Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure

○ H. OGUZ, ○○ H.H. HADIMLI, ○○○ V. KURTOGLU and ○○O. ERGANIS

Departments of
○ Pharmacology and Toxicology,
○○ Microbiology,
○○○ Animal Nutrition and Nutritional Diseases,
Faculty of Veterinary Medicine, University of Selçuk, Konya, Turkey

Correspondence to : Dr. Halis Oguz, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selçuk, 42031, Kampüs, Konya, Turkey.
E-mail: haoguz@selcuk.edu.tr.

SUMMARY

In this study, total aflatoxin (AF) and a natural zeolite (clinoptilolite ; CLI) were added to the broiler feed and development of humoral immunity against Infectious Bronchitis (IB) and Newcastle Disease (ND) was evaluated. A total of 576 1-d-old Ross broiler chicks (96 per each) were housed in six treatment groups [Control, CLI (15 g/kg diet), 50 ppb AF, 50 ppb AF plus CLI, 100 ppb AF, 100 ppb AF plus CLI] and fed for 42 days. Compared to controls, the antibody titres of IB were determined significantly lower (p < 0.05) in 50 and 100 ppb AF fed chicks from 20 to 42 days of age. The ND titres were also significantly lower (p < 0.05) in 100 ppb AF fed chicks, while no significant differences were seen in 50 ppb AF group compared to controls (p > 0.05). The addition of CLI to the AF-containing diets (50 and 100 ppb) significantly ameliorated (p < 0.05) the adverse effect of AF on humoral immunity. The single addition of CLI to the AF-free diet had no adverse effects in chicks, except the IB titres on 42nd day.

KEY-WORDS : aflatoxin - broiler - humoral immunity - zeolite - clinoptilolite - prevention.

RÉSUMÉ

Évaluation de l’immunité humorale du poulet de chair durant une exposition chronique à l’aflatoxine (50 et 100 ppb) et au clinoptilolite. Par H. OGUZ, H.H. HADIMLI, V. KURTOGLU et O. ERGANIS.

Dans cette étude, le développement d’une immunité humorale contre la bronchite infectieuse (IB) et la maladie de Newcastle (ND) a été étudié après addition à la ration des volailles d’aflatoxines (AF) et d’une zéolite naturelle (clinoptilolite ; CLI). 560 poussins Ross de 1 jour ont été répartis en 6 groupes (témoin, CLI (15 g/kg d’aliment, 50 ppb AF, 50 ppb AF + CLI, 100 ppb AF, 100 ppb AF + CLI) et nourris pendant 42 jours. Les titres en anticorps anti-IB ont été significativement plus bas (p < 0,05) pour les lots recevant 50 et 100 ppb AF de 20 à 42 jours après l’administration. Les titres pour la ND ont été aussi significativement plus bas (p < 0,05) pour les poulets recevant 100 ppb AF alors qu’aucune différence n’a été observée pour les groupes recevant 50 ppb AF par rapport aux témoins (p > 0,05). L’addition de CLI aux rations contenant AF (50 et 100 ppb) a amélioré de façon significative (p < 0,05) l’effet néfaste de l’AF sur l’immunité humorale. Une seule addition de CLI à un régime dépourvu de AF n’a aucun effet défavorable sur les poussins exceptés sur les titres anti-IB au 42ème jour.


Introduction

Aflatoxins (AF), potent mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus, are a major concern in poultry production. As well as the carcinogenic, mutagenic, teratogenic and growth inhibitory [12] effects of AF, their immunotoxic effects have also economic importance for the broiler industry and these have been well investigated using higher levels of AF (100 to 2000 ppb) in broilers [2, 5, 7].

AF in feed has been shown to adversely influence immune responses and thus increase the susceptibility of poultry to bacterial, viral and protozoan diseases [5, 17]. AF also cause a dose-related regression in the size of both thymus and bursa of Fabricius, which are the primary determinants of immunocompetence, depletion the functional cells [15] and decrease the antibody production [4]. Prophylactic immunization against the major infectious diseases in poultry, such as Infectious Bronchitis (IB), Newcastle Disease (ND) and Infectious Bursal Diseases (IBD), is vital to safeguard against these infections [2, 21]. Studies have shown that AF is an immunosuppressant of widespread nature in feed and feedstuffs, and exposure of poultry to subclinical doses of AF have been shown to cause infection, even among immunized birds in field situations [7].

Since the beginning of 1990s, the adsorbent-based studies have been performed for removing AF from contaminated feed and minimizing the toxicity of AF in poultry [9].
Zeolites [10], bentonites [12] and clinoptilolite [13, 14] were preferred because of their high binding capacities against AF and their reducing effect on AF-absorption from the gastrointestinal tract [10]. Therefore, the purpose of the present study was to evaluate the humoral immunity of broilers given low levels of AF (50 and 100 ppb) in one broiler period, and further investigate the possible preventive role of dietary adsorbent (CLI, 15 g/kg) on investigated values.

Materials and methods

CHICKENS AND DIET

Five hundred and seventy-six 1-d-old both sexes Ross broiler chicks were obtained from a commercial hatchery. Individually weighed chicks were divided at random into six groups each consists of 96 animals. The chicks were housed in heated batteries under fluorescent lighting and were fed a commercial food starter (maize and soybean based, 230 g protein, 13.80 MJ ME/kg) up to 21 d, a grower (215 g protein, 13.60 MJ ME/kg) diets up to 42 d. Chickens consumed the diets and water ad libitum. The starter and grower diets both were tested [8] for possible residual AF before feeding, and there were no detectable levels present (detection limit 1 µg/kg feed, recovery of the extraction method 95 %).

EXPERIMENTAL DESIGN

The experimental design consisted of six dietary treatments. 1) CONT: Basal diet ; 2) CLI: Basal diet plus 15 g clinoptilolite (CLI)/kg diet ; 3) 50 ppb AF: Basal diet plus 50 µg total aflatoxin (AF ; the composition given below)/kg diet ; 4) 50 ppb AF + CLI: Basal diet plus 50 µg AF plus 15 g CLI/kg diet ; 5) 100 ppb AF: Basal diet plus 100 µg AF/kg diet ; 6) 100 ppb AF + CLI: Basal diet plus 100 µg AF plus 15 g CLI/kg diet.

CLINOPTILOLITE

The commercial CLI (CLI/NUT-1000™), which is a member of heulandite-stilbite group, was provided from Incal Biotechnology and Mining Ltd., Izmir, Turkey and its chemical formula is KNa3Ca2 (Si29Al7) O72.32H2O by X-ray powder diffraction. The composition of fine-grained powder (D50 : 9.68 µm) is nearly 65.7 % SiO2, 10.9 % Al2O3, 3.0 % K2O and 2.6 % CaO. The loss of ignition at 1050 °C is 14.1 %. It also exhibits a MOHS’s hardness of about 3.5 to 4, a bulk density of 1.422 g/cm3, a packed density of 2.145 g/cm3, a poor density of 0.42 g/cm3 and a specific surface area of about 13.900 g/cm3. The point of mollifying is 1300 °C (Enteco Lab., Germany).

AFLATOXIN

The AF was produced from Aspergillus parasiticus NRRL 2999 culture (USDA, Agricultural Research Service, Peoria, IL) via fermentation of rice by the method of SHOTWELL et al. [19] with minor modifications by OGÚZ and KURTOĞLU [12]. Briefly, 100 g of sterile polished rice was inoculated with 1 ml of resuspended spores (1.5x10⁹ spores/ml) of Aspergillus parasiticus NRRL 2999 (USDA, Agricultural Research Service, Peoria, IL), placed in incubator at 28 °C and fermented for 5 d. Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The rice powder was then analyzed for AF content by the method of SHOTWELL et al. [19] and measured on TLC (Thin Layer Chromatography)-densitometer (Camag-III, Basel, Switzerland) on a TLC spots at 325 nm excitation and 425 nm emission wavelengths (TLC Plates from Merck ; other equipment from Desega ; the AF standards from Makor Chemical Ltd. Box 6570, Jerusalem, Israel). The amount of aflatoxin (AFB1, B2, G1 and G2) in the fermented rice was measured and it was found 108 mg/kg rice powder. The AF within the rice powder consisted of 72.51 % AFB1, 14.05 % AFB2, 9.78 % AFG1 and 3.66 % AFG2 based on total AF in the rice powder [detection limit : 1 µg AF/kg rice powder ; recovery of the extraction method : 92 %]. The rice powder was then calculated in order to provide the required level of AF (50 and 100 ppb ; µg/kg) in feed and incorporated into the basal diet. The safety measures were taken to avoid direct contact in all steps of production, grinding, analyses and mixing with feed.

VACCINATION AND SEROLOGY

Before all chicks (1-d-old) were separated into groups, the blood samples were taken and the maternal antibody titres against IB and ND were measured by Hemagglutination-Inhibition Test (HI) as described by ERGANIS and İSTAN-bulluoglu [3]. All chicks were vaccinated on the 1st d of age with Newcastle-Bronchitis vaccines (B1 type, La Sota strain, Massachusetts type, Intervet International B.V. Boxmeer, Holland) by aerosol-spray. On the 17th d broilers were orally vaccinated with ND+IB vaccines (La Sota strain, Massachusetts type, Intervet International, B.V. Boxmeer, Holland). Blood samples were collected interval 10 days from each treatment groups from randomly selected 10 broilers. (Other chicks in the groups were used for performance investigations published previously [14]). The sera samples were then separated and stored at -20 °C until used. The antibody titles against IB and ND were detected using HI test [3]. The hemagglutination antigens of IB and ND were provided from Poultry Diseases Research and Vaccine Production Institute, Manisa, Turkey.

STATISTICAL ANALYSIS

When the chicks reached 42 d of age, the feeding trial was terminated. The data for antibody titles for IB and ND were grouped according to collected days and expressed as mean ± pooled standard errors of means. The results obtained were statistically analysed using Duncan’s multiple range test [20]. Statements of statistical significance are based on p < 0.05.

Results

As seen in Tables I and II, the feeding AF at level of 50 and 100 ppb in the ration significantly reduced the antibody production against IB in broilers from 20 to 42 days of age. The ND titles were determined significantly lower in 100 ppb AF
fed chicks from 10 to 42 days, while no significant differences were determined in 50 ppb AF group. The addition of CLI to the AF-containing diets (both 50 and 100 ppb) significantly ameliorated the adverse effects of AF on antibody production. However, the addition of CLI to the AF-free diet did not adversely affect the investigated values, except the IB titres at 42nd day.

**Discussion**

Surveying studies have demonstrated that the AF levels in broiler food ranged from 5 to 100 ppb in Turkey [1, 11] and some other countries [7, 16]. However, generally, the determined values cumulated less than 50 ppb in broiler feed in these studies. The immunotoxic effects of AF in poultry have
been well-documented. Therefore, we particularly aimed to assess the impact of 50 and 100 ppb AF, which naturally occurred in field conditions, on antibody production in a growing period in the present study.

Because contamination of different ingredients used in poultry feed with AF seems to be widespread, extra prophylactic measures should be considered. These may include quality control of feed and the addition of some immunomodulant or non-nutritive sorptive materials in the diet that help to reduce the absorption of AF from the gut [6, 7, 18]. Recent studies have demonstrated that CLI was effective for reducing of AF-toxicity by the studies of growth performance [12, 14], haematological-serum biochemical [13] and macroscopic-histopathological [14] analyses.

In the present study, 50 ppb AF-treatment caused significantly lower (p < 0.05) antibody titres against IB of broilers (Table I, II). The effect of 50 ppb AF on ND titres was seen only 10th day of the experiment (p < 0.05). However, 100 ppb AF treatment significantly affected and inhibited the antibody production both IB and ND (p < 0.05) in the trial period. A dose-dependent inhibition in humoral immunity and antibody production was seen in this study. Our results agree with the other studies [4, 5, 7, 9] those performed on the immunotoxic effects of AF with 100 to 2500 ppb AF levels. It is important to consider poor humoral immunity in chicks by these AF-levels (50 and 100 ppb) in our study. Because these levels can be found in broiler feed in field conditions and the low levels show no significant clinical signs in broilers during the short periods. For example, the body weight gains have been determined previously in the other report [14] as 1658.46 g (Control), 1657.71 g (CLI), 1661.66 g (50 ppb AF), 1690.52 g (50 ppb AF + CLI), 1496.36 g (100 ppb AF) and 1518.38 g (100 ppb AF + CLI) for the chickens fed for 42 d. There were no statistically significance in the mortality values among the groups [14]. The immunosuppressive effect of AF has been related to its direct inhibition of protein synthesis [13], including those with specific functions such as immunoglobulins IgG and IgA, inhibition of migration of macrophages [9], interference with the haemolytic activity of complement, reduction of number of lymphocytes [5] through its toxic effect on the bursa of Fabricius [15] and impairment of cytokines formation by lymphocytes [4]. Our findings also show that CLI significantly ameliorated the adverse effect of AF (p < 0.05) on the investigated values. CLI has been known as binding the AF molecules in gas- trointestinal tract and precluding their absorption and also can alleviate the toxicity also of AF in poultry [10,12]. This finding is also in agreement with other studies that performed with different doses of CLI and different parameters [13, 14, 15].

These results clearly demonstrated that 50 and 100 ppb AF-treatment significantly affected the humoral immunity against IB and ND, and the simultaneous addition of CLI to the AF-containing diet provided significant reduction on the immunotoxic effects of AF. These improvements should contribute to a solution of the AF problem in broiler chickens, when used with mycotoxin management practices.

References


11. — NIZAMLIOGLU F. : Determination of aflatoxin B1, B2, G1 and G2 in feeds and feedstuffs which were brought to Konya Province laboratory, Veterinarnir, 1996, 4, 7-24-45.


