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Full Length Article

Immunological and Therapeutic Evaluation of Wheat (*Triticum aestivum*) Derived Beta-glucans against Coccidiosis in Chicken

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Abstract

This study was carried out to determine the immunomodulatory and therapeutic effects of wheat derived beta-glucans (purified) against avian coccidiosis. Briefly, beta-glucans from wheat bran were extracted and purified using standard procedures. A total of 250 broiler chicks (day-old) were reared at experimental station, Department of Parasitology, University of Agriculture, Faisalabad for this study. At 7th day of their age, birds were subdivided into five equal groups (n=50). Groups A, B and C were orally administered with graded doses of purified beta-glucans (50, 100 and 150 mg/kg of body weight) for three consecutive days; whereas, positive control (group D) was administered with vitamin-E at the dose rate of 87mg/kg; while negative control (group E) was served with phosphate buffered saline (PBS solution). At 14th day post administration of graded doses, 30 chickens from each group were randomly selected and used to monitor cellular and humoral immune responses, while remaining birds (n=20) in each group were challenged with mixed species of genus *Eimeria* for therapeutic evaluation. Overall, chickens in group A administered with 50 mg/kg b.wt. purified beta-glucans showed significantly higher immune responses in terms of enhanced humoral and cell mediated immunity as compared to those of other beta-glucans administered and E groups. While, the immune responses showed by group A were comparable with group D. After challenge with *Eimeria*, the groups A and D also showed maximum weight gains with low oocyst counts and maximum percent protection against lesions in both caecum and intestine. On the other hand, a minimum daily weight gain with increased number of oocysts in chickens was observed in control group (PBS) during therapeutic evaluation. These findings suggest that the beta-glucans derived from wheat may have immunomodulatory and therapeutic effects against *Eimeria* infection in chickens.

Keywords: Coccidiosis; Wheat; Beta-glucans; Immunomodulators

Introduction

Coccidiosis is an important protozoal disease of genus *Eimeria*, leading to high mortality and poor growth rate in poultry production, causing huge economic and health losses of about three billions US dollars per year (Dalloul and Lillehoj, 2006). In chickens, enterocytes are the primarily infected cells by *Eimeria* species, leading to bloody diarrhea and severe enteritis (Lillehoj and Trout, 1996). Moreover, immunosuppression may occur due to sub clinical form, followed by secondary diseases. For the prevention and control of coccidiosis, anticoccidials and vaccines are major contributors; however, their use has been described with uneven success (Basu and Aaldar, 1994). Resistance development in target parasites, high cost, and toxic effects of chemotherapeutic drugs are the main constraints. Therefore, for the prevention of coccidiosis, current circumstances demand alternatives. Natural and synthetic biological compounds may considered as appropriate agents (Patwardhan and Gautum, 2005), to sustain bird's health without affecting their performance (Awais *et al*., 2011). Plant based biological active compounds are thought to be promising candidates due to their availability and powerful efficacy with no or minimal residual effect in consumers (Patwardhan and Gautam, 2005). To cure health related issues, almost 64% of human population has been using plant based drugs, worldwide (Farnsworth, 1990). In this regard, one of the important sources is cereals. In humans and other animal models, various studies have shown that dietary cereal fibers may have a great effect on different physiological parameters (Neyrink *et al.,* 2008). Cereals based carbohydrates are mainly reported as antitussive, antioxidant, antimutagenic, anti-inflammatory, anticancerous and immunomodulators (Zhou *et al*., 2010). Cereals, fungi and yeast cell wall contain beta-glucans as principle structural component, with molecular weight of 2000 kDa approximately. These polysaccharides enable the host to develop resistance against viral, parasitic, fungal and bacterial diseases, by enhancing immune system, lysosomal enzyme activity, phagocytosis and IL-1 production (Estrada *et al*., 1997a).

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So, this study enumerates the immunomodulatory and therapeutic effects of wheat derived purified beta-glucans and its subsequent protection against *Eimeria* infection in chickens.

Materials and Methods

Procurement and Pre-treatment of Brans

Wheat was processed to obtain bran following the method of Akhtar *et al*. (2012). Briefly, the bran soaked for 1 h in de-ionized water at 4°C was passed through 200 mm mesh. After that, bran was washed thrice with 5–6 weight by volume (w/v) of water to eliminate the excessive starch. Then, water was evaporated (up to 10%) by heating at 50°C for about 12 h and bran was grounded to powder and then again passed through 60 mm mesh. The bran (de-starched) obtained was stored at 4°C for further use.

Extraction of Bio-molecules (Beta-glucans) from Cereals

Before extraction of bio-molecules the preserved bran was subjected to electric mill to obtain optimum particle size. The extraction of beta-glucans was performed by following the steps as described by Carr *et al*. (1990) with certain changes. All the bran (100 gm) was suspended in 4% Sodium Hydroxide (NaOH; 4900 mL) solution and was kept at room temperature for 18 h and then centrifuged at 6000 g for 15 min. The pH of the supernatants was adjusted to 4.5 with Hydrochloric acid (HCL; Sigma, USA); followed by 2nd centrifugation at the same speed and time. The supernatants thus collected were subjected to enzymatic digestion with α-amylase (Avonchem, UK) at 96°C for one hour. The solution was then cooled to room temperature followed by the addition of ethyl alcohol (Sigma, USA), and incubated at 4^{o}C for overnight. The extracted beta-glucans (crude) was collected by centrifuging at 6000 g for 15 min.

Purification of Beta-glucans

The purification of crude beta-glucans was carried out using ammonium sulphate precipitation method (Li *et al*., 2006). Briefly, the beta-glucans extracts were first placed in water and then centrifuged at a speed of 15,000 g for 40 min to eliminate residues. Ammonium sulphate (50%, w/v) was added to the supernatants by stirring to obtain an ending concentration of ammonium sulphate 25% (w/v). Further, the precipitates were centrifuged at the same speed for 25 min and then re-dissolved in water. The whole procedure was repeated once and finally the pellets were placed in water. Then, an equal volume of 100% propan-2-ol (IPA) was added slowly and the precipitates were collected by centrifugation (15,000 g, 25 min). As a final step, beta-glucans pellets were air dried with temperate warming.

Preparation of *Eimeria* Infective Stage

The unsporulated oocysts of *Eimeria* species (local isolates) were harvested from naturally infected chicken guts collected from poultry sales point/ shops and outbreak cases at farms of Faisalabad. They were allowed to sporulate in 2.5% potassium permanganate (Reid and Long, 1979). Finally, the concentration of sporulated oocysts was adjusted to 65000–70000 per 5 mL of PBS through McMaster counting technique.

Experimental Design

Overall, 250 day-old chicks (broiler; Hubbard) were raised in specific pathogens free (SPF) environment at experimental unit, Department of Parasitology, University of Agriculture, Faisalabad. At 7th day of their age, birds were separated into five equal groups (n=50). Group A, B and C were administered with graded doses of purified beta-glucans (50, 100 and 150 mg/kg of body weight) orally, for three consecutive days. While group D (positive control) received vitamin-E at the dose rate of 87 mg/kg, and group E was served with phosphate buffer saline (PBS; negative control). Moreover, all the birds were inoculated with the routine vaccine.

On day 14th post administration, thirty chickens were used to monitor humoral and cellular immune responses, while twenty were challenged, with mixed *Eimeria* species for therapeutic evaluation.

Immunological Evaluation

Humoral immune response: To monitor humoral immune response, the antibodies level against sheep red blood cells (SRBCs) was quantified by following the method of Yamamoto and Glick (1982) with certain changes as described by Qureshi and Havenstein (1994). Briefly, chickens were injected through intramuscular route with SRBCs (5%; 1 mL/chicken) after 14th day of beta-glucans administration. Similarly, a booster injection was given to chickens at 14th day post first injection. Antibodies containing sera were obtained at 7th and 14th day post first and second injections. Finally, microplate hemagglutination assay was performed for anti-SRBCs antibodies examination.

In vivo **Lympho-proliferative Response to Phytohaemagglutinin-P (PHA-P):** Lymphoblastogenesis was quantified as mentioned by Corrier (1990). In brief, administered and control birds were injected intradermally with Phytohemagglutinin-P (Sigma®, USA) (100 µg/100 mL/chicken) between the last two (fourth and third) digits of the right foot and the left foot served as control. The interdigital skin thickness was measured at 24, 48 and 72 h post administration of PHA-P with the help of screw gauge. Finally, the lyphoproliferative response was calculated with the formula:

Lympho-proliferative response =

(response of right foot, injected with PHA-P) – (response of left foot injected with PBS solution)

Dinitrochlorobenzene (DNCB) Test

To monitor the delayed-type hypersensitivity reaction dinitrochlorobenzene (DNCB) test was used as explained by Blumink *et al*. (1974). In brief, 2% DNCB dose (0.1 mL) prepared in acetone was applied on 4 cm^2 area of skin. The change in skin thickness was monitored (mm) at 24 and 72 h post injection, with the use of digital vernier caliper.

***In vitro* cell mediated response to concanavaline-A (Con-A):** *In vitro* lymphoblastogenic response of chicken lymphocytes to Con-A was measured in both the administered (A, B and C) and control groups (D and E) at 7^{th} and 14^{th} day post adminitration according to the method described by Qureshi *et al.* (2000). Briefly, the peripheral blood lymphocytes (PBLs) were separated using Histopaque-1077 (Sigma®, USA) according to manufacturer's instructions. The cells were washed thrice with PBS and adjusted to a concentration of 3×10^6 cells/mL of RPMI-1640 growth medium (Sigma-Aldrich®, USA), supplemented with fetal calf serum (Sigma-Aldrich®, USA) (7%) inactivated at 56ºC and 1 µg/mL gentamicin was added. A 100 µL suspension containing 3×10^6 PBLs from each experimental group were added to five wells (in duplicate) of a flat bottomed microtitration plate (96-wells; Medium binding, polystyrene, Flow Labs., UK), followed by the addition of Con-A (Sigma®, USA) (25 µg/100 µL) to five wells. Whereas, other five wells without Con-A served as unstimulated controls. The plates were incubated at 41ºC in the presence of 5% CO_2 and 50–70% humidity. Finally, the optical density (OD) was read using a plate reader (BioTek-MQX200, USA) at 540 nm wavelength and mean OD value was calculated using the following formula:

$$\text{Mean OD value} = \frac{\text{Con A-stimulated} - \text{unstimulated}}{\text{Unstimulated}}$$

Weekly Weight Gains

Birds from each group (A, B, C, D and E) were randomly selected and weighted on each week to monitor the effect of purified beta-glucans on their body weight. At 42^{nd} day of their age (35^{th} day of administration) final weights were tabulated in administered and control birds.

Therapeutic Evaluation

Remaining twenty birds in each group were challenged with 65000–70000 oocysts (sporulated) of mixed *Eimeria* species (local isolate) at 14^{th} day post-inoculation of polysaccharides. Increase in body weight from day 3^{rd} to 11^{th} and oocysts per gram (OPG) of droppings from day 4^{th} to 12^{th} post challenge were calculated (Ryley *et al*., 1976; Akhtar *et al*., 2012). Further, organ to body weight ratios and the percent protection against GIT lesions were evaluated with the formula of Singh and Gill (1975).

$$\text{Percent Protection against lesions} = \frac{\text{Average lesion score (IUC)} - \text{Average lesion score (IMC)}}{\text{Average lesion score (IUC)}} \times 100$$

IUC= infected untreated control
IMC= infected medicated control

Statistical Analysis

Duncan's multiple range (DMR) and one way analysis of variance (ANOVA) tests were executed for statistical significance with appropriate software (SAS, 2004). On the whole, differences were considered significant at 5% confidence level.

Results

Immunological Evaluation

Humoral immune response

Antibody response to sheep red blood cells (SRBCs): In control (PBS) and administered (vitamin-E; beta-glucans) chickens, humoral immune response was detected by haemagglutination test in terms of antibody titers (geomean titers; GMT) to sheep RBCs on day 7^{th} and 14^{th} after first and second injections of SRBCs.

Total anti-SRBCs antibody titer: Among beta-glucans treated groups, the highest geomean titre ($p<0.05$) was observed in chickens of group A at 7^{th} (64) and 14^{th} (82) day of post primary (PPI) and post secondary injections (PSI), respectively (Fig. 1). However, the GMT in chickens of group A was lower than that of positive control group, which showed 74.6 and 96 at both the 7^{th} and 14^{th} day of PPI and PSI, respectively.

IgM anti-SRBC antibody titer: At 7^{th} and 14^{th} day PPI, IgM anti-SRBCs antibody titer (GMT) was higher ($p<0.05$) in group D (vitamin-E; 26.6) and group A (50 mg/kg; 21.3), as compared to control group E (PBS; 10.7). Similar response in these groups was observed at 7^{th} and 14^{th} day PSI. However, in all the groups, maximum values of IgM titers ($p<0.05$) were observed on day 7^{th} PPI and minimum on day 14^{th} PSI (Fig. 1).

IgG anti-SRBC antibody titer: At 7^{th} day PPI, IgG anti-SRBCs antibody titer (GMT) was higher ($p<0.05$) in group B (100 mg/kg); whereas, at 14^{th} day PPI, IgG anti-SRBCs antibody titer (GMT) was higher ($p<0.05$) in group A. Similarly, at 7^{th} and 14^{th} day PSI, IgG anti-SRBCs antibody titer (GMT) was higher ($p<0.05$) in group A. However, in all the groups, maximum values of IgG titers were observed on day 14^{th} PSI and minimum on day 7 PPI, (Fig. 1).

Cell Mediated Immune Responses

***In vivo* lympho-proliferative responses to Phytohaemagglutinin-P (PHA-P):** Cell mediated immune response in terms of *in vivo* lympho-proliferative response

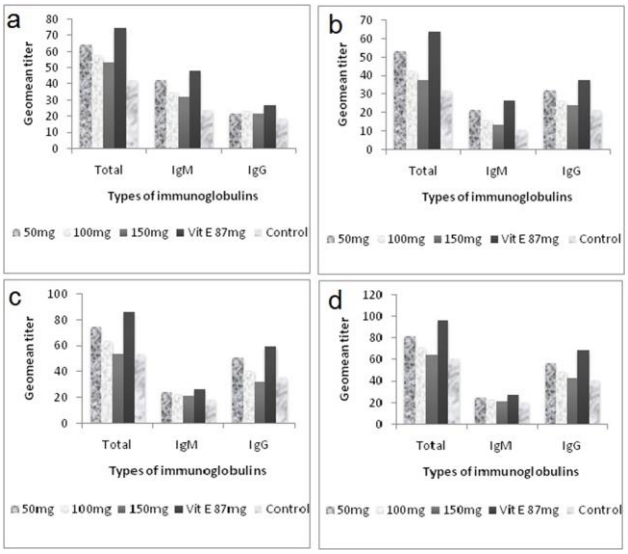

Fig. 1: Antibody response (geomean titres) to sheep RBCs. Level of immunoglobulins in administered and control groups at 7th day post primary injection (a), 14th day post primary injection (b) 7th day post secondary injection (c) and at 14th day post secondary injection (d). The group treated with 50 mg/kg b.wt of purified wheat beta-glucans showed highest level of immunoglobulins among beta-glucans administered groups

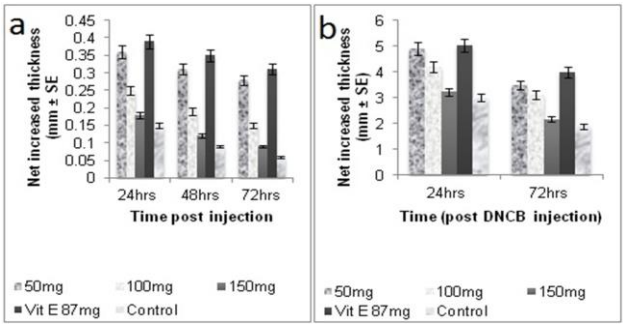

Fig. 2: *In vivo* lympho-proliferative response to PHA-P and DNCB. In response to T-cell mitogens PHA-P (a), a maximum toe thickness was observed in chickens of group administered with 50mg/kg b.wt of purified wheat beta-glucans at 24 h as compared to those of other polysaccharide treated groups. Similar response was observed with DNCB (b) at 24 and 72 h post injection

was assessed by measuring amplitude of toe-web swelling (mm±SE) at 24, 48 and 72 h post PHA-P injection in all the administered and control groups. The maximum swelling was observed at 24 h post PHA-P injection in both administered and control groups. Among treated groups, maximum swelling (0.36±0.03) was recorded in group A ($p<0.05$) as compared to control group E (0.15±0.02). However, the lympho-proliferative response to PHA-P by Group A was comparable ($p>0.05$) with that of positive control (Fig. 2a).

Cell mediated immune response (Mean±SE) to Dinitrochlorobenzene (DNCB): *In vivo* Dinitrochlorobenzene (DNCB) test was used to examine the delayed-type hypersensitivity reaction in broiler chickens of all groups (mm±SE) at 24 and 72 h post DNCB injection. The maximum swelling on the skin ($p<0.05$) was measured at 24 h (5.03±0.9) in group D and group A (4.89±1.2) as compared to group E (3.01±1.3), which was served with PBS solution. Although, the skin thickness was less at 72 h but a similar response pattern was recorded (Fig. 2b).

***In vitro* lympho-proliferative response to Concanavalin-A (Con-A):** On day 7th and 14th post administration of purified beta-glucans, similar *in vitro* lympho-proliferative response to Con-A was observed. Among different groups, group D and A, which were served with vitamin-E and 50 mg/kg of purified beta-glucans, respectively showed higher response ($p<0.05$) as compared to that of –ve control group (E) (Fig. 3).

Weekly Weights (g±SE) Post Administration of Beta-glucans

At 42nd day of bird's age (35th day of beta-glucans administration), all the chickens were individually weighed (g±SE) for final values to see the effects on weight gain. Among beta-glucans administered groups, maximum ($p<0.05$) weight gain was detected in group A (2022±16.1) and B (1990±20.2) as compared to group E (1942±16.7; PBS). The difference was statistically higher ($p<0.05$) between administered and control groups (Fig. 4).

Therapeutic Evaluation

Oocysts count: The oocysts per gram (OPG) of droppings were observed from 4th day to 12th day post-challenge and results were presented in terms of mean OPG of droppings (mean±SE), which was significantly higher ($P<0.05$) in control group (group E) than administered groups. On 9th day, OPG count was significantly lower ($p<0.05$) in group D and A as compared to other administered and control groups (Fig. 5).

Daily weight gain: From day 3rd to 11th post-challenge, daily weight gain (g±SE) was monitored. Results showed an increased daily weight gain ($p<0.05$) in group A than that of other two groups received beta-glucans, which was also comparable with that of group D. However, the control group showed significantly lower weight gain ($p<0.05$) than that of all administered groups (Fig. 6).

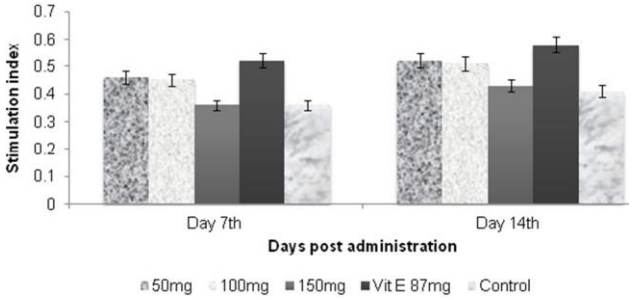

Fig. 3: *In vitro* lympho-proliferative response to Concanavalin-A. In response to Con-A (a T-cell mitogen), a maximum lympho-proliferative response at 7th day was observed in group A and B among beta-glucans treated groups with no significant difference. The same pattern was observed at 14th day post administration of beta-glucans.

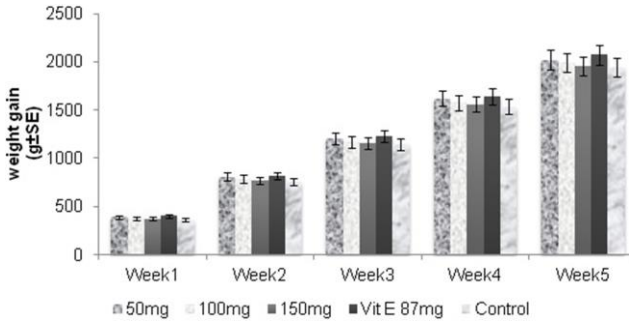

Fig. 4: Weekly weights (g±SE) post administration of beta-glucans. Birds from each group (A, B, C, D and E) were randomly selected and weighted on each week (5 weeks; post administration) to monitor the effect of purified beta-glucans on their body weight. At 42nd day of bird's age they were individually weighted for final values. Maximum ($p<0.05$) weight gain was detected in group D (vitamin-E) and A (50mg/kg b. wt)

Organ-body weight ratios: After 12th day of challenge, organ-body weight ratios of lymphoid organs including ceacal tonsils, thymus, spleen and bursa were evaluated. Organ-body weight ratios of all the lymphoid organs in all administered and control chickens were statistically similar ($p>0.05$) (Fig. 7).

Lesion scoring: Lesion scoring (scale 0 to 4) of dead and survived chickens was monitored by sacrificing the birds during and on 12th day post-challenge. All the chickens administered either with vitamin-E or purified beta-glucans showed relatively lesser ceacal and intestinal lesions as compared to control group (E). The postmortem findings of control group (PBS) also revealed severe hemorrhagic lesions on ceaca and intestine and most of them were found filled with blood

Ceacal lesion scoring: Out of twenty chickens, which were subjected to therapeutic evaluations, chickens in group A

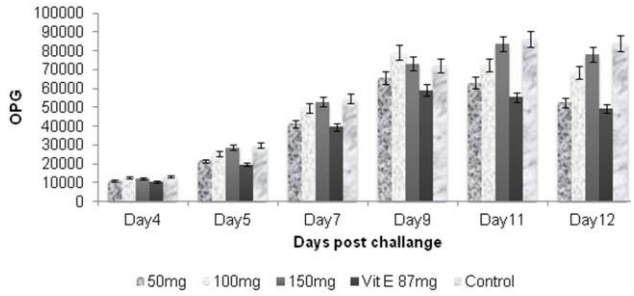

Fig. 5: Oocysts count in faeces of chickens after challenge with *Eimeria* species. Oocysts per gram of droppings were observed from 4th day to 12th day of challenge experiment. Highest count was observed at 9th and 11th days and the maximum output was in group E and C. While, the oocyst counts in chickens of group A were comparable with those of group D (positive control)

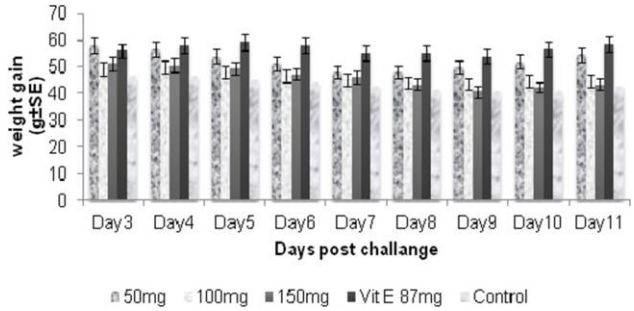

Fig. 6: Daily weight gains in chickens post *Eimeria* challenge. Daily weight gains from day 3rd to 11th were monitored during the challenge experiment with *Eimeria* species. The groups A and D showed maximum weight gains. A Minimum weight gain was observed in Group E

showed the maximum protection ($p<0.05$) against ceacal lesions (37.5%) as compared to that of other beta-glucans administered groups. However, maximum protection in terms of less lesion score was observed in chickens of group D (40%), which was statistically similar ($p>0.05$) to that of group A. On the other hand, chickens in group E showed the least protection with high lesion score (16.25%) (Table 1).

Intestinal lesion scoring: Likewise, intestinal score were also measured. The protection against lesions was 36.25 and 32.5 percent in group D and A, respectively. While, the protection against lesions was 17.5 percent in PBS served group (E) (Table 1).

Discussion

Natural components have traditionally been reported as immunomodulators and therapeutics that exclusively amend the immune system of an organism (Masihi *et al.*, 1992; Masihi, 1994). Cereals are an important source of biologically dynamic compound(s) that usually contain

Table 1: Lesion scores and percent protection against lesions in administered and control chickens after challenge with *Eimeria* species

Groups (n=20)	Lesions scoring of birds					% protection against lesions
	0	1	2	3	4	
	Caeca					
A	0	4	4	7	5	37.5[a]
B	0	3	6	5	6	32.5[b]
C	0	3	4	5	8	27.5[c]
D	0	4	8	4	4	40[a]
E	0	0	3	7	10	16.25[d]
	Intestine					
A	0	3	6	5	6	32.5[b]
B	0	3	3	9	5	30[bc]
C	0	2	3	9	6	26.2[c]
D	0	2	9	5	4	36.25[a]
E	0	1	3	5	11	17.5[d]

Score cards: 0 = no lesions, 1 = few lesions, 2 = moderate lesions, 3 = Extensive lesions, 4 = Bloody intestinal/ceacal contents;
Groups: A= 50 mg of beta-glucans, B = 100 mg of beta-glucans, C = 150 mg of beta-glucans, D = +ve Control (vitamin E), E = -ve Control

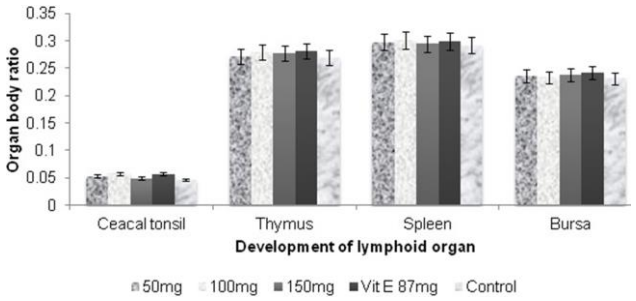

Fig. 7: Organ-body weight ratios. On day 12th post challenge, birds were evaluated for their organ to body weight ratios. Ratios of all the lymphoid organs were statistically similar ($p>0.05$) in all the groups

glycosides, terpenoids, phenols, polysaccharides and alkaloids (Wills *et al*., 2000). A wide range of biological effects have been manifested by cereals such as immunostimulants (Akhtar *et al*., 2012), antithrombosis, anti-inflammatory, anti-oxidant and anti-stress activities (Zhou *et al*., 2010). These pronouncements demanded for additional investigations on cereals to explore their immunostimulatory and therapeutic potential against parasitic diseases. Therefore, the current study was planned to demonstrate the immunomodulatory effects of cereals, *Triticum aestivum* derived beta-glucans and the therapeutic potential against avian coccidiosis. In common, various *in vivo* and *in vitro* assays have been used to demonstrate the cell mediated and humoral responses in different animal models (Qureshi *et al*., 1986; Corrior, 1990; Qureshi and Miller, 1991). In the current study, non-pathogenic sheep red blood cells (SRBCs) were chosen to demonstrate the effects of beta-glucans on humoral immune response (Saxena *et al*., 1997; Kundu *et al*., 1999). Microplate haemagglutination assay was performed to conclude the anti-SRBCs antibodies titers in chickens at day 7th and 14th after primary and secondary inoculations, Chickens administered with purified beta-glucans were found to have higher total, IgM and IgG anti-SRBCs antibody titers as compared to control group (PBS; -ve control), demonstrating the potential of beta-glucans as immunostimulator. The same kind of higher immune levels by polysaccharides were observed previously (Li *et al*. 1982; Salvin, 2003; Hikosaka *et al*., 2007). The results are also consistent to the previous findings of similar studies on plant derived polysaccharides where orally administered chickens with beta-glucans resulted in higher antibody response against SRBCs (El-Abasy *et al*., 2003). Enhanced humoral response in terms of increased number of antibody-producing cells and higher serum antibody levels were observed in radiation induced immunocompromised chickens administered with plant extracts (Amer *et al*., 2004).

Similarly, wheat derived polysaccharides showed stimulatory effects on the production of antibodies (Akhtar *et al*., 2008). Regarding humoral immune response, reliable stimulatory results had also been demonstrated in chickens by Maslog *et al*. (1999).

To demonstrate, *in vivo* cell mediated immune response in chickens, use of phytohemagglutinin-P (PHA-P), dinitrochlorobenzene (DNCB) and human gamma globulin (Hγg) are the most routinely used methods due to their accuracy, simplicity and ease to perform. Therefore, in the present study, the classical toe web assay and dinitrochlorobenzene assays were performed to demonstrate the effects of beta-glucans on cellular immune responses. The higher cellular immune responses in administered birds might be due to the delayed type hypersensitivity and/or stimulatory effects of biomolecules on macrophages that may lead to increase in thickness of toe web in response to PHA-P and DNCB (T-cell mitogens). Further, it could be assumed that increased population of lymphocytes might be responsible for the activation of immune retorts (Akhtar *et al*., 2008). On day 7th and 14th post administration of beta-glucans, *in vitro* lympho-proliferative response was detected in both administered and control groups by lymphoblastogeneic response of chicken peripheral blood lymphocytes (PBLs) to Con-A. Higher response was observed in beta-glucans administered groups as compared to control group (PBS). It is suggested that lympho-proliferative response might be due to specific receptors present on PBLs surface, come in direct contact with Con-A and go through mitotic division. Further, Con-A stimulated the PBLs which produced interleukine-1 by monocytes in PBL fraction, which further stimulated the proliferation of lymphocytes (Qureshi *et al*., 2000).

Effects of cereals derived beta-glucans were also evaluated on the development of lymphoid organs. Results showed no difference in administered and control groups. These results were in accordance to the report of Amer *et al*. (2004) who reported a non-significant

difference in the relative lymphoid weights in administered and control groups.

Growth promoting effects of beta-glucans were evaluated with respect to feed efficiency ratios and body weight gains on weekly basis from week 1st to 5th post administration of wheat derived purified beta-glucans. The results showed higher live body weights in chickens administered with vitamin-E and beta-glucans as compared to control group (PBS; -ve control) of chickens, indicating better feed utilization in experimental groups. El-Abasy *et al*. (2002; 2004) also demonstrated the related conclusion with higher body weights and lower feed utilization in plant administered groups as compared to control. Similar findings were also reported in immunosuppressed chickens exposed to X-ray radiation (Amer *et al*., 2004). Overall, the results of the present study suggest that beta-glucans (purified) may be responsible for the improvement of food utilization with higher weight gains. Moreover, growth promoting effects in this study might be correlated with prebiotic property of dietary fibers that may enhance the growth of gut microbiota (Saeed *et al*., 2011).

Therapeutic effects of beta-glucans derived from other cereals had been presented previously against *Eimeria* species (El-Abasy *et al*., 2003; Akhtar *et al*., 2008). In present study, significantly increased oocysts numbers were recorded in control group than those of administered with purified beta-glucans. The chickens in control groups were observed depressed, lethargic and dull with ruffled feather and irregular feed and water intake (Personal observations) that may lead to alteration in gut homeostasis, altered metabolism and lower body weight gains, (McKenzie *et al*., 1987; Adams *et al*., 1996; Kettunen *et al*., 2001). The pathogenic *Eimeria* species seriously affect the enteric microenvironment and ultimately cytokines production, which lead to physiological changes and increased hemorrhagic lesions (Allen, 1997). In this respect, lesion scoring in all the groups was monitored using the method of Johnson and Reid (1970) on a scale of 0 to 4. In the present study, maximum chickens in control groups showed severe lesions (3–4) in caecum and intestine. While, chickens administered with beta-glucans attained mild to moderated lesions (1.0-2.0) with higher percent protection. Lower oocysts counts and less harms to the enteric mucosa in all the administered chickens may present the participation of immune effecter components in preventing the progression of the life cycle of parasite (Lillehoj, 1989). These lower counts and fewer lesions in administered chickens may also indicate the anti-inflammatory effects of beta-glucans (Estrada *et al*., 1997b). These expressed properties may be a reason of improved daily weight gains in administered groups from 3rd to 12th day after infection.

Conclusion

The present study may conclude that beta-glucans derived from wheat had immunostimulatory effects on the broiler chickens with enhanced cellular and humoral immune responses.

The beta-glucans also had protective effects against *Eimeria* infection and significantly improved weight gains of chickens with few lesions and oocyst score. Further studies are needed to evaluate the immune responses at molecular levels to better understand the induced immunity types in chickens.

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