Treatment and Clinicobiochemical Observations of Cows Affected with Fat Necrosis

Yoshiko MOTOI, Shinichiro KINNO, Kyuko MINAMINO, Koichi SHIMBAYASHI, and Chuzo USHIMI

National Institute of Animal Health, Yatabe, Tsukuba, Ibaraki 305, and
Mizusawa Livestock Hygiene Service Center, Mizusawa, Iwate 023, Japan

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Of three groups of cows affected with fat necrosis (FN) in the field group 1 was administered with a daily dose of 300 g of the adlay, Coix Lachryma-jobi, group 2 with a daily dose of 150 g of substance S from soybeans containing vitamine E, and group 3 with a combination of a daily dose of 150 g of the adlay and the same dose of substance S for about 4 consecutive months. One-half of the cows of each group was loaded intravenously with arginine attempting at reducing the period of treatment. The disappearance rate of necrotic fat masses was 17%, and the reduction in size of these masses was evident in 67% of treated animals from the results of rectal palpation. In the affected cows the amount of secretion of glucagon in response to the loading with arginine, decreased while the glucagon level after treatment was essentially the same as in the healthy ones. The free fatty acid level in the affected cows exhibited an abnormal pattern of secretion before and after treatment as compared with that in the healthy ones. The results suggested a disordered endocrine function of the pancreas in FN affected cows.—Key words: Arginine, Cow, Fat necrosis.


Judging from its incidence, bovine fat necrosis (FN) among cattle which are of good breed and easy to raise occurs readily among those which are obese, having been fed too much concentrate in the growing stage and after reaching maturity. Thus cattle quality and inappropriate feeding and management have been reported as important causes of FN [14, 15]. On the other hand, it has been suggested [10] from the results of analysis of the necrotic portion of the adipose tissue affected with FN that mobilization of fat, hydrolysis of glyceride, and disturbance in biosynthesis may be cause. The mobilization of fat from the adipose tissue and biosynthesis of fat into this tissue are controlled by nerves and hormones. There have been, however, no papers dealing with FN published on the hormonal control of lipid metabolism.

It is already known that the plant, adlay or Coix Lachryma-jobi, is effective for the treatment of FN [22]. The mechanism leading to the therapeutic effect is, however, unknown. Recently, it was shown that the adlay was rich unsaturated fatty acid. It was also shown that like this plant, substance S contained large amount of unsaturated fatty acid. This substance is a by-product of soybean oil extraction and contains vitamine E. Katsumata et al. [10] and Shimbayashi et al. [23] reported that the necrotic portion of adipose tissue contained more saturated fatty acid than the normal portion. Therefore, it was assumed that a constant supply of such unsaturated fatty acid might have some therapeutic effect on FN.

An experiment to treat FN was carried out by continual administration of the adlay and substance S to verify their effects. Moreover, an attempt was made to stimulate the secretion of fat-mobilizing hormone by loading the patient with amino acid, so that the
period of successful treatment might be shortened. The responses of hormones and lipid components in the blood to the loading of amino acid were also investigated. Thus, the clinicobiochemical and pathological condition of FN in cattle was observed.

MATERIALS AND METHODS

Animal: Thirty breeding cows of the Japanese Black breed, each weighing 300–400 kg, were used for treatment. They had been raised on farm in the southern part of Iwate prefecture. They showed some clinical symptoms as anorexia, sclerous feces, and constipation. Rectal palpation revealed the presence of necrotic tissue masses of various aspects around the desk of the colon, rectum, and the kidney. Eight healthy cows of the same breed were used as controls.

Treatment: The 30 cows were first subjected to the arginine infusion test to observe the hormonal response before treatment. Then they were divided equally into three groups. Group I received a daily dose of 300 g of adlay (preparation name: Yokuinin). Group 2 received a daily dose of 150 g of substance S, and group 3 received a daily dose of 150 g of the adlay with a daily dose of 150 g of substance S for the period of treatment about 4 consecutive months. One month after the beginning of the treatment, each group was further divided equally into two subgroups, A and B. Subgroup A was infused with arginine to shorten the period of treatment at intervals of 2 weeks or 4 weeks and subgroup B was used as control against subgroup A. At the end of the experiment, all cows in subgroups A and B received arginine infusion to observe the hormonal response after the treatment. Arginine test was performed five times on subgroup A and two times on subgroup B during the experimental period. Rectal palpation was performed again in all the cows at the end of the treatment to observe the aspects of the necrotic tissue masses. During the experimental period, amounts of concentrated feed stuff fed to the cows were reduced to two-third of that fed before the experiment. The eight healthy cows received infusions of arginine at the same time as the affected cows, at the beginning and at the end of the treatment to examine seasonal changes.

Arginine infusion and blood sampling: Arginine used for loading was 40% aqueous solution of L-arginine hydrochloride. After it was adjusted to pH 6.8–7.0 and sterilized, 400ml (400 mg/kg of body weight) was infused into the jugular vein in the course of about 15 min. A vascular cannula was fixed beforehand in the opposite jugular vein. Blood samples were collected into heparinized blood sampling tubes seven times before infusion and 10, 20, 30, 60, 90, and 120 min after infusion. Plasma was separated at low temperature immediately after collection.

Items and methods of analysis: Pancreatic glucagon was subjected beforehand to the procedure to inhibit proteolysis, in which 1000 units of aproptinin were used per ml of blood. After isolated in plasma, it was analyzed by radioimmunoassay [2] with antibody G 42 [19]. Insulin was estimated by radioimmunoassay [16]. Wako test kit methods were used for the following; free fatty acid (NEFA), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), monoamine oxidase (MAO) and glutamic oxaloacetic transaminase (GOT), but total lipid (TL) was assayed by sulfophospho-vanillin method [6]. Plasma glucose was estimated by glucose oxidase method [7], lipase by the method of Kurooka et al. [12]. Amylase was estimated by Caraway's method [4] and total serum protein by the refractometric method, and the A/G ratio by cellulose acetate membrane electrophoresis, and vitamin E (V.E) by Abe and Katsu's method [1].

Fatty acid analysis of adlay and substance S: A 2:1, v/v mixture of chloroform: methanol was added to 20 g of Yokuinin and 1 g of substance S. The resulting mixture was homogenized by the Waring blender and fil-
terminated. Then the solvent was evaporated under reduced pressure. The subsequent steps followed the procedures mentioned in the previous paper [23]. Finally, analysis was performed by gas chromatography under the same conditions [23].

Date obtained from the present investigation were subjected statistical analysis using the t test.

RESULTS

Fatty acid compositions of substances used: Table 1 shows fatty acid compositions of the adlay and substance S. The adlay contained as much as 80% of such unsaturated fatty acids as oleic and linoleic acids. The ratio of unsaturated to saturated fatty acids in the adlay was 5.14. Like the adlay, substance S contained oleic and linoleic acids as the main unsaturated fatty acids. It contained 64% unsaturated fatty acids. The ratio of unsaturated to saturated fatty acids was lower than in the adlay.

Therapeutic experiment: Table 2 shows the results of the therapeutic experiment. The ratio of disappearance of necrotic tissue masses in the cows shown to be 17% by the results of rectal palpation conducted after treatment. These masses were softened and reduced in size and symptoms were alleviated in 66.7% of the cows treated. Generally, satisfactory therapeutic results were obtained from all groups. The time required for the treatment resulting in a complete recovery was not distinctly shorter in subgroup A, which had been loaded with arginine, than in subgroup B in each group.

Blood components: Table 3 presents changes in each component of blood in the healthy cows and the affected ones before the beginning and the end of the therapeutic experiment. Levels of NEFA, TL and V.E were significantly higher after treatment than before. The NEFA levels were essentially the same in the affected cows after treatment as in the healthy ones. TL levels were higher in the affected cows after treatment than in the healthy ones. MAO was higher in activity value in the affected cows before and after treatment than in the healthy ones.

Responses of the hormones and the blood components after arginine infusion: Fig.1 ex-

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Table 1. Compositions of fatty acid in Coix Lachryma-jobi and substance S

<table>
<thead>
<tr>
<th></th>
<th>Coix Lachryma-jobi</th>
<th>Substance S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauric acid</td>
<td>Trace</td>
<td>0.62</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>Trace</td>
<td>1.24</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>13.85</td>
<td>24.05</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>1.87</td>
<td>3.77</td>
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<tr>
<td>Total</td>
<td>15.72</td>
<td>29.68</td>
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<tr>
<td>Unsaturated fatty acid</td>
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</tr>
<tr>
<td>Isopalmitic acid</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>25.94</td>
<td>24.72</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>53.67</td>
<td>31.94</td>
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<tr>
<td>Elaidic acid</td>
<td>Trace</td>
<td>5.74</td>
</tr>
<tr>
<td>Total</td>
<td>80.84</td>
<td>63.62</td>
</tr>
<tr>
<td>Others</td>
<td>4.68</td>
<td>6.70</td>
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<tr>
<td>Ratio of unsaturated saturated fatty acid</td>
<td>5.14</td>
<td>2.14</td>
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Table 2. Therapeutic effects of Coix Lachryma-jobi and substance S for cows affected with fat necrosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Administered substance</th>
<th>Daily dose of substance (g)</th>
<th>Subgroup</th>
<th>Number of cow</th>
<th>Time of arginine infusion</th>
<th>Condition of masses of necrotic fat after treatment</th>
<th>Rate of reduction (%)</th>
<th>Rate of disappearance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coix Lachryma-jobi (Yokuinin)</td>
<td>300</td>
<td>A</td>
<td>5</td>
<td>5</td>
<td>40 (2/5)</td>
<td>0 (0/5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>5</td>
<td>2</td>
<td>80 (4/5)</td>
<td>0 (0/5)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Substance S</td>
<td>150</td>
<td>A</td>
<td>5</td>
<td>5</td>
<td>80 (4/5)</td>
<td>0 (0/5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>5</td>
<td>2</td>
<td>40 (2/5)</td>
<td>60 (3/5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Coix Lachryma-jobi and Substance S</td>
<td>150</td>
<td>A</td>
<td>5</td>
<td>5</td>
<td>80 (4/5)</td>
<td>20 (1/5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>5</td>
<td>2</td>
<td>80 (4/5)</td>
<td>20 (1/5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td>66.7 (20/30)</td>
<td>17.0 (5/30)</td>
<td></td>
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</table>

Table 3. Changes in blood chemical compositions before and after treatment of fat necrosis cows

<table>
<thead>
<tr>
<th>Fat necrosis cows (n=30) before-treatment</th>
<th>Pancreatic Glucagon (ng/ml)</th>
<th>Insulin (μu/ml)</th>
<th>NEFA* (μEq/l)</th>
<th>Glucose (mg/dl)</th>
<th>Total lipid (mg/dl)</th>
<th>HDL-C** (mg/dl)</th>
<th>Lipase (u)</th>
<th>Amylase (K-u)</th>
<th>GOT (μ)</th>
<th>MAO* (μ)</th>
<th>A/G</th>
<th>Vitamin E (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>0.067</td>
<td>8.6</td>
<td>265</td>
<td>62.5</td>
<td>202.7</td>
<td>(8.1)</td>
<td>(11.1)</td>
<td>(4.1)</td>
<td>(3.2)</td>
<td>(22.0)</td>
<td>(11.8)</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>(0.018)</td>
<td>(2.9)</td>
<td>(28)</td>
<td>(5.8)</td>
<td>(8.1)</td>
<td>(11.1)</td>
<td>(4.1)</td>
<td>(3.2)</td>
<td>(22.0)</td>
<td>(11.8)</td>
<td>(5.9)</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>0.058</td>
<td>10.9</td>
<td>400</td>
<td>64.3</td>
<td>238.9</td>
<td>(8.9)</td>
<td>(14.0)</td>
<td>(4.3)</td>
<td>(2.3)</td>
<td>(18.3)</td>
<td>(9.6)</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>(0.011)</td>
<td>(3.1)</td>
<td>(26)</td>
<td>(9.4)</td>
<td>(8.9)</td>
<td>(14.0)</td>
<td>(4.3)</td>
<td>(2.3)</td>
<td>(18.3)</td>
<td>(9.6)</td>
<td>(4.9)</td>
</tr>
<tr>
<td>Normal cows (n=8) after-treatment</td>
<td>X</td>
<td>0.054</td>
<td>10.9</td>
<td>394</td>
<td>72.8</td>
<td>210.1</td>
<td>(18.1)</td>
<td>(3.6)</td>
<td>(9.6)</td>
<td>(5.6)</td>
<td>(6.8)</td>
<td>(14.0)</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>(0.014)</td>
<td>(2.3)</td>
<td>(55)</td>
<td>(8.6)</td>
<td>(18.1)</td>
<td>(3.6)</td>
<td>(9.6)</td>
<td>(5.6)</td>
<td>(6.8)</td>
<td>(14.0)</td>
<td>(6.6)</td>
</tr>
</tbody>
</table>

a) Free fatty acid.
b) Triglyceride.
c) High density lipoprotein-cholesterol.
d) Monoamine oxidase.
e) There are significant difference between e and f (p<0.01), and between g and h (p<0.05), but not significant between g and i, and between h and i.

hribits the patterns of response of pancreatic glucagon, insulin, NEFA, and plasma glucose to arginine infusion in the healthy cows and the affected cows before and after treatment. Pancreatic glucagon and insulin in the healthy cows infused with arginine showed a rapid increase, reaching a peak 20 min after infusion, and began to decrease gradually at 60 min and returned to the preinfusion level 120 min after infusion. Before treatment the affected cows presented a pattern of response similar to that of the healthy cows, but the amount of secretion was generally small. It was significantly smaller in the affected cows up to 30 min after infusion than in the same ones after treatment and in the healthy ones. The amount and pattern of secretion were almost the same in the affected cows after treatment as in the healthy cows with a little difference between groups. NEFA decreased transiently in level in the healthy cows after arginine infusion, but had higher level in these cows 120 min after infusion than before. No affected cows, however, showed
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Fig. 1. Responses of pancreatic glucagon, insulin, free fatty acid and plasma glucose after loading with arginine in cows affected with fat necrosis. Upper figures represent the responses before (●) and after (○) treatment of affected cows and lower figures represent the responses of normal cows. Arginine infusion time (——) was about 15 min. Standard deviation are shown by vertical bars.

this pattern of response before or after treatment. In this healthy cows the plasma glucose response was reverse to that of NEFA. The plasma glucose level was highest 10–30 min after infusion, decreasing gradually, and lower than the preinfusion level 120 min after infusion. The affected cows presented the same changes as the healthy cows, before and after treatment. Fig. 2 presents the patterns of response of TL, TG, and HDL-C to arginine infusion before and after treatment. In the healthy cows TG levels decreased 10 min after infusion, but showed no changes later. The affected cows exhibited the same pattern of TG response as the healthy ones before and after treatment, except that they presented a more marked decrease in TG level 60 min and later after infusion. The response of HDL-C was almost the same in the affected cows as in the healthy ones before and after treatment. No seasonal influence was seen upon the pattern of response of any component in the healthy cows.

There was no outstanding difference in the pattern of response of any component to arginine infusion between any two treated groups.

DISCUSSION

The authors experienced on farms cattle affected with fat necrosis feel into a state of anorexia and became weakness increasingly, and then some of them died, unless they were given any treatments. Therefore it is considered that affected cows are unable to recover from the disease by only treatment and reduction of food intake and some other treatments such as drugs are need. Some methods have been reported for the treatment of FN in cattle [3, 5]. Shimada et al. [22] mentioned that the adlay and V.E were effective for the treatment of FN. It is unknown the mechanism by which the adlay has a therapeutic effect on FN. It has been demonstrated experimentally that the adlay has antispasmodic and analytic effect [11] and cell damaging activity
Shimada et al. [22] pointed out that this plant had a series of action to stimulate lipolysis and removal of extraneous substance from the body and to improve the hepatic function, and asserted that these actions improved the constitution of the affected animal with FN. The present investigation revealed that both adlay and substance S had a therapeutic effect on FN. It was then assumed that some actions other than those mentioned above might be effective for the treatment of FN. The results of analysis indicated that adlay and substance S had a large proportion of unsaturated fatty acids. It is known that the ratio of unsaturated to saturated fatty acids is lower in necrotic portions than in normal portions of the adipose tissue in patients with FN. It has been reported [21] that a constant supply of unsaturated fatty acids increases the content of these acids in body fat and milk fat in the ruminant. Therefore, it was presumed that unsaturated fatty acids continually supplied by feeding adlay and substance S might have a therapeutic effect on the necrotic portion of the adipose tissue. It was also considered that V.E contained in substance S might act as an antioxidant of fat and prevent peroxide from being produced in the adipose tissue and blood. In the present investigation, arginine was infused to each subgroup A, in addition to the treatment with adlay and substance S, to stimulate the secretion of glucagon and the mobilization of fat, so that the time required for recovery of the disease might be shortened. Nevertheless, there was no distinct reduction in time, as compared with subgroup B. The result is interpreted to mean that the action of insulin...
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may offset that of glucagon which mobilizes fat, since arginine infusion stimulates the secretion of insulin which synthesizes fat, as well as that of glucagon, and that the effect of mobilizing fat may be transient, since arginine is infused over a short time. In future, it is necessary to study the kind of amino acid to be infused and the time for infusion.

It was reported [14, 15, 22] that FN was caused by hyperadipose in adult cattle and obesity resulted from feeding an excess of concentrate to young animals. On the other hand, it was reported from the analysis of necrotic adipose tissue that lipid was reduced, neutral fat solidified fatty acid saturated, and connective tissue proliferated in cattle with FN [10]. The results mentioned above suggested that the disturbance in lipid metabolism might have participated in the occurrence of FN. Lipid metabolism is controlled by hormone in cattle in the same manner as in monogastric animals [24]. Therefore, if the pancreatic secretory function or the hepatic function is disturbed, hormonal control and lipid metabolism will also be disturbed. It is known that glucagon, which is a fat-mobilizing hormone, is secreted when amino acids such as arginine, glycine, methionine, and lysine are used for loading. Glucagon is distinctly secreted especially when arginine is used [18]. It was clarified in the present investigation that the healthy cows showed a definite pattern of response when infused with arginine and that the affected cows secreted smaller amount of pancreatic glucagon and insulin in response to arginine infusion, presenting an abnormal pattern of appearance of NEFA. These results suggested that there might be an abnormality in the endocrine function of pancreas. After treatment, the amount of hormone secreted in response to treatment was essentially the same in the affected cows as in the healthy ones. On the other hand, the level of NEFA after treatment was almost the same in the affected cows as in the healthy ones. The pattern of appearance of NEFA, in response to arginine infusion, in the affected cows, differed from that in the healthy ones inspite of recovery of hormonal secretion. In particular, there was no increase in the levels of NEFA in the affected cows after arginine infusion. These results were presumed to have been obtained by the influence of degeneration of lipid in the adipose tissue. In future, it is necessary to clarify the relationship between the pathological aspect and the endocrine function of the pancreas through examination to judge the severity of symptoms of FN and changes in response of hormone and lipid components in the course of treatment. It is considered that the increase in concentration of TL and V.E after treatment may have been induced by the large amounts of fat and this vitamin contained in the adlay and substance S administered. MAO is known to be an enzyme which reflects liver cirrhosis and other disorders causing tissue fibrosis [9, 13]. In the present investigation MAO activity was higher in the affected cows before and after treatment than in the healthy ones. No abnormalities were detected by any other liver function test. Talking this result, it is assumed that the increase in MAO activity levels may have been brought about by the influence of fibrosis an fibrocytogenesis in the necrotic portion of the adipose tissue.

No papers have been published to deal with the relationship between FN and the endocrine function of the pancreas. That condition in the pancreas may have been previously caused by feeding young animal with an excess of concentrate or by obesity in adult cattle. Morrow [17] mentioned that in the cause of the fat cow syndrome, inadequate feeding caused liver disfunction and a disordered condition of fat metabolism and provoked postpartum disease. It was reported that a disordered condition due to endocrine gland in the played a role in the occurrence of ketosis, which is a representive disease of the fat cow syndrome [20]. In an obese animal
the enhanced adipose tissue suppresses blood capillaries and may cause a circulatory disturbance. It was pointed out that when the adipose tissue fell in anaerobic condition with its blood supply disturbed, C18 acid increased and C18:1 acid decreased [8]. In the present investigation concentrate was decreased during the treatment to reduce body weight somewhat, and unsaturated fatty acids were supplied continually during the treatment. These steps were presumed to be effective enough to prevent the deposit of fat excess and to restore the endocrine function of pancreas.

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viral verruca in Coix Lachryma-jobi (part 2). Biochemical analysis of cell damaging substance

要 約

牛の脂肪壞死症の治療試験と臨床生化学的可見について：元井健子・金野慎一郎・南野久晃・新
林恒一・牛見忠蔵（農林水産省家畜衛生試験場，水沢家畜保健衛生所）牛の脂肪壞死症の野外発生例30
頭を10頭ずつの3群に分けて治療試験を試みた。第1群にはハトムギ（ヨクイシ）を300 g、第2群
には大豆由来のビタミンE含有物（S）を150 g、第3群にはハトムギ150 gとS150 g混合物を毎日約
4ヶ月間投与して臨床所見および直腸検査による腹腔内脂肪壞死塊の状態を観察した。さらに各群の半
数例はアルギニン負荷することにより、治療期間の短縮を試みた。その結果、ハトムギあるいはS投
与により壊死塊消失率17％、壊死塊縮小率67％が確認された。アルギニン負荷によるネルカゴン、イ
ソスリンや脂質成分の応答を調べると、健康牛では一定の応答パターンを示したのに対して病牛はグル
カゴンの応答分泌量が低いかがわかり、治療後グルカゴンはほぼ正常値に回復したのに対して、塩類
脂肪酸は治療後も異常パターンを示した。以上から本症における腸内分泌機能異常が示唆された。