Effect of fructooligosaccharide on humoral immunity induced by infectious bursal disease vaccine and some hematological parameters during aflatoxicosis in broiler chickens

Sheimaa Abdel redha Makii Alaa Abdul Aziz Abed Sadiyah Yassir Ofei
Coll. Vet. Med. / Univ. of AL-Qadisiyah Coll. Biotech. / Univ. of AL-Qadisiyah
email: alla.abed@qu.edu.iq
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Abstract

The study was conducted to investigate the positive effects of prebiotic Fructo-oligosaccharide (FOS) on humoral immunity induced by infectious bursal disease (IBD) vaccine and some hematological parameters of broilers with and without feeding aflatoxin contaminated diet. A total number of 120 one day old unsexed Hubbard broiler chicks were used in this study till 42 days end of experiment, the chicks were randomly assigned according to diet supplementation into four groups (30 chicks for each) as follows: G1 Basal diet, G2 was fed basal diet plus prebiotic (FOS) 0.25g/kg, G3 have been treated with $AFB_1$100 µg/kg, and G4 have been treated with $AFB_1$100 µg/kg plus prebiotic (FOS) 0.25g/kg. Results of G2 showed a significant enhancement of the immune status, while hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb) and packed cell volume (PCV) were within normal values. G3 showed that AFB_1 at 100 ppb broiler diet cause significant ($P<0.05$) decrease in antibody titers against (IBD) vaccine and decrease in hematological parameters mentioned above, while G4 obviously showed the capability of prebiotic (FOS) to minimize the undesirable effects of aflatoxinB_1 on immunity and hematology. This study proved the importance of prebiotic (FOS) as feed additive in improvement the immune response of the broiler chickens and minimizes the adverse effects of AFB_1 on antibody titer and some hematological parameters.

Key words: Fructooligosaccharide, humoral immunity, infectious bursal disease vaccine, hematological parameters, aflatoxicosis, broiler Chickens

تأثير فركتواوليغوسكرايد على المناعة الخلطية للقاح مرض جراب فابريشيا الخمجي وبعض معايير الدم أثناء التسمم بذيفان افلا في دجاج اللحم

شيماء عبد الرضا مكي علاء عبد العزيز عبد سعدية ياسر عوفي
كلية الطب البيطري/ جامعة القادسية كلية التقانات/ جامعة القادسية

الخلاصة

أجريت الدراسة للتحقق من التأثير الايجابي للبادئ الحيوي (فركتواوليغوسكرايد) على مستوى المناعة الخلطية ضد لقاح مرض التهاب جراب فابريشيا الخمجي وبعض معايير الدم لفروج اللحم المغذى على عليقة اعتيادية او عليقة ملوثة بذيفان افلا بي 1، استخدمت في هذه الدراسة 120 فرخة غير مجنسة لدجاج اللحم نوع هبرد بعمر يوم واحد ولغاية عمر 42 يوم ، تم توزيع الافراخ عشوائيا وفقاً للنظام الغذائي المتبع إلى أربع مجموعات (30 فرخة لكل مجموعة) على النحو التالي: المجموعة الاولىG1 غذيت على عليقة اساسية بدون إضافات ، المجموعة الثانية G2 غذيت على عليقة اساسية مضاف اليها 0.25 غم من مادة فركتواوليكوسكرايد لكل كغم علف ، المجموعة الثالثة G3 غذيت على عليقة اساسية مضاف اليها100 مايكروغرام ذيفان افلا لكل كغم علف ، المجموعة الرابعة G4 غذيت على عليقة اساسية مضاف اليها كل من مادة فركتواوليكوسكرايد و ذيفان افلا بنفس المقادير السابقة. أظهرت نتائج المجموعة الثانية ان اضافة البادئ الحيوي (فركتواوليغوسكرايد) إلى العليقة أدى الى زيادة معنوية في معيار الاجسام المضادة ، في حين أن المعايير الدموية المفحوصة (عدد خلايا الدم الحمراء والبيضاء وتركيز الهيموغلوبين وحجم الخلايا المرصوصة) كانت ضمن القيم الطبيعية ، كما بينت نتائج المجموعة الثالثة أن ذيفان افلا بتركيز100 جزء في البليون قد تسبب وبصورة معنوية ($P <0.05$) في خفض مستوى الأضداد ضد لقاح مرض التهاب جراب فابريشيا الخمجي مع انخفاض في المعايير الدموية السابقة الذكر،

ومن خلال ملاحظة نتائج المجموعة الرابعة تبين قدرة الفركتواوليغوسكرايد على الحد من الآثار الغير مرغوب فيها من قبل ذيفان افلا على مستوى المناعة ومعايير الدم. بينت الدراسة أهمية البادئ الحيوي (فركتواوليغوسكرايد) كإضافة غذائية مهمة في تحسين الاستجابة المناعية لدجاج اللحم وتقليل الاثر السلبي لذيفان افلا على معايرة الاجسام المناعية والصورة الدموية.

الكلمات المفتاحية: فركتواوليغوسكرايد ، المناعة الخلطية ، مرض جراب فابريشيا الخمجي ، معايير الدم ، ذيفان افلا ، دجاج اللحم.

Introduction

Two of the most commonly studied prebiotic oligosaccharides are Fructooligo-saccharides (FOS) and Mannanoligosaccharides (MOS), prebiotic (FOS) are found naturally in some cereal crops (1), and may extracted from the blue agave plant as well as fruits and vegetables such as bananas, onions, chicory root, garlic, asparagus, and leeks (2). (FOS) are non-digestible oligosaccharides, composed of glucose-$(fructose)_n$ with β−2→1 linkage between the fructose monomer units, resist hydrolysis by intestinal enzymes, due to *β*-linkages between fructose monomers (3, 4), but are fermented extensively by microflora in the lower digestive tract. The use of prebiotic (FOS) in poultry nutrition has attracted considerable concern, basically because it might act as a modulator of intestinal bacterial community and fermentation end-products, in addition (FOS) could stimulate the function of the immune system (5), and partially or completely reversed the adverse effect of aflatoxin (AF) on performance, biochemistry hematology and immune responses of birds, thus improve host health (6, 7). Aflatoxins are contaminants produced as a result of fungal metabolism (8), which grow on many kinds of cereals and still considered a major universal problem facing the poultry industry (9). One of the most important hazardous effects of (AF) metabolites that it can reacts contrary with many cell proteins, which may leads to inhibition of protein synthesis causing immunosuppression and low weight gain of chickens (10), leading to large economic losses. The aims of the study was to investigate the effects of supplementing (FOS) with or without feeding aflatoxin contaminated diet of the broiler chickens, through the evaluation of the immune response (Abs titer) against (IBD) vaccination and some hematological parameters.

Materials and methods

ELISA test

For evaluation antibody titers (humoral immunity) against Infectious Bursal Disease (IBD), ProFLOK IBD ELISA kit (Synbiotics-USA) was adopted according to the manufacturer directions**.**

Preparation of aflatoxin contaminated diet

Standard aflatoxin B1 (AFB1) was obtained from (Sigma-Aldrich Chemical Corporation, USA) in a sealed vial of 5 milligrams as white powder; the biological source is *Aspergillus flavus*. Pure crystalline (AFB1) was incorporated into the diets, by dissolving (AFB1) in chloroform (11), at dose of 1 mg/10 mL (12), and then 1 ml of AFB1 solution completed to 250 ml of chloroform then the solution mixed with 250 grams of ground feed, then mixed gradually into the basal diet to provide the desired level of AFB1 (100 ppb of AFB1/kg of feed). The toxin level or concentration in whole diet was calculated using Neogen ELISA kit (Neogen Corporation, USA) with XL800 reader, in accordance to the instructions of the manufacturer.

Aflatoxin extraction for ELISA assay

The diets were analyzed for aflatoxin content using a direct competitive ELISA kit Veratox® assay manufactured by Neogen Corporation, USA. Briefly, aflatoxin was extracted by vortexing 5 g of sample that has been ground to a fine particle size of whole feed components with 50 mL of a 70:30 methanol/water solution, and then filtered through a Whatman Filter #1. The filtrate was diluted if necessary before being sampled and mixed with enzyme-labeled toxin (conjugate), the mixed solution is transferred to antibody-coated wells, where free toxin and conjugate compete for antibody binding sites. The unbound conjugate and other soluble phase substances are then rinsed away and a substrate was

added, color develops as a result of the presence of bound conjugate, red stopping reagent was added and the color of the resulting solution was observed, absorbance (OD650) readings of the samples are compared with (OD650) readings of the controls, all these steps were in accordance with the manufacturer's instructions**.**

Fructooligosaccride

A commercial product of 50 gm. plastic container of Fructooligosaccride was obtained from (Sigma-Aldrich Chemical Corporation, USA), extracted from chicory (*Cichorium intybus*), the dosage used in this study was 0.25g/kg feed, according to the instructions of the manufacturer.

Infectious Bursal Disease (IBD) vaccine

For active immunization of broiler chickens against (IBD), freeze–dried inter-mediate plus type vaccine (Winterfield 2512, G61, 10^2EID50 / dose) (CEVAC® IBD L), France, was used in this study.

Broiler Chicks

A total of 120 unsexed 1-day old Hubbard Classic chicks were housed in a clean, disinfected and environmentally controlled house, wood shavings litter (5-8cm thickness) was used and kept almost dry throughout the experimental period, the chicks were kept under radiant gas brooders, the relative humidity was maintained between 60 and 70% and checked by hygrometer, with continuous lighting. The experiment lasted for 42 days of age. Each experimental group of the birds received its specific diet and water *ad libitum*. Chicks were divided into four treatment groups of 30 birds per group as follows: Group 1 was fed basal diet, group 2 was fed basal diet plus prebiotic (FOS) 0.25g/kg, group 3 was fed diet with $AFB_1$100µg/kg, and group 4 was fed diet treated with AFB1100µg/kg plus prebiotic (FOS) 0.25g/kg. Chicks were vaccinated against Newcastle Disease by eye drops at 1 and 27 days old with clone vaccine and by drinking water with LaSota vaccine at 12 days old, while Infectious Bursal Disease vaccine, Winter field strain were given in drinking water at 14 and 28 days old.

Blood samples

At 21, 35 and 42 days of age five birds from each group were taken randomly and blood was obtained via wing vein puncture and 2.5-3 ml of blood were collected, then the collected blood was divided into two parts, first part of collected blood was immediately transferred to tube containing EDTA with vigorous shacking (13), this part of un coagulated blood sample, has been submitted for hematological evaluation (RBC, WBC, Hb and PCV), using automatic fully digital hematology analyzer, BC 3000 Plus, Shenzhen Mindray Bio-Medical Electronics Co., Ltd. China. Second part of collected blood was transferred into the plain centrifuge tube, clotted blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum and kept frozen at (-20°C) till analysis (14).

Statistical analysis

All data were expressed as means ± standard error of the mean, analysis was done by using one way analysis of variance (ANOVA), ($P<0.05$) was considered significant (15), the data were analyzed by SPSS computing program.

Results

Detection of aflatoxin in diet

According to the results of ELISA assay, the level of aflatoxin in basal diet was 4µg/kg feed, while the level of (AFB_1) was 100ppb in contaminated feed**.**

Immune response

Table (1) showed the effects of dietary prebiotic (FOS) and (AFB_1) on humoral immune response after IBD vaccination of broiler chickens, the results demonstrated that, at 21 days old of age, antibody titers against IBD vaccine of (G2) and (G4) showed no significant difference at ($P\geq0.05$) compared to control and with each, although the result of the second group was higher numerically, followed by forth group, while (G3) showed a significant ($P<0.05$) decrease in comparison to all other groups. At 35 day old of age, antibody titers against IBD vaccine of (G2) showed significant increased at ($P<0.05$) in comparison to all other groups, but (G3) showed a significant ($P<0.05$) decrease in comparison to all other groups, while (G4) showed no significant ($P\geq0.05$),

but numerically lower than control group (G1), but increased significantly at (P<0.05) in comparison to (G3). While the comparison between the different two of ages revealed that the Ab titer of (G1), (G2) and (G4) increased more at 35 days of age than at 21 days of age, on the contrary, the Ab titer became lower at age 35 days than at age 21 days in (G3).

Table (1): ELISA antibody titer post IBD vaccination (Mean ± SE)

Groups				
Days	G1	G2	G3	G4
21	7414 ±21.39a	9843.33 ±995.78a	4132 ±833.8b	8526.33 ±2157.35a
35	10235.66 ±513.29a	13151 ±439.04b	3346.33 ±958.36c	9265.33 ±751.52a

values in the same raw with different superscripts are significantly different. (P<0.05) (n=5).

Hematological Parameters

Table (2) exhibited the effects of dietary prebiotic (FOS) and (AFB_1) on hematological parameters, the results demonstrated that no significance difference were found in the total RBC and WBC counts, Hb and PCV between control (G1), (G2) and (G4), while (G3) showed significant (P<0.05) decrease in comparison to all other groups.

Table (2): Effect of Fructooligosaccharide on some hematological parameters

Groups / 42 days				
Hemato-logical parameters	G1	G2	G3	G4
WBC x 10^9/L	18.255 ± 5.97a	16.945 ± 1.99a	15.988 ± 4.12 b	17.071 ± 2.98a
RBC x 10^{12}/L	2.69 ± 0.08a	2.63 ± 0.08a	2.26 ± 0.09b	2.59 ± 0.05a
Hb g/dl	12.05 ± 0.2a	12.11 ± 0.26a	10.56 ± 0.42b	11.83 ± 0.28a
PCV%	38.4 ± 0.75a	37.8 ± 0.79a	32.36 ± 1.27b	37.48 ± 0.89a

Values in the same raw with different superscript were significantly different (P<0.05) (n=5).

Discussion

According to the ELISA assay, the level of (AFB_1) in basal diet was (4µg/kg feed) this amount of (AFB_1) obviously under the international regulatory limits for poultry feed (> 20µg/kg) (16), and according to (17) who conclude that, it cannot be said whether 50 ppb AF level causes aflatoxicosis in broilers as no significant difference was observed compared to the control group, for that we assume this amount was negligible and had no adverse effects on health of broiler chickens of control group (G1) fed basal diet, thus no effect on immunity or hematology results that will be used for comparison with the results of the treated groups of study, while the level of (AFB_1) contaminated diet was (100ppb) which indicated that the preparing method and feed mixing was correct and precisely done. In immune response; The results showed that the antibody titer against (IBD) vaccine significantly increased in group which have been fed diet containing prebiotic (FOS) (G2) as compared with the control group (G1) and other groups (G2 and G4), these results were in agreement with (18, 19) who reported that the addition of prebiotics to the diet of broilers chicks stimulating the humoral immune response against (IBD) vaccination. The results showed a weakness of post vaccine immune response (low Abs titer) in (AFB_1) supplemented diet group (G3), this result was in agreement with (20) who showed that a significant reduction in the titers of antibodies following vaccination against (IBD) in broilers ingested aflatoxin contaminated diet at level of 100-200 ppb/kg, this significant decrease (P<0.05) in the antibody titer values in our study is clear indication of immunodepressing effects of (AFB_1) on humoral antibody response, this adverse impact of (AFB_1) could be attributed to the regressive development of the thymus and the bursa of Fabricius (21, 22), and due to inhibition of DNA and protein synthesis by aflatoxin through deficiency of amino acid transport and m-RNA transcription, resulting in lowered or reduced level of antibody production or titer (23). With respect to the results of (G4), it revealed high antibody titers against (IBD) vaccination as compared to (G3), this result was in agreement with (24, 25) who suggested that prebiotic decreases the immuno-suppressive

effect of mycotoxins and stimulates the immune response and subsequently result in higher levels of the antibody in response to the vaccine. Our results showed that the humoral immunity was better in (G1), (G2) and (G4) than (G3) at two periods of testing after both first and second vaccination, although the Abs titer is more better at 35 days than 21 days of age within (G2) and (G4) this result might be due to the effect of prebiotic (FOS), and because the birds became more immunocompetent with aging, we noticed same result was recorded of (G1) it can be explained according to the previous interpretation that the chickens became more immunologically competent with aging, and because the adverse effect of aflatoxin on immune organs and immune cells (lymphocytes), thus immune response, the opposite occur in the Ab titer in (G3) at age 35 days that become more lower than at age 21 days. The results of hematological measurements of (G2) showed no significant difference with (G1) and (G4), these results were in agreement with (26, 27) who stated that the dietary prebiotic did not have significant effects on heterophil, monocyte and lymphocyte percentages, the heterophil: lymphocyte (H/L) ratio or RBC proliferation, but these results were in disagreement with (28, 29) who showed that the prebiotic supplemented group showed the total leukcocytic count, RBC and Hb were significantly increased in the broilers fed on prebiotics supplemented diet. The results of (G3) showed significant decreased in WBC and RBC counts, Hb and PCV as compared with other groups, these results were in agreement with that of (30, 31, 32, 33) who have been reported that AF in broiler diet caused decrease in PCV and Hb levels and RBC count which related to adverse effects of aflatoxin on liver, and/or due to decrease the intestinal ability for iron absorption, and thus decrease hematological values, although (AFB_1) causes hepatic toxicity, but also has toxic impact on the kidney function thus reduce the secretion of the hemopoietin hormone, this hormone stimulate the synthesis of WBC and RBC in bone marrow, so reduce the secretion of this hormone may affects or decrease the synthesis of WBC and RBC (31). Aflatoxin B_1 group treated with prebiotic (FOS) (G4) showed significant difference compared to (AFB_1) treated group (G3), these results may indicate that, prebiotic (FOS) could reduce the adverse effect of (AFB_1) on WBC, RBC, Hb and PCV, these results were in agreement with (34) who have been observed that the addition of prebiotic to aflatoxin containing diet significantly recovered the adverse impacts of aflatoxin on hematological values of broilers blood. In conclusion; results are declare that the prebiotic (FOS) can enhance immune response and protect the blood components within normal values from the deleterious effects of (AFB_1). The toxic effects of (AFB_1) can be diminished by prebiotic (FOS) as feed additive with significant degrees of protection.

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