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Effect of *Saccharomyces cerevisiae* and esterified glucomannan on residues of Ochratoxin A in kidney, muscle and blood of laying hens

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ABSTRACT - The *in vivo* capability of *Saccharomyces cerevisiae* (SC), and of an esterified glucomannan (EGM) to reduce the oral bioavailability of ochratoxin A (OTA) added to a basal diet for laying hens was checked over a 12 week period. The residues of OTA in kidney, muscle and blood were studied. Eighty-four Isa Brown laying hens were divided into 6 experimental groups, fed 6 different diets: 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* and all the animals were clinically observed. At the end of the experimental period and immediately before the hens were euthanized, blood samples were collected; kidneys, and muscle were sampled. The ochratoxin A was checked using a HPLC flourometric method. During the trial all the hens were healthy. All the biological matrices of the OTA administered hens were positive to the mycotoxin; the recorded levels were very low and decreased in the order: kidneys > blood > muscle.

Key words: Ochratoxin A, Laying hens, Blood and tissues, Residues.

INTRODUCTION - Ochratoxin A (OTA) is a kidney toxin and a possible genotoxic, immunotoxic, and cancerogenic compound. It is a relatively stable molecula and it occurs in a wide range of food crops including cereals, coffee, grapes, cocoa beans and pulses (European Commission, 2002). OTA usually develops after harvest due to storage problems (Lancey and Magan, 1991). It is produced by a number of fungal species including especially *Penicillium verrucosum* and *Aspergillus ochraceus* (SCOOP, 2002). Epidemiological data suggesting an association of OTA with the aetiology of diseases in humans, e.g. Balkan Endemic Nephropathy (BEN), and with an increased incidence of tumours of the upper urinary tract (Dietrich et al., 2005). Significant sex and species differences have been observed in the sensitivity to the OTA nephrotoxic action, with swine being most sensitive. Poultry are generally 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 way (Bauer *et* al., 1988). In a gavage experiment, in which broilers were given OTA at the dose of 350 µg/kg b.w./day/28 days, no adverse effects were observed, only the histological examination revealed signs of alteration (Biro *et* al., 2002). Several are the methods to decontaminate feed/food from mycotoxins; the addition of adsorbents to animal feed to bind the mycotoxins in the gastrointestinal tract seems to reduce their gastrointestinal absorption (Ramos *et* al., 1996). 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, for their ability to adsorb mycotoxins (Yiannikouris *et* al., 2003). The in vivo adsorption efficacy of an autoclaved Saccharomyces cerevisiae and of an EGM was studied evaluating the carry-over of OTA added to basal diet for laying hens.

MATERIAL AND METHODS - Eighty-four Warren-Isa Brown laying hens (1.8 kg mean body weight) were randomly 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 tested by HPLC (Simonella *et al*., 1990) to ensure that it contained no residual ochratoxin A. During the 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. At the beginning of the trial and immediately before the slaughter, samples of blood were collected. At the end of the experiment, all the animals were slaughtered and kidneys, and superficial pectoral muscle were collected. HPLC fluorometric method was used to check OTA (Cirilli *et al*., 1986; Simonella *et al*., 1990). 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. Animal care and experimental procedures were conducted according to Directive 86/609/EEC (1986). The study was performed according to ISO 9001:2000 requirements. Differences between treatments were compared using the Student's *t*-test (paired data); a difference with $P<0.05$ was considered to be statistically significant.

RESULTS AND CONCLUSIONS - During the 12 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. The basal diet was negative for OTA when subjected to HPLC analysis. The HPLC method used to check OTA in the considered biological matrices was characterised by high specificity, and accuracy, as well as by satisfactory LOD and LOQ. All the biological matrices from the OTA administered hens were positive to the mycotoxin; the recorded levels were very low and decreased in the order: kidney > blood > muscle (Table 1). Our data agree with the results of several experiments in hens, and confirm that no direct relation between the concentration in the feed and the residue levels could be established (Reichmann *et al*., 1982; Niemiec *et al*., 1994). In a similar trial, swine were orally administered OTA with the diet at the same concentration of the present study, but for a shorter period of time. The levels of OTA recorded in kidney, blood and muscle were higher than the present ones (especially for blood and kidney) and decreased in the order: blood > kidney > muscle (Zaghini *et al*., 2006). Renal proteins have been demonstrated to have a strong affinity for OTA. Our data show how *Saccharomyces cerevisiae* and esterified glucomannan do not exert any particular effect in adsorbing OTA and reducing its oral bioavailability.

Table 1. Levels of OTA (ppb) in blood and tissues of laying hens orally administered OTA. Mean values±SD.

Groups	0-OTA	SC-OTA	EGM-OTA
Kidney	2.47±1.10	2.93±1.71	2.27±0.78
Muscle	0.31±0.22	0.40±0.38	0.45±0.28
Blood	1.06±0.48	0.89±0.21	1.21±0.56

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