

GIZZEROSINE INDUCED PATHOMORPHOLOGICAL LESIONS IN BROILER CHICKS

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ABSTRACT

Gizzerosine (GIZZ) is well known toxic substance spontaneously produced during fish meal heating process. Because of its action on H₂-receptors of histamine, it stimulates gastric juice secretion and induces gizzard erosions (GE). Its pathological effects on other organs have not been described yet. In this work pathological effects of GIZZ on different chicken organs were investigated.

INTRODUCTION

A toxic substance causing gizzard erosions (GE) in chicken, named gizzerosine (GIZZ), is produced spontaneously during the fish meal heating process by the reaction between histamine and protein in the fish meal (Okazaki et al., 1983; Masumura et al., 1985). Until now there were no pathomorphological descriptions of GIZZ effect in any other chicken organs. In the experiment the gizzerosine's pathological effect on various broiler chicken organs was investigated.

MATERIAL AND METHODS

In the experiment 220 commercial mixed-sex ROSS 1-day old broiler chicks were used. After 5 days of accommodation, at the end of the first week of life, the birds were divided into 3 groups. Group A received 100% of commercial broiler starter diet. A day before the first sacrifice 50% of commercial mash had been replaced with unheated fish meal (0.65 ppm GIZZ) in group B and with heated fish meal (1.15 ppm GIZZ) in group C. Every day during 5-day period fourteen chicks from each group were sacrificed under chloroform anesthesia. The birds were necropsied and organ samples were taken for histopathological examination and stained with hematoxylin-eosin stain (HE) and using Periodic acid Schiff-reagent (PAS) method. Samples from paraffine blocks were deparaffined in xylol and fixed in 4% glutaraldehyde and 2% osmium tetroxide in cacodylate buffer. The samples were dehydrated in graded ethanol series, passed through propylene oxide, and embedded in Epon 812. Semithin sections were stained with toluidine blue and appropriate areas selected for ultrastructural study. Ultrathin sections were prepared with diamond knives, stained with uranyl acetate and lead citrate and examined under a Philips 400 transmission electron microscope. For scanning electron microscopy (SEM) the conductivity of the sample surface was obtained by thin gold layer. Gizzerosine quantity in fish meal was determined by Dr. Toshio Wada, Japan Food Research Laboratories (authorized by the Japanese Government), Tokyo.

RESULTS

Necropsy of sacrificed animals did not reveal notable differences between treated groups. There were no remarkable histopathological changes except in gastrointestinal organs. The prevalent changes of gizzard were mild to severe cuticle erosions. The edema of proventriculus lamina propria with/without local fibroplasia was confirmed in group A and C. In group C multiple vacuoles in the lamina propria were found on the 5th day of the experiment (Fig. 1). Histopathological changes of duodenum, jejunum, ileum and caecum are displayed in Table. In group C the appearance of uniformly dispersed, multiple vacuoles in the lamina propria of duodenum, jejunum, ileum and caeca was the most striking finding toward the end of the experiment (Fig 2). The vacuoles were divided by thin, mutual walls, with pyknotic or crescent nuclei pushed aside (Fig 3). There were no PAS-positive material in vacuoles

of the lamina propria. Very thin, slight PAS-positive filaments were observed in numerous vacuoles of intestinal mucosal epithelium (Fig. 4). TEM analysis of the chicken intestine yielded secondary damaged cells in lamina propria. In their cytoplasm numerous empty spaces were visible. These cavities were of diverse shapes and different sizes, with no membranes. In some of them the nuclei were pushed toward the cellular edge (Fig. 5). Similar cavities were also found in intercellular spaces. SEM analysis confirmed the presence of multiple empty spaces in intestinal tissue (Fig. 6).

DISCUSSION

The most striking histopathological findings were discovered in intestine. Toward the end of the experiment the multiple vacuoles, uniformly dispersed in the lamina propria of duodenum, jejunum, ileum and caeca, occurred in group C (Table 1). We could not find any connection between appearance of vacuoles in lamina propria and subepithelial edema with subsequent epithelial desquamation (group A and B). The last changes could be induced by shock. In order to elucidate the origin of this vacuolar appearance, the samples were stained with PAS-reagent. The mild PAS-positive reaction was found in the mucosal epithelium vacuoles but not in the lamina propria. However, the "macrophage-origin" was then excluded. Very similar forms were observed in lamina propria of the proventriculus on the 5th day in group C. The predominant lesions of proventriculus were edema of the lamina propria with locally manifested fibroplasia, and occasionally local mucosal atrophy. These lesions as well as the cuticle erosions in gizzard were present during the whole experiment, regardless of different GIZZ concentrations in diet. Kubena et al. (1976) emphasized that the severity of intestinal lesions was greater than that of the gizzard lesions in broilers fed with various levels of fish meal. However, gizzard and intestinal lesions were not described precisely, and the causative compound of these lesions has not been discovered then. The possible connection between histopathological findings in chick intestine in group C and gizzerosine effect, could be found in the vacuolar appearance common in all parts of the intestine toward the end of our experiment. According to TEM analysis the observed cavities had no membranes or defined walls and it could not be confirmed with certainty that these were postmortal changes. On the other hand, their shape, arrangement and, particularly deformed nuclei pushed towards the cellular edge suggested that they were caused by a degenerative process. As the origin of these vacuolar forms was not determined here, and the identical changes were not described in literature until now, this will be the main subject of our future investigation.

LITERATURE

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Organ	A					B					C				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Duodenum	▽	▽	◻	◻	◻	◻	⊖	◻	◆	▽	◆	*	◆	◆	◆
Jejunum	▽	▽	▽	▽	▽	⊖	⊖	⊖	⊖	⊖	⊖	◆	◆	▽	◆
Ileum	▽	◻	⊖	◻	⊖	⊖	⊖	◻	▽	▽	⊖	◆	◆	◆	◆
Caecum	⊖	◻	◻	⊖	⊖	◻	⊖	◻	⊖	⊖	◆	▽	⊖	◆	◆

- ⊖ mucosal epithelium desquamation
- ▽ edema of lamina propria
- ◻ subepithelial edema
- ◆ fibroplasia of lamina propria
- ⊖ no visible or remarkable changes
- * mucosal atrophy
- ◆ multiple vacuoles in lamina propria

A - C - groups of chicks I - V - day(s) of sacrifice

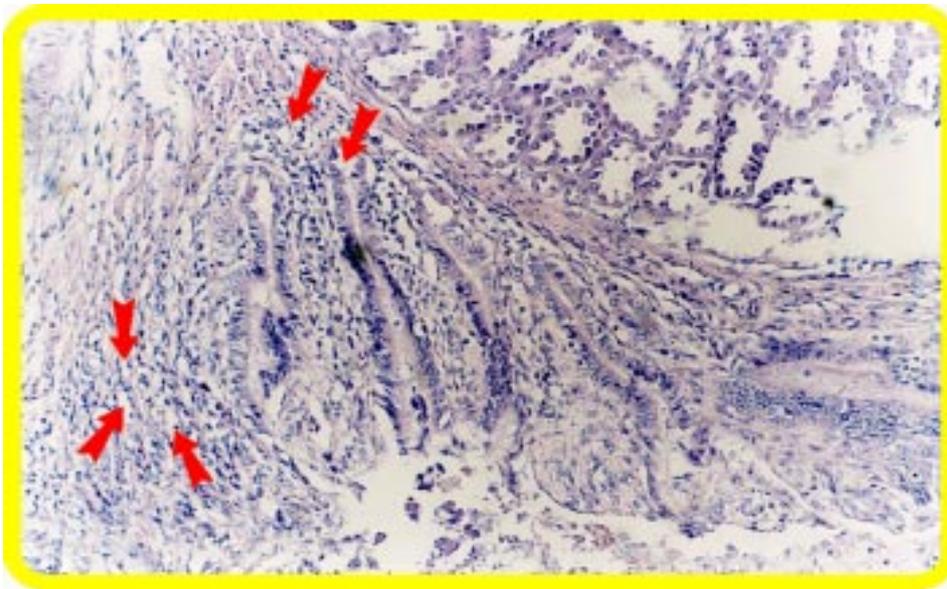
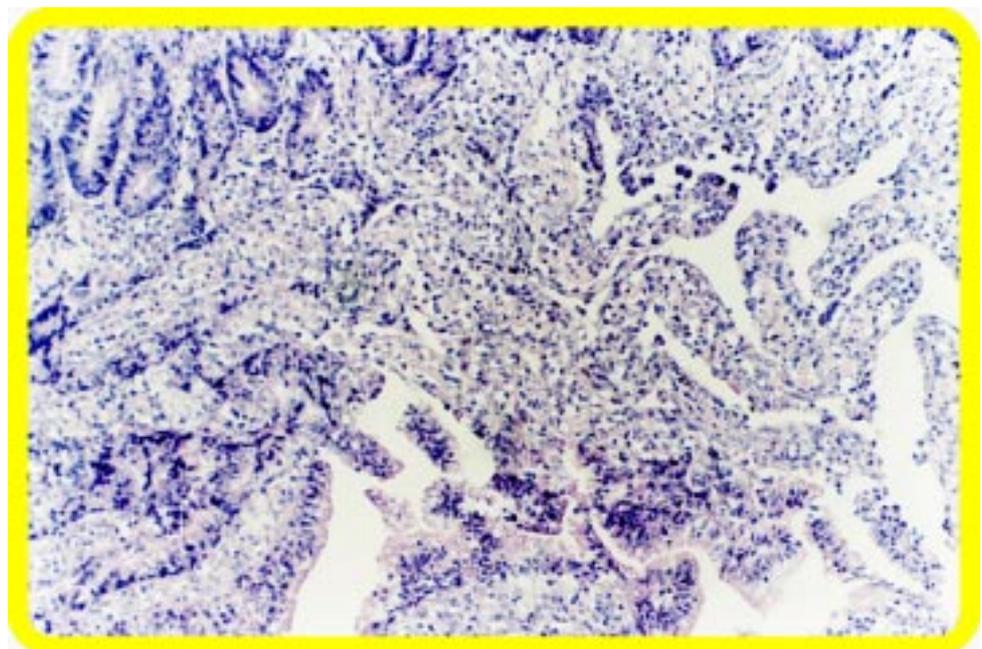


Fig 1. Proventriculus. Note the edema and vacuoles in the lamina propria (arrows). HE, 200 x

Fig 2. Jejunum. Multiple vacuoles dispersed in the lamina propria. HE, 200 x



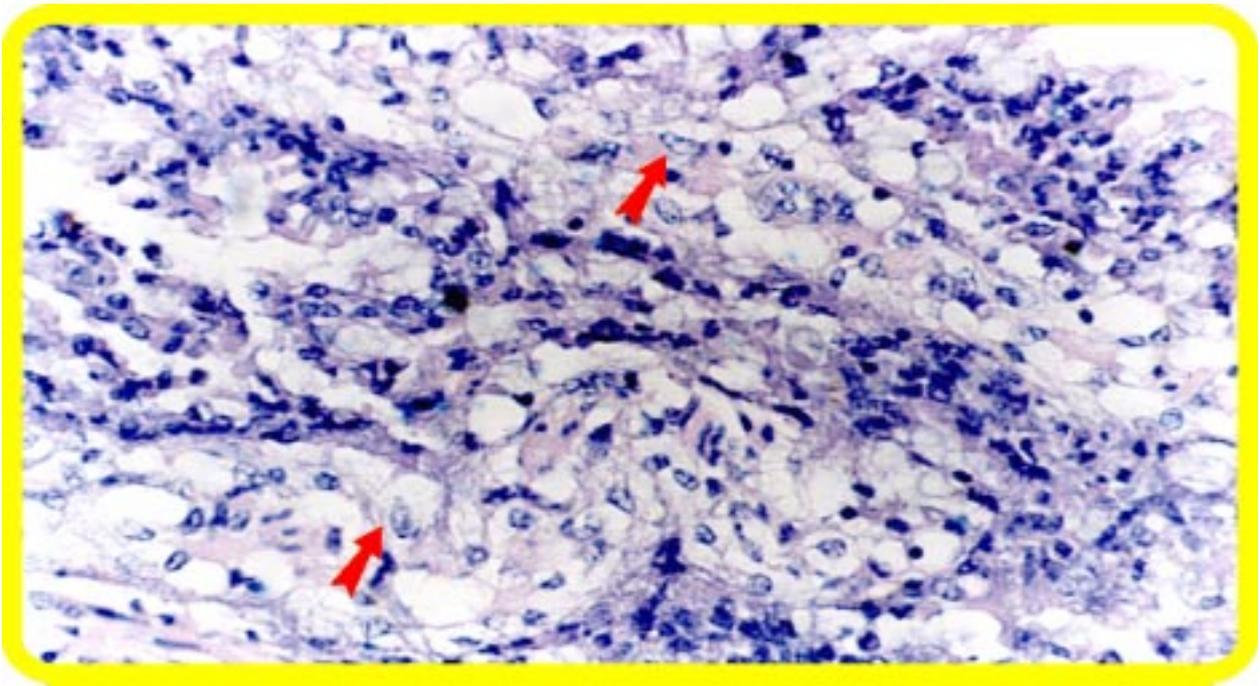


Fig 3. Jejunum. Vacuolar appearance in the lamina propria. Note the nuclei pushed aside (arrows). HE, 630 x

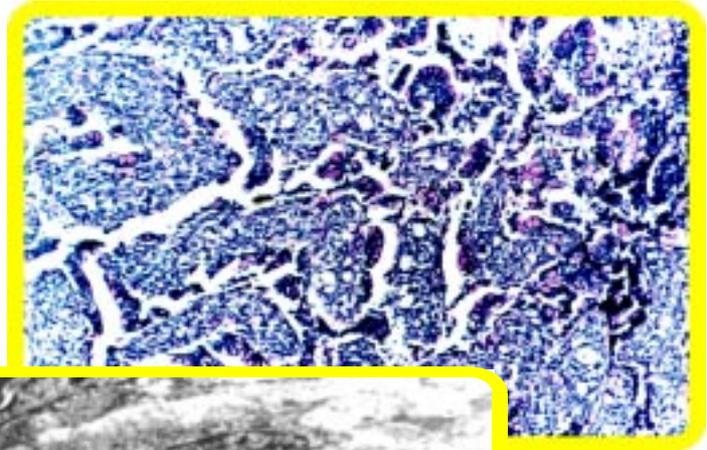


Fig 4. Jejunum. Mild PAS positive reaction in mucosal epithelium. PAS, 200 x

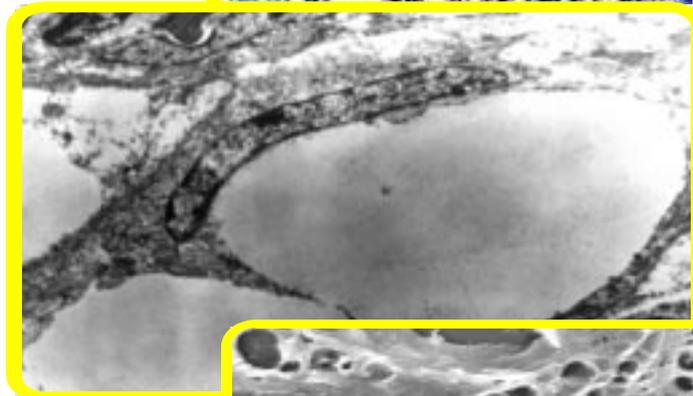


Fig 5. Ileum (TEM). Note the nucleus pushed toward the cellular edge. 9200 x

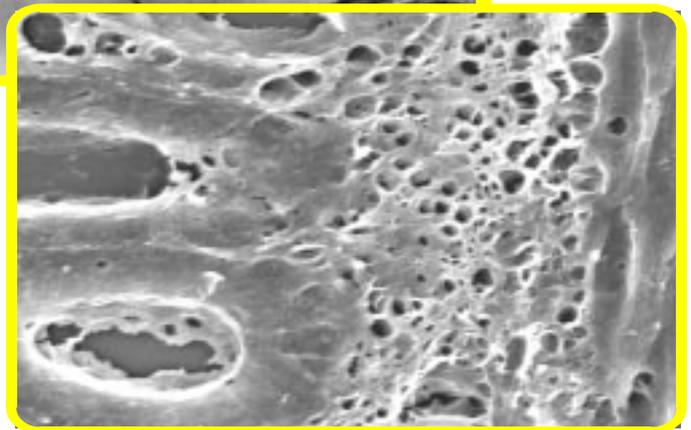


Fig. 6. Duodenum (SEM). Empty spaces in lamina propria. 900 x