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Effect of dietary yeast cell wall preparation on innate immune response in broiler chickens

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Immunomodulator stimulates leucocytes-particularly cells of the monocyte/macrophage system and thereby modulates, and most often potentiates, the immune system of the body (Seljelid 1990). Glucan and mannan are the main components of yeast cell wall (YCW) and obtained from pure culture of yeast, *Saccharomyces cevisiae*. ß-D- glucan is the major component of yeast cell wall and has shown to stimulate non-specific immune responses. Yeast ß-glucan enhances the immune responses in fish (Hiss *et al.* 2003, Ganguly *et al.* 2010) and cattle (Persson Waller *et al.* 2003). However, information regarding the effect of dietary administration of yeast cell wall preparation on immune responses in birds is limited. In the present study we evaluate the non-specific immune responses, viz. production of oxygen and nitrogen species, lymphoproliferation and IL-2 (cytokine) production in broiler birds following YCW treatment.

*Experimental bird:*Day old chicks (60), procured from a local commercial hatchery, were acclimated for 7 days before starting experimental feeding. On seventh day they were divided into 3 diet groups (2 treatments and control). Each diet group further divided into 2 replicate groups of 10 chicks each.

Experimental diets: Yeast cell wall (YCW) prepared commercially from *Saccharomyces cevisiae* was added @ 0.4 g and 0.8 g/kg control feed containing maize, soybean, groundnut cake (GNC), mineral mixture and vitamin as per standard.

Experimental design: On seventh day onward, the birds of treated groups were fed diet mixed with yeast cell extract (YCW) @ 0.4 g and 0.8 g/kg feed respectively and control groups were fed control feed (without YCW) for 15 days. After 15 days, treated groups were fed control diet for 20 days. Birds were allowed to take *ad lib.* feed throughout the experiment. The period of observation started after 15 days YCW administration (0 DPT) with 10 days interval, viz. 0, 10th, and 20th DPT.

Immunity study: Non-specific cellular immunological responses of treated and control birds were observed to assess immunostimulatory role of YCW in broiler chicken. Non-specific immunity was assessed in randomly selected (3) treated and control birds from each replicate groups on the aforementioned days.

Neutrophil functional assay/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: For isolation of PBMC, heparinized blood was first diluted with PBS (0.01 M, pH 7.2) at 1:1 ratio and then 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 4000 g (Chung and Secombes 1988). Viability of the cells was determined by Trypan blue dye exclusion method.

In vitro *nitrite production assay:* Nitrite production by PBMC 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 by 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).

Lymphoproliferation assay: The colorimetric –[4,5–dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide (MTT) 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 (SI) was calculated as per the following formula:

$$SI = \frac{\text{Mean optical density of lymphocyte walls with mitogen}}{\text{Mean optical density of negative control walls}} - 1$$

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Cytokine bio-assay: IL-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 (SI) was calculated as described above.

Statistical analysis : The results of each experiment were expressed as the mean±standard error and analyzed with SPSS 10.0 Windows software. One-way analysis of variance (ANOVA) was used to test the significance between control and experimental groups with probability level of 0.05.

Neutrophil functional assay (NBT) : The production of oxygen radicals in broiler birds fed both the dose of YCW as measured by NBT test was significantly higher on 10th DPT than control birds (P<0.05) (Fig.1). However, the birds fed 0.4 g/kg diet YCW had significantly higher (P<0.05) superoxide radical production by neutrophils throughout the experimental period.

In vitro *nitrite production assay:* On 0 and 20th DPT nitrite production by monocytes did not vary (P>0.05) among treatment and control group, however on 10th DPT birds fed 0.4 g YCW had peak and significantly (P<0.05) higher nitrite production compared to control group birds (Fig.2).

Lymphoproliferation assay: Upon Con A stimulation the *in vitro* lymphocyte proliferation in broiler birds, expressed as stimulation index is shown in the Fig. 3. Significant differences (P<0.05) of lymphocyte proliferation from control group were found on 0 day and 10th day of post treatment in both the dose level.

Cytokine assay: The *in vitro* cytokine production (IL-2) is expressed as stimulation index. The *in vitro* function of IL-2 at 1:2 dilution of YCW fed and control birds are shown in the Fig. 4. The stimulation index of YCW fed birds were significantly higher than control group (P<0.05) on 0 day of post treatment.

In vertebrates, the immunomodulating abilities of ß-glucans are thought to stem from their ability to activate leukocytes, but there is some confusion about their precise biological effects (Brown and Gordon 2003). In the present study, immunostimulatory role of *Saccharomyces cevisiae*

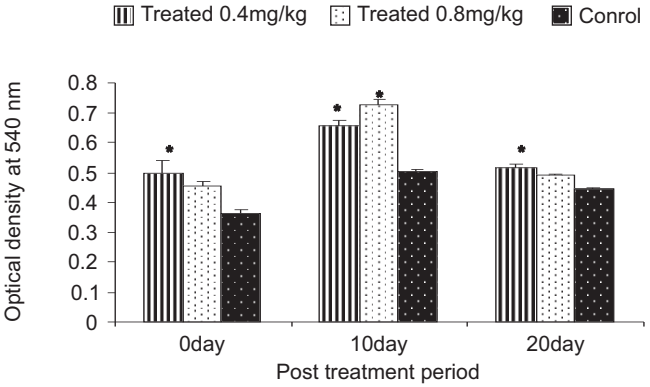

Fig.1. Assessment of superoxide anion production by blood leukocytes in YCW fed broiler birds at 0, 10th and 20th day (s) post treatment by NBT reduction test, Data presented as means±SEM (n=6), asterisks indicate statistically significant difference (P<0.05) compared to the control at a particular period.

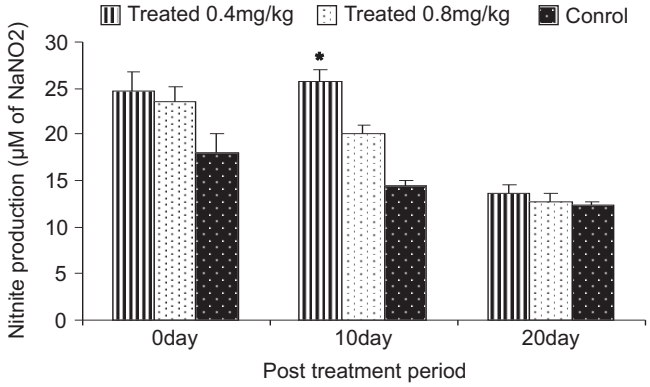

Fig. 2. *In vitro* NO_2^- production by blood monocyte in YCW fed broiler birds at different days post treatment, Data presented as means±SEM (n=6), asterisks indicate statistically significant difference (P<0.05) compared to the control at a particular period.

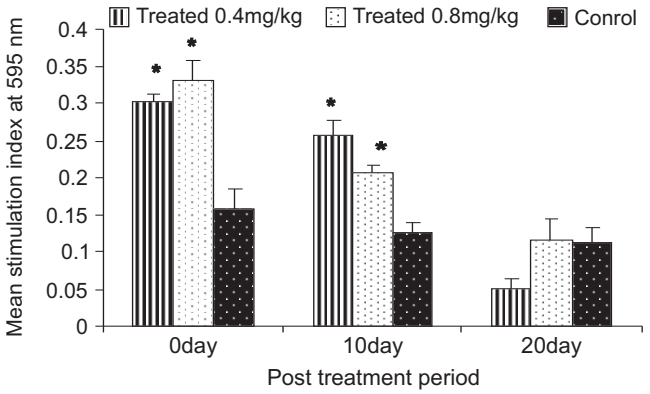

Fig.3. *In vitro* lymphoproliferation in YCW fed broiler birds at 0, 10th and 20th day (s) post treatment, Data presented as means±SEM (n=6), asterisks indicate statistically significant difference (P<0.05) compared to the control at a particular period.

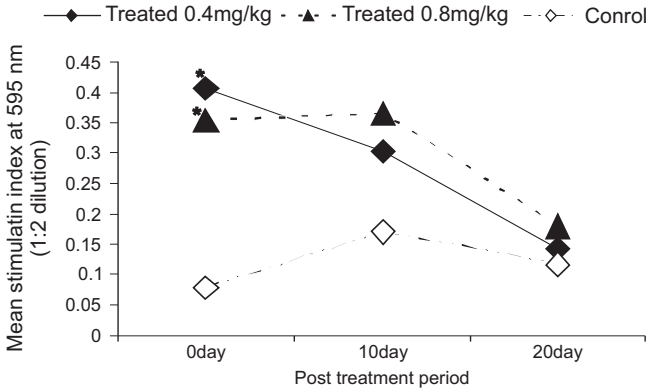

Fig.4. *In vitro* cytokine production by PBMC in YCW fed broiler birds at different days post treatment at different dilutions, Data presented as means±SEM (n=6), asterisks indicate statistically significant difference (P<0.05) compared to the control at a particular period.

cell wall was assessed in 2 different doses in terms of cellular immune effector activity. Lowry *et al.* (2005) showed that the use of yeast glucan enhanced oxidative respiratory burst in chicken. Guo *et al.* (2003) observed glucan enhanced the lymphocyte proliferation in cattle. Oral administration of yeast glucan enhanced the cytokine production in mice (Vetvicka *et al*. 2002, Tsukada *et al.* 2003). Our result corroborated the previous findings. The enhancement of oxygen radicals, nitrite, cytokine (IL-2) production and lymphoproliferation of broiler birds might be related to the improvement of cellular response following the oral administration of yeast cell wall preparation from *Saccharomyces cevisiae*.

SUMMARY

The present study was carried out to evaluate the effect of dietary supplementation of yeast cell wall preparation in 1-week-old broilers @ 0.4 g or 0.8 g/kg feed for 15 days and then control diet for 20 days on innate immune response. Non-specific immunity was assessed in randomly selected treated and control birds at the end of the experimental feeding period ('0' day post treatment) by performing *in vitro* neutrophil, monocyte and lymphocyte functional assay. In both the dose groups, superoxide anion production by neutrophil increased and then decreased to '20' DPT. The increase was significant in treated birds compared to control birds. *In vitro* nitrite production by monocyte was higher in treated birds than control. In the treatment group *in vitro* non-specific lymphocyte proliferation and IL-2 production initially increased and then decreased abruptly. But, in the treatment group *in vitro* non-specific lymphocyte proliferation and IL-2 production decreased gradually. Our findings showed that 15 days oral administration of yeast cell wall preparation improved innate immune responses in the chicken (broiler).

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