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The Effect of Low-dose Ochratoxin A (OTA) Fed in Ducks on Blood Haematological Profiles and Histopathological Alterations

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ABSTRACT

Contamination of mycotoxins in feed results in poor production of livestock and is a health hazard. Animal feed is reportedly prone to be contaminated with mycotoxins such as ochratoxin. In poultry production, ochratoxicosis may reduce growth rate, increase susceptibility to diseases by reducing immune responses and increasing mortality rate. Few studies on ochratoxicosis in duck, particular with exposure at a low level, have been reported. In this report, haematological and blood chemistry profiles and pathological changes in duck upon feeding of low doses of Ochratoxin A (OTA) are described. A total of 48 ducks (15 days old) were administered feed contaminated with OTA at 0, 50, 125 and 250 μg kg^{-1} for six weeks. The mortality rate was zero and no significant effects on feed consumption and body weight were observed at the end of the treatment period. In addition, OTA contamination in this experiment had no effect on red blood cell production. As a result, no haematological or blood chemistry profiles were identified as specific indicators of OTA exposure. However, depletion of heterophils, lymphocytes and eosinophils following OTA exposure was noted. Thus, OTA contamination has a tendency to suppress certain immune cells in duck even at low levels of exposure.

Key words: Ochratoxin A, duck, haematological, toxicity, mortality rate, immune cell

INTRODUCTION

Mycotoxins are secondary metabolic compounds produced by fungi. They are capable of initiating a toxic response in organisms in the animal kingdom. Contamination of mycotoxins in feed results in poor production of livestock and is a health hazard. Two main genera of fungi, *Aspergillus* and *Penicillium* which mostly develop during storage, may produce harmful mycotoxins such as aflatoxin and ochratoxin (Akande *et al*., 2006). Ochratoxin is commonly detected in animal feed when it is improperly stored. As with other major mycotoxins, ochratoxin has been detected worldwide in human blood and several food commodities, such as cereal, coffee, wine, spices, meat products, swine kidney and milk (Van Egmond and Speijers, 1994; Araguas *et al*., 2005; Matrella *et al*., 2006; Boudra *et al*., 2007; Iheshiulor *et al*., 2011). Ochratoxin is divided into three categories which are Ochratoxin A (OTA), Ochratoxin B (OTB) and Ochratoxin C (OTC). Among them, OTA is the most toxic and potently affects kidney; however, animals

may vary in their sensitivity to it (Iheshiulor *et al*., 2011). It is also suspected of being linked to the occurrence of Balkan Endemic Nephropathy (BEN) (Puntaric *et al*., 2001; Bamias and Boletis, 2008). The International Agency for Research on Cancer (IARC) classified OTA as a possible carcinogen in the category of Group 2B. Moreover, it is considered to cause a wide range of toxicological effects including nephrotoxic, hepatotoxic, neurotoxic, immunotoxic, genotoxic and teratogenic effects in several species of mammals (Creppy, 2002; O'Brien and Dietrich, 2005; Abdel-Wahhab and Kholif, 2008).

Ochratoxicosis adversely affects animal production, particular in the swine and poultry industries. Poultry feed is reportedly prone to be contaminated with mycotoxogenic fungi and mycotoxins, especially aflatoxin and ochratoxin (Fraga *et al*., 2007). In poultry production, ochratoxicosis may reduce growth rate, increase susceptibility to diseases by reducing immune response and increasing mortality rate (Gibson *et al*., 1989; Verma *et al*., 2004). Although the effects of OTA toxicity may not be focused on the immune system, suppression of immune function by OTA in chickens has been reported (Politis *et al*., 2005). Feeding chickens with OTA may promote anaemia by reducing concentrations of red blood cells and haemoglobin (Elaroussi *et al*., 2006). Furthermore, alteration of chicken serum chemistry profiles such as total protein, albumin, globulin, urea nitrogen, glycerides, potassium, uric acid and creatinine levels caused by OTA have been documented (Huff *et al*., 1988; Sreemannarayana *et al*., 1989). OTA has also been noted to cause leucopenia and lymphopenia in broilers (Benneth and Klich, 2003). The induction of microscopic change in kidney and liver by ochratoxicosis in broilers has also been documented (Stoev *et al*., 2002).

Currently, global duck production is rising and development of duck meat products is increasing (Huda *et al*., 2011). The Food and Agriculture Organization (FAO) estimated that the global production of duck meat was more than 4 million tons. In ducklings, acute liver damage due to OTA was also reported (Theron *et al*., 1966). Despite OTA being commonly detected in poultry feed at a low level, only few studies on ochratoxicosis in duck have been reported (Theron *et al*., 1966; Burns and Maxwell, 1987). Therefore, the aims of this study were to obtain basic data on the haematological and blood chemistry profiles and histopathological changes in duck following chronic exposure to low-level of ochratoxin A via contaminated feed.

MATERIALS AND METHODS

A total of 48 one-day-old ducks (Campbell) were kept under similar environmental conditions in which feed and water were provided *ad libitum*. The experimental procedures with animals were in accordance with the "Ethical Principles for the Use of Animals for Scientific Purposes", issued by the National Research Council of Thailand (NRCT). Feed was produced by a standardized protocol of the Institute of Animal Feed, Kasetsart University. Prior to the preparation of contaminated feed, the presence of major mycotoxins including aflatoxin B1, ochratoxin A and deoxynivalenol was tested by immunoaffinity with values found to be below 2, 5 and 25 μg kg^{-1} feed, respectively. Feed contaminated with OTA was prepared by dissolving OTA standard (Sigma Chemical Co., St. Louis, USA) in methanol and placing it into a small portion of feed (fine sieved) and then allowing it to evaporate. Thereafter, the contaminated portions were mixed into feed at OTA concentrations of 50, 125 and 250 μg kg^{-1} feed. After two weeks, 15-day-old ducks were divided equally into four groups: control (T0) and treatment groups (T1, T2 and T3). Then, ducks were continuously fed for six consecutive weeks.

Blood samples were taken from the jugular vein three times at 2, 4 and 6 weeks after the start of feeding on the contaminated feed. Blood samples were kept in EDTA for haematological testing

and serum samples were obtained from centrifuged whole blood. Haematological profiles were manually determined which included Pack Cell Volume (PCV), haemoglobin (Hb), Plasma Protein (PP), Red Blood Cell (RBC), White Blood Cell (WBC) and differential count (%) of the following: heterophil, eosinophil, basophil, lymphocyte and monocyte. Serum collected samples were quantified for three blood chemistry profiles: Aspartate transaminase (AST), uric acid and Creatine Kinase (CK). Post mortem examinations were performed on each duck at the end of the study. Histopathological findings of kidney, liver and spleen which were fixed in 10% neutral-buffered formalin, were observed. Fixed tissues were embedded in paraffin and stained with haematoxylin and eosin by a standard protocol and then microscopically evaluated. The statistical analysis was undertaken using nonparametric Dunnett's two-sided test for all parameters with differences considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effect of OTA on haematological parameters: As shown in Table 1, variations in certain data were noted. Although the PCV value at four weeks after the treatment was significantly different between T0 and T1 but it was not different in groups T2 and T3. However, the RBC count showed no difference among these groups. Hence, OTA at a low dose had no effect on RBC production. This is in contrast to previous studies in which birds received higher doses of OTA (Mohiuddin *et al*., 1993; Stoev *et al*., 2002; Elaroussi *et al*., 2006). Total White Blood Bell (WBC) count within the highly contaminated feeding groups (T2 and T3) had a tendency to decline with increasing time of exposure. Certain parameters showed significant associations with the level of WBC (Table 2). The difference of WBC values between the control group and T2 and T3 may describe by WBC differential count. A change in heterophils in circulation after OTA exposure was observed. In majority of birds, heterophils are the second most abundant leukocytes in the peripheral blood (Maxwell and Robertson, 1998). As known, heterophils play a crucial role in the pathogenesis of inflammation and bacterial infection in birds. Ochratoxicosis may induce inflammatory responses.

Some reports on studies of broilers stated that OTA may promote inflammatory chemotaxis and increase the heterophil level (McNulty, 1991; Moura *et al*., 2004). However, in our experiment, long-term feeding on OTA by ducks showed a trend of decreasing heterophils which

Table 1: Haematological parameters

Group	Time (week)	PCV (%)	Hb (g dL^{-1})	RBC ($\times10^6$ μL^{-1})	PP (g dL^{-1})	WBC ($\times10^3$ μL^{-1})
T0	2	32.50±2.56	8.82±0.75	2.04±0.25	3.77±0.29^{a1}	37.18±11.96^{a1}
	4	32.50±3.85^{a1}	9.30±0.99	2.44±0.40	3.90±0.33	39.18±12.57
	6	32.25±2.96	9.15±0.76	2.55±0.29	3.95±0.20	46.25±12.29
T1	2	33.20±2.09	9.23±0.80	2.26±0.46	3.37±0.24^{a2}	48.15±10.27
	4	28.40±2.50^{a2}	8.29±0.79	2.04±0.49	4.04±0.45	36.75±42.89
	6	32.40±4.42	9.25±0.82	2.65±0.48	3.78±0.34^{b1}	52.80±21.55^{b1}
T2	2	34.30±1.56	9.80±0.66	2.20±0.24	3.66±0.32	51.30±11.39^{a2}
	4	29.40±2.17	8.76±0.63	1.90±0.22	3.98±0.33	39.55±18.54
	6	29.40±3.74	8.59±1.05	2.50±0.61	4.18±0.23^{b2}	40.65±49.21
T3	2	33.10±2.80	9.06±0.85	1.99±0.47	3.62±0.31	50.60±10.52^{a2}
	4	30.80±3.22	9.01±1.30	2.00±0.62	4.30±0.53	41.55±19.9
	6	32.40±2.83	9.39±0.74	2.31±0.73	4.20±0.44^{b2}	34.25±8.07^{b2}

Different superscript letters and number indicate significant differences between groups and times, respectively at $p<0.05$, PCV: Packed cell volume, Hb: Haemoglobin, PP: Plasma protein, RBC: Red blood cell, WBC: White blood cell

Table 2: Differentiated white blood counts (%)

Group	Time (week)	Heterophil	Eosinophil	Basophil	Lymphocyte	Monocyte
T0	2	19.95±9.09^{a1}	2.18±0.97^{a1}	1.10±0.67	7.08±3.09	0.08±0.15^{a1}
	4	25.99±14.41	1.91±1.77	0.87±1.04	6.38±2.50	0.41±0.49^{b1}
	6	26.54±12.98	3.37±1.98	1.93±1.07^{a1}	7.97±3.49	0.69±0.63
T1	2	26.24±12.02	5.77±3.24^{a2}	2.20±1.40	9.87±4.99	0.68±0.49
	4	21.04±4.76	2.34±1.88	1.66±0.82	5.43±1.96	0.12±0.19
	6	23.91±9.39	2.49±2.24	1.09±0.55	6.74±4.40	0.48±0.58
T2	2	28.10±11.39	7.59±4.01^{a2}	2.01±1.52	7.98±4.90	1.09±1.16^{a2}
	4	26.59±16.07	1.74±1.35	0.88±0.67	4.82±1.73	0.04±0.14^{b2}
	6	25.74±5.06	1.35±0.68	0.84±0.92^{a2}	5.68±2.23	0.47±0.39
T3	2	31.58±8.12^{a2}	4.47±1.74	1.67±1.00	8.32±4.31	0.33±0.41
	4	25.77±15.95	2.89±2.44	1.10±0.69	4.79±1.93	0.00±0.00^{b2}
	6	22.22±74.55	1.93±1.25	0.75±0.81^{a2}	5.37±2.27	0.47±0.41

Different superscript letters and number indicate significant differences between groups and times, respectively at $p<0.05$

was particularly marked in the highest contamination group (T3). This phenomenon may need further investigation including evaluation on bone marrow and lymphoid organs in order to provide an explanation.

Interestingly, when considering all the treatment groups, OTA exposure had a tendency to reduce lymphocytes and eosinophils. In accordance with previous studies, depletion of lymphocytes and eosinophils may occur as a result of ochratoxicosis (Moura *et al*., 2004; Elaroussi *et al*., 2006). Moreover, histological changes in lymphoid organs, reduction of humoral and cellular immune responses or increasing sensitivity to bacterial and viral infection upon OTA exposure in birds has been reported (Singh *et al*., 1990; Stoev *et al*., 2002; Kumar *et al*., 2004; Elaroussi *et al*., 2008). Hence, failure of immune response from vaccination may occur. Additionally, Al-Anati and Petzinger (2006) stated that the immunosuppression mechanism by ochratoxicosis remains unclear but it was supposed that OTA may inhibit protein and DNA synthesis of immune system cells. As shown in Table 2, basophils can be observed more common in the peripheral blood of birds than in mammals. The function of basophils in birds is still unclear. Total plasma protein in ducks in our study showed variation. This is in contrast to a previous report that chickens fed with OTA at 400-800 μg kg^{-1} had decreased serum protein and presented hypoproteinaemia (Elaroussi *et al*., 2006). Effect of OTA on blood chemistry profile.

As a result, none of the blood chemistry parameters was shown to be specific to ochratoxicosis in poultry. Table 3 shows certain tested blood chemistry parameters including AST, uric acid and CK in ducks. The AST values, also known as SGOT, reflect injury of liver, skeleton, heart and renal tubules. Generally, increasing AST is linked to liver and skeletal damage. Although, the values showed a similar trend to CK, there was no statistical significance among groups at all tested times. The CK parameter is primarily specific for muscle injury in avian species. In addition, it is useful for distinguishing between hepatic and non-hepatic damage inducing AST elevation. In our study, a note worthy finding is that AST and CK increased rapidly after the initial period of exposure (2 weeks) in the treatment groups. These changes may be associated with hepatic cell and renal tubule injury that occurred at an early stage (as seen at the microscopic level). Uric acid is an end-product of amino acid metabolism and excreted primarily via renal tubules into faeces as a dry mass. Serum uric acid may be elevated due to its reduced excretion as a result of kidney

Table 3: Blood chemistry profiles

Group	Time (week)	AST (U L^{-1})	Uric acid (mg dL^{-1})	CK (U L^{-1})
T0	2	31.20±15.36	7.94±2.97	886.40±177.33^{a1}
	4	22.80±8.56	4.45±2.18^{a1}	820.90±273.44
	6	23.62±5.85	5.67±4.97	970.12±183.75
T1	2	61.87±33.16	5.53±2.41	1436.00±257.18^{a2}
	4	35.60±18.45	7.95±1.02^{a2}	826.80±418.31
	6	27.70±10.58	5.65±2.06	1077.40±394.04
T2	2	60.00±34.87	5.80±2.61	1605.50±348.15^{a2}
	4	31.20±11.87	7.39±1.29^{a2}	864.40±228.42
	6	25.80±6.66	6.75±1.43	958.20±375.04
T3	2	64.37±47.86	7.66±2.01	1495.37±296.77^{a2}
	4	31.20±11.58	8.11±1.47^{a2}	880.30±369.49
	6	27.10±14.16	5.76±2.31	1119.90±195.63

Different superscript letters and number indicate significant differences between groups and times, respectively at $p<0.05$, AST: Aspartate transaminase, CK: Creatine kinase

dysfunction. Although, uric acid is not a sensitive and specific parameter for renal function in poultry, the fluctuation of uric acid concentration in the treatment groups may indicate impairment of renal function due to OTA. This finding is in contrast to the results of Huff *et al.* (1975), who recorded that uric acid in broilers was increased 38-48% following exposure to a high dose of OTA at 4-8 μg g^{-1}. Chicken fed with high level of OTA contamination resulted in significant increase in Serum Glutamic Oxaloacetic Transaminase (SGOT), Glutamic Pyruvic Transaminase (SGPT), uric acid and creatinine (Elaroussi *et al.*, 2008). In general, the alterations of clinical blood chemistry parameters among groups were correlated with OTA exposure in a dose-dependent manner. An increasing of certain parameters at early stage of exposure in this study was noted.

Effect of OTA on histopathological alteration: At the end of the experiment, ducks in each group were sacrificed and kidney, liver and spleen were harvested for microscopic observation. The effects of OTA on kidney were renal tubule lesions with mild to moderate degeneration and/or coagulative necrosis of renal tubular epithelial cells in 1, 7 and 8 ducks of groups T1, T2 and T3, respectively. Moreover, liver lesion apparently showed mild fatty degeneration and coagulative necrosis of hepatocytes in the treatment groups. No spleen lesion was presented. Nevertheless, the results clearly showed that OTA was a primary cause of renal tubules damage induction at the microscopic level (particular 7 and 8 ducks in higher dose groups) which may be associated with variation in the levels of haematological or biochemical parameters. Unfortunately, an experimental error led to insufficient data for Feed Conversion Ratio (FCR) record. However, at the end of the experiment, no statistically significant difference in weight was seen among the groups (data not shown). Moreover, the mortality rate of duck in the experiment was zero.

CONCLUSION

We found evidence of variation in haematological and serum blood chemistry levels due to oral administration of different low concentrations of OTA. However, neither haematological nor serum blood chemistry profiles could specifically indicate ochratoxicosis in duck. It can also be concluded that administration of a low dose of OTA in the present study had no effect on red blood cell production. It is worth noting that WBC had a tendency to decline over exposure time, especially

in the cases of heterophils, lymphocytes and eosinophils. Increased numbers of histopathological alterations in kidney cells of higher dosed groups were presented. Thus, OTA contamination in feed has a tendency to suppress certain immune cells in duck, even at a low dose of exposure.

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