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Authors: Boston, Sarah, Wobeser, G., and Gillespie, M.

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CONSUMPTION OF DEOXYNIVALENOL-CONTAMINATED WHEAT BY MALLARD DUCKS UNDER EXPERIMENTAL CONDITIONS

Sarah Boston,[1] G. Wobeser,[2] and M. Gillespie[3]

[1] Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4, Canada

[2] Canadian Cooperative Wildlife Health Centre, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4, Canada

[3] Wildlife Branch, Manitoba Natural Resources, 1495 St. James Street, Winnipeg, Manitoba R3H 0W9, Canada

ABSTRACT: Captive mallards (*Anas platyrhynchos*) were fed wheat containing 5.8 ppm deoxynivalenol (DON, vomitoxin) from an outbreak of *Fusarium graminearium* head-blight that occurred on grain crops in Manitoba, Canada, during 1993. There was no evidence of taste aversion to this grain during a 10-day palatability trial. No significant differences were detected in serum protein, calcium, glucose, creatine kinase, aspartate aminotransferase or uric acid levels, blood packed cell volume, or body or organ weight, between ducks fed contaminated wheat and those fed uncontaminated wheat during a 14-day feeding trial. No gross or microscopic lesions were detected in birds fed contaminated wheat for 14 days. Based on these results, ducks will consume grain containing moderate levels of DON and short-term exposure to this grain will not result in obvious adverse effects.

Key words: Mycotoxin, *Fusarium* sp., wheat, deoxynivalenol, vomitoxin, taste aversion, mallard, *Anas platyrhynchos*.

INTRODUCTION

In 1993, as a result of cool wet weather during the growing season, there was widespread infection of grain crops in Manitoba, Canada, and North Dakota (USA) with the fungus *Fusarium graminearium* that causes head-blight or tombstone disease. This fungus produces the mycotoxin deoxynivalenol (DON, vomitoxin) that can cause reduced food intake, vomition, and weight loss in domestic mammals (Marasas and Nelson, 1987). No information was available on the effect of DON on wild waterfowl although poisoning by mycotoxins has been reported in waterfowl and sandhill cranes (*Grus canadensis*). (Windingstad et al., 1989). The identity of the toxin or toxins causing disease in sandhill cranes was not established, other than that it was associated with *Fusarium* spp. Smith et al. (1990) suggested that low-level consumption of mycotoxins (unspecified) might impair the resistance of wild waterfowl to infectious disease. Wyatt (1991) reviewed effects of DON on poultry and concluded that it: "may not pose any severe threat to poultry health and productivity." We tested hypotheses that contamination of grain with DON would not affect its consumption by ducks and that consumption of DON-contaminated grain would have no ill effects on ducks.

MATERIALS AND METHODS

Two experiments were done: a palatability test to determine if ducks would consume DON-contaminated wheat (DW), when given a choice between it and control (uncontaminated) wheat (CW), and a feeding trial to determine if consumption of DW would result in clinical or pathological effects on ducks. Fifty mallard ducks, approximately 1 yr of age, including 25 males and 25 females, from Whistling Wings, Hanover, Illinois (USA) were used. Ducks were banded and randomly selected by band number for allocation to experimental groups. Ducks were housed in indoor pens in the Animal Care Unit at the Western College of Veterinary Medicine University of Saskatchewan, Saskatoon, Saskatchewan, Canada, and exposed to 16 hr light–8 hr dark per day.

The DW was spring wheat grown in one location in Manitoba during 1993. It had been examined by the Canadian Grain Commission, Winnipeg, Manitoba, Canada, and found to contain 10% visibly abnormal kernels. The CW used as a control in both experiments was spring wheat, purchased commercially in Saskatoon, Saskatchewan, that had no visibly affected kernels. Samples of DW and CW analyzed by the Canadian Grain Commission, using an enzyme linked immunosorbent assay for

DON with a detection limit of 0.5 parts per million (ppm) (Veratox, Neogen Corporation, Lansing, Michigan, USA), were found to contain 5.8 ppm and no detectable DON, respectively.

Four groups of five ducks were used in Experiment 1. Groups A and B contained females, and groups C and D contained males. Ducks were housed in two rooms, with a pen containing a group of males and a pen containing a group of females in each room. Each pen contained a source of running water, a dish of granite grit and three identical 10-1 upright, cylindrical, metal poultry feeders. One feeder contained CW, one contained DW, and the third contained a mixture of equal volumes of DW and CW. Feeders were placed in a row at the end of each pen opposite the water source. The location of the feeders within the row was determined by prior random draw and changed each day. The feeding trial lasted 10 days. The weight of grain consumed from each feeder was measured daily. At least 1.5 kg of grain was in each feeder at all times to ensure that adequate grain was available should the ducks choose to eat from only one feeder. The amount of different grains consumed and the amount consumed from different feeder locations were compared using analysis of variance (ANOVA) (Zar, 1984). Ducks were observed through an observation window for signs of clinical illness, such as changes in activity level, and for any unusual behavior related to any of the grain types.

For Experiment 2, 30 mallards were divided into groups of five, with three groups of males and three of females. Prior to the trial, all ducks were weighed: the mean weights of the groups were not significantly different. Ten males and ten females fed exclusively DW were housed in one room with males in one pen and females in another. The ten control ducks fed CW were housed in a separate room with males in one pen and females in another. Each pen contained a feeder, a source of running water located at the opposite end of the pen to the feeder, and a dish of granite grit.

Ten ducks (group A) were fed DW for 7 days and 10 (group B) were fed DW for 14 days. The control group (C) was fed CW for 14 days. To reduce bias that may have occurred if the contaminated grain was unpalatable or caused appetite suppression, control ducks received approximately the same weight of feed per duck as did the ducks of the same sex receiving DW. After day 1, the control groups (male and female) received the weight of grain per duck consumed by the birds of the same sex fed DW on the previous day. Birds were observed for behavior with respect to their eating habits and signs of clinical illness.

Blood samples collected from the wing vein of all ducks on day 0, from group A on day 7, and from groups B and C on day 14, were analyzed in the Clinical Pathology Laboratory, Department of Veterinary Pathology, Western College of Veterinary Medicine. Total protein was measured by refractometer (Reichert TS meter, Leica, Deerfield, Illinois). Calcium, glucose, creatine kinase (CK) and aspartate aminotransferase (AST) were measured using an automated chemical analyzer (Abbot Spectrum Series II, Abbot Laboratories Ltd., Missisauga, Ontario, Canada) and reagents supplied by the manufacturer. Uric acid was measured on the same machine by a colorimetric enzymatic technique with reagents supplied by DD Diagnostic Chemicals Ltd., Charlottetown, Prince Edward Island, Canada. Values from blood samples taken on day 7 from group A, and day 14 for group B and C were compared to values for the same group on day 0. Because the variance differed significantly between some groups, the Wilcoxon paired-sample test was used for all comparisons (Zar, 1984). Blood values from groups B and C on day 14 were compared using a *t*-test (Zar, 1984)

Birds in group A were killed on day 7 and birds in groups B and C were killed on day 14 by carbon dioxide asphyxiation. All birds were necropsied. The gonads (right testicle in males), liver (without the gall bladder), spleen, right kidney, and bursa of Fabricius were weighed. Samples of gonad, liver, spleen, heart, kidney, lung, esophagus, proventriculus, duodenum, pancreas, ileum, ceca, bursa of Fabricius, thymus, brain, thyroid, salt gland, and pectoral and gastrocnemius muscles were fixed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin, sectioned at 6μm, stained with hematoxylin and eosin, and examined microscopically. Liver sections from some birds were stained with the periodic acid Schiff (PAS) reaction and frozen sections of liver from the same birds were stained with oil red O (ORO) (Luna, 1968).

On days 0, 7, and 14, all birds were weighed. Beak length was measured at necropsy so that a condition index (body weight/beak length ratio) could be calculated. Body weight, organ weight as a percentage of the body weight, organ weight divided by beak length, and the condition index were compared among groups using a one-way ANOVA, followed by adjusted *t*-tests with *P* values corrected by the Bonferroni method (Instat, Graphpad Software, San Diego, California, USA) to detect differences between means. In all statistical tests, $P < 0.05$ was assumed to be significant.

TABLE 1. Total amount (g) of wheat containing deoxynivalenol (DON), a mixture of DON-contaminated and control wheat, and control wheat consumed by mallards over a 10-day feeding period.

Group[a]	DON wheat	Mixed wheat[b]	Control wheat
A (females)	1,630	1,200	780
B (females)	2,625	155	915
C (males)	1,675	821	1,425
D (males)	2,515	1,185	865
Total	8,445	3,360	3,955

[a] Each group contained 10 ducks.
[b] Mixture of equal amounts by volume of DON-contaminated and control wheat.

RESULTS

Each of the four groups of ducks in Experiment 1 consumed a greater total weight of DW than of either CW or the mixture, and the total amount of DW grain consumed by the four groups was more than double that of either of the other grain types (Table 1). For three of the groups, the average daily consumption of the food types was not significantly different (Table 2) but one group of females (group B) consumed significantly more of the DW than of either of the mixture or CW (ANOVA, Bonferroni $P = <0.05$, <0.01, respectively). When data for all groups were pooled, the mean weight of DW consumed per day was not significantly different from that of either CW or the mixed grain.

Clinical signs of illness were not observed in any of the ducks. All males and five of the females gained weight over the 10-day period, four females remained at the same weight as on day 0, and one female lost weight (990 g to 915 g). The feeders were large enough so that five ducks could feed at the same time and all of the birds within each group ate simultaneously. Usually, one duck would start toward a feeder and the other four would follow. If a duck was positioned between two feeders, it would sometimes alternate between the two.

In Experiment 2, there were no significant differences among the groups in any of the blood parameters at day 0. There was no significant difference between males and females within the groups, or collectively, in any of these parameters on day 0, so the values for males and females within groups were pooled for analysis. There were very few changes in the blood parameters tested over the course of the feeding trials; at least one group had a significant change in three parameters (Table 3). The mean total protein declined in all groups between the first and second blood tests; however, the change was significant only in the control group. In all of the groups, CK values were greater at the second bleeding than at day 0; the increase was statistically significant in groups A and C. The AST values also rose over the trial period in all three groups but the increase was significant only among the control birds.

Body weights of male and female ducks were analyzed separately. There was no significant difference among the groups on days 0, 7 or 14. There was no significant change in body weight within any of the groups over the trial period. None of the

TABLE 2. Average daily consumption (g) of wheat containing deoxynivalenol (DON), a mixture of DON-contaminated and control wheat, and control wheat by mallards over a 10-day feeding period.

Group[a]	DON wheat	Mixed wheat[b]	Control wheat
A (female)	163.0 ± 117.0[c]	120.0 ± 128.8	78.0 ± 54.8
B (female)	262.5 ± 228.6	15.5 ± 24.8	91.5 ± 180.2
C (male)	167.5 ± 177.3	82.1 ± 107.5	142.5 ± 175.1
D (male)	251.5 ± 187.8	118.5 ± 114.2	86.5 ± 108.5

[a] Each group contained 10 ducks.
[b] Mixture of equal amounts by volume of DON-contaminated and control wheat.
[c] Mean ± SD.

TABLE 3. Comparison of those blood parameters in which there was a significant change in at least one group of mallards during a feeding trial with deoxynivalenol-contaminated and uncontaminated wheat.

Parameter	Group	Day 0	Day 7	Day 14	*P* value[a]
Total protein (g/l)	A[b]	45.7 ± 3.4[c]	43.6 ± 2.5	ND[d]	>0.05
	B[e]	48.0 ± 6.0	ND	44.8 ± 3.7	>0.05
	C[f]	47.3 ± 1.5	ND	43.2 ± 3.9	0.027
Creatine kinase (μ/l)	A	769 ± 277	1,165 ± 538	ND	0.049
	B	743 ± 156	ND	902 ± 221	>0.05
	C	734 ± 257	ND	1,124 ± 174	0.049
Aspartate aminotransferase (μl)	A	19.1 ± 11.8	24.4 ± 11.1	ND	>0.05
	B	17.4 ± 5.7	ND	21.3 ± 3.9	>0.05
	C	17.6 ± 5.7	ND	29.8 ± 13.3	0.004

[a] Wilcoxon signed rank test for pairs.
[b] A—group fed deoxynivalenol-contaminated grain for 7 days.
[c] Mean ± SD.
[d] ND—not done.
[e] B—group fed deoxynivalenol-contaminated grain for 14 days.
[f] C—group fed uncontaminated grain for 14 days.

condition index, organ weights, organ weights as a percentage of body weight, or organ weights divided by the beak length was significantly different among the groups at the time of necropsy, except for liver weight. The average percent of liver weight to body weight was 1.8% in group A, compared to 1.5% in group B and 1.6% in group C. Groups B and C were significantly different from group A, (ANOVA, Bonferroni $P = <0.001$ and <0.05, respectively).

Female ducks in group C of Experiment 2 appeared hungry throughout the trial. They finished their feed on 11 of the 14 days of the trial and only 10 to 60 g of uneaten grain remained on the other days. They also began to eat as soon as the feeder was refilled. Males in group C did not show this characteristic; they finished their feed on 6 of the 14 days and did not always eat immediately after their feeder was refilled.

No significant changes were found on gross or histological examination of the birds, other than the occurrence of diffuse, foamy vacuolation within hepatocytes of seven of the ten ducks from group A. Vacuoles in liver from three of these birds were PAS-negative and ORO-positive, indicating that they contained lipid.

DISCUSSION

Our first objective was to determine if ducks would voluntarily eat wheat that contained *Fusarium graminearium* blighted kernels. The ducks in this trial did not avoid eating the contaminated grain and the total amount of DW consumed was greater than that of the mixture or CW in each of the groups. The lack of taste aversion to DON-contaminated feed is similar to that observed in chickens (Moran et al., 1987) and it is likely that ducks would consume contaminated grain in the field.

Consumption of DW had no apparent adverse clinical or pathological effects on mallards over the 2-wk feeding period, when compared to birds fed CW. Many of the same parameters have been monitored in chickens fed DON-contaminated feed without detectable effects. Kubena et al. (1985) and Huff et al. (1986) reported mild anemia and Kubena et al. (1987) found decreased uric acid and glucose in chickens fed DON-contaminated feed. However, the amount of DON in the feed and the length of exposure to the mycotoxin were greater in the experiments with chickens than those used with the ducks: 18 ppm for 35 days (Kubena et al., 1985); 16 ppm for 21 days (Huff et al., 1986); and 18 ppm for 140 days (Kubena et al., 1987).

The decline in serum total protein in all groups over the trial may have been a result of the relatively low protein content of grain. The occurrence of hepatic lipidosis in some birds fed DW for 7 days might have resulted from reduced food intake and mobilization of fat associated with the dietary change. This change was not present in the ducks fed DW for 14 days. Farnworth et al. (1983) reported increased hepatic lipid in chickens fed a diet with 0.35 ppm DON; however, birds given greater levels of DON in the same study had less hepatic lipid, and this change has not been reported in other studies. The increased levels of CK and AST in all groups during the trial was likely a result of minor muscle injury during handling, blood collection, and confinement. The average values on day 0 were similar to baseline values reported for mallards (Dabbert and Powell, 1993). Values from the second bleeding were lower than those reported for mallards that had received controlled handling without any observable complications (Dabbert and Powell, 1993). No microscopic evidence of muscle injury was detected in any of the birds.

Crops with a similar level of contamination to the wheat used in this study were widespread in southern Manitoba in 1993. Since mallards in the prairie provinces of Canada obtain more than 90% of their energy needs during autumn from grain (Clark and Sugden, 1990), we felt that the level of exposure to DW we used was similar to what might be experienced by migrating ducks. We were reluctant to restrict birds to an all grain diet for longer than 14 days because grain is deficient in specific nutrients (Delnicki and Reinecke, 1986). Based on our results, we propose that wild mallards will consume DON-contaminated grain of the type found in southern Manitoba in 1993 and that short-term consumption of this grain is unlikely to have serious effects on the birds. The problem of DON-contamination of grain was more serious than usual in Manitoba in 1993 but infection of cereal crops with *Fusarium* sp. is frequent and widespread (Gordon, 1952), so the results of this study may be applicable over a wider area.

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