# EFFECT OF ANTIOXIDANT PREPARATION "OXYNIL" ON HEALTH STATUS AND PRODUCTIVITY OF LAYING HENS FED NATURALLY MOULDED FEED.

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**Summary.** Mycotoxins are unavoidable contaminants in foods and feeds and are a major problem all over the world. Low levels of mycotoxin contamination are responsible for reduced efficiency of poultry production and increased susceptibility to infectious diseases. Inclusion of synthetic antioxidants into poultry feeds might protect birds from some adverse effects of mycotoxins. In this study, added to the diet of laying hens even a relatively small amount of fusariotoxins and ochratoxin A contaminated mixed feed resulted in reduced birds' performance, egg production and egg quality. Moulded feed supplements did not cause noticeable adverse effects on laying hens health status when fed for 50 days. Inclusion of the synthetic antioxidant preparation "Oxynil" into mycotoxin-contaminated diet resulted in improved feed intake, egg production and higher levels of carotenoids' in egg yolk. "Oxynil" was also able to reduce elevated plasma uric acid and glucose concentrations in birds fed mycotoxin-contaminated diet. However, "Oxynil" did not demonstrate an expected protective effect on hens, expressed in plasma levels of natural antioxidants such as vitamins A and E, and a lipid peroxidation marker MDA. The use of synthetic antioxidant "Oxynil" as a prophylactic measure against mycotoxicosis is debatable. Further investigations is required.
**Keywords:** fusariotoxins, ochratoxin A, lipid peroxidation, laying hens, health, "Oxynil".

# ANTIOKSIDANTINIO PREPARATO „OXYNIL" ĮTAKA VIŠTŲ DEDEKLIŲ, LESINTŲ NATŪRALIAI SUPELIJUSIAIS LESALAIS, SVEIKATOS BŪKLEI IR PRODUKTYVUMUI

**Santrauka.** Mikotoksinai – neišvengiami maisto produktų bei pašarų teršalai, keliantys problemų visame pasaulyje. Nedidelis mikotoksinų kiekis lesaluose sąlygoja paukštininkystės produkcijos nuostolius, padidėjusį paukščių jautrumą infekciniams susirgimams. Sintetinių antioksidantų priedai lesaluose galėtų apsaugoti paukščius nuo neigiamo mikotoksinų poveikio. Atliekant šį eksperimentą (trukmė – 50 dienų), dedeklių vištų lesaluose esant netgi nedideliam fuzariotoksinų bei ochratoksino A kiekiui, gautam iš natūraliai supelijusių lesalų, paukščiai sulesė mažiau, priaugo mažiau svorio, dėjo mažiau ir prastesnės kokybės kiaušinių. Paukščių, lesintų lesalais su sintetinio antioksidanto „Oxynil" priedu, lesalų pasisavinimo ir produktyvumo rodikliai beveik nesiskyrė nuo kontrolinių vištų, o kiaušinių tryniuose buvo daugiau karotinoidų negu vištų, lesintų lesalais be priedų, tryniuose. „Oxynil" priedas taip pat sumažino vištų, lesintų mikotoksinais užterštais lesalais, plazmos šlapimo rūgšties ir gliukozės koncentracijas, tačiau preparatas nepasižymėjo antioksidantiniu poveikiu, išreikštu natūralių plazmos antioksidantų, vitaminų A ir E bei lipidų peroksidacijos žymens, malondialdehido (MDA) lygiais. Sintetinio antioksidanto „Oxynil", kaip mikotoksikozių profilaktikos priemonės, vartojimas yra diskutuotinas. Pageidautini tolimesni tyrimai.
**Raktažodžiai:** fuzariotoksinai, ochratoksinas A, lipidų peroksidacija, vištos dedeklės, sveikatingumas, „Oxynil".

**Introduction.** Mycotoxins are unavoidable contaminants in foods and feeds and are a major problem all over the world (D'Mello *et al*., 1999). The number of mycotoxins known to induce signs of toxicity in mammalian and avian species exceeds 300 (Fink-Gremmels, 1999) and is steadily increasing. The most significant mycotoxins in naturally-contaminated foods and feeds are aflatoxins, ochratoxin A (OA), zearalenone, T-2 toxin, deoxynivalenol (vomitoxin, DON) and fumonisins (Devegowda *et al*., 1998), and in many cases these mycotoxins can be found in combination contaminated feed (Garalevičienė *et al*., 2003).

Among all mycotoxins, those from *Fusarium* species are considered to be important contaminants of poultry feed. Trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages (D'Mello *et al*., 1999). Co-contamination of animal feedstuffs by aflatoxins and OA (Huff and Doerr, 1981), T-2 with OA (Chandrasekaran, 1996) and T-2 toxin with other *Fusarium* metabolites (Bata *et al*., 1983) has been reported in field conditions.

Acute mycotoxicosis outbreaks are rare events in modern poultry production. However, low levels of mycotoxin contamination, which very often are not detected, are responsible for reduced efficiency of production and increased susceptibility to infectious diseases. The problem is further complicated since in many cases molecular mechanisms of their action have not been fully elucidated. Biochemical changes in mycotoxicosis vary greatly and lipid peroxidation is regarded as one of the most important consequences of mycotoxicosis (Mezes *et al*., 1999). Enhanced lipid peroxidation due to OA has been reported by Omar *et al*. (1990), Hoehler and Marquardt (1996), Hoehler *et al*. (1997), Gautier *et al.* (2001). Effects of T-2 toxin and DON on lipid peroxidation were tested by Karppanen *et al*. (1989), Rizzo *et al.* (1994), Atroshi *et al*. (1997), Dvorska and Surai (2001).

Since lipid peroxidation plays an important role in mycotoxin toxicity, a protective effect of antioxidants is expected (Galvano *et al*., 2001). Protective effects against lipid peroxidation caused by mycotoxins were attributed to various antioxidant compounds. Antioxidants are

organic molecules of either synthetic or natural origin, which can avoid or delay the progress of oxidative rancidity. Their ability to do this is based mainly on their phenol-derivated structure. Hundreds of synthetic and natural antioxidants (vitamins A and E, ascorbic acid, CoQ10, selenium, antioxidant enzymes, various plant extracts) have been evaluated for antioxidant efficacy. Of the synthetic products, only five have found widespread use: butylated hydroxyanisole (BHA), butylated hydroxytoluoene (BHT), propyl gallate (PG), tertiarybutyl hydroquinone (TBHQ) and ethoxyquin (EQ).

Inclusion of synthetic antioxidants into poultry feeds might protect birds from some adverse effects of mycotoxins. The aim of this study was to test the protective effects of the synthetic antioxidant Oxynil (*Nutri-Ad International,* Belgium) when laying hens were fed naturally mycotoxin-contaminated feeds. In our previous study, Oxynil tended to decrease the concentration of malondialdehyde (MDA) in hen plasma and liver and showed no negative effects on laying hens health, productivity and egg quality (Garalevičienė, 2000).

**Materials and methods.** Animals and housing. Scientific investigations were made following the provisions of Law of Republic of Lithuania Nº 8-500 on Protection, Keeping and Use of Animals of November 6, 1997 ("Valstybės žinios", Nº 108, 28 11 1997) and of the by-laws, i.e. orders of State Veterinary Service of the Republic of Lithuania: On Breeding, Care, Transportation of Laboratory Animals (Nº 4-361, 31 12 1998) and Use of Laboratory Animals for Scientific Tests (Nº 4-16, 18 01 1999).

Feeding experiment was performed at the Lithuanian Veterinary Academy. Three groups of 45 week old Hisex Brown hens (n=10) were fed a basal diet with different supplements. The birds for the trial had been selected from a private poultry farm. Groups were formed from hens selected to have similar weights.

The hens were housed in individual cages on a wire netting floor with stationary feeding troughs and drinking bowls. Feed and water were consumed *ad libitum*. The trial, including two weeks period for adaptation to the basal diet, continued for 9 weeks. Body weight was recorded weekly whereas laying performance was recorded daily. Blood samples were taken from the wing vein after adaptation to the basal diet, at the 1st day of starting feeding experimental diets, then at days 20 and 50 of the experimental period. Eggs for analyzing were collected at the end of the adaptation period and at the end of the trial, in week 9. A set of 10 randomly selected eggs was used for each group.

Experimental diets. A naturally moulded commercial mixed feed was obtained from a private farmer. Moulded feed was dried, ground and analysed for the presence of ochratoxin A and trichothecenes as described below. After that, moulded feed was mixed thoroughly with the basal diet distinguished to the both experimental groups to constitute 10% of the basal diet. The final diets that consisted of 100% of the basal diet for the control group and from 90% of the basal diet and 10% of the moulded mixed feed for the experimental groups, were re-analysed for the presence of OA and trichothecenes DON, NIV, HT-2 toxin and T-2 toxin.

Feed analyses. One kg of final feed for each group was dried for14 h at 65°C and ground using 1.0 or 1.5 mm sieves. The concentration of OA was measured on VICAM-Series-4 fluorometer V1.0 (Vicam LP, Watertown, MA, USA) following the guidelines of manufacturers. The limit of detection was 1 μg/kg feed. Analyses of trichothecenes in the final diets were performed following the method described by Pettersson (1993). Feeds were extracted with acetonitrile-water (84+16, v/v), purified on charcoal aluminium celite columns (Romer, 1986) and derivatized either with Trisil TBT and pentafluoropropionic anhydride PFPA (Pierce, Rockford, IL, USA). GC was performed on a Hewlett-Packard GC 5890 Series II, equipped with a $^{63}$Ni electron capture detector and a DB-5 capillary column (30m x 0.32mm ID) with helium as a gas carrier and splitless injection mode. The concentrations of type B and type A trichothecenes were calculated from the determination of silyl derivatives and PFP derivatives, respectively. The detection limit was 10 μg/kg feed for DON, NIV, HT-2 toxin and 50 μg/kg feed for T-2 toxin.

Vitamin A in the final diets was determined fluorometrically, according to Lithuanian State Standard 30417-96 (1999).

Egg analyses. Quality of eggs was determined according to a Lithuanian State Standard LST 977 (1993). Determination of vitamin A in egg yolk at week 2 and 9 was performed as reported by Garalevičienė *et al*. (2001).

Blood analyses. Blood samples (n=10 per treatment, days 1, 20 and 50) were collected using intravenous cannulas Venflon 22G-0.8/25mm (Ohmeda, Sweden) into Eppendorf tubes, using EDTA and into heparinized tubes. After filling up, the heparinized tubes were centrifuged at 1600xg for 10 min. to obtain plasma for immediate analyses. Two ml of each plasma sample were frozen at –20°C for later analyses of vitamins A and E. In fresh plasma, the activities of aspartate transaminase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1) and gamma glutamyl transferase (GGT, EC 2.3.2.2), and the concentrations of total protein, albumin, cholesterol, glucose, total and direct bilirubin, creatinine and uric acid in plasma were determined on the automatic analysers "Monarch 2000" and "Opera" (Boehringer Mannheim, Germany). The concentrations of Na, K and Cl were determined on the analyzer "ILYTE Na, K, Cl system" (Boehringer Mannheim, Germany). Morphological blood parameters, as red blood cells (RBC), white blood cells (WBC), platelets (PTL), haemoglobin (Hb), hematocrit (HCT), mean corpusculum volume (MCV), mean corpusculum haemoglobin (MCH) and mean corpusculum haemoglobin concentration (MCHC) were measured on the analyzer "CELL DYN 3500" (Abbott GmbH Diagnostics, Wiesbaden-Delkenheim, Germany). Vita-mins A, E and malondialdehyde (MDA) in the thawed plasma were analysed on a fluorometer "Hitachi MPF-2A" following the method described by Thompson *et al*. (1973). The concentrations of vitamins A, E and MDA in liver were analysed on a fluorometer "Hitachi MPF-2A" according to Taylor *et al.* (1976).

Statistical methods. All calculations were made using SAS® computational package (SAS Institute, 1988). The repeated measures test was performed using the model: $y_{ijk}=\mu + a_i + b_j + ab_{ij} + ac_{ik} + \varepsilon_{ijk}$, with $a_i$ as a treatment (i=1, 2, 3), $b_j$ – a period (j=1, 2, 3), $ac_{ik}$ – a hen

within treatment (k=1, 2…10), $ab_{ij}$ – a treatment/period interaction, $\varepsilon_{ijk}$ – a random error. The $a_i$ and $b_j$ were fixed values and $ac_{ik}$ was a random and used as an error for $a_i$.

**Results.** After the dilution of experimental diets with the 10% supplement of moulded feed the nutritional content did not differ between the control and both experimental diets. None of the analysed mycotoxins in the control diet exceeded the limits of detection (Table 1).

Table 1. **Composition of the basal diet (commercial mixed feed for laying hens)**

| Ingredients [g/kg] | | Chemical composition [g/kg] | |
|---|---|---|---|
| Wheat | 320 | Dry matter | 898 |
| Barley | 90 | Organic matter | 798 |
| Maize | 200 | Crude protein | 167 |
| Sunflower crumbs | 130 | Crude fibre | 45 |
| Soya crumbs | 115 | Lysine | 6.9 |
| Sun flower oil | 38 | Methionine and cystine | 6.0 |
| Limestone | 95 | Tryptophane | 1.9 |
| Sodium chloride | 2 | Ca | 33 |
| Premix[1] | 10 | P | 5.0 |
| Oxynil[2] | 0.125 | Vitamin A [IU/kg] | 15000 |
| | | AME[3] [MJ/kg] | 11.3 |
| | | Ochratoxin A[4], µg/kg | 16 |
| | | Deoxynivalenol[5], µg/kg | 218 |
| | | Nivalenol[6], µg/kg | 87 |
| | | T-2 toxin[7], µg/kg | 324 |

[1]Composition per kg premix: 160 g Ca; 95 g P; 2 g methionine; 6 g cystine; 1 500 000 IU vit. A, 450 000 IU vit. $D_3$, 3.0 g vit. E, 200 mg vit. $B_1$, 600 mg vit. $B_2$, 1.2 g Ca-panthothenate, 5.0 g adenine, 3.0 g nicotinic acid, 400 mg vit. $B_6$, 2.0 mg vit. $B_{12}$, 100 mg folic acid, 6.0 mg biotin; 12.0 g Mn, 11.0 g Zn, 1.5 g Cu, 7.0 g Fe, 200 mg I, 50 mg Co, 7.0 g BHT;

[2]Oxynil was added only to the 2nd experimental diet;

[3]Apparent metabolizable energy (AME), [MJ/kg] was calculated according to a formula: 0.01551CP + 0.03431EE + 0/01669 starch + 0.01301 sugar (Commission Directive 86/174/EEC);

[4,5,6,7] Concentrations of mycotoxins found in the 1st and the 2nd experimental diets. The basal diet was found to be free from analysed mycotoxins.

Table 2. **Effects of experimental diets on the performance of laying hens at 45 to 51 weeks of age (Mean ± SE)**

| Diets | Control | 1st experimental | 2nd experimental |
|---|---|---|---|
| Performance parameters (n=10) | | | |
| Initial live weight, Day 1 [g] | $1689^{a}$±60 | $1706^{a}$±61 | $1685^{a}$±73 |
| Final live weight, Day 50 [g] | $1673^{a}$±52 | $1655^{a}$±55 | $1669^{a}$±71 |
| Feed intake [g/day] | $139^{a}$±7.2 | $119^{b}$±6.9 | $132^{a}$±8.1 |
| Egg production [g/day] | $56.1^{a}$±3.9 | $43.7^{b}$±4.2 | $49.9^{ab}$±2.8 |
| Digestibility of nutrients [%, n=5] | | | |
| DM | $64.2^{a}$±1.2 | $64.8^{a}$±1.5 | $63.5^{a}$±2.0 |
| OM | $68.9^{a}$±1.8 | $69.2^{a}$±2.1 | $67.1^{a}$±1.7 |
| CP | $75.9^{a}$±1.2 | $73.9^{a}$±1.9 | $75.3^{a}$±1.3 |
| EE | $74.2^{a}$±1.4 | $75.1^{a}$±1.4 | $75.5^{a}$±2.1 |
| CF | $13.9^{a}$±1.2 | $13.2^{a}$±1.5 | $13.4^{a}$±0.9 |
| NFE | $82.5^{a}$±2.2 | $81.1^{a}$±2.4 | $81.9^{a}$±2.9 |
| Egg quality (n=10) | | | |
| Egg fresh weight [g] | $61.8^{a}$±2.3 | $58.4^{a}$±3.8 | $60.1^{a}$±3.4 |
| Shell percentage [%] | $10.8^{a}$±0.8 | $11.7^{a}$±1.1 | $11.6^{a}$±0.9 |
| Carotenoids in yolk [µg/g] | $18.3^{a}$±0.9 | $15.6^{b}$±1.3 | $17.9^{a}$±1.1 |

Values within rows with no common superscripts are significantly different (P<0.05)

The results on the performance parameters, nutrient digestibility and egg quality are presented in Table 2. The inclusion of the moulded, toxin-containing feed supplement caused 14% (p<0.05) lower feed intake and 22% (p<0.05) lower egg production in the 1st experimental group compared to the control birds. The same parameters were slightly lower in the 2nd exper-imental group fed the diet with supplement of both moulded feed and Oxynil

when compared to those of the control hens, however, the changes were not statistically significant. No differences between groups in nutrients digestibility and egg quality were noted.

The activities of hen's plasma enzymes are presented in Table 3. No treatment-dependent changes in GOT and GGT were observed, the activities of these enzymes varied depending on the experimental period. However, ALP was affected both by treatment and treatment-period interaction.

Table 3. **Effect of experimental diets on blood plasma enzymes for laying hens at 45 to 51 weeks of age (Mean ± SE, n=10)**

| Diets | Days | AST, [IU/l] | ALP, [IU/l] | GGT, [IU/l] |
|---|---|---|---|---|
| Control | 1st | 106.3±6.5 | 877.4±62.3 | 15.9±1.2 |
| | 20th | 188.6±6.0 | 1030±15.1 | 23.0±6.3 |
| | 50th | 137.3±8.3 | 913.5±133 | 71.2±14.9 |
| 1st experimental | 1st | 106.4±6.3 | 964.8±111.8 | 20.3±5.7 |
| | 20th | 201.0±11.5 | 1323.0±60.9 | 30.0±7.4 |
| | 50th | 142.3±6.1 | 1029.0±90.3 | 101.4±23.2 |
| 2nd experimental | 1st | 121.6±8.3 | 855.1±73.6 | 16.8±1.8 |
| | 20th | 183.6±8.2 | 869.6±61.0 | 28.9±9.0 |
| | 50th | 143.6±4.8 | 874.1±101.8 | 57.0±14.3 |
| LSD [P<0.05] | | 19.4 | 235.1 | 33.3 |
| CV [%] | | 14.5 | 26.7 | 90.8 |
| P value treatment | | 0.4227 | 0.0046 | 0.7056 |
| P value period | | 0.0001 | 0.1506 | 0.0001 |
| P value interaction | | 0.4149 | 0.0526 | 0.6331 |

Other biochemical plasma parameters are presented in Tables 4 and 5. Highly significant effects (P<0.001) of treatment period and interaction between treatment and period were observed on plasma glucose and uric acid concentration. Being high in all groups before the experiment, glucose and uric acid remained to be high only in the 1st experimental group at the end of the trial. Sodium was affected by treatment and both sodium and creatinine showed dependency upon period and treatment-period interaction. Other biochemical parameters were only affected by period with an exception of chlorine that was not affected at all.

Table 4. **Effect of experimental diets on other blood plasma parameters for laying hens at 45 to 51 weeks of age (Mean ± SE, n=10)**

| Diets | Days | Total protein [g/l] | Cholesterol [mmol/l] | Glucose [mmol/l] | Total bilirubin [μmol/l] | Direct bilirubin [[μmol/l] |
|---|---|---|---|---|---|---|
| Control | 1st | 49.4±2.9 | 3.12±0.34 | 29.6±0.8 | 3.19±0.21 | 1.37±0.25 |
| | 20th | 57.6±2.8 | 4.62±1.02 | 18.5±0.6 | 3.36±0.16 | 1.39±0.13 |
| | 50th | 43.8±1.9 | 1.93±0.09 | 14.3±0.5 | 2.65±0.12 | 1.19±0.14 |
| 1st experimental | 1st | 46.8±2.6 | 2.61±0.24 | 32.1±0.8 | 2.87±0.19 | 0.90±0.07 |
| | 20th | 60.5±4.0 | 3.51±0.34 | 29.1±0.7 | 5.70±2.48 | 1.38±0.14 |
| | 50th | 44.7±1.0 | 2.22±0.08 | 29.4±0.6 | 2.48±0.15 | 0.75±0.09 |
| 2nd experimental | 1st | 53.0±2.0 | 2.97±0.17 | 30.9±0.7 | 3.11±0.16 | 1.20±0.12 |
| | 20th | 59.2±2.2 | 3.47±0.70 | 16.8±0.4 | 3.02±0.41 | 1.24±0.09 |
| | 50th | 47.1±1.8 | 1.86±0.25 | 14.7±0.5 | 2.80±0.13 | 0.76±0.12 |
| LSD [P<0.05] | | 6.9 | 1.40 | 1.65 | 0.49 | 0.38 |
| CV [%] | | 14.9 | 50.4 | 7.98 | 18.7 | 38.9 |
| P value treatment | | 0.1960 | 0.2768 | 0.0001 | 0.2032 | 0.4013 |
| P value period | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| P value interaction | | 0.5890 | 0.1819 | 0.0001 | 0.4492 | 0.5870 |

Table 6 summarizes effects of experimental diets on plasma vitamins A, E and MDA. After feeding birds experimental diets for 20 days, plasma vitamin A increased in all groups followed by a decrease up to or below the primary level at the end of the experiment. The concentration of vitamin E changed similarly to vitamin A while MDA tended to increase gradually during the trial. However, a large variation (up to 66%) of the levels of vitamins A, E and MDA was noted.

**Discussion.** *Diets.* The inclusion of moulded barley supplements into experimental diets did not affect their nutritional value. According to McDonalds *et al*. (1988) and National Research Council (NRC, 1994) content of nutrients in diets was sufficient enough for laying hens. Rotter *et al.* (1989) found greater concentrations of CP, ash and ADF in moulded than in non-moulded barley due

to a decrease in bulk density of the barley and an apparent relative increase in the protein concentration but with little change in the absolute quantities. However, in the present study moulded supplements comprised only 10% of the diet, therefore no effects on the nutritional value were noted.

Table 5. **Effect of experimental diets on other blood plasma parameters for laying hens at 45 to 51 weeks of age (Mean ± SE, n=10)**

| Diets | Days | Creatinine [μmol/l] | Uric acid [μmol/l] | Na [mmol/l] | K [mmol/l] | Cl [mmol/l] |
|---|---|---|---|---|---|---|
| Control | 1st | 34.9±1.4 | 325.6±33.9 | 118.6±7.3 | 3.33±0.17 | 115.9±1.1 |
| | 20th | 28.7±3.0 | 160.0±10.0 | 148.3±1.5 | 4.1±0.07 | 112.2±1.3 |
| | 50th | 20.7±0.7 | 170.4±6.4 | 150.7±0.9 | 4.52±0.31 | 110.9±1.7 |
| 1st experimental | 1st | 34.4±1.9 | 360.3±36.1 | 129.4±6.6 | 3.47±0.13 | 114.7±3.1 |
| | 20th | 23.4±1.9 | 357.3±34.9 | 153.8±0.5 | 4.04±0.09 | 113.6±1.7 |
| | 50th | 24.9±1.7 | 368.9±14.1 | 149.8±0.9 | 4.37±0.34 | 112.3±2.2 |
| 2nd experimental | 1st | 38.3±1.92 | 333.9±43.11 | 142.3±2.0 | 3.93±0.09 | 112.9±0.7 |
| | 20th | 22.1±1.7 | 166.0±15.8 | 147.9±1.1 | 4.08±0.06 | 107.9±1.6 |
| | 50th | 22.7±1.1 | 148.3±7.3 | 148.3±0.5 | 4.36±0.29 | 110.1±2.2 |
| LSD [P<0.05] | | 4.45 | 71.6 | 9.50 | 0.51 | 1.62 |
| CV [%] | | 18.1 | 31.3 | 7.4 | 14.2 | 5.12 |
| P value treatment | | 0.9248 | 0.0001 | 0.0393 | 0.6786 | 0.1509 |
| P value period | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.1231 |
| P value interaction | | 0.0009 | 0.0002 | 0.0022 | 0.2827 | 0.6080 |

Table 6. **Effect of experimental diets on blood plasma vitamins A, E and malondialdehyde (MDA) for laying hens at 45 to 51 weeks of age (Mean ± SE, n=10)**

| Diets | Days | Vitamin A [μmol/l] | Vitamin E [μmol/l] | MDA [nmol/g] |
|---|---|---|---|---|
| Control | 1st | 2.33±0.11 | 41.9±4.7 | 5.60±0.42 |
| | 20th | 2.71±0.13 | 63.7±14.7 | 7.34±0.73 |
| | 50th | 1.86±0.15 | 59.4±4.6 | 12.6±2.12 |
| 1st experimental | 1st | 1.93±0.18 | 35.2±9.6 | 6.72±0.37 |
| | 20th | 2.46±0.23 | 51.7±16.2 | 7.19±0.91 |
| | 50th | 2.35±0.32 | 82.6±9.9 | 12.31±1.03 |
| 2nd experimental | 1st | 2.07±0.1 | 36.8±6.4 | 4.78±0.39 |
| | 20th | 2.46±0.42 | 70.3±25.9 | 6.88±0.37 |
| | 50th | 1.63±0.28 | 53.0±13.7 | 10.83±0.58 |
| LSD (P<0.05) | | 1.2 | 33.9 | 2.9 |
| CV [%] | | 56.2 | 66.3 | 41.0 |
| P value treatment | | 0.0009 | 0.0917 | 0.3834 |
| P value period | | 0.3767 | 0.0001 | 0.0001 |
| P value interaction | | 0.0059 | 0.0003 | 0.2818 |

*Performance parameters, nutrient digestibility and egg quality*. In the present study, consumption of the 1st experimental diet resulted in lower feed intake and decreased egg production compared to the control birds. Rotter *et al*. (1989) noted negative effects of diets containing moulded barley alone and in combination with 2 to 4 mg OA/kg on feed consumption and body weight gain in broiler chicks. Reduced feed intake found in the present study was similar to the findings of Hedman *et al*. (1995), who described a linear decrease in both feed consumption, feed reduction and weight gain after increasing the NIV concentration (3, 6 and 12 mg/kg diet) in broiler chicks, and to our previous studies when laying hens were fed diets containing either naturally moulded feed or up to 5 mg nivalenol/kg diet (Garalevičienė *et al*., 2001, 2002).

Feeding animals naturally moulded feed often results into higher negative effects caused by interactions between various mycotoxins (Rotter *et al*., 1989; Garalevičienė *et al*., 2001). There are a lot of studies analyzing these effects. The effects of T-2 toxin and ochratoxin A were additive in their effects on reducing body weight gain, serum protein and LDH activity (Kubena *et al*., 1989b). A synergistic interaction between T-2 toxin and DON was observed on chickens fed diets containing DON-contaminated wheat (16 ppm) and pure T-2 toxin at 4 ppm (Kubena *et al*., 1989a). Final body weights were significantly reduced by the DON/T-2 toxin combination but not by the toxins only. In a similar trial, broiler chicks were fed diets containing ochratoxin A (2 ppm) or DON (Kubena *et al*., 1988) where for most of the parameters evaluated there were significant interactions that were described as "less than additive". Rotter *et al*. (1991) described that the combined toxicity of any two

trichothecenes (DOM, 15-acetyl-DON and HT-2 toxin) was found to be additive. Raju and Devegowda (2000) evaluated the individual and combined effects of aflatoxin $B_1$, ochratoxin A and T-2 toxin on performance and health of broiler chickens. Significant interactions were observed between any 2 toxins for their additive effects on body weight, food intake. Aravind *et al.* (2003) studied the toxic effects of mycotoxins in naturally contaminated diet (aflatoxin $B_1$ 168 ppb, ochratoxin A 8.4 ppb, zearalenone 54 ppb and T-2 toxin 32 ppb) fed to commercial broilers. Compared with the control, the naturally contaminated diet significantly decreased body weight and feed consumption and resulted in poor feed efficiency.

Reduced nutrients digestibility was found in chicken (Hamilton *et al.,* 1988; Rotter *et al*., 1989) and laying hens (Garalevičienė *et al*., 2001) fed moulded grain but not in the present study where the inclusion of moulded feed into diets reached only 10%.

Deteriorated egg production and quality was noted when laying hens were fed diets containing naturally moulded barley (Garalevičienė *et al*., 2001), however, no negative effects on laying performance were noted feeding hens up to 5 mg nivalenol/kg diet (Garalevičienė *et al*., 2002). Prior and Sisodia (1978) and Niemiec *et al*. (1993, 1994) noted reduced egg production, egg shell thickness and strength due to 0.5 to 4.1 mg OA/kg diet. In contrast, DON had no adverse effect on egg production and quality (Hamilton *et al*., 1985). The administration of DON from naturally contaminated oats at dietary concentrations of 2,5, 3,1 and 4,9 mg/kg diet to laying hens for 70 days did not produce any effects on egg production, fertility, hatchability or perinatal mortality (Bergsjö *et al*., 1993).

*Blood plasma parameters*. Blood plasma parameters might be used for an additional estimation of toxic effects on live birds (Schiefer, 1990). It is assumed that elevated activities of serum or plasma enzymes such as ALP, AST, GGT, GLDH and LDH might indicate recent organ damage (Lumeij, 1997). However, the data on the effects of mycotoxins and moulded feed on serum or plasma enzymes are contradictory. Only increased plasma ALP activity was found in the hens fed the 1st experimental diet while either control feed (free from mycotoxins) or the 2nd experimental diet (containing mycotoxins and Oxynil supplement) were without effect on plasma enzymes. These findings are similar to our previous study (Garalevičienė *et al*., 2002), in which no effect on plasma enzymes (with an exception of increased ALP) were found in laying hens, fed 0, 1 and 3 mg NIV/kg containning diet. However, feeding laying hens diets containing naturally moulded barley, sharp increase of plasma ALP was found (Garalevičienė *et al*., 2001). In contrast, neither the studies of Hedman *et al*. (1995) nor Kubena *et al.* (1988) noted any effects observed on serum enzymes in chickens fed up to 12 mg NIV/kg feed and 16 mg DON/kg diet.

There are not any data concerning feeding of experimental hens before the trial, thus the presence of several mycotoxins in their feed, resulting in altered uric acid and glucose concentrations, is quite possible. Increased serum glucose after consumption of OA-contaminated diets in chicks has been reported (Kubena *et al*., 1988). Swamy *et al*. (2002) fed broiler chicks diets containing grains naturally contaminated with *Fusarium* mycotoxins for 56 d. Dietary inclusion of contaminated grains caused significant linear increases in serum uric acid concentrations. These data are similar to the results of this study. Despite very high variations, uric acid was high in all birds at the start of the experiment and then decreased in both control and the 2nd experimental group that received Oxynil in the diet. Feeding control birds a good quality diet during the trial resulted in decreased uric acid and glucose concentrations as well as in the 2nd experimental group of bird, receiving Oxynil. Hens from the 1st experimental group showed the same high plasma uric acid and glucose due to the effect of dietary mycotoxins. It is known, that kidneys are the organs most susceptible to ochratoxin A; the toxin can cause both acute and chronic kidney lesions in poultry (Holmberg, 1992). Principally OA acts on the first part of the proximal tubules in the kidney and induces a defect on the anion transport mechanism on the brush border of the proximal convoluted tubular cells and basolateral membranes. The defect leads to an increase in urinary volume and in specific gravity of the urine, increased urine pH, altered renal enzyme activity, increased blood urea nitrogen, increased water consumption, depressed body weight gain and feed consumption (Endou, 1986). It was shown that T-2 toxin inhibits hepatic protein synthesis, causing aminoacidemia (Meloche and Smith, 1995). As a result, there will be greater degradation of free circulating amino acids for energy utilization, leading to excess uric acid synthesis.

*Vitamins A, E and MDA*. T-2 toxin is fat soluble, thus it may be incorporated into cell membranes, potentially changing membrane structural and functional properties (Coulombe, 1993). Lipid peroxidation by T-2 toxin in the liver has also been identified as an important underlying mechanism of T-2 toxin-induced cell injury (Leeson et al., 1995; Hoehler and Marquardt, 1996) and DNA damage (Atroshi et al., 1997). However, Hoehler and Marquardt (1996) found that T-2 toxin was not always effective in stimulating lipid peroxidation in chickens.

The levels of vitamins A, E and MDA in plasma, found in the present study, showed a high variation thus being hard to evaluate. Dvorska and Surai (2001) fed quails the diet containing T-2 toxin (8.1 mg/kg feed) for 30 days. Inclusion of T-2 toxin in the quail diet was associated with a significant decrease in all studied antioxidants ($\alpha$- and $\chi$-tocopherols, ascorbic acid, retinal and retinyl esters. In the present study, there was no significant effect of Oxynil on reducing plasma MDA as a marker of lipid peroxidation. In all treatment groups, plasma MDA tended to increase during the trial.

It is not clear whether T-2 toxin stimulates lipid peroxidation directly by enhancing free radical production, or the increased susceptibility of tissues to peroxidation is a reflection of a compromised antioxidant system. It is possible that the decreased level of vitamin A in the egg yolk of hens from the 1st experimental group could be a reflection of the decreased intestinal absorption of fat-soluble nutrients due to T-2 toxin in the feed. Decreased antioxidant absorption from the diet is just one of the possible mechanisms of antioxidant depletion from the tissues (Dvorska and Surai, 2001).

**Conclusions.** Inclusion of even a relatively small amount of fusariotoxins and ochratoxin A contaminated mixed feed in the diet for laying hens resulted in reduced birds' performance, egg production and egg quality.

1. Moulded feed supplements did not cause noticeable adverse effects on laying hens health status when fed for 50 days.

2. Inclusion of the synthetic antioxidant preparation Oxynil into mycotoxin-contaminated diet resulted in improved feed intake, egg production and higher levels of carotenoids' in egg yolk. Oxynil was also able to reduce elevated plasma uric acid and glucose concentrations in birds fed mycotoxin-contaminated diet. However, Oxynil did not demonstrate an expected protective effect on hens, expressed in plasma levels of natural antioxidants such as vitamins A and E and a lipid peroxidation marker MDA.

3. The use of synthetic antioxidant Oxynil as a prophylactic measure against mycotoxicosis is debatable. Further investigations is required.

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