Studies on Gizzard Erosion-Inducing Substance in Fish Meal

By YASUKIYO UMEMURA

Feed Safety Research Division, National Institute of Animal Health
(Yatabe, Ibaraki, 305 Japan)

Since the study of Janssen[6], many reports on the gizzard erosion (GE) in broilers have appeared, and fish meal in the diet was considered to induce this lesion[1,2,7]. Among the constituents of fish meal, histamine, produced by the bacterial spoilage of fish, was suspected to be the cause[3,4,11]. In the past few years, GE has also widely occurred in broilers and layers in Japan[8], and its cause was investigated by many Japanese workers[5,9,10,12]. This paper reports on the toxicity of fish meal, especially the heat-treated, as well as chemically formed one, and on the attempt to purify the GE-inducing substance.

GE-score (GES)

Degree of the toxicity of the diet, which induced GE, was expressed as GES. Seven one-day-old male broilers were fed the testing diet for 6 days, and body weight of each chick was recorded every day. On the 8th day after hatching, chicks were sacrificed for macroscopical observation on the incidence of erosion in the lining of gizzard, and severity of GE was graded into five. Grades 1, 2 and 3 were for simple erosion. Grade 4 was for erosion with ulceration. Grade 5 was for the severest case with ulceration and perforation. Each grade was scored as the number it bore, and the sum of scores of seven chicks was shown as GES. As shown in Fig. 1, chicks with a severe erosion or ulceration in the gizzard began to decrease in body weight on 5th or 6th day, and, hence, a severe toxicity of the diet could be predicted before the sacrifice.

Toxicity of fish meal

To the standard diet (SD), which initially contained 5% of white fish meal, 15% of fish meals or other products were added. Feeding of fish meal of cod or whole meal of mackerel (M) was found to be non-toxic, as it did not induce any GE (GES=0). It is widely believed that fish meal of low moisture causes GE, as was noticed in many poultry farms. It was carried out, therefore, to heat the non-toxic fish meals in order to make them toxic, and heat-treatment (135°C, 3 hours) was proved to be effective in converting non-toxic M to toxic one (GES=14). In the case of fish meal of cod, however, the same treatment was non-effective. Thus it was suggested that some precursor contained in M might be turned into the toxic substance by heating. The precursor was soluble in water, because the water-washed M was non-toxic at all even after it was heated, and the water extract was potent enough to induce GE (GES=19) when it was mixed with milk casein and heated. On the contrary, heated M was still toxic even after washing with water, indicating that the toxic substance was insoluble in water. It was also confirmed that neither the precursor in M nor the toxic substance in heated M could be extracted with organic solvents. To determine the precursor of toxic substance, the water extract of M was analyzed, and large amounts of free amino acids, histamine and nucleotides were detected. These constituents were examined for their effect to produce toxicity. Among free amino acids, histidine was prominent, and it was found that a severe toxicity was produced

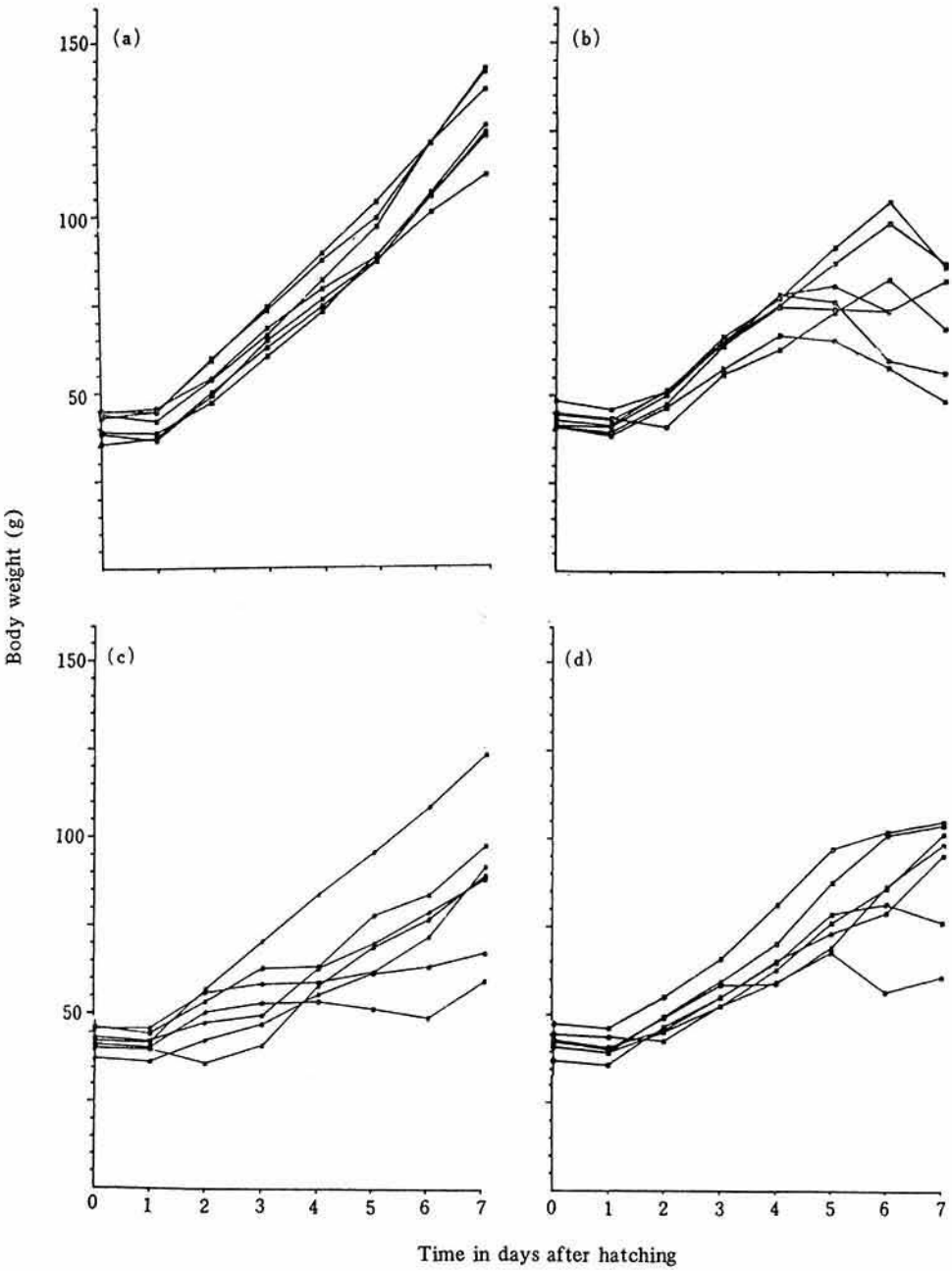

Fig. 1. Profile of body weight gain of broilers fed various diets
a) Whole meal of mackerel, GES=0.
b) Heated whole meal of mackerel, GES=14.
c) 2.4 g of histamine per one kg of diet, GES=2.
d) Heated mixture of casein-histidine (750 mg histidine/150 g casein/kg diet), GES=14.

when about the same amount of histidine as found in water extract of M was added to milk casein (750 mg/150 g casein/kg diet) and then heated (GES=14). Amino acid mixture, exclusive of histidine, did not show any effect when it was treated as described above. Histamine, even in a large amount (2.4 g/kg diet), could not induce a severe GE (GES=2), and, moreover, about five times amounts of histamine (225 mg/150 g casein/kg diet) as found in M showed only a low toxicity (GES=4) even after mixing with casein and heating. It seemed, therefore, that histamine in fish meal might not act as a main cause in inducing GE. Nucleotides had no effect in the induction of the toxicity. It was also proved that a negligible amount of histidine and no histamine were contained in the water extract of fish meal of cod.

Toxicity of heated mixture of protein-histidine

It was demonstrated that histidine was changed to the toxic substance when it was mixed with milk casein and heated. To examine the effect of protein to react with histidine, various kinds of proteins other than casein were used. Protein was mixed with histidine solution (750 mg histidine/150 ml, pH 5.5/150 g protein/kg diet), dried and heated. As shown in Table 1, diets which contained heated proteins without histidine were found to be non-toxic. Isolated soybean protein, gluten and ovalbumin showed severe toxicity when mixed with histidine and heated. In the case of gelatin, a somewhat milder toxicity was observed. On the other hand zein or yeast showed only a slight toxicity even when treated in the same way. It is considered that the nature of protein molecule is closely related to the production of GE-inducing substance. The difference of amino acid constituents of proteins, such as lacking of lysine and tryptophan in zein and tryptophan in gelatin, might have some effect. In the case of yeast, it is not assured whether yeast protein in the cell structure was mixed well with histidine in the present study. Among the proteins tested, milk casein was found to be easy in mixing with histidine solution and drying, therefore, casein-histidine mixture was used as the model of fish meal.

Table 1. Toxicity of various proteins

Heated protein	GES	
	+Histidine	−Histidine
Isolated soybean protein	20	0
Gluten	17	0
Zein	2	0
Yeast	3	0
Ovalbumin	15	0
Gelatin	7	0
Milk casein	18	0

Conditions of the preparation of heated casein-histidine

Histidine was dissolved in 150 ml of water. The solution was adjusted to pH 5.5, mixed with 150 g of casein, dried under reduced pressure, and heated at 135°C for three hours. The heated mixture was added to SD as to be one kg in total amount. As shown in Fig. 2, 30 mg of histidine was not effective in production of toxic substance. An increase in amount of histidine resulted in a rise of toxicity, and 750 mg of histidine was considered to be enough to produce a maximal

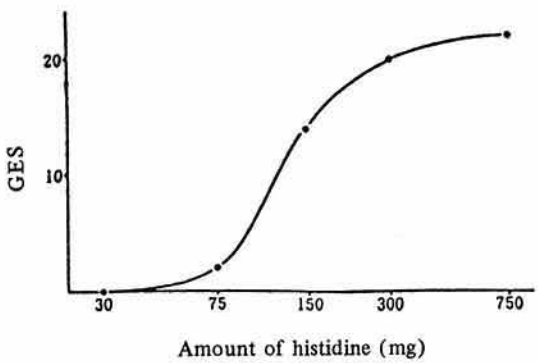

Fig. 2. Effect of amount of histidine mixed with casein before heating
A given amount of histidine was mixed with 150 g of casein (per kg of diet) and heated.

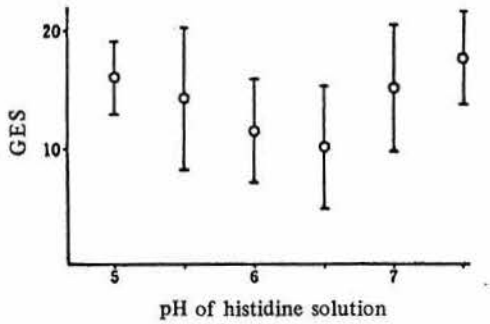

Fig. 3. Effect of changing pH of histidine solution.
Histidine solution of a given pH was mixed with casein and heated (750 mg histidine/150 ml/150 g casein/kg diet). The numbers of repeated experiments at pH 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were 5, 15, 5, 5, 5 and 3, respectively. The vertical line indicates the standard deviation.

toxicity. The effect of changing the pH of histidine solution to be mixed with casein was also examined (Fig. 3), and no significant difference was caused in the toxicity to induce GE by the changing pH value.

Heating condition to produce the toxicity was almost the same for the casein-histidine mixture as for M (Fig. 4). Heating at 110°C for three hours failed to produce the toxicity. Heating at 160° for three hours seemed to destroy the toxic substance once formed.

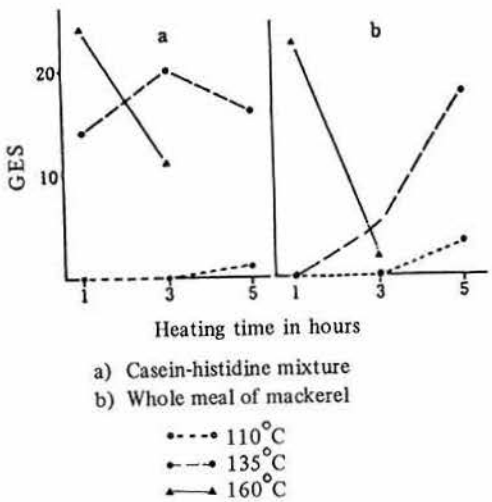

Fig. 4. Effect of heating conditions on toxicity

These results suggested that heating at higher temperature may accelerate the reaction between protein and histidine to produce toxicity, but that heating for a longer period may destroy the toxicity.

Purification of GE-inducing substance contained in heated casein-histidine mixture

With five volumes of 0.5 N-HCl, heated mixture was refluxed for 1.5 hours and centrifuged. In this condition, toxicity was observed only in the supernatant fraction, however, when refluxed for 30 minutes, the toxicity still remained in the residue. GE-inducing substance was precipitated from the supernatant fraction by adjusting to pH 3, and the resulting precipitate was solubilized at pH 6. This solution contained a heterogenous molecular weight component judging from gel filtration profile using Sephadex G-100. To the solution, which was obtained from 150 g of the starting casein (750 mg histidine), 250 mg of non-activated papain (1:350, Wako Chemical Co., Tokyo) was added and incubated at 37°C for 40 hours. After incubation, the reaction mixture was adjusted to pH 4 and the supernatant (S_P) was obtained. The toxicity was found in S_P. Increasing the amount of S_P in diet resulted in a rise of the toxicity, and 98 g (calculated as casein) of S_P, which was obtained from 450 g of starting casein, was proved to be toxic enough when it was added to one kg of diet. S_P was further purified by gel filtration using Sephadex G-25, and five fractions were obtained (Fig. 5). Only in the second fraction, which was considered to have the molecular weight of about 2,000–3,000, toxiicity was detected. Other fractions were non-toxic at all even when large amounts were fed to chicks (Table 2). In this examination, the fifth fraction was not tested, as this fraction contained only free amino acids in which trytophan was most abundant.

By the analyses with chromatography and electrophoresis, it was found that the second

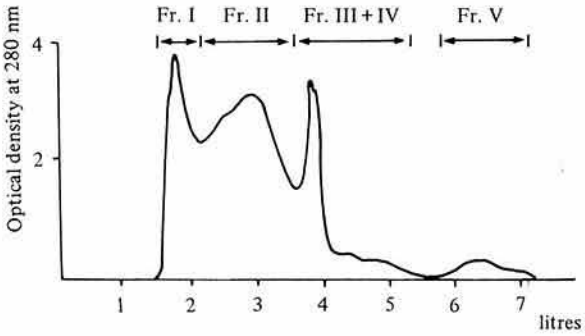

Fig. 5. Gel filtration of S_P

S_P, obtained from 90 g of the starting casein, was applied to a column (8×85 cm) of Sephadex G-25 and eluted with water with a flow rate of 18 ml/min

Table 2. Toxicity of various fractions obtained from heated casein-histidine

	Amount, added to one kg of diet	Amount (g) of starting casein	GES
Heated casein-histidine	138 g	150	14
Supernatant, obtained after acid hydrolysis	110 g	150	8
Precipitate, obtained from the supernatant	85 g	150	9
	33 E·L	150	2
	66 E·L	300	5
S_P	98 E·L	450	12
	131 E·L	600	17
Fractions, obtained after gel filtration			
Fraction I	14 E·L	450	0
	59 E·L	1,910	0
Fraction II	59 E·L	450	11
Fraction III + IV	18 E·L	450	0
	59 E·L	1,480	0
Fraction V	2.5 E·L	450	0

E·L: Optical density at 280 nm was multiplied by volume (litre)

fraction, in which GE-inducing substance was present, still contained several components. Further purification of the second fraction is now under investigation.

It is not known whether the GE-inducing substance contained in the second fraction of S_P is identical to that existed in heated M. It is, however, strongly suggested that they are the same or similar, because the presence of histidine and some kind of protein as well as the heat-treatment are necessary for the production of both substances.

After the identification of GE-inducing substance of heated casein-histidine mixture, it is intended to device a procedure for the screening of the fish meal which may induce GE.

The incidence of GE can be prevented by

the feeding of a diet which contains a small amount of fish meals, such as M. The process of fish meal production has also been improved to avoid over-heating. At present, in Japan, such commercial formula feeds for chicks are widely used, and occurrence of GE is scarcely observed in the field.

References

1) Avila, G. E. & Balloun, S. L.: Effects of anchovy fish meal in broiler diets. *Poult. Sci.*, **53**, 1372–1379 (1974).
2) Cover, M.S. & Paredes, F.: Perforating ventricular ulceration in young chickens. *Avian Disc.*, **15**, 609–610 (1971).
3) Harry, E. G., Tucker, J. F. & Laursen-Jones, A. P.: The role of histamine and fish meal in the incidence of gizzard erosion and proventricular abnormalities in the fowl. *Br. Poult. Sci.*, **16**, 69–78 (1975).
4) Harry, E. G. & Tucker, J. F.: The effect of orally administered histamine on the weight gain and development of gizzard lesions in chicks. *Vet. Rec.*, **99**, 206–207 (1976).
5) Horaguchi, H. et al.: Gizzard erosion and ulceration in broiler chicks. 2. Effect of fish meal. *Japan. Poult. Sci.*, **17**, 351–357 (1980) [In Japanese with English summary].
6) Janssen, W. M. M. A.: The influence of feeding on gizzard erosion in broilers. *Arch. Gefluegelkd.*, **35**, 137–141 (1971).
7) Johnson, D. C. & Pinedo, D. C.: Gizzard erosion and ulceration in Peru broilers. *Avian Dis.*, **15**, 835–837 (1971).
8) Kazama, T. et al.: Gizzard erosion and ulceration in broiler chicks. 1. Field outbreaks and reproduction of the disease. *Jap. Poult. Sci.*, **17**, 344–350 (1980) [In Japanese with English summary].
9) Miyazaki, S. & Umemura, Y.: Formation of gizzard erosion-inducing substance in heated casein-histidine mixture. *Natl. Inst. Anim. Health Q. (Jpn.)* **21**, 175–181 (1981).
10) Senda, H. et al.: Gizzard erosion and ulceration of broilers induced by fish meal in the feed. *J. Jpn. Soc. Poult. Dis.*, **16**, 69–75 (1980) [In Japanese].
11) Shifrine, M. et al.: Toxicity to chicks of histamine formed during microbial spoilage of tuna. *Appl. Microbiol.*, **7**, 45–50 (1959).
12) Umemura, Y. et al.: Properties of gizzard erosion-inducing substance in fish meal. *Natl. Inst. Anim. Health Q. (Jpn.)*, **21**, 52–60 (1981).

(Received for publication, January 22, 1982)