EFFECTS OF DIETARY AFLATOXIN AND SODIUM BENTONITE ON SOME HORMONES IN BROILER CHICKENS

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Abstract

In this study, 72 one-day-old, male, Avian race, broiler chicks were used. The birds were divided into 6 equal groups. While the first group was kept as a control, groups 2, 3, 4, 5 and 6 received for 45 d the feed containing 0.25% of sodium bentonite, 0.5% of sodium bentonite, 1 ppm of aflatoxin (AF) (approximately, 85% B1, 10% B2, 3% G1 and 2% G2), 0.25% of sodium bentonite with 1 ppm of AF, and 0.5% of sodium bentonite with 1 ppm of AF, respectively. At the end of the experiment, the animals were sacrificed, blood was collected and plasma was separated. Plasma triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), testosterone, and growth hormone (GH) levels were measured. A significant decrease was detected in plasma T3 and T4 levels in groups 2, 3, 4, 5 and 6, and a significant increase in plasma testosterone level in groups 2, 4, 5 and 6, compared to the control group. FA and sodium bentonite, which were given alone, were found to be effective on T3, T4, and testosterone contents. In the case of their combination, there was no certain evidence that the adsorbent ameliorated the effects of AF. Therefore these parameters could not be used in in vivo studies of binding the FA with sodium bentonite in broiler chickens.

Key words: broiler chickens, aflatoxin, sodium bentonite, hormones.

Aflatoxins (AFs) are poisonous compounds synthesised by genus of Aspergillus fungi (2, 11). The species of the fungi, frequently isolated from the feed and feedstuffs, are Aspergillus flavus and Aspergillus parasiticus (7). These species are also called the toxigenic fungi (19). In the presence of optimal conditions regarding temperature, humidity, O2 and CO2 in the feed, the fungi rapidly produce toxins and contaminate the feed (3, 15). The ingestion of such feed by poultry causes many adverse effects such as decrease in body weight gain, decrease in feed consumption and feed conversion rate, and damage of organs (8-10, 16, 18, 23, 26, 29). In order to prevent such negative effects, there has recently been a common practice of adding adsorbents into animal feed. Moreover, these compounds may be added into animal feed at certain rates and given to animals for protective purposes (12, 16, 18). The basic quality of these compounds is the high binding capacity due to their physico-chemical structures and irreversible binding properties (5, 31). On the other hand, certain amounts of AFs may be absorbed from the digestive tract because of incomplete binding (5). Sodium bentonite is one of the aluminosilicate compounds, used as adsorbent (29). Higher binding efficacy of this compound makes it the most preferred one, compared to others (5).

The objective of this study was to determine the effects of AF and bentonite, administered separately or in combination, on thyroid hormones, testosterone, and growth hormone. There have been few studies about the topic. However, we could not come across any study, concerning the effect of sodium bentonite given alone or in combination with FA. The obtained results will be a guide for in vivo binding efficacy trials with this compound in broiler chickens. It will be seen if these parameters could be used or not as references in the chickens.
Material and Methods

In this study, 72 one-day-old, male, *Avian* race chicks were used. The animals were weighed and divided into 6 groups with 12 animals in each by considering a homogenous weight distribution. A 24 h lighting system was applied and the animals had an access to feed and water *ad libitum*.

While the first group was fed the broiler ration, the groups 2, 3, 4, 5 and 6 were fed the ration containing 0.25% of sodium bentonite, 0.5% of sodium bentonite, 1 ppm of AF, 0.25% of sodium bentonite with 1 ppm of AF, 0.5% of sodium bentonite with 1 ppm of AF, respectively, for 45 d. At the end of the experiment, the animals were sacrificed and their plasma levels of triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), testosterone and growth hormone (GH) were determined. The analyses of hormones were done by electrochemiluminescence techniques, which were performed on Elecsys 2010 immunoassay analyser with Boehringer Manheim hormone kit. The method of Demet et al. was used in AF production (4), which is based on report of Shotwell et al. (32). The strain of *Aspergillus parasiticus* (NRRL 2999) was used to produce AF (4). Total AF levels in rice were detected by ELISA with Ridascreen® kit and its method. AF content in rice was determined by Nabney and Nesbit’s method (20). Subsequently, 85% B1, 10% B2, 3% G1 and 2% G2 were detected in rice flour, in average.

The data were evaluated as arithmetic means and standard deviation. A one-way-variance analysis was used and the significant differences between groups were determined by Duncan test. The statistical analysis was done on the SPSS 10.0 for Windows package program.

Results

The groups 2-6 which received sodium bentonite and AF in combination or alone displayed the decreases in the levels of T3, T4, and increases in GH and testosterone contents. The changes in TSH level occurred as either an increase or a decrease. The significant decreases were found in plasma T3 and T4 levels in groups 2, 3, 4, 5 and 6, compared to the control chickens (P<0.05). The significant increases were observed in plasma testosterone level in groups 2, 4, 5 and 6, compared to the control group (P<0.05). No significant difference was found between the groups concerning TSH and GH concentrations (P>0.05) (Table 1).

<table>
<thead>
<tr>
<th>Groups*</th>
<th>T3 (ng/mL)</th>
<th>T4 (µg/dL)</th>
<th>TSH (µIU/ml)</th>
<th>Testosterone (ng/dL)</th>
<th>GH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.32±0.13a</td>
<td>2.34±0.23c</td>
<td>0.36±0.03</td>
<td>11.83±2.18a</td>
<td>2.40±0.36</td>
</tr>
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<td>Group 2</td>
<td>1.19±0.16b</td>
<td>2.12±0.31b</td>
<td>0.34±0.02</td>
<td>25.33±4.98b</td>
<td>2.66±0.33</td>
</tr>
<tr>
<td>Group 3</td>
<td>1.07±0.13ab</td>
<td>1.91±0.27ab</td>
<td>0.41±0.08</td>
<td>16.45±2.09ab</td>
<td>2.71±0.30</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.91±0.05a</td>
<td>1.65±0.12a</td>
<td>0.41±0.29</td>
<td>19.90±1.78b</td>
<td>2.89±0.66</td>
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<tr>
<td>Group 5</td>
<td>1.05±0.07a</td>
<td>1.91±0.17a</td>
<td>0.36±0.01</td>
<td>22.62±2.56b</td>
<td>3.02±0.24</td>
</tr>
<tr>
<td>Group 6</td>
<td>0.94±0.17a</td>
<td>1.62±0.30a</td>
<td>0.41±0.06</td>
<td>31.43±8.01c</td>
<td>2.50±0.20</td>
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*group 1 - control; group 2 - sodium bentonite (0.25%); group 3 - sodium bentonite (0.5%); group 4 - AF; group 5 - sodium bentonite (0.25%) with AF; group 6 - sodium bentonite (0.5%) with AF

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*a, b, c* Differences are statistically significant in groups marked with different letters in the same column (P<0.05)

Discussion

In the present study, the statistically significant decreases were observed in the levels of blood T3 and T4 in all trial groups compared to the control group. No changes were detected in TSH levels. The mechanism concerning the decreases in plasma T3 and T4 levels may be associated with the decrease in blood iodine level. It is essentially significant in the synthesis of these hormones (1, 6, 17). Interestingly, the decreases in both hormone levels were not only observed in the group with received aflatoxin only, it was also observed in the groups which received feed with sodium bentonite and in the groups which received both compounds. It is obvious that AF causes damage in the digestive system (14). The damage which developed in the epithelium of the digestive tract, may reduce the absorption of dietary iodine from the digestive tract. Thus, iodine levels in the bloodstream may decline, compared with its normal limits. This may indirectly hinder the synthesis of T3 and T4 in the thyroid gland. The reason for these declines in the hormone levels in the groups which received adsorbent material alone could be the irreversible binding of iodine by bentonite, subsequently, its disrupted absorption from the digestive tract. In fact, sodium bentonite is a compound that possesses high binding capacity (5).
On the other hand, the other mechanism connected with the decreases of both hormone levels may be related to the changes which probably develop during the transport of these hormones in the bloodstream. An example for that is a significant reduction of plasma protein content by AF. (13). This decrease may trigger another decrease concerning the rates of protein-bounded T3 and T4, therefore, may lead to increased free hormone levels in the blood. Free hormones (not bounded by plasma proteins) diffuse quickly into tissues and are rapidly eliminated by the liver as soon as they come (28). In both cases, the plasma levels of the mentioned hormones may decline. This may be primary reason for the decrease which was much more higher in the group that received only AF. Yet, the decrease did not occur rapidly in the groups which received sodium bentonite only (groups 2 and 3) and in the groups which received sodium bentonite as a combination with AF. The absence of statistically significant difference among three groups proved that this effect is not the primary mechanism for the decrease in the hormone levels.

Another mechanism is the change in the metabolic rate of the hormones in tissues. T4 is converted into T3 via 5-deiodinase in tissues (1). By this conversion, T4 level progressively decreases, in contrary, T3 level increases in bloodstream. In fact, most of T3 in blood is formed as a consequence of this conversion (1, 21, 22). The decreases in T3 and T4 levels in all trial groups, renders the suspected hypothesis concerning the changes in tissue-enzyme level, compared to the control group. If both compounds which are added into feed alone or combined accelerate the conversion rate of these hormones (this may result in the increase in 5-deiodinase activities, involved in this conversion), there should be a decrease in blood T4 level (it was detected in the present study). In contrary, the T3 level should display an increase. However, this increase was not seen in the present study. On the other hand, regarding the decrease in the mentioned enzyme activity, there should be an increase in T4 and a decrease in T3 levels. The results showed that none of them happened. Consequently, it could be suggested that neither of the components had a direct effect on the conversion of the hormones which takes place in the tissues (conversion of T4 into T3).

Moreover, parallel to changes concerning T3-T4 hormones, no significant difference was observed in plasma levels of TSH. In fact, the decrease in plasma levels of T3 and T4 also causes an increase in the synthesis and release of TSH (22, 30). However, the results of the present study did not confirm that (no statistically significant difference was found between the groups). The emphasised reason may be related to T3-sensitive nuclear receptors in the group which received AF, because any situation may inhibit the stimulation of T3-sensitive nuclear receptors in tissues and the thyroid gland, subsequently, disrupts TSH secretion by the adenohypophysis. AFs are known to cause lipid peroxidation (10, 27). Essentially, intermediate and end products harmful for the cell membrane and other intracellular organelles may be formed following this reaction. If this reaction is severe enough to be not compensated by intracellular defense mechanism, it may result in damage to the cell and, unfortunately, lead to its death. This is the effect, which may reduce the sensitivity of the above-mentioned receptors of T3. Structural alterations in the receptor, which originates from this peroxidation, may be responsible for this pathway. It is not possible to suggest the same situation for the groups which received only sodium bentonite in the feed. Although adsorbent significantly reduced the absorption of vitamin E in the digestive tract (25), there is no evidence to suggest that it causes the peroxidation and cellular damage directly.

LH-RH and LH, which are secreted by the hypothalamus and pituitary gland, respectively, control the synthesis of testosterone and its levels in blood. Testosterone is synthesized in the Leydig’s cells from cholesteryl esters and cholesterol. Cholesterol is converted into pregnanolon by protein kinases, finally, other microsomal enzymes and various intermediate reactions contribute to it in order to transform testosterone (22). Therefore, the increases in enzyme activity and in the level of the primary products (cholesteryl esters, cholesterol) may also indirectly change the level of this hormone in blood. However, the change is also necessary concerning the other mechanisms (LH-RH, LH) which control its synthesis and release, otherwise, this change could not take place. An increase in testosterone level was detected in the study. This increase was observed in all trial groups. It was reported that AF reduced testosterone level by damaging testicular cells (24). Intriguingly, a decrease, not an increase, was observed in our study. This increase, although not definite, may be originated from the possible increase of protein kinase activity caused by AF. These changes were observed in the animals which received sodium bentonite only. The reason for that could not be clarified. The increase was even much more distinctive in the group 6 which received combination of both compounds.

The growth hormone is effective in increasing the protein synthesis (22). In the present study, no significant difference was found, regarding the growth hormone in all groups, compared to control. This clearly proved that AF given separately or in combination with sodium bentonite did not make either a positive or negative effect on the mentioned hormone. In conclusion, these hormones should not be taken into consideration as parameters which are used to detect the efficacy of such AF binding agents as sodium bentonite in in vivo trials on broiler chicks. Another conclusion is that the binding agents should not be added into animal feed in arbitrary intention. Again, the lack of similar studies which are related to this topic, does not allow to evaluate comparatively of the obtained data. However, the present study may serve as a reference for future investigations. Nevertheless, the obtained results revealed that many detailed investigations have to be done to clarify the effect of both studied compounds on these hormones as well as their mechanisms in broiler chickens.
References


