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Evaluation of biological response modification and immunotherapeutic activities of barley-derived arabinoxylans against coccidiosis in commercial broilers

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Abstract: Plant-derived polysaccharides have been reported for diverse biological response modification (BRM) and immunotherapeutic (IT) activities in animals by activating their defense systems to perform better immunogenically against invading pathogens. Keeping this in view, this study was conducted to assess the BRM and IT activities of barley (*Hordeum vulgare* L.)-derived arabinoxylans (AXs) against coccidiosis in broilers. Significantly enhanced ($P < 0.05$) in vivo and in vitro lymphoproliferative responses to T-cell mitogens were revealed in broilers administered barley AXs when compared to the control group. Humoral immune response in terms of antibody titers against sheep erythrocytes was also found higher ($P < 0.05$) in AX-administered chickens. Furthermore, AX-administered chickens also showed higher weekly weight gains and improved feed conversion ratios as compared to controls. Results of the challenge experiment showed that the percentage of protection and daily weight gains were statistically higher ($P < 0.05$) whereas mean oocysts per gram of droppings and lesion scores were significantly lower ($P < 0.05$) in the chickens administered barley AXs as compared to controls. In conclusion, barley-derived AXs led to better growth performance and elicited humoral and cellular immune responses in commercial broilers that persisted against coccidial infection in broiler chickens.

Key words: Biological response modification, arabinoxylans, immunotherapy, avian coccidiosis

1. Introduction

Herbs and parts of different plants have been used as medicinal sources for the treatment of different ailments since early ages (1). The literature reveals that more than 64% of people globally use botanical drugs for the treatment of different ailments (2) and on average more than 50% of synthetic drugs have been isolated from different plants or herbs (3). In this regard, cereals are one of the major sources of different drugs or chemical substances with different pharmacological aspects. Cereals contain various bioactive compounds, mainly polysaccharides, phenols, terpenoids, glycosides, and alkaloids (4,5). In the last few years, a variety of biomolecules, especially polysaccharides, have been extracted and used by different researchers to demonstrate the biological mechanisms of action for their pharmacological activities (6). Among cereals, barley (*Hordeum vulgare* L.) is one of the important cereal grains belonging to the grass family Poaceae (7). It contains different biological compounds such as vitamin E, the B-complex, minerals, and phenolic compounds (8). In Pakistan, it is being cultivated on 75,000 ha and its annual production is 70,000 t (9). Barley is frequently used in human and animal food, for the production of certain beverages and fermentable products, and in the malt processing industry (10). Arabinoxylans (AXs), nonstarch polysaccharides, are important components of cereal fibers including barley (11). AXs are mainly present in the bran portion of cereals in varying concentrations ranging from >4% to <10% (12). AXs are nondigestible oligosaccharides, which are protected from the digestive effect of the small intestine in humans with complete and/or incomplete fermentation in the large intestine (13). These carbohydrates help to maintain the normal environment of the colon and possibly contribute to human health by eliminating the risk of chronic diseases (13). Many nondigestible oligosaccharides are considered to be prebiotics that improve the health/efficiency of the host by stimulating the proliferation and/or activity of one or a few favorable bacterial species in the colon (14). It has been established that AXs have immunomodulatory and protective effects against avian coccidiosis; they also increased the daily weight gain in broiler chicks (4). AXs enhanced the immune response and downregulated the enterotoxigenic *Escherichia coli* response (15). Barley-

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extracted AXs have strong immunomodulatory effects against bowel cancer (16) and enhance the phagocytic activity of macrophages. Furthermore, they also have positive impacts on leukocyte number and spleen cell proliferation (17). Other biological activities of AXs include remarkable increases in thymus growth, spleen index, and antitussive activity (17,18). Previous studies also revealed antioxidant, antitumor, and prebiotic properties of AXs (17,19).

The poultry industry in Pakistan still lacks proper concern regarding different infectious ailments and coccidiosis is one of the major concerns. Previously, studies have been conducted on different formulations of herbs and plants for a good remedy or therapy to control this difficult disease. Although different plants have been studied on the basis of their immunomodulatory effects against coccidiosis, no such work has been conducted on barley-derived AX polysaccharides. Keeping in view the diverse activity of AXs from different sources, this study was conducted to investigate the biological response modification (BRM) and immunotherapeutic (IT) activities of barley-derived AXs against coccidiosis in chicken.

2. Materials and methods

2.1. Procurement and pretreatment of barley bran

Barley (*Hordeum vulgare* L.) grains were procured from a local market in Faisalabad, Pakistan, and processed to obtain destarched bran following the method of Akhtar et al. (4). The bran thus obtained was stored at 4 °C until further use.

2.2. Extraction of AXs

Polysaccharide from the destarched bran was extracted following the method of Zhou et al. (20) with minor modifications. Briefly, dried barley bran powder (100 g) was mixed with 1.5 L of 0.15 N NaOH and 0.5% H_2O_2 (v/v) for 90 min at 80 °C. The mixture was cooled to room temperature (26 °C) and centrifuged (314 × *g* for 30 min). The supernatant thus collected was neutralized with 0.2 N HCl (pH 4.5) followed by centrifugation (314 × *g* for 30 min) to concentrate up to one-fourth of the actual volume under decreased pressure. The concentrate was precipitated with the help of ethanol (65%), preceded by centrifugation (314 × *g* for 30 min at 4 °C). The sediment was dissolved in water and centrifuged (314 × *g* for 30 min at 4 °C). The supernatant was again precipitated by ethanol (65%) and the same procedure was repeated. The fractions thus obtained as sediments were used as the alkaline extract of polysaccharides and freeze-dried until further use.

2.3. Infective material

Sporulated oocysts of mixed *Eimeria* species, *E. tenella*, *E. acervulina*, and *E. necatrix*, maintained in the Immunoparasitology Laboratory of the University of Agriculture, Faisalabad (UAF), Pakistan, were used for the challenge experiment in this study. The infective dose was adjusted to 6.5–7.0 × 10^4 sporulated oocysts per 5 mL of phosphate-buffered saline.

2.4. Experimental design

A total of 200 one-day-old broiler chicks (Hubbard strain) were purchased from a local hatchery and raised in a coccidia-free environment at the Animal House of the Faculty of Veterinary Science, UAF. All the chickens were fed anticoccidial-free feed and water ad libitum. Chickens of all groups were vaccinated against endemic infection according to the local vaccination schedule. During the experimental trial, all procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of UAF.

At 7 days of age, chickens were randomly divided into four equal groups, A_1, A_2, A_3, and A_4 (n = 50 in each group), and administered barley AXs for 3 consecutive days (i.e. at 7, 8, and 9 days of age) as follows:

Group A_1 = Barley AXs @ 100 mg/kg of body weight (BW)

Group A_2 = Barley AXs @ 200 mg/kg of BW

Group A_3 = Barley AXs @ 300 mg/kg of BW

Group A_4 = Negative control with no treatment

2.5. Evaluation of BRM activities of barley AXs

On day 14 following AX administration, 30 chickens of each group were used for immunological evaluation and the remaining 20 birds were used for a therapeutic trial. Lymphoproliferative response to phytohemagglutinin-P (PHA-P) and concanavalin-A (Con-A) and antibody response to sheep red blood cells (SRBCs) were used to detect the cell-mediated and humoral immune responses, respectively.

Classic toe-web assay was used to quantify the in vivo lymphoblastogenic response as described by Corrier (21), whereas in vitro response was determined according to the methodology described by Qureshi et al. (22).

Anti-SRBC antibody titers were detected using a microplate hemagglutination test (23) with minor modifications (24).

2.6. Weekly weight gains and feed conversion ratios (FCRs)

Chickens from all groups were weighed individually every week after the administration of AXs. Feed given to each group was also recorded on a weekly basis and the data thus obtained were used to calculate the FCRs using the following formula:

FCR = Feed consumption (g) / body weight gain (g)

2.7. Immunotherapeutic evaluation

Twenty chickens from each group were infected with 6.5–7.0 × 10^4 sporulated oocysts of mixed species of the genus *Eimeria* (local isolates *E. tenella*, *E. acervulina*, and *E.*

necatrix) on day 14 following AX administration. Chickens of all groups were monitored for body weight gain per day and oocyst counts using the McMaster counting technique (25) from day 3 to 12 following the challenge with *Eimeria* species. During the challenge experiment, chickens of each group were also monitored for mortality. Dead chickens were scored for lesion scoring and surviving chickens in all groups were also slaughtered and scored (26). The percentage of protection against lesions was determined using the following formula:

Percent protection against lesions = Average lesion score (IUG) – average lesion score (IMG) / average lesion score (IUG) × 100

Here, IUG = infected untreated group and IMG = infected medicated group.

Chickens of each group were separately weighed and slaughtered on day 12 following the challenge. Lymphoid organs like the bursa of Fabricius, thymus, spleen, and cecal tonsils were excised and weighed. The data were presented as percent organ to body weight ratios.

2.8. Statistical analysis

Data thus collected were statistically analyzed through one-way ANOVA and Duncan's multiple range tests using statistical analysis software (27). The difference in mean values of different groups was considered significant at $P < 0.05$.

3. Results

3.1. Evaluation of BRM activity

3.1.1. In vivo lymphoproliferative response to PHA-P

The maximum lymphoproliferative response in terms of toe-web swelling was recorded at 24 h after injection of PHA-P both in AX-administered and control groups, followed by those recorded at 48 and 72 h after injection. At 24 h, a significantly higher response ($P < 0.05$) was detected in experimental chickens administered barley AXs at dose rates of 200 and 300 mg/kg of BW when compared with the control group. At 48 h, irrespective of the dose, all experimental groups revealed statistically higher responses ($P < 0.05$) as compared to the control, whereas, at 72 h only the chickens administered with AXs at a dose rate of 300 mg/kg of BW showed statistically higher responses ($P < 0.05$) as compared to the control group (Figure 1a).

3.1.2. In vitro lymphoproliferative response to Con-A

At day 7 after the administration of barley AXs, lymphoproliferative response to Con-A was significantly higher ($P < 0.05$) in group A_3 (0.81 ± 0.01), followed by group A_2 (0.72 ± 0.00), A_1 (0.41 ± 0.11), and A_4 (0.24 ± 0.04), respectively. A similar response was detected on day 14 after the administration of barley AXs (Figure 1b).

3.1.3. Antibody response to SRBCs

At day 7 post primary injection (PPI) of SRBCs, total anti-SRBCs titers (geomean titers) were higher in chickens administered barley AXs in graded doses when compared with the control group; however, a difference was also observed among the experimental groups administered graded doses of barley AXs. A similar response was detected on day 14 PPI of SRBCs. Moreover, results showed similar patterns of total anti-SRBC antibody titers on days 7 and 14 post secondary injection (PSI) of SRBCs like those on days 7 and 14 PPI of SRBCs. On the whole, the highest total antibody titers were detected in chickens of group A_2 administered barley AXs at a dose rate of 200 mg/kg BW (Table 1). A similar pattern of results was recorded for IgM anti-SRBC antibody titers (Table 1).

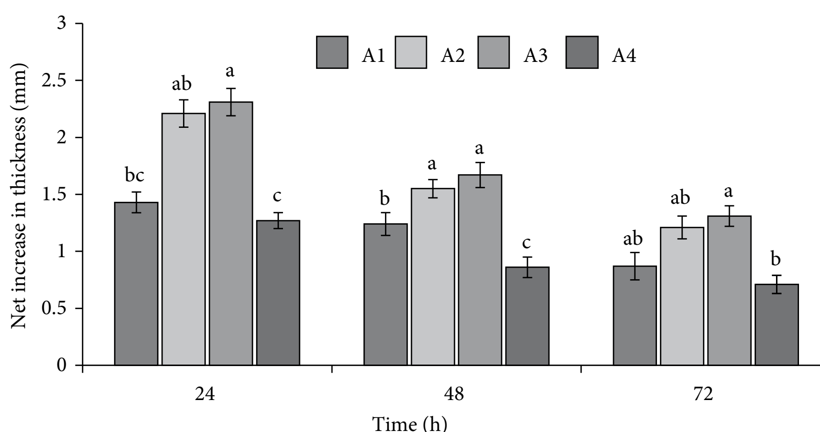

Figure 1a. In vivo lymphoproliferative response to phytohemagglutinin-P in experimental and control chickens.
Bars not sharing similar letters in the same time intervals differ significantly ($P < 0.05$).
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

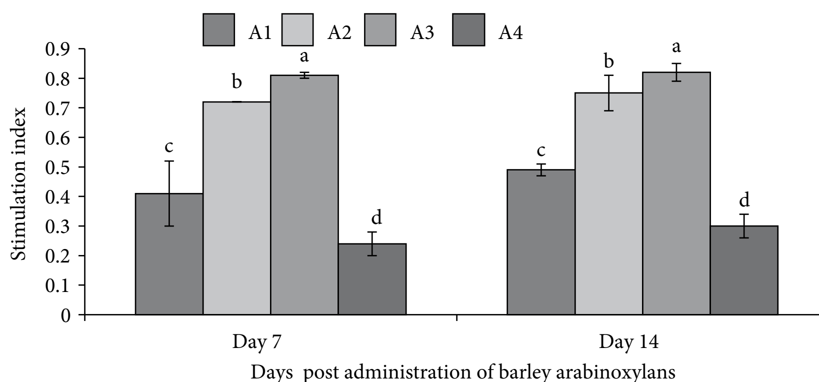

Figure 1b. In vitro lymphoproliferative response to concanavalin-A in experimental and control chickens.
Bars not sharing similar letters in the same time intervals differ significantly ($P < 0.05$).
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

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

Total anti-SRBC antibody titers				
Group	Day 7 PPI	Day 14 PPI	Day 7 PSI	Day 14 PSI
A_1	36.76	27.86	48.50	32.00
A_2	55.72	42.22	64.00	48.50
A_3	42.22	36.76	55.72	42.22
A_4	21.11	18.38	27.86	12.13
Immunoglobulin-M				
A_1	24.63	9.48	20.65	11.74
A_2	34.6	17.97	27.24	11.74
A_3	23.85	15.64	23.72	10.22
A_4	11.92	4.45	9.48	2.94
Immunoglobulin-G				
A_1	12.13	18.38	27.86	27.85
A_2	12.11	24.25	36.76	36.76
A_3	18.38	21.11	32.00	32.00
A_4	9.19	13.93	18.38	9.18

PPI = Post primary injection; PSI= post secondary injection.
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

On day 7 PPI of SRBCs, the IgG anti-SRBCs antibody titer was highest in group A_3 administered barley AXs (300 mg/kg BW), followed by those of groups A_2 and A_1, respectively. On days 14 PPI, 7 PSI, and 14 PSI of SRBCs, the patterns of IgG titers in the experimental and control groups were similar to those obtained for total anti-SRBC antibodies titers on the corresponding days (Table 1).

3.2. Effect on weekly body weight gains

The effect of barley AXs on live body weight gains was recorded on a weekly basis from week 1 to week 5 following the administration of barley AXs. The results showed significantly higher ($P < 0.05$) weight gains in chickens administered barley AXs as compared to those of the control group, whereas among the AX-administered groups, significantly higher ($P < 0.05$) response was detected in chickens of group A_2 when compared with those of groups A_1 and A_3 (Table 2).

3.3. Feed conversion ratios

FCR was measured on a weekly basis in all groups from week 1 to week 5 following the administration of AXs. All AX-administered groups showed improved FCRs as compared to controls. On the whole, the most improved weekly FCRs were detected in chickens of group A_1 (2.07), followed by those of group A_2 (2.08), group A_3 (2.11), and the control group (2.20).

3.4. Challenge experiment

3.4.1. Oocyst count

Results revealed significantly lower ($P < 0.05$) oocyst counts in experimental groups administered AXs as compared to controls. On the other hand, among the experimental groups, chickens of group A_2 (200 mg/kg BW) showed the lowest counts, followed by those of groups A_1 (100 mg/kg BW) and A_3 (300 mg/kg BW), respectively; the difference among experimental groups was statistically significant ($P < 0.05$) (Table 3).

3.4.2. Percent protection

The highest protection (65%) was recorded in chickens of group A_3 (300 mg/kg BW), followed by those of group A_2 (60%), group A_1 (55%), and the control group (35%). Chi-square analysis revealed a significantly higher protection rate ($P < 0.05$) in all AX-administered groups compared to the control, whereas the difference among AX-administered groups was statistically similar. In

Table 2. Weekly weight gains in chickens from week 1 to week 5 after administration of arabinoxylans in experimental and control chickens.

Week	Groups			
	A_1	A_2	A_3	A_4
1	395 ± 26.6^b	410 ± 4.56^a	405 ± 5.92^a	394 ± 4.56^b
2	596 ± 16.7^b	589 ± 6.52^b	610 ± 5.15^a	575 ± 11.2^c
3	980 ± 8.22^b	1005 ± 8.08^a	964 ± 8.72^c	910 ± 8.54^d
4	1450 ± 9.7^c	1530 ± 9.82^a	1480 ± 7.42^b	1370 ± 2.4^d
5	1885 ± 5.7^b	1930 ± 3.24^a	1830 ± 9.11^c	1650 ± 12.3^d

Means sharing similar letters in a row are statistically nonsignificant ($P > 0.05$).
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

Table 3. Oocysts per gram of droppings after challenge in experimental and control chickens.

Days after challenge	Groups			
	A_1	A_2	A_3	A_4
4	$17{,}338 \pm 45.18^b$	1261 ± 15.53^d	1410 ± 15^c	$35{,}643 \pm 174.69^a$
5	$21{,}831 \pm 39.95^b$	2972 ± 25.42^d	3699 ± 11^c	$51{,}459 \pm 178.84^a$
7	$191{,}755 \pm 205.9^b$	7100 ± 112.58^d	$11{,}994 \pm 216^c$	$205{,}203 \pm 719.03^a$
9	$221{,}606 \pm 171.85^b$	$24{,}491 \pm 232.77^d$	$41{,}559 \pm 317.08^c$	$316{,}010 \pm 280.15^a$
11	$312{,}691 \pm 184.37^b$	$22{,}167 \pm 159.9^d$	$18{,}281 \pm 157.8^c$	$352{,}602 \pm 530.45^a$
12	$305{,}752 \pm 97.2^b$	$13{,}315.67 \pm 343.22^d$	$14{,}104 \pm 176.01^c$	$345{,}407 \pm 238.7^a$

Means sharing similar letters in a row are statistically nonsignificant ($P > 0.05$).
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

general, it was observed that chickens maintained on AXs were relatively active with better feed and water intake as compared to the control group, in which chickens were lethargic with ruffled feathers and with less water and feed intake. Among postmortem findings, severe hemorrhagic lesions were found in the intestines of the birds of the control group while birds of AX-administered groups showed less severe lesions.

3.4.3. Lesion scoring

Both dead and surviving (sacrificed) chickens in all groups were scored for lesions on the intestines and ceca. The chickens were observed for lesion scoring on a scale of 0 to 4 on the basis of severity of lesions. Lower cecal lesion scores were recorded in groups administered with AXs when compared with the control group. The score of severe cecal lesions (3–4) was maximum (75%) in the control group, whereas in experimental groups the score for severe lesions ranged from 30% to 60%. The lowest score for severe cecal lesions was seen in group A_3 (AXs at a dose rate of 300 mg/kg BW). Similar to the cecal lesions, the score of severe intestinal lesions was also found maximum (80%) in the controls. Minimum severe intestinal lesions (3–4) were recorded in group A_3 (25%), followed by groups A_2 (35%) and A_1 (55%), respectively. Percentage protections against intestinal and cecal lesions are shown in Figure 2.

3.4.4. Daily weight gains after challenge

The effect of barley AXs on daily weight gain of chickens was recorded from day 3 to day 12 following challenge with mixed species of *Eimeria* and results are expressed as grams (±SE) (Table 4). The chickens of AX-administered groups showed significantly higher ($P < 0.05$) weight gains when compared to controls. Among the AX-administered groups, those administered with AXs at a dose rate of 200 mg/kg of body weight showed the highest daily weight gains followed by groups A_2 and A_3, respectively, and the difference was statistically significant ($P < 0.05$).

4. Discussion

Barley is one of the major cereal crops being cultivated throughout the world (9). It is mainly used for malting, beverages, and human and animal foods (7). It is mainly composed of starch (49.4%–66.2%), dietary fiber (13.6%–27.5%), and crude protein (9.3%–21.9%) (28). Dietary fiber contains variable amounts of polysaccharides like AXs and β-glucans. The AXs, nonstarch polysaccharides, are the primary components of the cell wall of different cereals including oat, barley, rice, rye, and wheat (29,30) and in barley bran their concentration is 4%–11% (12,28). Pharmacological activities of cereal-derived polysaccharides, especially AXs, decreased the glucose level in diabetes patients and increased fecal output (31). AXs also have prebiotic effects on the gastrointestinal tract by stimulating intestinal bacteria, which have beneficial effects on health (32,33). Immunomodulatory activities of AXs, either by enhancing the activity of natural killer cells (34) or reducing inflammation and pain, were also reported previously (18,35). Results of the present study revealed higher cellular immune responses in chickens administered barley-derived AXs as compared to those of the control group. The higher level of cellular immune responses in chickens administered a graded dose of AXs might be attributed to the enhanced effects on the phagocytic activity of macrophages that may cause increase in the thickness of the toe-web due to T-cell mitogens (36,37). Toe-web swelling in response to PHA-P in chickens due to enhanced delayed-type hypersensitivity suggested that the magnitude of immune response might be dependent on the function and number of lymphocytes (38,39). Therefore, it can be speculated that an increase in

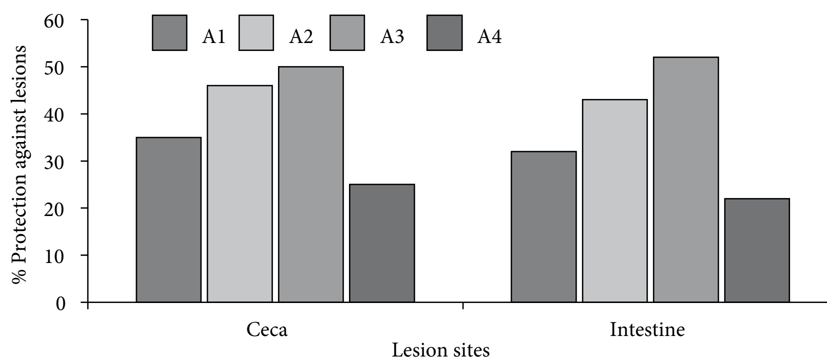

Figure 2. Percent protection against cecal and intestinal lesions following challenge in experimental and control groups.
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

Table 4. Daily weight gains from day 3 to day 12 after challenge in experimental and control chickens.

Days after challenge	Groups			
	A_1	A_2	A_3	A_4
2	33 ± 1.0[ab]	35.3 ± 1.53[a]	31.67 ± 1.53[b]	26 ± 1.00[c]
3	32.67 ± 1.53[b]	38.67 ± 1.53[a]	33.00 ± 2.00[b]	26.67 ± 2.08[c]
4	34.33 ± 1.53[b]	35.33 ± 1.16[a]	31.67 ± 1.53[b]	24.33 ± 1.16[c]
5	28.33 ± 1.53[c]	32.67 ± 1.53[a]	30.67 ± 2.08[b]	22.33 ± 1.53[c]
6	25.33 ± 1.53[c]	32.67 ± 1.53[a]	29.33 ± 2.08[b]	21.33 ± 1.53[d]
7	25.67 ± 1.53[b]	30.33 ± 1.53[a]	28.67 ± 1.53[b]	22.3 ± 1.16[d]
8	26.67 ± 2.52[b]	31.67 ± 2.08[a]	27.67 ± 1.53[b]	21.33 ± 1.53[c]
9	23.67 ± 2.52[b]	28.33 ± 0.58[a]	29.33 ± 1.5[a]	22.00 ± 1.00[b]
10	25.00 ± 3.0[a]	30.67 ± 2.08[a]	30.00 ± 1.00[a]	23.00 ± 1.00[b]
11	31.33 ± 2.52[a]	32.00 ± 2.00[a]	30.33 ± 1.53[a]	25.33 ± 0.58[b]
12	34.67 ± 2.08[a]	35.67 ± 0.58[a]	31.33 ± 0.58[c]	27.33 ± 2.08[c]

Means sharing similar letters in a row are statistically nonsignificant ($P > 0.05$).
A_1 = Barley-derived AXs @ 100 mg/kg of BW; A_2 = barley-derived AXs @ 200 mg/kg of BW; A_3 = barley-derived AXs @ 300 mg/kg of BW; A_4 = control.

the number and activity of lymphocytes in the lymphoid tissue may be responsible for the development of the improved immune response (40,41).

The birds administered barley AXs showed higher in vitro lymphoblastogenic response when compared with the control group. This higher response might be due to the fact that T-cells containing mitogen receptors undergo cell division upon coming into contact with Con-A (22). Furthermore, it could also be speculated that Con-A stimulated the peripheral blood leukocytes, which produced IL-1 by monocytes in the peripheral blood leukocyte fraction, which stimulated the propagation of lymphocytes (42). The higher humoral immune responses in barley AX-administered chickens might be due to stimulatory effects of polysaccharides on the classical complement pathway in serum by their interaction with antibodies (43,44). Results of the present study are consistent with previous findings of similar studies that oral administration of sugars resulted in higher antibody titers to SRBCs in chickens artificially inoculated with *Eimeria*, elevated antibody response to SRBCs in immunocompromised chickens, increased serum antibody responses against SRBCs and *Brucella abortus*, and increased number of plasma cells in PBLs, enteric leukocytes, and splenocytes (4,36,37,39,45). Results of this study showed higher body weight gains and improved FCRs in chickens administered graded doses of barley AXs as compared to those of the control group. The improved body weight gains and FCRs in AX-administered birds might be correlated with the ability of AXs to reduce the environmental stress and infectious insults by immunostimulation (37).

In the coccidiosis disease model, weight gain and oocyst count are two important parameters to detect the severity of the disease (4,39,46). In this study, increased weight gain and lower oocyst counts were recorded in AX-administered chickens, which indicated the resistance against *Eimeria* infection, although the direct relationship was not measured in the current study. Immune-enhancing effects of various native foods and herbal preparations on hosts against microbes and tumors have been reported to have a direct correlation with their ability to induce lymphocyte proliferation (47,48). In the case of coccidiosis, some medicinal foods and probiotic extracts have been reported as protective agents against various bacterial and parasitic infections by accelerating the cellular and humoral immunity against *Eimeria* (39,45,49). In this challenge experiment, a higher protection rate was detected in AX-administered groups as compared to controls. It has been reported that polysaccharides potentiate the complement system and antibodies to stop or minimize parasitic infestation, especially *Eimeria*, in chickens (43,44). Cereal-derived AXs have also shown some effects of protection by interacting with local immune responses (4,39), which might be responsible for activation of specific immunity against coccidiosis (45), thus ultimately resulting in lower oocyst counts. Furthermore, chickens

of the AX-administered groups were relatively active with higher weight gains as compared to the control group. Lower weight gains in control groups might be attributed to homeostasis imbalance in the gastrointestinal tract (50) leading to less feed intake, slower metabolism, and eventually lower weight gains (51–53). Furthermore, lower body weights might also be due to inflammation and maximum energy utilization against coccidial invasion and hence poor weight gains (39,54). On the other hand, higher body weight gains in AX-administered chickens might be correlated with the activity of AXs to stimulate the monocytes, natural killer cells, and neutrophils that release cytokines and enhance the leukocyte activity, thus efficiently improving the mechanism of host recovery (31). Previous studies also revealed the activity of polysaccharides to activate macrophages against different microorganisms and thus enhance protection against their pathogenic effects (55).

In conclusion, barley-derived AXs showed immunostimulatory and growth-promoting effects in broiler chickens and subsequently provided protection against *Eimeria* infection. Further studies are required for the elucidation of the exact mechanism(s) of action involved in such immunostimulatory activities. In the future, it would definitely help to investigate the possibility of the use of barley-derived AXs as effective nutraceuticals in the poultry industry.

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