Effect of industrial product IMBO® on immunosuppressed broilers vaccinated with Newcastle disease vaccine

O. G. Mohammadamin* and T. S. Qubih

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

The effect of IMBO was investigated on humoral immune response to Newcastle disease vaccines in broiler chickens. Haemagglutination inhibition test and enzyme-linked immunosorbent assay were used to assess the immune response. Results showed that although IMBO significantly enhanced humoral immune response to live Newcastle disease vaccine, it did not decrease post virulent NDV challenge mortality.

تأثير المنتج الصناعي ايمبو® على فروج اللحم المثبط مناعيا والملقح بلقاح نيوكاسل

اميد جرجيس محمدامين و طارق سالم قبع

فرع علم الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، موصل، العراق

الخلاصة

اجريت هذه الدراسة للبحث عن تأثير المعزز الحيوي المنتج ايمبو على الاستجابة المناعيةالخلطية للقاحات نيوكاسل في فروج اللحم.استخدم اختبار تثبيط التلازن و الاليزا لتقييم الاستجابة لمناعية. اظهرت الدراسة ان منتج ايمبو قد حسن الاستجابة المناعية الخلطية للقاح مرض النيوكاسل الحى بشكل ملحوظ لكن لم يقلل الهلاكات بعد التحدى بفايروس نيوكاسل الضارى.

Introduction

Severe outbreaks of Newcastle disease often occur in areas of° intensive poultry production, which is reasoned mainly to break down in immunity. Although poor vaccine quality is one of several possible factors that could lead to vaccination failures (1).The failure of protection usually results from: (i) mycotoxin and/or drug induced immunomodulation (ii) cold or heat stress (iii) infectious agents (iv) malfunctioning of the host defense mechanism and (v) presence of high titers of maternal antibodies (1-7). Immunostimulation of a bird may lead to increased antibody production, increased cellular immune responses,

and increased macrophage phagocytic ability which positively correlates with enhanced resistance to various viral and bacterial infections (8,9). Probiotics are defined as direct feed microbials or microbial cell preparations with a beneficial effect on the health and well-being of the host (10). Probiotic represent one of the most recent examples of natural substances that influence adaptive immune responses by activating the innate immune system (11), and enhancing the systemic antibody.response to some antigens in chickens (12). Recently, the beneficail effect of Biomin® C-X (*Enterotococcus faecium* + prebiotic+cell wall extract) on humoral immunity to Newcastle disease vaccine of commercial broilers was studied (13). This experiment was conductd to investigate the effect of Biomin®IMBO (Biomin G.T.I. GmbH., Ember AG-Austria; containing *Enterotococcus faecium* 5×10^{11} cfu /kg, prebiotic, cell wall

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and algae extracts) as a potential immunostimulator to enhance humoral immune response to live and killed Newcastle disease vaccines in broiler chickens.

Materials and methods

A total of 210 day-old Hubbard-Flex broiler chicks were procured from a local supplier. They were reared in cages in a separate rooms of the animal house, College of Veterinary Medicine, University of Mosul and fed *ad libitum* with a Hubbard-Flex recommended diet. Ambient temperature, lighting, ventilation and other environmental conditions fully met the requirements for management of Hubbard-Flex birds.

Biomin®IMBO

(Biomin G.T.I. GmbH., Ember AG-Austria, it contains *Enterotococcus faecium* 5×10^{11} cfu / kg, prebiotic, cell wall and algae extracts. IMBO was added to the feed free from antibiotics and administered throughout the study as recommended by the manufacturer 1.5g/kg.

Drugs

Cyclophosphamide (CPA) (Cycloxan® manufaured in India, Biochem Pharmaceutical Industries LTD) was procured from a local pharmacy. Day-old chickens of groups G2 and G5 were given 3 mg per chicken per day for 4 consecutive days intramuscularly into leg muscle (14).

Challenge virus

One day before challenge, birds in G1 splited randomely to two halves; negative control group (G1); left wthout challeneg and positive control group (G8) which submitted to challenge. At 39 days of age chickens were intramuscularly inoculated with virulent field NDV strain (obtained from the Microbiology Department, College of Veterinary Medicine, Mosul University). The virus titer was determined to be $1 \times 10^{6.5}$ EID_{50} / 0.1ml.

Sampling

On day 7(before vaccination 0, blood samples were taken from each group to assess the maternal immunity. Blood samples were taken at weekly intervals after vaccination and challenge.

Serological Test

Antibodies to NDV were quantitified by hemagglunation inhibition test (HI) using the diluted serum-constant virus procedure (15) and by indirect Enzyme-linked Immunosorbent Assay (ELISA).

Experimental design

Chickens were randomly divided to 7 groups with 30 birds each. These groups consisted of: 1) Non IMBO + Non CPA + Non challenge referred to as negative control (G1), 2) IMBO + CPA&L-K (G2), 3) IMBO + Non CPA&L (G3), 4) IMBO + Non CPA&L-K (G4), 5) Non IMBO + CPA&L-K (G5), 6) Non IMBO + Non CPA&L (G6), 7) Non IMBO + Non CPA&L-K (G7). Birds of all groups except negative and positive controls were vaccinated with live NDV (Cevac®Vitapest L; CEVA) at seven days old individually by oral route using 1 ml syringe. In addition, each bird in groups G2,G4, G5, and G7 was intramuscularly injected with 0.1 ml of killed NDV vaccine (Cevac®Broiler NDK) at seven days of age. Revaccination with live ND vaccine LaSota strain (Cevac® NEW L;CEVA) was done at 21 days of age by spraying.

Results

HI titer serum antibody response

According to figure (1), chicks contained maternal antibody level before vaccination and gradually declined to low levels with time. At 28 days of age, production of antibody detected in all groups except G1 group. The G2 (vaccine + IMBO+CPA) and G5 (vaccine + CPA) groups produced significantly (P<0.05) lower levels of antibody in comparison to other treatment groups indicating that immunosuppression occurred. Furthermore, no significant differences were found when the two groups were compared at different time points post booster vaccination. The data also revealed that GMT of G3 (live vaccine + IMBO) group was significantly (P<0.05) higher than G6 (live vaccine alone) group while GMT of G4 (live& killed vaccine + IMBO) group was statistically (P>0.05) not different compared with G7 (live& killed vaccines). Furthermore, when GMTs of G3 and G4 groups were compared no significant (P>0.05) difference were found.

ELISA titer immune response

The results of ELISA test are presented in figure (2). On day 28, only birds in G3 and G7 groups showed seroconversions, however their mean titers were not significantly (P>0.05) different. The data also demonstrated that the antibody titer of G3 was significantly higher (P<0.05) when compared with titer of G6, meanwhile the titer of G4 was not significantly (P>0.05) different when compared with G7. The titers of G2 and G5 groups remained low and did not differ signficantly.

Mortality

The post-challenge test results are shown in Table (1). The table shows that the protection rate in G2, G3,G4, G5, G6, G7 and G8 were 28.6, 85.71%, 96.29%, 31.8%, 83.87, 96.66%, and 0% respectively. No significant differences in mortality were found between probiotic fed and their corresponding control groups.
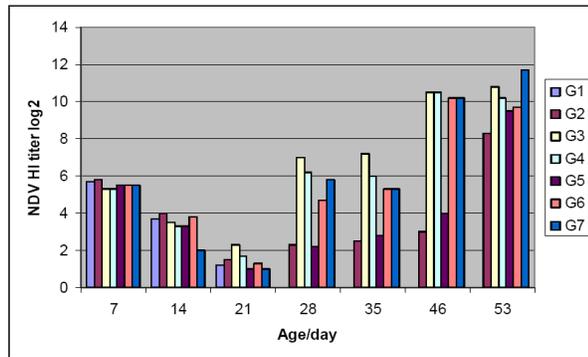
Fig. 1: Geometric mean HI antibody titer (log2) in chickens with or without IMBO supplementation.
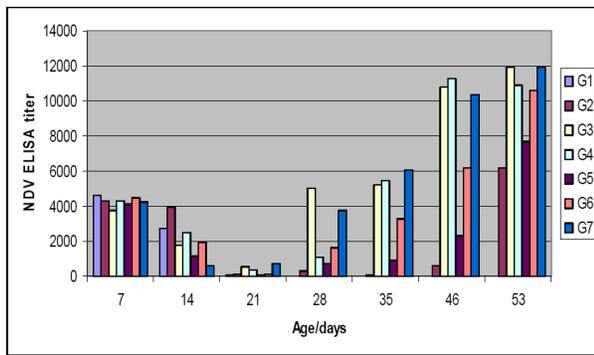

Fig. 2: Mean ELISA antibody titer in chickens with or without IMBO supplementation.

Table 1: Response to challenge with virulent Newcastle disease virus in chickens

Groups	No dead/No challenged	No survived/No challenged
Negative control group (G1)	0/14	14/14 [a] (100) [B]
IMBO,CPA &L-K vaccine (G2)	20/28	8/28 [c] (28.57)
IMBO&live vaccine (G3)	4/28	24/28 [a] (85.71)
IMBO&L-K vaccine (G4)	1/27	26/27 [a] (96.29)
CPA &L-K vaccine (G5)	15/22	7/22 [c] (31.81)
Live vaccine (G6)	5/31	26/31 [a] (83.87)
L-K vaccine (G7)	1/30	29/30 [a] (96.66)
Positive control group G8)	13/13	0 [b]

Discussion

In this experiment, significantly higher HI and ELISA titers were seen in birds received live NDV +IMBO(G3). This is in agreement with finding of (13), however, IMBO had no effect in birds immunized with live and killed vaccine G4. The data on the effect of probiotics on immunity are extremely controversial due to the variety of variables reported (16). More over(17) reported treating with just one bacterial type may not be as effective and increasing the types of bacteria in the mix could enhance the efficacy of probiotic functions. The post-challenge mortality rates observed in immunosuppressed (G2 and G5 groups) were higher compared with immune-competent birds (G3, G4, G6 and G7 groups). The protection rate did not differ significantly in G3 compared with G6 despite enhancement of humoral immune response which contradicts preivious report (18). The different results might be due to twofold increase in titer observed in latter study and in addition, they challenged birds orally with virulent NDV compared with IM challenge used in our study, in addition Leghorn male chickens were used compared to broilers in the present experiment. Under the conditions of this study, IMBO significantly enhaced humoral immune response to only live vaccine,and this is in agreement with (19), but did not restore immunity in immunosuppressed chickens and did not decrease post challenge mortality in immunosuppressed and immunocompetent broiler chickens.

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