ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 7 (6): 538-547, 2008
ISSN 1682-8356

Blood Biochemical Dynamics and Correlations in Laying Hens after Experimental Nickel Administration

Marcela Capcarova[1], A. Kolesarova[1], H. Arpasova[2], P. Massanyi[1],
N. Lukac[1], J. Kovacik[1], A. Kalafova[1] and M. Schneidgenova[1]
[1]Department of Animal Physiology, Slovak University of Agriculture in Nitra,
Tr. A. Hlinku 2, SK - 949 76, Slovak Republic
[2]Department of Poultry Science and Farm Animal Husbandry, Slovak University of Agriculture in Nitra,
Tr. A. Hlinku 2, SK - 949 76, Slovak Republic

Abstract: The concentrations of biochemical parameters (calcium, phosphorus, magnesium, sodium, potassium, glucose, total cholesterol, total proteins, triglycerides, alanine aminotransferase ALT, aspartate aminotransferase AST, gamma glutamyl transferase GGT and glutamatdehydrogenase GLDH) and their correlations in blood serum of Isa brown breed of laying hens after nickel administration were analyzed. Animals were divided into four groups (K, P1, P2, P3). Experimental hens (5 in each group) received nickel ($NiCl_2$) *per os* in drinking water in various dose (P1 - 20 mg $NiCl_2$ / L; P2 - 200 mg $NiCl_2$ / L; P3 - 2000 mg $NiCl_2$ / L of drinking water) for 28 days, control group - K (n = 5) did not receive nickel during experiment. Blood collection was realized at Day 0 (collection - control), Day 7 (collection 1), Day 14 (collection 2), Day 21 (collection 3) and Day 28 (collection 4). Significant decreases ($P<0.05$) of magnesium and triglyceride between control (1.74±0.28 mmol/L and 20.92±8.13 mmol/L) and P3 group (0.91±0.37 mmol/L and 8.04±8.49 mmol/L) were found. Nickel had only slight effect on other parameters of energy, enzymatic and mineral profile as the results were not significant. Positive high correlation between Ca-ALT (r = 0.71), P-ALT (r = 0.74) and total proteins (TP) - ALT (r = 0.77) in the control group was detected. Negative high correlation between P-K (r = -0.75) and Mg-K (r = -0.73) in group P1 and positive high correlation between P-cholesterol (r = 0.74), Na-ALT (r = 0.69) and cholesterol-triglycerides (TG) (r = 0.87) in group P1 was noted. Positive high correlation between Ca-ALT (r = 0.71) and cholesterol-TG (r = 0.91) in the group P2 and positive high correlation in P3 group was found: Ca-TG (r = 0.69), Mg-TG (r = 0.67), Na-AST (r = 0.69) and glucose-AST (r=0.71).

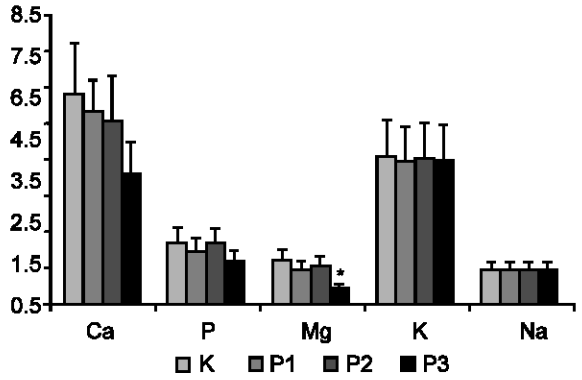
Key words: Blood, nickel, hen, biochemistry, correlation

Introduction

The vast industrial use of nickel has led to environmental pollution by the metal and its by-products during production, recycling and disposal. Nickel is a known hematotoxic, immunotoxic, hepatotoxic, pulmotoxic and nephrotoxic agent. Allergic skin reactions are common in individuals who are sensitive to nickel (Das and Buchner, 2007). Systemic contact dermatitis from nickel has been reported from a number of sources including medical devices and following experimental oral exposure (De Medeiros *et al*., 2008).

Nickel is a constituent part of all organs of vertebrates. Its absorption can be controlled. Low nickel offers reduced growth; this is particularly true of intra-uterine development. Nickel deficiency is accompanied by histological and biochemical changes and reduced iron resorption and leads to anaemia (Anke *et al*., 1984). Nickel is highly mobile in soil, particularly in acid soils. There is little evidence that nickel compounds accumulate in the food chain. Nickel is not a cumulative toxin in animals or in humans. Almost all cases of acute nickel toxicity result from exposure to nickel carbonyl (Barceloux, 1999). Nickel compounds are carcinogenic to human and are potent inducers of kidney and lung tumours in experimental animals (Lee *et al*., 2001) and induce genotoxicity and oxidative stress through the generation of reactive oxygen species (Lee, 2006). Since nickel has low mutagenic potential, it may act predominantly through epigenetic mechanisms, including down-regulation of tumour suppressor genes (Kowara *et al*., 2004). Soluble nickel compounds are likely human carcinogens (Davidson *et al*., 2005, Costa *et al*., 2005, Davidson *et al*., 2006).

According to Caicedo *et al*. (2007), nickel induced the most DNA damage and was the most apoptotic metals tested and reduces cell viability (Au *et al*., 2006). Exposure of animals and humans to different metal components through contaminated drinking water can result in a wide range of adverse clinical conditions (Jadhav *et al*., 2007). Nickel amount in organism of rats caused alterations in lipid metabolism (Stangl and Kirchgessner, 1996). As a result of dietary supplementation of 50 and 500 mg/kg, nickel accumulated in the kidneys, ribs, heart and liver of



Ca - calcium, P - phosphorus, Mg - magnesium, K - potassium, Na - sodium, K - control group, * P<0.05; Na/100.

Fig. 1: Effect of nickel on the parameters of mineral profile of laying hens.

animals (Bersenyi *et al.*, 2004). Cempel and Janicka (2002) found a very high increase in nickel levels in the kidney and then lung and serum of all exposed rats. In the liver, spleen and brain, the metal accumulation was lower. In experiment with rats significant differences between the number of leukocytes for the nickel-implanted animals and the nickel-free and control groups after 14 days of implantation were found. The histopathologic findings did not show differences between groups (Pereira *et al.*, 2008).

As it is written by Szilagyi *et al.* (1991), Ni-deficient goats had significantly lower enzyme activities in the heart (AST, ALT). Electron micrograph showed degeneration of cardiac and skeletal muscle in the Ni-deficient animals. Nickel deficiency elicited changes primarily in the heart and these resulted in depressed activity of several enzymes. Nickel deficiency results in lower activities of different dehydrogenases and transaminases and above all, of alpha-amylase and particularly affects carbohydrate metabolism. A marked decrease in metabolism was observed in the case of the energy sources fat, glucose and glycogen. Nickel therefore performs a vital function in metabolism: It is an essential element (Anke *et al.*, 1984).

Gilani and Marano (1980) observed the toxicity and teratogenicity of nickel chloride in the chicken embryos. The results of their study indicated that nickel chloride was teratogenic. The data of authors Wilson *et al.* (2001) indicates that adding 25 mg/kg of dietary nickel to a poultry diet have a positive influence on bone strength characteristics and performance. Oscar *et al.* (1995) indicated that an adequate level of Ni was present in the basal diet to promote optimal growth performance and carcass quality of broilers. Bersenyi *et al.* (2004) stated that supplementation of the diet with 50 mg Ni/kg had slight but non-significant beneficial effects on the growth performance of broiler chickens. The results of serum biochemistry were confirmed by a mild or moderate form of pathological focal fatty infiltration of the liver in broilers. Dietary supplementation of 50 mg Ni/kg slightly improved the body weight gain and had a beneficial effect on the feed conversion efficiency in broiler chickens. However, nickel added at a level of 500 mg/kg significantly reduced the body weight gain by 10% and resulted in significantly worse feed conversion efficiency. The activity of AST was increased insignificantly by dietary supplementation of 500 mg Ni/kg, indicating damage of the liver parenchyma.

Target of this study was to estimate blood biochemical dynamics and subsequently correlations between studied parameters of mineral, energy, enzymatic profile in laying hens in relation to experimental administration of nickel in tree different doses.

Materials and Methods

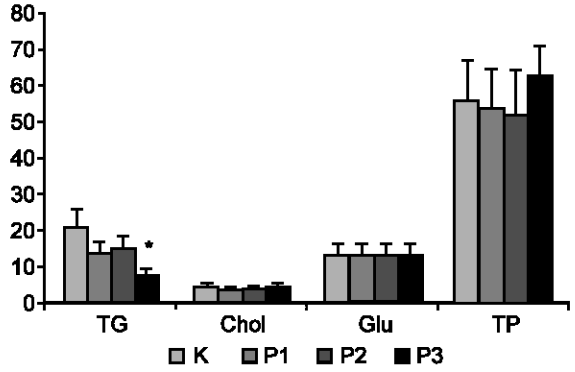
In this study the blood serum of adult laying hens, Isa Brown breed (n=20), was analyzed in order to find the effect of nickel when giving to the drinking water. Animals were divided into four groups (K, P1, P2 and P3). Experimental groups received nickel ($NiCl_2$, Sigma Chemicals, MO, USA) *per os* in drinking water in various dose (P1 - 20 mg $NiCl_2$/L; P2 - 200 mg $NiCl_2$/L; P3 - 2000 mg $NiCl_2$/L) for 28 days. The control group - K (n=5) did not receive nickel. Hens were fed with feed mixture HYD - 10 *ad libitum* and were place in metal cages according to farm technologies. Blood of hens was acquired from *venae basilica* at the ninth month of egg production by macromethod. Blood collection was realized at Day 0 (collection - control), Day 7 (collection 1), Day 14 (collection 2), Day 21 (collection 3) and Day 28 (collection 4). The blood serum was separated from whole blood by centrifugation at 3000 rpm for 30 minutes and samples were stored at -18°C. Biochemical parameters of mineral profile (calcium, phosphorus, magnesium, potassium and sodium) and of energy and enzymatic profile (glucose, total cholesterol, total proteins, triglycerides, alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP, gamma glutamyl transferase GGT and glutamatdehydrogenase GLDH) were measured by semi-automated clinical chemistry analyzer Microlab 300 (Vilat Scientific, Dieren, The Netherlands).

The statistical associations between concentrations of biochemical parameters of mineral, energy and enzymatic profile after nickel administration were analyzed using correlation analysis. To compare the results the analysis of variance, Students' t-test and Duncan's test were used to calculate basic statistic characteristics and to determine significant differences between experimental and control groups.

Table 1: Correlation between the concentrations of blood biochemical parameters in hens during the experiments

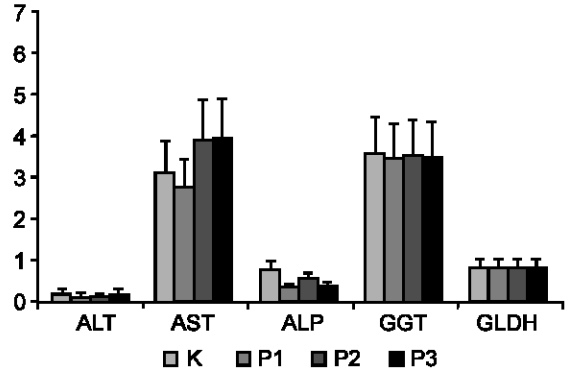
	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.38	0.50	0.17	0.07	-0.09	-0.20	-0.30	0.19	0.19	0.14	0.19	0.61	0.12
P		1	0.34	0.07	-0.06	0.05	-0.19	-0.19	0.16	0.24	-0.03	0.28	0.47	-0.02
Mg			1	-0.09	-0.08	-0.19	-0.14	-0.22	0.11	0.11	0.08	0.20	0.53	0.13
Na				1	0.05	0.12	0.07	0.20	0.29	-0.12	-0.15	0.34	0.01	0.23
K					1	-0.22	-0.19	0.01	0.13	-0.15	0.14	0.01	0.28	0.08
TP						1	-0.21	0.10	0.05	0.16	-0.26	0.26	-0.02	-0.05
GL							1	0.18	-0.09	-0.18	0.01	-0.06	-0.31	-0.31
AST								1	0.07	-0.19	-0.04	0.01	-0.27	0.11
ALT									1	-0.15	-0.03	0.12	0.25	0.07
GGT										1	-0.12	0.04	0.16	0.01
ALP											1	-0.01	0.03	-0.06
CHOL												1	0.54	0.15
TG													1	0.02
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.



TG - triglyceride, Chol - cholesterol, Glu - glucose, TP - total proteins, K - control group, * P<0.05.

Fig. 2: Effect of nickel on the parameters of energy profile of laying hens.



ALT - alanine aminotransferase, AST - aspartate aminotransferase, ALP - alkaline phosphatise (ALP/100), GGT - gamma glutamyl transferase, GLDH - glutamatdehydrogenase, K - control group.

Fig. 3: Effect of nickel on the parameters of enzyme profile of laying hens.

Results

Figure 1 showed concentrations of mineral profile (calcium, phosphorus, magnesium, potassium and sodium) in blood serum of hens after administration of nickel to the drinking water.

The average concentrations of calcium in the serum were 6.26±1.01 mmol/L in the control group, 5.87±0.87 mmol/L in group P1, 5.57±1.38 mmol/L in P2 and 4.15±1.59 mmol/L in P3 group. We noticed decrease of this parameter in each experimental group with nickel supplement in comparison with control group, mainly in group with highest nickel concentration in drinking water, however, results were no significant (P>0.05). The average level of phosphorus in blood serum was 2.21±0.52 mmol/L in the control group, 2.00±0.43 mmol/L in P1, 2.22±1.26 mmol/L in P2 and the lowest in P3 group 1.74±1.05 mmol/L. Results did not confirm the effect of these nickel doses on the level of phosphorus in blood serum of hens (P>0.05). In the group of control animals was the highest concentration of magnesium 1.74±0.28 mmol/L, in others groups the concentrations of magnesium were lower 1.47±0.52 mmol/L in group P1, 1.60±0.46 mmol/L in P2 and 0.91±0.37 mmol/L in P3 group. The statistic evaluation showed the significant decrease of magnesium (P<0.05) in P3 group versus control group. The highest content of potassium was found in control group 4.67±0.14 mmol/L. Others concentrations were lower 4.50±0.17 mmol/L in group P1, 4.58±0.17 mmol/L in P2 and 4.54±0.16 mmol/L in P3. In this case no significant differences (P>0.05) were found among the groups. The highest concentrations of sodium was 148.64±2.01 mmol/L in the control group, followed by 149.55±1.97 mmol/L in P3, 148.73±1.85 mmol/L in P2 and the lowest 148.31±2.53 mmol/L in P1. Results did not acknowledge (P>0.05) the effect of these nickel doses on the concentration of sodium in blood serum of hens.

Table 2: Correlation between the concentrations of blood biochemical parameters in hens of control group

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.57	0.31	0.63	0.06	0.42	-0.03	0.13	0.71	0.22	0.15	0.37	0.53	0.27
P		1	0.55	0.16	0.33	0.64	-0.33	-0.06	0.74	-0.24	-0.17	0.21	0.43	0.04
Mg			1	0.08	-0.18	0.21	-0.07	0.05	0.29	-0.32	0.10	0.44	0.25	0.28
Na				1	-0.03	-0.08	0.40	0.12	0.54	0.16	0.49	0.60	0.30	0.64
K					1	0.40	-0.34	-0.20	0.34	-0.02	-0.24	0.01	0.55	-0.33
TP						1	-0.13	0.26	0.77	0.04	-0.37	0.34	0.45	-0.27
GL							1	-0.00	0.09	0.23	-0.05	0.24	-0.38	0.07
AST								1	0.13	0.56	-0.35	0.18	0.17	0.01
ALT									1	0.10	-0.22	0.24	0.23	0.10
GGT										1	-0.05	0.32	0.02	0.13
ALP											1	0.03	-0.21	0.49
CHOL												1	0.50	0.26
TG													1	0.06
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

Table 3: Correlation between the concentrations of blood biochemical parameters in hens of group P1 (dose + 20 mg $NiCl_2$/L of drinking water)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.25	0.17	0.36	0.14	0.33	-0.15	-0.06	-0.05	-0.04	-0.12	0.18	0.14	-0.12
P		1	0.26	-0.10	-0.75	0.23	-0.26	-0.27	-0.13	0.22	0.10	0.74	0.61	-0.38
Mg			1	-0.17	-0.73	0.59	-0.00	-0.21	-0.16	0.09	-0.07	0.13	0.02	0.24
Na				1	0.30	0.19	-0.33	-0.30	0.69	0.37	-0.24	0.10	0.28	0.07
K					1	-0.25	-0.08	-0.05	0.17	0.05	-0.25	-0.27	0.15	0.07
TP						1	-0.48	-0.09	-0.15	0.39	-0.40	0.19	0.24	0.25
GL							1	-0.14	0.02	-0.29	0.05	-0.20	-0.23	-0.13
AST								1	0.74	-0.16	-0.09	-0.11	-0.33	0.41
ALT									1	0.01	0.01	0.21	0.01	0.28
GGT										1	-0.19	0.24	0.39	-0.10
ALP											1	-0.02	-0.10	-0.29
CHOL												1	0.87	-0.50
TG													1	-0.32
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

In Fig. 2 the average levels of parameters of energy profile (triglycerides, cholesterol, glucose and total proteins) in blood serum are plotted.

The concentration of triglyceride was 20.92±8.13 mmol/L in control group, 13.97±7.09 mmol/L in group P1, 15.09±8.54 mmol/L in group P2 and 8.04±8.49 mmol/L in group P3. After evaluation of average values of whole period of experiment we found the significant difference of this parameter ($P<0.05$) between control and P3 group. The other results were not significant ($P>0.05$). The highest concentrations of total cholesterol was found in the control group 4.32±1.73 mmol/L. Lower values were found in other groups 3.55±1.23 mmol/L in group P1, 3.56±1.01 mmol/L in P2 and 4.27±1.13 mmol/L in P3 group. The differences were no significant ($P>0.05$). The values of glucose ranged similarly in all groups. In control group we measured 13.38±1.0 mmol/L glucose, 13.71±0.86 mmol/L in group P1, 13.34±0.72 mmol/L in P2 and 13.58±1.1 mmol/L in P3. The results indicated no significant ($P>0.05$) differences among the groups. The concentrations of total proteins were 55.80±6.59 mmol/L in control group, 54.53±7.57 mmol/L in group P1, 52.65±4.64 mmol/L in group P2 and the highest 63.35±11.84 mmol/L in group P3. In this case we found no significant differences ($P>0.05$) among the groups of hens.

Figure 3 presents concentrations of selected enzymes ALT, AST, ALP, GGT and GLDH in serum.

The highest concentration of ALT was found in control group 0.18±0.09 µkat/L. In other groups the content of this parameters was 0.12±0.04 µkat/L in P1, 0.14±0.05 µkat/L in P2 and 0.15±0.16 µkat/L in P3 group, without significant differences ($P>0.05$). P3 group was the group with the highest concentration of AST 4.06±1.58 µkat/L, followed by P2 group with 3.95±1.94 µkat/L, control group with 3.18±0.56 µkat/L and finally P1 group with 2.85±0.34 µkat/L. Evaluation of this parameter brought no significant differences among the groups ($P>0.05$). The highest concentration of ALP was found in control group of hens 80.4±29.61 µkat/L. The second highest

Table 4: Correlation between the concentrations of blood biochemical parameters in hens of group P2 (dose + 200 mg $NiCl_2$/L of drinking water)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.39	0.12	0.55	-0.13	0.07	-0.37	-0.49	0.71	-0.24	-0.42	0.53	0.57	0.51
P		1	0.22	-0.05	-0.17	0.52	-0.55	0.06	0.62	0.32	-0.47	0.21	0.47	0.39
Mg			1	0.29	-0.07	-0.03	-0.08	0.10	0.60	-0.27	-0.14	0.44	0.43	0.18
Na				1	-0.08	-0.18	-0.17	-0.28	0.36	-0.29	-0.56	0.26	0.13	0.39
K					1	-0.27	-0.06	0.06	-0.10	0.01	0.36	-0.35	-0.24	0.36
TP						1	-0.48	0.37	0.22	0.44	-0.35	0.19	0.23	0.00
GL							1	-0.03	-0.47	-0.15	0.34	-0.22	-0.35	-0.56
AST								1	-0.26	0.35	0.40	-0.51	-0.50	-0.10
ALT									1	-0.01	-0.40	0.40	0.51	0.59
GGT										1	0.12	-0.42	-0.28	-0.20
ALP											1	-0.27	-0.26	-0.17
CHOL												1	0.91	0.24
TG													1	0.26
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

Table 5: Correlation between the concentrations of blood biochemical parameters in hens of group P3 (dose + 2000 mg $NiCl_2$/L of drinking water)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.44	0.47	-0.17	-0.09	0.11	-0.23	-0.51	0.19	0.52	0.13	0.33	0.69	0.27
P		1	0.23	0.21	0.31	-0.05	0.10	-0.07	0.08	0.60	-0.10	0.27	0.34	0.05
Mg			1	-0.61	-0.07	-0.05	-0.11	-0.28	-0.04	0.53	-0.26	0.28	0.67	-0.07
Na				1	0.08	0.32	0.23	0.69	0.31	-0.46	-0.09	0.16	-0.43	-0.27
K					1	-0.51	-0.32	0.12	0.21	-0.37	0.41	0.19	0.13	0.21
TP						1	-0.15	0.03	-0.22	0.31	-0.23	0.14	-0.04	-0.10
GL							1	0.71	-0.12	-0.37	0.02	-0.26	-0.35	-0.48
AST								1	-0.22	-0.46	-0.02	0.12	-0.22	-0.38
ALT									1	-0.24	0.03	-0.23	0.23	-0.08
GGT										1	-0.21	0.47	0.57	0.17
ALP											1	-0.19	0.04	0.00
CHOL												1	0.49	0.58
TG													1	0.11
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

content of ALP was in P2 group and that was 55.46±28.89 μkat/L. Lower concentrations were found in P1 group 38.93±28.62 μkat/L and in P3 group 37.65±23.64 μkat/L. On the contrary, in the case of GGT in the control group the lowest concentration (0.85±0.45g/L) was measured. The highest concentration was found in P1 group (1.19±0.58 g/L). In P2 group it was 1.09±0.61 g/L and in P3 group 0.88±0.7 g/L. The differences were no significant (P>0.05). In the small range and without significant differences (P>0.05) were values of GLDH in all groups. In the control group it was 0.19±0.11 g/L, in P1 group 0.17±0.14 g/L, in P2 group 0.23±0.16 g/L and in P3 group 0.2±0.12 g/L.

Correlations between the concentrations of blood biochemical parameters in hens are recorded in Tables 1-10. As it is shown in Tables, correlation analysis detected some significant relationships between the concentrations of biochemical parameters in blood serum. Any high correlations were not found between the concentrations of blood biochemical parameters in hens during the experiment (Table 1). Positive high correlation has been found between Ca-ALT (r = 0.71), P-ALT (r = 0.74) and total proteins (TP)-ALT (r = 0.77) in the control group (Table 2). Negative high correlation has been found between P-K (r = -0.75) and Mg-K (r = -0.73) in group P1 (Table 3). Positive high correlation has been detected between P-cholesterol (r = 0.74), Na-ALT (r = 0.69) and cholesterol-tirglycerides (TG) (r = 0.87) in group P1 (Table 3). Positive high correlation has been found between Ca - ALT (r = 0.71) and cholesterol-TG (r = 0.91) in the group P2 (Table 4). Positive high correlation between the concentration of blood biochemical parameters in group with highest nickel concentration in drinking water (P3) has been detected: Ca-TG (r = 0.69), Mg-TG (r = 0.67), Na-AST (r = 0.69) and glucose-AST (r = 0.71) (Table 5). Correlation analysis from individual blood coolection (control, blood collection 1, 2, 3, 4) is listed in Table 6-10. As shown in Table 6,

Table 6: Correlation between the concentrations of blood biochemical parameters in hens - control blood collection (Day 0)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.72	0.13	-0.16	-0.39	0.74	-0.27	0.23	-0.29	0.76	-0.29	0.56	0.45	-0.05
P		1	0.03	-0.06	-0.57	0.56	-0.27	0.26	-0.24	0.65	-0.20	0.41	0.29	-0.09
Mg			1	-0.18	0.09	0.10	-0.04	0.30	-0.43	-0.00	-0.45	0.04	0.08	0.33
Na				1	-0.02	-0.14	0.20	0.08	0.76	-0.22	-0.06	0.05	-0.14	-0.05
K					1	-0.37	-0.14	-0.05	0.16	-0.34	0.32	0.35	0.51	-0.16
TP						1	-0.77	0.47	-0.04	0.30	-0.48	0.52	0.33	0.37
GL							1	-0.32	-0.00	0.02	0.40	-0.57	-0.53	-0.32
AST								1	-0.06	-0.03	-0.57	0.09	0.00	0.46
ALT									1	-0.60	0.19	-0.10	-0.22	-0.10
GGT										1	0.05	0.22	0.19	-0.11
ALP											1	-0.37	-0.27	-0.28
CHOL												1	0.94	-0.15
TG													1	-0.24
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

Table 7: Correlation between the concentrations of blood biochemical parameters in hens - blood collection 1 (Day 7)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.10	0.44	0.20	-0.02	-0.03	-0.01	-0.60	0.46	0.33	-0.25	0.02	0.03	0.03
P		1	0.29	0.42	-0.22	0.35	-0.06	0.12	0.45	-0.05	-0.30	0.20	0.11	-0.14
Mg			1	0.00	-0.57	-0.02	-0.02	-0.54	0.37	0.26	-0.37	0.30	0.41	0.07
Na				1	-0.01	0.64	0.15	0.20	0.73	0.10	-0.17	0.68	0.10	0.48
K					1	0.09	0.03	0.31	-0.08	-0.25	-0.00	-0.10	0.28	0.17
TP						1	-0.55	0.32	0.73	-0.13	-0.29	0,27	0.38	-0.03
GL							1	-0.20	-0.16	0.33	0.34	0.17	-0.23	0.12
AST								1	-0.10	0.15	0.13	0.15	0.23	-0.11
ALT									1	-0.06	-0.33	0.06	0.36	0.24
GGT										1	-0.16	0.17	0.31	-0.24
ALP											1	0.18	-0.38	0.32
CHOL												1	0.39	0.66
TG													1	0.24
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

correlations between the concentrations of blood biochemical parameters in control blood taking were recorded: positive high correlations were between Ca-P (r = 0.72), Ca - TP (r = 0.74), Ca - GGT (r = 0.76), Na-ALT (r = 0.76), cholesterol-TG (r = 0.94) and negative high correlation was between TP-glucose (r = -0.77). Positive high correlations have been found between Na-cholesterol (r = 0.68) and TP-ALT (r = 0.73) in blood collection 1 (Table 7), Ca-Mg (r = 0.82), Ca-TG (r = 0.81), Mg - ALP (r = 0.71), Mg - TG (r = 0.81), K-ALP (r = 0.69), K - TG (r = 0.71) in blood collection 2 (Table 8), Ca - P (r = 0.73), Ca - Mg (r = 0.83), Ca - TG (r = 0.77), P - Mg (r = 0.79) and P-TG (r = 0.72) in blood collection 3 (Table 9). Further positive high correlation has been detected between P - ALT (r = 0.89), P - TG (r = 0.75), Mg - TG (r = 0.74), TP-GGT (r = 0.67), ALT-GGT (r = 0.80), ALT-TG (r = 0.79), cholesterol-TG (r = 0.78) in blood collection 4 (Table 10). Negative high correlation has been found between P-AST (r = -0.67) and TP-glucose (r = -0.70) in the last blood collection (Table 10).

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 some parameters after nickel administration to the drinking water for hens were measured.

Supplementation of nickel did not significantly influence the concentration of calcium in blood serum of hens; even through we obtained decrease of these parameters in each experimental group in comparison with control group. M'Bemba-Meka *et al.* (2006, 2007) explained that nickel induced destabilization of cellular calcium homeostasis. The calcium channel current of cells is inhibited by high concentrations of nickel (Seward and Henderson, 1990). According to Hiramo *et al.* (1994) content of calcium in rats was increased at 2 - 3 days post-instillation of nickel.

Nickel deprivation increased the urinary excretion of phosphorus and affects phosphorus metabolism

Table 8: Correlation between the concentrations of blood biochemical parameters in hens - blood collection 2 (Day 14)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.56	0.82	-0.16	0.54	-0.43	-0.59	-0.51	0.31	0.56	0.54	0.24	0.81	-0.29
P		1	0.53	-0.36	0.53	-0.21	-0.46	-0.36	-0.03	0.44	0.40	0.40	0.62	-0.18
Mg			1	-0.12	0.60	-0.37	-0.59	-0.40	0.40	0.33	0.71	0.18	0.81	-0.05
Na				1	0.12	0.57	0.03	0.49	0,27	0.51	-0.39	0.43	0.01	0.15
K					1	-0.49	-0.64	-0.60	0.66	0.22	0.69	0.42	0.71	0.21
TP						1	0.30	0.15	-0.20	-0.31	-0.21	0.33	-0.13	-0.26
GL							1	0.34	-0.41	-0.58	-0.39	-0.27	-0.53	-0.25
AST								1	0.31	-0.51	-0.22	-0.16	-0.47	0.42
ALT									1	0.29	0.36	0.29	0.43	0.57
GGT										1	-0.03	0.04	0.33	-0.17
ALP											1	0.22	0.65	-0.01
CHOL												1	0.62	0.03
TG													1	-0.12
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

Table 9: Correlation between the concentrations of blood biochemical parameters in hens - blood collection 3 (Day 21)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.73	0.83	0.02	-0.17	-0.40	-0.22	-0.30	0.56	-0.04	0.30	-0.12	0.77	0.29
P		1	0.79	-0.15	0.01	-0.16	-0.29	-0.37	0.65	0.47	0.14	0.20	0.72	0.39
Mg			1	-0.46	-0.41	-0.38	-0.25	-0.29	0.52	0.23	0.27	-0.10	0.62	0.21
Na				1	0.11	-0.15	-0.06	0.19	0.04	0.01	-0.25	0.52	0.17	-0.24
K					1	0.06	-0.52	-0.05	0.34	0.00	-0.25	0.44	0.22	0.29
TP						1	0.06	-0.30	-0.39	0.18	-0.30	0.04	-0.33	-0.40
GL							1	0.15	-0.59	-0.38	-0.25	-0.25	-0.43	-0.20
AST								1	0.19	-0.09	-0.14	-0.02	-0.46	-0.23
ALT									1	0.35	0.07	0.33	0.77	0.11
GGT										1	0.07	-0.36	-0.08	0.26
ALP											1	-0.30	0.08	-0.0
CHOL												1	0.46	-0.37
TG													1	0.04
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

(Nielsen, 2006). We found the lowest concentration of phosphorus in group with the highest supplement of nickel (1.74±1.05 mmol/L) versus control group (2.21±0.52 mmol/L), but differences were not significant. Sidhu *et al.* (2005) reported that nickel administration to rats has resulted in a significant increase in concentrations of phosphorus in liver tissue.

Magnesium, an essential metal, that is important in the normal functioning of DNA, has been shown to interact with some of the toxic heavy metals in respect to biochemical and molecular mechanisms and in altering the tumorigenic process (Littlefield *et al.*, 1994). In our experiment the supplement of nickel significantly ($P<0.05$) decreased the content of magnesium in P3 group of hens in comparison with control group. According to Nielsen *et al.* (1993) nickel affected growth of rats and number of variables associated with calcium and magnesium metabolism.

Nickel did not affect the level of potassium in blood of hens. The concentrations in experimental groups with nickel supplement were slightly lower as in control group, but not significantly. In experiment of Sidhu (2004a) the levels of potassium was found to be significantly suppressed following nickel treatment. To the contrary, when zinc was given to nickel-treated rats, the concentration of potassium was not significantly different from that of normal controls.

Our results did not prove the effect of nickel on the concentration of sodium in blood serum of hens. The results of Bwititi and Ashorobi (1998) show increase of sodium in plasma of rats after nickel supplementation.

After nickel treatment we found decreased of cholesterol level content in blood serum of hens in groups with nickel supplement, but differences were no significant. Similar results were obtained with chicken (Bersenyi *et al.*, 2004). By contrast, Das and Gupta (1997) recorded significant increase of testicular cholesterol in rats after nickel administration. Nickel-treated rats showed a significant increase in serum low-density lipoprotein-cholesterol, total cholesterol, triglycerides and a significant decrease in serum high-density lipoprotein-cholesterol. In the liver, nickel sulphate caused a loss of

Table 10: Correlation between the concentrations of blood biochemical parameters in hens - blood collection 4 (Day 28)

	Ca	P	Mg	Na	K	TP	GL	AST	ALT	GGT	ALP	CHOL	TG	GLDH
Ca	1	0.54	0.59	0.21	-0.16	0.23	-0.24	0.08	0.57	0.33	-0.17	0.26	0.55	0.54
P		1	0.63	-0.53	0.11	0.42	-0.47	-0.67	0.89	0.46	-0.10	0.45	0.75	0.34
Mg			1	-0.05	-0.22	0.04	0.08	0.21	0.72	0.04	-0.24	0.52	0.74	0.49
Na				1	-0.13	-0.11	0.10	0.60	-0.31	-0.03	-0.46	-0.47	-0.46	0.15
K					1	-0.25	0.44	0.53	0.04	-0.45	0.15	-0.58	-0.41	-0.55
TP						1	-0.70	-0.50	0.42	0.67	-0.50	0.60	0.31	0.42
GL							1	0.59	-0.34	-0.69	0.12	-0.33	-0.18	-0.27
AST								1	-0.20	-0.43	-0.15	-0.54	-0.25	0.03
ALT									1	0.80	-0.30	0.51	0.79	0.56
GGT										1	-0.31	0.49	0.38	0.56
ALP											1	-0.20	0.00	-0.64
CHOL												1	0.78	0.50
TG													1	0.36
GLDH														1

Ca - calcium, P - phosphorus, Mg - magnesium, Na - sodium, K - potassium, TP - total proteins, GL - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, CHOL - cholesterol, TG - triglycerides, GLDH - glutamatdehydrogenase.

normal architecture, fatty changes, extensive vacuolization in hepatocytes, eccentric nuclei and Kupffer cell hypertrophy (Das *et al.*, 2006). Eastin and O`Shea (1981) found no significant differences in plasma triglyceride and cholesterol of mallards after nickel treatment. According to Bersenyi *et al.* (2004) content of triglyceride in chicken blood after nickel administration was not altered. We found the significant decrease of triglyceride ($P<0.05$) between control and group with the highest level of nickel supplement.

The values of glucose ranged similarly in all groups (13.34 - 13.71 mmol/L). No significant differences among groups were found. Bwititi and Ashorobi (1998) concluded that their results showed increase in plasma glucose of rats. Chronic nickel chloride administration induced hyperglycaemia possibly through reduction in blood insulin levels and could be toxic to renal function. The results of Das and Das (2004) indicate that nickel influences the expression of genetic information by reducing hepatic DNA, RNA and protein concentration in rats. Total protein content of rats after nickel administration decreased (Das and Dasgupta, 1997). In our experiment we found no significant differences of this parameter among the groups of hens.

In blood nickel increased the activities of ALT to 330% (Mishra *et al.*, 1990). The similar results are published by Sidhu *et al.* (2004a). Activity of serum ALT was increased significantly following nickel treatment to normal rats (Sidhu *et al.*, 2004b). We observed no significant differences of ALT or GLDH concentration in serum of hens after nickel administration as well as Bersenyi *et al.* (2004) in rats. In our experiment P3 group with highest content of nickel was the group with the highest concentration of AST, followed by P2 group, control group and finally P1 group. Differences were no significant. In experiment realized by Mishra *et al.* (1990) nickel increased the activity of aspartate transaminases to 240% of the background level. Altered AST activity and concentration of triglyceride caused by nickel supplementation are indicative of the damage of the liver parenchyma of chicken (Bersenyi *et al.*, 2004).

We found the highest concentration of ALP in control group without nickel supplementation. In others group the concentrations of this parameter were lower, however not significantly. Also Bersenyi *et al.* (2004) did not observe any changes in this parameter in chicken blood after nickel treatment. But Hirano *et al.* (1994) introduced that alkaline phosphatase activity of rats was significantly decreased after instillation of nickel. Lung tissue ALP activity was also decreased by nickel. Because nickel does not inhibit ALP directly, the decrease in ALP activity is probably due to functional changes of type II cells. But Sidhu *et al.* (2004a) found increase of ALP activity in rats subjected to nickel treatment. In another experiment Sidhu *et al.* (2005) also observed a significant elevation of hepatic alkaline phosphatase activity in rats with nickel supplement.

Mishra *et al.* (1990) observed in kidney increased activity of GGT after nickel administration to rats and in the liver decreased activity of GGT. We observed increase of gamma-glutamyl transferase in blood in P1, P2 and P3 group with nickel supplement in comparison with control group without nickel supplement, but differences were not significant. Bersenyi *et al.* (2004) noted no changes or significant differences of GGT in chicken blood after nickel treatment.

Correlation analysis detected some significant relationships between the concentrations of biochemical parameters in blood serum. Positive high correlation has been found between Ca - ALT ($r = 0.71$), P-ALT ($r = 0.74$) and TP-ALT ($r = 0.77$) in the control group. Negative high correlation has been found between P-K ($r = -0.75$) and Mg-K ($r = -0.73$) in group P1. Positive high correlation has been detected between P - cholesterol ($r = 0.74$), Na-ALT ($r = 0.69$) and cholesterol-TG ($r = 0.87$) in group P1. Positive high correlation has been found between Ca-ALT ($r = 0.71$) and cholesterol-TG ($r = 0.91$)

in the group P2. Positive high correlation between the concentration of blood biochemical parameters in group with highest nickel concentration in drinking water (P3) has been detected: Ca-TG (r = 0.69), Mg-TG (r = 0.67), Na-AST (r = 0.69) and glucose-AST (r = 0.71). Magnesium and zinc, but not calcium, were also found to attenuate the acute toxic effects of nickel, indicating a possible correlation between prevention of acute effects and reduction in tumorigenicity (Kasprzak *et al.*, 1987). Fakayode and Olu-Owolabi (2003) found strong, positive correlations between the levels of metals, among others also nickel, in the feeds and the corresponding levels of metals in the chicken eggs. Bednarska and Laskowski (2008) found a significant positive correlation between nickel concentration in food and internal body concentration of nickel and a negative correlation between nickel exposure and the respiration rate of ground beetle. Morgan and Rouge (1984) found in nickel workers that atmospheric insoluble nickel correlated with both urinary nickel (correlation coefficient = 0.86, p = 0.02) and serum nickel (correlation coefficient = 0.87, p = 0.02). Some positive correlation was found between nickel and pathological spermatozoa in animals (Zemanova *et al.*, 2007).

Conclusion: In conclusion, levels of biochemical parameters and their correlations in blood serum of Isa brown breed of laying hens after nickel administration were analyzed. Significant decreases of magnesium and triglyceride between control and P3 group were found. Nickel had only slight effect on other parameters of energy, enzymatic and mineral profile as the results were not significant. There were found some positive and negative high correlations among monitored parameters.

Acknowledgment

We would like to express our gratitude to Ing. Peter Cupka for technical assistance and to Ing. Shubhadeep Roychoundry for English corrections. This study was supported by APVV project 0299-06 and VEGA scientific grant 1/0696/08.

References

Anke, M., B. Groppel, H. Kronemann and M. Grun, 1984. Nickel-an essential element. IARC Sci. Publ., 53: 339-65.

Au, A., J. Ha, M. Hernandez, A. Polotsky, D.S. Hungerford and C.G. Frondoza, 2006. Nickel and vanadium metal ions induce apoptosis of T-lymphocyte Jurkat cells. J. Biomed Mater Res. A., 79: 512-521.

Barceloux, D.G., 1999. Nickel: Clin. Toxicol. J. Toxicol., 37: 239-258.

Bednarska, A.J. and R. Laskowski, 2008. Effects of nickel and temperature on the ground beetle Pterostichus oblongopunctatus (Coleoptera: Carabidae). Ecotoxicol., 17: 189-98.

Bersenyi, A., S. Gy Fekete, M. Szilagyi, E. Berta, L. Zoldag and R. Glavits, 2004. Effects of nickel supply on the fattening performance and several biochemical parameters of broiler chickens and rabbits. Acta. Vet. Hungarica, 52: 185-197.

Bwititi, P.T. and R.B. Ashorobi, 1998. Effects of chronic oral nickel chloride administration on glycaemia and renal function in normal and diabetic rats. Afr. J. Health Sci., 5: 198-201.

Caicedo, M., J.J. Jacobs, A. Reddy and N.J. Hallab, 2007. Analysis of metal ion-induced DNA damage, apoptosis and necrosis in human (Jurkat) T-cells demonstrates Ni(2+) and V(3+) are more toxic than other metals: Al (3+), Be (2+), Co (2+), Cr (3+), Cu (2+), Fe (3+), Mo (5+), Nb (5+), Zr (2+). J. Biomed Mater Res. A, 2007.

Cempel, M. and K. Janicka 2002. Distribution of nickel, zinc and copper in rat organs after oral administration of nickel (II) chloride. Biol. Trace Elem. Res., 90: 215-226.

Costa, M., T.L. Davidson, H. Chen, Q. Ke, P. Zhang, Y. Yan, C. Huang and T. Kluz, 2005. Nickel carcinogenesis: Epigenetics and hypoxia signaling. Mutat Res., 592: 79-88.

Das, K.K. and S. Dasgupta, 1997. Alteration of testicular biochemistry during protein restriction in nickel treated rats. Biol. Trace Elem. Res., 60: 243-249.

Das, K.K. and S.N. Das, 2004. Studies on the role of ascorbic acid on nickel induced hepatic nucleic acid concentrations in rats. J. Basic Clin. Physiol. Pharmacol., 15: 185-195.

Das, K.K., A.D. Gupta, S.A. Dhundasi, A.M. Patil, S.N. Das and J.G. Ambekar, 2006. Effect of L-ascorbic acid on nickel-induced alterations in serum lipid profiles and liver histopathology in rats. J. Basic Clin. Physiol. Pharmacol., 17: 29-44.

Das, K.K. and V. Buchner, 2007. Effect of nickel exposure on peripheral tissues: Role of oxidative stress in toxicity and possible protection by ascorbic acid. Rev. Environ. Health, 22: 157-173.

Davidson, T.H., H. Chen, M.D. Garrick, G. D'Angelo and M. Costa, 2005. Soluble nickel interferes with cellular iron homeostasis. Mol. Cell. Biochem., 279: 157-162.

Davidson, T.L., H. Chen, D.M. Di Toro, G. D'Angelo and M. Costa, 2006. Soluble nickel inhibits HIF-prolyl-hydroxylases creating persistent hypoxic signaling in A549 cells. Mol. Carcinog., 45: 479-489.

De Medeiros, L.M., A.F. Fransway, J.S. Taylor, M. Wyman, J. Janes, J.F. Fowler Jr. and R.L. Rietschel, 2008. Complementary and alternative remedies: An additional source of potential systemic nickel exposure. Contact Dermatitis, 58: 97-100.

Eastin, W.C. Jr. and T.J. O'Shea,1981. Effects of dietary nickel on mallards. J. Toxicol. Environ. Health, 7: 883-892.

Fakayode, S.O. and I.B. Olu-Owolabi, 2003. Trace metal content and estimated daily human intake from chicken in Ibadan, Nigeria. Arch. Environ. Health, 58: 245-251.

Gilani, S.H. and M. Marano, 1980. Congenital abnormalities in nickel poisoning in chick embryos. Arch. Environ. Contam. Toxicol., 9: 17-22.

Hirano, S., T. Shimada, J. Osugi, N. Kodama and K.T. Suzuki, 1994. Pulmonary clearance and inflammatory potency of intratracheally instilled or acutely inhaled nickel sulfate in rats. Arch. Toxicol., 68: 548-554.

Jadhav, S.H., S.N. Sarkar, M. Aggarwal and H.C. Tripathi, 2007. Induction of oxidative stress in erythrocytes of male rats subchronically exposed to a mixture of eight metals found as groundwater contaminants in different parts of India. Arch. Environ. Contam Toxicol., 52: 145-151.

Kasprzak, K.S., M.P. Waalkes and L.A. Poirier, 1987. Effects of essential divalent metals on carcinogenicity and metabolism of nickel and cadmium. Biol. Trace Elem. Res., 13: 253-273.

Kowara, R., K. Salnikow, B.A. Diwan, R.M. Bare, M.P. Waalkes and K.S. Kasprzak, 2004. Reduced Fhit protein expression in nickel - transformed mouse cells and in nickel-induced murine sarcomas. Mol. Cell. Biochem., 255: 195-202.

Lee, S.H., J.G. Choi and M.H. Cho, 2001. Apoptosis, bcl2 expression and cell cycle analyses in nickel (II)-treated normal rat kidney cells. J. Korean Med. Sci., 16: 165-168.

Lee, S.H., 2006. Differential gene expression in nickel (II)-treated normal rat kidney cells. Res. Commun. Mol. Pathol. Pharmacol., 119: 77-87.

Littlefield, N.A., B.S. Hass, S.J. James and L.A. Poirier, 1994. Protective effect of magnesium on DNA strand breaks induced by nickel or cadmium. Cell. Biol. Toxicol., 10: 127-135.

Llamas, A. and A. Sanz, 2008. Organ-distinctive changes in respiration rates of rise plants under nickel stress. Plant Growth Regulation, 54: 63-69.

M'Bemba-Meka, P., N. Lemieux and S.K. Chakrabarti, 2006. Role of oxidative stress, mitochondrial membrane potential and calcium homeostasis in human lymphocyte death induced by nickel carbonate hydroxide in vitro. Arch. Toxicol., 80: 405-420.

M'Bemba-Meka, P., N. Lemieux and S.K. Chakrabarti, 2007. Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. Arch Toxicol., 81: 89-99.

Misra, M., R.E. Rodriguez and K.S. Kasprzak, 1990. Nickel induced lipid peroxidation in the rat: Correlation with nickel effect on antioxidant defence systems. Toxicol., 64: 1-17.

Morgan, L.G. and P.J. Rouge, 1984. Biological monitoring in nickel refinery workers. IARC Sci. Publ., 53: 507-20.

Nielsen, F.H., E.O. Uthus, R.A. Poellot and T.R. Shuler,1993. Dietary vitamin B12, sulfur amino acids and odd-chain fatty acids affect the responses of rats to nickel deprivation. Biol. Trace Elem. Res., 37: 1-15.

Nielsen, F.H., 2006. A mild magnesium deprivation affects calcium excretion but not bone strength and shape, including changes induced by nickel deprivation, in the rat. Biol. Trace Elem. Res., 110: 133-150.

Lu, H., X. Shi, M. Costa and C. Huang, 2005. Carcinogenic effect of nickel compounds. Mol. Cell. Biochem., 279: 45-67.

Mishra, M., R.E. Rodriguez and K.S. Kasprzak, 1990. Nickel induced lipid peroxidation in the rat: Correlation with nickel effect on antioxidant defense systems. Toxicol., 64: 1-17.

Oscar, T.P., D.M. Mitchell, D., H.M. Engster, B.R. Malone and W.M. Watson, 1995. Growth performance, carcass composition and pigmentation of broilers fed supplemental nickel. Poult. Sci., 74: 976-982.

Pereira, C.V., E. Kaminagakura, P.R. Bonan, R.A. Bastos and L.J. Pereira, 2008. Cellular, humoral and histopathologic analysis in rats implanted with orthodontic nickel brackets. Angle Orthod., 78: 114-119.

Seward, E.P. and G. Henderson, 1990.Characterization of two components of the N-like, high-threshold-activated calcium channel current in differentiated SH-SY5Y cells. Pflugers Arch., 417: 223-230.

Sidhu, P., M.L. Garg, P. Morgenstern P.J. Vogt, T. Butz and D.K. Dhawan, 2004a. Role of zinc in regulating the levels of hepatic elements following nickel toxicity in rats. Biol. Trace Elem. Res., 102: 161-172.

Sidhu, P., M.L. Garg and D.K. Dhawan, 2004b. Protective role of zinc in nickel induced hepatotoxicity in rats. Chem. Biol. Interact., 150: 199-209.

Sidhu, P., M.L. Garg, P. Morgenstern, J. Vogt, T. Butz and D.K. Dhawan, 2005. Ineffectiveness of nickel in augmenting the hepatotoxicity in protein deficient rats. Nutr. Hosp., 20: 378-385.

Stangl, G.I. and M. Kirchgessner, 1996. Nickel deficiency alters liver lipid metabolism in rats. J. Nutr., 126: 2466-73.

Szilagyi, M., M. Anke and I. Balogh, 1991. Effect of nickel deficiency on biochemical variables in serum, liver, heart and kidneys of goats. Acta. Vet. Hung., 39: 231-238.

Wilson, J.H., E.J. Wilson and P.L. Ruszler, 2001. Dietary nickel improves male broiler (Gallus domesticus) bone strength. Biol. Trace Elem. Res., 83: 239-249.

Zemanova, J., N. Lukac, P. Massanyi, J. Trandzik, M. Burocziova, M.P. Nad, M. Capcarova, R. Stawarz, M. Skalicka, R. Toman, B. Korenekova and D. Jakabova, 2007. Nickel Seminal Concentrations in Various Animals and Correlation to Spermatozoa Quality. J. Vet. Med., 54: 1-6.