

Effect of Low Levels of Dietary Aflatoxin B₁ on Laying Japanese Quail

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ABSTRACT In the present study, 256 7-wk-old Japanese quail were randomly distributed into four experimental groups (64 birds per group) and given rations containing 0 (controls), 25, 50, or 100 (g aflatoxin B₁ (AFB₁)/kg feed for 168 d. Each treatment consisted of four replicates of 16 quail. Egg production and individual egg weight were checked daily. Feed consumption and feed use were determined weekly. Eggs laid in the last day of each 28-d laying period were collected and subjected to individual analysis for specific gravity, Haugh units, shell thickness and percentage eggshell. Results showed that average egg production, feed use, and body weights were not affected ($P > 0.05$) by AFB₁. However, feed consumption was lower ($P < 0.05$) for groups fed 50 or 100 μg AFB₁/kg. Egg weight was significantly lower ($P < 0.05$)

only for groups exposed to 50 and 100 μg AFB₁/kg. Average egg specific gravity, Haugh units, and shell thickness were not affected ($P > 0.05$) by AFB₁. Percentage eggshell was higher ($P < 0.05$) in the group fed the ration containing 100 μg AFB₁/kg. Treatment associated lesions were observed only in the liver. Hepatic cell vacuolation with fatty infiltration was observed in all liver samples of quail fed AFB₁-contaminated rations. Bile duct proliferation and trabecular disorder were found only in livers of quail on the 100- μg AFB₁/kg treatment. Results indicated that chronic exposure to AFB₁ at levels above 50 μg /kg could adversely affect quail performance, emphasizing the importance of controlling aflatoxin contamination in quail rations.

(Key words: aflatoxin B₁, quail, egg, quality, toxicity)

2002 Poultry Science 81:976–980

INTRODUCTION

Aflatoxins are hepatotoxic metabolites produced by storage fungi of the genus *Aspergillus*, particularly *A. flavus* and *A. parasiticus*, during growth on a number of food and feed materials (Coulombe, 1991). Eighteen different aflatoxins have been identified, and the major ones are B₁, B₂, G₁, and G₂, with aflatoxin B₁ (AFB₁) being the most common and toxic compound (Hsieh and Atkinson, 1991). Effects of aflatoxins are dose- and time-dependent, and two distinct forms of aflatoxicosis, namely acute and chronic, can be distinguished depending on the dose and length of time of exposure (Leeson et al., 1995). Acute toxicity is clinically characterized by depression, anorexia, icterus, hemorrhages, and death (Pier, 1992). The liver is the main target organ, and the major histologic lesions in quail include hepatic cell vacuolation with fatty infiltration and bile duct hy-

perplasia (Sawhney et al., 1973). Chronic aflatoxicosis is generally associated with poor performance and usually results from regular low-level dietary intake of aflatoxins. In poultry, AFB₁ causes immunosuppression and decreased body weight gain and feed utilization. Reduced egg production and egg weight are the most reported manifestations of aflatoxicosis in layers (Leeson et al., 1995).

Susceptibility to aflatoxins can vary considerably between different poultry species (Arafa et al., 1981). Moreover, the dose-response relationship is markedly altered by intraspecies factors, such as age, sex, and breed (Pier, 1992). In quail, previous studies indicate that Japanese quail appear to be more resistant than Bobwhite quail (Ruff et al., 1992).

The available data on aflatoxin toxicity in quail, however, are mainly derived from short-term studies using high levels of toxin added to feeds, without specifying which of the four naturally occurring aflatoxins is present. Sawhney et al. (1973) reported that Japanese quail

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Received for publication September 1, 2001.

Accepted for publication February 28, 2002.

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Abbreviation Key: AFB₁ = aflatoxin B₁.

fed a diet containing 2 to 6 mg/kg AFB₁-equivalent for 6 wk showed adverse changes in feed conversion, egg production, egg weight, hatchability, and exterior and interior quality of eggs. Johri et al. (1990) demonstrated that dietary AFB₁-equivalent at 300 to 750 µg/kg for 100 d caused reduction in feed consumption, egg production, and hatchability of fertile eggs.

Continuous ingestion of low levels of aflatoxins in feeds may not cause any apparent physiological damage, but it can markedly impair the performance of birds, which would result in significant economic losses (Jones et al., 1982). There is very little information on the effect of low dietary levels of AFB₁ on the performance of laying quail, except for a report by Bintvihok et al. (1993), who observed decreased egg production after 12 wk exposure of Japanese quail to AFB₁ at levels of 50 to 200 µg/kg.

In Brazil, the quail egg industry is concentrated in the southeast region, particularly in the state of São Paulo, which accounts for nearly 70% of the 4.8 million eggs produced daily in the country. The potential impact of low levels of AFB₁ on quail performance and egg production parameters is not well understood. Therefore, the aim of the present work was to verify the effects of long-term toxicity of AFB₁ in young laying quail that were fed rations containing low levels of the toxin.

MATERIALS AND METHODS

Experimental Design and Quails

Two hundred fifty-six laying Japanese quail (*Coturnix coturnix japonica*) were purchased from a local commercial grower at 5 wk of age. The birds were placed in two batteries of eight wire cages each (16 birds per cage) and provided with linear feed-troughs and V-shaped troughs for running water. Initially, the quail were allowed 2 wk to adapt to the cages and during that period were fed a conventional maize and soybean meal basal diet, formulated to meet all the nutritional requirements of laying quail according to specifications of the National Research Council (1994). After this period, the quail were randomly assigned to one of four dietary treatment groups. Each treatment group consisted of four replicate pens, each containing 16 birds. There were two replicate pens per treatment in each battery. The dietary treatments were: 0 (control), 25, 50, and 100 µg AFB₁/kg feed. The study was conducted in the Aviculture Section of the University of São Paulo.² A lighting schedule began with 16 h of light at 35 d of age with increases of 30 min per wk until 18 h of light was reached at 63 d of age. This final schedule was maintained throughout the remainder of the study. The treatment rations were given ad libitum for 168 d (six 28-d laying periods). All laying quail were individually weighed in the first day of each

28-d laying period and monitored daily for signs of morbidity and mortality. All eggs were recorded, collected, and individually weighed on a daily basis. Feed consumption and feed use were determined weekly.

Aflatoxin Production and Diet Preparation

AFB₁ used in the experiment was produced in the laboratory of Mycotoxins of Biomedical Science Institute (University of São Paulo²), using toxigenic strains of *Aspergillus flavus* IMI-190³. A small fragment of an *A. flavus* colony, in Czapeck agar at 25 C, was inoculated in the center of a Petri dish with coconut agar (Lin and Dianese, 1976). Incubation was carried out at 25 C for 10 d, and cultures were assayed for aflatoxins as described by Lin and Dianese (1976). The coconut agar cultures were extracted with chloroform (30 mL chloroform per 10 g culture) by shaking for 30 min. The content was filtered through a Whatman #1 filter paper and evaporated to dryness. The purification was made with hexane and partitioned with chloroform (Sabino et al., 1989). Quantification was achieved by densitometry according to Scott (1990). The chloroform solutions were placed in flasks, kept in a water bath at 60 C until complete evaporation of the diluent, and subsequently resuspended in 1 mL of sterile maize oil (Almeida et al., 1996) that had been previously tested for the presence of aflatoxins.

AFB₁ test concentrations were obtained using sterile maize oil as the diluent, and appropriate amounts of these solutions were added to the basal diet to obtain the required levels of mycotoxin. Final mixtures were homogenized in a horizontal-helicoidal mixer (Marconi). To guarantee a balanced diet for all treatments, AFB₁-oil mixtures were substituted for maize oil (0.5% vol/wt) in the feeds. The concentrations of AFB₁ in final mixtures were confirmed by analyzing 1-kg samples following procedures proposed by Soares and Rodrigues-Amaya (1989). Additionally, the basal diet was screened and found to be free of the following mycotoxins: aflatoxins, ochratoxin A, and zearalenone. The assay detection limits were 2.0 µg/kg for aflatoxins, 5.0 µg/kg for ochratoxin A, and 55.0 µg/kg for zearalenone. Fumonisin B₁ and B₂ were detected at levels below 1.0 mg/kg in all diets.

Determination of Egg Quality

All eggs laid in the last day of each 28-d period were collected, weighed, and subjected to the following determinations: specific gravity (Hamilton, 1982), albumen height, percentage eggshell, and shell thickness (Potts and Washburn, 1974). Albumen height data were transformed to Haugh units, according to Stadelman and Cotterill (1986).

Histopathology

At the end of the trial, six quail from each treatment group were anesthetized with ether, euthanatized by

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TABLE 1. Effect of aflatoxin B₁ (AFB₁) on quail-day egg production, feed consumption, feed conversion, egg weight, and mortality of laying quail¹

AFB ₁ in ration (μg/kg)	Egg production (%)	Feed consumption (g/bird/d)	Feed use (g feed/g eggs)	Egg weight (g)	Mortality (n)
0 (control)	87.11 ± 9.83 ^a	28.69 ± 2.17 ^a	3.12 ± 0.39 ^a	10.67 ± 0.24 ^a	0/64
25	85.85 ± 7.63 ^a	28.69 ± 1.72 ^a	3.17 ± 0.32 ^a	10.62 ± 0.25 ^a	1/64
50	87.69 ± 9.11 ^a	27.57 ± 1.81 ^b	3.02 ± 0.39 ^a	10.53 ± 0.21 ^b	1/64
100	86.82 ± 8.33 ^a	27.76 ± 1.85 ^b	3.10 ± 0.35 ^a	10.51 ± 0.21 ^b	2/64

^{a,b}Means within a column with no common superscript differ statistically ($P < 0.05$).

¹Results are reported as means ± SD, for four replicates of 16 quail each.

cervical dislocation, and necropsied for gross lesions. Liver, spleen, and gizzard samples were collected in 10% neutral buffered formalin. Tissue sections 5 μm thick were stained with hematoxylin and eosin and were used for microscopic histological evaluation (Thomson, 1990).

Statistical Analysis

The results (cage means) were subjected to one-way ANOVA (Snedecor and Cochran, 1967), and treatment means were compared by the Tukey test, using the SAS general linear model (SAS Institute, 1992). Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The results of feeding AFB₁-contaminated diets on average egg production, feed consumption, feed utilization, egg weight, and mortality of laying quail over six, 28-d periods are presented in Table 1. Egg production was not affected ($P > 0.05$) by dietary treatments. Feed utilization values were also similar among the treatments ($P > 0.05$). Although Bintvihok et al. (1993) reported decreased quail egg production after 12 wk exposure to 50 μg AFB₁/kg, it appears that egg production and feed utilization are only adversely affected when Japanese quail are exposed to much higher concentrations of AFB₁ (Sawhney et al., 1973). In an experiment conducted by Johri et al. (1990), the minimum concentration that decreased egg production was 500 μg/kg over 100 d of exposure, although this level represents the sum

of all aflatoxin fractions. In this study, feed consumption was lower ($P < 0.05$) for groups fed 50 and 100 μg AFB₁/kg, when compared to controls or the group fed 25 μg AFB₁/kg. The decrease in feed consumption started during the first 28-d period and remained lower for groups fed 50 and 100 μg AFB₁/kg until the end of the trial (Figure 1). These findings were also observed by Johri et al. (1990) for concentrations of aflatoxins greater than 300 μg/kg.

Average egg weight decreased ($P < 0.05$) only for groups exposed to 50 and 100 μg AFB₁/kg. The decrease in egg weight became evident during the second 28-d period (Figure 2) and was more marked with increasing AFB₁, indicating a dose-dependent response. These results are similar to data presented by Sawhney et al. (1973) and Johri et al. (1990) for greater than 2 mg/kg and 500 μg AFB₁/kg, respectively.

Table 2 shows the effects of dietary treatments on average egg specific gravity, Haugh units, percentage eggshell, and shell thickness. Specific gravity values and Haugh units were similar among the experimental groups. According to Leeson et al. (1995), aflatoxins affect egg formation through an impairment of the normal mobilization of fat from the liver to the ovary. Sawhney et al. (1973) demonstrated that albumen height is adversely affected when Japanese quail are exposed to greater than 2 mg AFB₁-equivalents/kg feed, which is much higher than the concentrations used in the present experiment.

Eggs from the group receiving 100 μg AFB₁/kg feed had a higher percentage eggshell ($P < 0.05$), especially

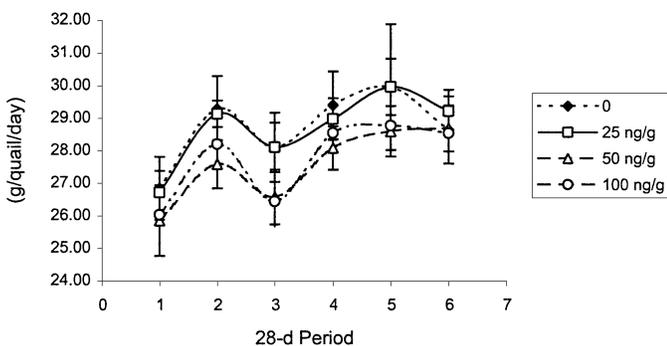


FIGURE 1. Effect of aflatoxin B₁ on feed consumption. Compared with controls, quail fed diets containing 50 or 100 μg AFB₁/kg had lower feed consumption during the six 28-d periods.

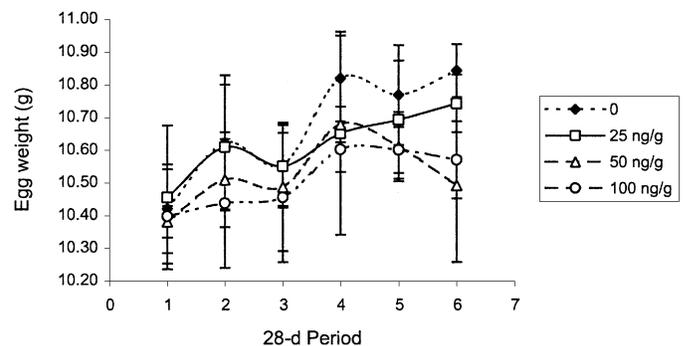


FIGURE 2. Effect of aflatoxin B₁ on quail egg weight. Compared with controls, average egg weight decreased with increasing concentrations of AFB₁, which became more evident after the second 28-d period.

TABLE 2. Effect of aflatoxin B₁ (AFB₁) on egg specific gravity, Haugh units, percentage eggshell, and shell thickness of laying quail¹

AFB ₁ in ration (μg/kg)	Specific gravity (wt/vol)	Haugh units	Eggshell (%)	Shell thickness (X 10 ⁻² mm)
0 (control)	1.070 ± 0.001 ^a	85.41 ± 2.42 ^a	8.07 ± 0.09 ^a	32.1 ± 1.2 ^a
25	1.070 ± 0.001 ^a	85.34 ± 2.46 ^a	8.04 ± 0.21 ^a	31.8 ± 1.6 ^a
50	1.070 ± 0.002 ^a	84.50 ± 2.06 ^a	8.13 ± 0.23 ^{ab}	31.9 ± 1.5 ^a
100	1.071 ± 0.002 ^a	84.87 ± 3.17 ^a	8.22 ± 0.24 ^b	32.2 ± 1.6 ^a

^{a,b}Means within a column with no common superscript differ statistically ($P < 0.05$).

¹Results are reported as means ± SD, for four replicates of 16 quail each.

during the third and fourth 28-d periods (Figure 3). However, shell thickness values were very similar for all the treatments, indicating that this variable was not significantly affected by the 168-d exposure to AFB₁ at the concentrations used in the present investigation. Our results are in agreement with those obtained by Washburn et al. (1985), who worked with laying hens fed rations containing 5,000 μg of total aflatoxin (sum of all fractions) per kilogram of feed. The authors observed a significant reduction in egg weight, together with normal shell deposition, which increased the respective values for percentage eggshell and shell strength.

Mortality during the 168-d experiment consisted of one quail in each of the 25 μg/kg and 50 μg/kg treatments and two quail in the 100 μg AFB₁/kg treatment. No histological lesions were observed in control quail. Lesions associated with AFB₁ were only observed in the liver. Mild to moderate hepatic cell vacuolation with fatty infiltration was observed in all liver samples of quail fed AFB₁-contaminated rations, which became more marked with increasing AFB₁ levels. Bile duct proliferation and trabecular disorder observed in liver samples of quail fed the 100 μg/kg treatment, including the two quail that died during the experiment, could be considered as typical lesions of aflatoxicosis, as previously reported in quail fed AFB₁-equivalents greater than 500 μg/kg (Arafa et al., 1981; Johri et al., 1990).

In the present study, experimental intoxication of laying quail with rations containing AFB₁ at 25, 50, or 100 μg/kg feed did not significantly affect quail-day egg production and feed use. However, it was observed that

chronic administration of AFB₁ at greater than 50 μg AFB₁/kg decreased feed consumption and egg weight ($P < 0.05$), which might cause significant losses in productivity of quail chronically exposed to the aflatoxin. In surveys carried out in southern and southeastern states of Brazil, aflatoxins have been reported in feed materials, such as maize, at average levels of 79 and 35 μg/kg, respectively (Sabino et al., 1989). The results of our experiment indicate that in this range of naturally occurring levels of aflatoxin there is the potential for a significant economic impact on the quail industry.

ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil (Grant Number 1999/00700-3) for financial support.

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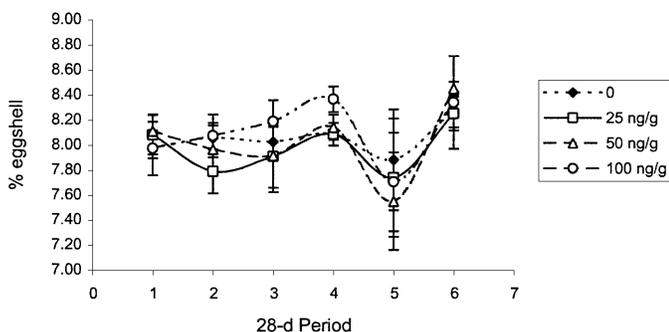


FIGURE 3. Effect of aflatoxin B₁ on percentage eggshell. Compared with the other treatment groups, eggs from the group receiving 100 μg AFB₁/kg feed had a higher percentage of eggshell, especially during the third and fourth 28-d periods.

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