Indian Journal of Animal Sciences **82** (7): 745–748, July 2012

Orally administered β- glucan of edible mushroom (*Pleuratus florida*) origin upregulates innate immune response in broiler

I PAUL[1], D P ISORE[2], S N JOARDAR[3], I SAMANTA[4], U BISWAS[5], T K MAITI[6], S GANGULY[7] and S K MUKHOPADHAYAY[8]

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 094 India

Received: 23 August 2011; Accepted: 4 February 2012

ABSTRACT

With the aim of assessing the effect of purified β-glucan from an edible mushroom (*Pleuratus florida*) as an immunomodulator on the innate immune responses in broiler chicken (40), purified mushroom glucan was administered orally to 1-week-old broiler chicks (20) @ 20 mg/kg feed for 15 days and then switch back to control diet. Similar number of birds were kept separately as control with normal feeding regime. Non-specific immunity and protective ability were assessed in treated and control birds at the end of the experimental feeding period (0 day) by performing neutrophil, macrophage and lymphocyte functional assay *in vitro* and challenged with virulent field isolate of Ranikhet disease. Superoxide anion production by neutrophil, *in vitro* non-specific lymphoproliferation and IL-2 production were increased gradually up to 10 days post treatment and the increase was highly significant ($P<0.05$) compared to control birds. *In vitro* nitrite production by macrophage was found to be high in treated birds. Also mushroom glucan as a feed supplement significantly provided protection against Ranikhet disease. The result showed the potentiality of β-glucan (mushroom origin) as an immunostimulant in poultry.

Key words: β-glucan, Chicken, Immunomodulator, Mushroom, Ranikhet disease

Immunomodulator stimulates leucocytes, particularly cells of the macrophage system and modulates and potentiates the immune system of the body (Seljelid 1990, Ganguly *et al.* 2009; 2010). It has been recommended earlier that the constant addition of immunomodulators to feed is beneficial for prevention of diseases (Onarheim 1992, Ganguly *et al.* 2009, 2010). One of such immunostimulant compound is β-glucan, polymers of glucose which consists of a linear backbone of β-1, 3 linked D-glucopyranosyl residues having varying degree of branching from the C_6 position (Bohn and Bemillier 1995). β-glucans are major structural components of yeast, mushrooms and fungal mycelia. Supplementation of β- glucan in diets increase the macrophage phagocytic activity, PHA-P- (phytohaemagglutinin-P) mediated lymphoproliferative response and also humoral response (Guo *et al*. 2003). β-glucan provides significant protection against pathogen as a feed additive by upregulating phagocytosis, bacterial killing, and oxidative burst in chicken (Lowry *et al*. 2005). In the mammalian system, action of β-glucan is mediated through toll-like receptors (TLR) and dectin-1 (Brown and Gordon 2003). In the present work evaluation was carried out for short term dietary influence of a purified β- glucan, prepared from an edible mushroom, on the innate immunity and disease resistance of broiler birds.

Present address: [1,2,3,4] [1](indira.vet@gmail.com) Department of Veterinary Microbiology,[5]Department of Veterinary Epidemiology and Preventive Medicine, [8]Department of Veterinary Pathology, Faculty of Veterinary and Animal Sciences.

[7]AICRP-PHT (ICAR) Kolkata, Department of Fish Processing Technology, Faculty of Fishery Sciences.

[6]Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721 302 India.

MATERIALS AND METHODS

Experimental bird

Forty (40 no.) day old chicks were procured from a commercial hatchery. They were kept for seven days before starting experimental feed. On 7th day they were divided into 2 diet groups (MG, mushroom glucan; treated and control). Each group contained 20 chicks.

Experimental design

On 7th day onward, the experimental birds of MG treated group was fed purified mushroom glucan (MG) @ 20mg/kg feed and control group were fed control feed (containing maize, soyabean, ground nut cake, mineral mixture and vitamins) for 15 days. After 15 days the treated groups were switched back to control diet for 20 days. Birds were allowed to take *ad libidum* feed throughout the life span. The period of observation was started from the last MG administration

(0 day) with 10 days interval, viz. 0 day, 10th day, and 20th days post treatment. Non-specific immunity was assessed in randomly selected treated and control birds on the aforesaid days.

After 10 day from last experimental feeding, 8 numbers of birds (32-day-old) were randomly selected from each group and maintained in two separate cages. Birds were challenged intranasally with virulent field isolate of RD (Ranikhet disease) virus (10^6 EID (effective infective dose) 50/ml dose). The birds were kept in observation for 10 days.

Neutrophil functional assay/ nitro-blue tetra zolium (NBT) assay

As a measure of non-specific immune response, oxidative radical production by neutrophils was determined by the NBT assay as per Siwicki *et al*. (1998).

Isolation of peripheral blood mononuclear cell (PBMC) from blood

Heparinised blood was diluted with phosphate buffer saline at 1:1 ratio. For isolation of PBMC, diluted blood was layered onto Hisep at the ratio of 1:3 (1 part of Hisep and 3 part of cell suspension) and centrifuged for 30 min at 1200 rpm (Chung and Secombes 1988). The solution was mixed well and a drop was placed on a Neubauer counting chamber for viability and enumeration of viable cells by the Trypan blue exclusion method.

In vitro *nitrite production assay*

Nitrite production by leukocytes was assessed using Griess reagent (Green *et al.* 1982). The cell number was adjusted to 2×10^7 viable cells /ml by diluting with RPMI-1640 growth medium. The optical density was measured in a plate reader at 570 nm and the molar concentration of nitrite in the supernatants was determined from standard curves obtained using known concentration of $NaNO_2$ (Joardar *et al*. 2003).

Lymphocyte transformation test (LTT)

PBMC suspension was adjusted to 2×10^6 cells /ml in RPMI-1640 growth medium. 100 µl of Con A at a concentration of 10µg/ml was added to 100 µl of cell suspension into each well of 96–well tissue culture plate and incubated at 37°C for 72 h containing 5% CO_2 tension. The colorimetric –[4,5–dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay described by Daly *et al*. (1995) was used to determine the proliferation of blood lymphocytes. The formazan development was read at 595 nm (Daly *et al.* 1995) using a plate reader. Stimulation index was calculated as per the standard method.

Cytokine bio-assay

PBMCs were separated and the cell count adjusted to 5×10^6 cells/ml in RPMI-1640 growth medium. The cells were placed in 96 wells flat bottom plates and stimulated with Con-A (20µg/ml). IL-2 (interleukin-2) activity in the culture supernatants was measured according to the method of Lafferty *et al*. (1980) with some modifications. The formazan development was read at 595 nm (Daly *et al.* 1995) using a plate reader. Stimulation index was calculated as per the standard method.

Statistical analysis

The results of each experiment are expressed as the mean±standard error and analysed by one-way analysis of variance (ANOVA) to test the significance between control and experimental groups.

RESULTS AND DISCUSSION

The production of oxygen radicals of MG fed broiler birds were measured by NBT test was significantly higher on 10^{th} day than control values ($P<0.05$) [Table 1]. In MG group the O.D. value was increased on '0' day and in 10^{th} day post treatment. Statistically significant ($P<0.05$) differences with control group were observed in the NBT activities among all the experimental days.

The *in vitro* nitrite production of broiler birds of MG fed and control groups by PBMC upon LPS (lipo-polysaccharides) stimulation after 72 h of incubation are shown in Table 2. Nitrite production was increased in 0 day MG fed group than control group.

The *in vitro* lymphocyte proliferation upon Con A stimulation of broiler birds fed with dietary MG were expressed as stimulation index. The values of stimulation index of broiler birds of MG fed group and control group are shown in the Table 3. In MG fed group showed highest S.I. value on 10^{th} day and it significantly differs from control group ($P<0.05$).

Table 1. Assessment of super oxide production by blood leukocytes of MG fed broiler birds at 0, 10^{th} and 20^{th} day post-treatment by NBT reduction test

Group	0 Day	10^{th} Day	20^{th} Day
Mushroom glucan	0.486 ±0.0326*	0.690 ±0.0199*	0.512 ±0.0017*
Control	0.363 ±0.0118	0.592 ±0.0080	0.443 ±0.0046

#Data are expressed as OD Value±SEM; *Significant differences ($P<0.05$) observed between control and treated group

Table 2. *In vitro* NO_2 production assay of mushroom glucan fed broiler birds at different days post treatment

Group	0 Day	10^{th} Day	20^{th} Day
Mushroom glucan	25±00	15.67±0.67	15.33±4.33
Control	24±2.08	14.33±0.67	12.33±0.33

Data are expressed as $NaNO_2$ (µM)±SEM.

Table 3. *In vitro* lymphoproliferation assay# of MG fed broiler birds at 0, 10th and 20th day post treatment

Group	0 Day	10th Day	20th Day
Mushroom glucangroup	0.295±0.0199	0.678±0.0130*	0.404±0.0026*
Control group	0.202±0.0257	0.127±0.0008	0.113±0.0014

Data are expressed as mean stimulation index (SI)±SEM; *significant (P<0.05) differences between rows.

Table 4. *In vitro* cytokine production by PBMC of MG fed broiler birds at different days post-treatment

Dilution	0 day (SI)	10th day (SI)	20th day (SI)
1:2	0.238±0.0015*	0.672±0.0007*	0.347±0.0009*
1:4	0.106±0.0271	0.616±0.0011*	0.105±0.0008*

Data are expressed as mean stimulation index (SI)±SEM; *Significant (P<0.05) differences between rows.

Table 5. *In vitro* cytokine production by PBMC of control broiler birds at different days post-treatment

Dilution	0 day (SI)	10th day (SI)	20th day (SI)
1:2	0.079±0.0225	0.179±0.0055	0.118±0.0003
1:4	0.049±0.0150	0.166±0.0237	0.063±0.0011

Data are expressed as mean stimulation index±SEM; *Significant (P<0.05) differences between rows.

Table 6. Percent protection in Mushroom Glucan fed broiler birds after virulent RD virus challenge

Group	No. of birds challenged	No of dead birds	No of protected birds	Percent protection (%)
Mushroom glucan	8	2	6	75
Control	8	8	0	0

The *in vitro* function of IL-2 at 1:2 and 1:4 dilutions of MG fed and control broiler birds group are represented in the Tables 4 and 5, respectively. The function of IL-2 at 1:2 dilution of MG fed group is significantly higher than control group (P<0.05) on each experimental days. In MG fed group stimulation index at peak level at 10th day of post treatment and then decreased at 20th day.

All birds (8) of control group died showing clinical symptoms within five day after challenge with a virulent field isolate of RD virus. Six birds (75%) are protected in MG fed group after viral challenge. Two birds were died showing clinical symptoms after seven days of viral challenge. All died birds of control group present characteristic pathognomonic post mortem lesions of RD such as petechial hemorrhages in proventiculus and hemorrhages in ileo-ceacal junction. In MG fed group the post mortem lesions of died birds were not so pronounced like control group. The numbers of dead, protected birds and protection % are shown in Table 6.

Previous studies showed that infections caused by *Staphylococcus aureus* and *Eimeria vermiformis* in mice can be prevented by β-glucan administration (Yun *et al.* 2003). Experimental respiratory challenge with *Escherichia coli* in broiler chicks can also be prevented by β-1,3/1,6 glucan derived from *Saccharomyces cerevisiae* (Huff *et al*. 2006). Rice *et al.* (2005) showed that dietary administration of glucan to rat enhanced survivability against *Staphylococcus aureus* infections. Orally administered yeast β-glucan to mice could reduce the mortality in anthrax infections (Vetvicka *et al*. 2002).

ACKNOWLEDGEMENTS

The authors are thankful to Hon'ble Vice-Chancellor, West Bengal University of Animal and Fishery Sciences, Department of Veterinary Epidemiology and Preventive Medicine, Faculty of Veterinary and Animal Sciences, Kolkata (WBUAF) and Department of Biotechnology, Indian Institute of Technology, Kharagpur, India for providing necessary facilities to carry out this original research work.

REFERENCES

Bohn J A and BeMiller J N. 1995. (1 3)-β-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate polymers* **28**: 3–14.

Brown G D and Gordon S. 2003. Fungal beta-glucans and mammalian immunity. *Immunity* **19**: 311–15.

Chung S and Secombes C J. 1988 Analysis of events occurring within teleost macrophages during the respiratory burst. *Comparative Biochemistry and Physiology* **89**: 539–44.

Daly J G, Olivier G and Moore A R. 1995. A calorimetric assay for the quantification of brook trout (*Salvelinus fontinalis)* lymphocyte mitogenesis. *Fish and Shellfish Immunology* **5**: 266–73.

Ganguly S, Paul I and Mukhopadhayay S K. 2009. Immunostimulants-their significance in finfish culture. *Fishing Chimes.* **29** (7): 49–50.

Ganguly S, Paul I and Mukhopadhayay S K. 2010. Immunostimulant, probiotic and prebiotic – their applications and effectiveness in aquaculture: A Review. *Israeli Journal of Aquaculture-Bamidgeh* **62** (3): 130–38.

Guo Y, Ali R A. and Qureshi M A. 2003. The influence of beta-glucan on immune responses in broiler chicks. *Immuno-pharmacology and Immunotoxicology* **25**: 461–72.

Huff G R, Huff W E, Rath N C and Tellez G. 2006. Limited Treatment with *b*-1,3/1,6–Glucan Improves Production Values of Broiler Chickens Challenged with Escherichia coli**.** *Poultry Science* **85**: 613–18.

Joardar S N, Ram G C and Goswami T K. 2003. *Veterinary Immunology and Immunopathology* **93**: 61.

Lafferty K J, Prowse S J and Al-Adra A. 1980. An improved assay for interleukin2 (lymphocyte growth factor) produced by

mitogen activated lymphocytes. *Australian Journal of Experimental Biology and Medical Science* **58**: 533–44.

Lowry V K, Farnell M B, Ferro P J, Swaggerty C L, Bahl A and Kogut M H. 2005. Purified beta-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella* Enterica serovar Enteritidis. *International Journal of Food Microbiology* **98**: 309–18.

Onarheim A M. 1992. Now a yeast extract to fortify fish. *Fish Farmer* **15**: 45.

Rice P J, Adams E L, Ozment-Skelton T, Gonzalez A J, Goldman M P and Lockhart B E. 2005. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *The Journal of Pharmacology and Experimental Therapeutics* **314**: 1079–86.

Seljelid R. 1990. Immunomodulators-medicine for the 90s. *Pathogenesis of wound and biomaterial associated infections* (Eds. Wadstrom T, Eliasson I, Holder I and Ljungh A.). Springer-verlag Berlin. 107–13.

Siwicki A K, Kiczaka W, Moranda M and Studnicka M. 1998. Immunostimulatory effects of dimerized lysozyme (KLP-602) on the non-specific defence mechanisms and protection against furunculosis in salmonids. *Veterinary Immunology and Immunopathology* **61**: 369–78.

Vetvicka V, Terayama K, Mandeville R, Brousseau P, Kournikakis B. and Ostroff G. 2002. Orally-administered yeast beta-1,3-glucan prophylactically protects against anthrax infection and cancer in mice. *The Journal of the American Nutraceutical Association.* **5** (2): 1–5.

Yun C H, Estrada A, Van Kessel A, Park B C and Laarveld B. 2003. Beta-glucan, extracted from oat, enhances disease resistance against bacterial and parasitic infections. *FEMS Immunology and Medical Microbiology* **35**: 67–75.