Turk J Vet Anim Sci
30 (2006) 41-45


Research Article

# Effects of Dietary Aflatoxin and Hydrate Sodium Calcium Aluminosilicate on Triiodothyronine, Thyroxine, Thyrotrophin and Testosterone Levels in Quails

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Received: 30.07.2003

**Abstract:** This study was performed on 80 male 14-day-old Coturnix coturnix japonica breed quails. The quails were divided into 8 groups with 10 animals in each as 1 control and 7 trial groups. While the control group was fed a commercial basal ration, groups 2-8 received, respectively, 2.5 g/kg feed hydrate sodium calcium aluminosilicate (HSCAS), 5.0 g/kg feed HSCAS, 10.0 g/kg feed HSCAS, 2.5 ppm aflatoxin (AF $B_1$ 78.30%, AF $B_2$ 14.60%, AF $G_1$ 4.50%, AF $G_2$ 2.60%); 2.5 ppm AF with 2.5 g/kg feed HSCAS, 2.5 ppm AF with 5.0 g/kg feed HSCAS, and 2.5 ppm AF with 10.0 g/kg feed HSCAS, respectively, for 21 days. At the end of the trial, blood samples were taken from the animals and triiodothyronine ($T_3$), thyroxine ($T_4$), thyrotrophin (TSH) and testosterone levels in the blood were measured. Statistically significant increases were detected in $T_3$ levels in groups 3 and 4 and significant decreases in groups 5 and 6, while there were significant increases in $T_4$ levels in groups 2, 4, 5, 7 and 8 and significant decreases in blood testosterone levels in all trial (groups 2 to 8) groups compared to the control (group 1).

**Key Words:** Aflatoxin, HSCAS, hormone, quail

## Bıldırcınlarda Triiodotronin, Tiroksin, Tirotropin ve Testosteron Düzeyleri Üzerine Yemle Verilen Aflatoksin ve Hidrate Sodyum Kalsiyum Alüminosilikatın Etkisi

**Özet:** Çalışma, 80 adet, erkek Coturnix coturnix japonica ırkı, 14 günlük bıldırcınlar üzerinde gerçekleştirildi. Bıldırcınlar, biri kontrol diğer yedisi deneme olmak üzere sekiz gruba ayrıldı ve her bir grupta 10 hayvan bulunduruldu. Kontrol grubuna ticari rasyon verilirken deneme gruplarından Grup 2, 3, 4, 5, 6, 7 ve 8'in yemlerine sırasıyla 2,5 g/kg yem HSCAS; 5,0 g/kg yem HSCAS; 10,0 g/kg yem HSCAS; 2,50 ppm aflatoksin (Af $B_1$ % 78,30, Af $B_2$ % 14,60, Af $G_1$ % 4,50, Af $G_2$ % 2,60); 2,50 ppm AF+2,50 g/kg yem HSCAS; 2,50 ppm AF+5,0 g/kg yem HSCAS; 2,50 ppm AF+10,0 g/kg yem HSCAS katıldı ve 21 gün süreyle verildi. Denemenin sonunda, hayvanlardan kan alındı ve kanda triiodotronin ($T_3$), tiroksin ($T_4$), tirotropin (TSH) ve testosteron düzeyleri ölçüldü. Sonuçta, kontrol grubuna göre istatistiksel olarak, $T_3$ düzeyinde Grup 3 ve 4'de önemli bir artış, Grup 5 ve 6'da önemli bir düşüş; $T_4$ düzeyinde Grup 2, 4, 5, 7 ve 8'de önemli bir artış; testosteron düzeyinde bütün deneme gruplarında (Grup 2-8) önemli bir düşüş tespit edildi.

**Anahtar Sözcükler:** Aflatoksin, HSCAS, hormon, bıldırcın

## Introduction

Aflatoxins (AFs) are natural contaminants of feed and feedstuffs (1). Poultry are most sensitive to these toxins (2). Although ducks are claimed to be the most sensitive poultry animals (3), sensitivity tests carried out on quails revealed that these animals may be easily affected by AFs present in feed (4,5). The severity of poisoning by AFs depends on the age, sex and species of the animal, the amount being exposed to and duration of exposure. The vitamins, minerals and antibiotics present in feed are among the factors that change the severity of poisoning. In addition, the amount of protein in the feed composition is closely related to poisoning (6,7). The liver is one of the organs most affected by AFs (8). They have various effects on other organs (9). The most suitable method to bind AFs in digestive tract is to add adsorbents to animals' feeds at certain rates, which hinder their absorption and alleviate their adverse effects (10,11). These compounds bind to AFs irreversibly in the digestive tract, and reduce the rate of AF absorbed and released into systemic circulation (12,13).

In this study our aim was to determine whether AF and hydrate sodium calcium alumino silicate (HSCAS), which were given to quails alone and in combination at certain doses for 21 days, had any effect on thyroid hormones and testosterone. Previously, no study was carried out to find the effects that may occur in hormones when AF and HSCAS are given alone and in combination in quails. For this reason, the effects that occurred in animals due to subacute exposure to both compounds were evaluated and it was determined whether one or more parameters used in in-vitro efficacy tests were determinative criteria concerning the efficacy of the HSCAS in quails. This study will also be a guide for determining the risk of any poisoning based on these parameters long before the appearance of clinical symptoms in AF poisonings in quails.

## Materials and Methods

Eighty male 14-day-old *Coturnix coturnix japonica* breed quails were used. The quails were divided into 8 groups, 1 control and 7 trial groups. While the animals in the control group were fed a commercial basal ration, 2.5 g/kg feed HSCAS, 5 g/kg feed HSCAS, 10 g/kg feed HSCAS, 2.5 ppm aflatoxin, 2.5 ppm AF with 2.5 g/kg feed HSCAS, 2.5 ppm AF with 5 g/kg feed HSCAS, 2.5 ppm with 10 g/kg feed HSCAS, respectively, were given to the animals in groups 2-8 for 21 days. The study was performed in separated quails, cages that were equipped with 24 h lighting, free access to water and feed, at 27-29 ºC on a daily basis. On day 21, blood samples were taken from the animals and triiodothyronine ($T_3$), thyroxine ($T_4$), thyrotrophin (TSH) and testosterone levels in the blood were determined. The detection of plasma $T_3$, $T_4$, TSH and testosterone levels was performed in a Boehringer Mannheim Elecsys 2010 brand immunoassay analyzer using in vitro electrochemiluminescence (ECL). AF was added to the feed according to Demet et al.'s (14) method based on the method of Shotwell et al. (15). The species of AF in the rice was detected based on the method described by Roberts and Patterson (16), according to the method described reported by Şanlı et al. (17). It was determined that rice flour contained AF $B_1$, $B_2$, $G_1$, $G_2$ and $G_2$. Their rates were calculated according to Nabney and Nesbit's method (18). The rates for AF $B_1$, $B_2$, $G_1$ and $G_2$ were, respectively, 78.30%, 14.60%, 4.50% and 2.60%. Total AF level in rice flour was detected in an ELISA apparatus using a Ridascreed® total AF kit and according to the method suggested in the kit's instructions. Accordingly, 84.68 ppm total AF was detected in rice flour with AF. The data were evaluated as arithmetic means and standard deviations; the significance of the groups was detected by one-way variance analysis. Duncan's test was used to determine differences between the groups (using SPSS 10.0 for Windows).

## Results

Significant differences were detected in the parameters except for TSH levels in the groups that received adsorbent and AF alone and in combination for 21 days. Compared to the control group, significant increases were detected in $T_3$ levels in groups 3 and 4 and a significant decrease in groups 5 and 6, while there were significant increases in $T_4$ levels in groups 2, 4, 5, 7 and 8, and significant decreases in testosterone levels in all trial groups (groups 2 to 8) (Table).

## Discussion

Thyroid hormones are among the major hormones playing important roles in the protection of the physiological balance of the body (19). Malfunctions in these hormones directly affect the general situation of the living being (20). Firstly, it was evaluated whether AF had any effect on thyroid hormones. There are numerous studies about animals that were given AF in feed and thus seriously affected (9-11,21). In this study, when an evaluation was performed in terms of thyroid hormones, a significant decrease was detected in $T_3$ levels and a significant increase in $T_4$ levels in the groups that received AF alone compared to the control group. No statistically significant difference was found in TSH levels. The results showed that $T_3$ and $T_4$ might have been greatly affected by AF. However, it is clear that this effect did not occur directly through TSH because insignificant changes were observed in the trial groups concerning this hormone level compared to the control. However, changes in $T_3$ and $T_4$ levels must cause a change, even though indirectly, in TSH levels since a decrease in blood $T_3$ level directly stimulates $T_3$-sensitive nuclear receptors in the thyroid gland thus causing TSH synthesis and release (22,23). An increase in blood TSH level accelerates the absorption of iodide from the digestive tract and its diffusion into the thyroid gland (23,24). Iodide that is diffused into the

Table. $T_3$, $T_4$, TSH and testosterone levels in control and experimental groups.

| Groups* | $T_3$ (ng/ml) | $T_4$ (µg/dL) | TSH (µIU/ml) | Testosterone (ng/dl) |
|---|---|---|---|---|
| Group 1 | 1.70 ± 0.23[c] | 0.65 ± 0.18[a] | 0.22 ± 0.06 | 109.01 ± 15.86[a] |
| Group 2 | 1.69 ± 0.24[c] | 1.20 ± 0.21[b] | 0.27 ± 0.08 | 84.63 ± 11.54[b] |
| Group 3 | 1.91 ± 0.24[d] | 0.81 ± 0.12[a] | 0.24 ± 0.04 | 74.50 ± 13.00[c] |
| Group 4 | 1.93 ± 0.23[d] | 1.62 ± 0.33[d] | 0.22 ± 0.05 | 62.23 ± 7.81[c] |
| Group 5 | 1.22 ± 0.18[a] | 1.41 ± 0.10[c] | 0.23 ± 0.07 | 29.70 ± 11.69[e] |
| Group 6 | 1.44 ± 0.17[b] | 0.74 ± 0.10[a] | 0.20 ± 0.09 | 50.60 ± 15.33[d] |
| Group 7 | 1.59 ± 0.15[bc] | 1.15 ± 0.13[b] | 0.21 ± 0.05 | 47.72 ± 6.87[d] |
| Group 8 | 1.71 ± 0.19[c] | 1.04 ± 0.13[b] | 0.23 ± 0.10 | 37.55 ± 9.10[e] |

[a, b, c, d, e] Means within the same column with different letters are statistically significant ($P < 0.05$).

* Group 1, control; Group 2, 2.5 g/kg feed HSCAS; Group 3, 5.0 g/kg feed HSCAS; Group 4, 10.0 g/kg feed HSCAS; Group 5, AF; Group 6, AF + 2.5 g/kg feed HSCAS; Group 7, AF + 5.0 g/kg feed HSCAS; Group 8, AF + 10.0 g/kg feed HSCAS.

thyroid gland forms a complex with a thyroglobulin molecule. Following that, every one or two thyroglobulin molecules unite and so $T_3$ and $T_4$ synthesis occurs. In this way, $T_3$ and $T_4$ levels in the blood are kept at a certain level (23-25). However, in our study, the lack of a significant increase in TSH levels might have been caused by the reduction in the sensitivity of the receptors in the thyroid gland due to AF. It is known that AFs cause lipid peroxidation in cells (26). Reactive oxygen species, which cause lipid peroxidation and whose formation is accelerated by AFs, may led to conformational changes in receptors. Such changes may also hinder $T_3$'s binding to these receptors and the activation of the intracellular messenger system. Hence, a physiological response may not develop in the body concerning changes in blood $T_3$ levels. On the other hand, the reason for an increase in $T_4$ and a decrease in $T_3$, which were observed in the group that received AF alone compared to the control group, might have been a slowdown in the conversion of $T_4$ to $T_3$ in peripheral tissues. While some of the $T_3$ diffused into the bloodstream is synthesized in the thyroid gland, a major proportion of it occurs as a result of conversion of $T_4$, synthesized in the thyroid gland, into $T_3$ in peripheral tissues. 5'-deiodinase is the enzyme primarily responsible for this conversion. On the other hand, malic enzyme and 6-phosphogluconate dehydrogenase also take part in this process. These enzymes convert NAD into NADP, which is responsible for this conversion (23). It is possible that AF limits the conversion of $T_4$ into $T_3$ by causing the changes, mainly in 5'-deiodinase as well as in other enzymes activities, that were mentioned above. As a result, while the $T_3$ level decreases, the $T_4$ level may increase. It is very likely that this mechanism has effects concerning the changes in the blood levels of these hormones. On the other hand, in the groups that received feed containing both AF and HSCAS significant changes were observed in $T_3$ and $T_4$ levels, compared to the groups that received AF alone. These changes were an increase in $T_3$ and a decrease in $T_4$. The decrease in $T_3$ and the increase in $T_4$ revealed that HSCAS was bound to AF in the digestive tract. The fact that neither of these parameters ($T_3$ and $T_4$) were close to the values of the control group and that there was a statistically significant difference between most of the groups indicated that the binding was not complete and a certain proportion of AF diffused into the blood and exerted its effect. Interesting results were obtained in the groups that received adsorbent alone. Some of them exhibited significant increases in both $T_3$ and $T_4$ levels. However, some researchers reported that sodium bentonite altered the levels of some minerals in the body. It was stressed that it caused an increase in the absorption of some and a decrease in the absorption of others (27). Similarly, the adsorbent causes an increase in the absorption and this may lead to an increase in the synthesis of both hormones.

Secondly, blood testosterone levels change as a result of malfunctions and extreme damage to Leydig cells, where testosterone is synthesized (28). It is known that

AF causes damage to the testes (29). When blood testosterone level was evaluated, a significant decrease was detected in the groups that received AF alone compared to the control group. This decrease also indicates an important malfunction in the testes. Of the groups that received AF with HSCAS, an increase was detected in testosterone levels, compared to the group that received AF alone. This increase also indicates that HSCAS was bound to AF. In fact, although an increase was observed in testosterone levels in the groups that received AF with HSCAS alone, compared to the group that received AF alone, this increase never reached the value of the control group. A significant decrease was detected in testosterone levels in the groups that received certain doses of HSCAS alone, compared to the control group. The decrease in testosterone level in the group received HSCAS only, although not certain, may be related directly or indirectly to the fact that the adsorbent bound to some compounds or caused an increase in the absorption of the others mentioned above.

In conclusion, AF given at a dose of 2.5 ppm for a subacute period affects blood $T_3$, $T_4$ and testosterone levels negatively. It is quite likely that the main mechanism for AF to change $T_3$ and $T_4$ levels was not directly through the hypothalamus, hypophysis or thyroid gland but decreases in the activities of enzymes such as mainly 5'-deiodinase, malic enzyme and 6-phosphogluconate dehydrogenase, which are responsible for the conversion of $T_4$ into $T_3$, might have played an important role in the changes of $T_3$ and $T_4$ levels in peripheral tissue. As a result, the $T_4$ level increased and the $T_3$ level decreased. The decrease in testosterone level in the group that received AF alone, compared to the control group, showed that AF caused damage to Leydig cells of the testes. On the other hand, the statistically significant differences in hormone levels of the groups that received AF with adsorbent, compared to the group that received AF alone, revealed that HSCAS bound to AF in the digestive tract but this binding was not completed. It was concluded that the determinations of $T_3$, $T_4$ and testosterone levels with other parameters (yield from quail feed performance, histopathological findings, biochemical parameters) will be important in in-vitro efficacy trials with HSCAS in quails against AF and an evaluation can be made based on the levels of these parameters ($T_3$, $T_4$ and testosterone) in the blood, long before the appearance of poisoning symptoms that may result from this toxin. Nevertheless, the data obtained show that more detailed research is needed to determine the exact effects of these 2 compounds on these hormones in quails.

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