Short Communication

Aflatoxin Effect on Humoral and Mucosal Immune Responses Against Infectious Bronchitis Vaccine in Broilers

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Abstract

The aflatoxin effect on humoral and mucosal immunity against infectious bronchitis (IB) vaccine was studied in 225 one-day-old chicks. The chicks were divided in 3 equal groups. Treatment groups included group A: chickens fed a basal diet, and group B: chickens fed 3 ppm productive aflatoxin in a basal diet. The chickens in groups A and B were vaccinated against IB (at one and 10 days old) by H120 live attenuated vaccine. For group C, chickens did not receive aflatoxin and were not vaccinated against IB. All chickens received experimental diets from 3 to 28 days old, continuously. At 28 days old, all chickens were slaughtered. After blood sampling, serum was prepared for measuring serum IgG titer against IB vaccine by Elisa method. Moreover, the heads were collected for nasal-tracheal lavage for assaying IgA against IB vaccine in mucosa of respiratory tract. The measurement of serum IgG against IB vaccine was done by commercial IBV ELISA kit. The assaying of mucosal IgA against IB vaccine was done by IBV ELISA plate and specific goat anti-chicken IgA. Results indicated that the chickens receiving aflatoxin showed less serum IgG and mucosal IgA titers than the others. Therefore, it seems that aflatoxin can affect mucosal immunity in the upper respiratory tract as well as the systemic immune response against IB vaccine.

Keywords: aflatoxin, bronchitis, chicken, mucosal immunity

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Introduction

Aflatoxins are secondary metabolites of various *Aspergillus* species. Foods of cereal grain origin demonstrate the most susceptible commodities for contamination with aflatoxin (Herzallah, 2013). Poultry can be exposed to high concentration of aflatoxin through feedstuffs, which will then lead to large economic losses (Hoerr, 2013). Generally, aflatoxicosis in poultry is characterized by mortality, decreased growth rates, and increased susceptibility to other diseases (Rangsaz and Gholami-Ahangaran, 2011; Gholami-Ahangaran and Zia-Jahromi, 2013; Gholami-Ahangaran et al., 2015). A practical approach to detoxification is the use of sorbents in the diet which will absorb aflatoxin in the gastrointestinal tract of poultry and reduce bioavailability and toxicity (Gholami-Ahangaran and Zia-Jahromi, 2013; Gholami-Ahangaran et al., 2015). However, the contamination of foods with aflatoxin is usually latent. Therefore, the control of this toxicity is very difficult.

Aflatoxin can be a main immunosuppressive agent (Hoerr, 2013) that can influence the efficacy of immune response in poultry. The majority of studies in this field focused on the effect of this mycotoxin on humoral and cellular immune system. Presently, there is no scientific research into the effect of aflatoxin on mucosal immunity against infectious bronchitis (IB) vaccines in chickens. Therefore, this research analyzed the humoral and mucosal respiratory immune responses against IB vaccine in chickens suffering experimental aflatoxicosis.

Materials and Methods

Aflatoxin production: Aflatoxin provided by *Aspergillous parasiticus* (PTCC: 1850) belonged to Iranian Scientific and Industrial Researches. Aflatoxin was produced according to the Shotwell method (Shotwell et al., 1996) on maize. Productive aflatoxin was assessed by competitive ELISA kit (Romer Co., USA).

Experimental design: A total of 225 one-day-old broiler chicks (Ross strain) were randomly divided into three groups by three replicates of 25 chicks in each separated pen during the 28-day experiment. Basal diet based on corn-soybean was balanced in accordance with the recommendation by National Research Council (1994).

Treatment groups included group A: chickens fed a basal diet, and group B: chickens fed 3 ppm productive aflatoxin in a basal diet. The chickens in groups A and B were vaccinated against IB (at one and 10 days old) by live attenuated H120 IB vaccine (Razi Vaccine and Serum Research Institute, Karaj, Iran). For group C, chickens did not receive aflatoxin and were not vaccinated against IB virus. The maize containing aflatoxin was added to the experimental basal diet of group B to the amount of 3 ppm aflatoxin. In the groups that did not receive aflatoxin, the same amount of uncontaminated maize (without aflatoxin) was added to the basal diet. All treatment groups received the experimental diets throughout the growing period from hatch until 28 days old. Feed and water were supplied *ad libitum* to all groups and 24-hour light was used throughout the experiment.

Mucosal and systemic immunoglobulin Assay: Blood samples from all chickens at 28 days of age were collected from wing vein for measurement of serum immunoglobulin G (IgG) titer against IB vaccine. The assay of serum IgG titer against IB vaccine was performed by commercial IBV ELISA kit (Synbiotecs Corporation, California, USA) according to instructions of the manufacturer.

After blood sampling, all of the chickens were slaughtered and samples of trachea and head were separated immediately. The mucosal surface of trachea and nasal were washed with one ml of phosphate buffer saline (PBS) containing bovine serum albumin (BSA) three times. Immediately after washing, the extracted liquid was centrifuged and the supernatant was collected (Tamura et al., 1989; Takada and Kida, 1996; Gholami-Ahangaran, 2011). To measure the level of mucosal IgA against IB vaccine, the lavage samples were tested by commercial ELISA kit (Synbiotics Corporation, California, USA). Due to the lack of commercial kits for measuring IgA, a commercial IBV ELISA kit was used instead, but the conjugated HRP goat anti-chicken IgG was replaced by conjugated HRP goat anti-chicken IgA. The goat anti-chicken IgA conjugated to horseradish peroxidase was purchased separately (Cat. No. A30-103P, Bethyl Laboratories, Montgomery, Texas, USA).

IgA titer in mucosal respiratory lavage was determined according to Gelb et al. (1998) and Thompson et al. (1997). For this purpose, mean optical density (OD) of negative control was calculated and three times of standard deviations were added to the OD of negative control. Then, to calculate the titer of each sample, the OD of negative control and positive-negative threshold was used (Gelb et al., 1998; Thompson et al., 1997). In this study, IgA titer in each sample was calculated manually based on negative control and positive-negative threshold. The IgA titers were expressed based on log 2. The IgG titers were calculated using the KPL software program.

Statistical Analysis: All data were analyzed using the one away ANOVA method by SAS software (SAS Institute, 2001). Significant differences among the treatment groups were recognized at $p < 0.05$ by Tukey test.

Results and Discussion

The mean systemic IgG and mucosal IgA titers in the chickens receiving aflatoxin were significantly lower than those in the chickens fed uncontaminated diet and vaccinated. Moreover, the chickens fed aflatoxin and vaccinated possessed higher systemic IgG and mucosal IgA titers than the chickens unvaccinated.

In this study, the reduction in systemic IgG and mucosal IgA titers against IB vaccine in the chickens receiving aflatoxin could be caused by aflatoxin effects on the immune system. The suppression of immune system caused by aflatoxin in poultry has already been reported (Hoerr et al., 2013),

but there is no report on the effects of aflatoxin on mucosal immunity in respiratory organ against IB vaccine.

There are some reports that indicate that aflatoxin can increase sensitivity of birds to viruses (Hoerr, 2013) and aflatoxin can cause adverse effects on production of serum antibodies against ND virus, pasteurellosis and IBD (Azzam and Gobal, 1998). Moreover, there are some evidences of aflatoxin effect on histopathologic feature of thymus, spleen and bursa of Fabricious in chickens. Thymic aplasia, splenic atrophy and lymphoid depletion in bursa can demonstrate the effect of aflatoxin on cellular immune response in chickens (Arulmozhi and Koshy, 2011). Ibrahim et al. (2000) showed that both percentage and mean of phagocytic activities were decreased significantly in chicks fed 2.5 ppm aflatoxin. Furthermore, leucopenia in chickens following aflatoxin toxicity at the level of 3 ppm demonstrated cellular immunosuppression of aflatoxin (Gholami-Ahangaran and Zia-Jahromi, 2014). The effect of aflatoxin on interferon, complement and serum proteins (Giambrone et al., 1978), subsequent liver damage and inhibition of protein synthesis (Gholami-ahangaran et al., 2015) are the possible causes of immunosuppression. It seems that the effect of aflatoxin on the immune system is influenced by the dose and duration of the use of contaminated diet with aflatoxin. For example, Kouwenhoven (1993) investigated the systemic antibody production following vaccination against Newcastle disease in chickens fed aflatoxin. In that study, 0.2-0.5 ppm of aflatoxin B1 could not change the systemic immune response to the ND, *Salmonella pullorum* and *Pasturella multocida* but higher dose (0.6-10ppm) could suppress the systemic antibody response to Salmonella and sheep RBCs. The result of the present study matches that of Ibrahim et al. (2000), who clarified that 2.5 ppm aflatoxin had negative effect on ND antibody formation. However, the effects observed in the present study may be the result of the high dietary aflatoxin contamination.

The overall results of this study showed that 3 ppm aflatoxin can reduce humoral and mucosal immune responses. Therefore, the control of mycotoxins as immunosuppressive agents helps improve immunity responses to vaccines.

Table 1 Serum IgG and mucosal IgA titers against IB vaccine in different groups

Treatments	A	B	C
Serum IgG Titer	1961.00±352.66[a]	403.20±407.93[b]	88.50±71.44[c]
Mucosal IgA Titer	4.92±1.65[a]	3.15±1.68[b]	0.27±0.45[c]

A: Chickens receiving basic diet and vaccinated against IB; B: Chickens receiving aflatoxin in diet and vaccinated against IB; C: Chickens receiving basic diet and not vaccinated against IB

* Data presented as Mean±SD

[a,b] Different words in each row represent significant differences between groups ($p < 0.05$).

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บทคัดย่อ

ผลกระทบของอะฟลาท็อกซินต่อการตอบสนองทางภูมิคุ้มกันแบบสารน้ำและเยื่อเมือกต่อวัคซีนต้านหลอดลมอักเสบในไก่กระทง

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ลูกไก่อายุ 1 วัน จำนวน 225 ตัว ได้ถูกแบ่งเป็น 3 กลุ่ม กลุ่มละจำนวนเท่ากัน เพื่อศึกษาผลกระทบของอะฟลาท็อกซินต่อภูมิคุ้มกันแบบสารน้ำและแบบเยื่อเมือกต่อวัคซีนป้องกันโรคหลอดลมอักเสบ (IB) กลุ่มทดลองประกอบด้วยกลุ่ม A คือ ไก่ที่ได้รับอาหารปกติ กลุ่ม B คือ ไก่ที่ได้รับอะฟลาท็อกซิน 3 ppm ในอาหาร ไก่ในกลุ่ม A และ B ได้รับวัคซีนต้านโรค IB (เมื่ออายุ 1 และ 10 วัน) โดยใช้วัคซีนเชื้อเป็นอ่อนกำลัง H120 กลุ่ม C คือ ไก่ที่ไม่ได้รับอะฟลาท็อกซิน และไม่ได้รับวัคซีนต้านโรค IB ไก่ทั้งหมดได้รับอาหารสำหรับการทดลองตั้งแต่วันที่ 3 ของการเจริญเติบโตจนถึงอายุ 28 วันอย่างต่อเนื่อง เมื่อไก่อายุ 28 วัน ไก่ทุกตัวได้ถูกการุณยฆาต หลังจากเก็บตัวอย่างเลือด ซีรั่มได้ถูกแยกมาเพื่อวัดระดับ IgG ต่อวัคซีนต้านโรค IB ด้วยวิธี ELISA นอกจากนั้น ส่วนหัวได้ถูกเก็บเพื่อทำการล้างช่องจมูกและหลอดลมและวัดระดับ IgA ในเยื่อเมือกของทางเดินหายใจต่อการตอบสนองต่อวัคซีน IB การวัด IgG จากซีรั่มต่อวัคซีน IB อาศัยชุดตรวจ ELISA ต่อ IBV ที่มีจำหน่าย การตรวจ IgA จากเยื่อเมือกต่อวัคซีน IB ใช้ IBV ELISA plate และ IgA จากแพะที่จำเพาะต่อ IgA ของไก่ ผลการทดลองบ่งชี้ว่าไก่ที่ได้รับอะฟลาท็อกซินมีระดับ IgG ในซีรั่มและ IgA ในเยื่อเมือกต่ำลงกว่ากลุ่มอื่น โดยรวม ดูเหมือนว่าอะฟลาท็อกซินมีผลต่อภูมิคุ้มกันแบบเยื่อเมือกในทางเดินหายใจส่วนต้นและภูมิคุ้มกันทางระบบต่อการได้รับวัคซีน IB

คำสำคัญ: อะฟลาท็อกซิน หลอดลมอักเสบ ไก่ ภูมิคุ้มกันทางเยื่อเมือก

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