Histopathology of Gizzard Erosion in Young Broiler Chickens Due to Fish Meal in the Diets

Chitoshi ITAKURA, Yuriko HAKOTANI, Masatomo GOTO,
Toshiyuki SAITO* and Kazuhiko ISHII*

*Department of Veterinary Pathology and *Department of Veterinary Pharmacology,
Faculty of Agriculture, Tottori University, Koyama, Tottori-shi, Tottori 680*

(Received for publication November 17, 1980)

Abstract. Outbreaks of gizzard erosion occurred in many flocks of young broiler chickens in Japan in 1978 and 1979. The lesions were identical to those caused experimentally by a diet containing 15% mackerel fish meal which was heated at 140°C for three hours. As a result, it was postulated that the naturally occurring cases were due to certain fish meals in the diets fed. The gizzard of young broiler chickens affected naturally and experimentally with the disorder was histopathologically examined. The initial gizzard lesions were widening and loosening of the lining with swelling of the chief and surface cells. With time, alterations of the lining became predominant, showing a trellis-like appearance containing many desquamated surface cells. Later, erosion and ulcer occurred in the depths of the fold. In these changes an excess secretion of the gizzard glands was noticed for the histopathogenesis.

A malady characterized by gizzard erosion has been reported in young broiler chickens in Yugoslavia [4, 18], Peru [12], Mexico [1], Venezuela [1], Brazil [1], the United States of America [15], West Germany [3], Austria [5], and Holland [11] from 1969 to 1976. The main clinical symptoms were reduced feed intake, listlessness and black vomit with low mortality. The main gross lesions were a thickened, warty or bark-like appearance of the gizzard lining with erosion and ulcer, enlargement and flaccidity of the proventriculus, dark brown to black watery content of the crop, proventriculus and gizzard, intestinal catarrhal exudate, and increased difficulty in peeling the gizzard lining in the processing plants. Certain batches of fish meal, especially Peruvian meal in the diets were implicated as the cause, and the condition could be reproduced by feeding diets with high con-

centration of fish meal to broiler chicks [4, 7, 11, 15].

In Japan, outbreaks of the disease quite similar to those described above were seen in many flocks of young broiler chickens in 1978 and 1979, and resulted in a serious economic problem [10, 14, 17]. It was determined that the etiological factor was present in certain fish meals in the diets [9, 13, 17].

This paper describes the histopathological findings of the gizzard erosion of young broiler chickens, both field and experimentally induced cases, and discusses the histopathogenesis of the lesion.

Materials and Methods

Materials for the present study consisted of young broiler chickens affected naturally and experimentally with gizzard erosion. The natural cases were collected from three poultry farms (groups A, B

and C), each of which was fed respectively. The experimental cases were composed of four groups (groups D, DC, E and EC) including the controls.

A. Natural cases

Group A: The disorder occurred in two flocks consisting of 9,500 and 6,000 chickens, respectively, in November, 1978. The main clinical signs, which were noticed from about 7 days of age, were anorexia, listlessness, crouching with ruffled feathers, undernutrition and stunt. The mortality of each flock was 6.3% up to 7 days of age and 12.0% up to 20 days of age, respectively. Nine birds, 9 to 21 days of age, were pathologically examined from this group.

Group B: The disease was observed in a flock consisting of 14,600 birds which were fed a starter diet up to 21 days of age and a rearing diet after 22 days of age. After 6 weeks of age marked individual difference in growth and bloody diarrhea were seen in some birds with a mortality of 1.2% from 38 to 51 days of age. Four birds were submitted to pathological examination at 50 days of age from this group.

Group C: The disorder was recognized in a processing plant in July, 1979. At this time, 53 days of age, the gizzard lining of most chickens in this flock was thickened and roughened, frequently with erosion, and could not be peeled easily. Three birds were pathologically examined from this group.

B. Experimental cases

Group D: Since hatching, male broiler chicks were fed the same formula starter diet as that used on the poultry farm of group A. Each five bird was necropsied at intervals of 5 days from 6 to 41 days of age. During the experimental period the initial sign, lassitude, started at about 17 days of age. At and after 20 days of age some birds showed anorexia and diarrhea, and tended to crouch. The histamine level in the diet used for this group was determined by a fluorometric assay. The level was 85.14 ± 3.07 (mean $\pm$ S.E.) $\mu\text{g/g}$, about three and a half times the average level (23.04 ± 2.06 $\mu\text{g/g}$) in four kinds of the normal formula starter diets.

Group DC: This group served as the control of group D and was fed a normal formula crumb starter diet. Although each five chick was necropsied at the same intervals as in group D, two birds at each time were histopathologically examined.

Group E: Since hatching, mixed-sex chicks were fed an experimental starter diet containing 15% mackerel fish meal which was heated at 140°C for three hours. Each two to five bird was necropsied, principally at intervals of 2 days from 3 to 16 days of age. During the experimental period, the initial signs, brown soft feces or diarrhea and spiritless-

ness, started at 2 days of age. At and after 6 days of age the chicks tended to crouch showing slight decrease in feed intake, black brown diarrhea, and stunt with ruffled feathers. Individual difference in growth, decrease in feed intake and undernutrition were prominent at and after 9 days of age. The body weight of each bird at 16 days of age was about half the control.

Group EC: This group served as the control of group E and was fed a normal formula starter diet. Fourteen birds were examined pathologically at almost the same intervals as in group E.

Tissues from the birds necropsied were fixed in 10% buffered formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin. Selected sections from the gizzard were stained with periodic acid-Schiff (PAS). This paper describes histopathological findings of the gizzard and duodenum. Cross sections were made from three regions (anterior, middle and posterior) of each gizzard and from the middle region of the U-shaped loop of the duodenum.

Results

Essential changes of the gizzard in both natural and experimental groups, except the control groups, were similar in character, although variable in degree. Therefore, the findings are described together.

A. Macroscopical findings

The gizzard lesions appeared first in the anterior region (proventricular-gizzard junction) and tended to be widespread along the folds to the middle and posterior regions. The incipient lesion was discoloration of the gizzard lining. With time, the gizzard lining was thickened with prominent folds and roughened, showing a warty or bark-like appearance. Later, the lesions were associated with sporadic to multiple, minute or cleft-like erosions which occurred mainly in the depths of the folds (Fig. 2). In the most advanced cases, the whole areas of the gizzard lining were involved, in which there were scattered petechial or ecchymotic hemorrhages and small ulcers. The gizzard showing such lesions was slightly small in size and filled with dark brown to black watery content.

The incipient lesion, discoloration, of the gizzard lining mentioned above was also frequent, sometimes with minute erosions, in the control groups (Fig. 2). There was intestinal catarrhal inflammation in some birds. The dark brown to black watery content was also observed in the crop, proventriculus and small intestine.

B. Histopathological findings

The gizzard lesions were graded roughly into three, mild, moderate and severe.

Mild lesion (Fig. 4): In the normal chickens the lining was almost completely homogeneous and stained deeply with eosin (Fig. 3). In the affected birds the lining was slightly widened, stained somewhat poorly with eosin, and loosened (Fig. 4). The vertical and horizontal striations were obvious, showing a trellis-like appearance. Swelling of the chief cells was present in almost all areas of the glandular tubules under the lining involved. Swelling of the surface cells was comparatively marked, frequently with masses of supranuclear mucin granules. Occasionally, between the glandular layer and the lining there was a little mucous secretion stained poorly with hematoxylin. These lesions were localized in the anterior region.

Moderate lesion (Figs. 5-8): Widening, rarefaction and loosening (trellis-like appearance) of the lining were severer than in the mild lesions (Figs. 5 and 6). Especially, the vertical and horizontal striations were obviously distinguished with many vacant spaces between the horizontal striations. Desquamated surface cells were contained within the spaces. In some cases the lining was widened strikingly and contained thin secreted substance stained poorly with eosin (Figs. 6 and 7). Occasionally, focal lesions consisting of cell debris and liquefaction were observed in the lower parts of the lining, and frequently de-

stroyed the surrounding lining, resulting in erosion (Fig. 8). These focal lesions were in the depths of the folds, and in the underlining lamina propria there were edema and rarely hyaline thrombi in the capillaries. The glands under the lining with the moderate lesions revealed almost the same changes as those under the mild lining lesions (Fig. 7). These alterations were distributed in the anterior and middle regions, though severer in the former.

Severe lesion (Figs. 9-12): This type of lesion was widespread in most regions of the lining. The most prominent alterations were seen under the lining or in the upper part of the glandular layer, in which there were retention of a thin secreted substance from the chief cells, and many desquamated cells consisting mainly of the surface cells (Figs. 9-11). Some of the lesions were associated with hemorrhage and a few, or numerous, heterophils. The glandular layer decreased markedly in height. The remaining chief and surface cells were swollen, some of which were flattened lining the dilated glands. The lamina propria was edematous, sometimes with heterophils.

The lining was markedly widened and loosened, containing many desquamated cells and heterophils when in the presence of bacterial clumps. Occasionally, the lining was pushed up by the secreted substance from the glands and looked like to be separated from the glandular layer.

More frequently such erosion as observed in the moderate lesions was seen. In addition, ulcer was recognized in two birds, 50 and 53 days of age, of groups B and C and in five birds, 16 or more days of age, of groups D and E. The ulcer occurred in the depths of the folds, penetrated the muscle layer with sharply defined margins, and contained necrotic cellular debris with bacterial clumps (Fig. 12). Heterophilic

Fig. 1. Incidence and severity of gizzard lesion.

□; mild ▨; moderate ■; severe

and histiocytic reactions were present in the bed of the ulcer.

The incidence and severity of the gizzard lesions in each group examined are set out in Fig. 1. In the natural cases birds having the severe lesions were most numerous, followed by those with the moderate lesions, and those having the mild lesions very few. In the experimental cases the lesions were severer in group E than in group D. In the controls birds having the mild and moderate lesions were seen and more in group EC than in group DC.

In the duodenum there were focal lesions consisting of mucous or desquamative catarrh, hemorrhage, loss of the villi and necrosis of the superficial mucosal membrane (Fig. 13). The lesions were observed in six of twelve birds examined in groups A, B and C, in two of forty chicks examined in group D, and in five of twenty-two cases examined in group E. The birds showing such duodenal lesions were associated with the moderate or severe lesions in the gizzard.

Discussion

The clinical signs and gross lesions observed in both the natural and experimental cases in this study were quite similar to those in the condition which was caused, or thought to be caused, by fish meals in the diets [1, 3-5, 9-15, 17, 18]. Judging from the occurring aspects of the natural cases, the starter diet is suspected as the cause in group A and the rearing diets in groups B and C. When a formula fish meal, which did not have the potency to induce the gizzard erosion, was heated at 130°C for 5 hours, it acquired an ability to produce the disorder [9]. In this study the gizzard lesions could be produced by feeding a fish meal heated at a high temperature. It is possible that the treatment of fish meal in the processing plants in some way alters the product nature.

An excess secretion of the gizzard glands is suggested for the histopathogenesis of the gizzard lesions in this study. The excess secretion apparently produces various changes of the gizzard lining, and then erosion and ulceration. As mucous or desqua-

mative catarrh was obvious in the gizzard glands, the changes might proceed in the same process as a catarrhal inflammation in other organs.

Catarrhal changes have been described in previous studies on the gizzard lesions due to fish meals [1, 13, 17], although it was not suggested for the histopathogenesis. Almost the same gizzard lesions as seen in the present study were produced in chicks receiving diets with histamine [6, 7, 19] and diets with high levels of copper sulphate [2, 16]. Fisher et al. [2] observed massive desquamation from the inter-crypt epithelial surface resulting in the virtual replacement of horizontal striations with dead cells and cell debris in the gizzard lesions due to administration of copper sulphate. From the histological findings, they suggested that the term "gizzard gland hyperplasia" was perhaps more appropriate to describe the fundamental pathological change in the gizzard. Harry and Tucker [6] reported that the gizzard lining was disrupted by the cavitation associated with desquamation of disintegrated cells from the glandular layer in the gizzard lesions caused by administration of histamine. The gizzard lining is formed with secretion from both chief and surface cells [8]. An excess secretion of the latter cells was paramount in the present cases, which is considered to result in loosening of the lining, especially the horizontal striations.

Hyperactivity or increased secretion of the proventriculus was linked with the gizzard erosion [1, 5, 7]. Halama [5] deduced that the role of fish meal rather was associated with a stimulation of the secretion of pepsin and hydrochloric acid by the glandular epithelium of the proventriculus. In the present study the gizzard lesions were more frequent and severer in the anterior region which is rich in endocrine cells. This

might suggest that the endocrine cells play a role in the production of the gizzard lesions.

Gizzard erosion occurs frequently in normal young chicks. Fisher et al. [2] called such lesions as "natural" erosion. The mild lesions of the gizzard seen in the present cases may have nothing to do with fish meal effects. It should be emphasized that both mild and moderate lesions in the gizzard of the controls were localized and not widespread.

Acknowledgments. This work was supported in part by Grant-in-Aid (456901 and 556201) from the Ministry of Education, Science and Culture, Japan.

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要 約

飼料中の魚粉に起因する肉用種若鶏の筋胃糜爛の病理組織学: 板倉智敏・箱谷百合子・五藤精知・斉藤俊之*・石井和彦* (鳥取大学農学部家畜病理学教室, *同薬理学教室)——1978年から1979年にかけて、国内の肉用種若鶏に筋胃糜爛が多発した。著者らはこれらの筋胃を病理組織学的に検索した。その病変は140°Cで3時間加熱処理した鯖魚粉15%を含む実験飼料を、肉用種初生雛に給与することにより発現した変化と同一であった。したがって、自然発生の原因は、給与された飼料中の魚粉にあると見なされた。自然例および実験例の筋胃の初期病変は、ケラチン様層の増幅と粗鬆化ならびに筋胃腺の主細胞と表層細胞の腫大であった。病変の進行とともに、ケラチン様層の水平層紋は著しく明瞭になって棚状構造を示し、多数の剝離表層細胞を含んでいた。このようなケラチン様層の変化に後続し、筋胃皺襞の凹地に糜爛ならびに潰瘍が発現した。以上の所見のうち、筋胃腺の分泌過剰が病変形成に対し重要視された。

Explanation of Figures

- Fig. 2. Gross lesions of gizzard and proventriculus. The upper one (group EC) is control and the lower one (group E) affected. In the latter the gizzard lining is markedly thickened and roughened with some minute erosions. The proventriculus is flaccid with mucous exudate on the mucosal membrane. Note the discoloration of the anterior and middle regions with prominent folds in the control. Both cases are 16 days old.
- Fig. 3. Normal gizzard mucous membrane. The lining is stained homogeneously with eosin. Group DC. 6 days old. Hematoxylin and eosin staining (H.E.). $\times 270$.
- Fig. 4. Gizzard mucous membrane of a mild lesion case. The lining is slightly widened and rarefied, the horizontal and vertical striations being distinguished with difficulty. Both chief and surface cells are swollen. Group E. 3 days old. H.E. $\times 270$.
- Fig. 5. Gizzard mucous membrane of a moderate lesion case. Vacant spaces, often containing desquamated surface cells, are frequent between the horizontal striations of the widened lining. Group E. 9 day old. H.E. $\times 92$.
- Fig. 6. Gizzard mucous membrane of a moderate lesion case. The lining is conspicuously widened, loosened with a trellis-like appearance, and rarefied. Group D. 31 days old. H.E. $\times 78$.
- Fig. 7. High-power magnification of the lining and glands of Fig. 6. Both chief and surface cells are swollen. Secreted substance is rarefied in the glandular tubules and the lining. Group D. 31 days old. H.E. $\times 276$.
- Fig. 8. Gizzard mucous membrane of a moderate lesion case. A focal liquefactive lesion with cell debris is present in the lower part of the lining, and the surrounding lining is destroyed (initial erosion). Group E. 5 days old. H.E. $\times 154$.
- Fig. 9. Gizzard mucous membrane of a severe lesion case. The lining is markedly widened and loosened, containing many desquamated cells. Between the lining and glandular layer there is active desquamation of surface cells with slight hemorrhages, and the other secreted substance is scant though vertical striations are distinguishable. Group E. 9 days old. H.E. $\times 40$.
- Fig. 10. Gizzard mucous membrane of a severe lesion case. Between the lining and glandular layer there are numerous desquamated surface cells and vertical striations composed of thin secreted substance. The glandular layer is decreased in height and the glandular tubules are filled with thin secreted substance. Group E. 11 days old. $\times 135$.
- Fig. 11. Gizzard mucous membrane of a severe lesion case. Between the lining and glandular layer there are many heterophils, desquamated cells and vertical striations composed of thin secreted substance. Group D. 31 days old. H.E. $\times 135$.
- Fig. 12. Gizzard mucous membrane of a severe lesion case. There is ulcer which penetrates the muscle layer with sharply defined margin and is associated with histiocytic and heterophilic reactions in the bed. Group D. 41 days old. H.E. $\times 51$.
- Fig. 13. Duodenal mucous membrane showing mucous and desquamative catarrh with slight hemorrhages. Group E. 11 days old. H.E. $\times 158$.