Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Effect of Aflatoxin B1 on Moulard Duck's Natural Immunity

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ARTICLE HISTORY (13-302)

Received: July 04, 2013
Revised: May 16, 2014
Accepted: August 03, 2014

Key words:
Aflatoxin B1
Complement system
Lysozyme
Mycotoxin NG

ABSTRACT

The absence of studies for the impact of aflatoxin B1 (AFB1) on natural immunity in ducks, motivated us to investigate its influence on the concentrations of the blood serum lysozyme and alternative pathway of complement activation among Moulard ducks. For the purpose of the study two separate experiments were conducted. The first one was among 40 duck babies divided into four groups: control group (not treated); – fodder was not supplemented with aflatoxin B1; group two- fodder + 0.5 mg/kg aflatoxin B1; group three – fodder + 0.8 mg/kg aflatoxin B1 and group four – fodder + 0.5 mg/kg aflatoxin B1 + 2 g/kg fodder Mycotox NG. The second experiment was also conducted by the same way but the blood was bled on the 50th day. The obtained results showed that the period of treatment has significant effect to the blood serum lysozyme concentrations. Lysozyme concentration in group second decreased from 4.42 to 2.16 mg/L and in group three from 3.92 to 2.70 mg/L. Results showed influence of the toxin also on the alternative pathway of complement activation. When applied in low doses and shorter periods it has immune stimulation effect, while applied in higher doses and longer periods it suppresses the alternative pathway of complement activation (APCA). Complement activity was decreased from 727.99 CH50 to 625.91 CH50 in second group and from 816.74 CH50 to 601.61 CH50 in group three. Mycotox NG has immune stimulation effect when applied for 15 days but in all other experimental groups it did not show such effect.

To Cite This Article: Valtchev I, T Koynarski, L Sotirov, Y Nikolov and P Petkov, 2015. Effect of aflatoxin B1 on Moulard duck's natural immunity. Pak Vet J, 35(1): 67-70.

INTRODUCTION

Good state of health among all animals is the most valuable strategy for any farmer. Nowadays farm animals are forced to grow rapidly under conditions that do not satisfy all their nutritional requirements. The main factors that contribute for the good animals' health are the two primary factors of the humoral natural immunity - blood serum lysozyme and complement system. Authors from all around the globe report species as well as breed, sex and season variations for the above mentioned factors (Semerdjiev *et al*., 2010; 2011; Sotirov *et al*., 2011a, 2011b). The impact of different infectious agents and some toxins have also been observed (Ghosh *et al*., 1991; Stoyanchev *et al*., 2010; Koinarski *et al*., 2012). The influence of aflatoxin B1 (produced by some toxigenic fungi: *Aspergilus flavus, Aspergillus parasiticus, Aspergilus nomius*) to animals' natural immunity is still unclear to medicine (Nazir *et al*., 2014).

Chen *et al.* (2014) let us know that feeding of 0.11 to 0.21 mg of AFB_1/kg impaired classical and alternative complement pathways in the duckling serum. Detailed information for toxico-pathological effects of ochratoxin A and aflatoxin A is supplied by Hassan *et al.* (2012a, 2012b, 2012c), Ahmad *et al*. (2012) and Khan *et al*. (2013). Pier (1992) states that the immune system is sensitive to aflatoxin. The researcher observed lower complement production and reduction in the phagocytic activity. Observed complement activity was significantly decreased ($P<0.05$) by aflatoxin.

Liu *et al.* (2002) treated porcine alveolar macrophages with Fumon is in B1 and aflatoxin B1 with 50 and 100 ng/ml, respectively. The research team detected roughly 55% (for the Fumonisin B1) and 36% (for the aflatoxin B) lower phagocytic activity 24 hours post challenge. Hameed *et al*. (2013) let us know that ochratoxin A induced pathological alterations which are dependent upon dose and duration of exposure. Hassan *et*

al. (2012b) concluded that production performance, pathological alterations and serum biochemical changes determined became more severe with increase in dietary levels of OTA. Similar data reported Khan *et al*. (2010) and Ahmad *et al.* (2012) in White Leghorn birds treated with different doses of AF and OTA but when vitamin E or Silymarin was added some of studied traits were ameliorated. El Miniawy *et al*. (2014) reported that the immunosuppression caused by aflatoxin increased the severity of lesions caused by H9N2 avian influenza virus and allowed the virus to be disseminated to more organs.

As seen so far, investigations for the aflatoxin B1 impact to natural immunity in birds are quite rare. The total absence of studies for this toxin impact among ducks, motivated us to investigate its influence to the concentrations the blood serum lysozyme and alternative pathway of complement activation among Moulard ducks.

MATERIALS AND METHODS

Birds and design of experiment: For the purpose of the study two separate experiments were conducted. The first one used 40 Moulard duck babies divided into four equal groups. For the first 10 days of the experiment, baby Moulard ducks were fed with appropriate fodder according to their age (Table 1). After mixing the fodder with aflatoxin B1, control samples were collected to determine if the target doses were reached. In the second stage of the experiment (day 11 to 21) the fodder was supplemented with aflatoxin B1(extracted from *Aspergilus flavus* – Sigma-Aldrich, Germany) in the following concentrations: group one (control group) – fodder was not supplemented with aflatoxin B1; group two (experimental group) – fodder + 0.5mg/kg aflatoxin B1; group three (experimental group) – fodder + 0.8 mg/kg aflatoxin B1 and group four (experimental group) – fodder + 0.5 mg/kg aflatoxin B1 + 2g/kg fodder Mycotox NG (SevaSante Animal, France). Blood samples were obtained aseptically from thewing vein (*v. ulnarisprofunda*) via vacuum tubes. On the 21st day from the beginning of the experiment blood samples were obtained from each duck without anticoagulant to analyze the concentration of the blood serum lysozyme and APCA.

The second experiment was also conducted among baby Moulard ducks which were divided into four groups having seven ducks in each. Groups were identical with the ones from the first experiment, but were challenged with the same concentrations of aflatoxin B1 and Mycotox NG till the 42nd day from the beginning of the study. Blood for analysis for this experiment was taken on the 50th day from the experiment's beginning.

Blood collection and analysis: Blood was obtained aseptically from *v. ulnarisprofunda* to determine serum lysozyme concentrations and APCA activity. Serum were extracted and used for determination of lysozyme concentrations following the method described by Lie (1985). The other factor of natural immunity – alternative pathway of complement activation was determined according to method of Sotirov (1991). The obtained data was processed by one way ANOVA with fixed effects of the factor using Statistica 6.0 (StatSoft Inc.).

RESULTS

Results from the first experiment, presenting the influence of the aflatoxin B1 to blood serum lysozyme concentrations are exhibited on Table 2. Despite the slightly higher levels of serum lysozyme among the experimental groups, we should point out the lack of significant differences between control and challenged groups for the 15 day challenge. These results unambiguously show the absence of immune stimulation by the used toxin substances. The levels of blood serum lysozyme obtained from the second experiment, where animals were challenged for 40 days with the same aflatoxin B1 and Mycotox NG (just for group 4) were relatively similar. Even though we see some trend for higher concentrations of serum lysozyme in favor of the experimental groups, statistically significant differences among the four duck groups were not obtained. The relatively high mean for group 4 is a result of one animal (sample 23) with extremely high lysozyme concentration (17.66 mg/L). The logical explanation of this phenomenon is the different genetic potential that each animal carry in its genome.

Comparing the results for each group in both experiments we obtained significant differences in favor of the first experiment data ($P<0.05$), where animals were challenged for only 15 days. Due to the high variation among the chronically challenged group (CV = 106.76%), differences between the double challenged groups from both experiments were not obtained.

Table 3 presents the results for the alternative pathway of complement activation among the ducks from both experiments. As seen from the table, there is definite increase of the complement activity depending on the aflatoxin B1 challenge dose, which indicates the immune stimulation effect for the two week challenge period. Significant differences between group 1 and 2 were not obtained. Contrary the differences between groups 2 and 3 were highly significant ($P<0.001$), between groups 3 and 4 ($P>0.05$); between group 1 and 3 ($P<0.001$); between groups 1 and 4 ($P<0.001$) and between groups 2 and 4 ($P<0.05$). The highest compliment activity was observed among the animals from group 4.

The presented data for the chronically challenged birds shows insignificant trend for decrease of complement activity from group 1 to group 4. Comparing the results for the APCA between the two experiments, we obtained some significant results in favor of the first one ($P<0.01–0.001$). These results indicate that the aflatoxin B1 could have immune stimulation effect, when used for short periods of time (15 days), but when used for longer periods (40 days) it has a definite suppressive impact.

DISCUSSION

The influence of aflatoxin B1 to innate immune response is observed in species like fish, where the natural immune factors are one of the primary protection mechanisms against pathogenic bacteria. Innate immune response is considered as main protective mechanism against broad range of pathogens. Saurabh and Sahoo (2008) state that it is much more essential for fish

Table 1: Composition and nutritional value of compound feed for Moulard baby ducks

Components	Compound feed (%)		
	Starter 0-4 Weeks	Grower 4-6 Weeks	Finisher 7-8 Weeks
Corn seed	50	48	52
Wheat	20	30	24
Soybean meal	17	6	10
Sunflower meal	6.54	9.8	5.8
Wheat bran	-	3	5
Fish meal	3	-	-
Lysine-L	-	0.23	0.13
Methionine	0.078	0.07	-
Salt	0.25	0.25	0.258
Dicalcium phosphate	1.1	0.8	0.8
Chalk	1.1	0.938	1.1
Vitamin and mineral premix	1.00	1.00	1.00
Energy and nutrient contents			
Metabolic energy, MJ/kg	12.1	12.1	12.1
Crude protein, %	18.6	15.6	15
Crude fiber, %	3.9	4.2	3.9
Crude fat, %	3.0	3.2	3.0
Calcium, %	0.89	0.81	0.66
Phosphorus, %	0.68	0.65	0.56
Lysine, %	0.88	0.74	0.70
Methionine+Cysteine, %	0.74	0.63	0.54

Table 2: Blood serum lysozyme concentration (mg/L) in Moulard baby ducks challenged with different doses of Aflatoxin B1 and Mycotox NG for 15 (n=10 in each group) and 40 (n=7 in each group) days

Group	Challenged for 15 days		Challenged for 40 days	
	Mean±SE	VC%	Mean±SE	VC%
I	3.57±0.49	43.6	1.99±0.29	38.7
II	4.42±0.62	44.4	2.16±0.43	52.9
III	3.92±0.39	31.9	2.70±0,36	35.6
IV	3.82±0.42	34.7	5.29±2.13	106.8

Table 3: APC Aactivity (CH50) in blood serum obtained from Moulard baby ducks challenged with different doses of Aflatoxin B1 and Mycotox NG for 15 (n=10 in each group) and 40 (n=7 in each group) days

Group	Challenged for 15 days		Challenged for 40 days	
	Mean±SE	VC%	Mean±SE	VC%
I	696.5 ± 17.6^{c}	8.0	622.3±15.9	6.8
II	728.0 ± 17.6^{ad}	7.6	625.9±10.4	4.4
III	816.7 ± 14.0^{ab}	5.4	601.6±11.3	4.9
IV	876.5 ± 35.0^{bcd}	12.6	605.6±14.1	6.1

Values bearing superscript a,c and b,d differ at $P<0.001$ and $P<0.05$, respectively.

compared with mammals. As part of the innate immunity the lysozyme is known for its opsonic nature and the lytic ability against both Gram negative and Gram positive bacteria. It is considered that lysozyme frequently collaborate with complement system in process of destruction of pathogenic agents.

Sahoo and Mukherjee (2001, 2002, 2003), El-Boshy (2008) report that the targeted aflatoxin decreases the level of serums bactericidal activity, lysozyme level and neutrophil oxidative activity, when used in a dose of 1.25 mg/kg among Indian Carp. Same authors claim that some substances like Levamisole, α-tocopherol, vitamin C and beta-1,3glucans applied in appropriate doses could reduce or even eliminate the immunosuppressive effect of aflatoxin.

Many authors reported that mycotoxins can suppress immune responses due to hepatotoxicity, atrophy of immune organs during development, suppression of cell-mediated immunity and result in increased susceptibility to infection (Corrier, 1991; Dietert and Golemboski, 1994; Surai and Mezes, 2005). Other investigations showed that mycotoxins exert direct effects on the local gastrointestinal immune system, including reduction of epithelial integrity and intestinal barrier function, reduced enterocyte proliferation, altered cytokine production and altered numbers of IgA-producing cells (Bouhet and Oswald, 2005). It is important to note that the immunosuppressive effects of mycotoxins depend on dosage, route of administration, animal species, age and sex.

Newest data in this field (Yunus *et al.,* 2011) indicates biphasic nature of the aflatoxin B1 effects to natural immunity. Aforementioned authors report increase or decrease of the humoral immunity in broiler chicken depending on the challenge dose and the length of the exposure to the toxin.

Analyzing our results and combining them with the results of the above-mentioned authors, we could see that both the period of time and the dose have influence to the immune response. We established that higher doses and longer periods of challenge have suppressive effect to complement system, while lower doses and shorter periods of challenge stimulate the immune system.

Hassan *et al.* (2012a) report for immunosuppressive risks in chicks that could be exposed to OTA *in ovo*. The lympho blast ogenic responses of the chicks hatched from OTA-contaminated eggs in response to PHA-P administration were significantly lower at 24, 48, and 72h after PHA-P injection when compared with responses by control chicks. The percentage of abdominal macrophages displaying phagocytosis of SRBC, the number of SRBC/macrophage, and nitrite production were each significantly lower in cells from chicks in the OTA-administered groups. Total Ab, IgG, and IgM titers against SRBC showed significant reductions in the groups that had been hatched from eggs injected with the higher doses of OTA (as compared with titers associated with chicks in control eggs). The same authors suggested that there were immunosuppressive effects from OTA in the progeny obtained from breeder hens kept on OTA-contaminated diets (Hassan *et al*., 2011b). One year later Hassan *et al*. (2012c) confirm the immunosuppressive effect of OTA in male WL chicks regarding functional impairment in some of the components of the immune system. In general the following authors (Hassan *et al*., 2011a, 2012d; Ahmad *et al.*, 2012, Khan *et al*., 2013; Hameed *et al.*, 2013) let us know that ochratoxin A and aflatoxin A induced pathological alterations and suppression of immune system which are dependent upon dose and duration of exposure.

Conclusion: Blood serum lysozyme concentration is significantly lower in Moulard baby ducks challenged with aflatoxin B1 for 40 days, compared with the lysozyme concentration in baby ducks challenged for 10 days period. The APCA activity has increased significantly for challenge period of 10 days, while applied for 40 days, the APCA activity has decreased in all challenged groups. Otherwise the effect of aflatoxin B1 over this indicator depends on both challenge dose and period of treatment. Mycotox NG has immune stimulation effect when applied for 15 days but in all other experimental groups it did not show such effect.

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