body weight were significantly higher in iodine-fed diet growing male goats [20].

Blood Minerals

Given that selenium is transmitted to the baby through the placenta to the embryo, as well as milk and colostrum, even in livestock faced with a lack of need for selenium transfer through active pairs, priority is given to supplying the fetus [22]. The results of the present experiment were in agreement with the report of Awawdeh and Talafha [23], which stated that selenium supplementation in the ewes' diet and subsequent parturition of ewes after 4 weeks increased the concentration of selenium in the blood of lambs. Experimental data of Hefnawy et al. [24] and Gunter et al. [25] showed a high positive correlation between selenium concentration in serum of ewes and lambs ($r = 0.879$, $r = 0.798$, $r = 0.74$). The transfer of selenium through the placenta is one of the primary sources of supply of selenium to lambs before colostrum. In the Hefnawy et al. [22] experiment, selenium transfer from mother to kids was affected by organic selenium doses. Erdoğan et al. [26] also reported that the use of slow-release pellets containing selenium, copper, zinc, cobalt, phosphorus, manganese, and iodine in the late stages of pregnancy (60 days before parturition) for ewes increased serum selenium levels in their lambs. In the present study, the group receiving 0.3 mg selenium day⁻¹ increased serum selenium concentration compared to zero selenium levels. Therefore, the slow-release pellet of this study has been able to significantly overcome the deficiency of selenium in the lambs and increase blood selenium levels and improve their function. In general, the results of this study showed that the traditional method of ewes production that are not used in mineral supplements, offering slow-release selenium pellets, or just injections of a dose of vitamin E and selenium in the late pregnancy period, can provide the daily intake of nutritionally the essential trace elements increase their performance and prevent problems caused by deficiency of these elements. Jalilian et al. [27] reported that selenium concentrations increased significantly in the plasma of squirrel ewes and their lambs supplemented with selenium. The results of Davis et al. [28] showed that plasma selenium levels in lambs were affected by selenium concentration of ewes dietary and ranged from 74 to 775 $\mu\text{g L}^{-1}$. However, no lamb had a selenium level close to or above 1400 $\mu\text{g L}^{-1}$, suggesting that sheep poisoning symptoms appear at this level [28]. Hall et al. [29] reported that selenium injection into selenium-deficient Herford heifers

Table 6 Some blood hematological parameters of the lambs born to mothers receiving slow-release pellets containing iodine and selenium at the age of 10, 60 and 90 days

Treatments	Red blood cell (10^{12} L^{-1})	Hemoglobin (g dL^{-1})	White blood cell (10^9 L^{-1})	Lymphocyte (%)
Selenium (0) iodine (0)	16.50b	9.15	18.50	52.24
Selenium (0) iodine (0.4)	17.30b	10.20	20.14	46.78
Selenium (0.3) iodine (0)	17.60a	9.95	18.68	48.12
Selenium (0.3) iodine (0.4)	17.54a	10.05	18.25	50.35
SEM	0.16	0.18	0.46	0.80
10 days of age	17.45	9.20	17.20	55.95a
60 days of age	17.20	9.90	16.85	48.20ab
90 days of age	16.82	10.25	16.40	40.70b
SEM	0.16	0.85	0.18	1.53
P value				
Treatment	0.001	0.81	0.51	0.87
Selenium levels	0.001	0.94	0.45	0.48
Iodine levels	0.46	0.41	0.31	0.85
Selenium \times iodine	0.69	0.80	0.63	0.80
Age	0.49	0.01	0.32	0.02
Treatment \times age	0.30	0.33	0.13	0.07

Means without a common letter within the same column differ ($P < 0.05$)

SEM standard error of the mean

increased the concentration of selenium in their calves. Wallace et al. [30] stated that selenium injection had no effect on the selenium concentration of young calves born to mothers who received enough selenium daily, but selenium concentrations in calves born of mothers who were suffering from selenium deficiency increased [31]. Therefore, it can be concluded that the present experimental ewes feeding on the rangelands of this study are probably marginal selenium deficiency, which has shown a positive response to the slow release of selenium pellet due to lack of selenium in soil and herbaceous plants. Further studies are needed to clarify this issue the effect of selenium concentration and the dominant herbaceous plants of these regions are needed. Also, Kachuee et al. [32] showed that the supplementation of maternal selenium to during late pregnancy on selenium, zinc, copper, and iron concentrations in Khalkhali goats and their kids can affect and correlate with Cu, Zn, and Fe levels, and this effect depends a lot on the chemical or physical variety of Se supplementation.

Blood Enzymes

In the study of Pavlata et al. [33], a significant positive correlation was found between the concentration of selenium and the concentration of glutathione peroxidase enzymes. However, in the present study, glutathione peroxidase activity was a reflection of serum selenium serum

concentration in lambs, and exactly, the same trend was observed. Free radicals are constantly produced in physiological activity and their production increases under stress conditions.

The main sources of free radical production in cells include the electron transfer chain in mitochondria, the enzymes of xenobiotic metabolism, and immune cells, and in particular immune cells to kill pathogens, produce free radicals that selenium prevents arachidonic acid peroxidation, thereby eliminating cells and protects tissues from damage to free radicals [34]. In lambs with white muscle disease, the activity of the enzyme creatine phosphokinase is up to 100 times higher, because the cell membrane damage causes the inoculation of enzymes to enter the cell [35]. In the present study, no significant effect was found in the activity of creatine phosphokinase enzymes among the recipient groups of different levels of selenium.

Similarly, Alimohamady et al. [36] have no significant effect on creatine phosphokinase in lambs fed with basal diet containing 0.06 mg selenium per kg dry matter (control) or base diet plus 0.2 mg Se per kg DM. In contrast to the results of this study, there are reports that selenium supplementation [37] and selenium injections [38] that showed a decrease in serum creatine phosphokinase activity in sheep fed with a base diet were effective in selenium deficiency. This difference may be due to the type of ration consumed, the growth rate and physiological conditions of the livestock being studied.

The level of glutathione peroxidase activity is one of the indicators for assessing the status of selenium in the body [21]. In a study, Kendall et al. [7] reported a significant increase in the activity of glutathione peroxidase in lambs receiving a slow-release pellet containing zinc, selenium, and cobalt, which is consistent with the results of this study. Considering that, the amount of selenium in this study was equal to the minimum marginal margin of selenium (0.06–0.0 mg per kg DM) [35]. Therefore, it can be concluded that the administration or offering of a slow-release pellet containing selenium with sufficient and continuous supply of this element resulted in the optimum level of glutathione peroxidase activity. Since that creatine kinase (CK), formerly known as creatine phosphokinase, is an intracellular enzyme whose increase in blood plasma indicates muscle tissue damage due to stress, infection, and the antioxidant deficiency such as selenium [28]. Reducing the activity of this enzyme in pellet receiving ewes can be related to selenium provision in the effect of pellet consumption. This experiment is consistent with several reports that the effect of adding selenium orally and injector on the decrease in the activity of this enzyme in the target livestock with selenium deficiency [37, 38]. Nudda et al. [39] reported that iodine supplementation and selenium supplementation reduced blood urea nitrogen. A possible explanation for reducing the concentration of blood urea nitrogen and subsequently the urine nitrogen in the milk after increasing the levels of iodine may be an interaction between iodine and the activity of some ruminal microbial species. In ruminant animals, an irritant effect associated has shown with iodine doses for digestion of cellulose by ruminal microorganisms [40]. Therefore, it can be assumed that iodine may partially contribute to the digestion of the ruminal microbial protein and, as a result, reduce the production or consumption of ammonia. Consequently, there may be a decrease in the concentration of ammonia from the rumen to the blood in the iodine-supplemented groups.

Thyroid Hormones

The results of all evaluated traits showed that over time, the concentration of nitrogen, creatine phosphokinase, and glutathione peroxidase activity increased significantly ($P < 0.05$). Hefnawy et al. [41] also reported that injection three doses of selenium at 8 and 5 weeks before and 1 week after parturition for pregnant ewes could maintain a higher concentration of T_3 hormone in the lambs born until 8 weeks of age than the control treatment. Rock et al. [42] reported a higher concentration of T_3 hormone in lambs born of mothers receiving selenium than lambs born of control mothers. Also, the addition of selenium to the diet of goats [5] and pregnant cows [43] increased the concentration of T_3 hormone in lambs and calves. Maia et al. [44] reported that selenium deficiency reduces the activity of the deiodinases, which increases the

serum concentrations of T_4 hormone. Therefore, reducing the T_4 in the lambs produced by mothers receiving 0.3 mg day^{-1} of selenium per day compared to zero selenium represents a more appropriate supply of selenium and, therefore, increased the deiodinases activity. However, considering the significant interaction between selenium levels and iodine levels, one cannot rely solely on the main effects. In this regard, by the effect surveying of treatment, it is determined that lambs born of mothers receiving iodine alone compared to control treatment significantly increased the concentration of T_4 . This case may be due to increased iodine titration for T_4 synthesis in the thyroid.

Selenium has the most essential role in the production, activity, and metabolism of thyroid hormones after iodine, and it has been shown that selenium concentration in the thyroid gland is high and there is a close relationship between the amount of this element in the body and the production and activity of thyroid hormones [45]. In addition to the role of selenium anti-oxidation in thyroid protection, this element also exists in the structure of the dinosaurs responsible for the transformation of T_4 into a form with more activity and bio-filtration, T_3 [46].

Blood Hematological Parameters

Very limited works have been carried out to examine of the effect of iodine supplementation on the hematological parameters of ewes. However, consistent with the results of this study, Rajendran et al. [47] showed that iodine supplementation did not affect the hematological characteristics of goats; although, in the present study, iodine supplementation increased the serum RBC level. Pattanaik et al. [48] reported that supplementation of iodine in the diet increased the concentration of hemoglobin in goats. The researchers said that increasing hemoglobin concentration in iodized goats was associated with better protein conditions in their bodies, which could be caused not only as a result of the consumption of nitrogen but also as a result of raw protein intake. The effects of selenium supplementation on hematological parameters in livestock are controversial. Selenium hematopoietic effects can be related to the protective effect of the cell membrane and intracellular organelles by the antioxidant effects of selenium, thereby increasing the half-life of red blood cells and leukocytes [38]. In accordance with the results of this study, the results of Alimohamady et al. [36] showed that selenium supplementation did not affect the hematological parameters of lambs. Mohri et al. [38] did not show a significant result on white blood cell counts between treatments with selenium and vitamin E injections in 70-day-old lambs. Faixova et al. [37] reported that the use of oral selenium for 3 months in lambs resulted in lower levels of white blood cells in experimental groups than in the control group.

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