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Research article

EFFECT OF ORGANIC SELENIUM AND IODINE SUPPLEMENTATION ON SELENIUM AND THYROID HORMONES STATUS OF LACTATING EWES AND LAMBS

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The aim of this research was to determine the influence of 56-days (from day 4 to day 60 post partum) of simultaneous supplementation of selenium (Se) and iodine (I) enriched *Chlorella* algae on blood and urine Se concentrations and thyroid hormone levels in ewes and lambs. The study included 18 Šumava sheep ewes and their lambs divided into three groups (CON, HSe, HSeI). Selenium and I content in the diet of ewes measured 0.2 and 0.7 mg (CON), 0.4 and 0.7 mg (HSe), 0.4 and 1.3 mg (HSeI) per kg of dry matter. During the experimental period a significantly higher blood serum Se concentration was determined in ewes of the HSeI group ($P < 0.001$) and urine ($P < 0.01$). A significantly higher ($P < 0.001$) blood serum Se concentration was found also in lambs of the HSeI group. The highest Se concentration in the blood serum was recorded in ewes (128.0 μg/L) and lambs (74.2 μg/L) on the 26th day of the experiment. The results demonstrate a higher utilization of Se administered in the organic form accompanied by a higher intake of I applied also in the organic form. Plasma thyroid hormone levels in ewes were not explicitly affected by higher Se and I supplementation. A higher Se concentration in the lambs' blood serum led to significantly higher ($P < 0.05$) T3 levels up to 30 days post birth.

Key words: *Chlorella* algae, iodine, selenium, sheep, thyroxine, triiodothyronine

INTRODUCTION

Iodine (I) and selenium (Se) are micronutrients essential for normal thyroid function and play a vital role in maintaining good health in animals. Iodine is a component of thyroxine (T4) and triiodothyronine (T3) hormones. Selenium in selenoenzymes iodothyronine deiodinases plays a crucial role in the activation or inactivation of both thyroid hormones [1,2]. Moreover, Se is found as selenocysteine in the catalytic centre of enzymes protecting the thyroid gland from free radical damage [3,4].

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Several studies have examined how dietary I or Se intake affects Se status and thyroid gland function in different animal species. For example, I intake in the diet for growing kids (0.14 – 0.29 mg per head/day) along with I administration per os (0.2 – 0.3 mg per head/day) led to a significant decrease in Se concentration in the blood (88 μg/L) and glutathione-peroxidase (GSHPx) activity in comparison with the group without I supplementation. No significant differences were found in T3 or T4 levels [5].

Numerous studies have demonstrated the relationship between Se supplementation, its concentration and GSHPx activity in different tissues. For example, Misurova et al. [6] found out, that *per os* Se supplementation (0.43 mg per head/day) significantly increased Se concentration in the blood (177 μg/L) and GSHPx activity in goats. Chadio et al. [7] and Travnicek et al. [8] confirmed these results in ewes and lambs.

The form of I and Se supplementation in animal diets can be either inorganic [9] or organic [10]. It is generally accepted that the inorganic form is not utilized as efficiently as the organic form [6]. Moreover, organic forms of Se are absorbed and retained more readily by ruminants than inorganic forms [11]. Therefore, recent studies have focused on comparing both these forms [12,13].

Although there is enough information available on the effects of either Se or I deficiency on the development of some human and animal diseases, there is far less information about their mutual interactions [14]. There is a lack of studies about the impacts of co-administration of Se and I in sheep, particularly for organic forms.

The aim of the present study was to evaluate Se status and thyroid hormone levels in ewes and their lambs during administration of I and Se in the organic form.

MATERIALS AND METHODS

Animal, feeding and experimental design

The 56-day experiment was conducted at the Faculty of Agriculture of The University of South Bohemia in České Budějovice. The Animal Care and Use Council of the University of South Bohemia approved the use and treatment of animals in this study.

Eighteen pregnant ewes of Šumava sheep (dual-purpose breed) were divided into three groups (control – CON; experimental – HSe, HSeI; with 6 animals in each group), which were balanced for parity (4) and mean weight (54.5 ± 3.0 kg). The ewes were stabled in a conventional pen (1.6 m2 / head) with free access to water.

The composition of the feed ration is shown in Table 1. All ewes received an identical diet during the pregnancy period and up to the first 3 days post partum. From the 4th to the 60th day post partum (experimental period) the ewes received the feed with a different addition of I and Se enriched algae *Chlorella* supplement. The supplement contained 109.4 mg I and 27.0 mg Se per 1 kg dry matter (DM) for group CON, 109.4 mg I and 54.3 mg Se for group HSe, and 211.1 mg I and 54.3 mg Se for group HSeI.

Table 1. Intake of iodine (I) and selenium (Se) per ewe/day in the feed and supplement dry matter (DM)

Period	Groups		Feed*			Supplement†			Total content			
			DM	Se	I	DM	Se	I	Se	I	Se	I
			(kg)	(mg)		(kg)	(mg)		(mg)		(mg/kg DM)	
BE	CON	(n = 6)	1.3	0.1	0.2	0	0	0	0.1	0.2	0.08	0.15
	HSe	(n = 6)	1.3	0.1	0.2	0	0	0	0.1	0.2	0.08	0.15
	HSeI	(n = 6)	1.3	0.1	0.2	0	0	0	0.1	0.2	0.08	0.15
E	CON	(n = 6)	1.7	0.1	0.3	0.009	0.2	1.0	0.3	1.3	0.2	0.7
	HSe	(n = 6)	1.7	0.1	0.3	0.009	0.5	1.0	0.6	1.3	0.4	0.7
	HSeI	(n = 6)	1.7	0.1	0.3	0.009	0.5	1.9	0.6	2.2	0.4	1.3

BE – before experiment (pregnancy period and the first 3 days *post partum*);
E - experiment (4 to 60 days *post partum*); DM = dry matter; *1500 g meadow hay, 240 g lucerne granules and 270 g oat groats; † Se and I enriched alga *Chlorella*

The algae were produced by heterotrophic cultivation at the Microbiological Institute of the Czech Academy of Sciences in Třeboň [15].

The average birth weight of lambs (n = 18, 6 per group) was 4.6 ± 0.5 kg and at the end of the experiment (on the 60th day of the lambs' age) the weight was 15.4±1.8 kg. The lambs were housed with their mothers. They were fed only with maternal milk.

Sampling and analysis

Urine samples were collected from the ewes on the day of parturition (within 24 hours *post partum*), and on day 60 *post partum* with a bladder catheter.

Blood samples were taken from the *vena jugularis* on the day of parturition (within 24 hours *post partum*) and on day 10, 30, and 60 *post partum* in ewes, on the day of birth, and on day 10, 30, 60 in lambs. In total, 36 urine samples, 72 blood samples from ewes, and 72 blood samples from lambs were collected.

Selenium concentration was determined in the blood serum and urine by neutron activation analysis and spectrofluorimetry according to Kvicala et al. [16].

Iodine in the ewes and lambs blood plasma was determined on the basis of alkaline ashing by a spectrophotometric method according to Sandell-Kolthoff [17].

Plasma concentrations of total T3 and T4 hormones were assessed by commercial radioimmunoassay kits (Immunotech, Prague, Czech Republic).

Dry matter content of the feed ration was determined after drying at 105°C for 24 hours in a forced air-drying oven.

Statistical analysis

The data were statistically analysed using the program Statistica CZ 6.1 (Statsoft CZ). Dependent variables were estimated using the following (ANOVA) model: $Yijk = \mu +$

Gi + Pj + εijk, where Yijk = Se concentrations in blood serum and urine (μg/L), T3, T4 levels in blood plasma (nmol/L); μ = mean; Gi = group (i = CON, HSe, HSeI); Pj=period of experiment (j = 1, 10, 30, 60 days), and εijk = residual error. Tukey and Student t-test were used for the comparison of groups and period of experiment.

RESULTS

Blood serum selenium concentration in ewes and their lambs

The effect of dietary I and Se on serum Se concentration in ewes is shown in Fig. 1A. The addition of both microelements into the feed ration led to a Se concentration increase in the blood serum of ewes, especially in the HSeI group. A significant increase of Se in the blood serum (83.5 μg/L; P<0.001) was determined on day 10 *post partum* (the 6th day of the experiment) in the group with a high I and Se supplementation. The increase of Se continued until day 30 *post partum* (128.0 μg/L; P<0.001) when the concentration the in the serum was by 197 % higher compared to the initial values. On the last day of the experiment (day 60 *post partum*) a further increase of Se concentration was not measured. In the control group (CON) with recommended norm intake of both microelements, the increase of Se in the blood serum during the whole experiment was gradual until the end of the experiment. Significantly lower (P<0.001) values of Se concentration were detected from day 10 *post partum* in comparison to the HSeI group. On this day the increase of Se amounted to 22.7 % (51.9 μg/L; P<0.01) and at the end of the experiment 74.9% (74.0 μg/L; P<0.001). Similar changes in blood serum Se concentration were detected in the HSe group, in which only Se was added to the feed ration. During the whole experiment significant differences between the CON and the HSe group were not recorded.

Before the experiment a markedly lower Se concentration in the lambs' blood serum of all groups (CON 28.7; HSe 28.2; HSeI 30.0 μg/L) was observed (Fig. 1B). Selenium and I supplementation of ewes led to a gradual increase in serum Se concentration in their lambs (Fig. 1B). Concerning the dynamics of Se concentration in lambs, in the

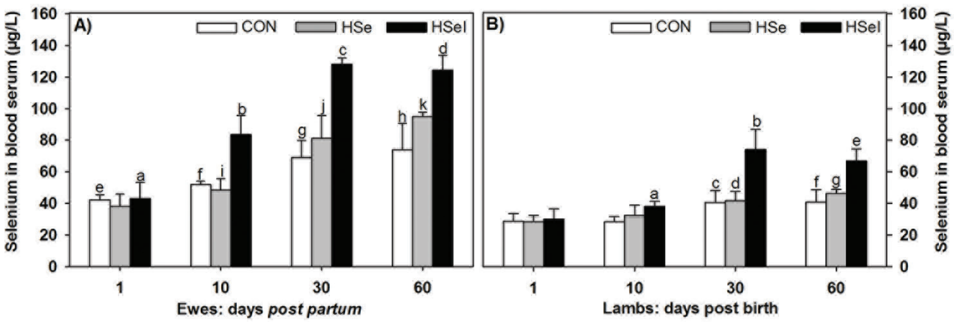

Figure 1. Selenium concentration in sera of **A)** ewes and **B)** their lambs with different I and Se intake from 4 days post partum or birth.
CON (control group; 0.2 mg Se+0.7 mg I/ kg DM; n = 6), **HSe** (0.4 mg Se+0.7 mg I/ kg DM; n = 6); **HSeI** (0.4 mg Se+1.3 mg I/kg DM; n = 6); Results are expressed as mean ± standard deviation; **A)** $^{a:b;\ b:c;\ f:b;\ i:b;\ g:c;\ j:c;\ e:f;\ h:d;\ k:d;\ f:g}$ (P<0.001), $^{e:f}$ (P<0.01); **B)** $^{a:b;\ b:c;\ b:d;\ e:f;\ e:h;}$ (P<0.001)

HSeI group a rapid and significant (P<0.001) increase from the 10th (38.0 μg/L) to the 30th day of the lambs' age (74.2 μg/L) was observed, which was followed by a slight and insignificant (9.9%) decrease on the 60th day. Also the Se concentration was significantly (P<0.001) higher in lambs of HISe group.

Selenium content in the ewes' urine

Before the experiment no significant differences were observed in the ewes' urine Se content (CON 16.4; HSe 15.8; HSeI 16.0 μg/L). At the end of the experimental period a significantly higher (45.8 μg/L; P< 0.01) urine Se content was recorded in the HSeI group compared to group CON (21.2 μg/L) and HSe (28.5 μg/L).

Levels of thyroid hormones in blood plasma

Until the beginning of the experiment no significant differences in blood plasma levels of T3 and T4 were observed (Fig. 2). During the experimental period a gradual decrease of T3 level was recorded, which reached a statistical significance for all groups on the 60th day *post partum* (CON, HSeI (P<0.01); HSe (P<0.05). Plasma T4 level in all ewes showed a tendency to decrease as well, which temporarily reached statistical significance (P < 0.05) only on the 30th day post partum in the control group (CON) with the recommended norm intake of Se (0.2 mg Se/kg DM).

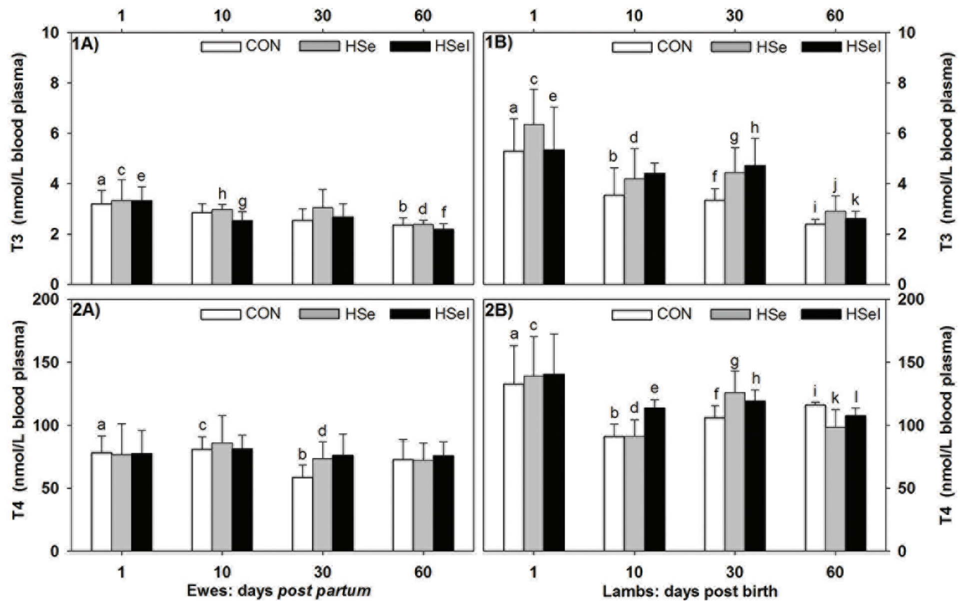

Figure 2. Effect of different I and Se intake on plasma **1)** T3 and **2)** T4 levels of **A)** ewes and **B)** their lambs supplemented from 4 days post partum or birth .
CON (control group; 0.2 mg Se+0.7 mg I/ kg DM; n = 6), **HSe** (0.4 mg Se+0.7 mg I/ kg DM; n = 6); **HSeI** (0.4 mg Se+1.3 mg I/kg DM; n = 6); Results are expressed as mean ± standard deviation; **1A)** $^{a:b;\ e:f}$ (P<0.01); $^{c:d;\ e:g;\ h:g}$ (P<0.05); **1B)** $^{a:b}$ (P<0.01); $^{c:b;b:d}$ (P<0.05); **2A)** $^{a:i;c:j;\ f:i;\ g:j;\ h:k;}$ (P<0.01); $^{a:b;\ c:d;\ e:k;\ g:f;\ h:f}$ (P<0.05); **2B)** $^{c:d;e:b;e:d}$ (P<0.01); $^{a:b;\ c:d;\ e:k;\ g:f;\ h:f;\ i:k;\ i:l\ ;c:k}$ (P<0.05)

Similarly as in ewes, no significant differences were observed in lambs blood plasma T3 and T4 levels before the experiment (Fig. 2). From 4 to 60 days of age lambs of the control group recorded a linear and significant ($P<0.01$) decrease in plasma T3 levels. In the experimental groups of lambs the decline was slow and significant at the end of the experiment (HSe ($P<0.01$); HSeI ($P<0.05$)). Among the high Se supplemented groups (HSe, HSeI) significantly higher blood serum Se concentration in the HSeI group was accompanied by an insignificantly higher plasma T3 level up to the 30th day post birth. In the case of T4 lower values were observed in all groups of lambs at the end of the experiment, but statistically significant ($P<0.05$) was only in the HSe group (Fig. 2). Higher Se supplementation of ewes (HSe, HSeI) resulted in a significantly higher ($P<0.05$) T4 level on the 30th day of the age of lambs and significantly lower ($P<0.05$) on the 60th day of age compared to the lambs of ewes supplemented with the recommended intake of Se (CON). A significant difference ($P<0.01$) between experimental groups was observed only on the 10th day after birth.

DISCUSSION

The optimal concentration of Se in the blood serum of ewes is 120-150 μg/L [18] while values from 25-50 μg/L are considered deficient [19]. In the submitted study the concentration of Se in the blood serum of ewes before the experiment was not optimal in any of the three groups (42.3; 38.1; 43.1 μg/L) as a result of its low concentration in the basic feed ration (0.08 mg/kg DM). During the experiment launched on the 4th day *post partum* the ewes in the control group (CON) were given the recommended norm amount of I (0.7 mg/kg DM) and Se (0.2 mg/kg DM). In the HSe group the adequate intake of I and Se was raised by 100%. In the third group (HSeI) the intake of I concurrently was raised by 85.7% and Se by 100% in comparison to NRC requirements [20].

An increased blood serum Se concentration in the ewes with high I and Se (HSeI) intake to the 60th day *post partum* shows the influence I on Se utilisation and is in accordance with Guyot et al. [9] and Aghwan et al. [21], who found a synergistic relationship between I and Se metabolism when both microelements were supplemented simultaneously. Higher I supplementation in HSeI group also led to a significant ($P< 0.01$) increase of Se content in urine compared to the group HSe.

In the newborn lambs low serum Se concentrations (CON 28.7; HSe 28.2; HSeI 30.0 μg/L) were observed. Ghany-Hefnawy et al. [22] reported that such a low serum Se status reflected insufficient levels of Se in the diet of mothers and its low transplacental transfer. At the beginning of the experiment the changes in lambs' serum Se concentrations were recorded, which corresponded to the dynamics of Se in their mothers.

A significant increase of Se concentration in the blood serum of ewes and their lambs corresponds to the dynamics of I concentration in the blood plasma of lambs (Fig.

3B). Excessive I supplementation of lactating ewes led to a high I concentration in milk. Such excessive I intake disrupts the oxidative and antioxidative balance in the thyroid gland [23]. To compensate for this imbalance a proportional amount of selenoenzyme GSHPx is required. It can be assumed that a high I concentration in the milk led to a higher Se utilisation in suckling lambs of the HSeI group. In addition, in this group no significant Se decrease after 30 days post birth was associated with a significant decrease of I in the blood plasma (Fig. 3B), which was caused by the decrease of I concentration in milk with progressing lactation of the ewes.

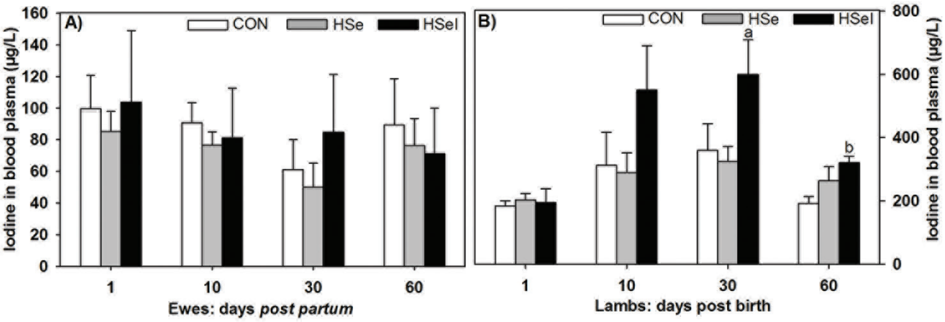

Figure 3. Iodine concentration in blood plasma of **A)** ewes and **B)** their lambs with different I and Se intake from 4 days post partum or birth. **CON** (control group; 0.2 mg Se+0.7 mg I/ kg DM; n = 6), **HSe** (0.4 mg Se+0.7 mg I/ kg DM; n = 6); **HSeI** (0.4 mg Se+1.3 mg I/kg DM; n = 6); Results are expressed as mean ± standard deviation; **B)** [a:b]($P<0.001$)

Awadeh et al. [24] and Bik [25] found out that Se supplementation has a positive effect on T3 level in cows and ewes. In our experiment a higher nutritional dosage of Se did not result in a significantly higher plasma T3 level. The same findings with the same result were reported by Qin et al. [26], who did not find any influence of Se supplementation on blood serum T3 level in Cashmere goats. When comparing the T3 (Fig. 2) level between experimental groups (HSe, HSeI), which differed only in I intake, during the experimental period in the HSeI group a lower T3 level was recorded ($P< 0.05$) only the 10th day post partum, although this group had significantly ($P < 0.001$) higher Se concentrations in the blood serum (Fig. 1A). The lower plasma T3 level of the HSeI group was probably due to the higher I concentration in the blood plasma (Fig. 3A) as reported by Wichtel et al. [27]. However, this result is contrary to Guyot et al. [9], who reported a higher blood T3 level in cows with high I and Se supplementation. Guyot et al. [9] also observed in their study with combined I and Se supplementation in cows, that a high I (5.45 mg/kg DM) and Se (0.45 mg/kg DM) diet resulted in a decrease of blood T4 level. In our trial high I and Se supplementation did not lead to significant decrease of blood plasma T4 level. These findings are in agreement with Qin et al. [26], who reported that blood serum T4 level was significantly enhanced ($P< 0.05$) only by increasing I supplementation, but no interactions between I and Se were observed for T4 levels.

In lambs a decrease of thyroid hormones level was recorded until 60 days of age. Such decrease is consistent with the results of Head et al. [28] and Hewnavy et al. [29] and corresponds to ontogenetic dynamic thyroid hormones levels in blood plasma [30]. In contrast with the results of Chadio et al. [7] and Kumar et al. [31] who did not notice any effect of Se supplementation on lambs thyroid hormone levels, in our experimental period higher Se supplementation in ewes of HSe and HSeI group resulted in significantly higher ($P<0.05$) lamb plasma thyroid hormones levels up to 30 days post birth. After the 30th day post birth, a significant decline ($P<0.05$) only in plasma T4 level was observed compared to the group with the recommended intake of Se (CON).

CONCLUSIONS

Our results show that in Se deficient lactating ewes and their offspring a higher supplementation of Se and I in an organic form led to its higher utilisation, manifested by a significantly higher Se concentration in the blood serum. In addition, higher supplementation of organic I lead to significantly higher Se content in the ewes' urine. The ewes thyroid hormone levels showed did not seem to be explicitely influenced by higher Se and I supplementation. But a higher Se concentration in the lambs' blood serum affected the postnatal dynamics of the thyroid hormones within 30 days after birth.

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EFEKTI SUPLEMENTACIJE ORGANSKIM SELENOM I JODOM NA STATUS SELENA I TIREOIDNIH HORMONA OVACA U LAKTACIJI I NJIHOVE JAGNJADI

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Cilj ispitivanja je bio da se odredi uticaj istovremene suplementacije tokom 56 dana (od 4-og do 60-og dana post partum) selenom (Se) i jodom (I) obogaćenim *Chlorella* algama, na koncentraciju selena u krvi i urinu i na nivo tireoidnih hormona kod ovaca i njihove jagnjadi. U studiju je bilo uključeno ukupno 18 Šumava ovaca i njihove jagnjadi podeljenih u tri grupe (CON, Hse, HseI). Sadržaj selena i joda u ishrani je iznosio: 0,2 i 0,7 mg (CON), 0,4 i 0,7 mg (HSe), 0,4 i 1,3 mg (HSeI) po kg suve materije. Tokom oglednog perioda signifikantno više koncentracije Se u krvi (P< 0,001) i urinu (P< 0,01) su izmerene kod ovaca u HSeI grupi. Signifikantno više koncentracije Se u serumu (P< 0,001) su izmerene kod jagnjadi u HSeI grupi. Najviša vrednost koncentracije Se u krvi izmerena je kod ovaca (128,0 μg/L) i jagnjadi (74,2 μg/L) 26-tog dana ogleda. Rezultati pokazuju viši stepen iskoristljivosti organski vezanog Se istovremeno aplikovanog sa organski vezanim I. Nivoi tireoidnih hormona u plazmi ovaca se nisu

eksplicitno menjali pri višoj suplementaciji selenom i jodom. Više vrednosti koncentracije Se u krvnom serumu jagnjadi su dovele do signifikantno (P< 0,05) viših nivoa T3 do 30-og dana nakon rođenja.