

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

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Summary

Selenium and iodine status in calves at different age periods (3, 6 and 9 months old) and heifers (12 months) at two farms (A-Kovin, and B-Vrbas) in previously defined iodine and selenium deficient areas of Serbia was determined. Plasma selenium concentrations, determined by AAS, in calves and heifers from both farms were very low, ranging from 1.58 to 9.42 $\mu\text{g/L}$. Plasma GSH-Px activity was low in 3 month old calves: 8.4 ± 5.2 and 16.1 ± 4.3 $\mu\text{kat/L}$ in farm A and B, respectively, and significantly higher in 12 month old heifers: 39.0 ± 6.2 and 40.8 ± 9.8 $\mu\text{kat/L}$ in farm A and B, respectively. Plasma T_4 levels in all calves and heifers were relatively high, ranging between 57.1 and 102.9 nmol/L. 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 months old calves in farm B, where the level was significantly higher than in the other two groups. A statistically significant correlation was found between plasma GSH-Px activity and $T_3:T_4$ ratio in the 6 months old calves from farm A ($r^2 = 0.80$), as well as in combined group of 6 and 9 months old calves from farm B ($r^2 = 0.68$).

Key words: selenium, iodine, deficiency, calves, heifers.

Several agriculturally important areas in Serbia are having been recognized to be iodine and selenium deficient (Jovanović et al. 1996; Sinadinović and Han, 1995; Mihailović et al. 1994). In a number of goitrogenous parts in Serbia the incidence of clinically manifested hypothyroid cases in humans fell dramatically during the '60-ies when iodine prophylaxis was introduced. 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 lower performance. The result of such joint selenium and iodine deficiency is reflected in constant and relevant economic losses.

Three iodothyronine deiodinases, types D1, D2 and D3, which catalyze deiodination of thyronines contain in their catalytic sites selenium in the form of selenocysteine. Enzymes ID1 and ID2 catalyze 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). However, it has been shown in rats that even severe and prolonged nutrition lacking in selenium does not significantly affect thyroidal ID1 activity (Arthur et al. 1990; Bates et al. 2000). It is interesting to note that at the same time extrathyroidal ID1 activities, in the liver,

kidney and muscle are significantly decreased (Beckett et al. 1992). Thus, it can be assumed that both iodine and selenium status of animals should be sufficient to ensure normal thyroid function and plasma thyronine status.

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

The experiment was conducted on 64 iodine and selenium unsupplemented calves and heifers (Holstein × Friesian crossbreeds) at two dairy farms situated near towns of Kovin (Farm A) and Vrbas (Farm B) in northern Serbian province Vojvodina.

Four groups of 8 calves, were formed at each farm on the basis of calves' age and described as follows: Group I: age 0-3 months; Group II: age 4-6 months; Group III: age 7-9 months; and Group IV: age 10-12 months (heifers).

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

Plasma thyronine concentrations (T_4 and T_3) were determined in duplicate samples using standard commercial RIA kits (INEP-Beograd). Selenium concentration was determined by hydride generation atomic absorption spectrophotometry-AAS (Welty et al., 1987). Glutathione peroxidase (GSH-Px – EC. 1.11.1.9) activity was analyzed spectrophotometrically (Günzler et al., 1974).

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 samples and lower than 0.05 ppm in >67% samples.

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

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

	Farm A				Farm B			
	Group I (0-3 months)	Group II (4-6 months)	Group III (7-9 months)	Group IV (10-12 months)	Group I (0-3 months)	Group II (4-6 months)	Group III (7-9 months)	Group IV (10-12 months)
	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
	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* ($\times 10^{-2}$)							
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

Plasma T_4 levels (Table 1) in all calves and heifers were relatively high, between 57.1 and 102.9 nmol/L. Thyroxine levels distribution among age groups on two 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) lower compared to group I (102.9 ± 24.3 nmol/L). 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 in farm B, where the level was significantly higher.

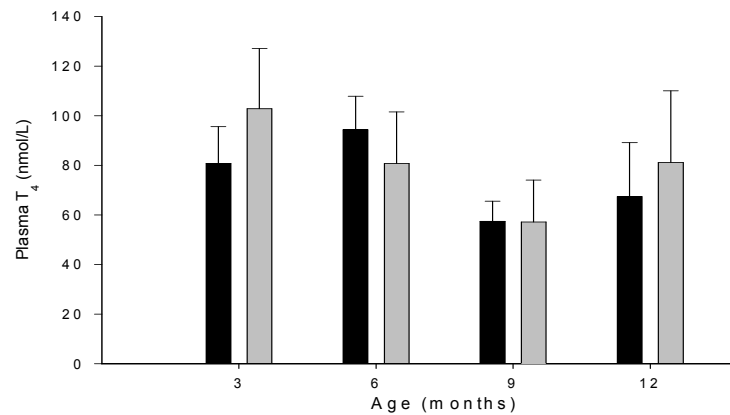


Fig. 1. – Plasma T_4 concentrations (nmol/L) in calves/heifers during first 12 months of age

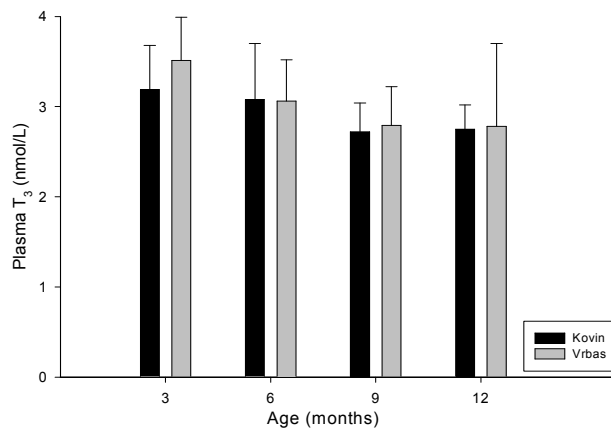


Fig. 2. – Plasma T_3 concentrations (nmol/L) in calves/heifers during first 12 months of age

A significant 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 ($r^2 = 0.68$) as well as in combined groups II and III from farm B (Figure 3).

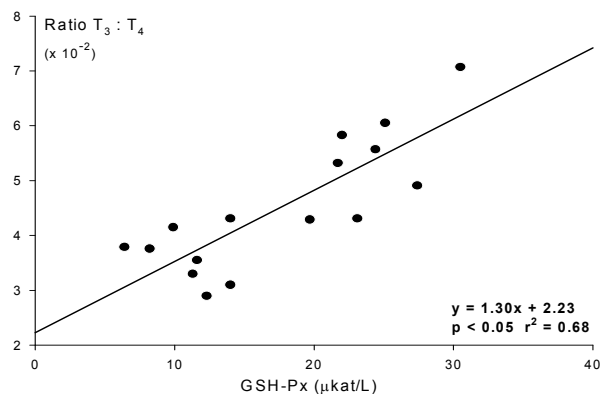


Fig. 3. – Correlation between blood plasma GSH-Px activity and $T_3:T_4$ in calves from farm B, aged 6 – 9 months

Discussions

Selenium concentrations in feedstuffs on the territories surrounding farm A ($n=14$) and farm B ($n=17$) were lower than 0.1 ppm in >90% of samples and lower than 0.05 ppm in >67%. Those values can be considered marginally deficient by 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 cattle (Gerlof, 1992). Selenium concentrations in plasma of calves from Kovin and Vrbas were very low, from 1.58 to 9.42 $\mu\text{g/L}$, reflecting low selenium intake through milk and feeds. McDowell et al. (2001) in an experiment carried out in Florida, with selenium content in feeds similar to ours, measured plasma selenium levels of selenium unsupplemented calves to be from 10 to 30 $\mu\text{g/L}$. Pehrson et al. (1999) determined higher values of plasma selenium (32 to 81 $\mu\text{g/L}$) in 3 week 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 (Group I): 8.4 ± 5.2 and 16.1 ± 4.3 $\mu\text{kat/L}$ in the farm A (Kovin) and B (Vrbas), respectively, and significantly higher in 12 month old heifers (Group IV): 39.0 ± 6.2 and 40.8 ± 9.8 $\mu\text{kat/L}$, respectively, but still very low. Similar

increase in selenium content and selenoenzymes in blood plasma and tissues had been recorded by Bates et al. (2000) in growing rats.

GSH-Px activity is regarded to be a good functional expression of selenium status (Smith et al. 1988). Plasma $T_3:T_4$ ratio are a measure of selenoenzyme ID1 activity, predominantly in liver, kidney and muscle. Enzyme ID1 in these tissues account for about 80% of circulating T_3 (Beckett et al. 1992). A significant correlation was found between plasma GSH-Px activity and $T_3:T_4$ ratio in the calves of Group II from farm A ($r^2 = 0.80$) as well as in combined Groups II and III from farm B ($r^2 = 0.68$) (Figure 3). These correlations indicate that dependency of thyroxin activation on selenium status in extrathyroidal tissues could be of particular importance in calves aged 4-9 months.

Selenium concentrations in feedstuffs on the territories surrounding farm A (n=14) and farm B (n=17) were lower than 0.1 ppm in >90% of samples and lower than 0.05 ppm in >67%. Those values can be considered marginally deficient by the categorization given by Kubota et al. (1967).

Thyroxine levels distribution among age groups on two farms were consistent. Plasma T_4 levels in all calves were relatively high, between 57.1 and 102.9 nmol/L. In plasma of calves from the farm A (Kovin) 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 calves from farm B (Vrbas) in group II (80.8 ± 20.7 nmol/L) and III (57.1 ± 17.0 nmol/L) lower compared to group I (102.9 ± 24.3 nmol/L). At the farm A Sinadinović et al. (1982) described a case of severe hypothyroidism with an outbreak of massive congenital goiter in calves. Pezi et al. (2003) found T_4 levels of only 41 – 55 nmol/L in plasma of Friesian cows during lactation period. Kallfelz et al. (1973) detected T_4 levels 54 – 111 nmol/L in sera of adult cows, while Awadesh et al. (1998) measured T_4 from 90 – 128 nmol/L in plasma of lactating cows supplemented with different Se levels, and 87 – 103 nmol/L in their suckling calves.

Plasma T_3 levels in calves ranged from 2.72 to 3.51 nmol/L and did not vary significantly, except for Group I in farm B (Vrbas), where the level was significantly higher than in the other groups. Our results for plasma T_4 and, specially for T_3 levels 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 lower plasma T_4 and T_3 levels before iodine supplementation ($T_4 = 47$ nmol/L, $T_3 = 1.36$ nmol/L). Triiodothyronine (T_3) levels we measured were 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.

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