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REGULAR ARTICLE

EFFECT OF DIETARY NICKEL SUPPLEMENTATION ON CONTENT OF MINERAL ELEMENTS IN RABBIT *TESTES*

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ABSTRACT

This study reports the effect of dietary nickel (Ni) on the accumulation of magnesium (Mg), potassium (K) and copper (Cu) in *testes* of rabbits. Broiler rabbits (*Oryctolagus cuniculus*) of experimental groups were fed a granular mixture with addition of various concentrations of Ni (E1 – 17.5 g $NiCl_2$ per 100 kg of feed mixture, group E2 - 35.0 g $NiCl_2$ per 100 kg of feed mixture). Group of rabbits withou Ni addition served as control (C). After the 90-days experimental period biological material *(testes*) was taken from the animals. Samples were analyzed by the atomic absorption spectrometry (AAS) method. The concentrations of Mg, K and Cu in *testes* in groups with dietary Ni supplement were not influenced Ni and differences among the groups remained insignificant (P>0.05).

Keywords: Magnesium, potassium, copper*, testes*, rabbits

INTRODUCTION

Nickel (Ni) is a heavy metal present in all elements of the environment. It is the fifth most widespread element on Earth. In chemical compounds, it has a few oxidation states, −1 being the lowest and +4 the highest. However, the most common state of Ni is +2 [Ni(II) (**Valko et al., 2005**). On the basis of the results from research **Murawska-Ciałowicz et al. (2012)** the mechanism of toxic activity of Ni(II) in the testes is oxidation-based. This research indicates the differences in the level of stress in animals that either have or lack protamine 2. In case of exposure to Ni(II), Reactive Oxygen Species (ROS), which are responsible for DNA damage, were produced in the testes. The release of Ni into the environment occurs from various sources: metallurgy and refining industries, coal combustion, diesel and fuel oil, sewage. Nickel and its compounds are bioaccumulated by human body by different routes – inhalation, ingestion and dermal **(Cempel and Nikel, 2006).** Ni is accumulated mostly in bones, parenchymal organs, myocardium, skin, hair **(Kabata-Pendias and Pendias, 1999)**. **Grabeklis et al. (2011)** investigated the composition of various biological matrices sampled from workers of chemical plant, exposed to various metals, including Ni. In the occupationally exposed workers, the content of Ni in hair, whole blood and blood plasma, as well as urine was higher. Our previous studies **Kalafova et al. (2011a, 2011b, 2011c, 2011d, 2012a**) reported that peroral administration of Ni or combination of Ni and Zn affect some production and metabolic parameters as well as the content of mineral elements in some organs of rabbits. The aim of this study was to investigate the effect of dietary Ni on the accumulation of Mg, K and Cu in *testes* of rabbits.

MATERIAL AND METHODS

Animals

In the present study, adult male rabbits (*Oryctolagus cuniculus*, Californian breed, broiler line) were used. Rabbits (n=15) were obtained from an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic. Rabbits (age: 4 months, weighing 3.5–4 kg) were housed in individual flat-deck wire cages (area 0.34 m^2) under a constant photoperiod of 14h of day-light.The temperature (18–20°C) and humidity (65 %) of the building were recorded continually using thermograph positioned at the same level as the cages. The animals were healthy and their condition was judged as good at the commencement of the experiment. In this animal study institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of the Slovak Republic.

Experimental design and diets

Rabbits were randomly divided into 3 groups (n=5 in each group). Rabbits were fed with a granular feed mixture *ad libitum* (KKV1). The experimental groups (E1 and E2) received Ni addition in the diet for 90 days (Table 1). The group that received a diet without Ni served as a control group.

Table 1 Design of experimental intervention

Group	C	E1	E2
Ni inclusionin g.100 kg^{-1} of FM	-	17.5	35.0

FM – Feed mixture, C – control group, E1, E2 – experimental groups

Procedures

The samples were analyzed for concentration of magnesium (Mg), potassium (K) and copper (Cu) using the atomic absorption spectrophotometry (AAS) method (wavelength for Mg 285.2 nm, K 766.5 nm, Cu 324.8 nm). Biological material (*testes)* was taken from animal organisms with chromo-nickel surgical instruments. Prepared samples were dried until dry mass was obtained. To obtain the dry mass, small pieces of tissue with the weight of 0.050 to about 1.000 g were placed on a Petri's dish and put into the thermostat regulated dryer at 60°C for 24h, next, the dryer temperature was set to 105°C. The samples were regularly weighted day by day until the loss of their mass was unnoticeable. Dried samples were mineralized by wet mineralization. In the process of wet mineralization all dry material of each sample was placed in separate mineralization tubes, dissolved by adding 2mL of concentrated HNO_3-$HClO_4$ mixture in the proportion 4:1 and heated in a thermostat digestion block at 120°C for 90 min. The resulting solution was diluted to 10 mL with demineralised water. All element concentrations are expressed on wet-weight basis in $\mu g.kg^{-1}$. The recovery of the method was 96–98 % and the reproducibility was better than 1 %.

Statistical analysis

To compare the results the analysis of variance, one-way ANOVA test were applied to calculate basic statistic characteristics and to determine significant differences among the groups. Statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA) was used. Differences were compared for statistical significance at the level $P<0.05$.

RESULTS AND DISCUSSION

Nickel is a widely distributed metal that is industrially applied in many forms **(Lu et al., 2005)**. It is an essential mineral element that may accumulate to toxic levels in soils due to anthropogenic activities **(Llamas and Sanz, 2008)**. In our experiment the concentrations of Mg, K, and Cu after nickel administration to the feed in *testes of* broiler rabbits were measured. The concentrations of Mg, K and Cu in *testes* after dietary inclusion of Ni are presented in Table 2. The analysis of our data demonstrated no significant differences ($P>0.05$) among the groups. **Miranda et al. (2009)** evaluated Cr, Ni and Cu accumulation in cattle raised in a serpentine area in Southwest Europe (Galicia, NW Spain). Samples of liver, kidney and muscle of 41 animals aged 8-12 months were collected at slaughter. Representative samples of soil and forage were taken from 10 farms. Accumulation of Cr in animal tissues was generally low and within the normal range. However, 20 % of the animals had toxic levels of Ni in kidney and 32 % of the animals had liver Cu levels above the acceptable range. Serpentine soils had a significant effect on Ni and Cu accumulation in cattle, and a relatively high percentage of the animals showed tissue levels of Ni and Cu indicative of risk of toxicity. The results of **Das et al. (2002)** indicate that nickel sulfate affects the steroidogenic enzymes, causing alteration in the formation of testosterone in both dietary groups, which was manifested in the elevated cholesterol and ascorbic acid level with decreased activities of steroidogenic enzymes in *testes* of adult rats. However, these alterations were reversible in both groups of animals fed normal protein diets and protein-restricted diets. **Toman et al. (2012)** reported a serious, time-dependent changes in the *testes*, mainly in the *germinal epithelium*, after a peroral intake of Ni. The results of **Toman et al. (2003)** showed significant decreas in the body weight in male mices after 12 weeks of Ni administration. After peroral administration of Cd and Ni in diet **Toman et al. (2005)** recorded changes in the testes of mice. **Martiniakova et al. (2009)** reported that peroral administration of Ni (35 g $NiCl_2$ per 100 kg of feed mixture) or supplementation with a combination of Ni and Zn (35 g $NiCl_2$ and 30g $ZnCl_2$ per 100 kg of the mixture) affects the microstructure of compact bone tissue in young rabbits. **Bersenyi et al. (2004)** found that supplementation with 50 and 500 mg Ni (as $NiCl_2$) per kg of feed resulted in accumulation of Ni in kidneys, bones, heart and liver of rabbits. Our previous studies **Kalafova et al. (2012b)** determinated the effect of dietary Ni on the accumulation of cadmium (Cd) and zinc (Zn) in *testes* and *epididymis* of rabbits. The concentrations of Cd and Zn in *testes* and *epididymis* in groups with dietary Ni supplement were not influenced.

Table 2 Concentration of Mg, K and Cu in *testes* of broiler rabbits

	C	E1	E2
Magnesium	1788,39±356,06	1510,56±495,78	1802,72±551,80
Potassium	41012,29±36113,02	43868,32±17880,25	56770,93±20463,46
Copper	10,82±2,67	8,08±1,11	8,81±1,95

C – control group, E1, E2 – experimental groups; results are expressed as mean±SD ($\mu g.g^{-1}$), differences among the groups were not significant (P>0.05)

CONCLUSION

In conclusion, the inclusion of Ni to the diet for male broiler rabbits had no effect on the concentration of Mg, K and Cu in *testes.* The study in the field of environmental pollution and the effect of various elements on animal organisms with be worthy of further investigations.

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