NOTE

Avian Pathology

Distribution of Protoporphyrin in Female Broiler Chickens Affected with Protoporphyria

Yutaka UEDA1), Yoshiyuki AOHAGI1) and Satoshi NAKAHARA1)

1)Tottori Prefecture Poultry Meat Hygiene Association, 1291–7 Kotake, Daisen-cho, Saihaku-gun, Tottori 689–3203, Japan

(Received 15 April 2005/Accepted 6 September 2005)

ABSTRACT. One hundred thirty-seven broiler chickens at a poultry meat processing plant had dark green to black livers. Thirty-one chickens of these were collected at random and examined pathologically and biochemically. All of thirty-one chickens were female. The chickens showed mild retarded growth and a remarkable atrophy of the gallbladders. Microscopically, the livers showed dark brown pigments in the Kupffer cells, hepatocytes, and portal triads. These pigments showed birefringence with a Maltese-cross pattern under polarized light. Hyperplasia of the cholangioles, fibrosis, and infiltration of inflammatory cells were present in the portal triads. All the examined samples showed the same dark brown pigments in alveolar walls of the lungs. A high concentration of protoporphyrin was detected in affected livers, marrow, and feces (489, 104, and 116 µg/g wet wt., respectively) by biochemical assay.

KEY WORDS: chicken, liver, protoporphyrin.


The porphyrias are a group of disorders caused by abnormalities in the biosynthesis pathway of heme [12]. It has been reported in various animals and humans [8, 12, 15, 16]. There are several different types of enzyme defect in the heme synthetic pathway [12]. Protoporphyria is one of them. It is a defect in the ferrochelatase enzyme, the last enzyme in the heme synthetic pathway. It is hereditary and clinically characterized by photosensitization in humans and bovine [3, 16]. Experimentally, it is induced in mice and pigs by drugs [5, 14]. In birds, accumulation of protoporphyrin (PP) in the liver has been reported previously [4, 6, 17, 18], but these reports have only described a small number of broiler chickens. This report describes the pathological and biochemical outline of the protoporphyrinic disorder based on many affected chickens.

About 1,200,000 broiler chickens were inspected at a poultry meat processing plant between February and May 2004. Of these chickens, one hundred thirty-seven chickens had dark green to black livers. These came from several farms, but their parents were distributed from a common Cobb breeder farm. Thirty-one (54–63 days old) of these one hundred thirty-seven chickens were collected at random and examined.

The thirty-one chickens were examined grossly, including sex confirmation. The weight of fifteen chickens and the length of the gallbladders of seventeen chickens were measured. These came from several farms, but their parents were distributed from a common Cobb breeder farm. Thirty-one (54–63 days old) of these one hundred thirty-seven chickens were collected at random and examined.

The thirty-one chickens were examined grossly, including sex confirmation. The weight of fifteen chickens and the length of the gallbladders of seventeen chickens were measured. As a control for these measurements, sound chickens from the same lots and that were the same sex as the affected chickens were also measured in the same manner.

All thirty-one affected chickens were female, while males and females were distributed evenly in the population. Many of them showed mild retarded growth. The weights of the affected chickens were between 2,050 g and 2,616 g (average 2,381 g), compared with control chickens with weights between 2,520 g and 3,246 g (average 2,840 g). There was a significant difference by Student’s t-test (P<0.01). The livers of affected chickens were slightly enlarged, uniformly dark green to black on both the outer and cut surfaces. Many of the gallbladders were atrophic (Fig. 1). The length of affected gallbladders was 10 mm–26 mm (average 17 mm), compared with control gallbladders with a length of 30 mm–45 mm (average 36 mm). There was a significant difference by Student’s t-test (P<0.01). No significant lesions were found in other organs.

The livers of thirty-one chickens were microscopically examined, including observation using a polarizing microscope. Extensive tissues from five chickens were also microscopically examined, including the liver, spleen, kidney, lung, duodenum, pancreas, proventriculus, gizzard, cecum, ileum, ovary, thigh muscle, femur, marrow, bursa of Fabricius, and skin. These tissues were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, and sectioned at 5 µm. The sections were stained with hematoxylin and eosin (HE), and some of them were stained using Hall’s bile method, Berlin blue, and periodic-acid-

Fig. 1. An affected liver (left) and a control liver (right). The affected liver was uniformly greenish black. The gallbladder (arrow) was atrophic.
Dark brown pigments were present in all livers. These pigments were within the Kupffer cells, hepatocytes, dilated bile canaliculi, and portal triads, which were large massive to fine granular. The Hall’s bile method, Berlin blue, PAS stains of the pigments were negative. Under polarized light, the pigments showed birefringence with a Maltese-cross pattern (Fig. 2). In the portal triads, hyperplasia of cholangioles, fibrosis, and infiltration of mononuclear cells and heterophils were present in varying degrees. In the lungs, all the examined samples showed the same dark brown, large massive pigments in the alveolar walls, but their deposition produced little tissue reactions (Fig. 3). No significant lesions were found in other tissues.

Table 1. PP concentration of affected and control chickens (µg/g wet wt.)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Affected chickens (average)</th>
<th>Control chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>Liver</td>
<td>230–810 (489)</td>
<td>ND–0.42</td>
</tr>
<tr>
<td>Spleen</td>
<td>7.4–27.0 (19.8)</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.1–14.7 (10.2)</td>
<td>ND</td>
</tr>
<tr>
<td>Lung</td>
<td>12.7–49.2 (28.8)</td>
<td>ND</td>
</tr>
<tr>
<td>Marrow</td>
<td>83–136 (104)</td>
<td>ND–0.37</td>
</tr>
<tr>
<td>Skin</td>
<td>0.33–0.55 (0.43)</td>
<td>ND</td>
</tr>
<tr>
<td>Feces</td>
<td>54.5–194 (116)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not detected.

Deposition of PP pigments was also found in the lungs of all five affected chickens. There was one chicken that had pigments in the lungs in a previous report [18]. In bovine, it has been suggested that because bovine excrete adequate amounts of bile acids to maintain efficient PP excretion in bile, accumulation of PP in the liver is prevented [2]. Thus, severe hepatic disease has not been reported [3, 19]. Because broiler chickens are bred indoors with little sunlight, it is possible that photosensitization is low. Characteristically, liver disease is the main symptom in chickens.

Deposition of PP pigments was also found in the lungs of all five affected chickens. There was one chicken that had pigments in the lungs in a previous report [18]. In bovine, it has been suggested that because bovine excrete adequate amounts of bile acids to maintain efficient PP excretion in bile, accumulation of PP in the liver is prevented [2]. Thus, severe hepatic disease has not been reported [3, 19]. Because broiler chickens are bred indoors with little sunlight, it is possible that photosensitization is low. Characteristically, liver disease is the main symptom in chickens.

In the present study all of the thirty-one chickens collected at random were female. In previous reports also, all of the six chickens checked for sex were female to date [4, 17, 18]. It is thought that this disorder tends to affect females. Because the chicken sex chromosome is heterozygous for females and homozygous for males, contrary to mammals [7, 13], recessive traits tend to be expressed in...
females in the case of sex-linked inheritance. The cause of this disease is not clear, but considering this remarkable tendency, the possibility of sex-linked inheritance should be considered as a cause.

Mild retarded growth and weight loss were recognized in this study. These have been little reported in previous papers. Retarded growth might have been difficult to recognize because the degree was mild. Retarded growth and weight loss not only causes destruction of the liver, but also a decrease in poultry meat productivity.

Remarkable atrophy of the gallbladder was also recognized in the present study. This has not been described previously. This could mean a depression of the bile flow. Hepatocellular damage by crystalline deposits of PP might cause impairment of biliary excretion, as Bloomer [1] described in humans.

No cases of an enzyme defect in the heme synthetic pathway have been proved in birds. But, it is known that a defect of the ferrochelatase enzyme leads to an excess of PP in humans and bovine [3, 16]. The liver and marrow are main organs in heme synthesis [12]. The present study clarified that a large quantity of PP was accumulated in the liver and marrow. Therefore, there might have been a defect of the ferrochelatase enzyme in affected chickens.

The affected chickens excreted a large quantity of PP in their feces. Taking this fact into consideration, diagnosis of this protoporphyric disorder might be possible before necropsy. This could provide a great deal of information concerning this disorder.

ACKNOWLEDGMENTS. We wish to thank Dr. Akinori Shimada and Dr. Takehito Morita of the University of Tottori for assistance with the observations using the polarizing microscope.

REFERENCES