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ORIGINAL ARTICLE

Effects of clay on fat necrosis and carcass characteristics in Japanese Black steers

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ABSTRACT

Twenty 10-month-old Japanese Black steers were used to evaluate the effects of clay on fat necrosis and carcass characteristics. Ten steers (Clay group) were fed the clay (50 g/day) during 10–30 months of age. The other 10 steers (Control group) were not fed it. There was no significant difference in body weight or average daily gain between the two groups ($P > 0.05$). The occurrence of fat necrotic mass in the Clay group (30%) was lower ($P < 0.05$) than that in the Control group (90%) at slaughter. The size of necrotic masses in the Clay group was smaller ($P < 0.05$) than that in the Control group. There was no significant difference in the marbling score, beef color, Longissimus muscle area or subcutaneous fat thickness between the two groups. These results suggest that the clay prevented the occurrence of fat necrosis and did not affect the carcass characteristics in Japanese Black steers.

Key words: *carcass characteristics, clay, fat necrosis, Japanese Black steer.*

INTRODUCTION

Fat necrosis in cattle is generally characterized by masses of necrotic tissue in the fat deposits of the abdominal cavity. These masses compress the colon, rectum and urinary organs, and occasionally give rise to intestinal stenosis (Shimada *et al*. 1978). About 1000 beef cattle per year have been disused due to fat necrosis in Japan (Ministry of Agriculture, Forestry and Fisheries 2013). The economic loss arising from this disease is huge in Japanese Black cattle. The pathogenesis of bovine fat necrosis may be related to obesity (Shimada & Morinaga 1977; Oka *et al*. 1988; Katamoto *et al*. 1996), genetics (Abe *et al*. 1998) and fescue toxicosis (Stuedemann *et al*. 1985), but it is poorly understood. It was reported that adlay, phytosterol and isoprothiolane were effective in treating the disease (Shimada *et al*. 1979, 1988; Motoi *et al*. 1984; Oka *et al*. 1988). However, the findings on the therapeutic effects of adlay were not consistent (Oka *et al*. 1988; Shimada *et al*. 1988). In addition, phytosterol and isoprothiolane cannot be given to fattening cattle because isoprothiolane increases lipolysis of adipose tissues (Tanaka & Ohtani 1989), and phytosterol and isoprothiolane inhibit lipid deposition into adipocytes (Katamoto *et al*. 1991) and slim cattle (Oka *et al*. 1988; Shimada *et al*. 1988).

Clays have specific structures of porous aluminosilicate layers, and have been widely used in humans and animals for the prevention and/or treatment of mycotoxin-mediated diseases and diarrhea, and as antibacterial agents (Lindemann *et al*. 1993; Schell *et al*. 1993; Carretero 2002; Papaioannou *et al*. 2005; Williams & Haydel 2010; Queiroz *et al*. 2012). In some farms, feeding on certain types of clay has reduced the size of fat necrotic masses in cattle.

In the present study, we thus examined the effects of clay on fat necrosis and carcass characteristics in Japanese Black steers.

MATERIALS AND METHODS

Animals and dietary treatments

Twenty 10-month-old Japanese Black steers, which were derived from the same sire (Fukutoshidoi), were used in this experiment. Our preliminary research data indicated that the incidence of fat necrotic masses was higher in cattle derived from Fukutoshidoi than in those derived from the other Japanese Black sires in Hyogo Prefecture. The steers were obese because their fattening indexes, (body weight/withers height) × 100, at the age of 10 months were higher than 250. The cattle with fattening indexes of more than 250 at the age of 10 months are defined as obese in the Tajima strain of Japanese Black cattle (Oka & Iwamoto 2007). They were

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cared for according to the guide for the care and use of experimental animals of Hyogo Prefectural Technology Center of Agriculture, Forestry and Fisheries. The steers were divided into two groups: Control group ($n = 10$) and Clay group ($n = 10$). The clay (Ushikin; Maegawa Sangyo, Hyogo, Japan) used in this study had been mined on Awaji Island, and is used as a raw material for Awaji roof tiles. The clay was granulated to powder containing 4% moisture. The results of chemical analysis of the clay are shown in Table 1. The steers in the Clay group were fed the clay (50 g/day) during 10–30 months of age. The concentrate diets were fed twice a day, and were restricted to achieve the intended growth rate during 10–17 months of age and were then given *ad libitum* during 18–30 months of age. The concentrate diet during 10–14 months of age consisted of 50% flaked corn, 40% wheat bran and 10% soybean meal. During 15–22 months of age it consisted of 15% rolled barley, 50% flaked corn, 30% wheat bran and 5% soybean meal. During 23–30 months of age it consisted of 25% rolled barley, 50% flaked corn, 20% wheat bran and 5% soybean meal. The roughages were restricted and fed twice a day. Timothy hay was fed during 10–12 months of age and the amount decreased from 4 to 3 kg/day. Wheat straw was fed during 13–30 months of age and the amount decreased from 3 to 1 kg/day. The animals were housed in pens and individually accessed feed *ad libitum* by the use of electronic gates (American Calan Inc., Northwood, NH, USA). Water and trace-mineralized salt were freely available. Body weight, withers height and heart girth of steers were measured every month. The steers were slaughtered at the age of 30 months.

Examination of fat necrosis

The masses of necrotic fat were determined by rectal palpation every month after 20 months of age. The masses of necrotic fat in all intra-abdominal fat were investigated at slaughter. The volume of masses per head is shown as the sum of each mass volume (length × wide × height).

Carcass analyses

The carcasses were chilled for 48 h, and their meat quality was evaluated between the sixth and seventh ribs by official Japanese graders in accordance with the Japan Meat Grading Standards (JMGA 1988). Fat samples were scraped off the Longissimus muscle and subcutaneous adipose tissue at the sixth to seventh rib and perinephric adipose tissue with a slide glass. Samples were stored in sample tubes at −40°C until they were used for fatty acid analysis (Oka *et al*. 2002).

Table 1 Chemical composition of the clay

Chemical composition		Concentration† (%)
Silicic acid	SiO_2	66.8
Aluminum oxide	Al_2O_3	19.2
Ferric oxide	Fe_2O_3	5.4
Potassium oxide	K_2O	2.6
Magnesium oxide	MgO	2.2
Calcium oxide	CaO	1.3
Sodium oxide	Na_2O	1.2
Titanium oxide	TiO_2	0.7
Manganese oxide	MnO	0.1

†Expressed as % of dry matter basis.

Blood sampling and analyses of blood constituents

Blood samples were collected from the jugular vein during 13.00–14.00 hours every 2 months. The plasma was obtained by centrifugation and frozen at −40°C until assayed. Plasma levels of urea nitrogen, total cholesterol, triglyceride, glutamic-oxyorthosilicate (GOT) and gamma-glutamyl transpeptidase (γ-GTP) were measured using an autoanalyzer (Model DRI-CHEM 5500; Fuji Film, Tokyo, Japan). Plasma levels of free fatty acid were determined using a commercial test kit (NEFA-C test; Wako Pure Chemical Industries, Osaka, Japan). The plasma levels of vitamin A and vitamin E were measured by high-performance liquid chromatography (Abe *et al*. 1977, 1979).

Statistical analysis

Student's *t*-test or Welch's *t*-test was used to compare the mean values of feed intake, body weight, withers height, heart girth, average daily gain (ADG), carcass characteristics, blood constituents, and number and volume of masses of necrotic fat between the groups. The significance of the difference between the two means was tested by Student's *t*-test if the variance was uniform or by Welch's *t*-test if it was not. Chi-squared test was used to compare the incidences of fat necrotic mass between the groups. Differences were considered significant at $P < 0.05$.

RESULTS

Feed intake, body weight and ADG

The Clay group had greater ($P < 0.05$) dry matter intake (DMI) of concentrate than the Control group at 18–30 months of age and during the experimental period (Table 2). There was no significant difference in the DMI of roughage between the two groups. The total digestible nutrient (TDN) intake in the Clay group was higher ($P < 0.05$) than that in the Control group at 18–30 months of age (Table 2). There was no significant difference in the body weight or ADG during the experimental period between the two groups (Table 3). There was also no significant difference in

Table 2 Feed intakes of steers in the two groups

Item	Control		Clay	
	Mean	s.d.	Mean	s.d.
Dry matter intakes (kg)				
Concentrate				
10-17 months of age	1028	54	1038	54
18-30 months of age	2078	231	2308	199*
Total	3106	263	3345	231*
Roughage				
10-17 months of age	544	22	548	18
18-30 months of age	366	20	359	33
Total	909	38	907	46
TDN intake (kg)				
10-17 months of age	1152	55	1162	53
18-30 months of age	1960	204	2156	173*
Total	3112	237	3318	204†

*$P < 0.05$, †$P < 0.10$. TDN, total digestible nutrients.

Table 3 Body weight and average daily gain (ADG) of steers in the two groups

Item	Control		Clay	
	Mean	s.d.	Mean	s.d.
Body weight (kg)				
10 months of age	288.7	11.1	287.1	11.6
17 months of age	458.7	24.3	454.4	18.9
30 months of age	647.6	66.5	668.6	52.0
ADG (kg/day)				
10–17 months of age	0.81	0.08	0.80	0.07
18–30 months of age	0.50	0.14	0.57	0.10
Total	0.61	0.11	0.65	0.08

ADG, average daily gain.

withers height and heart girth during the experimental period between the two groups.

Blood constituents

The plasma concentration of total cholesterol was higher ($P < 0.05$) in the Clay group than in the Control group at the age of 18 months (Fig. 1). The plasma concentration of triglyceride was higher ($P < 0.05$) in the Clay group than in the Control group at the age of 20 months (Fig. 1). There was no significant difference in the plasma levels of urea nitrogen, free fatty acid, GOT, γ-GTP, vitamin A and vitamin E between the two groups.

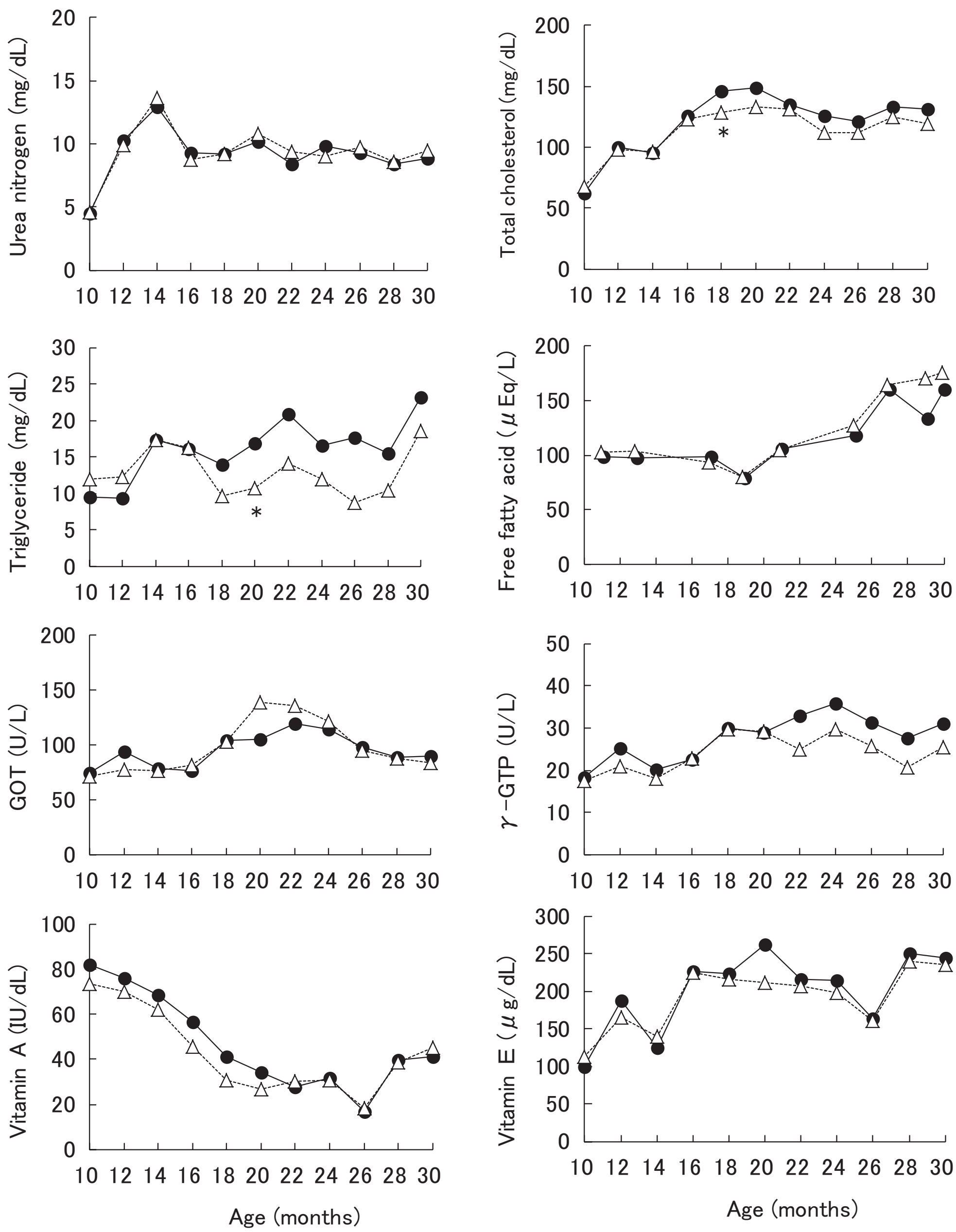

Figure 1 Blood constituents of steers in the clay group (●) and the control group (△). *$P < 0.05$.

Table 4 Incidence ratio of fat necrotic mass of steers in the two groups

Item	Control	Clay
Mean number of masses per head	3.7	0.7*
Incidence ratio (%)		
Perirectal fat	80	30*
Mesenteric fat	50	10
Perirenal fat	40	0*
Total	90	30**

*$P < 0.05$, **$P < 0.01$.

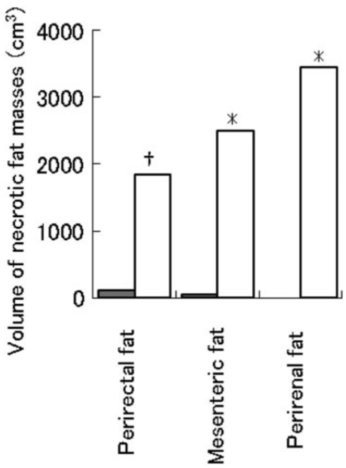

Figure 2 Volumes of necrotic fat masses of steers in the clay group (■) and the control group (□). *$P < 0.05$, †$P < 0.10$.

Incidence and size of necrotic fat masses

A fat necrotic mass in a steer of the Control group was found by rectal palpation at the age of 20 months. Subsequently, fat necrotic masses in the Control group gradually increased in number and were found in five steers in the Control group at the age of 30 months. On the other hand, a fat necrotic mass in a steer of the Clay group was found by rectal palpation at the age of 30 months.

The incidence of necrotic fat mass in the Control group at slaughter was extremely high (90%) and varied among the different sites (80% in perirectal fat, 50% in mesenteric fat and 40% in perirenal fat) (Table 4). The incidence of necrotic fat mass in the Clay group (30%) was lower ($P < 0.05$) than that in the Control group (Table 4). The number of necrotic fat masses in the Clay group was also lower ($P < 0.05$) than that in the Control group (Table 4). The volume of necrotic masses in mesenteric and perirenal fat was smaller ($P < 0.05$) for the Clay group than for the Control group (Fig. 2).

Carcass characteristics

There was no significant difference in carcass weight, marbling score, beef color, Longissimus muscle area, rib thickness, subcutaneous fat thickness or yield estimated percentage between the two groups ($P > 0.05$) (Table 5). The percentages of linoleic acid (18:2), linolenic acid (18:3) and polyunsaturated acids in intramuscular, perirenal and subcutaneous fat were higher ($P < 0.05$) in the Clay group than in the Control group (Table 6). There was no significant difference in the percentages of saturated acids and monounsaturated acids between the two groups ($P > 0.05$).

Table 5 Carcass characteristics of steers in the two groups

Item	Control		Clay	
	Mean	s.d.	Mean	s.d.
Carcass weight (kg)	398.3	43.5	412.6	33.3
Marbling score (BMS no.)†	5.9	1.0	6.3	0.9
Beef color (BCS no.)‡	3.4	0.5	3.7	0.5
Longissimus muscle area (cm^2)	47.9	5.7	51.2	5.0
Rib thickness (cm)	6.5	0.8	6.6	0.7
Subcutaneous fat thickness (cm)	2.6	0.6	2.6	0.6
Yield estimated percentage (%)	72.8	1.0	73.1	1.0

†Beef marbling standard numbers (JMGA 1988). ‡Beef color standard numbers (JMGA 1988).

DISCUSSION

The development of fat necrotic masses in steers of the Control group was indispensable to investigate the preventive effect of clay on fat necrosis in fattening steers. It was suggested that the pathogenesis of bovine fat necrosis is related to genetics (Abe *et al.* 1998) and obesity (Shimada & Morinaga 1977; Oka *et al.* 1988; Katamoto *et al.* 1996). Before the present study, we investigated the incidence of fat necrotic mass in progeny of sires in Hyogo prefecture. The steers derived from a sire that had a high incidence of fat necrotic mass were used in order to develop fat necrotic masses. In addition, the steers used in this study were obese as defined by their fattening indexes being higher than 250 at the age of 10 months (Oka & Iwamoto 2007). They were fed a relatively large amount of the concentrate from the beginning of the experiment. As a result, the steers of the Control group had fat necrotic masses at a high rate.

The TDN intake in the Clay group was higher than in the Control group at 18–30 months of age because two steers in the Control group developed anorexia caused by fat necrosis. The plasma concentrations of total cholesterol and triglyceride tended to be lower in the Control group than in the Clay group from the age of 18 months. It was considered that the TDN intake affected the levels of total cholesterol and triglyceride.

The incidence of necrotic mass was lower in mesenteric fat than in perirectal fat. Shimada *et al.* (1978) reported that the incidence of necrotic fat mass was highest in colon mesenteric fat among several fat tissues. They investigated the cattle that showed

Table 6 Fatty acid composition of Longissimus muscle fat, subcutaneous fat and perirenal fat in the two groups

Item	Control		Clay	
	Mean	s.d.	Mean	s.d.
Subcutaneous fat (%)				
Myristic acid (14:0)	2.23	0.23	2.24	0.14
Myristoleic acid (14:1)	2.31	0.42	2.24	0.31
Palmitic acid (16:0)	21.99	0.86	22.23	0.56
Palmitoleic acid (16:1)	7.96	0.89	7.90	0.71
Stearic acid (18:0)	6.42	0.83	6.04	0.74
Oleic acid (18:1)	56.84	1.45	56.52	0.98
Linoleic acid (18:2)	2.17	0.32	2.72	0.61*
Linolenic acid (18:3)	0.09	0.02	0.11	0.03*
SFA	30.63	1.07	30.51	0.92
MUFA	67.11	0.94	66.65	1.15
PUFA	2.26	0.33	2.84	0.63*
Longissimus muscle fat (%)				
Myristic acid (14:0)	2.54	0.20	2.33	0.16*
Myristoleic acid (14:1)	1.09	0.13	1.04	0.09
Palmitic acid (16:0)	25.97	1.32	25.00	1.61
Palmitoleic acid (16:1)	4.37	0.41	4.35	0.36
Stearic acid (18:0)	11.22	1.45	11.10	1.06
Oleic acid (18:1)	52.92	1.66	53.76	1.98
Linoleic acid (18:2)	1.82	0.24	2.34	0.45**
Linolenic acid (18:3)	0.07	0.01	0.09	0.02*
SFA	58.38	1.82	59.14	2.38
MUFA	39.73	1.71	38.43	2.43
PUFA	1.89	0.25	2.43	0.47**
Perirenal fat (%)				
Myristic acid (14:0)	1.99	0.33	1.91	0.33
Myristoleic acid (14:1)	0.53	0.20	0.52	0.16
Palmitic acid (16:0)	21.86	1.67	21.43	1.83
Palmitoleic acid (16:1)	2.71	0.51	2.86	0.44
Stearic acid (18:0)	20.05	3.47	19.73	2.87
Oleic acid (18:1)	50.95	3.58	51.05	3.40
Linoleic acid (18:2)	1.85	0.25	2.40	0.65*
Linolenic acid (18:3)	0.07	0.01	0.09	0.03*
SFA	43.89	3.99	43.08	3.64
MUFA	54.20	3.99	54.43	3.63
PUFA	1.92	0.26	2.49	0.67*

*$P < 0.05$, **$P < 0.01$. MUFA, monounsaturated fatty acids (sum of 14:1, 16:1 and 18:1). PUFA, polyunsaturated fatty acids (sum of 18:2 and18:3). SFA, saturated fatty acids (sum of 14:0, 16:0 and 18:0).

clinical signs such as anorexia, diarrhea and constipation. Such clinical signs are exhibited when necrotic fat masses compress the intestines. Therefore, they found a lot of necrotic masses in mesenteric fat around the colon. In the present study, the two steers that showed anorexia had necrotic mesenteric fat masses compressing the intestines.

There was no significant difference in the marbling score, beef color, Longissimus muscle area or subcutaneous fat thickness between the two groups. These results suggest that the clay did not affect the carcass characteristics in Japanese Black steers.

The percentage of monounsaturated fatty acid in beef fat affects the flavor of beef (Sakuma *et al*. 2012; Suzuki *et al*. 2013). Because there was no significant difference in the percentages of monounsaturated acids of Longissimus muscle fat between the two groups, it was suggested that administration of the clay did not affect the flavor of beef.

The level of saturated fatty acids was higher and that of unsaturated fatty acids was lower in necrotic fat than in normal fat from affected cattle (Rumsey *et al*. 1979; Shimada 1979). From these results, the causative factor of fat necrosis was thought to initiate a change in the composition of fat within normal fat cells (Rumsey *et al*. 1979). In the present study, there was no significant difference in the percentages of saturated acids and monounsaturated acids between the two groups because the samples from normal fat were used for fatty acid analysis. Katamoto *et al*. (1996) also reported that there was no significant difference in the fatty acid composition of normal fat between the heifers with necrotic fat masses and normal heifers, and suggested that increased saturation in the fatty acids of fat deposits was not the initial cause of bovine fat necrosis.

The percentages of polyunsaturated fatty acids in the carcass fat were higher in the Clay group than in the Control group. We reported that the percentages of linoleic acid in intramuscular, intermuscular and subcutaneous fat were higher in the steers fed the low roughage/concentrate ratio diet than in those fed the high ratio diet during 16–30 months of age (Oka *et al*. 2001). In the present study, the intake ratio of roughage/concentrate in the Clay group was lower than that in the Control group at 18–30 months of age. It is likely that the roughage/concentrate ratio affects the percentages of polyunsaturated acids.

The mechanism of the effect of the clay on fat necrosis is unclear at this time. Further study is necessary to clarify this mechanism.

In conclusion, the present results suggest that clay prevented the occurrence of fat necrosis and did not affect the carcass characteristics in Japanese Black steers.

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