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Full Length Article

Evaluation of *Avena sativa* Derived Arabinoxylans as Native Biological Response Modifiers in Broilers

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Abstract

This study was aimed to assess the immunomodulatory effects of *Avena sativa* (oat) derived arabinoxylans (AXs) in chickens. The extracted AXs were evaluated for immunological and immunotherapeutic activities against coccidiosis in chickens. Oat derived AXs significantly enhanced (P<0.05) the *in vitro* and *in vivo* lympho-proliferative responses to T-cell mitogens in chicken. Moreover, significantly higher (P<0.05) humoral immune retorts in terms of antibody titres (GMT) to sheep red blood cells (SRBCs), Newcastle disease and Infectious bursal disease vaccines were also detected in AXs administered chickens; whereas, the difference in organ-body weight ratios was statistically non-significant between AXs administered and control chickens (P>0.05). Results of the challenge experiment revealed that the daily weight gains and percent protection were statistically higher (P<0.05); while, mean oocysts per gram of droppings and lesion scores were lower (P<0.05) in the AXs administered chickens than the control group. In conclusion, oat derived AXs had immunopotentiating potential that persisted against coccidial infection in chickens.

Keywords: *Avena sativa*; Biological response modification; Coccidiosis; Chicken

Introduction

Recently, plant derived polysaccharides have gained much significance in the biomedical amphitheatre due to their broad therapeutic spectrum in addition to immunomodulatory activities (Wasser, 2002). Most of the natural/ plant originated compounds are comparatively less toxic due to their compatibility with the body tissues and hence, do not cause significant side effects, as with the use of synthetic drugs being used as immunomodulators (Schepetkin and Quinn, 2006). Under the circumstances, plant derived polysaccharides may be good alternatives as they have been widely reported for therapeutic activities including antitumor, wound-healing, radioproteictive hematopoietic, anti-atherosclerotic and immunostimulatory properties (Lazareva *et al*., 2002). It is considered that the plant derived polysaccharides modulate the innate immunity (Schepetkin and Quinn, 2006; Khaliq *et al.*, 2016).

In current era, there has been developed a considerable interest in the polysaccharides rich extracts from different cereals because of their immunomodulatory and therapeutic activities in different animal models (Yun *et al*., 2003). In this regard, several experimental and clinical studies have been conducted against various infections/diseases in human beings with promising results (Schepetkin and Quinn, 2006). In this regard, very few studies have been reported in animals but no published data on any avian species is available.

Keeping in view, the current study was designed to evaluate the oat bran derived arabinoxylans (AXs) as native BRMs in broilers and to see their subsequent protective effect against *Eimeria* infection. Results of this study will provide opportunity to identify natural BRMs that may exhibit beneficial immunostimulatory properties in chickens and the novel immunotherapeutic agents against *Eimeria* infection.

Materials and Methods

Procurement and Processing of Oat Bran for Extraction of Arabinoxylans (AXs)

Oat (*Avena sativa*) procured from local market was processed to obtain bran following the method of Akhtar *et al*. (2012). Polysaccharides (AXs) from the de-starched bran were extracted as described by Zhou *et al*. (2010). The fraction thus obtained was used as alkaline extract of AXs and freeze dried for further use.

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Infective Material

Mixed *Eimeria (E.)* species (including *tenella, necatrix, maxima and acervulina*) maintained at Immuno-parasitology Lab, University of Agriculture, Faisalabad, Pakistan (UAF) were administered orally at the rate of 6.5×10^4 sporulated oocyts per bird to induce infection in the experimental birds.

Experimental Design

Two hundred broiler chicks (One-day-old) were procured from hatchery and reared at experimental poultry shed, Parasitology Department, UAF. The birds were offered anti-coccidial free diet and water *ad libitum*. At 7th day of age, birds were randomly distributed into four equal groups (n= 50) and the following treatments were administered orally for three consecutive days (Akhtar *et al*., 2012) as follows:

Group B_1= Oat derived AXs @ 100 mg/kg of body weight (BW)/day
Group B_2= Oat derived AXs @ 200 mg/ kg of BW/day
Group B_3= Oat derived AXs @ 300 mg/ kg of BW/day
Group B_4 = Control group (administered with phosphate buffered saline)

Chickens in all the four groups were routinely vaccinated for immunization.

Immunological Evaluation

On 14th day post administration of AXs, thirty chickens from each group were used for immunological investigations and the remaining 20 birds for therapeutic evaluation against *Eimeria* infection.

Antibody Response to SRBCs

Anti-SRBC antibody titers (GMT) were monitored by haemagglutination assay according to Qureshi and Havenstein (1994) research methodology.

Lymphoproliferative Responses to Phytohaemagglutinin-P (PHA-P) and Concanavalin-A (Con-A)

In vitro lymphoproliferative response to Con-A was calculated by using the methodology described by Qureshi *et al.* (2000); whereas, *in vivo* lymphoblastogenic response to PHA-P was detected by using the method described by Corrier (1990).

Immunotherapeutic Evaluation

Remaining twenty birds in each group were challenged with sporulated oocysts of mixed *Eimeria* species (65,000-70,000 sporulated oocysts/ 5 mL; local isolates as mentioned above) on 14th day post-administration of oat derived AXs. All the challenged groups were observed for daily body weight gains, from day 3rd to 12th post-challenge. Oocysts per gram of droppings (OPG) of all the groups were calculated by using McMaster counting technique on daily basis from day 3rd to 12th post challenge. Mortality was observed in all groups and; chickens (both survived and dead) from all the groups were scored for lesions on caeca and intestines (Johnson and Reid, 1970). On day 12th post-challenge, the birds were killed humanely and lymphoid organs were isolated and weighed. The data obtained was presented as percent organ-body weight ratios.

Results

Immunological Evaluation

***In vivo* lymphoroliferative response to PHA-P:** The maximum response was detected at 24 h post-PHA-P injection in both oat AXs administered and control groups followed by those at 48 and 72 h, respectively. Maximum swelling (mm±SE) (0.677±0.034) was recorded in group B_2 administered with oat AXs (200 mg per kg of BW) after 24 h followed by group B_3 (0.665±0.035), B_1 (0.585±0.043) and control group (0.511±0.042), respectively. A non statistical difference was ($p>0.05$) found between all the experimental groups offered with graded doses of oat bran AXs; whereas, this difference was statistically higher ($p<0.05$) in AXs administered groups as compared to control. A similar response was recorded at 48 and 72 h after PHA-P injection (Fig. 1).

***In vitro* lymphoproliferative response to Con-A:** On day 7th and 14th post administration of oat bran AXs, *in vitro* lymphoproliferative response was detected in experimental and control groups in terms of lymphoblastogenesis by chicken peripheral blood lymphocytes (PBLs) against Con-A. On both day 7th and 14th post administration of oat bran AXs, significantly higher ($p<0.05$) response was detected in the PBLs of chickens offered with graded doses of oat bran AXs, when compared with control group. On the other hand, among the treatment groups, administered with graded doses of AXs, the lymphoblastogenic response was similar statistically ($p>0.05$) (Fig. 2).

Humoral Response to SRBCs

Total anti-SRBCs antibody titre: At 7th day post primary injection (PPI), total anti-SRBC antibody titres were higher in chickens offered with oat derived AXs, when compared with those of control group. Overall, group B_2 (Oat AXs @ 200 mg per kg of BW) represented the better achievement of results in terms of maximum titers (GMT 55.71) to sheep RBCs. At day 14th PPI of sheep RBCs, birds of group B_2 and B_3 revealed higher response than chickens of group B_1 and control; while the difference between group B_2 and B_3 was negligible. Total anti-SRBC antibody titers were higher in chickens of AXs administered groups at day 7th post secondary injection (PSI) than control group. Similar trend

Table 1: Antibody titers to sheep red blood cells in arabinoxylans administered and control chickens

Total anti-SRBCs antibody titer				
Group	Day 7 PPI	Day 14 PPI	Day 7 PSI	Day 14 PSI
B_1	42.22	36.75	48.50	55.71
B_2	55.71	48.50	64.00	73.51
B_3	48.50	42.22	55.71	64.00
B_4	32.00	27.85	42.22	48.50
		Immunoglobulin-M		
B_1	24.82	16.00	24.25	18.38
B_2	32.76	21.11	27.85	24.25
B_3	28.52	18.37	24.25	24.25
B_4	18.81	12.13	18.37	16.00
		Immunoglobulin-G		
B_1	16.00	19.93	24.25	35.50
B_2	21.11	26.30	34.70	46.88
B_3	18.37	22.89	30.21	37.64
B_4	12.12	15.10	22.89	30.50

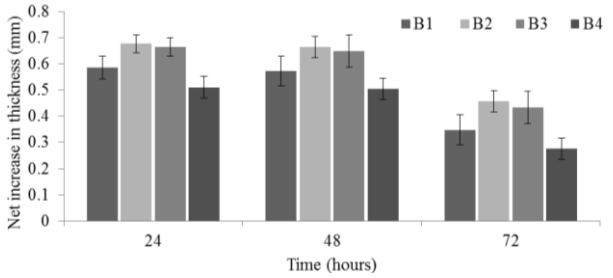

Fig. 1: *In vivo* lymphoproliferative response to Phytohaemagglutinin-P in arabinoxylans administered and control chickens

B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

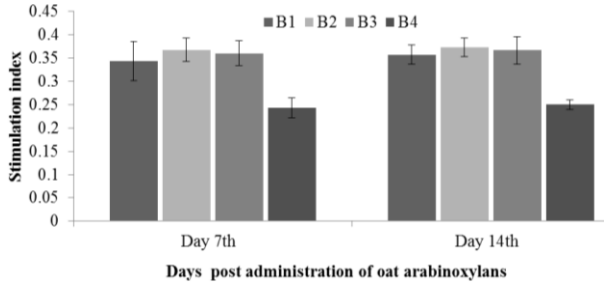

Fig. 2: *In vitro* lymphoproliferative response to Concanavalin- A in arabinoxylans administered and control chickens

B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

was observed in total anti-SRBC antibody titers at day 14th PSI (Table 1).

IgM anti-SRBC antibody titer: At 7th and 14th day PPI, IgM titers were higher in chickens managed on oat AXs compared to control group. On day 7th PPI, group B_2 revealed the maximum response (GMT 32.76) followed by B_1 (24.82), B_3 (28.52) and control group. On day 7th PSI, about 50% of the total Igs, IgM were observed. Among all the groups, maximum titers were recorded in groups B_2 and B_3. The minimum levels of IgM were observed at 14th day PSI in all the groups (Table 1).

IgG anti-SRBC antibody titer: At day 7th PPI, anti-SRBC IgG titers were higher in oat AXs offered groups than control group. Group B_2 revealed the maximum immune response (GMT 21.11) followed by those of group B_1 (16.00) and B_3 (18.37). At day 7th PSI, about 50% of total Igs were IgG. The peak of IgG titres were observed at day 14th PSI. The higher anti-SRBC IgG titers were found in AXs administered groups when compared with control group (Table 1).

Effect on the Growth of Lymphoid Organs

Organ-body weight ratios of lymphoid organs including caecal tonsils, bursa, spleen and thymus, were observed on 35th day post-administration of oat AXs. On the basis of the statistical analysis, the organs to body weight ratios of chickens of AXs administered and control groups were statistically similar (P>0.05).

Challenge Experiment

Oocyst count: The OPG values were observed from day 4th to 12th day post challenge and results showed statistically elevated (P<0.05) OPG values in chickens of control group, when compared with those administered with oat derived AXs. Among AXs administered groups, OPG values were significantly lower (P<0.05) in group B_2 and B_3 when compared with group B_1 (Fig. 3).

Percent protection: Percent protection was highest in group B_2 (60%) followed by group B_3 (55%) and B_1 (45%) while minimum in control group D (30%). Post-mortem findings of control group revealed hemorrhagic lesions on caeca and intestine and most of them filled with blood (Table 2).

Lesion scoring: Chickens (both dead and survived) of AXs administered and control groups were scored for lesions on intestine and caeca. Relatively lower caecal lesion scores were recorded in groups offered with oat AXs than control group. The score of severe caecal lesions (3–4) was maximum (80%) in control group; whereas in experimental groups the score for severe lesions ranged from 45–65%. The lowest score for severe caecal lesions was demonstrated by group B_2 administered with oat AXs (200 mg/kg of BW) (Table 3). Similar to the caecal lesions, the score of severe intestinal lesions was also recorded highest (75%) in control group. The minimum severe intestinal lesions (3–4) were recorded in group B_2 (45%) followed by those of group B_3 and B_1 (65%, each) respectively (Table 3).

Daily weight gain: Effect of oat AXs on daily weight gain of chickens was recorded from day 2nd to 12th post challenge with mixed species of *Eimeria* and results are expressed as

Table 2: Per cent mortality and protection in arabinoxylans administered and control chickens post challenge with Eimeria species

Group (n)	Dose administered	Mortality (%)	Protection (%)
B_1 (n=20)	100 mg /kg of B. Wt.	55^a	45^b
B_2 (n=20)	200 mg /kg of B. Wt.	40^a	60^b
B_3 (n=20)	300 mg /kg of B. Wt.	45^a	55^b
B_4 (n=20)	Control	70^b	30^a

Different letters in a particular column are presenting a non-significant difference (P>0.05).
B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

Table 3: Lesion scoring in arabinoxylans administered and control chickens post challenge with Eimeria species

Group (n)	Caecal lesion Scoring of Birds				
	0	+	++	+++	++++
B_1 (n=20)	0	3	4	5	8
B_2 (n=20)	0	5	6	4	5
B_3 (n=20)	0	4	5	6	5
B_4 (n=20)	0	0	4	5	11
	Intestinal lesion Scoring of Birds				
B_1 (n=20)	0	3	4	7	6
B_2 (n=20)	0	5	6	5	4
B_3 (n=20)	0	3	4	10	3
B_4 (n=20)	0	1	4	5	10

B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

Mean (gms)±SE. The chickens of experimental groups administered with oat AXs showed significantly higher (P<0.05) weight gains as compared to control group (B_4). Among the AXs administered groups, those administered with AXs (200 mg//kg of BW; B_2) showed highest daily weight gains followed by groups B_3 and B_1 (Table 4).

Discussion

The plants, herbs and their products have traditionally been reported as immunomodulators and therapeutics. Upto 19th century; the isolation, identification and characterization of their dynamic compound(s) did not get much attention (Phillipson, 2001). About 64% humans uses ayurvedic and plant drugs in different health related problems (Farnsworth, 1990) overall about 50% synthetic medicines have been derived from plants and their products (Anonymous, 1988). Specifically immune system of an organism is modified by vaccination and non-specifically by immunomodulators (Masihi *et al*., 1992, 1997; Masihi, 1994).

Cereals are an important source of biologically active compounds containing mainly glycosides, terpenoids, phenols, polysaccharides and alkaloids (Wills *et al*., 2000). Extensive studies on dietry fibres expressed the utilization of whole grain, bran and readily fermentable carbohydrate compounds of whole cereal grains have much impact on many physiological biomarkers in humans (Salvin, 2003;

Table 4: Daily weight gains post challenge with *Eimeria* species in Arabinoxylans administered and control chickens

Days	Group			
	B_1	B_2	B_3	B_4
2	$29.33±0.57^b$	$33.00±2^a$	$32.33±1.527^a$	$28.00±1.00^b$
3	$31.00±1^a$	$33.33±.57^a$	$33.00±2.645^a$	$30.00±1.73^a$
4	$30.33±1.15^a$	$32.33±0.57^a$	$32.66±1.52^a$	$30.33±0.57^a$
5	$29.6±1.52^{ab}$	$32.00±1^a$	$30.33±2.08^{ab}$	$28.33±0.58^b$
6	$27.33±1.53^{bc}$	$31.00±1.73^a$	$29.66±1.15^{ab}$	$26.33±0.57^c$
7	$26.6±1.15^b$	$30.33±0.58^a$	$29.66±0.57^a$	$25.00±1^c$
8	$25.3±0.58^c$	$30.33±0.57^a$	$28.66±1.16^b$	$23.33±1.52^d$
9	$23.3±0.57^c$	$30.00±1^a$	$27.66±0.58^b$	$21.66±0.57^d$
10	$24.66±1.52^b$	$31.00±1^a$	$31.33±2.08^a$	$22.00±1.64^b$
11	$26.3±0.58^b$	$32.66±0.57^a$	$31.66±0.58^a$	$24.00±0.73^c$
12	$27.6±0.57^b$	$33.67±0.58^a$	$33.00±1^a$	$25.67±1.08^b$

Different letters in a particular row are presenting a non-significant difference (P>0.05).
B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

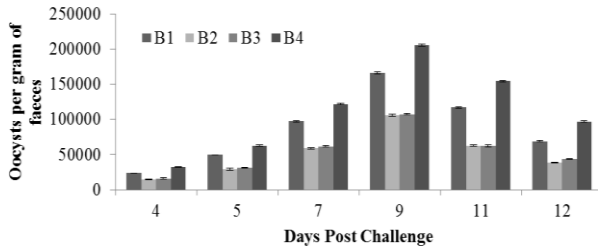

Fig. 3: Oocysts per gram of droppings post-challenge in arabinoxylans administered and control chickens
B_1 = Oat derived arabinoxylans @ 100 mg/kg of BW; B_2 = Oat derived arabinoxylans @ 200 mg/kg of BW; B_3 = Oat derived arabinoxylans @ 300 mg/kg of BW; B_4 = Control

Neyrinck *et al*., 2008; Kendall *et al*., 2010).

Oat is one among the major cultivated cereal crops in the world. These are used for malting, beverages and for human & animal food. Major components of these cereals include starch, dietry fibers and crude protein (Oscarsson *et al*., 1996). Dietry fiber contains variable amount of polysaccharides like beta-glucan and AXs. Both are non-starch polysaccharides (NSP), and the main components of cereals cell wall; including, oat, barley, rice, rye and wheat (Ebringerova *et al*., 2005).

Chemically AXs are two pentose sugars; α-L-arabinofuranose that is attached to the beta- (1,4) linked xylans backbone (Ahmed *et al*., 2013). Pharmacological activities of cereals derived polysaccharides especially AXs decreased the glucose level in diabetes patients and increased the fecal output (Lo *et al.*, 2005). The AXs also have the prebiotic effects by stimulating intestinal bacteria, which have beneficial effects on health (Broekaert *et al.*, 2011). Immunomodulatory activities of AXs either by stimulating natural killer cells (Akhtar *et al.*, 2012) or reduced inflammation and pain had been reported previously (Hoentjen *et al.*, 2005). Various *in vivo* and *in*

vitro tests are being used to evaluate the lymphocyte proliferation responses in various animal models (Qureshi *et al*., 1986; Corrier, 1990; Qureshi and Miller, 1991). In chicken, use of phytohemagglutinin-P (PHA-P) and human gamma globulin (Hγg) are the most routinely used methods to demonstrate the lymphoproliferative responses because of its simplicity, accuracy and ease to perform. In the current study, toe web test was performed to measure the effect of AXs on cellular immune response in terms of lymphoproliferative response in chickens as compared to control. The results revealed statistically higher ($P< 0.05$) lymphocyte proliferation response in chickens inoculated orally with oat derived AXs, when compared with control group. Results of the study indicated the highest cell mediated immune response in the form of thickness of toe web skin in response to PHA-P injection in birds inoculated with oat derived AXs than control group. The higher level of cellular immune response in chickens injected with graded doses of the AXs may be attributed to the enhanced effects on the phagocytic activity of macrophages that may cause increase in the thickness of toe web due to T-cell mitogen (El- Abasy *et al*., 2003; Akhtar *et al*., 2008). 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 depend on the function and number of lymphocytes (El-Abasy *et al*., 2002). Therefore, it can be speculated that an increase in the function and number of lymphocytes in the lymphoid tissue may be responsible for the development of the enhanced immune response.

The results of organ-body weight ratios revealed non-significant ($P>0.05$) difference between challenge and control groups. Awais *et al*. (2011) have earlier reported similar response in an analogous immunostimulatory study in chicken model.

Effect of oat derived AXs on the antibodies mediated immune response in chickens was measured by using SRBCs as antigen to measure the T-cell response (Kundu *et al*., 1999). Total anti-SRBCs antibody titers (GMT) were higher in oat AXs administered chickens when compared with control group both at 7th and 14th day post injection of SRBC. On the whole, results of the study revealed significantly higher antibody titres (Total Igs, IgM and IgG) in chickens administered with AXs. It can be speculated that the OAT derived AXs stimulated the humoral arms of the immune response that enhanced the antibody titres. The results of this study strongly supported the findings of previous studies reporting the immunostimulatory effect of cereal derived polysaccharides by the activation of host defence mechanisms (Chihara *et al.*, 1992).

Challenge Experiment

In challenge experiment, birds of the AXs administered and control groups were challenged with mixed species of genus *Eimeria* on day 14th post administration of AXs. The severity of infection was assessed on the basis of weight gain and OPG values (Idris *et al*., 1997). Increased weight gain and lesser OPG values were recorded in AXs administered chickens indicated the resistance against *Eimeria* although the direct relationship was not measured in the current study.

Coccidiosis in chickens caused severe consequences on the immune system and may affect the immune organs including spleen, bursa, thymus, caecal tonsils (Shatshneider and Parri, 1976; Rashid, 2004; Rizwan *et al.*, 2016). Although it has been reported that coccidiosis did not affect the lymphoid organs (Venkatratnam *et al*., 1985). A number of cereals and plants derived polysaccharides have been used as immune modulators or as therapeutic agents or as anti-inflammatory agent (Ghoneum and Golappudi, 2005; Wang and Zang, 2010). Further, polysaccharides derived from different plants have also been reported to activate macrophages to cytotoxicity against microorganisms and thus lead to enhanced protection (Luettig *et al*., 1989).

In case of coccidiosis, some medicinal foods and probiotics have been reported to provide immunity against the infection by stimulating the specific cellular and humoral immunity against *Eimeria* (Dalloul *et al*., 2003; Gabriel *et al*., 2006). In the present study, highest protection was recorded in AXs administered chickens as compared to those of control group. The protective efficacy of AXs could be linked with local immune responses, which may intrigued specific immune response to *Eimeria* infection (El-Abasy *et al*., 2004; Akhtar *et al*., 2008). However, a small fraction of chickens in control group might be protected due to self-resolving nature of Eimeria infection (Sharma, 1991). In present study, oocyst count was significantly higher ($P<0.05$) in control group with ruffled feathers and lethargic conditions as compared to AXs administered groups, taking better feed and water intake. Further, daily weight gains and protection rates were higher in AXs administered chickens in comparison with control group. Alterations in gut homeostasis, change in physiology, intestinal inflammation and diversion of energy from growth to antigen encountering due to coccidiosis could be the possible reason which ultimately led to poor weight gain in control group (Klasing *et al*., 1987; Adams *et al*., 1996). Polysaccharides (AXs) might be involved in enhancing the activity of monocytes, natural killer cells and neutrophils that released cytokines and enhanced the leukocyte activity, which could efficiently improved the mechanism of host recovery (Lo *et al*., 2005). Many plant derived polysaccharides have been reported to activate the macrophages against different microorganism and so enhanced the body protection (Luettig *et al*., 1989). The administration of AXs might also be involved in mediating the release of inflammatory mediators including IL_6, IL_{10} and IFN-γ, an important constituent of cell mediated immune response, thus improving the host immune system (Yun *et al.*, 2000; Lee *et al.*, 2007; Yamazaki *et al.*, 2008; Sung *et al*., 2009). Increased INF-γ level could be linked to protective immune response to *Eimeria* infection (Lillehoj and Trout, 1996; Yun *et al*., 2000). Higher level of IL-10 and

IL-6 could be linked with increased production of antibodies by triggering the activity of B-cells that enhanced body's immune system by phagocytosis and by destructing the extracellular pathogens (Roitt *et al*., 1989; Abbas and Lichtman, 2001). A statistically non-significant difference was detected on the development of lymphoid organs in chickens of AXs administered and control groups. Similar findings have been reported in some previous studies (Awais *et al*., 2011; Akhtar *et al.*, 2012; Kaleem *et al*., 2014).

Conclusion

Oat bran derived AXs had immunostimulatory effects in the broiler chickens that subsequently provided protection against *Eimeria* infection. Further studies are required for structural and functional elucidation of oat derived polysaccharides, AXs and their mechanism(s) responsible for immunomodulatory properties in chicken. In future perspectives, it would definitely help to investigate the possibility of the use of oat derived polysaccharides as neutraceuticals.

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