

Necropsy Findings of Carcasses and Histopathology of Liver in Aflatoxicosis Epizootics of Duck

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Abstract

The overall death for the said period was 7080, of which 627 ducks were died of due to hepatitis and aflatoxicosis for a period of 5 years since 2011-12 to 2015-16. The hepatitis and hepatitis on aflatoxicosis found to be increasing trends in the duck farm. The average hepatitis along with aflatoxicosis during this period calculated to be 8.85% of total deaths. The clinical signs of aflatoxicosis were impetance, retarded growth, loss of body weight, dehydration, indigestion, roughed feathers and skinny, lameness and ataxia, cyanosis, convulsion, voice change to hiss, drop of egg production, egg bound, ocular discharge, blindness, huddle together, nervous signs and pendulous abdomen. Pathological lesions liver and kidney with haemorrhagic liver, fatty infiltration with enlargement necrotic patches, some cases of white calcified spot in liver, weak and thin liver with blackish hue also observed. Liver decolourized to yellowish and mottled. Kidney showed enlargement with white spotted calcified zone, haemorrhagic pale and white spotted in colour. Gastrointestinal tract mostly found empty with mucous secretion and thin of gastric lumen. Cardiac size revealed atrophic, ischemic, rounded and thin musculature. The concentration of aflatoxons in feed rages from 20-30ppb.

Keywords: Aflatoxicosis; Duck; Necropsy; Carcass; Histopathology; Epizootics; Prevalence

Introduction

Aflatoxins are poisonous and carcinogenic chemicals produced by several species of fungi like *Aspergillus flavus* and *Aspergillus parasiticus*. There are several chemical compounds of aflatoxins namely B1 and B2, G1 and G2, G1 and M1 and M2. The aflatoxicosis is a serious problem in of animal production and human nutrition. The susceptibility with aflatoxins varies in species of animals. The ducks and rabbits are most susceptible animals in comparison to other animals like turkey, cat, pig, chicken, sheep and goat [1]. The aflatoxicosis is highly economically significant disease that occurs in poultry through feed and supplements contamination. Due to cumulative accumulation of toxins in liver and muscle that causes retarded growth and reduce feed efficiency. It also impairs the normal health of the birds, and causes liver and kidney damage. The ducks, particularly ducklings are 200 time more susceptible than chicken [2].

Acute symptoms of aflatoxicosis include hepatitis with diffuse degeneration in liver parenchyma with enlarged cell nuclei, fatty changes and extensive proliferation of bile duct. In acute cases, there may be edema, altered digestion, absorption, metabolism and eventual death. In chronic cases, there is impaired metabolism, stunted growth, immunity problems, and cirrhosis and epatic cancer [3]. Although ducks is most susceptible animal for the condition but reporting of duck aflatoxicosis is rare. Duck population is second to chicken in the poultry industry in Asian countries but unlike chicken duck is much resistant to may infectious diseases. Aflatoxins undergoes an extensive transformation into different metabolites in the liver with the presence of Hepatic enzymes (the cytochrome P450, higher in duck) to the metabolically active metabolite exo-AFB1-8, 9-epoxyde (AFBO) to exert its toxicity. Metabolites of aflatoxins bind cellular compounds (proteins, DNA and RNA) to influence normal cellular activities, and is considered the active form responsible for the carcinogenicity and mutagenicity [4].

The mortality pattern of ducks in an organized farm revealed continuous contribution of deaths due to hepatitis with clinically similar to hepatitis with aflatoxicosis. A study was undertaken to evaluate the extent of hepatitis and aflatoxicosis in the duck population in the present experimental farm for the year 2012-16.

Materials and Methods

Clinical Signs Develops

A number 3500 standing duck population in different age group is reared with standard health and nutritional protocol. Routine vaccination with duck plague vaccine and duck cholera vaccine are given. The duck cholera vaccination used to do four month interval after five weeks of age while duck plague once in a year. The feed ingredients for different age groups are given as per their need for effective good health and productivity. The clinical signs provided by each age group of ducks including ducklings of up to 5 weeks, grower up to 20 weeks and adults above 20 weeks were recorded. The clinical signs under observation were inapetance, retarded growth, body condition, dehydration status, indigestion, feathers roughness, lameness, cyanotic condition, convulsions, change in voice, drops in egg production, egg bound, ocular blindness, huddling, nervous signs, pendulous abdomen and heart conditions.

Feed Analysis for Aflatoxin Contamination

As duck is very sensitive to aflatoxins even in very low concentration (10-20 ppb) of aflatoxins in feed can cause damage to liver. A number 8 feed samples from different farm houses were collected and sent for screening of aflatoxins content to Department of Animal Nutrition, Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. The department adopted high performance thin layer chromatographic (HPTLC) method to estimate feed contamination level of aflatoxin.

Necropsy Study

Routine necropsy study was accomplished within 8-10 hours of deaths as per standard protocol [1]. Clinical signs and external appearance of carcass were notes for preliminary identification of death cases due to aflatoxicosis. Disease diagnosis was confirmed based on characteristic pathological lesions revealed in different organs like liver, lungs, heart, gall bladder, bile duct, kidney and abdominal ascites along with clinical signs and concentration of aflatoxin in feed. Animal suffering from aflatoxicosis with diffuse degeneration in parenchymal cells, fatty changes of parenchymatous organs, enlarged cell nuclei, and extensive bile duct proliferation, changes in kidney and lungs were also be seen. The data generated was statistically analyzed as described by Snedecor and Cochran [5].

Histomorphology

Tissue samples were collected from liver from the carcass died of suspected aflatoxicosis with characteristic pathological lesions of enlarged and palor of liver, distended gall bladder and pendulous abdomen due to ascites. During autopsy a smaller piece of liver was collected in 10% formal saline for histopathological tissue section and microscopic observation as per standard protocol [6].

Results

Prevalence of Aflatoxicosis

Confirmation of aflatoxicosis made based on clinic-pathological signs, histopathology and, concentration of feed more than 20 ppb. A total of 7080 ducks were died due to different ailments during this period of study for 5 years since 2011-12 to 2015-16. The average mortality was calculated to be 9.34%. The mortality found to regressing from the 2011-12 to 2015-16 except in the last year. Highest and least mortalities recorded 9.34 and 2.39% respectively in the year of 2011-12 and 2014-15. On the other hand, the hepatitis and hepatitis due to aflatoxicosis is increasing trends since 2011-12 to 2015-16. A total of 627 ducks died of hepatitis of which a number of 30 cases died of aflatoxicosis. Aflatoxicosis death diagnosed based on clinicopathological changes in organs, histopathological changes and feed contaminated with 20 ppb and above. The average hepatitis during this period calculated to be 8.85% of total deaths (Table 1). The proportion of death due to a single aetiology of hepatitis is alarming in duck production. Hepatitis along with aflatoxicosis in duck farming is big a constrains for economic propagation and production. Although duck is very resistant to many of the infectious diseases but they are very sensitive to hepatitis particularly due to aflatoxicosis. The causes of hepatitis in duck may be of several aetiological factors like duck hepatitis viral type 1, 2, 3, duck plague, viral inclusion body hepatitis, duck hepatitis B virus, parvovirus infection but except duck plague other diseases are very scanty or no report from India. Therefore, most of the hepatitis may be from aflatoxicosis origin [7]. Epizootics of duck aflatoxicosis and hepatitis with prevalent in duck farm also reported [8].

| Year | Hepatitis | Aflatoxicosis | Total | % hepatitis | Total death | Mortality |
|-----------|-----------|---------------|-------|-------------|-------------|-----------|
| 2015-2016 | 328 | 25 | 353 | 27.32 | 1292 | 3.17 |
| 2014-2015 | 156 | 5 | 161 | 14.41 | 1117 | 2.39 |
| 2013-2014 | 60 | 0 | 60 | 2.77 | 2162 | 5.23 |
| 2012-2013 | 33 | 0 | 33 | 1.86 | 1773 | 8.40 |
| 2011-2012 | 20 | 0 | 20 | 2.71 | 736 | 9.34 |
| Total | 597 | 30 | 627 | 8.85 | 7080 | |

Table 1: Year wise Mortality due to Hepatitis in ducks

Clinical Signs

Although a well balance diet was provided to each of the age group of duckling, grower and adults ones. Toxin binder also given in the feed to avoid intoxication of aflatoxicity. In ducklings clinically there appeared to be reduced growth at the earliest age of 2-3 weeks. Retarded growth along with inappetance, abnormal vocalizations and some cases with feather picking (Table 2). In few cases purple discoloration of legs and feet, ocular discharge with impaired vision, some cases of lameness were seen. The duckling persist with this clinical signs for few days to week. Neurological signs of ataxia, convulsions, torticollis and opisthotonus prior to death (Figure1). Those ducklings survived from aflatoxin contaminated feed to grower and adult stage showed progressive reduction of body weight, ruffled feathers, change in voice, digestive problem, chronic inanition, drastic reduction in egg production, egg bound and reduction in vigour and vitality (Figure 1).

| Clinical signs | Ducklings | Grower | Adults |
|--------------------------------|-------------------------|----------------------------|--|
| Inappetance | Mild to moderate | Moderate | Severe |
| Retarded growth | Moderate | Severe | Severe |
| Body weight loss | Stunted | Moderate | Progressive loss |
| Dehydration | Perceptible | Moderate | High |
| Indigestion | Mucoidwatery feces | Mild indigestion | Irregular fecal mass |
| Roughed feathers and skinny | Rudimentary growth | Thin feathers | Feather lustreless |
| Lameness and ataxia | Mild | Moderate | Drowning signs |
| Cyanosis | Bill, legs, feet purple | Bill softening | Blackening and soft |
| Convulsion | Occasional | Neck bending | Most of the cases |
| Change in voice | Mild depressive | Hiss sound | Weak hiss sound |
| Drop egg production | - | - | Severe drops |
| Egg bound | - | - | 2-4 eggs bound |
| Ocular discharge and blindness | Mucous secretion | Dried up mucous | Blindness with dry mucous |
| Huddle together | Flocks together | Huddle | - |
| Nervous signs | Mild wing-flapping | wing-flapping, torticollis | wing-flapping, torticollis |
| Pendulous abdomen | - | - | Bulging abdomen on ascites (female) |
| Heart | Rounded | Soft and thin musculature | Soft, thin musculature, hydropericardium |

Table 2: Clinical signs of ducklings and duck on aflatoxicosis



Figure 1: Duck showing torticollis and convulsion

Aflatoxin such is not very toxic but the metabolites of the aflatoxins are hepatotoxic. After Ingestion, aflatoxins absorb through GI tract even at very small doses and accumulated in liver. With the help of hepatic enzymes, the cytochrome P450, active metabolite *exo*-AFB1-8, 9-epoxyde (AFBO) is produced along with other several metabolites, which is very toxic. Metabolites of aflatoxins bind cellular compounds of proteins, DNA and RNA to influence normal cellular activities, and are considered to be the active form responsible for the carcinogenicity and mutagenicity. Ducks said to be 200 time more sensitive than chicken to convert aflatoxins to metabolites [9]. Due to destruction of liver structure and enzymetic activities, the metabolism of fat, protein and carbohydrate is severely impaired as a result hypoproteinemia and fatty changes occur. Imbalance production of bile and liver enzymes hampers digestibility and absorption through intestines, as result feed conversion ratio is drastically impaired [10]. The clinical signs observed corroborated with the reports [4,11]

Feed Contamination with Aflatoxin

A number 8 feed samples were tested for aflatonins through HPTLC methods revealed that all the sample contained aflatoxins ranging from 20- 30 ppb. Aflatoxins are very toxic to ducks even in smallest quantity. There are similar reports that even very small quantity can affect duck damaging the liver [10].

Pathomorphological Lesions

134 After absorption, liver is the target organ for accumulation and exert of toxic effects of Aflatoxins, impairing the metabolism of all simpler forms of nutrients. Inhibition of protein and nucleic acid synthesis in duck liver because of faster metabolism of aflatoxin in ducks than any other animals, moreover more amount of fat deposited in liver parenchyma as fatty change occurred. In ducklings, most of the pathological lesions observed in liver and kidney. Liver haemorrhage, fatty changes with enlargement necrotic patches are frequently observed, some cases of white spot with firm and consolidated consistency, weak and thin liver with blackish hue also observed. Decolourized, yellowish mottled and ascetic liver has also been observed (Figure 2).



Figure 2: Friable yellowish consolidated liver

In some cases, friable hard liver lobes were noted. Kidney showed enlargement with white spotted calcified zone and, haemorrhagic spots. The contents of gastrointestinal tract mostly found empty with mucous secretion and thin of gastric lumen. Cardiac size revealed atrophic, ischemic, rounded and thin musculature (Figure 3). Ocular lesions with dried up mucous secretion over periphery of eyes causing irritation leading to both unilateral and bilateral corneal opacity. The skin found to be more wrinkled, shedding of much feathers and dry appearance. Swollen abdomen with accumulation transudates due to ascites leading to pendulous abdomen (Figure 4). Due to severe muscular weakness of oviduct, the adult ducks could not deliver VI position.



Figure 3: Rounded and thin heart muscle



Figure 4: Pendulous abdomen due to ascites

As a result egg bound with 2-4 eggs in the oviductal area with salpingitis, impaction and deposition of cheesy materials were noted (Figure 5). The bile duct and gall bladder showed distended with diluted bile, more secretion of bile leads to blue coloration upto duodenum. Due to structural change in liver parenchyma friable liver with yellowish hue and consolidation occurs. Liver parenchymal changes with our observation also reported in ducks [12,13].

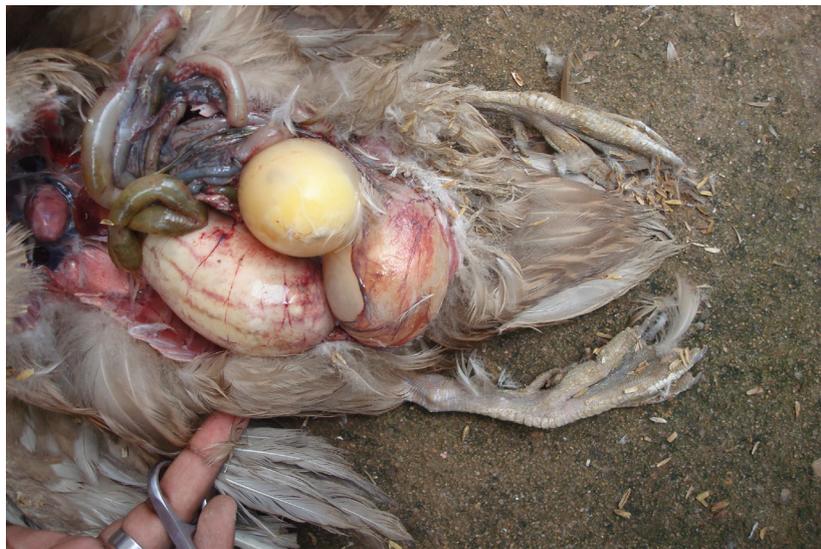


Figure 5: Egg bound with multiple eggs

Histopathology

Of the domestic birds, ducks are highly susceptible to aflatoxins particularly in the ducklings life of 3-5 weeks of age. A high death occurs due to aflatoxicosis affecting liver, kidney and muscular damage. The histopathological changes in liver and kidney in ducks are prominent with aflatoxin exposure. The pathological changes in liver are congestion and haemorrhages in the liver with soft and friable consistency were the predominant gross lesion.

Microscopically, fatty changes and cloudy swelling are cardinal signs in liver. Swelling and circular necrosis of liver (Figure 6). There were perilobular fibrosis with massive infiltration with red blood cells were observed (Figure 7). Proliferative changes with biliary hyperplasia also occurred. Kidney revealed degeneration and atrophy of glomeruli. Increased accumulation of fat in the liver and proliferation of the bile duct, perilobular fibrosis and infiltration with red blood cell in the liver parenchyma due to aflatoxicosis were also reported in duck aflatoxicosis (Figures 2,3,4,5,6 and 7) [14].

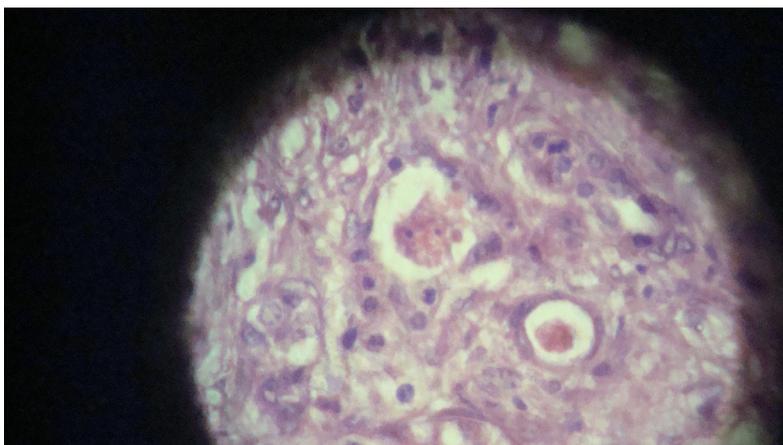


Figure 6: Swelling and necrosis of liver parenchyma

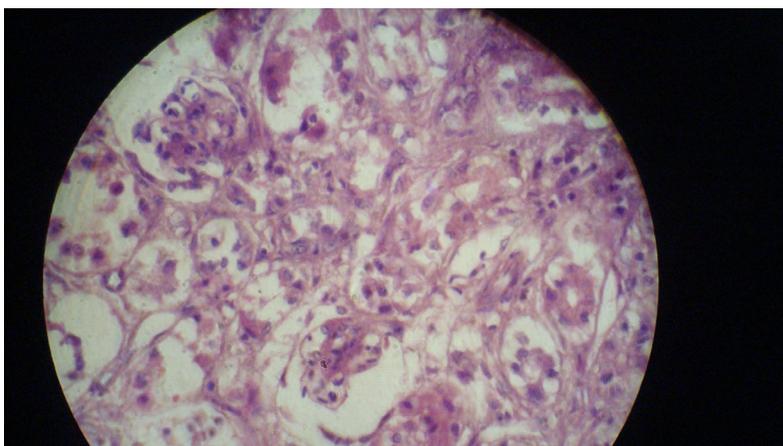


Figure 7: Perilobular fibrosis with infiltration

Conclusion

Aflatoxicosis in ducks is a very common fatal disease to hinder duck and egg production. The duck is very sensitive to minutes of aflatoxins particularly in the early life. The metabolites of aflatoxins are epoxides which binds larger molecule, like nucleic acids and nucleoproteins. The toxic effect of these metabolites is mutagenesis, carcinogenesis, teratogenesis, reduced protein synthesis, and immune-suppression. Reduced protein synthesis alters essential metabolic enzymes, structural proteins and production. As a result structural changes in liver along with increase in AST, ALT, bilirubin and bile acids.

The disease does not occur in isolated cases rather it affects whole flock as it is mainly due to feed origin. The disease can be diagnosed easily, clinically and at necropsy with involvement of liver, kidney, intestine and even heart. Once any flock is affected with aflatoxin, ameliorative intervention may be taken through several steps. Withdrawal of contaminated feed should be done immediately and new fresh feed may be given with toxin binder like glucomannan. Liver stimulant to be given to enhance the liver function properly. Care also is to be given to feed ingredients contamination and storage of feed. To provide toxin free feed avoid maize and dumpy feed for duck population.

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