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Studies on some feed additives and materials giving partial protection against the suppressive effect of ochratoxin A on egg production of laying hens

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ABSTRACT

The protective effects of various feed supplements against the harmful effect of ochratoxin A on egg production and sexual maturation of two-weeks old Plymouth Rock female chicks designed for laying hens were studied. A significant protective effect of the feed additives or materials: water extract of artichoke (WEA), sesame seed (SS), Roxazyme-G (RG) and L-β phenylalanine (PHE) against the suppressive effect of ochratoxin A (OTA) on egg production of laying hens was found. A similar protection was also seen on the toxic effect of OTA on various internal organs of the same hens. A significant protection was found against the decrease of the weight or the quantity of eggs as well as against the delay of the beginning of the laying period of chicks, both of which were provoked by ochratoxin A. These protective effects were strongest in chicks treated with SS or WEA, but were slightest in chicks treated with L-β PHE.

1. Introduction

Ochratoxin A (OTA) is a mycotoxin widely encountered in some components of animal feeds. Experimentally, the most obvious effects of OTA-contaminated feed on chicks are reduced rates of weight gain (Dwivedi and Burns, 1984; Stoev et al., 2000, 2002b), decreased egg production (Haazele et al., 1993), immunosuppressive effects (Stoev et al., 2000) and increased mortality. Feed additives or water supplements are often studied for their potential to ensure safely utilization of some mycotoxin-contaminated harmful feeds. It has been found that ascorbic acid supplementation to laying hen diet can partially reduce the toxic effect of nephrotoxic mycotoxin OTA as reported by Haazele et al. (1993). On the other hand, polyenzyme complement Roxazyme-G (RG), sesame seed (SS), water extract of artichoke (WEA) and L-β phenylalanine (PHE) were also found to protect against the toxic effect of the same mycotoxin (Stoev et al., 2002b). It is well known that part of the toxic effect of OTA is due to the structural homology with PHE, resulting in an inhibition of protein synthesis due to a competition for the specific t-RNA (Bunge et al., 1978; Creppy et al., 1979). This could explain the protective effects of PHE administration or its structural analogue aspartame, an artificial sweetener (Creppy et al., 1995; Belmadani et al., 1998). The L-β PHE, which is an essential amino acid, is usually used in 5:1 M ratio towards OTA as the higher doses (above 10:1 M ratio) provide only a slight protection against OTA, because of increasing the absorption of OTA from the stomach and intestine. In addition, the higher supplementation of pure PHE in diets contaminated with OTA also tended to create an amino acid imbalance, which reduced body weight gain and feed conversion efficiencies (Gibson et al., 1990; Bailey et al., 1990).

The other feed supplement SS is rich in proteins (above 20%) and relatively rich in L-β PHE (about 4.3%), and therefore it could be considered as feed material. Therefore, the SS can supply animals with PHE and thereby to protect against toxicity of OTA. By using SS-supplementation to feed, the increase of OTA-absorption from the stomach and intestine, which is usually provoked by pure PHE supplementation, could be avoided (Roth et al., 1988). The SS could also increase the energy metabolism of animals via ensuring enough quantity of PHE as it is well known that part of the toxic effect of OTA is due to its structural homology with PHE and the competitive inhibition of carrier proteins located in the inner mitochondrial membrane, which can disturbs intramitochondrial phosphate transport in the cells (Meisner and Chan, 1974).

The other putative antidote RG is a commercially available enzyme complement, which contains an enzyme complex derived from *Trichoderma longibrachiatum* consisting of cellulase, endo-β-1:3,1:4-glucanase, xylanase, amylase and pectinase. It has been developed especially to complement the digestive enzymes of pigs

Abbreviations: OTA, ochratoxin A; PHE, phenylalanine; RG, Roxazyme-G; SS, sesame seed; WEA, water extract of artichoke; LDWAT-group, low dosed group 1 without antidote treatment; WAT-group, group 2 treated with high doses OTA without antidote treatment; PHE-group, group 3 treated with high doses OTA and phenylalanine; RG-group, group 4 treated with high doses OTA and Roxazyme-G; SS-group, group 5 treated with high doses OTA and sesame seed; WEA-group, group 6 treated with high doses OTA and water extract of artichoke.

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and poultry, so that the non-starch polysaccharides in cereals and legumes are broken down into simpler molecules, which the animals can digest and utilize improving feed conversion efficiency, utilization of feed and energy metabolisability (Vranjes and Wenk, 1995; Um et al., 1998), thereby, counteracting OTA impairment of energy production in chicks (Stoev et al., 2002b).

WEA is prepared as a steam infusion of dried leaves of artichoke, which is a botanically defined natural product (Gahnian et al., 1995). It contained the following biologically active compounds: cynarine, flavonoids, cynaropicrin, dehydrocynaropicrin, grosheimin as well as a high content of calcium and ascorbic acid (Gahnian and Asenov, 1986; Stoev et al., 2002b). It was used because of its potent possibility to increase diuresis in animals suffering from cardiac and renal insufficiency, and thereby, to accelerate the urinary route of excretion of OTA (Stoev et al., 2002b). In addition, it is found that cynarine content in such extract decreases serum urea and lipids, improves diuresis and increases biliary secretion, which probably augments the hepatobiliary route of excretion of OTA (OTA is mainly eliminated via bile and urine) and thereby protects against OTA toxicity (Stoev et al., 2000, 2002b). It is also found that some medical preparations prepared from artichoke-extract (chophytol and chophytamine) as well as some components of the WEA as cynarine or flavonoids have a potent hepatoprotective effect against hepatotoxic damage (Gahnian et al., 1995; Cairela et al., 1973; Mantia et al., 1973), which can be provoked by OTA. On the other hand, the high content of calcium and vitamin C in such WEA could also protect against OTA toxicity (Stoev et al., 2000, 2002b), since some authors reported that ascorbic acid supplementation (300 mg/kg) to laying hen diet that contained OTA (3.0 mg/kg) partially reduced its toxic effect (Haazele et al., 1993).

This experiment is an extension of our former short term study on the protective effects of polyenzyme complement RG, SS, WEA and L-β PHE against the toxic effect of OTA on various internal organs, biochemical indices and body weight gain in stock chicks, published in Toxicology Letters (Stoev et al., 2002b). The purpose of this study was to explore further the protective effects of above mentioned feed additives and materials against the decrease of the weight or the quantity of eggs as well as against the delay of the beginning of the laying period of chicks, all of which appeared in consequence of the toxic effects of OTA.

2. Materials and methods

2.1. OTA production

A strain of *Aspergillus ochraceus* (isolate D2306, as used by Tapia and Seawright (1984)) was grown on sterilised shredded wheat (40 g) in 500 ml conical flasks, moistened by a 40% (v/w) addition of sterile water and incubated on a rotary shaker at 27 °C for 2 weeks (Harris and Mantle, 2001). The brown granular product was then sterilised at 80 °C for 1 h and stored at −20 °C until the study. A sample was analysed by HPLC with diode array detection for ochratoxin A and found to contain ~2 mg/g and relatively small component of the biologically-inactive deschloro-analogue ochratoxin B (Harris and Mantle, 2001). No other mycotoxins were present in this solid substrate fermentation process and the necessary dilution by approximately 10^3 when homogenised into chick ration made only a minimal addition of other components of the moulded shredded wheat substrate.

2.2. Experimental design

Specific pathogen-free female chicks (Plymouth Rock) were purchased at two-weeks of age, housed in wire floor cages (two chicks in each cage with more than 500 cm^2 per chick at the beginning of experiment; one chick in each cage with more than 1000 cm^2 per chick at later stages of sexual maturity) with continuous infra-red lighting at a temperature suitable for their age. Commercially prepared complete standard feed (Smesler, Stara Zagora, Bulgaria) suitable for the breed, age and utilization of the chicks according to the accepted standards and regulations of the country, which contained the defined concentrations of OTA with or without one of the putative antidotes, supposed to protect against the toxic effects of OTA, were available *ad libitum* during the whole experimental period as described in Table 1. The main ingredient of the feeds in later aging periods were maize (30–35%), wheat (28–30%), soya groats (11–12%), sunflower groats (9–10%), sunflower oil (2–3%), fish flower (around 3%), lucerne flower (2–3%), chalk (around 8–10%), dicalcium phosphate or other similar mineral supplements (2–3%), salt (0.1–0.3%), sodium bicarbonate (0.09%), lysine (0.05%), vitamin supplements, etc. The metabolizable energy of diet ranged between 2750 and 2850 kcal/kg, whereas the proteins of diets ranged betweens 15% and 16% at the later periods up to 20–21% at the initial growing periods. The female chicks were grouped in six experimental and one control groups (10 chicks in each group) and treated with various additives (supplements) via the feed as shown in Table 1.

The L-β PHE (Finomvegyszergyar, Budapest, Hungary) was used in 5:1 M ratio towards OTA as suggested by Gibson et al. (1990) and Bailey et al. (1990) (see Table 1).

RG (Hofmann La Roche, Grenzach-Wyhlen, Austria) was used in the suggested by the producer concentrations (Table 1).

The percentage of SS (Institute of Introduction of Plant Resources, Sadovo, Bulgaria) was conformable to the energy and ration balances of fed forages during various aging periods of the respective experimental animals in accordance to the standards and regulations of the country (Table 1). According to Horani and Daghir (1975) laying hens are efficient in energy utilization of proteins (above 20% proteins) from sesame seed supplementation and can keep the metabolizable energy values of diet (2747 kcal/kg). Such a SS-supplementation can increase significantly the quantity of PHE (bound with proteins) in feed – up to 3440 ppm.

The 5% total WEA (*Cynara scolymus L.*) was prepared as a steam infusion of dried leaves of artichoke (Gahnian et al., 1995) and was given as shown in Table 1.

Negative effects due to the tested above antidotes in the given concentrations have not been found so far as reported by some other authors (Gibson et al., 1990; Bailey et al., 1990; Vranjes and Wenk, 1995; Um et al., 1998; Gahnian et al., 1995; Cairela et al., 1973; Mantia et al., 1973; Horani and Daghir, 1975).

The Trakia University Animal Care Ethic Committee approved the study protocol and the chicks were housed, maintained and slaughtered in accordance with the Bulgarian Welfare Regulations.

2.3. Measurements of egg production

All chicks were examined for the beginning of the laying period, the quantities of their egg production and the weight of the eggs. The number of eggs from each laying hen was calculated during the first 10 days after beginning of the laying period of each pullet. The weight of the eggs was calculated only for the first 50 eggs from each group of laying hens after beginning of the laying period of each pullet from the respective group.

2.4. Histological examination

For histological examination of various internal organs 10 chicks from each group were slaughtered at the end of the experiment – at about 12-month age. Materials for histological examination were taken from kidneys, liver, heart, thymus, bursa Fabricii,