Scand. J. Lab. Anim. Sci. No. 4 . 1993 . Vol. 20

Concurrent exposure to lead, selenium or monensin effects on hepatic porphyrin levels in broiler chickens during acute toxicosis

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Introduction

Monensin (ionophore) is a anticoccidial agent which is highly effective against all species of coccidia in the field together with growth promoting properties. It is however occassionally toxic to animals (*Hausmann et al.* 1991, *Khan* 1991, *Novilla* 1992). Administration of selenium at therapeutic levels either prior to or during monensin intoxication has resulted in a partial protection and amelioration of the toxic effects of the drug (*Vleet et al.* 1983, 1985). On the other hand, administration of "safe" doses of monensin to lambs previously intoxicated with selenium has exacerbated the toxicity by increased mortality and elevated selenium content in various body tissues. There have been few reports on the toxic effects resulting from simultaneous administration of selenium and monensin. The influence of these factors on animal organs in the presence of lead is also very interesting (*Madej et al.* 1988, *Szarek & Khan* 1992, *Zawirska* 1981).

The increased levels of free erythrocyte proprotoporphyrin (FEP) as well as inhibition of erythrocyte alanine aminotransferase (ALAD) activity during lead toxicosis has been well documented in birds (*Beyer et al.* 1988, *Madej et al.* 1988). However, little information is available on avian porphyrin levels in liver tissue during lead toxicosis.

Concurrent administration of lead with selenium or other elements or organic compounds is known to result in a variable effect on toxicity (*Cerklewski & Forbes* 1976, *Levander et al.* 1977, *Carlson & Nielsen* 1985). There is only one report describing the changes in FEP levels and ALAD activity in erythrocytes of hens during concurrent administration of lead and selenium (*Madej et al.* 1988). There are no reports of the effects of concurrent administration of lead with selenium or monensin on liver porphyrin levels.

The influence that lead selenium and monensin in combination can have on the activity of porphyrin transformation is interesting, because it is possible to find these factors together naturally in the field (*Bayer et al.* 1988, *Beker et al.* 1992, *Hausmann & Sasse* 1991, *Matesić et al.* 1981, *Zantopoulus et al.* 1992).

Materials and methods

Broiler chicks two weeks of age (Astra B) were divided into groups (Table 1) and kept on a diet containing selenium (15 mg/kg as sodium selenite) (Na_2SeO_3), selenium plus vitamin E (15 mg Se + 200 mg α-tocopherol/kg), monensin (240 mg/kg) as sodium monensin (Elanco Laboratories, Italy) and lead (1200 mg/kg) as lead acetate ($Pb(C_2H_3O_2)_2 \cdot 3H_2O$) as outlined in Table 1. The birds in group OO were kept on a basal diet. After an adaption period of 12 days on the various diets, the birds had lead (2000 mg/kg body mass) administrated as a single oral dose in groups (SL, ESL, ML and OL).

The experiments were performed in the framework of project 4.100.201.

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Table 1. Outline of the experiment.

Groups	No. of birds	1st toxic substance in feed (mg/kg)	Adaption period (days)	2-nd toxic substance, 3 oral doses (mg/kg body weight)
SL	13	selenium (15)	12	lead (2000)
ESL	13	selenium (15) plus vitamin E (200)	12	lead (2000)
ML	13	monensin (240)	12	lead (2000)
LS	13	lead (1200)	12	selenium (8)
LM	13	lead (1200)	12	monensin (400)
LO	13	lead (1200)	12	none
OL	13	none	12	lead (2000)
OO	13	none	12	none

Birds in groups LS, LM and LO were orally dosed with selenium (8 mg/kg body mass), monensin (400 mg/kg body mass) and nothing respectively. Group OO was kept as control.

Outline of the experiment is introduced in Table 1.

At 24 hours post intoxication (p. i.) five birds were selected at random from each group and sacrificed. Liver was collected from each chicken, weighed and a portion was preserved in dry ice for porphyrin estimation. Porphyrins were extracted from liver tissue (*Zawirska* 1981). Uroporphyrin was eluted from chromatograms with 5 % HCl (*Chu & Chu* 1955). Qualitative chromatographic analysis of proto- and coproporphyrin was carried out using the methods described by *Eriksen* (1953, 1958). All porphyrins were assayed quantitatively by spectrophotometry. Results of porphyrin estimations were statistically analysed by the analysis of variance and Duncan's multiple range test at probability level 0.05 (*Steel & Torne* 1960).

Experimental results

During acute lead toxicosis the birds kept on basal food (group OL) showed increased levels of uro-, copro- and protoporphyrins in liver. These increases were also found in birds kept on diets containing selenium (group SL) and selenium plus vitamin E (group ESL).

A higher increase was recorded in the birds kept on the diet containing monensin (group

Table 2. Liver porphyrin levels (μg/g) in chicks of different groups 24 hours after inducing acute lead toxicosis (mean ± SD)*.

Group (dietary addition)	Lead[1] mg	Uroporphyrin x 10^{-2}	Coproporphyrin x 10^{-2}	Protoporphyrin x 10^{-2}	Total porphyrin x 10^{-2}
SL (Se)	2000	2.9 ± 1.0^{c}	3.6 ± 0.0^{b}	6.9 ± 0.7^{b}	13.4 ± 1.7^{b}
ESL (Se + E)[2]	2000	0.5 ± 0.6^{ab}	4.0 ± 1.3^{b}	5.2 ± 0.8^{b}	9.7 ± 2.7^{b}
ML (Mon)[3]	2000	1.8 ± 0.7^{bc}	5.6 ± 0.3^{c}	15.2 ± 2.3^{c}	22.6 ± 3.3^{c}
OL (–)	2000	1.3 ± 0.2^{ab}	6.3 ± 0.4^{c}	6.8 ± 1.9^{b}	14.4 ± 2.5^{b}
OO (–)	0	0.4 ± 0.3^{a}	1.9 ± 0.9^{a}	2.8 ± 0.4^{a}	5.1 ± 1.6^{a}

[1] - oral dose given per kg body mass.
[2] - selenium + vitamin E.
[3] - monensin.
* - values with different superscripts appearing in the same column are statistically significant ($P < 0.05$).

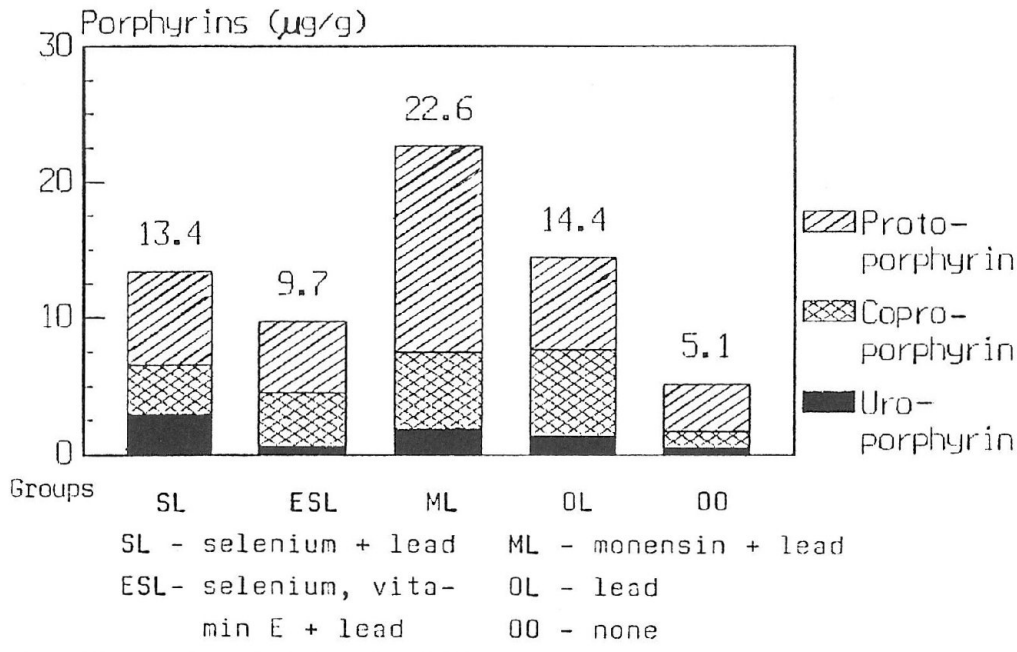

Fig. 1. Liver porphyrine levels in chicks of different groups during acute lead toxicosis at 24 hours post intoxication.

ML). Results of the liver porphyrin levels are shown in the Table 2 and Figure 1.

During acute selenium and monensin toxicosis in the birds kept on diet containing lead (groups LS and LM, respectively), a significant increase was recorded in uro- and coproporphyrin levels, respectively but the total porphyrin levels remained insignificant (Table 3).

Discussion

An understanding of porphyrin metabolism in various pathological conditions of the liver is the subject of much research (*Berenson et al.* 1992). It is well-known that lead causes inhibition of ALAD and ferrochalatase with subsequent increases in the precursors of herne (porphyrins) in tissues of the affected animals (*Doss & Tiepermann* 1978).

Table 3. Liver porphyrin levels (µg/g) in chicks of different groups 24 hours after acute intoxication with selenium or monensin (mean ± SD)*.

Group (dietary addition)	Se/Monensin mg[1]	Uroporphyrin x 10^{-2}	Coproporphyrin x 10^{-2}	Protoporphyrin x 10^{-2}	Total porphyrin x 10^{-2}
LS (Pb)	selenium	1.20 ± 0.0^{ab}	5.54 ± 2.5^{ab}	6.46 ± 2.9	13.20 ± 5.4
LM (Pb)	monensin	2.30 ± 1.0^{b}	3.10 ± 0.2^{ab}	5.57 ± 0.8	10.97 ± 2.0
LO (Pb)	0	0.40 ± 0.2^{a}	3.03 ± 1.1^{ab}	3.10 ± 0.1	6.53 ± 1.4
OO (–)	0	0.40 ± 0.3^{a}	1.20 ± 0.9^{a}	1.60 ± 0.4	3.20 ± 1.6

[1] - oral dose given per kg body mass one time only (Se - 8 mg, monensin - 400 mg).
* - values with different superscripts appearing in the same column are statistically significant ($P < 0.05$).

Increased levels of protoporphyrins and decreased ALAD activity in the erythrocytes of various species of birds have been well documented in experimental lead toxicosis (*Madej et al.* 1988, *Moore* 1990, *Turk et al.* 1992). Reports however have not mentioned increased levels of porphyrins in liver during lead toxicosis as observed in the present experiment. A further increase in the liver porphyrin levels in birds kept on diets containing monensin and selenium produced an enhancement of lead toxicosis. The role of selenium and monensin in inducing increased liver porphyrin levels is still not understood. The morphological and biochemical increased liver porphyrin level is vague. The morphological and biochemical changes recorded in the birds of different groups administered lead with selenium or monensin, suggested mitochondrial damage in hepatocytes with increased levels of lead in the liver (*Khan* 1991, *Szarek & Khan* 1992). These changes might have been responsible for pronounced disturbances in porphyrin metabolism of birds during lead toxicosis.

The findings in the present experiment demonstrate that liver porphyrin levels were elevated in chicks during acute lead toxicosis. Concurrent administration of selenium or monensin in the diets further increased porphyrin levels.

Acknowledgements

We thank Prof. Dr. B. Zawirska, head of Chair and Dpt. of Pathological Anatomy, Medical Acad. in Wroclaw for estimation of hepatic porphyrins.

Summary

Acute Toxicosis of selenium and monensin produced a significant increase in uro- and corpoporphyrin levels. Results also indicated that acute lead toxicosis increased liver porphyrins levels of broiler chickens. This was further aggravated when selenium or monensin were administrated concurrently. This experiment indicated presence of an interaction between lead and selenium or monensin in elevation of porphyrins in the liver tissue of the broiler chickens.

Sammendrag

Undersøgelser i slagtekyllinger over akutte forgiftninger af selen og monensin viste forhøjet uro- og corpoporfyrin niveau i leveren. Resultaterne fra undersøgelsen indicerede også at akut blyforgiftning forøgede leverporfyrin niveauet i slagtekyllinger. Dette blev yderligere underbygget, når selen eller monensin blev indgivet samtidig. Dette forsøg indicerer tilstedeværelsen af en sammenhæng mellem bly og selen eller monensin i forøgelsen af porfyriner i leveren hos slagtekyllinger.

Yhteenveto / K. Pelkonen

Akuutti seleeni- ja monensiinimyrkytys aiheutti erkitlä nousun uroivän- ja corpoporfyriinitasoissa. Tulokset viitaavat myös sijhen, että akuutti lyijymyrkytys nosti broilerikanoissa maksan porfyriinitasoja ja tämä nousu vielä lisääntyi, jos samanaikaisesti annettiin seleeniä ja monensiinia. Lyijyllä ja seleenillä tai monensiinilla näyttää olevat vuorovaikutus broilerikanojen maksan porfyriinimäärän lisääntymisessä.

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