

Epidemiological survey, general blood biochemistry, and histological examination of slaughtered heavy horse breeds with hemorrhage in the adipose tissue in the crest of the neck

Koji HARADA¹, Saori KANEMITSU¹, Kohei AKIOKA¹, Kazunari FUJITA¹, Yasunobu NISHI², Yasuho TAURA² and Naoki SASAKI^{2*}

¹Meat Safety Inspection Office in Kumamoto Prefecture, Kumamoto 861-1344, Japan

²Department of Large Animal Clinics, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-0841, Japan

Fifty-four slaughtered horses were classified into groups having adipose tissue in the crest of the neck with or without hemorrhage (AH and NH groups, respectively). Blood biochemical tests (Alb, TP, T-bil, GOT, GPT, LDH, T-cho, and BUN) and an epidemiological survey (age, gender, weight, origin, breed, BCS, CNS, and hoof disease) were performed. T-bil tended to be high, while the other parameters were normal. Weight, BCS, and CNS were higher in the AH group ($P < 0.05$). GOT was lower in the AH group ($P < 0.05$). It was suspected that the horses in the AH group had lipomatosis. It was assumed that the adipose tissue of the horses in the AH group contained damaged capillaries, and inflammation was confirmed based on evidence of macrophages and lymphocytes.

Key words: crest of the neck, lipomatosis, slaughtered heavy horse breeds

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The crest of the horse's neck (Fig. 1) contains white adipose tissue and is eaten raw like horse sashimi. Sometimes called the nuchal fat pad, there is approximately 5 kg of it per horse. The crest of the neck therefore trades at high prices. Because the "whiteness" of the crest of the neck is essential for its market value, a lack of adipose tissue means that the neck will be diverted to other uses, with concomitant large economic losses for the slaughterhouses.

Many horses at slaughterhouses in Kumamoto Prefecture have been fattened to increase their carcass weight and therefore contain ventral fat deposits along the nuchal ligament from the base of the neck to the second cervical vertebra. At the time of slaughter, hemorrhagic adipose tissue on the longitudinal cut surface of the crest of the neck can often be seen in the horses. In this study, epidemiological surveys, blood biochemical tests, and histological examinations were performed on horses to elucidate this issue.

From September to November 2020, epidemiological surveys, blood biochemistry, and histological examinations were performed on 54 horses (average age, 3.2 ± 2.3 years; average weight, 928.4 ± 140.2 kg) brought to slaughterhouses in Kumamoto Prefecture, Japan.

The horses underwent an antemortem inspection by a veterinarian prior to slaughter, which was conducted considering the welfare of the animal and comprised exsanguination and removal of limbs and internal organs. They were then passed through the slaughter process, where their internal organs were inspected and their carcasses were split along the back. Finally, the carcasses of the slaughtered horses were inspected by veterinarians, washed, and subjected to other processes before being refrigerated at 8°C.

The state of the fat deposits in the crest of the neck was evaluated by vertically cutting the surface of the crest of the neck, which was split longitudinally during the splitting process. The presence or absence of blood in the adipose tissue in the crest of the neck was evaluated. At the time of evaluation, five horses (average age, 3.0 ± 0.0 years; average weight, $1,012.6 \pm 105.1$ kg; gender, gelding; AH group) presented with blood in the adipose tissue in the crest of the neck, while 49 horses did not have blood in the adipose tissue in the crest of the neck (average age, 3.3 ± 2.4 years; average weight, 919.8 ± 140.5 kg; gender, 2

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*Corresponding author. e-mail: nsasaki@yamaguchi-u.ac.jp

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males, 20 females, 27 geldings; NH group). The slaughtered horses were subjected to epidemiological surveys and blood biochemistry tests using the methods described below. An overview of the horses is presented in Table 1.

Blood samples were collected at the time of exsanguination. They were collected using vacuum blood collection tubes containing a serum separator (Neotube NP-SP0725, Nipro Corp., Osaka, Japan) and transported to the Meat Safety Inspection Office in Kumamoto Prefecture at approximately 4°C in a cooler box. At the Inspection Office, serum was isolated from blood samples via centrifugation for 10 min at 3,000 rpm and used as the test material. The sera were then stored at -20°C for blood biochemical tests using SPOTCHEM EZ SP-4430 biochemistry tests according to the manufacturer's instructions (ARKRAY, Inc., Kyoto, Japan). The following parameters were measured: albumin (Alb), total protein (TP), total bilirubin (T-bil), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), total cholesterol (T-cho), and urea nitrogen (BUN). Serum glucose and insulin levels, which are diagnostic indicators for equine metabolic syndrome, could not be measured.

After the adipose tissue in the crest of the neck, with or without hemorrhage, was fixed in 15% neutral-buffered formalin, paraffin-embedded sections were prepared, and hematoxylin and eosin (HE) staining and Berlin blue staining were performed. The samples were examined under a microscope.

Age, gender, weight, origin, and breed were obtained from the horse farm supplier. Body condition score (BCS) was determined according to the 6-point scale (0 to 5) of Carroll and Huntington [5]. The cresty neck score, which was developed by Carter *et al.*, was evaluated using a score of 1 to 5 [6]. The accumulations of visceral and subcutaneous adipose tissue were evaluated by macroscopic examination during the splitting process. Hoof disease was evaluated grossly before slaughter and splitting.

Age, weight, BCS, CNS, and the results of the eight blood biochemical tests were compared according to the presence or absence of adipose tissue with hemorrhage in the crest of the neck. Numerical values are presented as the average \pm standard deviation. The Mann-Whitney *U* test was used to determine the differences between the groups. A χ^2 test was performed to evaluate relationships between the presence or absence of blood in the adipose tissue in the crest of the neck and gender, breed, and origin. A *P* value of less than 5% ($P < 0.05$) was considered significant.

Although none of the 54 slaughtered horses showed significant accumulations of visceral adipose tissue, the horses showed significant accumulations of adipose tissue in the neck and abdominal cavity. None of the 54 slaughtered horses presented any symptoms of disease, lameness,

Table 1. Outline of the 54 slaughtered horses

	Gender			Breed		Origin		
	M	F	Cast	HH	LN	FR	CA	DOM
AH group	0	0	5	5	0	3	1	1
NH group	2	20	27	46	3	16	24	9

M, male; F, female; Cast, castration; HH, heavy horse breeds; LN, light horse; AH group, horses having adipose tissue with hemorrhage; NH group, horses having adipose tissues without hemorrhage; FR, France; CA, Canada; DOM: domestic.

gait abnormalities, swelling of the coronet, or pain during the antemortem inspections; all horses had hooves with flattening of the sole and no rings on the hoof wall.

Adipose tissue with hemorrhage in the crest of the neck was painless to the touch and unencapsulated. The dorsal surface and longitudinal cut surface of adipose tissue in the crest of neck were white to white-yellow, slightly hard, and elastic, and the dorsal surface was rough. Macroscopically, the longitudinal cut surface of adipose tissue of the crest of neck from the second cervical vertebra (C2) to the base of the neck (C7), dorsal to the nuchal ligament, contained fat lobules. The size of the nuchal fat pad was approximately 2 mm \times 3 mm near the top of the vertex of crest of the neck and 4 mm \times 5 mm along the nuchal ligament.

Adipose tissue with hemorrhage was present symmetrically along the longitudinal cut surface and was recognized as a semi-dome shape centered around the border of the nuchal ligament to the base of the neck (Fig. 1b). Hemorrhage did not reach the subcutaneous tissue surface of the neck crest. Macroscopically, the color of the adipose tissue was reddish brown to dark red, depending on the horse. In the tissue images of the HE-stained adipose tissues with hemorrhage (Fig. 2), it was possible to identify normal and overgrown adipocytes with or without hemorrhage spots (Fig. 2a). Arteriovenous blood vessels were found in the stromal cell adipocytes. Adipocyte nuclei were small, and no nuclear variants were found. Moreover, some muscle tissue and collagen fibers were found, with or without hemorrhage spots. Macrophages and lymphocytes were partially confirmed in the hemorrhaged stromal cells of the adipocytes in the AH group (Fig. 2b). Berlin blue staining of adipose tissues revealed no hemosiderin granules in the stroma of the adipocytes.

The values for BCS and CNS were higher in the AH group than in the NH group ($P < 0.05$; Table 2). Additionally, GOT was lower in the AH group than in the NH group ($P < 0.05$; Table 3). On the other hand, T-bil was higher in the NH group than in the AH group, and all other biochemical parameters were within their normal ranges; there were no significant differences in these parameters in comparisons between the AH and NH groups. There was also no signifi-

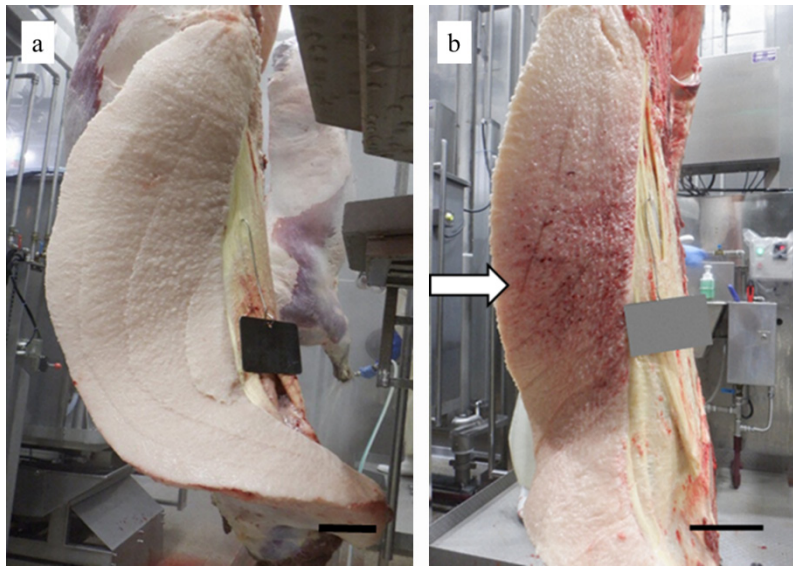


Fig. 1. Adipose tissue in the crest of the neck. (a) Normal adipose tissue. Bar=10 cm. (b) Adipose tissue with hemorrhage (white arrow). Bar=10 cm.

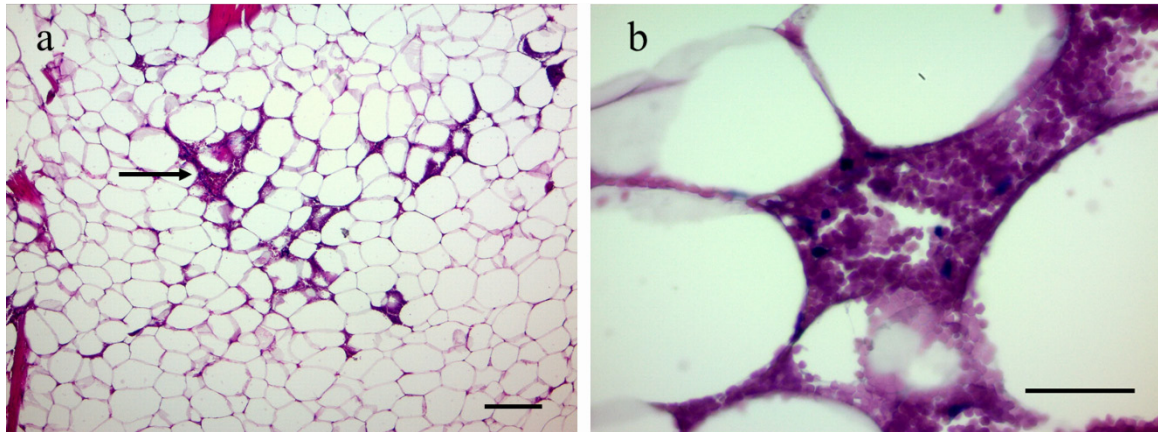


Fig. 2. Adipose tissue with hemorrhage. (a) Normal and overgrowth adipocytes with or without hemorrhage spots (arrow) can be seen. HE stain. Bar=200 μ m. (b) Macrophages and lymphocytes are partially confirmed in the hemorrhaged stromal cells. HE stain. Bar=50 μ m.

Table 2. Comparison of age, body condition score (BCS), crest of the neck score (CNS), and weight according to presence or absence of hemorrhage in the crest of the neck

	Age	BCS	CNS	Weight
AH group	3.0 \pm 0.0	5.0 \pm 0.0 ^a	4.6 \pm 0.5 ^a	1,012.6 \pm 105.1 ^a
NH group	3.3 \pm 2.4	4.2 \pm 0.7 ^b	3.7 \pm 0.7 ^b	919.8 \pm 140.5 ^b

AH group, horses having adipose tissues with hemorrhage; NH group, horses having adipose tissues without hemorrhage; age, years; BCS (1 to 5); CNS (1 to 5); weight, kg. Values with different letters (a and b) are significantly different ($P < 0.05$).

Table 3. Blood biochemical test

	AH group	NH group
TP (g/dl)	7.7 ± 0.2	7.9 ± 0.6
Alb (g/dl)	3.5 ± 0.2	3.4 ± 0.3
T-bil (mg/dl)	1.4 ± 0.4	1.7 ± 1.1
GOT (IU/l)	368.0 ± 9.7 ^a	462.0 ± 99.1 ^b
GPT (IU/l)	10 ^{>}	13.3 ± 4.0
LDH (IU/l)	644.0 ± 141.6	773.7 ± 286.7
T-cho (mg/dl)	98.8 ± 15.5	93.0 ± 15.4
BUN (mg/dl)	13.0 ± 2.3	13.6 ± 2.8

Values are presented as means ± SE. AH group, horses having adipose tissues with hemorrhage; NH group, horses having adipose tissues without hemorrhage. Values with different letters (a and b) are significantly different ($P < 0.05$).

cant difference in gender, breed, or origin between the AH and NH groups.

BCS is an indicator of obesity [2], and CNS is related to the state of general obesity [4, 11]. Based on the above findings, the horses in the AH group were obese.

Lipomatosis is infiltrative in humans and comprises a condition in which unencapsulated adipose tissue with indistinct organizational boundaries accumulates extensively; it often occurs in the head and neck and is painless [5]. Lipomatosis in the equine often occurs in subcutaneous tissue, is painless to the touch, and is unencapsulated, with infiltrating lesions [6, 8]. Based on our findings, the adipose tissues were presumed to be indicative of lipomatosis, the result of lipomatosis, or lipomas. Referring to the cresty neck score of Morales *et al.* [10], the crest contained adipocytes and muscle cells and was classified as grade 4, meaning “significant lipomatosis”.

Images of the adipose tissue with hemorrhage revealed a hemorrhaged stroma containing adipocytes, macrophages, and lymphocytes. In humans, inflammatory changes are caused by adipocyte enlargement in the capillaries in adipose tissue [10, 12] and blood coagulation [7, 13]. Adipocyte enlargement causes oxygen deficiency in adipocytes and vascular endothelial cells of adipose tissue, resulting in cell death [14]. From these findings, it was inferred that the adipose tissue of the crest of the neck and the capillaries in the adipose tissue contained inflammatory changes, blood coagulation, and damaged vascular endothelial cells. and that this was accompanied by oxygen deficiency of the adipocytes.

Adipose tissue with hemorrhage was present symmetrically along the longitudinal cut surface and was recognized as a semi-dome shape centered along the border of the nuchal ligament. Inflammatory changes and blood coagulation of the capillaries in the adipose tissue were not investigated in this study. An examination of distributed peripheral nerve

fibers and immunohistological procedures should have been carried out post-mortem.

No hemosiderin granules were found in the stroma of adipocytes in the adipose tissue with hemorrhage in the crest of the neck. Berlin blue staining utilizes the reaction of trivalent iron ions and is transformed by hemoglobin phagocytosed by macrophages, which disassemble the hemosiderin bound to the trivalent Fe^{3+} . Hence, hemosiderin dyed by Berlin blue staining indicates old hemorrhages. The results of the current study indicate that the hemorrhages were not old. Blood spots identified in the carcasses of horses, cattle, and lambs at the time of slaughter are considered defects and reduce the value of the carcasses. The use of electrical stunning results in blood spots caused by the rupture of capillaries due to muscle contraction caused by electrical stimulation and high blood pressure in cattle, sheep, and poultry [1, 3, 4]. Previously, blood spots were considered to result from the rupture of capillaries at the time of slaughter, but the mechanism behind the rupture of capillaries had not been fully elucidated to date. In this study, it was considered that muscle contraction due to electrical stimulation did not occur because a non-electrical stunning method was used at the time of slaughter and that the blood spots were caused by the rupture of capillaries that were already vulnerable due to lipomatosis.

In this study, the slaughtered heavy horses having adipose tissue with hemorrhage in the crest of the neck were thought to be obese. Images of the adipose tissue of these horses revealed that there were hemorrhages within the adipose tissue consisting of macrophages and lymphocytes as a result of inflammation. The adipose tissue in the crest of the neck was presumed to be indicative of lipomatosis, the result of lipomatosis, or a lipoma. In addition, macrophages and lymphocytes were partially confirmed in the stroma of adipocytes. Based on these findings, it is possible that there was mild inflammation in adipose tissue with hemorrhage.

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