Research article

Effect of probiotic on humoral immunity of broiler chickens vaccinated against Gumboro disease under experimental aflatoxicosis

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Abstract

*The aim of this study was to investigate the effects of supplementing broiler diets with probiotic live yeast, *Saccharomyces cerevisiae*, (SC) to alleviate the deleterious effect of Aflatoxin B₁ on the immunity of broiler chickens after Gumboro disease or Infectious Bursal Disease (IBD) vaccination. A total number of 120 unsexed Hubbard broiler chicks were used in this study. The chicks were randomly divided according to diet supplementation into four groups (30 chicks for each) from 1 day old to 6 weeks of age as follows, G1: Basal diet (control), G2: basal diet + SC 2g/kg, G3: basal diet + AFB₁100µg/kg, G4: basal diet + AFB₁100 µg/kg + SC 2g/kg. All groups were vaccinated with IBD vaccine at age of 14 and 28 days old. Blood samples were collected to test the immune response by ELISA test at 20 and 35 days old. The addition of SC to the broiler diet G2 revealed significant enhancement of the immune status. While the results of G3 showed that AFB₁ at 100 ppb broiler diet caused significant ($P<0.05$) decrease in the value of antibody titers of IBD immunity at 2 examined periods of age, although G4 AFB₁ plus 2g/kg of SC showed a Tangible role in minimizing effects of AFB₁. It is concluded that AFB₁ at 100ppb does have a negative effect on IBD vaccination while using SC had the ability to elevate the immune response after IBD vaccination with and without AFB₁ contaminated diet.*

Keywords: probiotic. Humoral immunity, IBD vaccination, Gumboro disease.

Introduction

Aflatoxin is still a great issue in poultry industries, Aflatoxin B₁ (AFB₁) recognized as one of the most important fungus toxins in poultry industries that damages different body organs (1). Outbreaks of IBDV were reported in vaccinated flocks associated with ingestion of aflatoxin-contaminated food (2, 3). The immune-depressive capability of AFB₁ considers as the clearest point of negative effects that exhausted the immune system by increasing susceptibility to many different pathogens of birds (4). Aflatoxin can reduce the size of bursa of Fabricius thereby reduce the immune response efficiency (5), which is considered as one of

diagnostic methods after the immune competency of birds with aflatoxicosis. Aflatoxin can also reduce complement fixation activity, which is the most sensitive area of cell-mediated immunity (CMI), it can cause general immune suppression in chickens leading to poor vaccination response (6). The degree of damages caused by AFB₁ on the immune response system of broilers depends on the concentration and duration of exposure to the aflatoxin and the age of the birds (7). Nowadays, the use of probiotics is a remarkable method of choice to enhance the health of birds through stabilizing gut flora (8), enhancement the

productivity, blood biochemistry (9), and *Salmonella* shedding rate (9, 10). In addition, these microorganisms detoxified mycotoxins through binding to their cell wall (11). An example of these microorganisms is *Saccharomyces cerevisiae* was found to be helpful in counteracting the adverse impacts of many mycotoxins (12). In addition, act as an immunomodulatory agent by activating specific and nonspecific host immune responses (13). Moreover, it increases CD3+, CD4+, and CD8+ T-lymphocyte counts (14, 15) reported that aflatoxin has adverse effects on broiler weight gain feed conversion ratio and immune response. Many other materials such as Fructooligosacchride (FOS) and Polyfam (toxin binding compound) could minimize the adverse impacts of aflatoxin B₁ on immunity of IBD or ND vaccine and on chicken's health (15, 16). Because of the latest epidemiological data signifies the intimate relationship between outbreaks of IBD and aflatoxin contaminated broiler diet and weakness of biosecurity measurement and management mistakes, many researchers showed that chicken exposed to the risk of immunosuppressant contaminated food, such as fungal toxins (aflatoxin) and infectious bursal disease (IBDV) which might lead to increase the susceptibility to other diseases and failure of vaccine response at the protective level (17, 18, 19). However, (20) showed that aflatoxin is more potent immunosuppressant than IBD virus. The aim of this study to reveal the protective effect of probiotic (SC) against the toxicity induced by aflatoxin contaminated diet on immune status after IBD vaccination.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 406

Aflatoxin preparation:

Pure crystalline AFB₁ (Sigma lious Chemical Co. USA) was incorporated into

the diets according to (21, 22) to provide the affecting level of AFB₁ (100 ppb /kg of diet).

Vaccines:

1- Gumboro Disease virus vaccine strain Winter field 2512 G-61 and France 2- Newcastle Disease virus vaccine (Lassota and clone 30 strain), USA.

Table (1): vaccination program

Vaccine type	Route of vaccination	Age/days
ND vaccine Clone 30 strain	Eye drops	1 and 27
Lassota strain	Drinking water	12
IBD vaccine	Drinking water	14
IBD vaccine	Drinking water	28

Broiler chicks:

A total of 120 unsexed 1-day old Hubbard Classic chickens were caged, numbered and divided into four groups of 30 birds per group. (G1: basal diet (control), G2: basal diet + SC 2g/kg, G3: basal diet + AFB₁100µg/kg and G4: basal diet + AFB₁100µg / kg + SC 2g/kg). Feeding additives were continued to be submitted to the chicks over the period of study that resembles the scenario of practical feeding conditions. Each experimental group of the birds received its specific diet and water ad libitum.

Probiotic:

Live yeast (*Saccharomyces cerevisiae*) (Daehan new pharm company, Korea) mixed with the ration at a rate of 2 g/kg (0.2 %).

Detection of Aflatoxin in diet:

A direct competitive ELISA (Veratox Kit, Neogen) XL800 reader was used to detect any aflatoxin in basal diet, and to record the exact concentrations in part per billion after incorporation of pure AFB₁ to the diet.

Immune response ELISA analysis:

For detection of maternal immunity and infectious Bursal Disease antibody titer in chicken serum samples, the procedure used in this test was performed according to the

manufacturer instructions listed in the ELISA Kit (Symbiotic-USA).

Statistical analysis:

Data were analyzed by one-way ANOVA for each triplicate or quintuplicate set using

SPSS (ver 17.0; SPSS, USA). Values for all parameters expressed as the mean $\pm$ the standard deviation (SD). P values < 0.05 were considered statistically significant.

Results

The results of serum samples of one-day old chicks of maternal immunity against IBD showed a good level of protective antibodies, the mean value was 7405. The results reported that at the age of 20 days old, there was significant elevation in the level of Abs for G1 (7414), G2 (8776) and G4 (6758.6) compare with G3 (4132), although there is a

significant difference between G2 (8776) and G4 (6758.6) table (2). Furthermore, at age of 35 days old, the results show there was significant increase at the level of Abs titer against IBD vaccination of G1 (10235.6), G2 (11779.3) and G4 (9603.3) compared with G3 (3346.3), although there is significant difference between G2 and G4 Table (2).

Table (2) ELISA Antibody titer

Age / days	G1	G2	G3	G4
Maternal immunity	Mean average for all groups = 7405 $\pm$ 15.84			
20	7414 $\pm$ 213.9^a	8776 $\pm$ 122.86^a	4132 $\pm$ 833.8^b	6758.6 $\pm$ 323.6 ^c
35	10235.6 $\pm$ 513.29^{ac}	11779.3 $\pm$ 215.76^a	3346.3 $\pm$ 958.36^b	9603.3 $\pm$ 275.1 ^c

Mean values in the same row with different superscripts characters are significantly different, ($p < 0.05$). 10 birds were used for each group. G: mean groups.

Discussion

Mycotoxins are metabolites contaminate crops, and when ingested can cause different health disorders in poultry such as liver destruction and increased susceptibility to diseases (25). Previous study showed that there was a significantly increased in the antibody titer against IBVDV in-group fed diet-containing SC comparing with the control group this is may be due to immune system enhancement or modulation (26). El-Naga (27) and many other researchers (28, 29, 20) indicated that the diet containing yeast give the highest level of globulin in broilers. In chickens supplemented with AFB₁ in their feed, showed decrease immune response after vaccination, could be attributed to the negative immunological impacts such as regression of development of the thymus and the bursa of Fabricious (30, 31). Whereas thymus, spleen, and bursa of Fabricious were the organs responsible for the humoral and cell-mediated immune response in broiler chickens (32). AFB₁ also cause a reduction in synthesis of

immunoproteins (31). In the current study, we report that there was no effect of aflatoxin on the antibody titers against IBD vaccination compared to control group with a significant variation at ($P < 0.05$) at 20 and 35 days old. Furthermore, it was clear continuing decreased of humoral immune response even with second vaccination (boosted dose) in chickens supplemented with AFB₁ in their feed. This may refer to the disaster impact of chronic aflatoxicosis. In this study obviously AFB₁ exerted its maximum effects during humoral response in the 2nd vaccination because of the long duration of administration i.e. chronicity. The retardation in the antibody titer values against IBD vaccine it seems to be an obvious evidence of immune-depressing effects of aflatoxicosis, this result was similar to the previous reports of (33, 34), in terms of dosage level of aflatoxin, our results appear to be similar to results of (4, 32) and (35), they have shown that the Abs titer was affected at the dose of (100 ppb) AFB₁ of

contaminated feed. Also (36) showed that a significant reduction in the titers of antibodies to IBDV, following vaccination in broilers chicks ingesting aflatoxin in diet at level of 100-200 ppb/kg, while our result disagreed with (37) who reported that there is no difference in the level of immune proteins when aflatoxin was fed. The low level of antibody titers may be the cooperation of many factors involved to weaken immune strength; this weakness was shown in devastating effects of AFB₁ on immune response against IBDV. This reduction of antibody titers was restrained the DNA and protein synthesis by aflatoxin through impairment of amino acid transfer and mRNA transcription, resulting in a lower level of protein production lead to decreased antibody production (33, 38). (39) they were shown that aflatoxin increases the specific activity of lysosomal enzymes in muscle and liver, this encourage break down, disintegration or degradation of antibodies, in addition to lymphocytic depletion of the bursa (40) and interfering or overlap with functions of lymphocytes and spleen (25), and general lymphocytopenia (41). All of these previous factors may act together decrease the immune response and increased susceptibility to different infectious diseases. In poultry, SC has been used to have positive effects against AFB₁ fed (42, 43), we can

conclude in this study that group fed diet containing SC plus AFB₁, the antibody titer against IBDV significantly increased compared to the control group. These results agreed with previous reports that found SC and other probiotics has been shown to bind aflatoxin (i.e. detoxification of AFB₁) and prevented absorption of aflatoxin from the intestine and improved the immune response (44, 45). While (46) they showed no such efficacy of SC to improve the immune status of birds; they showed that the SC had low effect on Abs titer against ND immune response. Moreover, Yeast has a well-balanced amino acid composition and high contents of lysine, methionine, and threonine, therefore, Yeast could somewhat contribute in the increasing amounts of amino acids especially methionine in the intestine and might play a crucial role in depressing devastating effects of aflatoxin in supplemented diets. (47) Showed high methionine level in diet may involve in minimizing the impact of aflatoxicosis in comparison to diets without amino acids supplementation. Finally, we conclude from the results of this study, that the AFB₁ contaminated diet had detrimental impacts on Abs titer, while SC had the ability to diminish these impacts, even more it could elevate the immune response after IBD vaccination.

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