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**REGULAR ARTICLE**

# ACCUMULATION OF IRON AND NICKEL IN *TESTES* AND *EPIDIDYMIS* OF BROILER RABBITS AFTER NICKEL PERORAL ADMINISTRATION

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## ABSTRACT

This study reports the effect of dietary nickel (Ni) on the accumulation of Ni and iron (Fe) in *testes* and *epididymis* 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 and epididymis*) was taken from the animals. The samples were analyzed for concentration of Ni and Fe using the atomic absorption spectrophotometry (AAS) method. The concentrations of Fe and Ni in *testes* and *epididymis* in groups with dietary Ni supplement were not influenced and differences among the groups remained insignificant (P>0.05).

**Keywords:** Nickel, iron, *testes*, *epididymis*, rabbits

## INTRODUCTION

Nickel (Ni) is a heavy metal present in parts of the environment. It is the fifth most widespread element on Earth. Ni (II) compounds are used in different industries and for producing everyday objects, also used in shipbuilding, chemical, electrochemical, and galvanizing industries. It is used for producing Ni-Cd batteries, stainless steel, bathroom fittings, coins, colourings, kitchenware, cutlery, surgical instruments, dental and orthopedic prostheses, artificial jewellery, and so forth. Thus, Ni exposure is a problem of the whole population and allergies to nickel are reported quite often (10% women, 1% men) (**Prystowsky et al., 1979; Das et al., 2008)**. Although the toxicity and carcinogenicity of nickel compounds in humans and experimental animals are well demonstrated, the underlying mechanisms of their action remain unclear (**Sunderman et al, 1985; Stohs and Bagchi, 1995**). The most plausible mechanism that may be operative *in vivo* is the generation of reactive oxygen species (ROS), which may initiate lipid peroxidation (LPO), oxidative damage of critical macromolecules such as proteins or DNA, and cell damage or death. LPO constitutes a free radical oxidation process in which polyunsaturated fatty acids of the cell membrane decompose to yield, among others, highly reactive lipid hydroperoxides, $H_2O_2$, hydroxyl radicals, and malondialdehyde (MDA) (**Pryor, 1985; Halliwell and Gutteridge, 1989**). Effects on reproduction and essential trace metal (especially Fe ) metabolism have been reported at levels as low as 5 $\mu g.g^{-1}$ in food or drinking water (0.2–0.4 $mg.kg^{-1}$) but these findings have not always been corroborated (**Outridge and Scheuhammer**, 1993). **Kalafova et al. (2011a, 2011b, 2011c, 2011d, 2012**) 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 Ni and Fe in *testes* and *epididymis* 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 inclusion in 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 Ni and Fe using the atomic absorption spectrophotometry (AAS) method (wavelength for Ni 232.0 nm, Fe 248.3 nm). Biological material (testis and epididymis) was taken from animal organisms with chromo-nickel surgical instruments. Preparation 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 to105°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

**Concentration of Ni in *testes and epididymis* of rabbits**

The concentrations of Ni in *testes and epididymis* after dietary inclusion of Ni are presented in Figure 2. The analysis of our data demonstrated no significant differences ($P>0.05$) among the groups. The increase values of Ni were observed in epididymis; however differences among the groups remained insignificant ($P>0.05$). **Toman et al. (2012)** reported a serious, time-dependent changes in the *testes*, mainly in the *germinal epithelium*, after a peroral intake of Ni. **Skalická et al. (2012)** investigated the occurence of cadmium (Cd), Ni and lead (Pb) in the muscle and liver of cattle from agricultural farm near an industriaal plant in Eastern Slovakia. The maximum levels of Cd, Pb and Ni were recorded in the liver (0.865; 2.324; 1.140 $mg.kg^{-1}$, respectively) and muscle (0.300; 0.854; 0.700 $mg.kg^{-1}$, respectively). It was concluded that the exposure to an industrial plant significantly increases the levels of contaminants in the muscle and organs of cattle, as the most susceptible livestock. The results of **Toman et al. (2003)** showed decreas in the body weight in male mices after 12 weeks of nickel administration. After peroral administration of Cd and Ni in diet **Toman et al. (2005)** recorded changes in the testes of mice.

**Concentration of Fe in *testes* and *epididymis* of rabbits**

In Figure 3 the average levels of Fe in *testes* and *epididymis* of rabbits are plotted. The average values of Fe in testis and epididymis were not influenced by dietary inclusion of Ni

(P>0.05). The results of **Cempel (2004)** indicated that nickel ingestion (300 and 1200 ppm in the drinking water) induced Fe accumulation in serum and some organs of rats. The highest amount of Fe was found in the liver of all exposed animals. Fe is more suitable for biological purposes, in comparison to other transition metals, such as copper (Cu) and Ni (**Comporti, 2002**). This metal participates in numerous lifetime processes that make body function possible and adequate. A variety of studies have demonstrated the ability of Fe to catalyze the formation of ROS and stimulate lipid peroxidation **(Stohs and Bagchi, 1995). Sunderman et al. (1985), Athar et al. (1987) and Misra, (1990)** recorded that administration of Ni resulted in enhanced lipid peroxidation and increased tissue iron levels. **Kalafova et al. (2011)** observed significantly lower values of Fe in the group of female rabbits with Ni administration when compared with control group and group with Ni and zinc (Zn) addition. Data about the mechanisms of action of nickel on the reproduction system is inconsistent. NiCl2 concentrations used in this study are relatively low as it is naturally present in the feed, or in their individual components, which did not adversely affect the accumulation of nickel and iron in testes and epididymis of rabbits.

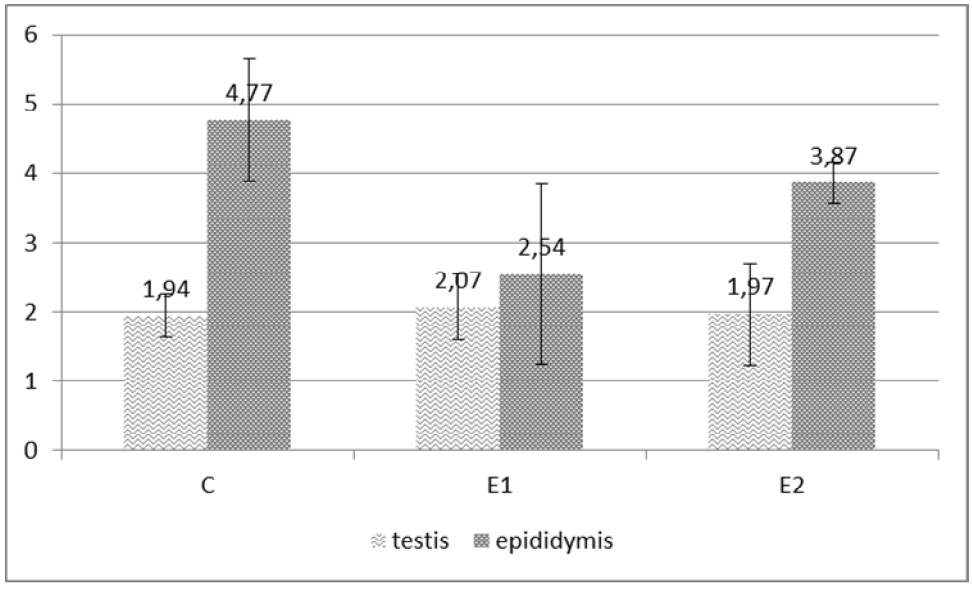


**Figure 2** Concentration of Ni in testes and epididymis of broiler rabbits ($\mu g.g^{-1}$)
C – control group, E1, E2 – experimental groups

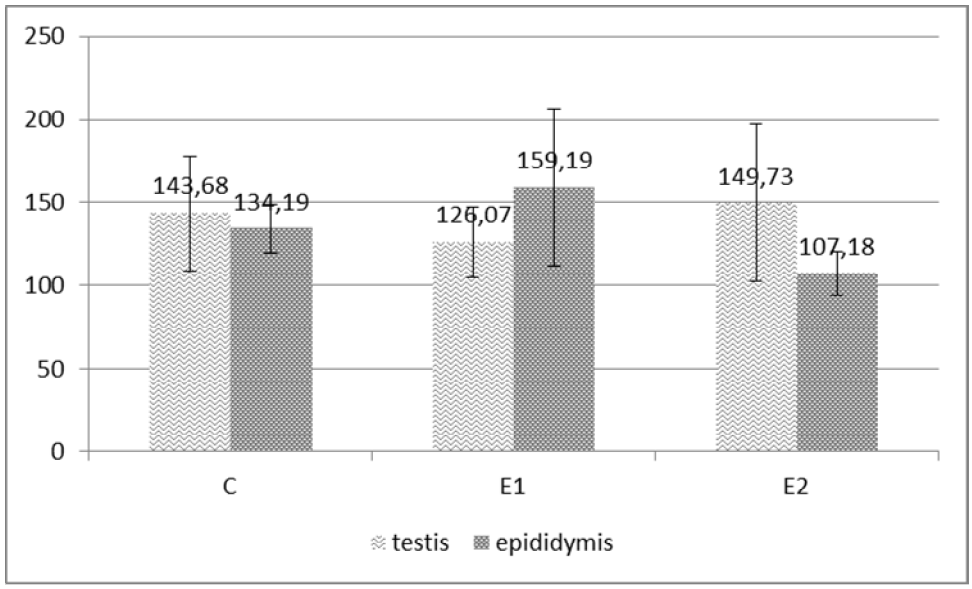


**Figure 3** Concentration of Fe in testes and epididymis of broiler rabbits (μg.g$^{-1}$)
C – control group, E1, E2 – experimental groups

## CONCLUSION

In conclusion, the inclusion of Ni to the diet for male broiler rabbits had no effect on the concentration of Ni and Fe in *testes* and *epididymis*. 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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