

**SELENIUM AND IODINE STATUS RELATIONSHIP IN CALVES AND HEIFERS FROM
SELENIUM AND IODINE DEFICIENT AREAS IN SERBIA**

JOVANOVIĆ I, PEŠUT OLIVERA, GVOZDIĆ D and STOJIĆ V*

**Faculty of Veterinary Medicine, Belgrade*

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The selenium and iodine status was determined in calves (3, 6 and 9 months old) and heifers (12 months) on two farms (A-Kovin, and B-Vrbas) in iodine and selenium deficient areas of Serbia. Selenium concentrations in feedstuffs on the territories surrounding farm A and B were lower than 0.1 ppm in more than 90% of the samples and lower than 0.05 ppm in more than 67% of the samples. Plasma selenium concentrations in calves and heifers from both farms were very low, ranging from 1.58 to 9.42 µg/L. Plasma GSH-Px activity was very low in 3 month old calves: 8.4 ± 5.2 and 16.1 ± 4.3 µkat/L on farm A and B, respectively, and significantly higher in 12 month old heifers: 39.0 ± 6.2 and 40.8 ± 9.8 µkat/L on farm A and B, respectively. Mean plasma T_4 levels in all groups of calves and heifers were relatively high, ranging between 57.1 and 102.9 nmol/L. Mean plasma T_3 levels in calves and heifers from both farms ranged from 2.72 to 3.51 nmol/L, and did not vary significantly, except for the 3-month-old calves on farm B, where the level was significantly higher than in the other groups. A significant correlation was found between plasma GSH-Px activity and $T_3:T_4$ ratio in the 6-month-old calves from farm A ($r^2 = 0.80$), as well as in the combined groups of 6-and 9-month old calves from farm B ($r^2 = 0.68$).

Key words: selenium, iodine, deficiency, calves.

INTRODUCTION

Three iodothyronine deiodinases, types D1, D2 and D3, which catalyze deiodination of thyronines, contain selenium in the form of selenocysteine in their catalytic sites. Enzymes ID1 and ID2 catalyze primarily outer-ring (5') deiodination and are responsible for bulk T_4 to T_3 conversion. Enzyme ID3 catalyzes inner-ring (5) deiodination rendering T_4 and T_3 inactive (Bates *et al.*, 2000). It has been shown in rats that even severe and prolonged Se deprivation does not significantly affect thyroidal ID1 activity (Arthur *et al.*, 1990; Bates *et al.*, 2000). However, extrathyroidal ID1 activities, in liver, kidney and muscle (Beckett *et al.*, 1992), are decreased. Therefore it is logical to assume that both iodine and selenium status of animals should be sufficient to insure normal thyroid function and plasma thyronine status.

Several parts of Serbia are recognized to be both iodine and selenium deficient (Jovanović *et al.*, 1996; Sinadinović and Han, 1995; Mihailović *et al.*, 1994). In eight goitrogenous parts in Serbia the incidence of severe hypothyroidism in humans fell dramatically during the '60-ies when iodine prophylaxis was introduced. Nevertheless, in some of those areas the incidence of hypothyroidism never fell below 20 - 30%, and in others it is on the rise again (Sinadinović and Han, 1995). In marginal iodine and/or selenium deficiency, such as we could expect in Serbia at present, only young animals would eventually show clinical signs of hypothyroidism, while adults would have overall poorer health/production performance. Therefore, the purpose of this study was to examine the relationship between selenium status and circulating thyronines in iodine/selenium unsupplemented calves and heifers from calving to 12 months of age, living in selenium and/or iodine deficient regions.

MATERIAL AND METHODS

Experimental animals

The experiment was conducted on 64 iodine and selenium unsupplemented calves and heifers (Holstein x Friesian crossbreds) at two dairy farms situated near the towns of Kovin (Farm A) and Vrbas (Farm B) in the northern Serbian province of Vojvodina. On the farm A Sinadinović *et al.* (1982) described a case of severe hypothyroidism with an outbreak of massive congenital goiter in calves. Four groups of 8 calves, were formed at each farm on the basis of age and denoted as follows: Group I: aged 0-3 months; Group II: aged 4-6 months; Group III: aged 7-9 months; and Group IV: aged 10-12 months.

Blood and feedstuff samples

Blood samples were taken from the jugular vein in heparinized tubes and centrifuged at 1500 x g for 15 minutes to obtain plasma. Each sample was divided into two: fresh plasma was utilized immediately for determination of selenium dependant GSH-Px activity, and the rest was frozen at -20°C for subsequent thyronine and Se analysis. Samples of feedstuffs for determination of Se content were collected randomly from the territory approximately 15-20 km around each farm.

Selenium and iodine status

Plasma thyronine concentration (T_4 and T_3) was determined in duplicate samples using standard commercial RIA kits (INEP-Beograd). Selenium concentration was determined using hydride generation atomic absorption spectrophotometry (Welty *et al.*, 1987). Glutathione peroxidase (GSH-Px – EC. 1.11.1.9) activity was analyzed spectrophotometrically (Günzler *et al.*, 1974) using a tertiary butyl hydroperoxide (TBH) concentration below 2,32 mM in order to determine only the activity of selenium dependant GSH-Px (Sankari, 1985).

Statistical analysis

The significance of differences between groups was calculated using Student's t-test, where $p < 0.05$ was considered statistically significant. Correlations were determined using linear regression.

RESULTS

Selenium concentrations in feedstuffs on the territories surrounding farm A ($n=14$) and B ($n=17$) were lower than 0.1 ppm in 90% of the samples and lower than 0.05 ppm in >67%.

Selenium concentrations and GSH-Px activities in plasma of calves and heifers from farms A and B are presented in Table 1.

Mean plasma selenium concentrations in calves and heifers from both farms were very low, ranging from 1.58 to 9.42 $\mu\text{g/L}$. It is evident that the plasma GSH-Px activity was very low in 3-month-old calves (Group I): 8.4 ± 5.2 and 16.1 ± 4.3 $\mu\text{kat/L}$ on farm A and B, respectively; and significantly higher in 12 month old heifers (Group IV): 39.0 ± 6.2 and 40.8 ± 9.8 $\mu\text{kat/L}$ on farm A and B, respectively.

Plasma thyroid hormone concentrations and their ratio are presented in Table 2.

Mean plasma T_4 levels in all groups of calves and heifers were relatively high, between 57.1 and 102.9 nmol/L. Thyroxine level distribution among the age groups on both farms (Figure 1) were consistent. In the plasma of calves from farm A, T_4 concentrations were significantly lower ($p < 0.05$) in group III (57.4 ± 8.2 nmol/L) compared to group II (94.3 ± 13.6 nmol/L), and in the calves from farm B in group II (80.8 ± 20.7 nmol/L) and III (57.1 ± 17.0 nmol/L) compared to group I (102.9 ± 24.3 nmol/L). Mean plasma T_3 levels in calves and heifers from both farms (Figure 2) ranged from 2.72 to 3.51 nmol/L, and did not vary significantly, except for group I on farm B, where the level was significantly higher than in the rest of the groups.

A significant positive correlation ($r^2 = 0.80$) was found between plasma GSH-Px activity and $T_3 : T_4$ ratio in the calves of group II from farm A, as well as in combined groups II and III ($r^2 = 0.68$) from farm B (Figure 3).

Table 1. Selenium status in blood plasma of calves and heifers during the first 12 months of life (n=8 for all groups); different superscript letters in one row denote significantly different ($p < 0.05$) values compared to the previous age group

Calf-Heifer group	Farm A				Farm B			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
	Plasma Se concentration ($\mu\text{g/L}$)							
M \pm SD	3.34 \pm 1.07	8.53 \pm 2.64 ^a	9.42 \pm 2.74 ^b	7.60 \pm 2.41 ^c	1.58 \pm 1.30 ^a	9.18 \pm 3.56 ^b	NA*	6.28 \pm 0.74 ^c
Range	2.3-5.4	4.9-13.4	7.0-12.4	4.3-9.6	1.0-3.9	4.5-13.1	NA	5.2-7.2
	Plasma GSH-Px activity ($\mu\text{kat/L}$)							
M \pm SD	8.4 \pm 5.2 ^a	25.9 \pm 8.1 ^b	29.5 \pm 6.1 ^b	39.0 \pm 6.2 ^c	16.1 \pm 4.3 ^a	11.2 \pm 2.4 ^b	24.9 \pm 2.9 ^c	40.8 \pm 9.8 ^d
Range	3.0-15.7	17.2-36.1	21.2-37.7	30.3-49.2	8.9-21.0	6.4-14.0	21.7-30.5	23.7-50.7

* NA – not analysed

Table 2. Thyroid hormones in plasma of calves/heifers during the first 12 months of life (n=8 for all groups); different superscripts in one row denote significantly different ($p < 0.05$) values compared to the previous age group

Calf-Heifer group	Farm A				Farm B			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
	Plasma T4 (nmol/L)							
M \pm SD	84.8 \pm 14.8 ^a	94.3 \pm 13.6 ^b	57.4 \pm 8.2 ^c	67.4 \pm 21.8 ^d	102.9 \pm 24.3 ^a	80.8 \pm 20.7 ^b	57.1 \pm 17.0 ^c	81.1 \pm 29.0 ^d
Range	66-116	74-116	50-76	43-102	74-154	42-101	38-87	41-126
	Plasma T3 (nmol/L)							
M \pm SD	3.19 \pm 0.49	3.08 \pm 0.62 ^a	2.72 \pm 0.32 ^b	2.75 \pm 0.27	3.51 \pm 0.48 ^a	3.06 \pm 0.46 ^b	2.79 \pm 0.43	2.78 \pm 0.92
Range	2.1-3.6	2.3-4.4	2.4-3.2	2.3-3.1	3.0-4.4	2.3-3.8	2.3-3.6	1.9-5.0
	T3 / T4 ratio* (x 10-2)							
M \pm SD	4.15 \pm 0.67 ^a	3.29 \pm 0.59 ^b	4.81 \pm 0.72 ^c	4.20 \pm 0.91 ^d	3.51 \pm 0.58 ^a	3.97 \pm 0.88 ^b	5.07 \pm 1.13 ^c	3.63 \pm 0.94 ^d
Range	3.10-5.15	2.47-4.14	3.95-6.15	3.13-5.29	2.52-4.21	2.47-5.48	3.22-7.07	2.14-4.81

* individual T₃ / T₄ data for each animal were considered for mean \pm SD calculation

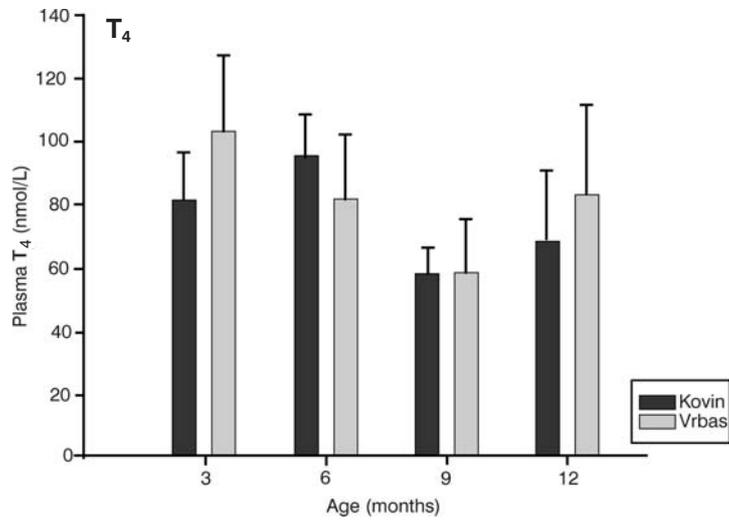


Figure 1. Plasma T₄ concentration (nmol/L) in calves/heifers during the first 12 months of age

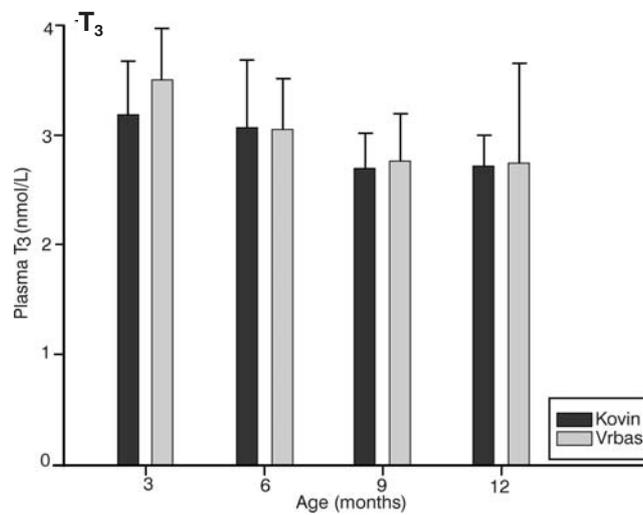


Figure 2. Plasma T₃ concentration (nmol/L) in calves/heifers during the first 12 months of age

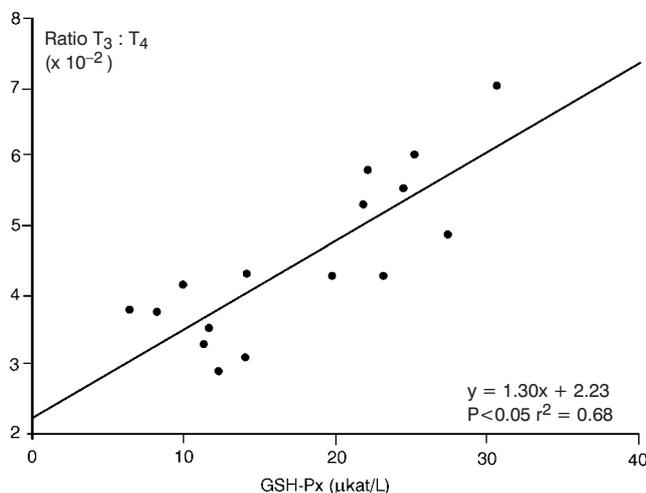


Figure 3. Correlation between blood plasma GPx activity and T₃:T₄ ratio in calves from farm B, aged 6 – 9 months

DISCUSSION

Selenium concentrations in feedstuffs on the territories surrounding both farms were lower than 0.1 ppm in more than 90% of the samples and lower than 0.05 ppm in more than 67%. Those values can be considered marginally deficient according to the categorization given by Kubota *et al.* (1967).

Plasma selenium concentration and GSH-Px activity are considered to be good indicators of short-term selenium status in ruminants (Gerlof, 1992). Selenium concentrations in plasma of calves from Kovin and Vrbas (Table 1) were very low, ranging individually from 1.0 to 13.4 µg/L and reflecting the low selenium intake through milk and feeds. McDowell *et al.* (2001) in an experiment carried out in Florida, with selenium contents in feeds similar to ours, measured plasma selenium levels of selenium unsupplemented calves ranging from 10 to 30 µg/L and in supplemented 20 – 70 µg/L. Pehrson *et al.* (1999) found plasma selenium concentrations from 32 to 81 µg/L in 22-day-old calves whose dams were supplemented with selenium.

Plasma GSH-Px activities on both farms (Table 1) were very low in 3-month-old calves but significantly higher in 12-month-old heifers, although still very low. A similar increase in selenium content and selenoenzymes in blood plasma and tissues was recorded by Bates *et al.* (2000) in growing rats.

Plasma T₄ levels (Table 2) in all calves were relatively high, between 38 and 154 nmol/L. Kalfelz and Erali (1973) detected T₄ levels of 54 – 111 nmol/L in sera of adult cows, while Awadesh *et al.* (1998) measured T₄ from 90 – 128 nmol/L in plasma of lactating cows supplemented with different Se levels, and 87 – 103 nmol/L in their suckling calves. On the other hand, Pezi *et al.* (2003) found T₄ lev-

els of only 41 – 55 nmol/L in plasma from Friesian cows during the lactation period.

Our results for plasma T_4 and, specially for T_3 levels (Table 2) correspond to those given by Sinadinović and Han (1995) for the same dairy farm in Kovin, after iodine supplementation ($T_4 = 98$ nmol/L, $T_3 = 2.41$ nmol/L), while the same authors found significantly lower plasma T_4 and T_3 levels before iodine supplementation ($T_4 = 47$ nmol/L, $T_3 = 1.36$ nmol/L). According to Beckett *et al.* (1989), in selenium deficient rats plasma T_4 levels were elevated together with largely unchanged T_3 , as a consequence of a marked decrease (>90%) in liver ID1 activity. In the calves from our study, ratios of circulating $T_3 : T_4$ were 0.0214 – 0.0395. Triiodothyronine (T_3) levels were somewhat below those given by Awadesh *et al.* (1998), who found plasma T_3 levels in young calves (up to 2 months of age) ranging from 3.2 to 8.1 nmol/L. However, when comparing the $T_3:T_4$ ratio ranging from 0.032 – 0.053, the difference appears to be smaller.

Significant positive correlations were found between plasma GSH-Px activity and $T_3:T_4$ ratio in some groups of calves from both farms (Figure 3). GSH-Px activity is generally regarded as a good functional expression of selenium status (Smith *et al.*, 1988), and plasma $T_3:T_4$ ratio as a measure of selenoenzyme ID1 activity, predominantly in the liver, kidney and muscle. The enzyme ID1 in these tissues accounts for about 80% of circulating T_3 (Beckett *et al.*, 1992). These correlations indicate that the dependence of thyroxine activation on selenium status in extrathyroidal tissues could be of particular importance in calves aged 4-9 months.

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Address for correspondence:
Doc. Dr Ivan B Jovanović
Department of Physiology and Biochemistry
Faculty of Veterinary Medicine,
Blvd. JNA 18, 11000 Belgrade,
Serbia & Montenegro

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STATUS SELENA I JODA KOD TELADI I JUNICA SA SELEN I JOD-DEFICITARNIH PODRUČJA SRBIJE

JOVANOVIĆ I, PEŠUT OLIVERA, GVOZDIĆ D i STOJIĆ V

SADRŽAJ

Cilj ovog rada bio je da se odredi status selena i joda kod teladi (3,6 i 9 meseci starosti) i junica (12 meseci starosti) sa područja Srbije koja su deficitarna sa jodom i selenom. Ogled je izveden na ukupno na 64 životinje, podeljene na 8 grupa (8 jedinki po grupi), na dve farme u različitim delovima Srbije (farma A-Kovin, farma B-Vrbas). Koncentracija selena u hranivima dobijenim sa područja

oko farmi A i B bila je niža od 0.1 ppm u više od 90% uzoraka, i niža od 0.05 ppm u više od 67% uzoraka hraniva. Koncentracija selena u plazmi kod teladi i junica sa obe farme bila je veoma niska, i kretala se u intervalu od 1.58 do 9.42 $\mu\text{g/L}$. Aktivnost enzima glutation-peroksidaze (GSH-Px) u plazmi kod teladi starosti 3 meseca je bila niska i iznosila je $8.4 \pm 5.2 \mu\text{kat/L}$ na farmi A i $16.1 \pm 4.3 \mu\text{kat/L}$ na farmi B, dok je kod junica starosti 12 meseci ustanovljena statistički značajno viša vrednost, koja je iznosila $39.0 \pm 6.2 \mu\text{kat/L}$ na farmi A i $40.0 \pm 9.8 \mu\text{kat/L}$ na farmi B. Koncentracija tiroksina (T_4) u krvnoj plazmi kod svih grupa teladi i junica bila je relativno visoka i kretala se u intervalu od 57.1 do 102.9 nmol/L. Koncentracija trijodtironina (T_3) u krvnoj plazmi svih teladi i junica kretala se u intervalu od 2.72 do 3.51 nmol/L, i samo je kod teladi starosti 3 meseca na farmi B koncentracija T_3 bila statistički značajno viša u odnosu na ostale grupe. Takođe je ustanovljena statistički značajna korelacija između aktivnosti enzima GSH-Px u plazmi i odnosa $T_3:T_4$ kod teladi starosti 6 meseci na farmi A ($r^2=0.80$), kao i kod kombinovanih grupa teladi starosti 6 i 9 meseci na farmi B ($r^2=0.68$).