KRMIVA

THE EFFECT OF SELENIUM ADDED TO FEEDSTUFFS ON ITS CONTENT IN TISSUES AND ON GROWTH OF RABBITS

UTJECAJ SELENA DODANOG KRMIVU NA NJEGOV SADRŽAJ U TKIVU I NA RAST KUNIĆA

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ABSTRACT

0.1 (A) and 0.3 ppm (B) respectively of selenium in the form of Na_2Se0_3 were added to the feed mixuter that was fed to female New Zealand White rabbits in the final stage of gestation and in lactation period. The addition of selenium in groups A and B had a positive effect on the increase of body mass of fetuses in the last week of gestation (K=36.25 %, A=57.8 %, B=56.4 %) and a significant effect on the development of body mass in young rabbits in group A (K=343.6 g, A=436.2 g, B=373.5 g). The content of selenium in mother's blood decreased in the second week to the lowest level, but in the third week it exceeded the values from the first week (K=1.06/0.74) 1.22, A=0.94/0.93/1.2, B=1.13/0.89/1.15 $\mu g.g^{-1}$ DM). In the liver of young rabbits the concentration of selenium was significantly higher in group B (K=1.97, A=1.89, B=2.18 $\mu.g^{-1}$ DM) at age 21 days, while at age 30 days it increased proportionally with the supply of selenium (K=1.95, A=2.20, B=2.54 $\mu.g^{-1}$ DM). In kidneys the situation was not so clear since the concentration of selenium was higher in comparison with liver, and the highest value was determined at age 30 days in group B (3.22 $\mu g.g^{-1}$ DM).

INTRODUCTION AND LITERATURE SURVEY

The role of selenium (Se) in human and animal nutrition has been intensively stressed in the last thirty years. Nevertheless, we have not found a lot of data in literature on the significance of selenium in nutrition of rabbits. Little information has been found on their insensibility to lack of selenium in a ration (Jenkins, 1979, cited by Cheeke, 1976), i.e. selenium dificiency does not cause diseases like muscular dystrophy, liver necrosis, fertility defect etc (Lee et al., 1979).

The aim of the present study was to establish the effect of selenium added in the form of Na_2SeO_3 to traditional feed mixtures that females were given in the last week of gestation until weaning, on the development of body mass of mothers and the young in the mentioned period and on the content of selenium in blood of mothers and the liver and kidneys of the young.

In human and in animal nutrition the most important is selenium organically bound to proteins (Girling, 1984). The main selenium compounds are selenocisthine, selenocistheine, selenomethionine and metilselenomethionine. In the common feedstuffs selenium ap-

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pears as selenomethionine. Selenium is not equally spread in the soil. In superficial layers selenium appears in quantities between 0.1 and 2 μg^{-1} (Girling, 1984), the highest values are 30 - 324 $\mu.g.^{-1}$ dry matter. Toxical quantities of selenium in soil can be found in areas with low precipitation and with alcaline reaction. In such conditions selenium transforms into selenates and into organic selenium compounds, so it becomes easily accessible to plants. In the soil with low pH value selenium is bound to aluminium and ferrous compounds, and therefore the loosening is slower. In acid and wet soil the supply of plants with selenium, and consequently of animals, is insufficient.

Feed plants like grass, wheat and weeds contain very small quantities of selenium. Only if they grow in selenium rich soil they contain about 50 ppm of selenium (Krajnović, 1983). Selenium deficiency becomes evident when its concetration in feedstuffs is lower than 0.1 ppm (Stekar, 1975; Krajinović, 1983, Girling, 1984 and others). Stekar and Muck (1971) found the following contents of selenium in domestic cereals: wheat 0.005-0.188, maize 0.00- 0.58, barley 0.011-0.084, oat 0.037-0.071, all mg/kg. Krajinović (1983) has found in corn 0.028 mg, oat 0.010, barley 0.06 mg, alfalfa 0.001-0.077 mg and in hay 0.016-0.024 mg/kg dry matter. Selenium dificiency is more pronounced in ruminants than in monogastric animals since microorganisms in rumen reduce selenium into insoluble forms. Monogastric animals can absorb up to 80 % and even 85 % of consumed selenium, while in sheep the utilization is only up to 35 % (Florkin and Stok, 1970). Nearly all selenium is absorbed in the small intestine and it quickly spreads into the body. It has been established that embryo can be supplied with selenium from the placenta which can cause its excessive growth (McConnell, 1970). Selenium passes into milk very quickly (McConnel and Roth, 1964). Two hours after injecting sodium selenite to the nursing bitch, an increase of selenium was observed in blood and milk. Inorganically applied selenium transformed into organically bound selenium and it bound the proteins in milk. In cows comparatively small proportion of the serum selenium came into milk (Conrad and Moxon, 1979). Concentration of selenium increases in serum and in milk in the first ten days of selenium supply but the highest values are reached in 40 days. In the organism selenium deposits in kidneys, in liver, in pancreas, spleen, intestine, lungs, in hair and wool. The quantity of selenium varies most in kidneys where it depends on the supply of selenium most evidently.

Nearly all biochemical activities of selenium are connected with the enzyme glutation-peroxidase (GSH-Px), i.e. this element is the essential compound of GSH-Px. GSH-Px activity is closely connected to vitamin E. Some selenium compounds can be replaced by vitamin E in medical treatment of certain diseases (Stekar, 1986). It can be stated that selenium deficiency does not cause such diseases in rabbits which are characteristic for some other animals (Lee et al., 1979). It was not possible to treat muscular dystrophy, which was caused by vitamine E deficiency, by extra selenium, but the application of vitamin E was successful. Similar results were obtained by other researchers as well. Experiments by Cheeke and Whanger (1976) showed that the established inefficiency of rabbits to deficient supply of selenium was not the consequence of enzyme glutation peroxidase lack in organs of rabbits with the exception of erythrocytes, which showed a certain deviation. Other authors (Lee et al., 1979) report that there are two forms of GSH-Px enyme. One of them does not depend on the presence of selenium. This form is always present in the organism and it acts upon hidroperoxieds. In comparison with rats the total activity of GSHPx enzyme in rabbits is twice as high. The part of selenium inedependent enzyme is higher too (43:35%). But this observation cannot explain the nondependence of rabbits on selenium deficiency GSH-Px can be found in kidneys and liver only.

MATERIAL AND METHODS

Female New Zealand White rabbits from practical breeding conditions were chosen for the trial. The animals were divided into three more or less equal groups which were fed by experimental feed mixtures in the last week of gestation and during the lactation period, lasting 30-days. The control group (n=6) did not receive additional selenium, Group A (n=7) received plus 0.1 ppm selenium with feed mixture and Group B (n=7) was given 0.3 ppm additional selenium in the form of Na_2SeO_3. Components of feed mixture and selenium are given in Table 1. Females were weighed at the beginning of the trial (7 days before the planned kindling) and just after it. The young were weighed on the day of birth and on 21st and 30th day of age. Blood was sampled once a week during the lactation period. Two females were chosen in each group to take 6 - 8 ml of blood sample from the ear vein. Before the sampling ears were washed with shampoo in order to prevent the contamination with selenium from the hair. 20 μl heparin was sucked into injection nee-

dle before sampling in order to prevent the blood from coagulating (Falnoga, 1986). Samples were stored in penicillin flasks which had been washed in aqueous solution of HNO_3 (1:1) and treated at high temperature on the gass flame. Afterwards they were frozen up to an average temperature -20°C and later up to -24°C.

Table 1: Chemical analysis of feed mixture and selenium per groups, mean values of samples at the begining and the end of the trial
Tablica 1: Rezultati kemijske analize krmne smjese i selena po skupinama prosjek uzoraka na početku i koncu pokusa

Component Sastav	Group - skupina		
	K	A	B
Dry matter,% Suha tvar,%	89,70	89,74	89,88
Crude protein,% Sirove bjelančevine,%	16.81	17.41	17.71
Crude fat,% Sirove masti,%	2.41	3.08	2.96
Crude fibre,% Sirova vlaknina,%	17.44	17.18	16.45
Se,ppm,difference to K Se,ppm,razl. do K	0.194	0.295*	0.480
	-	0.101	0.285

* jedno mjerenje - one measurment

The youngs were selected at age 21 and 30 days for kindney and liver analysis from the litters of which females' blood had been sampled. Treatments were the same as in blood sampling.

In feedstuffs, blood and tissue selenium was determined by radiochemical neutron activation analysis. Lyophilised samples were decomposed by saturated solution of $Mg(NO_3)_2$. The residue were exposed to 550°C and dissolved HCl, Se(VI) was reduced to Se(IV) and after 4-nitro-o-fenilendiamine had been added, we extracted the achieved chelate 5-nitro-2,1,3-benzoselenadiasol with CC14. The activity of gama isolated radionucleide ^{75}Se (1/2 = 120, E(#)=0.121, 0.136, 0.264 and 0.400 MeV) was measured with coaxical HP detector which was connected to a multichannel analyzer (Dermelj et al., 1985).

The achieved results were compared to the analysis of corresponding stadard reference materials (Table 8).

RESULTS AND DISCUSSION

There were no statistically significant differences in groups concerning the feed intake, which matched with reports by other authors (Stekar, 1975; Krajinović, 1984; Cheeke and Whanger, 1976 etc.). Results are shown in Table 2.

Table 2: An average daily feed intake of females and litter per week (g/day)
Tablica 2: Prosječna dnevna konzumacija ženki zajedno s leglom po tjednima (g/dan)

Trial,weeks* Tjedan pokusa	K	A	B	Sign.F
First week (last week of gestation) 1. tjedan (zadnji tjedan bređosti)	225.1	239.6	211.9	0.828
Second week (first week of lactation) 2. tjedan (1. tjedan laktacije)	239.8	315.7	266.9	0.465
Third week (second week of lactation) 3. tjedan (2. tjedan laktacije)	325.7	401.2	356.5	0.913
Fourth week (third week of lactation) 4. tjedan (3. tjedan laktacije)	330.9	430.6	370.6	0.147
Mean value Srednja vrijednost	280.4	346.7	301.5	

*: Last week of lactation was not statistically processed because it was not possible to distinguish gestatory females from non gestatory ones. Statistički nije obrađen zadnji tjedan laktacije, jer nije bilo moguće točno razlikovati ponovo bređe odnosno nebređe ženke.

Additional supply of selenium did not effect the development of body mass of pregnant females, while the body mass of embryos inceased significantly in this period (Table 3). The increase in body mass was as foolows: 36.25 % in control Group K, 57.8 % in Group A, and 56.4 % in Group B. Higher body mas of the new-born young in both experimental groups was due to more youngs per litter (Table 4). A statistically significant increase of an average body mass of youngs was determined at age 21 days, which was the period of the highest milk production, in group A in comparison with the control group K. The same ratio could be observed at age 30 days. More added selenium (+ 0.3 ppm) did not have any effect at all.

Table 3: Average masses of females in trial* (g)
Tablica 3: Prosječne mase ženki po skupinama u vrijeme pokusa* (g)

	K	A	B	Sign.F
Average mass at the beginning of trial (7 days before expected birth) Pros. masa na početku pokusa (7 dana pred predviđenim okoćenjem)	4133	4294	4093	0.563
Average mass on the day of birth (4-7 days after the beginning of the trial) Pros. masa na dan koćenja (4-7 dana nakon početka pokusa)	3827	3963	3860	0.798
Differences among weight of females Razlika između težina ženka	306	231	233	
Average litter mass on the day of birth Prosječna masa legla na dan koćenja	480	547.1	534.3	0.277
Gain of embryos during last days (7 days) before the birth,% % prirasta plodova u zadnjim danima (7 dana) pred koćenjem	36.25	57.8	56.4	

* Masses of females were not statistically proccesed because it was not posible to distinguish gestatory females from non gestatory ones.
Statistički nisu obrađene mase ženki, jer nije bilo moguće točno razlikovati ponovo bređe odnosno nebređe ženke.

Table 4: Average number and mass of the young per litter per groups
Tablica 4: Prosječna masa mladunaca i njihov broj u leglu po skupinama

Week Tjedan	No. of youngs Br. mlad.	$\bar{x}$ youngs /litter x mlad./ leglo	Litter mass (g) masa legla (g)	$\bar{x}$ weight of young, g x težina mlad. u g.	$\bar{x}$ gain g/day x prir. g/dan
Group K - Skupina K					
Day of birth Na dan koćenja	45	7.5	480.00	63.85	
					13.32
Age 21 days Na 21. dan starosti	40+2**	7	2383.3	343.6*a	
					34.3
Age 30 days Na 30. dan starosti	40+2	7	4302.5	652	
Mortality,% Smrtnost %	6.7				
Group A Skupina A					
Day of birth Na dan okoćenja	61	8.71	547.1	62.2	
					17.8
Age 21 days Na 21. dan starosti	47+2**	7	2931.4	436.2b	
					32.7
Age 30 days Na 30. dan starosti	47+2	7	4810.0	730.3	
Mortality,% Smrtnost %	19.7				
Group B Skupina B					
Day of birth Na dan okoćenja	66	9.34	534.3	56.5	
					15.1
Age 21 days Na 21. dan starosti	51+2**	7.57	2978.6	372.5a	
					30.8
Age 30 days Na 30. dan starosti	51+2	7.57	4722.9	649.4	
Mortality,% Smrtnost %	19.7				

* Sign. F (mass at age 21 days) = 0.016; Sign. F (masa na 21. dan) = 0.016
Sign. F (mass at age 30 days) = 0.062; Sign. F (masa na 30. dan) = 0.062
a, b Mean values labeled with the same letter do not differ significantly at 5 % risk (Duncan multiply range test);
Prosjeci označeni s istim slovom međusobno se ne razlikuju signifikantno pri 5% značajnosti
** In each group 2 young ones were taken for analysis; U svakoj skupini bila su uzeta po 2 mladunca za analizu.

Chemical analysis of selenium in blood of females showed a significant decrease of selenium in mother's blood in the second week of lactation (Table 5), which does not correspond to the data in literature (McConnell and Roth, 1964; Maus et al., 1980). Authors reported of the increase of selenium in blood and milk in bitches and cows during the lactation period if selenium was added. The decrease was most evident in the control group. In the third week of lactation the contents exceeded the starting values in all groups, more so in the control Group K and Group B. Differences among groups in the third week were not significant, neither were the average values from the first and the third week. Such development can be explained by higher transfer of selenium from blood into milk in the second week of lactation because of high milk production. We cannot prove it since we do not know the contents of selenium in milk of females.

Table 5: The average content of selenium in mother's blood during lactation (mg. kg^{-1} DM of lyophilised samples)
Tablica 5: Prosječni sadržaj selena u krvi majke u vrijeme laktacije (mg. kg^{-1} ST liofiliziranih uzoraka)

Week Tjedan	K	Difference Razlika	A	Difference Razlika	B	Difference Razlika	$\bar{x}$*
1	1.06		0.94		1.13		1.04a
2	0.74		0.93		0.89		0.86b
		-0.32		-0.01		-0.24	
3	1.22		1.12		1.15		1.16a
		+0.48		+0.19		+0.26	
4**			1.14				

* Mean values labeled with the same letter do not differ significantly at 5 % risk (Duncan multiply range test)
Prosjeci označeni s istim slovom se međusobno ne razlikuju pri 5 % značajnosti (Duncan multiply range test).
** contents of selenium in the fourth week of lactation were not statistically processed because samples from groups K and B were not analysed, and measuring was done only twice in each group
Sadržaj selena u četvrtom tjednu laktacije nisu statistički obrađeni jer su uzorci skupina K i B ispala pri analizi selena, u svakoj skupini pa su bila samo po dva mjerenja.

Chemical analysis of selenium in liver of young rabbits at age 30 days in comparison with the age 21 days (Table 6) showed a statistically significant increase of selenium in the liver in Group A and in Group B but not in the control Group K. Statistically significant differences were found among groups, selenium increased especially in Group B in comparison with Group K and Group A at the age 21 days. At the age 30 days a significant gradation from Group K over Group A to Group B was observed (1.95:2.20:2.54 μg^{-1} DM).

Table 6: An average content of selenium in liver of rabbits per groups (mg.kg^{-1} DM of lyophilised samples), n=2
Tablica 6: Prosječan sadržaj selena u jetrima kunića po skupinama (mg.kg^{-1} ST liofiliziranih uzoraka), n=2

Week Tjedan	K	A	B
Age 21 days 21. dan starosti	1.97	1.89	2.18
Age 30 days 30. dan starosti	1.95	2.20	2.54

Sign. F (week) = 0.189; Sign. F (tjedan) = 0.189
Sign. F (group) = 0.137; Sign. F (skupina) = 0.138

In the present research we have confirmed that kidneys are collectors for selenium supply as it was already stated by other authors (Krajinović, 1983; Florkin and Stotz, 1979, etc.). In Table 7 all the values shown are higher than in liver. Unexpectedly the lowest content was found in Group A and a comparatively high content in the control group. The comparison of both figures shows a significant increase of selenium in kidneys at regular supply.

Table 7: An average content of selenium in kidneys of the young per group ($\mu g.g^{-1}$ DM of lyophilised samples), n=2
Tablica 7: Prosječan sadržaj selena u bubrezima mladunaca po skupinama ($\mu g.g^{-1}$ ST liofiliziranih uzoraka), n=2

Week Tjedan	K	A	B	$\bar{x}$
Age 21 days 21. dan starosti	2.33	1.95	2.29	2.19A**
Age 30 days 30. dan starosti	3.14	2.23	3.22	2.86B
$\bar{x}$	2.735a*	2.09b	2.755a	

* Sign. F (week) = 0.011; Sign. F (tjedan) = 0.011
** Sign F (groups) = 0.043; Sign. F (skupina) = 0.043
a,b Mean values labelled with the same letter do not differ significantly at 5 % risk (Duncan multiply range test).
Prosjeci označeni istim slovom se međusobno ne razlikuju značajno pri 5 % značajnosti (Duncan multiply range test).
A,B Mean values labelled with the same letter do not differ significantly at 1 % (Duncan multiply range test).
Prosjeci označeni istim slovom međusobno se ne razlikuju značajno pri 1% značajnosti (Duncan multiply range test).

Table 8: Selenium results in standard reference samples ($\mu g.g^{-1}$ dry weight)
Tablica 8: Rezultati za selen u standardnim referentnim uzorcima ($\mu g.g^{-1}$ suhe težine)

Standard reference sample Standardni referentni uzorak	N		Selen-Selenium R± σ C.,V
NBS SRM 1577A Bovine liver Bubreg goveda	4	0.49± 0.02	0.71± 0.07
NBS SRM 1577 Bovine liver Bubreg goveda	10	1.01± 0.07	1.1± 0.1
IAEA H-8 Horse kidney Bubreg konja	6	4.36 ± 0.24	4.67± 0.35

R± σ : arachived results ± stand. deviation
dobiveni rezultati ± stand. devijacija
N : number of determinations
broj određivanja
C.,V. : values in the certificate
vrijednosti iz certifikata

CONCLUSION

Concerning the achieved results it can be stated that comparatively high quantities of selenium in the rations of female rabbits in the period of lactation have a positive effect on the increase of body weight of embryos in the last week of gestation and on the development of the young until age 21 days and partly until the end of lactation.

The established decrease of selenium in blood of females has not been explained so far. Further studies on dynamics of selenium content in milk are recommended.

It can be concluded that an optimum quantity of selenium in a ration for females and the young in lactation period is about. 0.30 ppm and supplement of the basic ration with selenium is reasonable only if a suitable analysis of feedstuffs is available.

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SAŽETAK

U krmnu smjesu koju su dobivale ženke bijele novozelandske pasmine u zadnjem tjednu bređosti i u laktaciji, što je već sadržavala 0.194 ppm selena (K), dodato je 0.1 (A), odnosno 0.3 ppm (B) selena u obliku Na_2SeO_3. Dodatak selena u skupinama A i B pozitivno je utjecao na povećanje mase plodova u zadnjem tjednu bređosti (K=36.25 %, A=57.8%, B=56.4%) te značajno na razvoj tjelesne mase mladih kunića u skupini A (K = 343.6 g, A = 436.2 g, B = 373.5 g). Sadržaj selena u krvi majke u drugom tjednu pao je na najnižu razinu, u trećem tjednu premašio je vrijednosti prvog tjedna (K = 1.06/0.74/1.22, A = 0.94/0.93/1.12, B = 1.13/0.89/1.15 $\mu g.g^{-1}$ ST). U jetrima mladih kunića koncentracija selena na 21. dan bila je značajno veća u varijanti B (K = 1.97, A = 1.89, B = 2.18 $\mu g.g^{-1}$ ST), na 30. dan statistički je postupno rasla razmjerno s dodatkom selena (K = 1.95, A = 2.20, B = 2.54 $\mu g.g^{-1}$ ST). U bubrezima slika je bila manje jasna, u usporedbi s vrijednostima u jetrima utvrđene su više koncentracije selena, najviša pak na 30 dan u skupini B (3.22 $\mu g.g^{-1}$ ST).

IZVLEČEK

Vpliv krmi dodanega selena na njegovo vsebnost v tkivih in na rast kuncev

V krmno mešanico, ki smo jo pokladali samicam pasme beli novozelandec v zadnjem tednu brejosti in v laktaciji in ki je že vsebovala 0.194 ppm selena (K), smo dodali 0.1 (A) oziroma 0.3 ppm (B) selena v obliki Na_2SeO_3. Dodatek selena je v skupinah A in B pozitivno vplival na povečanje mase zarodkov v zadnjem tednu brejosti (K=36.25 %, A=57.8 %, B=56.4 %) in značilno na razvoj telesne mase mladičev v skupini A (K=343.6 g, A=436.2 g, B=373.5 g). Vsebnost selena v krvi mater se je v drugem tednu znižala na najnižjo raven, v 3. tednu pa je presegla vrednosti 1. tedna (=1.06/0.74/1.22, A=0.94/0.93/1.12, B=1.13(0.89/1.15 $\mu g.g^{-1}$ SS). V jetrih mladičev je bila koncentracija selena na 21. dan značilno višja pri različiti B (K=1.97, A=1.89, B=2.18 $\mu g.g^{-1}$ SS), na 30. dan pa se je statistično stopnjevala sorazmerno z oskrbo s selena (K=1.95, A=2.20, B=2.54 $\mu g.g^{-1}$ SS). V ledvicah je bila slika manj jasna, v primjerjavi z vrednostmi v jetrih so bile ugotovljene višje koncentracije selena, najvišja pa na 30. dan v skupini B(3.22 $\mu g.g^{-1}$ SS).