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Studies on wheat bran Arabinoxylan for its immunostimulatory and protective effects against avian coccidiosis

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ABSTRACT

Wheat (*Triticum aestivum*) bran derived polysaccharides, Arabinoxylans (AXs), were evaluated for their immunostimulatory and protective efficacy against *Eimeria* infection in chickens. Humoral response revealed significantly higher ($P<0.05$) total Igs, IgG and IgM titers at days 7th and 14th post primary and secondary injections of sheep red blood cells in the experimental chickens administered with AXs as compared to those of control group. The percent protection and daily weight gains were significantly higher ($P<0.05$) in the chickens of experimental groups as compared to control; whereas, mean oocyst per gram of droppings and lesion scores were significantly higher ($P<0.05$) in control group as compared to chickens in the experimental groups. The differences in organ body weight ratio of all the lymphoid organs were statistically non-significant ($P>0.05$) between experimental and control groups except thymus and cecal tonsils. In conclusion, AXs showed both immunostimulatory and protective effects against coccidiosis in broiler chickens.

1. Introduction

Medicinal herbs and plants have remained the primary health care agents from the time immemorial before the advent of modern medicine. However, isolation and identification of the active compound(s) involved in such activities did not gain much momentum till 19th century (Phillipson, 2001). It is reported that more than 64% of the world's human population uses botanical/ayurvedic drugs to combat health problems (Farnsworth, 1990) and almost 50% of the synthetic medicines have been derived from, or patterned after chemicals of plant origin (Anonymous, 1988). In this regard, cereals are known to be one of the most important source of pharmacologically active, distinct but often overlapping classes of constituents mainly terpenoids, glycosides, alkaloids, phenols and polysaccharides (Wills, Kerry, & Morgan, 2000). Extensive studies on dietary fibers have revealed that the consumption of whole grain cereal foods, cereal bran and highly fermentable, non digestible carbohydrate components of cereal grains have a large impact on various physiological parameters in different experimental models including human beings (Kendall, Esfahani, & Jenkins, 2010; Neyrinck et al., 2011). Over the last few years, a number of beneficial properties of cereal derived bio-molecules in general and polysaccharides in particular have been demonstrated in various studies that increased the interest of researchers to know about the biological activity of natural carbohydrate polymers (Neyrinck et al., 2011; Paulsen, 2002).

Among cereals, wheat is an important source of dietary carbohydrates and proteins; and its bran is an abundantly available by-product, constituting 14–19% of the wheat grain (Adom & Lui, 2002). It is a rich source of fatty acids, tocopherols, polysaccharides and phenolic compounds. Wheat bran polysaccharides have been reported for antioxidant, immunostimulatory, anti-inflammatory, antitussive, anticancerous and antimutagenic (Anson et al., 2010; Brindzova, Mikulasova, Takacsova, Mosovska, & Opattova, 2009; Ferguson & Harris, 1999; Helsby, Zhu, Pearson, Tingle, & Ferguson, 2000; Prisenznakova, Nosalova, Hromadkova, & Ebringerova, 2010; Zhou et al., 2010) activities.

Keeping in view, the immunological activities of wheat bran in numerous animal models, the present study reports the effects of wheat bran polysaccharides, Arabinoxylans (AXs), on chicken's humoral immune profile and its protective efficacy against avian coccidiosis.

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2. Materials and methods

2.1. Procurement and pre-treatment of wheat bran

Wheat (*Triticum aestivum* L.) bran procured from local flour mill was used in the present study. Wheat bran (100 g) soaked in de-ionized water (1 L) was incubated for 60 min at 4 °C. After incubation, it was passed through 200 mesh followed by washings thrice with 5–6 volume (w/v) of de-ionized water to remove the starch. Water was removed (up to 10%) from washed bran suspension by heating at 50 °C for 12 h in the water bath. After air drying, bran was ground to powder by using the hammer mill and then passed through 60 mesh. The bran fraction thus collected was used on de-starched bran which was stored at 4 °C till further use.

2.2. Alkaline extraction of polysaccharides, Arabinoxylans (AXs)

AXs were extracted from de-starched bran by following the methodology of Zhou et al. (2010). Briefly, dried wheat bran powder (100 g) was mixed with 1.5 L of 0.15 N sodium hydroxide (NaOH) (containing 0.5% H_2O_2, v/v) for 90 min in water bath at 80 °C. The mixture was cooled to room temperature and centrifuged at 5000 × *g* for 30 min. The supernatant thus obtained was neutralized with 0.2 N hydrochloric acid (HCl), adjusted to a pH 4.5 and centrifuged again at 5000 × *g* for 30 min. The supernatant obtained after centrifugation was concentrated up to 1/4th of the original volume under reduced pressure in a rotary evaporator. The concentrate was precipitated by using ethanol (65%), followed by centrifugation at 5000 × *g* for 5 min at 4 °C. Supernatant was discarded and the sediment was dissolved in distilled water and centrifuged at 5000 × *g* for 30 min at 4 °C. Supernatant thus collected was again precipitated by ethanol as described above and freeze-dried. The dried fractions thus obtained were used as alkaline extract of AXs and its composition has been reported in the literature earlier (Zhou et al., 2010).

2.3. Infective material

Oocysts of mixed species of *Eimeria* (local isolates) collected from outbreak cases of coccidiosis were used in the present study. Contents from the positive guts were subjected to sporulation (Reid & Long, 1979) and after sporulation, oocysts were washed twice with phosphate buffered saline (PBS; pH 7.2) and subjected to McMaster counting technique to calculate the number of sporulated oocysts/milliliter of suspension (Ryley, Meade, Ifazalburst, & Robinson, 1976). The final concentration of suspension was adjusted to 7.0×10^4 sporulated oocysts per mL of PBS and placed in a sterilized screw capped bottle and stored at 4 °C till further use. Identification of the sporulated oocysts was made on the basis of their predilection site, morphometric analysis, sporulation time, number of sporozoites, presence of micropyle and shape index according to the keys given in the literature (Anonymous, 1984; Reid & Long, 1979). Analysis revealed the presence of four distinct *Eimeria* (*E.*) species viz. *E. tenella*, *E. maxima*, *E. acervulina* and *E. necatrix* in the final suspension used for challenge experiment.

2.4. Experimental design

A total of 120-day-old industrial broiler chicks (Hubbard) procured from local hatchery were reared on floor system under standard management conditions at Experimental Station, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. All the chicks were fed withdrawal feed and water ad libitum; and vaccinated following the routine vaccination schedule at study area (Awais, Akhtar, Muhammad, Haq, & Anwar, 2011). After 5 days acclimatization period, chicks were randomly divided into four equal groups ($n = 30$) and administered orally with graded doses of AXs dissolved in PBS (5 mL) for three consecutive days i.e. 5th, 6th and 7th day of age as follows:

Group A: AXs at a dose rate of 100 mg/kg of body weight/day
Group B: AXs at a dose rate of 200 mg/kg of body weight/day
Group C: AXs at a dose rate of 300 mg/kg of body weight/day
Group D: PBS 5 mL/day and served as control

On day 14th, half chickens ($n = 15$) from each group were separated to demonstrate the humoral response and remaining half were challenged with mixed species of genus *Eimeria* (local isolates).

2.5. Immunological evaluation

Sheep red blood cells (SRBCs) were used as T-dependent antigens to demonstrate the antibody response in experimental and control chickens following the procedure described by Yamamoto and Glick (1982) with minor modification as suggested by Qureshi and Havenstein (1994). In brief, on day 14th post administration of AXs, chickens were injected with SRBCs (5%) via intramuscular route (1 mL/chicken) followed by a booster injection at day 14th post primary injection. Blood was collected at days 7th and 14th post primary and secondary injections to separate the sera. All the sera samples were analyzed for total immunoglobulins (Igs), IgM (mercaptoethanol-sensitive) and IgG (mercaptoethanol-resistant) anti-SRBCs antibodies by microplate hemagglutination assay.

2.6. Effect on the body weight and development of lymphoid organs

After last blood sampling for immunological evaluation on day 49th of age, all the birds were individually weighed and lymphoid organs including bursa of fabricius, thymus, spleen and cecal tonsils were removed surgically and weighed. The results were expressed in terms of percent organ weights ratio relative to the live body weight (Giamborne & Closser, 1990).

2.7. Evaluation of protective efficacy against Eimeria infection

Protective efficacy of AXs was determined in remaining half chickens ($n = 15$) of the experimental groups in comparison with the control. On day 14 post administration of AXs, chickens in all the groups were challenged with 7.0×10^4 sporulated oocysts of mixed species of genus *Eimeria* (local isolates; mainly *E. tenella*, *E. acervulina*, *E. maxima* and *E. necatrix*). Chickens in all the groups were monitored for mortality from days 3rd to 12th post challenge in experimental and control groups and results were expressed in terms of percent protection (Akhtar et al., 2012).

Daily weight gain was calculated in chickens of both experimental and control groups from days 3rd to 12th post challenge. Oocysts per gram of droppings were also calculated in each group from days 3rd to 12th post challenge by using the modified McMaster counting technique (Ryley et al., 1976). The cecal and intestinal lesions of dead and survived chickens in all the groups were enumerated from days 6th to 9th post challenge (peak days of infection) and were scored on a scale from 0 to 4 (Johnson & Reid, 1970).

2.8. Assessment of elicited humoral response against Eimeria spp. in chickens administered with Arabinoxylans

The effect of AXs on specific immunity against *Eimeria* species (local isolates) used in the challenge experiment was assessed by enzyme linked immunosorbent assay (ELISA) following Garcia et al. (2006) with minor modifications (Awais et al., 2011). Briefly, on days 7th and 14th post challenge, blood was collected from the

chickens of each group to get sera samples for ELISA. The optical density (OD) of all the wells was read at 492 nm in an ELISA reader. The mean absorbance values were recorded and the OD value was calculated. Positive and negative control sera samples were run in each plate and the corrected OD value was calculated by using the formula as follows:

$$OD_{corrected} = \frac{OD_{Sample} - OD_{Negative\ control\ of\ plate}}{OD_{Positive\ control\ of\ plate} - OD_{Negative\ control\ of\ plate}}$$

2.9. Effect on the development of lymphoid organs post-challenge

Survived chickens in all the groups were individually weighed on day 12th post challenge and lymphoid organs were removed surgically and weighed; and results were expressed as organ-body weight ratios as described earlier (Section 2.6).

2.10. Statistical analysis

Data thus obtained were subjected to one-way analysis of variance (ANOVA) and statistical significance between different treatment groups was ruled out by using Duncan's multiple-range tests. Data on percent protection were analyzed using the Chi-square test. All the values were considered significant at $P < 0.05$.

3. Results

3.1. Immunological evaluation

Microplate hemagglutination assay was used to detect the antibody response in chickens of experimental and control groups on days 7th and 14th post primary (PPI) and secondary injections (PSI) of SRBCs. The results were expressed in terms of geomean titer. At day 7th PPI, total anti-SRBC antibody titers were significantly higher ($P < 0.05$) in chickens administered with graded doses of wheat bran Arabinoxylans (groups B and C) as compared to control group; whereas, chickens of group A had no significant difference ($P > 0.05$) from those in control group. As a whole, group B showed the best performance in terms of highest antibody titers (GMT: 36.76) to SRBCs. At day 14th PPI of SRBCs, chickens in group B and C showed significantly higher ($P < 0.05$) response as compared to those in group A and control; and the difference between groups B and C was statistically non-significant ($P > 0.05$) (Table 1). Total anti-SRBC antibody titers at day 7th PSI were significantly higher ($P < 0.05$) in experimental chickens as compared to control; however, the difference was statistically non-significant ($P > 0.05$) between the treatment groups. Similar trend in total anti-SRBC antibody titers was recorded at day 14th PSI (Table 1).

At days 7th and 14th PPI, anti-SRBC IgM titer was significantly higher ($P < 0.05$) in experimental chickens as compared to those in control group. At day 7th PPI, group B showed the highest response (GMT: 24.18) as compared to groups A and C; although the difference was statistically non-significant ($P > 0.05$) between the treatment groups A and C. On the other hand, at day 14th PPI, IgM response was significantly higher ($P < 0.05$) in groups B and C as compared to control group but group A showed no significant ($P > 0.05$) difference from control group. At days 7th and 14th PSI, anti-SRBC IgM titers (GMT: 32.00, 32.00, 27.86) were significantly higher ($P < 0.05$) in experimental chickens as compared to control group. At day 7th PSI, anti-SRBC IgM titers were significantly higher ($P < 0.05$) in all the experimental groups as compared to control; whereas, there was no statistical difference ($P > 0.05$) between the treatment groups. At day 14th, anti-SRBC IgM titers were higher in chickens of group B and C as compared to control whereas group A had no significant difference ($P > 0.05$) from control group.

At day 7th PPI, anti-SRBC IgG titers were significantly higher ($P < 0.05$) in experimental chickens as compared to control group; whereas, the difference was statistically non-significant ($P > 0.05$) between the treatment groups. On the other hand, there was no significant difference ($P > 0.05$) in IgG anti-SRBC antibody titer between the experimental and control groups on day 14th PPI except group C which showed a significantly higher ($P < 0.05$) response as compared to control. A similar trend was observed on days 7th and 14th PSI (Table 1).

3.2. Effect on body weight and development of lymphoid organs in non-challenged birds

Organ body weight ratio of lymphoid organs including thymus, spleen, bursa of fabricius and cecal tonsils were calculated in non challenged chickens and results revealed a statistically non-significant ($P > 0.05$) difference between treatment and control groups (data not shown). On the other hand, live body weight gain was significantly higher ($P < 0.05$) in groups B (1656.0 ± 52.88) and C (1671.2 ± 32.08) as compared to experimental group A (1595.8 ± 71.70) and control group (1456.0 ± 58.79); although group A had higher live body weight gain as compared to control group but the difference was statistically non-significant ($P > 0.05$).

3.3. Challenge experiment

Protective efficacy of the wheat bran polysaccharides, AXs, was determined in chickens of both the experimental and control groups. Percent protection was maximum in chickens of group B and C (60% in each) followed by group A (45%). On the other hand, significantly lower (25%) protection in control group as compared to all the experimental groups was recorded (Table 2).

Table 1
Antibody response to sheep red blood cells in experimental and control chickens.

Group	Day 7th PPI	Day 14th PPI	Day 7th PSI	Day 14th PSI
Total anti-SRBCs antibody titer				
A	16.00^{bc}	13.93^{ab}	32.00^{a}	32.00^{a}
B	36.76^{a}	27.86^{b}	32.00^{a}	36.76^{a}
C	21.11^{ab}	18.38^{a}	27.86^{a}	32.00^{a}
D	10.56^{c}	8.00^{b}	9.19^{b}	9.19^{a}
Immunoglobulin-M				
A	12.00^{ab}	7.55^{c}	16.00^{a}	9.19^{ab}
B	24.18^{a}	16.38^{a}	16.00^{a}	24.25^{a}
C	14.60^{ab}	9.97^{ab}	12.13^{a}	24.25^{a}
D	7.30^{b}	4.34^{c}	5.40^{b}	4.59^{b}
Immunoglobulin-G				
A	4.00^{bc}	6.06^{a}	16.00^{a}	24.18^{a}
B	10.56^{a}	10.56^{a}	18.38^{a}	27.86^{a}
C	6.06^{b}	8.00^{a}	15.11^{a}	24.25^{a}
D	3.03^{c}	3.48^{a}	3.48^{b}	7.55^{a}

Geo-means sharing similar letters in a column are statistically similar ($P > 0.05$).
A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control. PPI = post-primary injection; PSI = post-secondary injection

Table 2
Percent mortality and Lesion scores in experimental and control chickens.

Group	Protection (%)	Mean lesion score	
		Intestine	Ceca
A	45^{ab}	2.45	2.60
B	60^{a}	2.20	2.15
C	60^{a}	2.15	2.20
D	25^{b}	3.26	3.15

For mortality, percent values sharing similar letters in column are statistically non-significant ($P > 0.05$).
A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control.

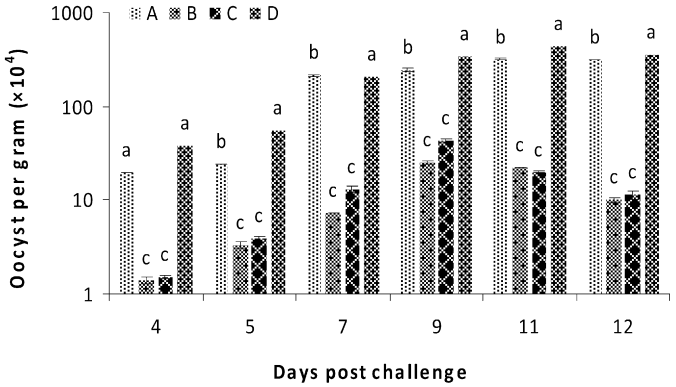

Fig. 1. Oocysts per gram of droppings post-challenge in experimental and control chickens. Bars sharing similar letters on each particular day are statistically non-significant ($P > 0.05$). A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control.

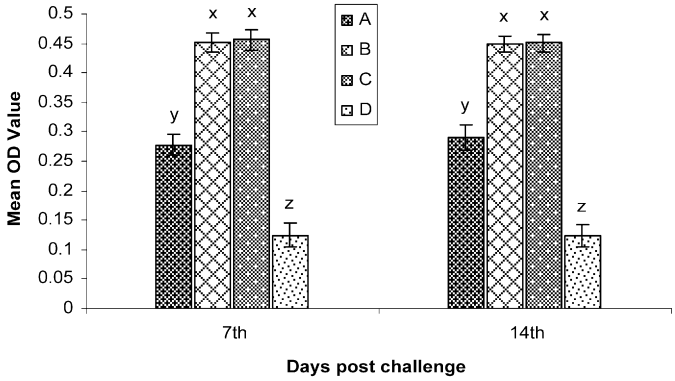

Fig. 3. Serum antibody titers on days 7th and 14th post infection with *Eimeria* species (local isolates). Bars sharing similar letters on each particular day are statistically non-significant ($P > 0.05$). A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control.

The oocysts per gram of droppings (OPG) were monitored from days 3rd to 12th post challenge and results showed that mean OPG in control group was significantly higher ($P < 0.05$) as compared to chickens in experimental groups. Moreover, OPG was significantly lower ($P < 0.05$) in groups B and C as compared to group A; whereas, the difference between groups B and C was statistically non-significant ($P > 0.05$) (Fig. 1).

Further, experimental chickens administered with AXs, showed relatively better feed and water intake; and showed least abnormal signs of coccidiosis while chickens in control groups were depressed, had ruffled feathers, and decreased their feed and water intake. Results of daily weight gain from days 3rd to 12th post challenge showed significantly higher ($P < 0.05$) weight gain per day in groups B and C as compared to group A and control group D and the difference between groups B and C was statistically similar ($P > 0.05$) (Fig. 2).

Lesion scoring (scale 0–4) of the survived and dead chickens showed that in group A, 45% of the chickens developed mild to moderate lesions (1.0–2.0) and 55% showed severe lesions (3.0–4.0). In group B, only 25% of the chickens had severe lesions; and 75% mild to moderate lesions and in group C, 30% chickens developed severe and 70% had mild to moderate lesions. On the other hand, 75% chickens in control group had severe lesions and moderate lesions (2.0) were also recorded in some of the chickens (20%) of control group. Means of cecal and intestinal lesion scores are shown in Table 2.

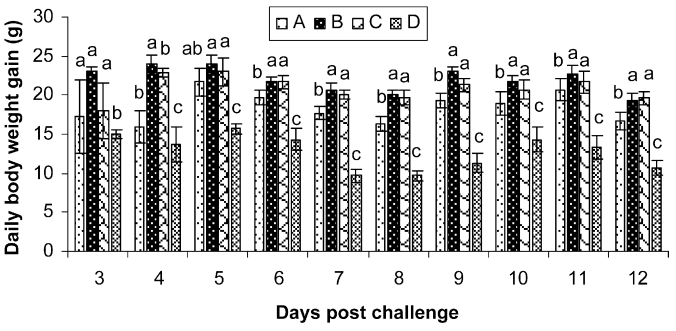

Fig. 2. Daily weight gains from days 3rd to 12th post challenge in experimental and control chickens. Bars sharing similar letters on each particular day are statistically non-significant ($P > 0.05$). A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control.

3.4. Effect on antibody titers against Eimeria species

The results of ELISA performed on sera samples collected from both AXs administered and control chickens are shown in Fig. 3 at mean absorbance level (492 nm). The results showed that mean antibody titers were significantly higher ($P < 0.05$) in chickens administered with AXs as compared to control group on day 7th post challenge. Moreover, titers were significantly lower ($P < 0.05$) in group A as compared to groups B and C; whereas, the difference between groups B and C was statistically non-significant ($P > 0.05$). A similar trend was observed on day 14th post challenge; however, all the groups showed higher OD values on day 14th as compared to those on day 7th post challenge.

3.5. Effect on the development of lymphoid organs post challenge

On day 12th post challenge, organ body weight ratios of lymphoid organs were calculated and results showed a non significant difference ($P > 0.05$) in organ-body weight ratio of spleen and bursa; whereas, organ body ratios of thymus and cecal tonsils were significantly higher ($P < 0.05$) in chickens administered with AXs as compared to control group (Table 3).

4. Discussion

Arabinoxylans (AXs) are considered to be one of the major dietary fiber components of many cereals including wheat, rice, oats, barley, rye and sorghum (Ebringerova, Hromadkova, & Heinze, 2005; Fincher & Stone, 1986). Their pharmaceutical application in human beings have been investigated by many researchers and shown to prevent and/or reduce the risk of different cancers (Gebruers et al., 2008) and treatment of some digestive disorders (Lu, Walker, Muir, & O'Dea, 2004). Several experimental studies suggested that among all the dietary fibers, wheat bran is a rich source of phytochemicals and shown to be most effective in cancer prevention and other digestive disorders in human beings (Kumar et al., 2011).

In wheat bran, AXs have been reported to increase splenocyte proliferation, promote SRBCs induced delayed hypersensitivity reaction and enhance macrophage phagocytic activity in animal models (Cao et al., 2010). In the current study, wheat bran AXs exerted stimulatory effects on antibody response in chickens. It resulted in higher total Igs, IgG and IgM anti-SRBC antibody titers on 7th and 14th days PPI and PSI of SRBCs as compared to control group. Significantly higher anti-SRBC antibody titers in AXs administered chickens indicated the alleviated humoral immunity which may be due to an increased animal's ability to resist

Table 3
Effect of Arabinoxylans on the development of lymphoid organs post challenge.

Groups	Thymus (Mean ± SE)	Spleen (Mean ± SE)	Bursa (Mean ± SE)	Cecal tonsils (Mean ± SE)
A	0.37 ± 0.027[a]	0.17 ± 0.014[a]	0.19 ± 0.016[a]	0.08 ± 0.003[a]
B	0.33 ± 0.022[ab]	0.14 ± 0.032[a]	0.18 ± 0.027[a]	0.06 ± 0.004[b]
C	0.38 ± 0.030[a]	0.16 ± 0.012[a]	0.19 ± 0.014[a]	0.07 ± 0.004[b]
D	0.27 ± 0.023[b]	0.14 ± 0.016[a]	0.16 ± 0.016[a]	0.04 ± 0.007[c]

Means sharing similar letters in a column are statistically similar ($P > 0.05$).
A = AXs (100 mg/kg of body weight); B = AXs (200 mg/kg of body weight); C = AXs (300 mg/kg of body weight); D = control.

physiological and infectious insults (Korte, Koolhaas, Wingfield, & McEwen, 2005). Further, cereal derived AXs may stimulate the immune responses by the activation of host defense potentiators, which regulate or augment the ability of host responsiveness to lymphocytokines or other intrinsic bioactive factors through maturation, differentiation or proliferation of the immune cells for host defense mechanism (Chihara, 1992). Moreover, consistent findings had also been reported with polysaccharides of bacterial origin on antibody production and delayed type hypersensitivity reaction in chickens (Maslog et al., 1999).

Organ-body weight ratios of lymphoid organs in chickens of experimental groups were statistically non-significant ($P > 0.05$) as compared to control group; although AXs administered chickens showed apparently higher live weight gain. The impact of AXs on live body weight gain of chickens can be corroborated with the previous findings of Hedge, Rai, and Goni (1978) who described that a diet containing wheat bran supported better growth than the corresponding unsupplemented diet. Growth promoting effects of wheat bran in poultry birds as feed supplement had also been reported by some workers (Darwazeh, 2010; Leeson & Summers, 2008) which support the findings of the current study.

In challenge experiment, chickens of both the experimental and control groups were challenged with mixed species of genus *Eimeria* on day 14th post-administration of AXs and phosphate buffered saline, respectively. Efficient protective effects in terms of increased weight gain and reduced oocyst shedding as indicators of bird's resistance to *Eimeria* infection were observed; although direct correlation between these two parameters was not recorded in the current study unlike the previous studies (Dalloul et al., 2005; Lee, Lillehoj, Park, Hong, & Lin, 2007). Some neutraceuticals and probiotics had also been reported to provide protection against certain infections by potentiating the cellular and humoral immune responses including *Eimeria* infection (Akhtar et al., 2012; Awais et al., 2011). In the present study, higher protection was recorded in AXs administered chickens which might have prevented the invasion or development of the coccidian parasites in the bird's intestine (Yun et al., 1997). Further, in response to AXs, certain immune mediators especially interleukin-2 had also been reported to release which might be considered responsible for countering the immunosuppressive effects of *Eimeria* infection in chickens (Banfield, Kwakkel, & Forbes, 2002). Such protective effects due to AXs might be correlated with the enhanced T-cell immune responses, characterized by interferon and interleukin-2 secretions against coccidiosis in chickens (Guo, 2003). Although, 25% protection in control group was also observed that might be due to the self-limiting phenomena occurred during the course of coccidial infection (Sharma, 1991).

Further, significantly lower ($P < 0.05$) oocyst count recorded in AXs administered chickens as compared to control, might be attributed to the better physical destruction of oocysts in the gizzard and intestine (Banfield & Forbes, 2001). Although, these findings contradicted with the previous studies of Gabriel, Mallet, Leconte, Fort, and Naciri (2006) who showed that whole wheat increased parasite development as indicated by higher lesion scores and oocyst per gram values in the droppings. Moreover, chickens of AXs administered groups were active with improved feed conversion efficiency which might be due to the fact that wheat bran AXs used as a fermentable substrate enhanced the growth of non-pathogenic, facultative anaerobes and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of the growth of enteric pathogens and enhancement of digestion and utilization of nutrients (Brisbin et al., 2008; Kabir, 2008).

In challenge experiment, survived and dead chickens were monitored for lesion scoring on a scale 0–4. Maximum (75%) chickens in control group showed severe lesions (3–4) and 25% developed mild to moderate lesions ($\leq$2.0). On the other hand, 70–75% chickens administered with AXs developed mild to moderated lesions (1.0–2.0) which might be due to decreased damage to cecal and/or intestinal mucosae. These findings suggested the involvement of some immune effector components present in the AXs which inhibited the development of the coccidian parasite (Lillehoj, 1989; Trout & Lillehoj, 1996). It has been reported that during coccidial infection, the cytokine metabolite milieu produced within the microenvironment of the bird's intestine, may lead to physiological alterations including vasodilation which caused increased hemorrhagic lesions (Allen, 1997). Further, protective effects of AXs can also be correlated with local immune responses that correspond with the onset of specific immunity to coccidial infection (Zhou et al., 2010). The higher antibody responses in AXs administered chickens can be suggested to be responsible for higher weight gain and growth rate.

Significantly higher body weight gain in chickens administered with AXs may be associated with growth factors present in the wheat bran polysaccharides (Banfield & Forbes, 2001). Significantly lower ($P < 0.05$) body weight gain in control group during coccidial infection might be due to the inflammatory immune responses occurred during coccidiosis which diverted energy from growth that affected the weight gain (Klasing, Laurin, Peng, & Fry, 1987). Growth promoting effects of wheat and its components in healthy as well as *Eimeria* infected chickens have also been reported previously (Cumming, 1987, 1992).

In the current study, elicited humoral response against *Eimeria* species (local isolates) used in challenge experiment was noted in chickens administered with AXs as compared to those in the control group. Recently, antibodies have been reported for having a key role in protective immunity against *Eimeria* infection (Wallach, 2010) in addition to their ability of efficiently hindering the development of *Eimeria* in the intestine (Rose, 1974). Smith, Wallach, Petracca, Braun, and Eckert (1994) had also reported a positive correlation between antibody titer and protection against coccidiosis. In similar studies, antibodies have been described for inducing partial protective passive immunity by blocking the growth, development and replication of coccidial parasite (Anwar et al., 2008; Crane et al., 1988; Hafeez, Akhtar, Javed, & Haq, 2007). In this study, therapeutic efficacy of AXs may be attributed to their stimulatory effects on the production of antibodies against experimentally induced coccidiosis and thus leading to higher weight gains and lower fecal egg count in chickens administered with AXs.

Results of the current study revealed that organ-body weight ratios for cecal tonsils and thymus were elevated in AXs

administered chickens as compared to control that might be due to the compensatory hypertrophy and/or cellular infiltration of lymphoid organs to enhance the production of immune cells by thymus in response to a need for increased activity during infection and recovery (Bettsille, 1986; Panda & Combs, 1964); although the exact phenomenon in this regard is still unknown.

In conclusion, the findings of the current study demonstrated that wheat bran derived AXs have the potential to stimulate the antibody mediated immune response in chickens and could be explored as a low cost alternative to allopathic drugs to prevent avian coccidiosis. Further studies are needed to elucidate the mechanism(s) of wheat bran AXs involved in immunological and therapeutic activities.

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