Effect of Dietary Lysine on Growing Pigs with Special Reference to Histopathological Studies on Costochondral Joint

Noboru KUBA¹⁾, Kohei MIYAWAKI²⁾, Fuminori FUNABASHI³⁾
and Masanori KOBAYASHI⁴⁾

*Department of Veterinary Pathology, Faculty of Agriculture, Gifu University,
Kakamigahara-shi, Gifu 504*

Senzo YAMANE⁵⁾

*Department of Animal Diseases, Faculty of Agriculture, Utsunomiya University,
Utsunomiya-shi, Tochigi 320*

Toshiji INAGUCHI

*Department of Pathology, School of Medicine, University of Kanazawa,
Kanazawa-shi, Ishikawa 920*

(Received for publication December 6, 1978)

Abstract. Two experiments, I and II, were carried out with 15 piglets (hybrids of Landrace × Yorkshire) approximately 60 days old. Six piglets were used for experiment I and the other nine for experiment II. The former were fed a high lysine diet, and the latter a low lysine diet for 4 months. DL-methionine and P/Ca ratio were formulated to meet the levels recommended by the National Research Council, U.S.A., in 1973. The nutrient contents and amino acids of the diet were made as much the same as possible for the two control groups of experiments I and II. The 9th right costochondral joint was selected in all the animals for the present study, because it exhibited more characteristic lesions than any other site examined. The results obtained are summarized as follows. (1) Growing piglets fed a high lysine diet revealed, as a whole, relatively mild disturbances in the ossification process of the costochondral joint. (2) Growing piglets fed a low lysine diet revealed, as a whole, marked disturbances in the ossification process of the costochondral joint.

Two experiments were carried out with growing piglets to evaluate the effect of dietary lysine on growth characteristics, main organs and bone, especially the costochondral joint (articulatio costochondralis).

The present paper deals exclusively with the histopathological findings of the 9th costochondral joint in 15 piglets. Two experimental groups were established. One of them was fed a high lysine diet and the

other a low lysine diet for about 4 months. Interesting results were obtained from these groups.

Materials and Methods

For the two experiments, I and II, 15 piglets of both sexes were used. They were hybrids of Landrace × Yorkshire of the same litter. They were weaned at 10 days of age. After that, synthetic milk A containing 1.25% of lysine was given until 39 days of age in both experiments, and synthetic milk

Present addresses: ¹⁾ Department of Laboratory Animal Science, School of Health Sciences, Fujita-Gakuen University, Toyoake-shi, Aichi 470-11; ²⁾ Animal Industry Experiment Station, Nagano-ken Agricultural Research Center, Shiojiri-shi, Nagano 399-07; ³⁾ Research Laboratories, Chikusan-Konosha Co., Ltd., Toyohashi-shi, Aichi 441-31; ⁴⁾ Kasugai-shi, Aichi 480-03; ⁵⁾ Sano City Office, Sano-shi, Tochigi 327

B containing 1.02% of lysine until 59 days of age in experiment I and until 51 days of age in experiment II.

Of the fifteen piglets, six were used in experiment I and nine in experiment II. All the piglets were fed twice daily in a restricted amount (Table 1).

Experiment I (high lysine diet): A total of 6 piglets 60 days old were used. A control group was set up with three of them weighing 13.2 to 19.1 kg, averaging 16.2 kg [23]. Basal diets A₁ and A₂ were fed daily in the first term (for 20 days, or from 60 to 79 days of age) and in the second term (for 104 days, or from 80 to 183 days of age), respectively [23]. The nutrient contents of the two basal diets are shown in Table 1. The crude proteins and amino acids in the diets are shown in Table 4. Diet A₁ contained 17.6* (17.3)% protein and 0.91* (0.89)% lysine for the first term, and diet A₂ 15.9* (16.1)% protein and 0.65* (0.69)% lysine (* Calculated value and, in parentheses, actually determined value). An experimental group consisted of the other three weighing 16.2 to 18.7 kg, averaging 17.8 kg [23], and fed daily basal diets A₁ and A₂ with addition to 0.3 percent L-lysine hydrochloride (Ln: >98.5% purity) (Tables 2 and 3).

Experiment II (low lysine diet): A total of 9 piglets 52 days old were used. An experimental group consisted of three animals weighing 12.4 to 16.8 kg, averaging 14.1 kg [23], and fed basal Diet B₁ and B₂ daily in the first term (for 28 days, or from 52 to 79 days of age) and in the second term (for the subsequent 105 days, or from 80 to 184 days of age), respectively [23]. The nutrient contents of basal diets B₁ and B₂ for each term are shown in Table 2. The crude proteins and the amino acids in each diet are shown in Table 4. Diet B₁ contained 15.8* (16.0)% protein and 0.59* (0.60)% lysine, and diet B₂ 15.2* (15.2)% protein and 0.80* (0.81)% lysine. A control group consisted of the other six animals (three barrows and three females) weighing more than 12 kg [23] to 19.1 kg, except two (10.6 and 11.3 kg), averaging 14.2 kg, and fed daily basal diets B₁ and B₂ with addition of 0.3 percent Ln (Tables 2 and 3).

DL-methionine (Mt: >95.0% purity) and Ca/P were formulated (Table 2) to meet the levels recommended by the National Research Council, U.S.A., in 1973 [24]. The two experiments were performed over a period from November 24, 1969, to April 17, 1970.

At the end of the experimental period the animals were sacrificed by total bleeding. The bones and main organs were removed and fixed in a 10 percent buffered formalin-physiological saline solution for more than 7 days. Bones were decalcified

with Hashimoto's electrolytic decalcifying kit. The rib block was limited to about 3 mm in thickness. A piece about 3–4 cm in length was adequate. It included the costochondral joint and extended from distal cartilage of 2–3 cm in length of the shaft. Decalcification was completed within 3 days. Paraffin and celloidin sections were prepared and stained with hematoxylin and eosin (H.E.), Azan stain, van Gieson's stain for collagenous fibers, PAS stain, and Gömörie's method of Bielschowsky-Marsch's silver impregnation for argyrophil fibers.

Bone specimens were collected from os frontale, mandibula, os metacarpale tertium proximale, and the 9th costochondral joint. All of them were collected from the right side of the body. Of these specimens, the 9th costochondral joint was collected from all the animals, because it exhibited the most characteristic and pronounced lesions. Any other bone or main organ was discarded from the description, because there were not so marked differences in it between the control and experimental groups.

Results

I. Findings of experiment I

A. Costochondral joint of control cases (C-1, 2 and 3)

Of the three cases, one (C-2) showed moderate swelling and the other two no noticeable changes (Fig. 1). In the longitudinal section of the joint, the free edge of the chondral layer facing the diaphysis took a form of convex lens (C-2). Microscopical findings are as follows.

a) Cartilaginous layer: Juvenile myeloid tissue developed newly to form islets here and there in the cartilaginous layer in one case (C-2). Homogenous streaks stained well with eosin (eosinophilic streaks) were scattered to run longitudinally among the columns of chondrocytes. They were PAS-positive and stained red and blue by van Gieson and Azan staining, respectively. Most of them started from small and medium-sized nutrient blood vessels and were connected with newly formed fine blood vessels. In one case (C-1) fissures varying in length were produced in the costochondral joint (Fig. 4). Some of them

Table 1. Diet per head per day

	Age (days)	Growing ration (kg)	Growing-fattening ration (kg)	Total (kg)
First term	52- 55	0.8	•	0.8
	56- 59	0.9	•	0.9
	60- 65	1.0	•	1.0
	66- 70	1.1	•	1.1
	71- 75	1.2	•	1.2
	76- 78	1.3	•	1.3
	79	0.6	0.7	1.3
Second term	80- 90	•	1.4	1.4
	91-100	•	1.6	1.6
	101-110	•	1.8	1.8
	111-120	•	2.0	2.0
	121-130	•	2.2	2.2
	131-140	•	2.4	2.4
	141-150	•	2.6	2.6
	151-160	•	2.8	2.8
	161-170	•	3.0	3.0
	171-180	•	3.2	3.2
	181-184	•	3.4	3.4

Remarks.

First and second term : See Table 2.

Pigs were penned in groups of three each for group feeding.

Table 2. Nutrient contents of basal diets

	Experiment I		Experiment II	
	First term Basal diet A ₁	Second term Basal diet A ₂	First term Basal diet B ₁	Second term Basal diet B ₂
	Growing (%) (60 to 79-day-old)	Growing-fattening (%) (80 to 183-day-old)	Growing (%) (52 to 79-day-old)	Growing-fattening (%) (80 to 184-day-old)
Crude protein	17.6	15.9	14.8	14.8
Ether extract	3.1	2.8	3.0	2.7
Crude fiber	4.8	5.5	5.5	6.0
Crude ash	7.4	7.3	6.9	7.1
Nitrogen-free extract	53.4	54.6	55.0	55.4
DCP	14.5	12.9	13.1	11.9
TDN	69.0	67.2	68.0	66.6
Lysine	0.91	0.65	0.59	0.50
Methionine	0.32	0.25	0.32*	0.27**
Ca/P	0.941/0.937	1.072/0.833	0.941/0.937	0.975/0.818

Remarks.

*, ** Contain 0.08% and 0.04% of DL-methionine added, respectively, to meet the levels recommended by N.R.C. (1973).

contained erythrocytes. Moreover, homogeneous areas varying in shape appeared discontinuously on the free edge of the layer of hypertrophic chondrocytes facing

the diaphysis. Some of them were zonal or spotty in shape (eosinophilic band or patch) (Figs. 4 and 5). All of them were remarkably eosinophilic, PAS-positive, stained yel-

Table 3. Contents of lysine of basal and experimental diets

Experiment I (high lysine diet)	Growing First term.....(60 to 79 day-old) ...	{ Basal diet A ₁ (control) Basal diet A ₁ +0.3% Ln (high lysine diet)
	Growing-fattening Second term.....(80 to 183 day-old) ...	{ Basal diet A ₂ (control) Basal diet A ₂ +0.3% Ln (high lysine diet)
Experiment II (low lysine diet)	Growing First term.....(52 to 79 day-old) ...	{ Basal diet B ₁ +0.3% Ln (control) Basal diet B ₁ (low lysine diet)
	Growing-fattening Second term.....(80 to 184 day-old) ...	{ Basal diet B ₂ +0.3% Ln (control) Basal diet B ₂ (low lysine diet)

Remarks.

Details of basal diets A₁, A₂, B₁ and B₂, see Table 2.

Ln : L-lysine hydrochloride.

Table 4. Crude proteins and amino acids in diets

		Diet for growing pigs (%)					Diet for growing-fattening pigs (%)				
		First term					Second term				
		Cp	Lys	Met	Cys	Try	Cp	Lys	Met	Cys	Try
Experiment I (high lysine diet)	Control group (basal diet A ₁ & A ₂)*	17.6 (17.3)	0.91 (0.89)	0.32	0.26	0.23	15.9 (16.1)	0.65 (0.69)	0.25	0.23	0.20
	Experimental group (basal diet A ₁ & A ₂)* + (0.3% Ln)	17.9 (18.2)	1.21*** (1.24)	0.32	0.26	0.23	16.2 (15.2)	0.95*** (0.81)	0.25	0.23	0.20
Experiment II (low lysine diet)	Control group (basal diet B ₁ & B ₂)**	16.1 (16.7)	0.89*** (0.91)	0.32	0.24	0.19	15.2 (15.2)	0.80*** (0.81)	0.27	0.24	0.20
	Experimental group (basal diet B ₁ & B ₂)**	15.8 (16.0)	0.59 (0.60)	0.32	0.24	0.19	14.9 (15.1)	0.50 (0.50)	0.27	0.24	0.20

Remarks.

*, **: See Table 2.

***: Added to 0.3% Ln (see Table 2).

First and second term: See Table 1.

The number in parentheses shows the determined value. Any other number indicates a calculated one.

For methionine see Table 2.

low and red by van Gieson and Azan staining, respectively, negative for the iron reaction, and free from any cell reaction. They had a fibrous or fibrillar structure inside (Fig. 6).

b) Layer of capillary invasion: This layer was uneven and presented severe congestion and scattered foci of hemorrhage (Fig. 5).

c) Bony trabeculae and bone marrow: Primary spongy bony trabeculae adjacent to the eosinophilic patches developed relatively well in two cases (C-1 and 3). It was less noticeable in the other case (C-2). Bony trabeculae were fine and rarefied, exhibiting an irregular arrangement. The bone marrow was dilated and characterized by hyperplasia of fibrous cells (osteogenic and

connective-tissue cells called collectively) and osteoclasts (Fig. 4).

B. Costochondral joint in experimental cases (E-4, 5 and 6)

There were no particular changes observed. The longitudinal section of the joint presented few abnormal changes (E-5) (Fig. 1). Microscopical findings are as follows.

a) Cartilaginous layer: Eosinophilic streaks were seen among the columns of chondrocytes in some areas. They had almost the same morphological and tinctorial properties as those found in the control group. In one case (E-4) eosinophilic patches appeared discontinuously on the free edge of the cartilaginous layer facing the diaphysis. No cell reactions were induced. In another case (E-6) those patches were very small and regarded as lesions indicating the early stage of disease. Small fissures were recognized around these patches (Fig. 8). When serial sections were examined, there was a long tape-like area adjacent to the external periosteum and along the costochondral joint. Its terminal portion was enlarged to show the appearance of ampule and buried between primary spongy bony trabeculae (Fig. 7). It exhibited the same tinctorial properties as the eosinophilic patch. Its ampule-like portion had an area stained slightly diffuse light red by van Gieson staining.

b) Layer of capillary invasion: This layer was affected with a little serious congestion in one case (E-6) (Fig. 8). There were little changes in another case (E-5) (Fig. 3).

c) Bony trabeculae and bone marrow: Primary spongy bony trabeculae adjacent to eosinophilic patches were short and fine and tended to be rarefied, though slightly (Fig. 8).

II. Findings of experiment II

A. Costochondral joint in control cases (C-104, 105, 106, 107, 108 and 109)

In one case (C-105), the joint was enlarged a little. In a longitudinal section of the joint, the free edge of the chondral layer facing the diaphysis was remarkably irregular and indented (C-104, 105, 107 and 108) (Fig. 2). Microscopical description was made separately on two groups; that is, group A (C-104, 107 and 108) in which eosinophilic patches (or bands) appeared and group B (C-105, 106 and 109) which was free from these patches.

Group A

a) Cartilaginous layer: Eosinophilic streaks running longitudinally among the columns of chondrocytes were found distributed thinly in all the three cases (C-104, 105 and 108) (Figs. 9, 10 and 15). They had almost the same morphological and tinctorial properties as those observed in experiment I. Besides, eosinophilic patches appeared on the free edge of the layer of hypertrophic chondrocytes. They contained the remains of many cavernous blood vessels (C-104, 107 and 108) (Figs. 9, 10, 14 and 16). The inside of these patches presented a fibrous or fibrillar appearance (Figs. 9, 12 and 16). It showed essentially the same morphological and tinctorial changes as that observed in experiment I.

b) Zone of capillary invasion: Congestion and hemorrhage were obvious (Figs. 13 and 14). Blood capillaries proliferated excessively in one area or another in the layer of hypertrophic chondrocytes. The new development of juvenile bone marrow tissue was noticeable (C-104 and 107). Besides, chondroclasts appeared in the vicinity of the walls of newly developed enlarged blood vessels of that layer (Figs. 10 and 11).

c) Bony trabeculae and bone marrow:

In every case primary spongy bony trabeculae tended to be fine, short or thin. Not a few fibrous cells were proliferated in dilated spaces among bony trabeculae (Figs. 11 and 16). Osteoclasts appeared clearly on the edges of bony trabeculae and in the bone marrow (Fig. 16).

Group B

Eosinophilic streaks running longitudinally among the columns of chondrocytes were many (C-105) or a few (C-106 and 109). Some of them reached the end of the diaphysis (C-105). Those streaks had almost the same morphological and tinctorial properties as those observed in group A. Congestion of blood capillaries was seen in two cases (C-105 and 109). In one case (C-105), primary spongy bony trabeculae were so fine and thin and ran so irregularly as to attract attention. Not a few osteoclasts appeared in the bone marrow (C-105 and 108).

B. Costochondral joint in experimental cases (E-101, 102 and 103)

Enlargement varied in severity with the case. It was severe in case E-103 (Fig. 2) and moderate in case E-101. In a longitudinal section of the joint, the free edge of the chondral layer facing the diaphysis was irregular and indented, and presented the appearance of convex lens as a whole in two cases (E-101 and 103) (Fig. 2). Microscopical findings are as follows.

a) Cartilaginous layer: In this layer nutrient blood vessels were congestive, the arrangement of cell columns was disturbed, and the columnar cell layer decreased in height (E-101 and 103). In all the cases eosinophilic streaks were found running longitudinally among the cell columns. They had almost the same morphological and tinctorial properties as those observed in the control cases. A small cyst was present in an area of the hypertrophic cell

layer of the cartilage (Fig. 21). The stroma among the cell columns varied in breadth, particularly distinctly in some areas (E-101 and 103).

Moreover, an eosinophilic band was produced on the free edge of the layer of hypertrophic chondrocytes facing the diaphysis. It extended all over the edge, except a small portion (E-103), over about three-fifths (E-101), or over about one-fifth (E-102) of the edge. This lesion varied in severity and extension with the individual case. In case E-103, it was composed of two or three layers piled one on top of another (Figs. 22 and 23). Small bud-like foci of calcification stained blue by Azan staining were scattered among these layers (E-103). In one case (E-101), the eosinophilic bands contained many areas where a fibrous or fibrillar structure was presented. Caverns were buried among these areas. They were the remains of degenerative blood vessels, large and small. In another case (E-102), an eosinophilic band generally appeared to be a broad plane with an indented edge. It contained a diffuse area of deposition of calcium stained light blue with hematoxylin.

b) The layer of capillary invasion: Hemorrhage varying in severity was found in all the cases. In extremely severe cases, foci of diffuse hemorrhage were scattered (Figs. 20 and 23), hemorrhage was rather extensive (E-101), or congestion was so intense along the whole edge of the layer of capillary invasion that hemorrhage seemed to follow congestion immediately (E-102). In some cases, chondroclasts occasionally appeared in areas adjacent to newly formed blood vessels in the layer of hypertrophic chondrocytes (E-101 and 103).

c) Bony trabeculae and bone marrow: In such area as adjacent to the eosinophilic bands, primary spongy bony trabeculae

were fine and became rarefied extensively (Figs. 22 and 23), exhibiting an irregular arrangement (Fig. 24). Intertrabecular spaces were dilated and fibrous cells proliferated noticeably (E-101 and 103) (Figs. 17, 18, 22, 23 and 24). Osteoclasts multiplied remarkably in the bone marrow (E-101 and 103). In two cases (E-101 and 103), islets of unresorbed cartilage were observed in the diaphysis (Fig. 19).

Discussion

Two experiments were carried out. Each of them was divided into two terms, the first and the second [23]. Growing pigs were fed a low lysine diet containing lysine to 0.60 and 0.50 per cent in the first and the second term, respectively, for about 4 months. There was an outstanding tendency for a serious disturbance to be induced in the process of ossification in the layer of osseous development of the costochondral joint. When a high lysine diet containing 1.24 and 0.81 per cent of lysine was fed in the first and the second term of experiment, respectively, no serious disturbance was recognized in the process of ossification in the layer of osseous development as a whole, but pathological changes tended distinctly to be generally mild or scanty. These results were similar to those obtained from rabbits by Moriwaki et al. [22]. In the present experiment discretion was used to maintain the level of crude protein and the composition of amino acids in the diet and the balance between phosphorus and calcium.

Attention should be paid to the following interesting results of the present experiment. When body weight was measured, case E-103 which manifested severer changes than any other case was found to be among the upper three showing heavy body weight of the 15 cases examined. This result was

contrary to expectation. It seemed to suggest that pathological changes of bone might not always be mild in those which exhibited satisfactory growth. Essentially the same result as this was stressed by Kimura [12] who had performed an experiment on starvation.

When growing rats were fed gliadin contained in wheat, as a source of protein, they failed to show any change in body weight for a month. Since this fact was reported by Henriques [9], many papers have been published to report that lysine is essential for growth [13, 18, 21, 25]. Harris et al. [8] mentioned that deficiency in lysine led to the arrest of growth, a decrease in calcification of bone, hypoproteinemia, anemia, a decrease in mitotic figures of spermatocytes, and a reduction in subcutaneous adipose tissue in rats. They found that these symptoms and changes were similar to those manifested at the time of fasting, and asserted that they had been caused by the inhibition of protein synthesis. After that, it was stressed by one author to another that protein played an important role in the process of ossification [1, 7, 10]. It was also pointed out that such changes as induced by fasting were remarkable in the costochondral joint [12]. Mack et al. [15] observed that growth was better and bone harder in schoolchildren fed a diet with addition of lysine than in those fed a diet with no addition.

In 1941, it was demonstrated that lysine was one of the essential amino acids for the growth of swine [19]. Since then, discussion has been made on the relationship between the amount of lysine contained in a diet and the growth of swine, but no conclusions have been drawn as yet [11, 16, 17, 24, 27]. Some authors asserted that no addition of lysine to a diet was effective for the treatment of fracture [6], but others stood by

their view that lysine was efficacious in curing fracture [20]. Besides, it was stressed that the addition of lysine to a tissue culture of osteoblasts was essential for the development of these cells [5]. It should be noted that a larger intake of ^{45}Ca was found in the epiphysis than in the diaphysis in rats [14].

Such pathological changes of interest as common to the experimental and control groups of the present experiment are mentioned below.

(1) Eosinophilic streaks: These streaks ran longitudinally mostly between the columnar and the hypertrophic cell layers. They seemed to be related to the circulatory disturbances, judging from their location and tinctorial and morphological properties.

(2) Eosinophilic bands or patches: The bands or patches appeared mainly on the free edge of the layer of hypertrophic chondrocytes facing the diaphysis. It was characteristic of them to be accompanied with hemorrhage. Judging from their location and tinctorial and morphological properties, the areas containing these eosinophilic bands or patches were regarded as necrotic foci. Their pathogenesis was presumed as follows. At the beginning of the course of disease hemorrhage, large and small in extent, may take place on account of circulatory disturbances in the layer of capillary invasion. Calcification may be disturbed in the layer of hypertrophic chondrocytes which is in the physiological process of decomposition. As chondrocytes proliferate continuously, this layer may increase in thickness. As a result, necrotic foci may be formed.

Recently, "leg weakness" of swine has come to be a current problem. In some papers were reported essentially the same changes as "eosinophilic streaks" and

"fibrous degeneration of cartilage" mentioned above [2, 3, 28, 30]. They were regarded as those of necrosis of the chondrocyte layer in those papers. On the other hand, Duthie et al. [3] asserted that the possibility that the disease in question might have been caused by PPLO infection could not be denied. Some previous authors [30] negated the presence of any etiological agent and asserted that such stress as loading with weight might be a cause of the disease in question. The etiology of this disease was unknown in some investigation [4]. Seiber et al. [26] found similar changes in the articular cartilage of cattle and ascribed the cause of these changes to standing for a long time.

No discussion will be made on the etiology of "leg weakness" in the present paper. It should be noted, however, that no anemia was observed and that hemorrhage and congestion were rather conspicuous in the layer of capillary invasion in the present experiment [29].

Acknowledgments: The authors are grateful to the Tanabe Seiyaku Co., Ltd., for cooperation extended at the beginning of this experiment and to the Kyowa Hakko Co., Ltd., for supply of L-lysine hydrochloride.

References

- [1] Bavetta, L. A., and Bernik, S. (1955). Lysine deficiency and dental structures. *J. Amer. dent. Ass.* **50**, 427-433.
- [2] Bullough, P. G., and Heard, T. W. (1967). Pathological lesions associated with the "leg weakness" syndrome in pigs. *Brit. vet. J.* **123**, 305-310.
- [3] Duthie, I. F., and Lancaster, M. C. (1964). Polyarthritis and epiphyseolysis of pigs in England. *Vet. Rec.* **76**, 263-273.
- [4] Fell, B. F., Jones, A. S., and Boyns, R. (1967). Lesions of distal ulnar epiphysis in wild East African pigs. *Vet. Rec.* **81**, 341.

- [5] Fisher, A. (1948). Amino acid metabolism of tissue cell *in vitro*. *Nature (London)* **161**, 1008.
- [6] Hagger, R., Kinny, T. D., and Kaufman, N. (1955). Bone healing in lysine-deficient rats. *J. Nutr.* **57**, 305-317.
- [7] Harada, M., and Horiguchi, G. (1955). Bone and nutrition (primary report). Experimental studies on changes of bone and bone marrow in hypoproteinemia. *Wakayama med. Report* **2**, 7-14.
- [8] Harris, H. A., Neuberger, A., and Sanger, F. (1943). Lysine deficiency in young rats. *Biochem. J.* **37**, 508-513.
- [9] Henriques, V. (1909). Lässt sich durch Fütterung mit Zein oder Gliadin als einziger stickstoffhaltiger Substanz das Stickstoffgleichgewicht herstellen? *Z. physiol. Chem.* **60**, 105-118.
- [10] Hori, S., and Niwa, K. (1951). Studies on the protein composition of bone in horses with osteomalacia. *J. Jap. vet. med. Ass.* **4**, 180-183 (in Japanese).
- [11] Jensen, A. H. (1973). Nutrient requirements of swine. *Feed Stuffs* **45**, 31.
- [12] Kimura, T. (1946). Experimental studies on starvation. II. Anatomopathological changes. *Medicina* **45**, 575-587 (in Japanese).
- [13] Kligler, D., and Krehl, W. A. (1952). Lysine deficiency in rats. II. Studies with amino acid diets. *J. Nutr.* **16**, 61-74.
- [14] Likms, R. C., Bavetta, I. A., and Posner, A. S. (1957). Calcification in lysine deficiency. *Arch. Biophys.* **70**, 401-412.
- [15] Mack, P. B., Vose, G. P., Kinard, C. L., and Cambell, H. B. (1962). Effects of lysine-supplemented diets on growth and skeletal density of preadolescent children. *Amer. J. clin. Nutr.* **11**, 255-262.
- [16] McWard, G. W., Becker, D. E., Norton, H. W., Terill, S. W., and Jensen, A. H. (1959). The lysine requirement of weanling swine at two levels of dietary protein. *J. Anim. Sci.* **18**, 1059-1066.
- [17] Magruder, N. D., Sherman, W. C., and Reynolds, W. M. (1961). Evaluation of supplemental lysine for practical swine rations. *J. Anim. Sci.* **20**, 573-577.
- [18] Mendel, L. B. (1915). Nutrition and growth. *J. Amer. med. Ass.* **8**, 524-531.
- [19] Merz, E. T., Shelton, D. C., and Beeson, W. M. (1941). The amino acid requirement of swine, lysine. *J. Anim. Sci.* **8**, 524-531.
- [20] Mihashi, Y., Tsunoda, H., and Ito, R. (1961). Curative effect of L-lysine on fracture caused by disturbances in Ca metabolism in dogs. *J. vet. Med.* No. 294, 25-27 (in Japanese).
- [21] Mitchell, H. H., and Smuts, D. B. (1932). The amino acid deficiency of beef, wheat, corn, oats, and soy beans for growth in the white rat. *J. biol. Chem.* **95**, 263-281.
- [22] Moriwaki, T., and Ishitani, R. (1964). Histo-pathological studies on nutrition of amino acid. *Kenkyuseika, Council Agri. Forest. Fisheries*, No. 20, 303-343 (in Japanese).
- [23] Niwa, T. (1964). Progeny testing method of meat productive performance of pigs in Japan—Progress of basic researches of their results—. *Ann. Report nat. Inst. Anim. Indust.*, 129-151 (in Japanese).
- [24] National Research Council, U.S.A. (1973). Nutrient Requirements of Domestic Animals. No. 2, Nutrient Requirements of Swine, 7th rev. ed., Washington, D.C.
- [25] Osborne, T. B., and Mendel, L. B. (1914). Amino acid in nutrition and growth. *J. biol. Chem.* **17**, 325-349.
- [26] Seibel, S., Dammrich, K., and Andreade, U. (1973). Überbelastungsschäden an der distalen Epiphysenfugenscheibe des Radius bei Mastbullen aus verschiedenen Aufstellungsformen. *Berl. Münch. tierärztl. Wschr.* **86**, 441-447.
- [27] Takahashi, S., Hirakata, Y., and Morimoto, H. (1968). Effects of adding lysine and methionine to low protein diets for pigs. *Ann. Report nat. Inst. Anim. Indust.* **16**, 31-36 (in Japanese with English summary).
- [28] Thurley, D. C. (1969). Changes in the epiphyseal cartilage of immature pigs without clinical lameness. *Path. vet.* **79**, 217-226.
- [29] Truett, J., and Trias, A. (1961). The vascular contribution to osteogenesis. IV. The effect of pressure upon the epiphyseal cartilage of the rabbit. *J. Bone Jt. Surg.* **43B**, 800-813.
- [30] Walker, T. B., Fell, F., Jones, A. S., Boyne, R., and Elliot, M. (1966). Observation on "leg weakness" in pigs. *Vet. Rec.* **79**, 472-479.

要 約

発育中のブタに対するリジンの影響，特に肋骨—肋軟骨関節の病理組織学的研究：久葉 昇・宮脇耕平・船橋史憲・小林正紀（岐阜大学農学部家畜病理学教室），山根仙造（宇都宮大学農学部家畜疾病学教室），稲口利次（金沢大学医学部病理学教室）——ランドレースとヨークシャーの交雑種15頭（約60日齢雄および雌を含む）を用い，実験Ⅰは6頭（内対照3頭），同Ⅱは9頭（内対照6頭），前者は高リジン飼料，後者は低リジン飼料を投与した．これら2実験の期間は，それぞれほぼ4ヶ月とした．DL-methionine および Ca/P 比は，N.R.C. (1973) の飼料標準に適合する様にこれを配合した．また，実験ⅠおよびⅡの両対照群の飼料成分およびアミノ酸が，それぞれ近似する様に努めた．実験はその終了と共に放血殺，今回の研究に対しては，検討した諸項目中，病変の最も顕著であった右側第9肋骨—肋軟骨関節を選択した．得られた結果は次の通りである．

1. 高リジン飼料を投与された発育中の豚群では，全体としてそれらの肋骨—肋軟骨関節の化骨機転障害が比較的軽度であった．

2. 低リジン飼料を投与された発育中の豚群は，全体として，それらの肋骨—肋軟骨関節の化骨機転障害が顕著であった．

Explanation of Figures

All photographs in the following plates are of tissue sections of the 9th right costochondral joint. Staining: Hematoxylin and eosin (H.E.). E, Experimental group; C, Control group.

- Figs. 1 and 2 are photomacrograph of each sagittal section of the costochondral joint of two pigs in experiments I and II. Both cases in the former are almost normal E-103 in the latter, however, reveals a striking swelling of the costochondral joint.
- Fig. 3. An almost normal costochondral joint. Chondrocytes are arranged in a neat and orderly fashion. E-5, $\times 40$.
- Fig. 4. Diaphyseal face of the cartilage showing a horizontal split and capillary invasion. Striking homogeneous eosinophilic areas (bands or patches) with hemorrhage are evident. C-1, $\times 100$.
- Fig. 5. Irregularity in the vicinity of the diaphyseal face of the cartilage with an eosinophilic horizontal band and marked hemorrhage. Unresorbed cartilage in the primary spongiosa is also evident. C-2, $\times 40$.
- Fig. 6. Fibrous degeneration or fibrillation of the eosinophilic band. No cellular reaction is evident in and around the lesion. C-2, $\times 100$.
- Fig. 7. Ampullaceous eosinophilic patch projecting from the distal face of the cartilage. van Gieson staining. E-6, $\times 200$.
- Fig. 8. Early lesions of eosinophilic patches developed on the free edge of the chondral layer facing the diaphysis. E-6, $\times 200$.
- Fig. 9. Irregularities on the line of the costochondral joint, eosinophilic bands accompanied with dilatated blood vessels without blood supply on the diaphyseal face of the cartilage. C-104, $\times 40$.
- Fig. 10. Newly formed primary vascular marrow tissue in the cartilage. Chondroclasts (arrow) are seen in the wall of a dilatated capillary. C-104, $\times 40$.
- Fig. 11. High-power magnification of part of Fig. 10. C-104, $\times 100$.
- Fig. 12. Fibrous degeneration of spurs of hypertrophic chondrocytes projecting from the diaphyseal face of the cartilage. C-104, $\times 100$.
- Fig. 13. Eosinophilic streaks associated with tongue-like overgrowth of the cartilage. C-107, $\times 40$.
- Fig. 14. Gross, tongue-like prolongation of a segment of the cartilage separated from the diaphysis by an eosinophilic horizontal band (necrotic focus) accompanying hemorrhage. C-107, $\times 40$.
- Fig. 15. Long eosinophilic streaks running through the cartilaginous cell columns. Note necrotic chondrocytes, increased intercellular matrix, and reactionless structures adjacent to streaks. C-108, $\times 40$.
- Fig. 16. A field of eosinophilic bands (necrotic foci) and rarefaction of trabeculae of the primary spongiosa. Note the fibrous degeneration of the bands with hemorrhage. C-108, $\times 200$.
- Fig. 17. Typical field of an eosinophilic horizontal band with hemorrhage in the zone of calcification and chondrocytic disintegration on the distal face of the cartilage. E-101, $\times 40$.
- Fig. 18. High-power magnification of a part of Fig. 17. Fibrous degeneration or fibrillation of eosinophilic horizontal bands can be seen. E-101, $\times 100$.
- Fig. 19. Isolated islets of unresorbed cartilage in the diaphysis. E-101, $\times 100$.
- Fig. 20. Eosinophilic horizontal band on the diaphyseal face of the cartilage. Hemorrhage is evident in the layer of capillary invasion. E-102, $\times 40$.
- Fig. 21. Cyst formation in the layer of hypertrophic chondrocytes. E-103, $\times 40$.
- Fig. 22. Typical field of disorganization, necrosis and hemorrhage on the diaphyseal face of the cartilage. Extensive rarefaction of trabeculae and fibrosis are evident. E-103, $\times 40$.
- Fig. 23. Typical lesions similar to those of Fig. 22. Areas of necrosis of the distal end of the cartilage are found in parallel bands stained with eosin. E-103, $\times 40$.
- Fig. 24. Extensive rarefaction and irregular arrangement of poorly calcified trabeculae (osteoid), and multiple osteoclasts in the primary marrow. E-103, $\times 40$.