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

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Abstract

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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 beneficial effect of Biomin® C-X (Enterococcus faecium + prebiotic+cell wall extract) on humoral immunity to Newcastle disease vaccine of commercial broilers was studied (13). This experiment was conducted to investigate the effect of Biomin®IMBO (Biomin G.T.I. GmbH., Ember AG-Austria; containing Enterococcus 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. Gmb., Ember AG-Austria, it contains Enterococcus 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® manufactured 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 split randomly to two halves; negative control group (G1); left without challenge 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 1×10^{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 hemagglutination 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 significantly.

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<sup>a</sup>         |
| IMBO, CPA & L-K vaccine (G2)| 20/28                 | 8/28<sup>c</sup>          |
| IMBO & live vaccine (G3)    | 4/28                  | 24/28<sup>a</sup>         |
| IMBO & L-K vaccine (G4)     | 1/27                  | 26/27<sup>a</sup>         |
| CPA & L-K vaccine (G5)      | 15/22                 | 7/22<sup>c</sup>          |
| Live vaccine (G6)           | 5/31                  | 26/31<sup>a</sup>         |
| L-K vaccine (G7)            | 1/30                  | 29/30<sup>a</sup>         |
| Positive control group G8   | 13/13                 | 0<sup>b</sup>             |

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 previous 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 enhanced 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.

References

1. Chaudhry K M, Chaudhry RA. Causes of vaccine failure. Vet Int. 1996;12:13-15
2. Li Y C, Ledoux D R, Bermudez A J, Fritsche K L, Rottinghaus G E. Effects of fumonisin B1 on selected immune responses in broiler chicks. Poult Sci. 1999;78:1275-1282.
3. Sharma J M, Kim I J, Rautenschlein S, Yeh H Y. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev Comp Immunol. 2000;24:223-235.
4. El-Lethey H, Huber-Eicher B, Jungi T W. Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. Vet Immunol Immunopathol. 2003;95:91-101
5. Shivachandra SB, Sah RL, Singh SD, Kataria J M, Manimaran K. Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl adenovirus serotype-4 (FAV-4) associated with hydropericardium syndrome. Vet Res Com. 2003;27:39-51
6. Kim Y, Brown TP, Pantin-Jackwood MJ. The effects of cyclophosphamide treatment on the pathogenesis of subgroup J avian leukosis virus (ALV-J) infection in broiler chickens with Marek’s disease virus exposure. J Vet Sci. 2004;5:49-58.
7. Muruganandan S, Lai J, Gupta PK. Immunotherapeutic effects of mangiferin mediated by the inhibition of oxidative stress to activated lymphocytes, neutrophils and macrophages. Toxicology, 2005; 215: 57-68.
8. Dugas B, Mercenier A, Lenoir-Wijnkoop I, Arnaud C, Dugas N and Postaire E. Immunity and probiotics. Immunol Today. 1999;20:387-390.
9. Maassen C B J D Laman W J Boersma and E Claassen. Modulation of cytokine expression by lactobacilli and its possible therapeutic use. In R. Fuller and G. Perdigon (ed.), Probiotics2001; 3: immunomodulation

10. Nemcová R. Criteria for selection of lactobacilli for probiotic use. Vet. Med. 1997; 42: 19-27.

11. Maldonado Galdeano C and Perdigo G. The probiotic bacterium Lactobacillus casei induces activation of the gut mucosal immune system through innate immunity. Clin. vaccine Immunol. 2006; 13: 219-226.

12. Haghighi H R Gong J Gyles C L Hayes M A Sanei B Parviz P Gisavi H Chambers J R and Sharif S H. Modulation of antibody-mediated immune response by probiotics in chickens. Clin. Diagn. Lab. Immunol. 12:1387-1392, by the gut microflora and probiotics. Kluwer Academic, Dordrecht, the Netherlands. 2005; PP: 176-192.

13. Al-Dalo N R A A. Effects of probiotics on some aspects of pathology and productivity of chicken broilers. A Ph.D. Thesis. College of Veterinary Medicine, University of Mosul. Mosul, Iraq. 2007

14. Sharma J M and Lee L F. Suppressive effect of cyclophosphamide on the T-cell system in chickens. Infect. Immun. 1977; 17:227-230.

15. Young M Alders R Grimes S Spradrow P Dias P da Silva A and LoboQ. Controlling Newcastle disease in Village Chickens: A laboratory manual. PP:72-85.2002

16. De Roos N M and Katan M B. Effects of probiotic bacteria on diarrhea lipid metabolism and carcinogenesis: A review of papers published between 1988 and 1998. Am. J. Clin. Nutr. 2000 71:405-411.

17. Sharif S. Probiotics Help Produce Safer, Healthier Chickens. University of Guelph Ontario Veterinary College. College News Published January 19. 2007; 338 Views

18. Zhang X Zhang X and Yang Q. Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle-disease. Vaccine. 2007;25:3254-3262.

19. Emad J K and Amjad H E. Study of some Impact of Enterococcus faecium As Probiotic on chick 2-NewCastle Disease Antibody and White blood cells. Iraqi J Vet Med 2007;31(1):78-86.