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Case Report
Effects of Mycotoxins on Meat Type Pekin Ducklings: A Case Report

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Abstract
Background and Objective: There is limited scientific data on the effects of mycotoxins on ducks. This case study was undertaken to explore the resultant effects of various levels of mycotoxins in a commercial environment. **Materials and Methods:** Elevated mortality on day 5 was observed in a group of ducklings that were part of an experimental feed test. Increased mortality was observed in 1 of the 4 feed treatment groups, including all 6 pens from that treatment out of the 24 randomly assorted pens for the entire trial. No increased mortality was observed in any of the pens from the other treatment groups. Six birds were randomly selected from all 4 treatments and removed from the feed trial for necropsy. **Results:** Ventriculus lesions were found in both the affected and unaffected groups on necropsy. No other gross lesions were observed. Affected pens had decreased growth, as well as evidence of compensatory gastrointestinal (GI) changes GI tract compared to unaffected pens. Feed from all treatments was analyzed for nutrient composition as well as mycotoxin levels. Various levels of Deoxynivalenol and Fumonisin were found in all of the diets. **Conclusion:** Severity of ventriculus lesions in the treatment groups appeared to increase with increasing Fumonisin levels in the feed with the clinically affected treatment group having the highest levels. Since data on the effects of specific mycotoxins as well as the interaction of mycotoxins is limited; this data could contribute to improve the understanding of the effects of mycotoxins on performance and livability of ducklings.

Key words: Aflatoxin, duck, fumonisin, histology, mycotoxin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mycotoxins, toxic metabolites produced by molds and fungi, associated with commercially manufactured feeds have been a major issue for commercial poultry producers for many years[1]. There are over 300 reported toxic fungal metabolites[2]. Three of the most common types of mycotoxins affecting poultry feed are: Aflatoxins, Fumonisins and Deoxynivalenol (DON). Mycotoxin levels in the feed vary and are dependent on several factors including weather conditions during the growing period of the crops mainly severely dry or wet growing seasons[3]. The effects of mycotoxins in commercially raised poultry has been extensively researched and allowable threshold limits are often set to avoid toxicity issues. However, ducks have been found to be more sensitive to mycotoxins than chickens or turkeys[4]. The duck industry comparative to other commercial poultry segments is small and little research has historically focused on the effects of mycotoxins on ducks[5]. Further, mycotoxin contaminated feed typically has more than one mycotoxin contamination[1], thus, determining the toxic level of a single mycotoxin in ducks is difficult. So, this study was conducted to explore the resultant effects of mycotoxins in a commercial environment.

MATERIALS AND METHODS

A research experiment evaluating different feed formulations in commercial ducklings was being conducted. The experiment included 2,880 straight run Pekin (Anas platyrhynchos domesticus) ducklings randomly divided between 24 pens (120 birds per pen). Birds were placed at the end of November with outside weather conditions being very cool. Higher than average mycotoxin levels had previously been reported from various feed mills and producers during this time period. All pens were in the same house and the house was environmentally controlled (±3°F). The pens were divided into 4 different dietary treatments all diets were pelleted corn/soy based (4 different levels of k/cals ± 20k/cal), with each treatment group represented by 6 randomly distributed replicate pens within the house. The treatments varied by level of dietary k/cals with only a 20 k/cal difference between highest and lowest treatment.

All groups had lower than expected mortality (below 0.5%) and no signs of disease through day 4 of age. In 6 of the 24 pens an average mortality of 5.97% was observed on the morning of day 5 and there was no mortality observed in the other 18 pens. In the afternoon observation the same 6 pens had an additional average mortality of 2.95% and all other pens had no mortality. Day 6 for the same 6 pens averaged 8.0% mortality. Evaluation of pens revealed numerous scattered dead birds all on their ventrum with legs outstretched. Several live birds in the affected pens were lethargic and ataxic when righted. Moribund birds vocalized but could not lift their head and leg movement/control was minimal and irregular. All affected pens were at this time identified as being on the same dietary treatment and subsequently switched to the control diet. On day 7 mortality had decreased to 2.13% on average in the affected 6 pens. The remaining birds were assessed and birds that were weak and lethargic (4.0%) were humanely euthanized. By day 8, the mortality rates returned to normal in all pens.

Necropsy: Six birds at 7 days of age were randomly selected from each of the 4 treatment groups. Body weights were collected on all selected bird. Intestines starting from the end of the duodenal loop and ending just before the attachment of the end of the ceca to the ileum were removed, weighed, measured for length and the serosal surface examined for lesions. Ventriculi from all birds were removed and rinsed with tap water for evaluation. Each ventriculus was scored 0-3 using the scoring rubric (Table 1 and Fig. 1).

Sections of select ventriculus and liver were collected and preserved in 10% neutral buffered formalin for histopathologic analysis.

Feed Analysis: All feed samples were sent to an outside laboratory[A] for analysis of nutrient composition, as well as mycotoxin testing. Briefly, for Vomitoxin analysis a 50 g sample was collected and homogenized with water and polyethylene glycol. Then sample was passed through an affinity column. Extracts were then analyzed using HPLC. For Fumonisin analysis, a second 50g sample was selected and homogenized with methanol. The sample was then passed through an affinity column and extracts analyzed by fluorometer.

Table 1: Ventriculus scoring rubric

Score	Lesion description
0	No grossly evident changes
1	Several small superficial koilin restricted erosions
2	1-2 ulcers extending through the koilin
3	Greater than 2 ulcers extending through the koilin with moderate ulceration of the underlying mucosa

Scoring rubric used for ventriculus scoring

[A]ATC Scientific, Little Rock, AK

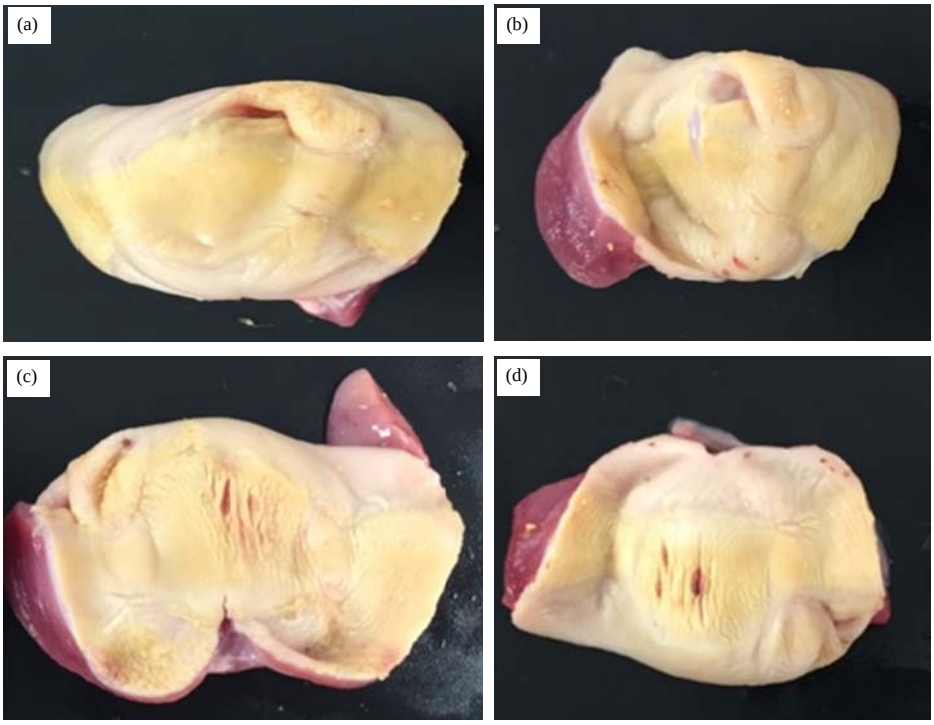

Fig. 1: Ventriculus lesions in 7 day old peking ducks
Examples of ventriculus showing lesions of scores: (a) 0, (b) 1, (c) 2 and (d) 3

Table 2: Gut health parameter measurements

Treatment	BW (g)	Relative GI wt	GI density score	GI efficacy score	N
Unaffected	317.94	8.0	0.24[A]	3.09[A]	18
Affected	199.33	8.0	0.17[B]	2.23[B]	6

[A,B]Superscripts within column denote significant difference at the ($p \leq 0.05$) level. Lack of superscript denotes no significant difference. Relative GI wts: (GI wt(g)/BW (g)×100), GI Density Score: GI wt(g)/GI Length (cm), GI Efficacy Score: BW (g)/GI Length (cm)

Table 3: Average ventriculus score and mycotoxin levels (ppb)

Treatment	Ventriculus score	Vomitoxin	Fumonisin
Unaffected (1)	0.50[C]	700	2000
Unaffected (2)	2.00[AB]	800	3000
Unaffected (3)	1.00[BC]	800	2000
Affected	2.33[A]	500	4000

[A,B,C]Superscripts within column denote significant difference at the ($p \leq 0.05$) level. Lack of superscript denotes no significant difference

Statistical Analysis: All data was analyzed using the One Way ANOVA feature of JMP 12[B]. Means were compared using Tukey-Kramer HSD analysis. Significance threshold was set at $p \leq 0.05$.

RESULTS

Necropsy: The feed treatment group experiencing high mortality had significantly lower body weights than the other 3 groups, (199.3g vs 317.94g). All groups had very similar relative GI weights [GI weight (g) / body weight (g)×100] (8.0 vs 8.0). However, there were notable differences in GI Density [GI wt (g) / GI length (cm)] between the affected vs unaffected groups (0.17 vs 0.24) as well as GI Efficacy [Body Weight (g) / GI length (cm)] (2.23 vs 3.09) (Table 2).

On gross necropsy, the ventriculi of several birds within all treatment groups had numerous mucosal ulcers with varying degrees of severity. These ulcers varied from pin point to more extensive coalescing foci forming linear tracks of ulcerated koilin with some extension to the underlying ventricular mucosal epithelium. The most extensive ulcers were up to 0.4 cm in length and were 0.2 cm deep. Average lesion score per feed treatment group are presented in Table 3.

[B]JMP version 12, SAS Institute Inc. Cary, NC

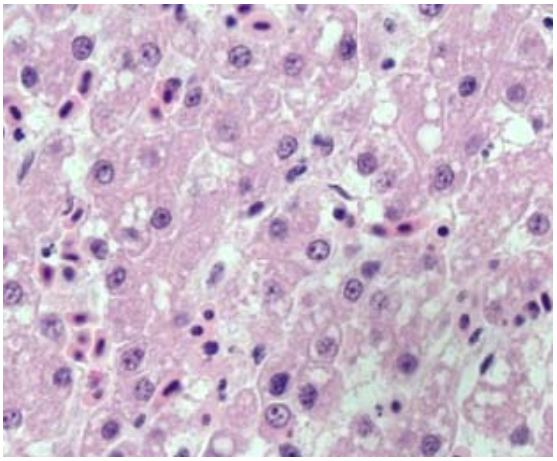

Fig. 2: 400X-liver, mild vacuolar change microvesicular type

Feed analysis: All proximate nutritional values were within acceptable limits. However, mycotoxins were found in the feed samples of all 4 treatments. The two prominent mycotoxins were DON (vomitoxin) with levels ranging from 500-800 ppb and fumonisin with levels ranging from 2,000-4,000 ppb (Table 3). All of the reported mycotoxin levels were at acceptable levels for chickens and turkeys[6].

Microscopic description: Sections of liver contain minimal to mild microvesicular vacuolar type change consistent with hepatic lipid degeneration (steatosis) (Fig. 2). Hepatocytes also contain glycogen type vacuolar changes (Fig. 2). In some sections there are small to moderate numbers of 3-4 micron diameter, round to oval, single nucleus containing protozoan parasites. These parasites are free within sinusoids, in regions of individual hepatocellular dropout and occasionally these organisms are phagocytized by Kupffer cells (Fig. 3).

All ventriculus sections have moderate generalized degeneration of the koilin with increased numbers of sloughed epithelial cells entrapped within the koilin layers. The surface koilin is irregular with deep fissures and clefts (Fig. 4). In more severely affected sections there are clusters of degenerate heterophils intermixed with cellular debris (Fig. 5). There are few small discrete areas of complete koilin loss and ulceration of the mucosal epithelium. In these regions there is replacement of the epithelial cells with clusters of degenerate heterophils, cellular debris and embedded foreign material (Fig. 5).

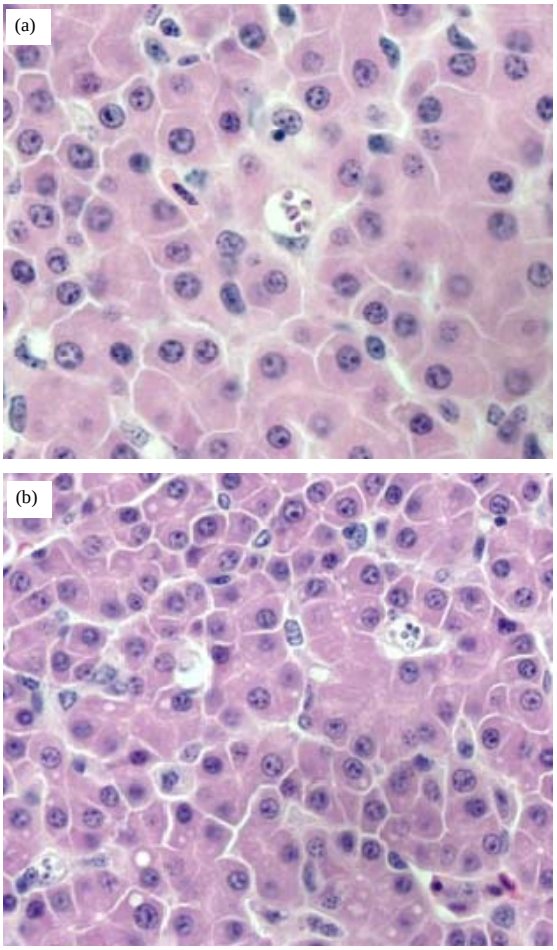

Fig. 3(a-b): (a) 400X-liver and (b) Phagocytized protozoa in Kupffer cells

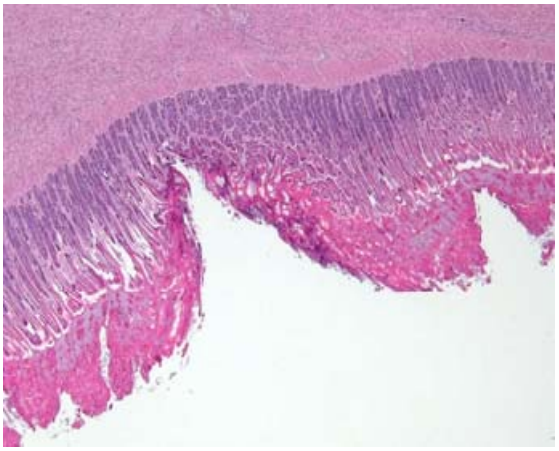

Fig. 4: 20X-Ventriculus, koilin fissure, thinning, loss and loss of mucosal epithelial cells. Notice embedded debris in these fissures

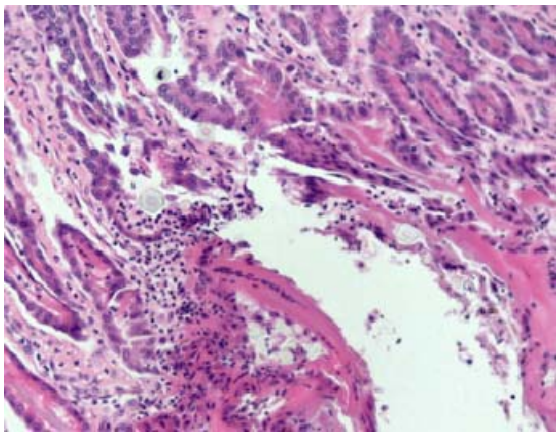

Fig. 5: 200X-Ventriculus, debris entrapped within fissure with sloughed epithelial cells and degenerate heterophils

DISCUSSION

Body weights between affected pens and other pens were significantly different. The affected pen had around a 118g lower body weight than the remaining pens. There were major differences observed when examining the intestinal tract. The affected group had significantly lower GI density score, as well as a decreased GI efficiency score (Table 2). These data suggest that there the intestinal function of these birds is being impaired. Genger *et al.*[7] presented similar data in turkeys, concluding that as intestinal function becomes impaired the birds respond by compensatory hypertrophy/hyperplasia of the intestines, in hopes to make up for the decreased intestinal functionality.

The mycotoxin report showed all 4 diets contained both DON and Fumonisin. Although the mycotoxin levels on feed analysis was within normal limits for chickens and turkeys, ducks are much more sensitive to mycotoxins. Davis *et al.*[8] reported mortality rates (20-50%) in ducks fed feed with similar mycotoxin composition as well as finding ventricular lesions all 4 of the treatments with varying severity.

Average ventriculus scores (by group) ranged from 0.5-2.33. However, there did appear to be a correlation between ventriculus score and Fumonisin levels, suggesting Fumonisin levels are directly associated with the mortality in the affected pen. It is also possible that there is an interaction between Fumonisin and DON levels in respect to severity of ventriculus lesions. Two of the groups had the same Fumonisin levels but different DON levels.

The constellation of changes in the ventriculus of the birds and in the livers are not classic for but have some features suggestive of mycotoxin containing feed. The lesions are mild and may represent low doses of mycotoxins. Alternatively the mycotoxins in these birds might be less likely to lead to gross and microscopic lesions. The lack of biliary hyperplasia in all sections may suggest early or low dose of mycotoxin or species deviation from classic findings associated with mycotoxins. Alternatively high dose of mycotoxins leading to death prior to biliary hyperplasia could explain not having this characteristic change.

The protozoan parasites within the liver in one bird are not able to further identified in histopathology sections. Additional molecular techniques are necessary for further differentiation and identification of these protozoa and is beyond the scope of this publication. The protozoan parasites are eliciting limited to no inflammation and mainly are associated with occasional single cell hepatocellular drop out and activation of regional Kupffer cells and likely had no significant resultant disease.

SIGNIFICANCE STATEMENT

This study suggests ducks are more sensitive than other commercial poultry species to mycotoxins especially when there are multiple mycotoxins present. Ducks from this study that were exposed to mycotoxins demonstrated decreases in growth, histological changes in the ventriculus as well as increased mortality. Based on this study duck producers should closely monitor mycotoxin levels of incoming ingredients to ensure optimal livability and performance.

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