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Absorption of ^{35}S-Thiamine in Jejunum and its Brain and Liver Content in Chickens on Different Selenium Level Diets

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The effect of 0.3, 3 and 10 mg SeO_2 added to chicken ration on *in vitro* absorption of ^{35}S-thiamine and its content in brain and liver have been studied.

Addition of SeO_2 inhibits thiamine absorption in chicken jejunum. With an increase of SeO_2 in the ration thiamine transport decreases. Higher doses of SeO_2 (3 and 10 mg) inhibit intestine alkaline phosphatase activity. A dose of 10 mg lowers total thiamine content in brain and liver, whereas 3 mg retains it.

Observations of numerous investigators have proved that selenium deficiency in chickens (22), turkey-poults (16) and ducklings (10) diet, has a negative effect on their development and health. The obtained data have shown that chickens receiving selenium deficient ration suffer exudative diathesis that increases their lethality. They can easily be cured by adding 0.1-0.2 mg selenium/kg forage (1). Furthermore the biological part of selenium in organism is determined by its significance for the intermediate metabolism of proteins, amino acids (8), phosphorus (11), activation or inhibition of enzymes (1, 4, 17). In definite doses, the element is considered as essential for the physiological and biochemical function of organism (1, 10, 20, 22).

In its chemical properties, selenium is closely related to sulphur and this is the basic reason for the existence of concurrent interrelations between the two elements. Their antagonism, on the other hand, has been successfully exploited for treatment of selenium toxicosis with sulphur compounds and sulphur containing amino acids (5, 6). DL-methionine included in mice ration increases selenium excretion with the urine (5). All those facts have led to the assumption of its possible participation in thiamine intermediate metabolism. Sulphur being a structural component of vitamin B_1 renders the hypothesis plausible.

The purpose of the present investigation was to study the effect of different doses of selenium dioxide included in chickens

diet on thiamine absorption and its content in brain and liver.

Materials and Methods

An experiment with newly hatched white Plymouth Rock male chickens, divided in four groups, was carried out. The components of their basic ration were balanced according to their nutritive values (table I). The rations of the groups differed only in the amount of added exogenous selenium oxide administered as follows:

I group (control), basic ration, without SeO_2; II, basic ration + 0.3 mg SeO_2/kg forage; III, basic ration + 3.0 mg SeO_2/kg forage, and IV, basic ration + 10.0 mg SeO_2/kg forage.

Selenium in the ration was determined colorimetrically with the method of CUMMINS *et al.* (3).

For examining thiamine absorption, 6 chickens were taken, from each group, at 45 days of age. The method of tissue accumulation (9), *in vitro*, was applied. After starving for 18 hours, with access to water, the chickens were decapitated and segments, 4 cm long, were separated from the proximal part of the jejunum and immediately rinsed with 0.9 % (w/v) NaCl. The mucosa was brought to the surface by turning the intestine inside out and then cut in sections 3-4 mm long. The sections were promptly placed in Erlenmeyer flasks, 100 ml, containing 20 ml Krebs-Hensleite buffer with 0.3 % glucose, pH 7.4, previously tempered and aerated. The incubation was carried out at 37° C for one hour in a schaker water bath (80 osc/min). At the beginning of the incubation 0.1 ml ^{35}S-thiamine solution (activity 14.7 μCi) was added to each flask. After the incubation the segments were rinsed with distilled water, dried, weighed, and homogenized with 15 % (w/v) trichloracetic acid. The homogeneous mixture was spun at 10,000 (rpm) for 10 minutes and the sediment rinsed twice with 5 % (w/v) trichloroacetic acid.

Table I. *Composition of the experimental (basic) ration.*
Selenium's content in the ration is 0.537 mg/kg forage.

Components	%
Maize meal	62.00
Soy-bean groats	29.20
Forage yeasts	2.00
Alfalfa meal	2.00
Chalk	1.00
Calcium diphosphate	1.70
Salt	0.30
Mixture of trace elements *	0.50
Vitamin premix **	1.00
L-Lysine	0.10
DL-Methionine	0.15
1 kg blend contains (g):	
Exchange energy	2,839 kcal
Raw protein	186.9
Raw fibres	39.1
Methionine and Cysteine	7.8
Lysine	11.9
Calcium	9.6
Total phosphorus	7.2

* The mixture of trace elements contains (%): ironic sulfate, 7.5; cuprous sulfate, 1.0; manganous sulfate, 12.5; zink sulfate, 10.0; cobalt sulfate, 0.1; potassium iodide, 0.05; manganese sulfate, 10.0; chalk, 68.85.

** 1 kg blend contains: vitamin A (acetate), 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 10 mg; vitamin K, 0.5 mg; Riboflavin, 3 mg; thiamine, 1.5 mg; nicotinic acid, 30 mg; calcium pantothenate, 10 mg; pyridoxine, 2 mg; choline chloride, 1,000 mg; vitamin B_{12}, 25 mg.

The radioactivity of the supernatant was measured with Packard-Tricarb Scintillation Spectrometer, model 3330, after addition of 0.5 ml of the sample to 10 ml of the scintillation solution (7.5 g PPO/ 2.5-diphenyloxazole and 0.74 g POPOP/ 1.4-Di-[2-(5-Phenyloxazolyl)] benzene in one liter of one part triton-x-100 and two part toluene).

In another trial with 6 chickens from each group, the content of total thiamine in brain and liver was determined (19).

Alkaline phosphatase in jejunum mu-

cosa was determined with the method of BODANSKY (2).

The results were statistically elaborated and P values determined with t Student's test.

Results

Thiamine absorption is influenced by the different levels of selenium in the ration; with the increase of its content the absorption decreased (table II).

The activity of intestine alkaline phosphate underwent changes as shown in figure 1: the lowest level of SeO_2 in the ration —0.3 mg did not exert substantial changes to the enzyme activity whereas quantities of 3 and 10 mg SeO_2/kg forage markedly depressed it ($P < 0.001$).

The addition of 0.3 mg SeO_2/kg forage did not lead to noticeable changes in the thiamine content in brain and liver. However, with raising of SeO_2 level up to 3 mg a significant increase of thiamine as in brain so in liver was observed ($P < 0.001$); on the contrary 10 mg of the compound strongly decreased its level in these tissues (table III).

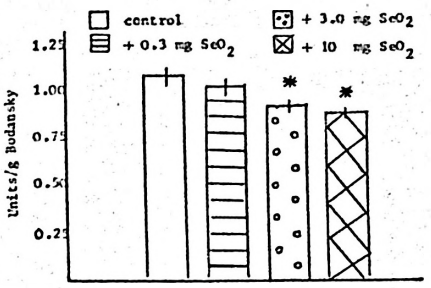

Fig. 1. *Influence of different levels of selenium oxide in the ration on intestine alkaline phosphatase activity in chickens jejunum.* Number of chickens per group, 6. * $P > 0.001$.

Table II. *Influence of different levels selenium oxide in the ration on absorption of ^{35}S thiamine in chickens jejunum.* Number of chickens per group, 6.

SeO_2 (mg/kg forage)	Radioactivity g/intestine wall (cpm/g)	P
—	57,588 ± 2,240	—
0.3	45,590 ± 2,450	<0.01
3.0	33,500 ± 1,828	<0.001
10.0	35,216 ± 1,985	<0.001

Discussion

The data analysis showed an existing relation between selenium content in the diet and thiamine absorption i.e. with the increase of selenium in the ration vitamin B_1 transport was inhibited. A similar relationship was observed with the effect of selenium on alkaline phosphatase activity in jejunum mucosa. In this case the enzyme inhibition at a higher selenium level in the ration is also characteristic. The activity of intestine alkaline phosphatase as shown by our previous investigations with chickens (12) and by those of SCHALLER *et al.* (21) and MATSUDA

Table III. *Content of thiamine in brain and liver of chickens receiving different amounts selenium oxide in the ration.* Number of chickens per group, 6.

SeO_2 (mg/kg forage)	Brain		Liver	
	µg/g fresh tissue	P	µg/g fresh tissue	P
—	1.478 ± 0.043		2.243 ± 0.051	
0.3	1.555 ± 0.068		2.197 ± 0.045	
3.0	1.766 ± 0.075	< 0.01	2.689 ± 0.049	< 0.001
10.0	1.071 ± 0.068	< 0.001	1.276 ± 0.060	< 0.001

et al. (15) is of a definite functional importance for the thiamine active transport. It was found that the lower level of the vitamine B_1 transport in chickens distal small intestine parts (12) correlated with the lower alkaline phosphatase activity or with its inhibition by L-phenylalanine, a specific inhibitor of the enzyme (14, 21), or by ethanol (15).

The ions of some metals effect the activity of the intestine enzymes, the alkaline phosphatase including. The blockade of their active site sulfhydryl groups inhibits the enzyme activity (23).

PONZ (18) has succeded in inhibiting glucose active transport by sodium selenite. In his opinion this is due to inhibition of enzymes carrying SH-groups in their active site by the selenium ion. With addition of free SH-group donors, glutation-SH or L-cysteine to the substrate a significant increase in thiamine transport in chickens small intestines was observed (13). LAZAROV (11) has showed that 5.5 mg SeO_2/kg forage inhibits the absorption of ^{32}P in chickens alimentary channel.

On the other hand we assume that selenium being an antagonist of sulphur displaces it from thiamine molecule and as a result selenium thiamine is built. Most probably this compound's transport through the intestinal epithelial cells membranes is impeded. JACOBSSON (7) has established that sulphur in methionine and cysteine molecules can be replaced by selenium whereby selenium-methionine and selenium-cysteine are formed. STANCHEV (unpublished data) has established that selenium-methionine absorption in chickens duodenum and ileum is lower than methionine absorption. Consequently the mentioned properties of selenium are of paramount importance for thiamine transport.

Thiamine content in brain and liver varies according to the quantity of selenium in the diet. A low SeO_2 level of 0.3 mg did not cause substantial changes in thiamine distribution in these tissues. The addition of 3 mg SeO_2, however, markedly increased total thiamine, whereas 10 mg decreased its content.

The results of this investigation showed that selenium participates in thiamine distribution in the different tissues. The optimal physiological doses rendering a positive effect, however, are in a very narrow range; the limit in the chickens ration being 3-4 mg SeO_2/kg forage, above which selenium intoxication occurs (1).

Resumen

Se estudia el efecto de SeO_2 (0,3, 3 y 10 mg/kg) añadido a la dieta, sobre la absorción *in vitro* de S^{35}-tiamina y su contenido en cerebro e hígado de pollo.

La adición de SeO_2 a la dieta inhibe la absorción de tiamina por yeyuno de pollo, así como la actividad de la fosfatasa alcalina intestinal (con 3 y 10 mg/kg de SeO_2). Disminuye el contenido total de tiamina en cerebro e hígado únicamente con la dosis de 10 mg/kg.

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