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A Critical Study of Dietary Administered Purified B-Glucan of Edible Mushroom (Pleurotus Florida) Provides Immunostimulation and Protection in Broiler Experimentally Challenged with Virulent Newcastle Disease Virus

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ABSTRACT

Immunostimulants enhance the body's resistance against various infections through increasing the oxidative activity of neutrophils, engulfment capacity of phagocytic cells and stimulating cytotoxic cells as necessary defence mechanisms. Many disorders could be treated using immunostimulants such as autoimmune diseases, viral infections and cancer (Shahbazi and Bolhassani, 2016). Several types of stimulants such as bacterial products, complex carbohydrates (e.g. glucans, schizophyllan, scleroglucan, lentinan, statolon, bestatin, acemannan), vaccines, immunoenhancing drugs (e.g. levamisole, isoprinosine, fluoro-quindone, avridine, polyribonucleotides), nutritional factors (e.g. vitamins, carotenoids, lipids, trace elements like selenium), animal extracts (e.g. chitosan from shrimp), cytokines (e.g. macrophage activating factor, interferon, interleukin-2, tumour necrosis factor) and plant extracts (e.g. Lectins, mitogens such as phytohaemagglutinin, concanavalin A) which have different mechanisms and functions (Galeotti, 1998). Immunostimulants activate different elements of the immune system in human and animals. They develop the non-specific immunotherapy and immunoprevention by stimulating the basic factors of the immune system including phagocytosis, properdin and complement systems, protective Secretory IgA antibodies, α- and γ-interferon release, T- and B-lymphocytes and synthesis of specific antibodies and cytokines. This also helps in the synthesis of pulmonary surfactant (Labh and Shakya, 2014).

Keywords : Neutrophils, Phytohaemagglutinin, Cytokines, Glucans, Schizophyllan, Scleroglucan, Lentinan, Statolon, Bestatin, Acemannan

INTRODUCTION

The infections of various aetiologies are considered the greatest challenge in the profit-making process of the modern poultry industry. At this backdrop, immunostimulants can play quite significant role in enhancing the body defence mechanisms of poultry birds leading to improved growth and production performance with reduced mortality. Both specific and non-specific immune responses of poultry have been shown to be

stimulated by β-glucans obtained from yeast cell wall and mushrooms (Vetvicka et al., 2002). Earlier, oral applications of various stimulants, viz. glucans, lactoferrin, levamisole, and chitosanhave been reported (Kamilya et al., 2008). Immunostimulatory role of edible mushroom (Pleurotus florida) was established in fish. It was observed that proteoglycan/glucan extracted from Pleurotus florida when used in feed, enhanced the activities of immune-effector cells in fish and the protection against pathogen (Aeromonas hydrophila) upon experimental challenge (Kamilya et al., 2008). In vitro immunostimulatory effect as well as adjuvanticity of mushroom glucan was reported in fish (Kamilya et al., 2008).

With this background, the objective of the present work was to study the immunomodulatory and protective role of dietary administered purified glucan obtained from edible mushroom (Pleurotus florida) in commercial broiler chicken against pathogenic infections, taking Newcastle disease virus (NDV) as a representative pathogen.

METHODS

Mushroom glucan

Glucan was isolated from the edible oyster mushroom (Pleurotus florida) by ethanol precipitation and ion exchange chromatography. The neutral concentrated polysaccharides were subjected to Sephadex G-100 gel permeation chromatography. The gel permeation produced three fractions. The second yielded the most polysaccharide, with a protein ratio of 24.6. It was frozen in a conical flask at – 20 °C, lyophilized and stored in desiccators at – 20 °C (Wasser, 2002; Kamilya et al., 2006).

Experimental bird

Broiler birds were used as experimental animals in the present study. The experimental design and procedure adopted was as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India, and was approved by the Animal Ethics Committee of the West Bengal University of Animal and Fishery Sciences (WBUAFS). Fifty (50)-day-old chicks were procured from a commercial hatchery of Kolkata (India). All chicks were vaccinated at the 5th day with Newcastle disease vaccine (F strain) (Institute of Animal Health and Veterinary Biologicals, West Bengal, India). On 7th day, they were divided into three groups (group A, B, C) on the basis of diet. A and B groups contained 20 chicks with replica of 10 chicks in each pen. Control group (C) had 10 birds with replica of 5 chicks in each pen.

Isolation of peripheral blood mononuclear cells (PBMC)

Isolation of Peripheral blood mononuclear cells was carried out from treated and control birds on 4th day post challenge (Chung and Secombes, 1988). The diluted blood samples were layered onto HiSep® (Himedia) at the ratio of 1:3 (1 part of HiSep and 3 parts of cell suspension) and centrifuged for 30 min at 1200 rpm. Then, the white blood cell interface layer was collected, transferred into clean sterile test tube and washed thrice. The cell viability of the isolated cells was enumerated by the Trypan blue exclusion method. In most of the cases, > 90% of the cells were viable.

Stock solutions for lipopolysaccharide (LPS) and concanavalin-A (ConA) Stock solution of LPS (Sigma, USA) was prepared at a concentration of 20 µg/ml of the growth medium Roswell Park Memorial Institute (RPMI)-1640 (Sigma, USA), filtered through a sterile membrane filter (0.2 µ) and stored at – 20 °C until use. Stock solution of concanavalin A (Sigma, USA) was prepared at the concentration of 20 µg/ml of the growth medium RPMI-1640, filtered through a sterile membrane filter (0.2 µ) and stored at – 20 °C until use.

Estimation of phagocytic activity

The adherent cells (macrophages) were separated from iIEL suspension of treated and control birds (on 4th day post challenge) on glass cover slips, methanol fixed and stained by Giemsa. The phagocytic activity (PA) of the cells was determined (Yoshida et al., 1993). cDNA preparation The PBMC and iIEL cells (2 × 106 cells/ml) were suspended in RPMI-1640 and 100 µl of cell suspension was dispended into wells of 96-well tissue culture plates. The final volume of the wells made up to 200 µl with ConA at a concentration of 10 µg/ml in positive control wells (in triplicate) and with RPMI-1640 growth medium in negative control wells (in triplicate).

Finally, it was incubated at 37 °C for 3 h with 5% CO2 tension. The cells were thoroughly mixed with the culture medium and the cell suspension was transferred to a 15- ml polypropylene tube. The tube was centrifuged at 400×g for 5 min to pellet the cells. The cells pellet was washed twice with 10 ml of PBS and was suspended in 1 ml of culture medium. The cell density was determined and it was adjusted using ice-cold PBS so that it falls within the range of 5000 cell/µl. The cDNA was prepared following the protocol (GeNei™, India).

Statistical analysis Data were analysed with SPSS 17.0 for Windows software and one-way analysis of variance (ANOVA). Means were compared by LSD post hoc test and a probability level of 0.05 was used.

RESULTS

Effect of virulent virus challenge All birds (four) of control group died showing clinical symptoms within 4 day after challenge with a virulent field isolate of ND virus. Two birds of group A died within 7 days after challenge. Two birds were protected in this group. Three birds were protected in group B after virus challenge. One bird died showing clinical symptoms after 7 days of viral challenge. All birds of control group showed characteristic post-mortem lesions as petechial haemorrhages in proventriculus and haemorrhages in ileo-coecal junction (Fig. 1). After NDV challenge, 100% mortality was observed in control birds within 4 days, whereas in treated birds 50% and 75% protection of challenged birds was observed in group A and group B birds, respectively (Table 1)

Neutrophil function assay

The super oxide anion production by blood leucocytes of group A (0.641 ± 0.01) and group B (0.721 ± 0.01) birds were significantly higher than the control birds (0.283 ± 0.04) when assessed on the 4th day of post challenge period (Fig. 2a).

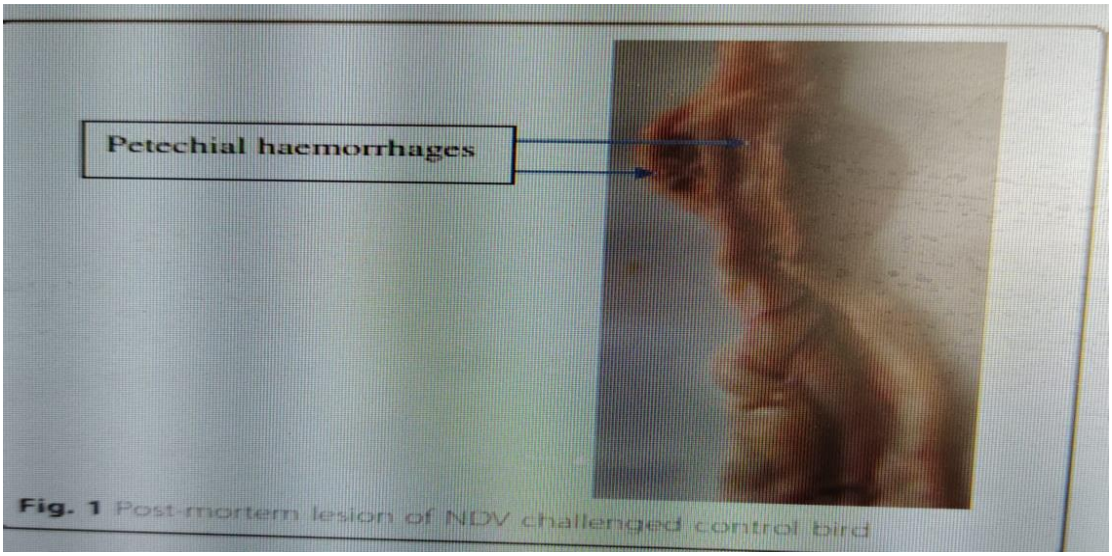


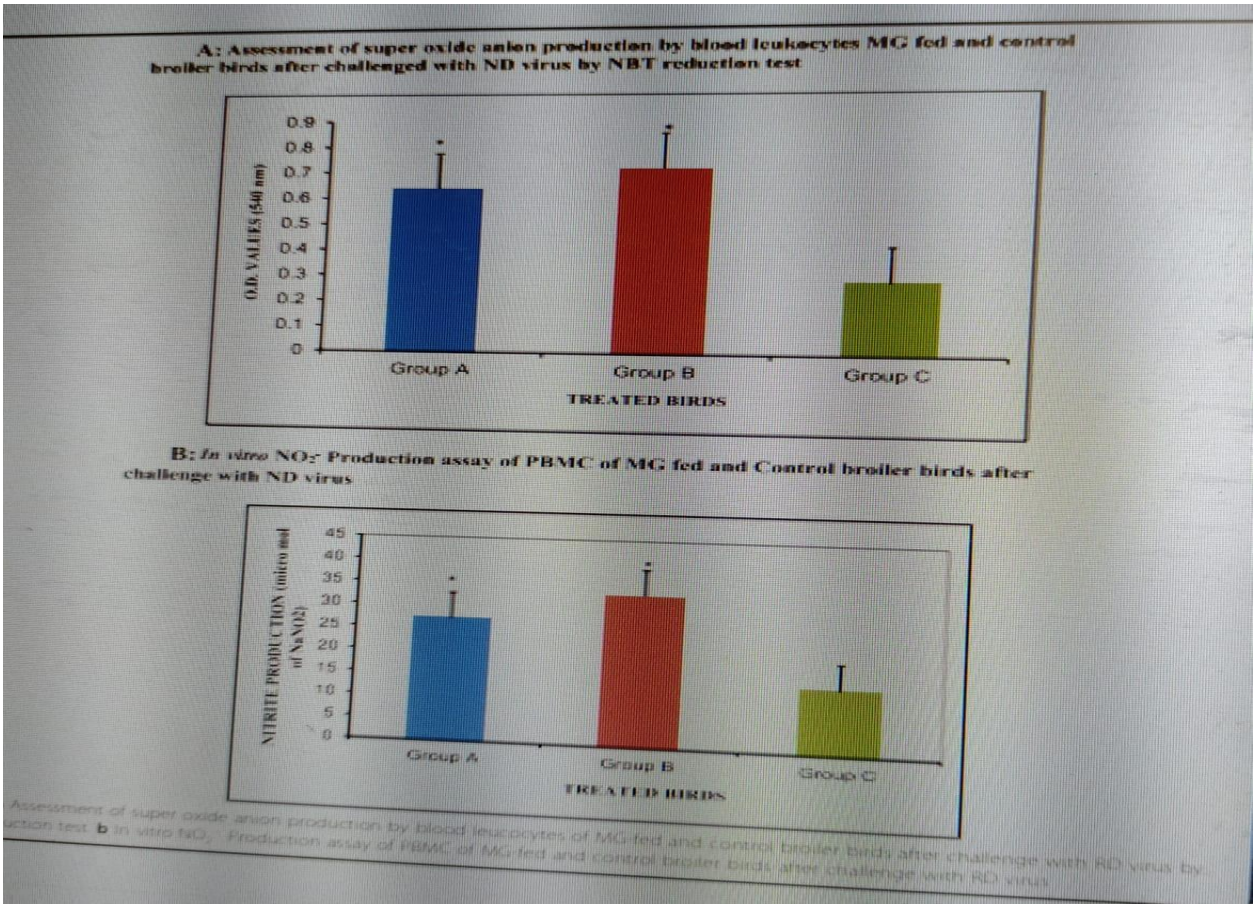
Fig. 1 Post-mortem lesion of NDV challenged control bird

Table 1 Percent protection in control and treated broiler birds after virulent ND virus challenge

Treat group	No. of birds challenged	No. of birds died	No. of birds protected	Protection (9%)
Group A (15 mg/kg feed)	4	2	2	50
Group B (30 mg/kg feed)	4	1	3	75
Group C (control diet)	4	4	0	0

In vitro nitrite production assay

The in vitro nitrite production of PBMC and iIEL of treated broiler birds was higher than control birds after challenge with virulent ND virus. Group A (27.33 ± 1.20 µl and 25.33 ± 2.02) and group B (33.66 ± 0.33 µl and 32.66 ± 0.33) birds showed higher production than control birds (14.00 ± 0.57 µl and11 ± 0.57) after challenge with virulent ND virus (Figs. 2b and 3).



MG-fed broiler birds after virus challenge are presented in Figs. 4 and 5. MG-fed groups showed significantly higher ($P < 0.05$) stimulation indices in both the cases, i.e. group A 0.371 ± 0.02 and 0.295 ± 0.02, and group B 0.428 ± 0.01 and 0.314 ± 0.01, respectively, than control birds (0.203 ± 0.01 and 0.135 ± 0.01) after the 4th day of virus challenge.

CONCLUSION

It is observed from the experiment that protective immunity against Newcastle disease was comparatively more in mushroom glucan-fed broiler chicken. When compared to the treatment regime, it was observed that in all the cases, broiler chicken fed with 30 mg mushroom glucan/kg feed showed better immunostimulatory (and protective) activities. Therefore, it is concluded that the use of 30 mg mushroom glucan/kg feed may be used for better output in terms of disease protection against Newcastle disease (ND) virus in broiler chicken.

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