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ALTERATIONS IN LIVER AND KIDNEYS OF CHICKENS FED WITH HIGH LEVELS OF SODIUM SELENITE OR SELENIZED YEAST

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The experiment was carried out with 260 chickens divided into 13 groups, for 6 weeks. All chicken groups were fed with commercial mixtures, and selenium was added in their feed in the form of sodium selenite or selenized yeast at following concentrations: 0, 2, 5, 10, 15, 20 or 30 mg Se/kg.

Three birds from each group were sacrificed on the 10th, 24th and 42nd days of the experiment. All internal organs were inspected and parts of the liver and kidneys were subsequently taken for pathohistological investigations.

In birds fed with 2 and 5 mg Se/kg feed in the form of sodium selenite neither pathomorphological nor pathohistological alterations in the liver or kidneys were noticed at any of the monitoring intervals. In birds fed with the higher rates of inorganic selenium (10, 15, 20 or 30 mg Se/kg feed) certain pathohistological alterations occured that were more marked with the higher concentrations of selenium in the feed or after longer duration of intake.

No alterations were noticed in the mentioned organs from chickens supplied with 2, 5, 10 or 15 mg Se/kg feed in the form of selenized yeast. Alterations of liver and kidneys were encountered only in birds treated with exceptionally high levels of organic selenium (20 or 30 mg Se/kg feed).

In chickens fed with 10, 15, 20 or 30 mg Se/kg feed in the form of Na_2SeO_3, the liver was enlarged and of a lighter coloration, while pathohistological evidence varied between intracellular edema and necrotic changes. In kidneys, edema of the renal tubules was noticed and at the highest levels vacuolization and ballooning dystrophy of cells with loss of nuclei was found.

In chickens supplied with 20 or 30 mg Se/kg feed in the form of selenized yeast, pathohistological changes were less marked than in those fed with the same amounts of Se in the form of Na_2SeO_3.

Key words: broilers, sodium selenite, selenized yeast, liver, kidneys

INTRODUCTION

In Se poisoning, specific dose-related, pathomorphological and pathohistological alterations occur in internal organs. In acute selenosis, haemorrhagic and necrotic degeneration of the liver is noticed,while the nuclei of hepatocytes are fragmented and decayed. In kidneys, parenchymatic degeneration occurs, and discharge collector tubules are impaired (Orstadius, 1960).

In subacute selenosis, the liver is enlarged, with focal necrosis and haemorrhage. The renal medulla is haemorrhagic, and obstruction of the ureter is present (Rosenfeld and Beath, 1964). Chronic selenosis differs from the acute and subacute forms by a substantially lower concentration of Se in all tissues. In this form of poisoning, the liver is cirrhotic and atrophic due to formation of fibrous tissue. The kidneys are atrophic and usually contain concrements in their discharge tubules. In chickens fed with 10 and 20 mg Se/kg feed, a variegated liver is encountered. Selenium is distributed through the whole organism, but the highest concentrations are found in liver and kidneys (Levander, 1986). Selenium at 6 mg Se/kg feed (in the form of Na_2SeO_3) led to an increase in its concentration in the liver, kidneys and muscles of broilers and hens (Moksnes and Norheim, 1982; Moksnes, 1983).

Madej *et al*. (1988) established that levels of 1,4 mg Se/kg body mass in the drinking water caused pathohistological lesions in parenchymatous organs in hens. Se was detected in the liver even in poultry fed with 1 and 5 mg Se/kg feed (Jensen, 1986).

Harry *et al*. (1988) found a visible focal necrosis or marked fibrous areas in the liver of waterbirds living in water that contained 140-1400 μg Se/L.

When chickens were given toxic doses of organic Se (8-13 ppm in Se-enriched corn), the predominant pathological changes were characterised by local necrosis in the liver, myocardial degeneration and convoluted tubule necrosis in the kidneys (Qi *et al*., 1992).

On the basis of the pathohistological alterations in the liver and kidneys of chickens fed with toxic levels of inorganic and organic selenium, these changes may be classified as chronic selenotoxicoses.

MATERIAL AND METHODS

A total of 260 chickens of the Hybro variety were divided into 13 groups for the experiment. They were fed with commercial mixtures containing 0, 2, 5, 10, 15, 20 and 30 mg added Se/kg in the form of sodium selenite or selenized yeast. The experiment lasted for 42 days. Three birds from each group were sacrificed on the 10th, 24th and 42nd days of the experiment. After the autopsy and detailed macroscopic inspection of the organs, parts of the liver and kidneys were taken for pathohistological analyses.

Sections of liver and kidneys were fixed in 10% neutral formalin and absolute alcohol. After that, they were embedded into paraffin and cut on a cryotome. Microtome sections 10 μm thick, were stained with by haematoxylin-

eosin, by the trichromic method according to Masson-Goldner, by the Best method for glycogen and with Sudan III for fats.

RESULTS

Pathoanatomical and pathohistological evidence in the sacrificed chickens depended on the Se dose received and the time of sacrifice. In chickens fed with meal containing more than 10 mg Se/kg (Na_2SeO_3) or more than 20 mg Se/kg (selenized yeast), specific pathohistological alterations were noticed. In the livers of chickens fed with meal containing 10 mg added Se/kg, intracellular edema, enlarged hepatocytes, light cytoplasm, which sometimes had a foamy appearance, were noticed on the 10th day of the experiment (Figure 1). Glycogen quantity was lower than in chickens receiving feed without added selenium. In kidneys, intracellular edema of the epithelial cells of proximal renal tubules appeared (Figure 2). Cells were enlarged with a weblike cytoplasm, and the tubular lumen was narrower. After the same interval, alterations of the liver and kidneys were more marked in chickens treated with 15 mg Se/kg feed. There were fatty vacuoles in the hepatocytes, and the nuclei were dislocated to the peripheral cytoplasm. In the kidneys, a more severe intracellular edema was encountered, and the lumina of some tubules were closed. High selenium levels (20 and 30 mg Se/kg feed)induced more intensive and more massive alterations in the liver and kidneys of the chickens. Fatty degeneration of the liver was present, nuclei were lacking, and fused fatty vacuoles had the appearance of fatty cisterns (Figure 3). Vacuolization and a ballooning appearance of cells without nuclei were found in the kidneys (Figure 4).

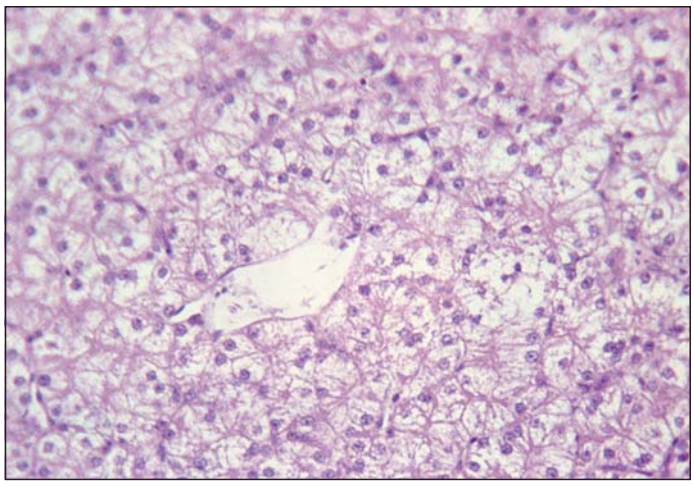

Figure 1. Liver of a chicken fed with a commercial mixture containing 10 mg added Se/kg in the form of sodium selenite and sacrificed after 10 days. Intracellular edema most intensive (HE, x 400).

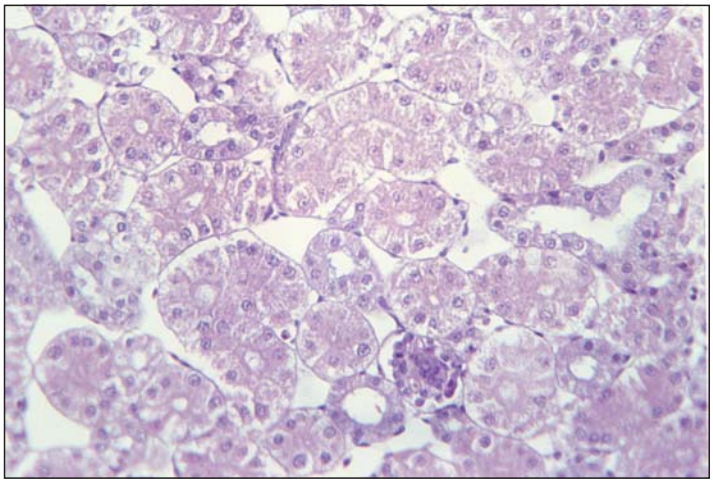

Figure 2. Kidney of a chicken fed with a commercial mixture containing 10 mg added Se/kg in the form of sodium selenite and sacrificed after 10 days. Intracellular edema in proximal renal tubules (HE, x 400).

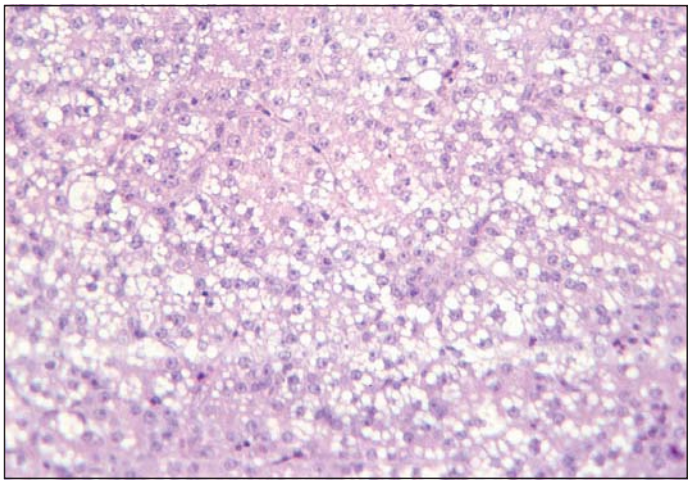

Figure 3. Liver of a chicken fed with a commercial mixture containing 30 mg added Se/kg in the form of sodium selenite and sacrificed after 10 days. Fatty degeneration most intensive (HE, x 200).

The most intensive pathohistological alterations were observed in chickens sacrificed on the 24th and 42nd days of the trial. Alterations of the necrobiotic type were noticed in the liver, especially in chickens treated with 30 mg Se/kg feed

(Figure 5). The epithelium of the renal tubules was filled with fatty drops, and in the glomerular area, a homogenous eosinophil content was noticed (Figure 6).

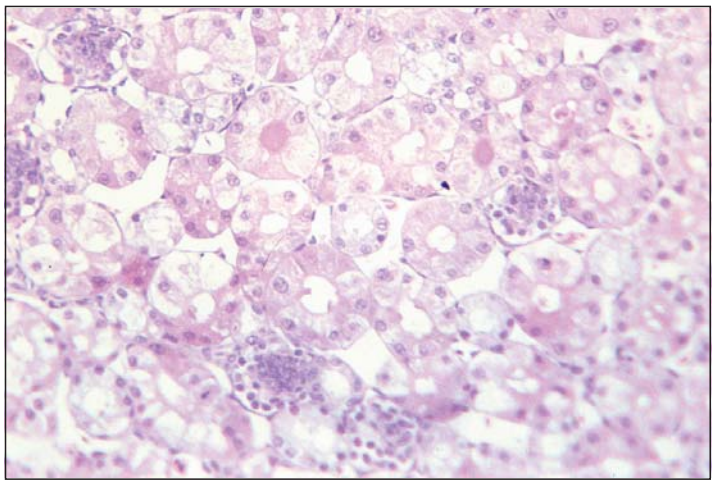

Figure 4. Kidney of a chicken fed with a commercial mixture containing 30 mg added Se/kg in the form of sodium selenite and sacrificed after 10 days.

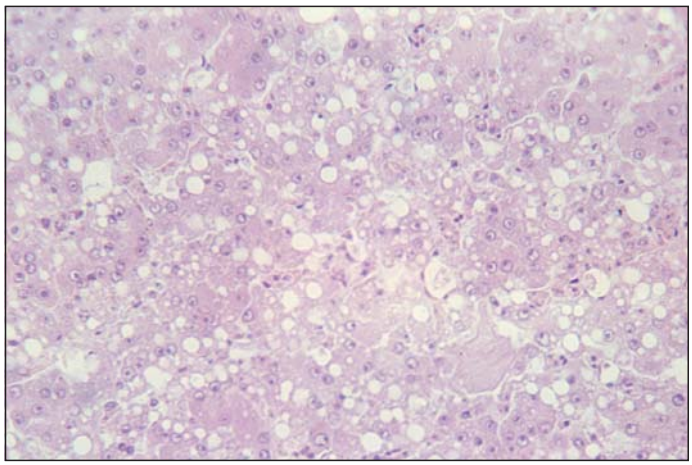

Figure 5. Liver of a chicken fed with a commercial mixture containing 30 mg added Se/kg in the form of sodium selenite and after sacrified 42. Fatty and necrobiotic degeneration (HE, x 200).

In chickens fed for 10 days with 20 or 30 mg Se/kg feed in the form of selenized yeast, intracellular edema of varying intensity was found in the liver, and edema of the proximal tubular epithelium was present in renal parenchyma (Figure 7,8).

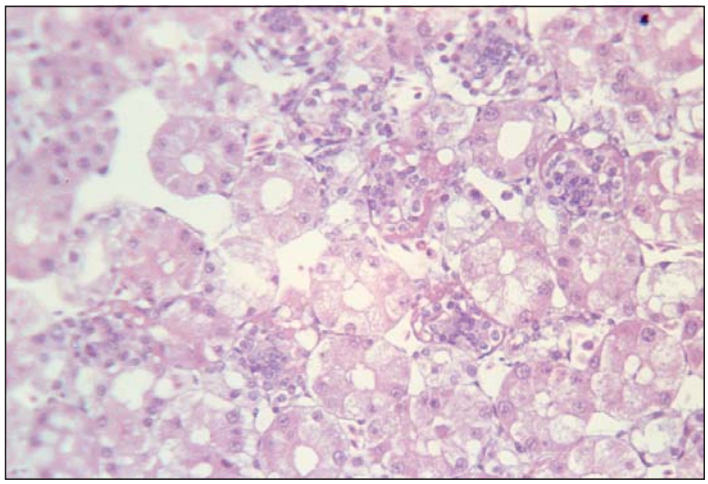

Figure 6. Kidneys of a chicken fed with a commercial mixture containing 30 mg added Se/kg in the form of sodium selenite and sacrificed after 42 days. Epithelium of the renal tubules with fatty drops and a homogeneous eosinophil content in the glomerular area (HE, x 200).

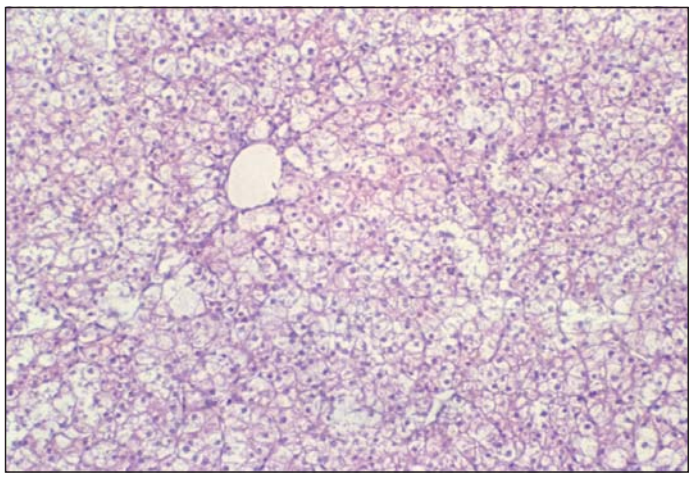

Figure 7. Liver of a chicken fed with a commercial mixture containing 30 mg added selenised yeast and sacrificed after 10 days. Intracellular edema (HE, x 200).

Alterations found after 24 days in organs of chickens fed with meal containing 20 mg added Se/kg feed were identical to those found after 10 days.

However, supplying 30 mg Se/kg feed caused more marked alterations in the liver of birds sacrificed after the same interval (24 days). Fatty infiltration was found, and intracellular edema of the renal tubule epithelium was present in the kidneys. On the last day of the experiment (42nd day), alterations in the organs were more intensive in both groups in comparison to previous intervals. Fatty infiltration and the presence of fatty vacuoles were found in the liver.

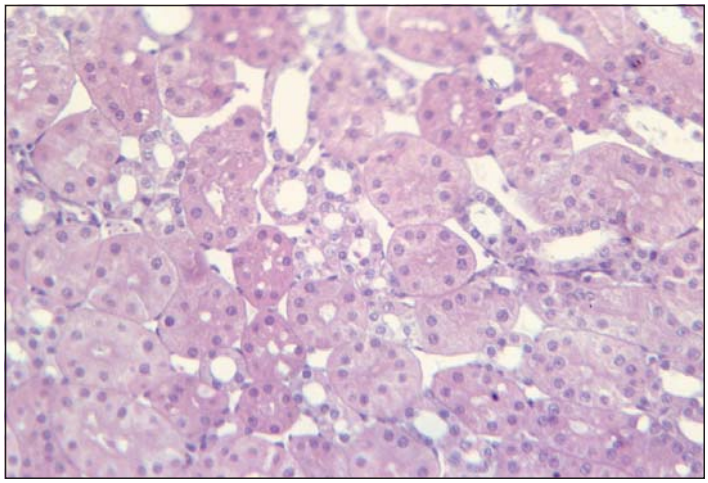

Figure 8. Kidneys of a chicken fed with a commercial mixture containing 30 mg/kg added selenised yeast and sacrificed after 10 days. Edema of the proximal tubular epithelium in rena parenchyma (HE, x 200).

DISCUSSION

We have found in our experiment that only the concentrations higher than 10 mg Se/kg feed (in the form of selenized yeast) led to specific pathoanatomical and pathohistological alterations in chicken liver and kidneys. In other organs, there were no visible macroscopic alterations. Certain authors (Rosenfeld and Beath, 1964; Jahn, 1975; El-Begearni and Combs, 1979 and Gary and Margaret, 1993) state that in chronic selenosis alterations in cardiac muscle, lungs and stomach, as well as the presence of yellowish fluid around certain organs are also encountered. The lowest levels of Na_2SeO_3 in our experiment, (2 and 5 mg Se/kg feed), did not induce any pathohistological changes in the mentioned organs. This indicates that these doses were not toxic for chickens.

In the liver of chickens fed for 10 days with mixtures containing 10 mg Se/kg, intracellular edema of moderate to severe intensity and reduction of glycogen granules in hepatocyte cytoplasm can be seen. Robins (1994) considers glycogen reduction as one of the most sensitive indicators and the most frequent

primary non-specific response to cell damage. Similar alterations were found by Jahn (1975) in broilers treated with high doses of selenium for several weeks. Thus,30 mg Se/kg feed led after 10 days to the appearance of fatty degeneration with visible fatty cisterns. Herigstad *et al*. (1973) found identical alterations in the liver of swine fed with high levels of selenium for several weeks. In chronic selenosis, Rosenfeld and Beath (1964) found enlarged hepatocytes, fatty vacuoles and necrobiotic areas in animal liver, which is similar to our results with chickens treated with 30 mg Se/kg feed in the form of Na_2SeO_3.

Histological lesions in mallards that died of selenosis were hepatocellular vacuolar degeneration progressing to centrolobular and panlobular necrosis (Green and Albers, 1997). Histological lesions in the surviving mallards included atrophy of lymphoid tissue, hyalinogranular swelling of hepatocytes, atrophy of seminiferous tubules and senescence of feathers (Green and Albers, 1997).

The most severe changes were found in the livers of birds supplied with 30 mg Se/kg feed for 24 and 42 days. Hepatocyte nuclei were pycnotic or lacking, and also unreactive necrobiotic areas were noticed. Harry *et al*. (1988) found focal necrosis and focal lymphocytic infiltration in coot liver. The water inhabited by these birds contained 140-1400 μg Se/L. Rosenfeld and Beath (1964) stated that in chronic selenosis parenchymatous degeneration occurs in the kidneys, as well as haemorrhage and early glomerulonephritis in renal tubules.

Equivalent levels of Na_2SeO_3 and selenized yeast do not exert the same toxic impact. Pathohistological alterations in the liver and kidneys of chickens fed with selenized yeast are less marked than those in animals fed with Na_2SeO_3. Zulian (1984) reported that selenized yeast was less toxic than Na_2SeO_3. However, Mihailović *et al*. (1993) found that Na_2SeO_3 and selenized yeast caused identical pathohistological changes in certain internal organs of swine. Pathohistological changes in the liver and kidneys were visible only at high doses of selenized yeast (20 and 30 mg Se/kg feed). Changes in the liver varied from intracellular edema and opaque cytoplasm, to fatty infiltration. An increase of Kupfer cell number was noticeable. Herigstad *et al*. (1977) also observed an increase of Kupfer cell number in swine fed with high doses of selenomethionine. Intracellular edema of moderate to severe intensity was found in the proximal tubules. Changes in organs are more noticable in animals which consume this feed for a longer time, but are less intensive than in animals fed with high doses of sodium selenite.

The molecular mechanisms of Se toxicity are not well defined. The primary targets of acute Se toxicity in food animal species are the cardiovascular, gastrointestinal and haematopoietic systems (Raisbeck, 2000). However, organic Se in high doses is also toxic, but SeMet does not produce free radicals when reacting with glutathione.

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PROMENE NA JETRI I BUBREZIMA PILIĆA HRANJENIH VISOKIM NIVOIMA NATRIJUM SELENITA ILI SELENIZIRANOG KVASCA

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SADRŽAJ

Ogled je izveden na 260 pilića Hybro provenijence podeljenih u 13 grupa, u trajanju od 6 nedelja. Sve grupe pilića hranjene su komercijalnim smešama, a selen je dodavan u hranu u formi natrijum selenita ili seleniziranog kvasca u količini od 0, 2, 5, 10, 15, 20 ili 30 mg/kg.

Po tri životinje iz svake grupe su žrtvovane 10., 24. i 42. dana ogleda. Izvršen je pregled svih unutrašnjih organa, a potom su uzimani delovi jetre i bubrega za patohistološka istraživanja.

Kod pilića hranjenih sa 2 i 5 mg Se/kg hrane u obliku natrijum selenita nisu uočene patomorfološke ni patohistološke promene na jetri i bubrezima ni u jednom od ispitivanih vremenskih intervala. Kod pilića hranjenih sa višim nivoima neorganskog selena (10, 15, 20 ili 30 mg Se/kg hrane) uočene su određene patohistološke promene koje su bile izraženije sa povećanjem koncentracije selena i dužinom konzumiranja.

U pilića koji su dobijali 2, 5, 10 ili 15 mg Se/kg hrane u obliku seleniziranog kvasca nisu ustanovljene promene na pomenutim organima. Promene na jetri i bubrezima su se javljale samo kod životinja tretiranih izuzetno visokim nivoima organskog selena (20 ili 30 mg Se/kg hrane).

U pilića hranjenih sa 10, 15, 20 ili 30 mg Se/kg hrane u obliku natrijum-selenita, jetra je bila uvećana i svetlije boje, a patohistološki nalaz se kretao od intracelularnog edema do nekrotičnih promena. Na bubrezima je uočen edem bubrežnih kanalića, a pri najvišim dozama ustanovljena je vakuolizacija i balonirajuća distrofija ćelija sa gubitkom jedara.

U pilića koji su dobijali 20 ili 30 mg Se/kg hrane u obliku seleniziranog kvasca, patohistološke promene su bile manje izražene nego kod onih hranjenih sa istim nivoima Se u obliku natrijum-selenita.