EFFECTS OF AFLATOXIN AND VARIOUS ADSORBENTS
ON PLASMA MALONDIALDEHYDE LEVELS IN QUAILS

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Abstract

One-day-old 120 quail chicks (Coturnix coturnix japonica) were used in the study. The quails were divided into 10 equal groups. Group 1 was maintained as the control group. Groups 2, 3, 4, 5, 6, 7, 8, 9, and 10 were administered 2.5 ppm of aflatoxin, 2.5 ppm of aflatoxin+0.5% hydrated sodium calcium aluminosilicate (HSCAS), 0.5% HSCAS, 2.5 ppm of aflatoxin+0.5% yeast cell wall+oxicinol+tymol, 0.5% yeast cell wall+oxicinol+tymol, 2.5 ppm of aflatoxin+0.5% aluminosilicate+organic acid, 0.5% aluminosilicate+organic acid, 2.5 ppm of aflatoxin + 0.5% ammonium salts+HSCAS, and 0.5% ammonium salts+HSCAS, including various specialties, respectively. All three compounds were given in feed for a period of 21 d. At the end of the study, blood samples were collected from the animals into heparinized tubes for the detection of plasma malondialdehyde (MDA) levels. According to the obtained results, statistically significant differences were found in the MDA levels in groups 2, 7, and 9, in comparison with the control group. The highest level of MDA was detected in the group that was administered only aflatoxin. On the other hand, the values pertaining to the groups, which were administered with toxin binders in association with aflatoxin, were assessed to be close to the values of the control group. The groups that were administered only toxin binders, displayed values very close to those of the control group.

Key words: quails, aflatoxin, malondialdehyde, adsorbents.

Aflatoxins synthesized by fungi pertaining to the Aspergillus genus have 4 main metabolites: B₁, B₂, G₁, and G₂. This number increases with metabolized products. Aflatoxins are compounds that are comprised of a coumarine nucleus (1, 2, 5). They are known to cause intoxication even upon intake of very low doses. The liver is an organ in which the toxin accumulates at the highest level following absorption. In poultry, aflatoxin B₁ is transformed into various metabolites by means of hydroxylation, hydration, demethylation, and epoxidation with microsomal enzymes (2, 4, 30). The toxic effects of the aflatoxin varies according to the susceptibility of poultry species, feed, age, the level of toxin intake, nutrition conditions, its transformation into non-toxic compounds, and capacity of hepatic microsomal enzymes (2, 21, 23, 29). Aflatoxins have various toxic effects on certain cells, tissues, organs, and systems (6, 7, 9, 12, 22). One of the most common methods used for the prevention of their toxicity, is the addition of adsorbents (hydrated sodium calcium aluminosilicate, clinoptilolite) into feed. These adsorbents irreversibly bind to aflatoxins in the digestive system and restrict their absorption. These adsorbents bind to aflatoxins due to their physicochemical properties (2, 10, 13, 24-28, 32). Aflatoxins are known to generate free radicals (8, 14). The most significant indicator of possible lipid peroxidation caused by free radicals is malondialdehyde (MDA), which induces the peroxidation itself, or is one of the peroxidation end products. Changes in tissue or blood MDA levels may point out the development and severity of peroxidation (11, 17, 19).

This study was aimed at the evaluation of effects of various dietary adsorbents and aflatoxin on plasma malondialdehyde levels in quails.

Material and Methods

Animals. One hundred and twenty one-day-old quail (Coturnix coturnix japonica) chicks were divided into 10 equal groups. Between the 1st and 21st d of the experiment, group 1 was provided with a basal diet, whereas groups 2, 3, 4, 5, 6, 7, 8, 9, and 10 were administered 2.5 ppm of aflatoxin, 2.5 ppm of aflatoxin+0.5% hydrated sodium calcium aluminosilicate (HSCAS), 0.5% HSCAS, 2.5 ppm of aflatoxin+0.5% yeast cell wall+oxicinol+tymol, 0.5% yeast cell wall+oxicinol+tymol, 2.5 ppm of aflatoxin+0.5% aluminosilicate+organic acid, 0.5% aluminosilicate+organic acid, 2.5 ppm of aflatoxin + 0.5% ammonium salts+HSCAS, and 0.5% ammonium salts+HSCAS, including various specialties, respectively. All three compounds were given in feed for a period of 21 d. At the end of the study, blood samples were collected from the animals into heparinized tubes for the detection of plasma malondialdehyde (MDA) levels. According to the obtained results, statistically significant differences were found in the MDA levels in groups 2, 7, and 9, in comparison with the control group. The highest level of MDA was detected in the group that was administered only aflatoxin. On the other hand, the values pertaining to the groups, which were administered with toxin binders in association with aflatoxin, were assessed to be close to the values of the control group. The groups that were administered only toxin binders, displayed values very close to those of the control group.

Key words: quails, aflatoxin, malondialdehyde, adsorbents.
yeast cell wall+oxicinol+tymol, 2.5 ppm of aflatoxin+0.5% aluminosilicate+organic acid, 0.5% aluminosilicate+organic acid, 2.5 ppm of aflatoxin+0.5% ammonium salts+HSCAS and 0.5% ammonium salts+HSCAS, respectively.

**Toxin production.** The method implemented for rice by Demet et al. (3), in accordance with the method reported by Shotwell et al. (33), was used for the production of aflatoxin. The level of the aflatoxin produced was measured in accordance with the method reported by Stroka et al. (34), and by using HPLC. Accordingly, the total level of aflatoxin was determined to be 73.96 ppm, whereas the levels of derivatives were measured as 83.88%, 9.51%, 6.18%, and 0.43% for B1, G1, B2 and G2, respectively.

**Measurements of MDA levels.** At the end of the 21st d of the experiment, the animals were euthanized; and blood samples were collected into heparinized tubes. The blood samples were centrifuged at 3000 rpm for 10 min, and the obtained plasma was transferred into separate tubes. The measurement of plasma MDA levels were performed in accordance to the method reported by Yoshioka et al. (36). A pink stain was found to form upon the stoichiometric reaction of MDA with thiobarbituric acid, and was detected at 535 nm, according to spectrophotometric measurements. The obtained absorbencies were placed into the equation that was calculated according to the standard curve prepared with 1,1,3,3 tetrathoxypropane, and were expressed as nmol/ml.

**Statistical analyses.** The data was presented in a form of arithmetical mean values and standard deviations. Differences between groups were analysed with the one-way analysis of variance (ANOVA) and Duncan tests, using the SPSS 10.0 statistical software package for Windows. Values were considered significant at P<0.05.

**Results**

Statistically significant differences (P<0.05) in the MDA levels between control group and groups 2, 7, and 9 were observed (Table 1). The differences among the remaining groups were insignificant. Upon assessed evaluation, the MDA levels in the group administered only aflatoxin was high, whereas MDA levels were reduced in the groups that were administered both aflatoxin and a toxin binder (Table 1). On the other hand, MDA levels in the groups that were administered only with absorbents, were found to be close to those of the control group.

**Discussion**

MDA is a product generated during the oxidative breakdown of certain macromolecules, and is found either in free form, or bound to certain tissue structures. Endoperoxides are generated as a result of changes that occur in the molecular structure of fatty acids during their breakdown, and MDA is generated during the breakdown of endoperoxides (17, 20, 31, 35). MDA is considered to be the most significant indicator of membrane lipid peroxidation, arising from the interaction of reactive oxygen species (ROS) with cell membranes. Upon uniting with thiobarbituric acid at a proportion of 1:2, MDA generates a product that is spectrophotometrically or fluorometrically measurable, and this constitutes the most common method of detecting lipid peroxidation (16-20).

A statistically significant increase in MDA level was demonstrated in the group that was administered only with aflatoxin, in comparison with the control group. This increase demonstrates that the administration of aflatoxin at a dose of 2.5 ppm for a period of 21 d causes lipid peroxidation in quails. As is known, peroxidation occurs as a result of the inability of the organism to compensate for the free radicals generated by aflatoxin. Due to the high susceptibility of lipid membranes to peroxidation, the free radicals easily peroxidated the lipid membranes, and MDA was generated as a final product of peroxidation. MDA also causes peroxidation itself, and accelerates peroxidation by the means of synergy with free radicals.

### Table 1
Malodialdehyde levels in quail blood plasma following aflatoxin (AF) and adsorbent treatments

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>2.152±0.056ab</td>
</tr>
<tr>
<td>Group 2 (2.5 ppm of AF)</td>
<td>3.150±0.167d</td>
</tr>
<tr>
<td>Group 3 (2.5 ppm AF+ 0.5 % HSCAS)</td>
<td>2.470±0.133nc</td>
</tr>
<tr>
<td>Group 4 (0.5 % HSCAS)</td>
<td>2.197±0.049bc</td>
</tr>
<tr>
<td>Group 5 (2.5 ppm of AF+ 0.5% yeast cell wall+oxicinol+tymol)</td>
<td>2.264±0.189abc</td>
</tr>
<tr>
<td>Group 6 (0.5 % yeast cell wall+oxicinol+tymol )</td>
<td>2.021±0.068a</td>
</tr>
<tr>
<td>Group 7 (2.5 ppm of AF+ 0.5% aluminosilicate+organic acid)</td>
<td>2.660±0.062bc</td>
</tr>
<tr>
<td>Group 8 (0.5 % aluminosilicate+organic acid)</td>
<td>2.234±0.074ab</td>
</tr>
<tr>
<td>Group 9 (2.5 ppm of AF+ 0.5% ammonium salts+HSCAS)</td>
<td>2.700±0.093c</td>
</tr>
<tr>
<td>Group 10 (0.5% ammonium salts+HSCAS )</td>
<td>2.265±0.062ab</td>
</tr>
</tbody>
</table>

abcd. Means within the same column with different letters are statistically significant (P<0.05).
Therefore, peroxidation can be defined as a chain reaction. The dysfunction or inadequacy of enzymatic and non-enzymatic mechanisms that inhibit peroxidation makes the occurrence of lipid peroxidation, in other words, oxidative stress, inevitable. On the other hand, examination of the groups that were administered aflatoxin and adsorbents in their diet, revealed the MDA levels of these groups was close to those of the control group. This suggests a decrease in the severity of peroxidation. The main mechanism of this decrease is directly related to the binding of aflatoxin to these absorbent substances in the digestive tract; and therefore, a decrease in the level of aflatoxin that passes into the systemic blood circulation. Based on the measured MDA levels, examination of the studied adsorbent substances has demonstrated the varying capacity of these substances to bind aflatoxin. The differences observed in their binding capacity were directly related to the physico-chemical properties of the utilized adsorbents. The obtained results, demonstrated the absorbent with the highest binding capacity to be oxicone+tyramin+micronized yeast and HSCAS aluminosilicate+organic acid, and ammonium salts+HSCAS displayed lower binding capacities. Absorbent substances were demonstrated not to cause oxidative stress individually, and to be inert in this respect, based on the closeness of the MDA levels in the groups that were administered with these substances, to those of the control group.

Amongst similar studies that were previously conducted on this subject in various animal species, one carried out by Eraslan et al. (14) demonstrated that the administration of aflatoxin at four different doses to broiler chickens for a period of 45 d, caused an increase in MDA levels. Eraslan et al. (8) also studied the effects of aflatoxin and the combination of aflatoxin and sodium bentonite in broiler chickens, and reported that the administration of aflatoxin at a dose of 1 ppm for 45 d increased the MDA levels of hepatic and renal tissues, whereas the administration of adsorbents decreased MDA levels. Eraslan et al. (15) also investigated acute intoxication in rabbits, and found aflatoxin to increase plasma MDA levels. Taking into consideration, in particular studies that were carried out in poultry species, aflatoxin is understood to cause an increase in MDA levels (8, 14, 15). The results of this study are in accordance with the results of a previous study, in which aflatoxin was administered in association with adsorbents. Since a similar study carried out on the effects of a combination of aflatoxin and toxin binders on plasma MDA levels in quails, was not encountered, this study bears significance with regard to being a reference for future studies.

In conclusion, aflatoxin causes lipid peroxidation in quails, when administered at the indicated dosage and for the indicated period. However, the used adsorbents did not cause lipid peroxidation themselves. Although at different rates, the utilized toxin binders are capable of binding aflatoxin and decreasing plasma MDA levels.

References

32. Rosa C.A., Miazza R., Magnoli C., Salvato M., Chiaccicera S.M., Ferrero S., Saenz M., Carvalho E.C., Dalcer A.: Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult Sci 2001, 80, 139-144.