BLOOD TRIIODOTHYRONINE, THYROXINE AND THYROID-STIMULATING HORMONE CONCENTRATIONS IN MULARD DUCKS WITH EXPERIMENTAL AFLATOXICOSIS

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Summary


The aim of the experiment was to evaluate the effect of aflatoxin B_1 applied either independently or in combination with Mycotox NG on blood plasma triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH) in mulard ducks. Four groups of 20 ten-day-old birds each were used. The control group received compound feed according to the species and the age. The feed of group II was supplemented with 0.5 mg/kg aflatoxin B_1, of group III – with 0.8 mg/kg aflatoxin B_1, whereas group IV received compound feed with 0.5 mg/kg aflatoxin B_1 and 2 g/kg Mycotox NG. Blood hormone concentrations were assayed on the 21st and 42nd day in samples collected from v. metatarsalis medialis. Lower blood T_3 and T_4 were established in groups II and III. The addition of 2 g/kg Mycotox NG to the feed of group IV had not a significant protecting effect against the adverse effects of aflatoxin B_1 on blood triiodothyronine and thyroxine concentrations.

Key words: aflatoxicosis B_1, ducks, Mycotox NG, thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3)

INTRODUCTION

Aflatoxins are secondary toxic metabolites produced by Aspergillus (A. flavus, A. parasiticus, A. nomius and A. pseudotamarii) fungi (CAST, 2003). They contaminate naturally cereal crops such as corn, wheat, sorghum, beans including peanuts and cotton seed (Gugnani, 2000). Poultry are extremely sensitive to these mycotoxins (Robens & Richard, 1992), and among them, ducks are the most sensitive species (Ostrowski-Meissner, 1983). The commonest aflatoxins in animal feeds and animal foodstuffs are AFB_1, AFB_2, AFG_1 and AFG_2. Aflatoxin B_1 is the most toxic and most commonly encountered in poultry feeds (FAO & WHO, 1997; CAST, 2003). The share of AFB_1 from total aflatoxins in naturally contaminated...
The removal of aflatoxins from contaminated feeds is the main problem when seeking efficient means of decontamination. During the last years, a number of studies aimed at finding appropriate methods to minimise production losses due to the presence of aflatoxins in compound poultry feeds. The methods for reduction of aflatoxins’ adverse effects include use of mould growth inhibitors, microbial inactivation, mechanical separation, heat and chemical inactivation, grinding, usage of nutrient supplements and mycosorbents (Yildiz et al., 2004). Most of these techniques are expensive, take a lot of time, and are unfeasible, inefficient and potentially dangerous. At present, the most popular method by reason of its easy application is the use of inorganic adsorbents. They bind aflatoxins and thus, decrease their absorption in the alimentary tract.

The purpose of the present study was to determine the effect of aflatoxin B1 independently or together with Mycotox NG on blood concentrations of some hormones (triiodothyronine, thyroxine and thyroid-stimulating hormone) in mulard ducks.

MATERIALS AND METHODS

The experiment was performed with eighty 10-day-old female mulard ducks divided in four groups of 20 birds in each:

- Group I – control. Mulards from the control group were fed pelleted starter, grower and finisher feeds according to their age, produced at Zoohraninvest, Stara Zagora.
- Group II – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B1.
• Group III – experimental. Mulards received the standard feed supplemented with 0.8 mg/kg aflatoxin B₁.
• Group IV – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B₁ and 2 g/kg Mycotox NG (Ceva Santé Animale, France).

Aflatoxin B₁ was produced by Aspergillus flavus (99% purity; Sigma-Aldrich, Germany). In experimental groups, the feed was ground before being mixed with aflatoxin for better homogenisation.

Blood samples were collected from v. metatarsalis medialis by the 21st and 42nd day of the trial in sterile heparinised vacutainers (FL medical, Italy) for analysis of blood triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH). Blood was centrifuged within 30 min from collection at 1500xg for 10 min. Plasma was separated, immediately frozen and kept at −20°C until analysed. The blood hormones were assayed on an automated analyser Elecsys 2010 (Roche Diagnostics) via electro-chemiluminescence immunoassay (ECLIA) and commercial test kits (Roche Diagnostics).

The experiment was approved by the Animal Ethics Commission at the Faculty of Veterinary Medicine, Trakia University (Permit 42.10.10.2011).

Data were statistically processed by one-way analysis of variance (ANOVA), and the level of statistical significance was determined by the Tukey-Kramer test (P<0.05).

RESULTS

The changes in blood concentrations of studied hormones (T₃, T₄ and TSH) are

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triiodothyronine (T₃, nmol/L)</th>
<th>Thyroxine (T₄, nmol/L)</th>
<th>Thyroid stimulating hormone (TSH, mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 days of age</td>
<td>42 days of age</td>
<td>21 days of age</td>
</tr>
<tr>
<td>I</td>
<td>4.20±</td>
<td>3.97±</td>
<td>46.06±</td>
</tr>
<tr>
<td></td>
<td>0.079</td>
<td>0.083</td>
<td>1.17</td>
</tr>
<tr>
<td>II</td>
<td>3.81±</td>
<td>3.62±</td>
<td>40.64±</td>
</tr>
<tr>
<td></td>
<td>0.057b</td>
<td>0.072b</td>
<td>1.26ba</td>
</tr>
<tr>
<td>III</td>
<td>3.54±</td>
<td>3.47±</td>
<td>32.90±</td>
</tr>
<tr>
<td></td>
<td>0.0741c,2a</td>
<td>0.0531c,2a</td>
<td>1.361c,2c</td>
</tr>
<tr>
<td>IV</td>
<td>4.07b</td>
<td>3.89±</td>
<td>45.75±</td>
</tr>
<tr>
<td></td>
<td>0.0552a,3c</td>
<td>0.0432a,3c</td>
<td>1.402a,3c</td>
</tr>
</tbody>
</table>

*Difference from control group I; bP<0.05; cP<0.01; dP<0.001; 1 – vs control group; 2 –vs group II; 3 – vs group III.
Blood triiodothyronine, thyroxine and thyroid-stimulating hormone concentrations in mulard ducks...

shown on Table 1. Blood T₃ and T₄ were statistically significantly lower in ducks from groups II and III by 21 and 42 days of age (P<0.05–P<0.001). On the 21st day, triiodothyronine attained 3.81±0.057 nmol/L in group II (P<0.01) and 3.54±0.074 nmol/L in group III (P<0.001) vs controls (4.20±0.079 nmol/L). Thyroxine concentrations on day 21 were 40.64±1.26 nmol/L (P<0.05) and 32.90±1.36 nmol/L (P<0.001) in groups II and III, respectively as compared to untreated group I (46.06±1.17 nmol/L).

By 42 days of age, the observed changes were more marked. Average blood T₃ in group II was 3.62±0.072 nmol/L (P<0.01) whereas in group III – 3.47±0.053 nmol/L (P<0.001). Respective T₄ concentrations were 37.28±1.94 nmol/L (group II; P<0.001) and 28.71±1.07 nmol/L (group III; P<0.001). T₃ and T₄ levels in 42-days-old control ducklings were 3.97±0.083 nmol/L and 47.32±1.01 nmol/L, respectively. In experimental group IV, the combined treatment with AFB₁ and 2 g/kg Mycotox NG resulted in insignificantly lower blood T₃ and T₄ levels compared to controls (P>0.05). At 21 days of age, the values were 4.07±0.055 nmol/L and 45.75±1.40 nmol/L, and at 42 days of age – 3.89±0.043 nmol/L and 43.69±0.88 nmol/L, respectively.

There were no statistically significant differences in blood TSH concentrations between control and experimental groups for both studied ages.

DISCUSSION

Thyroid gland hormones are essential for maintenance of systemic physiological equilibrium of living beings (Nakamura & Nakao, 1993; Bozakova & Popova-Ralcheva, 2007). The reduced secretion rate of these hormones has a direct effect on the general condition of organisms (Rose, 2000). Our results demonstrated that AFB₁ could influence blood T₃ and T₄ concentrations by decreasing them. Nevertheless, these changes were not directly related to TSH as seen from the insignificant alterations in TSH levels in all treated groups compared to controls. The changes in T₃ and T₄ should result in TSH alteration, even indirectly. Lower T₃ and T₄ concentrations stimulate thyroid gland T₃ and T₄ receptors, stimulating the synthesis and release of TSH (Noyan, 1993). Increased TSH levels stimulate the utilisation of iodine by the alimentary tract and its diffusion in the thyroid gland (Noyan, 1993; Markou et al., 2001). Iodine is accumulated in the thyroid and forms a complex with thyroglobulin molecules. Afterwards, each thyroglobulin molecule binds to one or two molecules and forms T₃ and T₄. This way, blood T₃ and T₄ levels are sustained (Noyan, 1993; Dunn & Dunn, 1999; Markou et al., 2001). The lack of substantial increase in blood TSH levels observed in this study could be probably due to the lower sensitivity of thyroid receptors caused by aflatoxin B₁ (Graczyk et al., 2002; Eraslan et al., 2006). Aflatoxins are reported to induce lipid peroxidation in cells (Rastogi et al., 2001). The damage of thyroid receptors has probably resulted from the aflatoxins-induced enhanced generation of reactive oxygen species, provoking lipid peroxidation.

Lower thyroid hormone levels indicate development of metabolic disturbances. The relationship between feed quality and blood thyroid hormone concentrations has been extensively studied. An essential regulatory function of these hormones on growth, energy utilisation and a number of vital functions is reported in chickens (Carew et al., 1998). Quails, treated orally
with 2.5 mg total aflatoxin (B₁, B₂, G₁ and G₂) per 1 kg feed over 21 days exhibited reduction of T₃ and increase in T₄ concentrations (Eraslan et al., 2006). Probably, these changes occurred consequently to the delayed conversion of T₄ into T₃ in peripheral tissues (only a small part of blood T₃ is synthesised in the thyroid gland; the major part comes from T₄, in particular 5-deiodinase liver T₄ conversion (Berry & Larsen, 1992). According to the same authors, aflatoxins limit this process by inhibition of 5-deiodinase, resulting in lower blood T₃ together with higher blood T₄ levels.

The lower blood T₃ and T₄ could be further attributed to lower iodine concentrations, participating in the synthesis of hormones (Dunn & Dunn 1999; Markou et al., 2001). The AFB₁-caused damage of alimentary tract epithelium could reduce the absorption of dietary iodine (Johri et al., 1990). Thus, blood serum iodine levels decrease and this could indirectly influence thyroid T₃ and T₄ synthesis.

The reduction of plasma proteins, involved in T₃ and T₄ transportation, has been outlined as a reason for lower blood levels of studied hormones (Jassar & Balvant 1993; Graczyk et al., 2002). Altered concentrations of thyroid gland hormones (T₃ and T₄) could be also attributed to the impaired morphology of the gland. The experimental patulin intoxication of rats (Selmanoglu & Kockaya, 2004) has resulted in impaired thyroid gland morphology (lymphoid cell infiltration with enlarged interfollicular interstitial tissue and degenerated colloid).

The supplementation of feeds with adsorbents is considered as one of the most reliable methods for reduction of adverse effects of mycotoxins on animals (Dakovic et al., 2005). The different clays, bentonites, zeolites, synthetic aluminosilicates and phyllosilicates are able to bind mycotoxins and thus, to reduce their deleterious effects on animals (Abdel-Wahhab et al., 1999). Adsorbents exhibit a high affinity towards binding aflatoxins in the gastrointestinal tract of birds decreasing their toxicity (Kececi et al., 1998; Eraslan et al., 2005, 2006). The adsorbent Mycotox NG contains bentonite (montmorillonite) and micronised yeast (Saccharomyces cerevisiae). Our data confirm previous data (Celyk et al., 2003; Eraslan et al., 2005; Shi et al., 2009) showing that these mycosorbents could decrease at a significant extent the toxic effects of aflatoxins and protect growing birds.

In conclusion, the results from the present study demonstrated clearly that the supplementation of the compound feed of mulards with increasing doses of aflatoxin B₁ (0.5 mg/kg or 0.8 mg/kg) resulted in lower blood plasma triiodothyronine and thyroxine concentrations. The addition of a mycosorbent had a partially protecting effect against the observed toxic effects.

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