褪色と高い脂質含量を特徴とする肝臓変性を有すると殺豚におけるDFD肉の発現

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Production of Dark Firm Dry Meat in Slaughtered Pigs with So-Called Liver Degeneration Characterized by Yellowish Discoloration and High Lipid Contents

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ABSTRACT. The livers which are discolored yellowish and have a large amount of lipids (liver degeneration) are frequently observed in slaughtered pigs. Dark firm dry (DFD) meat and liver degeneration have a common etiological agent of exhaustion in pigs before slaughter. The correlation between them was examined. In 65 cases of 77 slaughtered pigs with the degenerative liver, which contained more than 6.6% total lipids of liver wet weight, the carcasses showed early rigor mortis and the higher final pH above 6.0. R values of the muscles, which indicated the decrease of ATP, were higher. The meat had a DFD appearance and the muscle fibers had no tendency to shrink. These data showed that the pigs with liver degeneration produced DFD meat at a high rate. In 4 cases of 5 pigs exhausted experimentally by 53 hr fasting and hard exercise before slaughter, both liver degeneration and DFD meat were produced simultaneously. The livers of them were discolored and contained about 8.0% or more total lipids. The carcasses of them showed early rigor mortis and the higher final pH above 6.0. From these data, it was suggested that the exhaustion in pigs before slaughter caused both liver degeneration and DFD meat.—KEY WORDS: DFD meat, exhausted pigs, liver degeneration.

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The meat and liver quality of slaughtered pigs is greatly affected by the physiological conditions before slaughter [3, 6, 10, 12–17, 21, 24]. Dark firm dry (DFD) meat from pigs [3, 7, 10–13, 15, 20] is significantly deficient not only in flavour but also in meat hygiene [1, 2, 6, 19, 22]. DFD meat is produced from pigs which have been fasted for a long time, or which have been exhausted by shipping, discharging and long distance transportation [3, 10, 15]. DFD carcasses are characterized by higher final pH and early development of rigor mortis after slaughter [7, 11, 15, 20].

The livers which are discolored to dull yellow (Fig. 1) are frequently observed in slaughtered pigs [8, 16]. These livers are named so-called liver degeneration [8], and also called fading of hepatic parenchyma or fatty liver [18]. About 3 million slaughtered pigs were checked in Hokkaido from 1982 to 1987, and the incidence of liver degeneration was a high rate of 5.52%. Liver degeneration is mainly observed in the pigs which was transported from long distance and which were carried to a slaughterhouse in the previous day of slaughter [16]. In the pigs with liver degeneration, a large amount of lipid moved from adipose tissues to liver and accumulated there [17, 23]. Liver degeneration is considered to be caused principally by a long period of fasting and thirst from the last feeding on a farm to the time of slaughter, accompanied by much stressor [16, 17].

We have studied on DFD meat and liver degeneration in slaughtered pigs separately [12, 13, 15–17], and noticed the similarity between them. Exhaustion due to a long period of fasting and exercise is a common etiological agent for them. Therefore, in the present study, to confirm the correlation

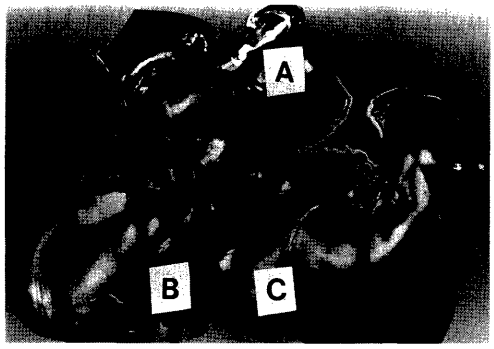

Fig. 1. Liver degeneration in slaughtered pigs (B and C). The livers are dull yellow. A is a normal liver.

between DFD meat and liver degeneration, the carcasses of the pigs which have the yellowish discolored liver with high lipid contents were examined. Furthermore, we tried to produce both of them experimentally in a same pig.

MATERIALS AND METHODS

Experimental animals: Females and castrated males of mixed-bred meat pigs, weighing each about 100 kg and appearing clinically healthy, were used. The pigs of Group I and Group II were usually slaughtered ones. Fifty pigs which had a normal dark red liver were assigned to Group I. Seventy-seven pigs, the livers of which were discolored yellowish, were assigned to Group II. The pigs of Group III and Group IV were from a herd of a farm. Five of them were assigned to Group III and Group IV comprised the other five. The pigs of Group III were fed on the farm and transported to the slaughterhouse by truck for 15 min. After 3 hr of holding, they were slaughtered. The pigs of Group IV were fasted on the farm for 26 hr and then transported with Group III. After one day of holding without feed and water, they were exercised under a repeated electrical stimulus for half an hour and then immediately slaughtered (53 hr fasting). The carcasses of each group were dressed by the routine procedure and chilled at 3°C within 40 min after slaughter.

Sampling of the livers and muscles: On evisceration (within 10 min of slaughter), the livers and other internal organs were examined macroscopically and the livers were collected. Samples of *Musculus (M.) longissimus dorsi* were collected at 90 min and 24 hr after slaughter from between the 5th and 6th *costae* of the carcasses, using with the tool devised by us [12, 13]. The samples of the livers and muscles were stored in liquid nitrogen. A part of the muscle sample was fixed in 10% buffered neutral formalin.

Determination of total lipid contents in the livers: The extraction of total lipids from livers was carried out with Folch's method [4]. Total lipids were extracted twice with chloroform-methanol (2:1, v/v), and the extracts pooled were washed with physiological saline solution. The extracts were evaporated and weighed. Total lipid contents were expressed as a percentage of liver wet weight.

Determination of the development of early rigor mortis in the carcasses: When rigor mortis breaks out in the warm carcasses which are hung on hooks, the characteristic form like Fig. 2 are appeared. The phenomenon of the ham swelling and the arm rising, and the trunk moving along with the arm was considered as early rigor mortis [14, 15]. In the present study, the development of early rigor mortis in the carcasses were determined by the observation at 90 min after slaughter.

Measurement of R value of the muscles: R value suggests the breakdown of adenine nucleotides to hypoxantine derivatives in muscles [7]. These were extracted with perchloric acid. Absorbance ratios of 250 nm/260 nm (R value) of this extracts were measured.

Measurement of temperature and pH of the carcasses: A thermometer (OMRON

Fig. 2. Early rigor mortis in carcasses of slaughtered pigs (left). The arrows show the ham swelling and the arm rising. Right is a normal carcass of the same size.

HC-100S) and the glass combination electrode (HORIBA 6326) of a pH meter (HORIBA H-7_{LD}) were inserted directly between the 5th and 6th *costae* into the middle of *M. longissimus dorsi* of the carcasses. The temperature and pH of the carcasses were measured at 10 min, 90 min and 24 hr after slaughter.

Morphological observation of the muscles: The cut cross-section surfaces of loins, between the 5th and 6th costae of the carcasses, were observed macroscopically 24 hr after slaughter. Subjective color morphology rating was performed for *M. longissimus dorsi*, which forms the heart of loin, using the pork color standard (National Institute of Animal Husbandry, Ibaraki); a 6-point scale, in order, from pale to dark. For light microscopy, paraffin sections of the muscles were stained with hematoxylin-eosin (HE). Volume density, which indicates the proportion of volume occupied by muscle fibers in the muscle tissue 24 hr after slaughter, was measured by point counting method [9].

RESULTS

Total lipid contents in the livers: The livers from the pigs of Group I and Group III had a original dark red color and a normal appearance. As shown in Fig. 3, total lipid contents of the livers of Group I and Group III were less than 6.0% of liver wet weight. The average total lipid contents of the livers of Group I and Group III were 4.94±0.54% and 5.19±0.49%, respectively. The livers from the pigs of Group II and Group IV were discolored yellowish (Fig. 4). Total lipid contents of the livers of Group II and Group IV were varied from 6.6% to 13.4% of liver wet weight (Fig. 3). The average total lipid contents of the livers of Group II and Group IV were 9.46±1.47% and 8.45±0.99%, respectively.

Occurrence of early rigor mortis in the carcasses: The results of the observations for the carcasses of each group at 90 min after slaughter were shown in Table 1. Early rigor mortis was observed in only a part of the carcasses of Group I at 90 min after slaughter. It had occurred in the greater part of the carcasses of Group II. Early rigor mortis

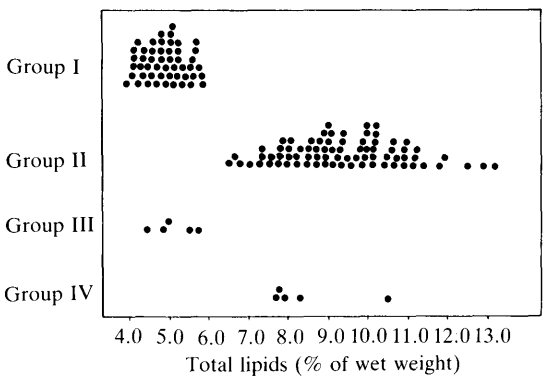

Fig. 3. Total lipid contents in livers.

Fig. 4. The livers of Group IV (B-E). B and C are discolored obviously. D and E appear a little dark because of hyperemia. A is a normal liver as a control. Cut surfaces of *lobus hepatis dexter lateralis*.

was not observed in the carcasses of Group III, but observed in all the carcasses of Group IV.

R value of the muscles: The average R values of the muscles of each group at 90 min after slaughter were shown in Table 1. The average R values of Group II and Group IV were higher than those of Group I and Group III, respectively.

pH and temperature of the carcasses: The pH of the carcasses at 24 hr after slaughter (the final pH) of each group were shown in Fig. 5. The final pH of all the carcasses of Group I and Group III were below 6.0. On the other hand, the final pH of the carcasses of Group II and Group IV varied from 5.5 to 7.0, although the greater part of those were above 6.0. So, Group II and Group IV were divided into two subgroups with the boundary of the final pH of 6.0, respectively. The proportion of the higher and the lower in the final pH of Group II, which belonged to Group II-1 and Group II-2, respectively, was 65 to 12. Similarly, the proportion of those in the final pH of Group IV, which belonged to Group IV-1 and Group IV-2, respectively, was 4 to 1. The pH and temperature of the carcasses of each group at various periods after slaughter were shown in Table 2. The pH of the carcasses of Group I and Group III decreased slowly after slaughter and reached about 5.7 fially at 24 hr after slaughter. On the other hand, the pH of the carcasses of Group II-1 and Group IV-1 decreased only a little after slaughter. The average final pH of Group II-1 and Group IV-1 were considerably higher compared with those of

Table 1. Occurrence of early rigor mortis in carcasses and R values of muscles at 90 min after slaughter

Groups	Occurrence of early rigor mortis	R value
Group I	7/50[a)]	0.88±0.05[b)]
Group II	70/77	0.93±0.09**[c)]
Group III	0/ 5	0.86±0.04
Group IV	5/ 5	1.10±0.10**

a) Number of occurrence/number of observation.
b) Mean±SD.
c) Significant differences between Group I and Group II, and between Group III and Group IV were indicated with *: $p<0.05$, **: $p<0.01$.

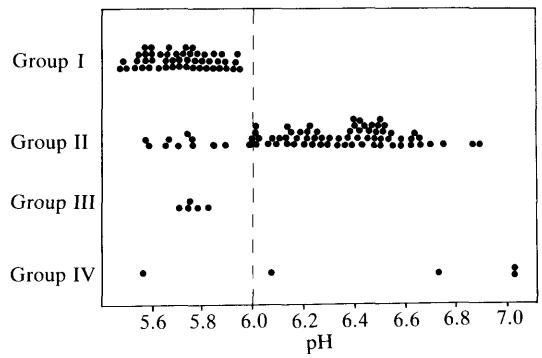

Fig. 5. pH of the carcasses at 24 hr after slaughter. From these data, Group II and Group IV were divided into two subgroups with the boundary of the pH 6.0, respectively.

Table 2. pH and temperature in carcasses at the various periods after slaughter

Groups	pH			Temperature(°C)
	10 min	90 min	24 hr	10 min
Group I	6.61±0.23[b)]	5.97±0.20	5.73±0.13	39.6±0.4
Group II[a)]				
1. pH≧6.0	6.67±0.22	6.37±0.19**[d)]	6.37±0.21**	39.5±0.6
2. pH<6.0	6.52±0.20	6.05±0.31	5.78±0.13	39.7±0.4
Group III	6.69±0.06	5.81±0.10	5.76±0.04	39.7±0.3
Group IV[a)]				
1. pH≧6.0	6.60±0.12	6.56±0.26**	6.71±0.45**	41.0±1.1*
2. pH<6.0	6.50[c)]	5.76	5.57	41.5

a) Group II and Group IV were divided into two subgroups with the boundary of pH 6.0 at 24 hr after slaughter.
b) Mean±SD.
c) Number of this subgroup is one, the value was shown.
d) Singificant differences between Group I and Group II, and between Group III and Group IV were indicated with *: $p<0.05$, **: $p<0.01$.

Group I and Group III, respectively. The temperature of the carcasses of Group IV was higher than those of the other groups at 10 min after slaughter.

Development of DFD meat in the carcasses of Group II and Group IV: The average color morphology ratings of *M. longissimus dorsi* of each group at 24 hr after slaughter were shown in Table 3. In Group I and Group III, *M. longissimus dorsi, i.e.*, the heart of loin, was light red, the color morphology rating being 2 to 4. These meats were of normal quality. In Group II-1 and Group IV-1, the meat color of *M. longissimus dorsi* was dark, the color morphology ratings being 3 to 6 (Figs. 6 and 7). These meats had a firm and dry appearance. In Group II-2 and Group IV-2, the meat color of *M. longissimus dorsi* was light red and pale red, respectively.

Microscopic observation of the muscles: Figs. 8 and 9 show the microscopic structures of *M. longissimus dorsi* specimens obtained from the carcasses of the Group I and Group IV-1, respectively, at 24 hr after slaughter. At 90 min after slaughter, in each group, muscle fibers kept their shape and were in contact with adjacent fibers (photos were not shown). At 24 hr after slaughter, in Group I and Group III, muscle fibers had shrunk, leaving gaps between the fibers, and the endomysium formed a network structure among the fibers (Fig. 8). On the other hand, in Group II-1 and Group IV-1, muscle fibers had shrunk only a little and were arranged closely, and the gaps between the fibers had a tendency to be narrow (Fig. 9). In Group II-2 and IV-2, muscle fibers had shrunk at 24 hr after slaugher. As shown in Table 3, the average volume densities of muscle fibers in the muscle tissues of Group II-1 and Group IV-1 were significantly higher than those of Group I and Group III, respectively.

Table 3. Subjective color morphology ratings of *M. longissimus dorsi* and volume densities of muscle fibers in the muscle tissues at 24 hr after slaughter

Groups	Color morphology rating	Volume density of muscle fibers
Group I	2.8±0.4[b)]	0.51±0.03
Group II[a)]		
1. pH≧6.0	4.5±0.9**[d)]	0.79±0.06**
2. pH<6.0	2.8±0.4	0.59±0.16
Group III	2.9±0.4	0.51±0.04
Group IV[a)]		
1. pH≧6.0	5.3±1.0**	0.90±0.05**
2. pH<6.0	1.5[c)]	0.47

a—d) See the foot-notes on Table 2.

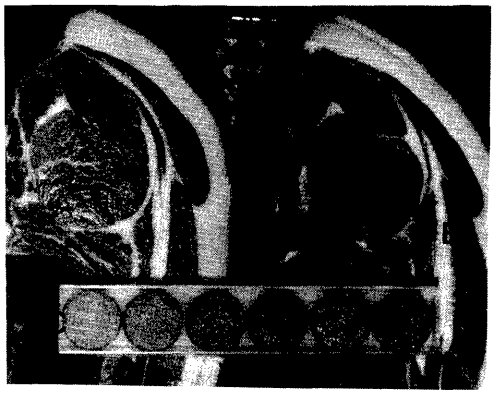

Fig. 6. Dark and dry appearance of the loin of Group II-1 at 24 hr after slaughter (B). A is a loin of Group I. Cut surfaces of the carcasses between the 5th and 6th costae. C is the pork color standard.

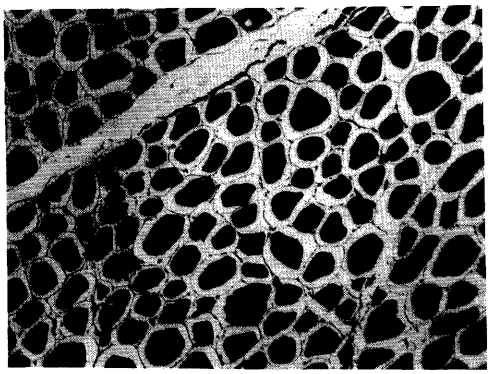

Fig. 8. Shrunk muscle fibers in Group I at 24 hr after slaughter. The endomysium forms a network structure among the fibers. *M. longissimus dorsi.* HE stain, ×40.

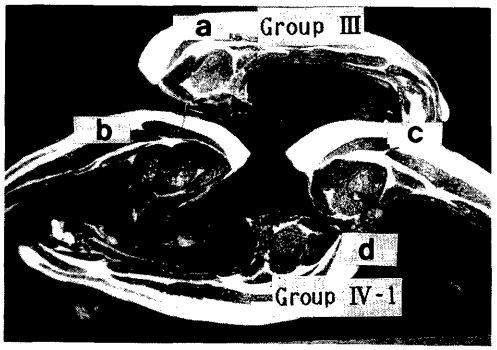

Fig. 7. Dark appearance of the loins of Group IV-1 at 24 hr after slaughter (b-d). a is a loin of Group III. Cut surfaces of the carcasses between the 5th and 6th costae.

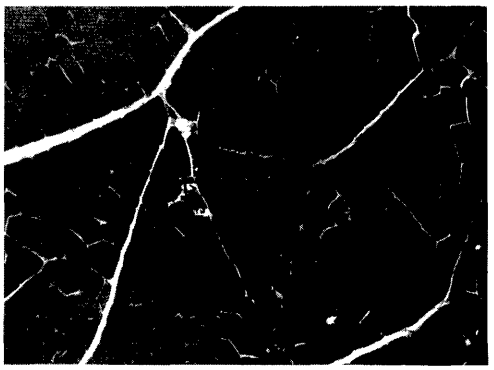

Fig. 9. Muscle structure of Group IV-1 at 24 hr after slaughter. Muscle fibers remain almost the original shape and are arranged closely. *M. longissimus dorsi.* HE stain, ×40.

DISCUSSION

Liver degeneration of slaughtered pigs which was the beginning of this study had been diagnosed histologically as a cloudy and hydropic swelling [8]. Mori *et al.* [17] reported the increase of triglycerides in the degenerative livers. Yonemichi *et al.* [23] reported peripheral or diffuse fatty changes of hepatic lobules in the degenerative livers. In the present study, there was no normal liver of slaughtered pigs that contained more than 6.0% total lipids. On the other hand, the degenerative livers contained more than 6.6% total lipids.

The qualities of the carcasses and muscles of the pigs with liver degeneration were greatly different from those of the pigs with a normal liver. In general, rigor mortis in porcine carcasses develops gradually during a period ranging from several hours to 24 hr after slaughter [10, 14, 15]. In fact, rigor mortis was observed in only a part of the carcasses of pigs with a normal liver at 90 min after slaughter. On the other hand, the greater part of the carcasses of the pigs with liver denegeration represented rigor mortis at 90 min after slaughter. Development of rigor mortis in carcasses depends on the

level of ATP in muscles [10]. R values of muscles indicate the breakdown of ATP [7]. DFD muscle has a higher R value and develops rigor mortis earlier because of the lower level of ATP [7, 12, 15, 19]. In the present study, the high R values of the muscles of the pigs with liver degeneration correlate to the development of early rigor mortis in the carcasses, and also predict the abnormality in meat quality.

In general, pH of the carcasses decrease slowly after slaughter because lactic acid was accumulated in muscles by post-mortem glycolysis [10, 12, 19, 21]. In fact, the pH of the carcasses of the pigs with a normal liver decreased slowly to the normal final pH of about 5.7 by 24 hr after slaughter. The meat from them had a normal appearance. In contrast, 65 cases of 77 carcasses of the pigs with liver degeneration had the higher final pH above 6.0 at 24 hr after slaughter. The cut surfaces of loins of these carcasses had a dark firm dry appearance.

During the process of conversion of muscle to meat, muscle fibers loose moisture and shrink slowly [10, 13]. This is caused by the precipitation of myoprotein, following the decrease of muscle pH and the approach toward the isoelectric point of myoprotein [5]. In the present study, the muscles of the normal final pH from the pigs with a normal liver showed the shrinkage of muscle fibers at 24 hr after slaughter. On the other hand, in the muscles of the higher final pH from the pigs with liver degeneration, muscle fibers had no tendency to shrink even at 24 hr after slaughter. The higher volume densities in these muscle tissues indicated the lower degree of shrinkage of muscle fibers. This abnormal phenomenon was considered to be due to the lack of the natural decrease in pH of the carcasses. It seems to be closely related with a dark firm dry appearance [13]. These data as to the carcasses and muscles of the higher final pH above 6.0, which were produced from the pigs with liver degeneration, agree with the reports concerning DFD porcine muscles [7, 12, 13, 15, 19, 20].

We succeeded in producing both liver degeneration and DFD meat in the same pig that was slaughtered after the exhaustion by a long period of fasting and hard exercise. The livers of the exhausted pigs lost their original color and turned dull yellow. Total lipid contents in the livers were increased. The carcasses of the exhausted pigs showed a rise in temperature and early rigor mortis. Four cases of five carcasses of them developed DFD meat, which had the higher final pH above 6.0 and a characteristic appearance and structure. These changes were not observed in the pigs which were untreated. It was considered that the lipids which increased in the livers of the exhausted pigs were mobilized from adipose tissue, which pigs had in abundance, under the stress condition of fasting and exercise. The rise in temperature and early rigor mortis of the carcasses could be explained by the hard exercise before slaughter. It was presumed that the natural decrease in pH did not occur in the carcasses of the exhausted pigs because only a small quantity of lactic acid was formed from muscle glycogen depleted before slaugher.

As mentioned above, it was found that the pigs with liver degeneration produced DFD meat at a high rate. It was suggested that the exhaustion by fasting and exercise in pigs before slaughter caused both liver degeneration and DFD meat.

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要　　約

褪色と高い脂質含量を特徴とする肝臓変性を有すると殺豚における DFD 肉の発現：前田博之・森千恵子・黒川美千子・湯浅　亮[1)]（旭川食肉検査事務所，[1)]酪農学園大学）――と殺豚に，褪色し脂質含量の増加した肝臓（肝臓変性）がしばしば見られる．黒っぽく硬く乾いた感じの DFD 肉は，高い最終 pH を特徴とし，品質低下が指摘されている．肝臓変性と DFD 肉の発現要因は，と殺前の豚の消耗疲労という点でよく類似している．そこで，両者の関連を調査検討した．正常肝の総脂質量は6.0％以下であった．正常肝を有すると殺豚では，枝肉の最終 pH は5.7付近まで低下し，肉色は淡赤色で，と殺後24時間の筋肉組織では筋線維の萎縮が認められた．肝臓変性肝の総脂質量は6.6％以上であった．肝臓変性肝を有すると殺豚では，枝肉は早い死後硬直を示し，ATP の分解を示す筋肉の R 値は高かった．この枝肉77例中65例は6.0以上の高い最終 pH を示し，肉色は暗赤色で，乾いた感じであり，と殺後24時間でも筋線維の萎縮は少なかった．これらのことは，肝臓変性豚の枝肉には高率に DFD 肉が発現することを示している．53時間の絶食と筋肉運動を実験的に負荷した豚では，肝臓は褪色し脂質含量は高かった．枝肉の早い死後硬直と筋肉の高い R 値が認められ，5 例中 4 例に DFD 肉が発現した．このことから，と殺前の長時間の絶食やストレスを伴う激しい筋肉運動による消耗疲労は，肝臓変性と DFD 肉の両方を惹起する原因となることが示された．