Ochratoxin A, esterified glucomannan and *Saccharomyces cerevisiae* in the diet for laying hens

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**Abstract.** The main topic of this experimental trial was to evaluate the *in vivo* ability of an esterified glucomannan (EGM), and of *Saccharomyces cerevisiae* to reduce the oral bioavailability of ochratoxin A (OTA) added to a basal diet for laying hens. The residues of OTA in the kidney were evaluated, and the effects of all the 3 compounds on egg quality and on some blood parameters were also considered. Eighty-four Warren-Isa Brown laying hens were divided into 6 experimental groups. Over a 12 week period, 6 different diets were administered: 0-0: basal diet; EGM-0: diet supplemented with 0.2% EGM; SC-0: diet supplemented with 0.2% SC; 0-OTA: diet supplemented with 0.2 ppm OTA; EGM-OTA: diet supplemented with 0.2% EGM and 0.2 ppm OTA; SC-OTA: diet supplemented with 0.2% SC and 0.2 ppm OTA. During the trial feed and water were provided *ad libitum*; feed consumption and egg production were recorded weekly and daily respectively. At the end of the trial, all the hens were euthanized and kidneys were collected to check the ochratoxin A using a HPLC fluorometric method. After the extraction (citric acid and dichloromethane), and the purification steps (Silica SPE-columns Isolute), the samples were injected into a HPLC Chromolith Performance RP-18 column (100x4.6 mm); 26% acetonitrile and 74% of a mixture water:acetonitrile:acethone:acetic acid 1% (79:7:7:7) went to make up the mobile phase (1 ml/min). The fluorometric detector was setted at 340 nm excitation and 460 nm emission wavelengths. During the trial all the hens were healthy, there was no significant effect on feed intake, weight gain, egg production or blood parameters. In the 3 groups administered OTA variations were observed in Haugh index and in the pH of the albumen. In the group EGM-OTA, a change in egg component ratios was observed. The HPLC method used to check OTA in kidney showed good detection limit, recovery, specificity, linearity and accuracy. All the kidneys of the hens administered OTA resulted positive for the toxin, the level of the residues was very low and similar between the 3 groups (mean values±SD; 0-OTA: 2.44±1.08 ppb; EGM-OTA: 2.27±0.78 ppb; SC-OTA: 2.93±1.71 ppb). This absence of differences may be due to the low doses of OTA added to the basal diet. Our results confirm the data available in literature about the tissue residues of OTA in hens and the lower sensitivity of chickens than pigs.

**Keywords:** laying hen; ochratoxin A; adsorbents; residues.

**Introduction**

The mycotoxin ochratoxin A (OTA) can be found in a large number of foods, mostly in cereal products, coffee, wine, grape juice, beer, vine-fruit, pulses and pork. As OTA is carcinogenic, as well as nephrotoxic, immunotoxic and teratogenic, it is important to keep human exposure to a *minimum*. OTA is produced by several fungi of the genera *Aspergillus* and *Penicillium*. In temperate climate zones the toxin is produced by *P. verrucosum* at below 30°C and down to 0.8 a.w, mostly in corn, wheat, barley and rye. Infections can occur at the pre-harvest and post-harvest stage, but post-harvest ochratoxin A formation predominates. The control of formation of the toxin requires adequate drying of grains prior to storage. The distribution of OTA in stored grains is very heterogeneous, making analysis and dietary exposure assessment of farm animals difficult (Larsen et al., 2001). As cereals are widely used in animal feed, and because OTA is relatively stable *in vivo*, it can also be found in some animal products, especially kidney and liver. Ochratoxin A is a potent renal toxicant in all animal species and has been associated with nephropathy.
Significant sex and species differences have been observed in the sensitivity to the OTA nephrotoxic action, with swine being most sensitive. Immunosuppressive effects have been reported in mice, rats, pigs, and chickens; while embryotoxic and teratogenic actions were underlined in rats and mice. In humans, intake of high amounts of OTA has been linked to Balkan endemic nephropathy and associated with an increased incidence of tumours of the upper urinary tract. Exposure to nephrotoxic doses is always associated with renal tumours in laboratory rodents (Bozic et al., 1995; Walker and Larsen, 2005). The poultry, in general, are less sensitive than pigs. When laying hens were administered feed naturally contaminated at levels of 1.3, 2.6 or 5.2 ppm, egg production decreased in a dose-dependent manner (Bauer et al., 1988). In a gavage experiment, in which broilers were given OTA at a dose of 350 µg/kg b.w./day/28 days, no adverse clinical effects were observed, only the histological examination revealed signs of alteration (Biro et al., 2002). After oral ingestion, in most species, OTA is absorbed primarily from the small intestine, then it binds itself to serum albumin which retards elimination by limiting glomerular filtration and thus renal excretion. In relation to serum half-life of OTA there are large interspecies differences, for example in poultry, the half-life is rather short (3.5-4 hours). Like in other species, the highest residual amounts of the toxin are found in the kidneys and livers (Hagelberg et al., 1989). There are controversies about transfer of OTA to eggs, however, this transfer is generally scanty. Ochratoxin A is a relatively stable molecule and is only partially degraded under normal cooking and fermenting processes. So, there is strong interest in decontamination technologies, many of them are ineffective. One of the most recent approaches involves the addition of adsorbents to animal feed to bind the mycotoxins in the gastrointestinal tract reducing their absorption (Ramos et al., 1996). Mannanoligosaccharides are derived from the cell wall of yeast (Saccharomyces cerevisiae); they have the ability to bind several pathogens and different chemical substances (i.e. aflatoxin B1 and zearalenone) in the gastrointestinal tract preventing their colonization or absorption respectively (Devegowda et al., 1996, 1998). Aravind et al. (2003) underlined that the addition of dietary esterified glucomannan (EGM) is effective in broilers to counteract in vivo toxic effects of feed naturally contaminated with aflatoxins, ochratoxin, zearalenone and T-2 toxin. On the other hand, another method for controlling mycotoxin hazards in animal husbandry is based on the use of specific yeast cultures, such as Saccharomyces cerevisiae (SC) strains, that are able to adsorb mycotoxins, thus limiting their gastrointestinal bioavailability (Yiannikouris et al., 2003). The purpose of this study was to evaluate the in vivo ability of an esterified glucomannan (EGM), and of a Saccharomyces cerevisiae strain (inactivated by autoclave) to reduce the oral absorption of ochratoxin A (OTA) added to a basal diet for laying hens. The residues of OTA in the kidney were evaluated, and the effects of all the 3 compounds on egg quality and on some blood biochemical parameters were also considered.

Materials and methods

Eighty-four Warren-Isa Brown laying hens were divided into 6 experimental groups. Over a 12 week period, 6 different diets were administered: 0-0: basal diet; EGM-0: diet supplemented with 0.2% EGM; SC-0: diet supplemented with 0.2% SC; 0-OTA: diet supplemented with 0.2 ppm OTA; EGM-OTA: diet supplemented with 0.2% EGM and 0.2 ppm OTA; SC-OTA: diet supplemented with 0.2% SC and 0.2 ppm OTA. The basal diet was formulated to contain all required nutrients, without added antibiotics or coccidiostats. Before any supplementation, the basal diet was tested by HPLC (Simonella et al., 1990) to ensure that it contained no residual ochratoxin A. The same method was also used to verify the OTA concentration in the experimentally contaminated diets. Supplementation with EGM or SC was done in place of corn. The EGM-0 and the SC-0 diets were introduced to take informations on the possible effects (positive and/or negative) of EGM and SC when fed alone. The hens were placed in a room maintained at a constant temperature (22°C) and a relative humidity of 75%. Lighting consisted of 16L:8D. Before the beginning of the experimental trial, all of the birds were clinically observed; feed and water were provided ad libitum; feed consumption and egg production were recorded weekly and daily respectively. Only for 0-0 and 0-OTA groups, immediately before the slaughter, samples of blood were collected. After centrifugation, ALT and AST activities, as well as bilirubin, calcium, cholesterol, and protein levels were evaluated on the plasma (Du Pont’s dimension automatic system setted at 37°C). At the end of the study, all the hens were euthanized and kidneys were collected to check the ochratoxin A using a HPLC fluorimetric method setted up and validated slightly modifying the method proposed by Simonella et al. (1990) [Standard Operating Procedure FT 10.01.021]. After the extraction (citric acid and dichloromethane), and the purification steps (Silica SPE-
columns Isolute), the samples were injected into a HPLC Chromolith Performance RP-18 column (100x4.6 mm); 26% acetonitrile and 74% of a mixture water:acetonitrile:acethone:acetic acid 1% (79:7:7:7) went to make up the mobile phase (1 ml/min). The fluorometric detector was setted at 340 nm excitation and 460 nm emission wavelengths. Egg quality (qualitative and physical traits) was evaluated at the beginning, and at the end of the trial. Traits were egg weight; percentages of yolk, albumen, and shell; Haugh Index (Haugh unit); color number (CN) (Scholtyssek, 1995); pH of albumen, and shell thickness.

The plan of this study was authorized by the Italian Ministry of Health on the basis of a previous judgment issued by the Ethic Scientific Committee of the Alma Mater Studiorum (Bologna, Italy), according to Directive 86/609/EEC (1986). The study was performed according to ISO 9001:2000 requirements.

The experimental data were subjected to variance analysis (SAS, 1987); differences between treatments were compared using the Student’s t-test (paired data); a difference was considered statistically significant if $P<0.05$.

Results and discussion

During the 12th week experimental period, all birds were healthy (no pathological macroscopical lesions were observed) and mycotoxin, EGM, and Saccharomyces (SC) had no apparent effects on feed intake, weight gain or egg production. In relation to this parameter, the group EGM-OTA represents an exception with an egg production (68.13 %) significantly ($P<0.05$) lower than groups 0-0 (82.24 %) and 0-OTA (87.50 %). Our data related to the egg production do not agree with the results reported by Niemec et al. (1994), they underline a dose-dependent decrease in egg production administering diets contaminated by 2-4 ppm of OTA to laying hens. Any significant differences were observed on biochemical blood parameters (Table 1), they agree with physiological mean values related to the animal species (Martini, 1981).

Table 1 Plasma biochemical parameters (mean values and SEM)

<table>
<thead>
<tr>
<th>Diets</th>
<th>ALT UI/l</th>
<th>AST UI/l</th>
<th>Bilirubin mg/100 ml</th>
<th>Calcium mM/l</th>
<th>Cholesterol mM/l</th>
<th>Proteins g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0</td>
<td>46.17</td>
<td>177.23</td>
<td>2.65</td>
<td>5.80</td>
<td>2.75</td>
<td>4.32</td>
</tr>
<tr>
<td>0-OTA</td>
<td>24.11</td>
<td>168.70</td>
<td>1.92</td>
<td>7.89</td>
<td>3.03</td>
<td>4.90</td>
</tr>
<tr>
<td>SEM</td>
<td>5.23</td>
<td>7.16</td>
<td>0.76</td>
<td>0.31</td>
<td>0.09</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Variations were observed in several egg quality parameters (Table 2). The Haugh index, and pH of albumen showed to be worse in all the intoxicated groups compared to the others. Even if the observed differences do not have a statistical significance ($P>0.05$), the considered adsorbents (EGM and SC) exert a positive effect reducing the negative action determined by the toxin. These data resembling those reported by Rizzi et al. (2004).

Table 2 Egg quality parameters at the end of the study (mean values and SEM).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Egg weight g</th>
<th>Yolk %</th>
<th>Shell %</th>
<th>Albumen %</th>
<th>pH</th>
<th>Haugh units</th>
<th>Shell thickness mm</th>
<th>CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0</td>
<td>65.54B</td>
<td>24.27B</td>
<td>11.37A</td>
<td>64.85</td>
<td>8.08B</td>
<td>88.86A</td>
<td>0.368A</td>
<td>10.77</td>
</tr>
<tr>
<td>EGM-0</td>
<td>69.51A</td>
<td>25.70A</td>
<td>10.91B</td>
<td>64.50</td>
<td>8.13B</td>
<td>87.27A</td>
<td>0.353A</td>
<td>10.52</td>
</tr>
<tr>
<td>0-OTA</td>
<td>65.48B</td>
<td>24.91B</td>
<td>11.26A</td>
<td>65.74</td>
<td>8.25A</td>
<td>81.38B</td>
<td>0.356A</td>
<td>10.64</td>
</tr>
<tr>
<td>EGM-OTA</td>
<td>65.30B</td>
<td>25.59A</td>
<td>10.38B</td>
<td>64.03</td>
<td>8.31A</td>
<td>85.00AB</td>
<td>0.325B</td>
<td>10.53</td>
</tr>
<tr>
<td>SEM</td>
<td>0.50</td>
<td>0.32</td>
<td>0.09</td>
<td>0.44</td>
<td>0.09</td>
<td>0.99</td>
<td>0.0023</td>
<td>0.06</td>
</tr>
</tbody>
</table>

| 0-0   | 65.55        | 24.27B | 11.37   | 64.85 AB  | 8.08B | 88.86A      | 0.352              | 10.26B |
| SC-0  | 65.68        | 25.88A | 11.14   | 62.98C    | 7.93C | 87.92A      | 0.353              | 10.97A |
| 0-OTA | 65.48        | 24.91B | 11.26   | 65.74A    | 8.25A | 81.38C      | 0.361              | 10.66B |
| SC-OTA | 63.97       | 25.48A | 11.02   | 63.51B    | 8.26A | 84.78B      | 0.354              | 10.48B |
| SEM   | 0.29         | 0.13   | 0.07    | 0.27      | 0.07 | 0.50        | 0.34               | 0.10   |

$A, B, C = P<0.01$
In the groups fed diets containing OTA, EGM and SC, a change in egg component ratios was observed: the yolk percentage increased, while the shell percentage and thickness decreased in groups containing EGM or in group EGM-OTA respectively.

The basal diet was negative for OTA when subjected to HPLC analysis. The HPLC method used to check OTA in kidney was characterised by a high specificity, and accuracy. The linearity was good in the range 1, 2, 5, 8, and 10 ppb (R² = 0.9999 and 0.9981 for the reference and the calibration curves respectively), as well as the percentages of recovery (mean value: 91.95 %; minimum value: 79.71 % for 1 ppb, maximum value: 99.47 % for 10 ppb). The detection limit was 1 ppb. All the kidneys of the hens administered OTA resulted positive for the toxin; the level of the residues was very low and similar between the 3 groups (mean values±SD; 0-OTA: 2.44±1.08 ppb [minimum value: 0.62 ppb; maximum value: 4.67 ppb]; EGM-OTA: 2.27±0.78 ppb [minimum value: 1.25 ppb; maximum value: 3.74 ppb]; SC-OTA: 2.93±1.71 ppb [minimum value: 0.88 ppb; maximum value: 6.14 ppb]). This absence of differences may be due to the low doses of OTA added to the basal diet. The choice of the OTA administered dosages born from the general levels of contamination of unprocessed cereals from different European countries (EC, 2002). Our results confirm the data available in literature about the tissue residues of OTA in hens and the lower sensitivity of chickens than pigs (Juszkiewicz et al., 1982; Reichmann et al., 1982; Niemiec et al., 1994).

Acknowledgements

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