Effect of ursodeoxycholic acid supplementation on growth, carcass characteristics, and meat quality of Wagyu heifers (Japanese Black cattle)

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ABSTRACT: Ursodeoxycholic acid (UDCA), which is used as a hepatic and digestive medicine in humans and domestic animals, was added to the diet of Wagyu beef cattle to investigate its effects on growth, carcass characteristics, and meat quality. The study involved 20 Japanese Black heifers. Animals were divided into the following 2 groups, a control group and a UDCA group (diet supplemented with UDCA), with each group containing 10 animals. The UDCA was administrated at a dose of 2.5 g/(animal/d) to each heifer 24 times over a period of 7 mo in the finishing period. The heifers were slaughtered at 29 mo of age, and carcass characteristics and meat quality were determined. Both the UDCA group and the control group showed similar ($P > 0.1$) final BW, fattening periods, and daily BW gain. Supplementation of UDCA significantly increased meat qual-ity grade ($P < 0.05$) and marbling ($P < 0.01$) and but did not show a significant ($P > 0.1$) effect on dressing percentage, fat thickness, rib thickness, or ribeye area. The percentage of ether extract in the LM was significantly greater ($P < 0.05$) in the UDCA group (43.2%) than in the control group (37.8%), whereas the percentage of moisture was significantly less in the former than the latter ($P < 0.05$). The L* (lightness) values of the muscles were greater ($P < 0.05$) in the UDCA group than in the control group. No significant differences ($P > 0.1$) were observed between groups in water-holding capacity, fatty acid composition, and vitamin E content of the LM or in intermuscular fat characteristics. Supplementation of the diet with UDCA can increase marbling without causing growth defects and can improve carcass characteristics in Wagyu cattle.

Key words: beef cattle, marbling, meat quality, ursodeoxycholic acid

INTRODUCTION

Marbling is an indication of high-quality beef, and fat quality has an effect on human health. In Japan and the United States, beef quality grade depends on the degree of marbling (JMGA, 1988; AMSA, 2001). A greater intake of SFA is associated with an increased risk of human health issues.

Wagyu beef has more marbling and a greater ratio of MUFA to SFA than does beef from traditional North American breeds (Sturdivant et al., 1992; May et al., 1993; Elías Calles et al., 2000). Controlling the vitamin A content in the feed is a technique widely used in Japan to increase marbling (Oka et al., 1998a,b; Adachi et al., 1999; Nade et al., 2003). However, the inability to carry out this technique appropriately sometimes leads to a vitamin A deficiency, which in turn causes addition health problems (e.g., dropsy, nyctalopia, arthritis). In addition, the use of this fattening method to increase marbling puts considerable pressure on the cattle and sometimes reduces the magnitude of BW gain because of impaired liver function and reduced appetite.

Bile acid is used to improve digestive function and to restore and strengthen liver function in humans and animals (Lazaridis et al., 2001; Lindor et al., 2004). Bile acid contains cholic acid, deoxycholic acid, urso-deoxycholic acid (**UDCA**), lithocholic acid, and che-nodeoxycholic acid. Ursodeoxycholic acid, which is synthesized chemically from the cholic acid present in bovine bile acid, has some beneficial properties when used as a medicine or supplement in human and animal diets (Trauner and Graziadei, 1999; Paumgartner and Beuers, 2002). Although UDCA is used in beef cattle production in Japan, few studies have been conducted using UDCA as an additive for beef cattle, and its effect on meat quality is unclear. The objective of this study was to investigate the effects of UDCA when added to the diet of Wagyu heifers during the finishing period.

Table 1. Chemical composition of the grain ration

Item	Amount, %
DM	90.36
CP	13.23
Crude fiber	5.10
Ether extract	3.49
Crude ash	3.10

MATERIALS AND METHODS

Animal care and use was according to the feeding guidelines for beef cattle approved by the Ministry of Agriculture, Forestry and Fisheries in Japan.

Animals and Management

The study used 22-mo-old Wagyu (Japanese Black; n = 20) heifers. The heifers were divided into a control group and a group that received dietary UDCA supplements (n = 10/group).

During the finishing period, each animal was fed 3 kg of grain ration (containing 46% wheat, corn, and grain sorghum; 46% hominy feed, wheat bran, and barley bran; 7% soybean meal; and 1% minerals; Table 1) daily and received rice straw ad libitum. In both groups, the intake of vitamin A was controlled to achieve high marbling (Oka et al., 1998a,b).

In the finishing period, a UDCA additive [50 g/ (animal/d); Urso-5%, DS Pharma Animal Health Co., Tokyo, Japan] containing 50 mg of UDCA/g was administered to the cattle 24 times over a period of 7 mo. The UDCA additive was mixed with the grain ration before feeding.

In the first month (i.e., at 22 mo of age), a 50-g dose of the UDCA additive (UDCA, 2.5 g/dose) was supplemented 6 times in anticipation of the first cumulative effect. To prevent ketosis in cattle, this dosage of UDCA additive is usually used in Japan. Between 23 and 28 mo of age, the UDCA additive was supplemented 3 times each month, whereas during the final month (i.e., at 29 mo of age), no UDCA was supplemented. The total dose of UDCA additive per animal was 1,200 g (i.e., 60 g of UDCA/period). The heifers were slaughtered at the age of 29 mo, when BW was approximately 700 kg, following the conventional method of feeding the cattle.

Carcass Characteristics

After cooling the carcasses, they were dissected at the sixth and seventh rib according to the Japanese meat grading system (JMGA, 1988). The LM area, rib thickness, subcutaneous fat thickness, beef marbling score, beef color score, carcass yield, and carcass quality grade were determined by Japanese professional meat graders. The beef marbling score was ranked from 1 to 12 with a standard model panel, in which greater scores corresponded to more marbling. The beef color score ranged from 1 (pale) to 7 (dark) on a standard model panel. The quality grade was ranked from 1 (inferior) to 5 (excellent) on the basis of the marbling, meat color, firmness, and texture of the meat and on the color and quality of the fat.

The carcass yield was estimated by the following equation (JMGA, 1988): carcass yield (%) = 69.419 + 0.130 × (LM area) + 0.667 × (rib thickness) − 0.025 × (cold left-side carcass weight) − 0.896 × (subcutaneous fat thickness). The viscera were observed by a veterinarian during sanitary inspection of the slaughterhouse.

Meat Quality

The color of the meat (LM, trapezius muscle, and semispinalis muscle) and that of the fat (subcutaneous and intermuscular fat) were measured using a colorimeter (CM-2006, Konica Minolta, Tokyo, Japan) and were expressed as L* (lightness), a* (redness), and b* (yellowness). The chemical composition (percentages of moisture and ether extract) of the LM, trapezius muscle, and semispinalis muscle was determined by AOAC methods (AOAC, 2003).

The water-holding capacity (**WHC**) was measured according to the revised method of Irie and Swatland (1992). Several samples (0.45 to 0.55 g) of the LM were cut to exclude the connective and vascular tissues and then weighed. Samples were wrapped in a membrane filter (Y100A047A, Advantec Co. Ltd., Tokyo, Japan), placed on glass beads in polycarbonate centrifuge tubes, and centrifuged at $5,000 \times g$ at 5°C for 1 h. Samples were weighed before and after centrifugation: WHC % = W2/W1 × 100, where W2 and W1 are weights after and before centrifugation, respectively.

The tocopherol concentration was determined using a procedure described in a previous report (Sugawara and Maekawa, 2000). We incubated 0.5 to 1.0 g of the minced sample (LM and intermuscular fat from the sixth to seventh rib) in 1% NaCl and 6% pyrogallol in ethanol. The mixtures were saponified with 60% NaOH at 70°C for 30 min, and then 1% NaCl was added and the mixture was extracted repeatedly with 10% ethyl acetate in hexane. After centrifugation at $3,000 \times g$ at 5°C for 5 min, 5% decane was added to the extracts and allowed to evaporate in a hot water bath. High-performance liquid chromatography (CTO-20A, Shimadzu, Kyoto, Japan) was used to quantify the tocopherol concentration using a column (Inertsil NH$_2$, 5 μm, 150 × 4.6 mm, GL Sciences Inc., Tokyo, Japan). The peaks were detected by fluorescence (excitation, 297 nm; emission, 327 nm).

The refractive index for intermuscular fat was determined with a digital refractometer (RX700α, Atago, Tokyo, Japan) at 50°C using the extracted fat (Irie et al., 2003). The refractive index was converted as follows: (n^d − 1.4500) × 10,000, where n^d is the refractive index. The melting point was measured by the capillary tube method (AOAC, 2003).

Table 2. Effects of supplemental ursodeoxycholic acid (UDCA) to Wagyu heifers on growth traits (mean ± SE)

Item	Control[1]	UDCA[1]
Initial BW, kg	263.0 ± 4.9	266.9 ± 7.3
Final BW, kg	701.0 ± 17.1	694.9 ± 31.7
BW gain, kg	438.0 ± 17.4	431.1 ± 28.9
Fattening period, d	579.5 ± 5.9	571.4 ± 2.3
Daily BW gain, kg	0.76 ± 0.03	0.76 ± 0.05

[1]n = 10.

The fatty acid composition of intermuscular fat and intramuscular fat (LM) was analyzed after esterification with 15% boron trifluoride-methanol (Nishioka and Irie, 2005) using a flame-ionization detector on a gas chromatograph (GC2014A, Shimadzu, Kyoto, Japan) equipped with a 60 m × 0.25 mm capillary column (DB-WAX122-7062, J & W Scientific, Folsom, CA). The column oven was maintained isothermally at 160°C. The injector and detector temperatures were 270°C. The flow rate of the carrier gas (He) was 51.8 mL/min. The individual fatty acids (C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3, C20:4) were identified by comparing the retention times of the sample peaks with those of known reference methyl esters. The weight percentage was determined from the peak areas with an integrator.

Statistical Analysis

Data from the 2 groups were compared using the 2-sided paired t-test, with $P < 0.05$ accepted as statistically significant. All analyses were conducted using the SPSS system (IBM Corporation, Armonk, NY).

RESULTS AND DISCUSSION

Body weight, BW gain, and daily BW gain during the experimental period are shown in Table 2. No differences ($P > 0.1$) in growth characteristics were observed between the control and UDCA groups (Table 2).

Carcass characteristics are shown in Table 3. No differences ($P > 0.1$) in yield grade, carcass weight, loin muscle area, belly thickness, and subcutaneous fat depth were observed between the 2 groups. The marbling score was significantly greater in the UDCA group than in the control group ($P < 0.01$).

This result showed that UDCA increased marbling in cattle without affecting the carcass composition. We propose that UDCA helped to emulsify the lipids present in the feed, after which the lipids were absorbed into the intestine; these lipids are mainly used as an energy source for intramuscular fat synthesis and stimulation of adipocytes in muscles. Andrae et al. (2001) showed that supplementation of finishing beef cattle diets with high-oil corn enhanced intramuscular lipid de-

position. Gilbert et al. (2003) reported that differences in carcass characteristics, adipose tissue cellularity, and lipogenesis were apparently caused by protected lipids rather than protected starch. Although acetate is well known to be a major source for fatty acid synthesis in ruminants, these reports and our results showed that lipids from the diet can also be a good source for intramuscular fat synthesis. On the basis of the increase in NE alone, however, we cannot explain why there was an increase only in intramuscular fat and not in subcutaneous fat. Details regarding the possibility of an increase in adipocytes are discussed below.

Recent studies (Makishima et al., 1999; Houten et al., 2006; Watanabe et al., 2006; Nguyen and Boucsarel, 2008; Trauner et al., 2010) have shown that bile acids are signaling molecules that can regulate triglyceride, cholesterol, energy, and glucose homeostasis. The hydrophilic bile acids (i.e., chenodeoxycholic, lithocholic, and deoxycholic acids) activate farnesoid X receptor, which suppresses lipid synthesis; the farnesoid X receptor, however, does not influence the hydrophobic UDCA (Makishima et al., 1999). Lithocholic, deoxycholic, and chenodeoxycholic acids activate **TGR5** (the G-protein-coupled bile salt receptor), which expends energy by promoting thyroid hormone activation (Watanabe et al., 2006); UDCA only minimally, however, activates TGR5 (Maruyama et al., 2002). Secreted bile acids are reabsorbed in the intestine, and the bile acid pool is kept at an approximately constant size by regulating synthesis. Thus, UDCA supplementation may indirectly increase intramuscular fat by decreasing the hydrophilic bile acids that suppress obesity.

The increase in intramuscular fat caused by UDCA supplementation is certainly related to the increased absorbance of fat from the feed. Increased NE, which increases marbling in cattle, can be achieved by increasing the lipid content of the diet (Pethick et al., 2004). Azain (2004) noted that fatty acids and their derivatives can produce hormone-like effects and regulate gene expression in preadipocytes. Further, Zhang et al. (2010) reported that serum lipids in vitro can convert

Table 3. Effects of supplemental ursodeoxycholic acid (UDCA) on carcass characteristics of Wagyu heifers (mean ± SE)

Item	Control[1]	UDCA[1]
Carcass weight, kg	443.5 ± 11.9	437.3 ± 20.7
Dressing percentage	63.2 ± 0.4	62.9 ± 0.4
Quality grade[2]	3.3 ± 0.2	4.1 ± 0.2*
Fat thickness, cm	3.7 ± 0.3	3.4 ± 0.4
Rib thickness, cm	7.9 ± 0.2	8.0 ± 0.3
Ribeye area, cm[2]	56.5 ± 1.4	60.6 ± 2.1
Marbling score[3]	4.2 ± 0.3	6.1 ± 0.5**
Color score[4]	4.1 ± 0.2	3.6 ± 0.2†

[1]n = 10.

[2]Quality grade: 1 = inferior; 5 = excellent.

[3]Marbling score: 1 = low; 12 = high.

[4]Color score: 1 = pale; 7 = dark.

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$.

bovine myogenic satellite cells to adipocytes. In Wagyu cattle, though it is not reported in the other breeds, Okumura et al. (2007) reported that intramuscular fat content continues to increase after 24 mo of age. Hence, it is possible to assume that UDCA increases the absorption of dietary lipids into the serum to increase NE, decreases the hydrophilic bile acids related to fat suppression, and subsequently increases lipids in the serum to increase the conversion of intramuscular adipocytes, instead of facilitating the direct accumulation of fatty acids.

The meat color score tended to decrease in the UDCA group compared with the control group ($P < 0.1$). It is certain that the difference in color originated from the white color resulting from increased intramuscular fat in the UCDA group.

The percentages of moisture and ether extract in the 3 muscles are shown in Table 4. The percentage of ether extract in the LM was significantly greater in the UDCA group than in the control group ($P < 0.05$). This result was consistent with the marbling data provided above. Cameron et al. (1994) reported that the marbling scores graded by official graders of the Japan Meat Grading Association were highly correlated with the percentage of intramuscular lipid.

Although the UDCA group had a greater percentage of ether extract in the LM than did the control group, it had a significantly smaller percentage of moisture in the LM than did the control group ($P < 0.05$). Although no significant difference was observed between the 2 groups in terms of the percentages of moisture and ether extract in the trapezius and semispinalis muscles, the mean percentage of ether extract was slightly greater in the UDCA group and the percentage of moisture was a slightly less in the control group.

The α-tocopherol content in muscle and fat did not differ ($P > 0.1$) between the 2 groups (Table 5). Vitamin E containing α-tocopherol is lipid soluble. It is unlikely that the lipids absorbed effectively into the body by UDCA led to an increased content of vitamin E in the tissue; therefore, vitamin E in the tissues was maintained even when there was an increase in intramuscular fat. The WHC of meat also did not differ ($P > 0.1$) between the 2 groups.

No significant differences ($P > 0.1$) were observed between the 2 groups in terms of fat characteristics, such as melting point, refractive index, or fatty acid composition (Table 5). Essential fatty acids, such as C18:2, did not increase ($P > 0.1$) after dietary supplementation with UDCA. Fatty acids in the diet are changed partly by bacterial isomerization and biohydrogenation in the rumen and partly by desaturation in the adipose tissue (Daley et al., 2010). It is unlikely that UDCA affected the bacterial action on fat and tissue desaturase. The fact that the fatty acid composition was the same in both the control group and the UDCA-supplemented group suggests that UDCA does not play a protective role for fatty acids in the rumen. Otherwise, one would expect less biohydrogenation of the fatty acids by the

Table 4. Effects of supplemental ursodeoxycholic acid (UDCA) on meat quality of Wagyu heifers (mean ± SE)

Item	Control[1]	UDCA[1]
Moisture, %		
LM	46.7 ± 0.7	42.9 ± 1.3*
Trapezius muscle	55.8 ± 1.5	54.7 ± 1.0
Semispinalis muscle	42.9 ± 1.4	41.3 ± 0.9
Ether extract, %		
LM	37.8 ± 1.0	43.2 ± 1.7*
Trapezius muscle	27.5 ± 1.9	29.2 ± 1.3
Semispinalis muscle	44.0 ± 1.9	46.5 ± 1.2
Water-holding capacity	77.8 ± 1.2	79.6 ± 1.0
Color[2]		
LM		
L*	43.0 ± 1.1	45.4 ± 1.8
a*	18.0 ± 0.8	16.5 ± 1.2
b*	15.0 ± 0.8	15.2 ± 0.4
Trapezius muscle		
L*	38.7 ± 1.2	42.0 ± 1.4†
a*	16.9 ± 0.9	16.9 ± 0.9
b*	12.6 ± 0.7	14.0 ± 0.6
Semispinalis muscle		
L*	40.1 ± 1.1	43.9 ± 1.3*
a*	18.8 ± 0.9	17.5 ± 0.9
b*	14.5 ± 0.6	14.8 ± 0.9
Subcutaneous fat		
L*	69.9 ± 1.7	68.7 ± 1.4
a*	0.1 ± 0.4	1.5 ± 0.9
b*	10.6 ± 1.1	13.6 ± 1.7
Intermuscular fat		
L*	74.6 ± 1.4	70.1 ± 2.4
a*	−0.5 ± 0.4	0.5 ± 0.8
b*	9.7 ± 1.0	10.8 ± 1.5
Vitamin E, µg/g		
LM	6.4 ± 0.5	7.4 ± 0.5
Intermuscular fat	10.9 ± 0.9	9.5 ± 0.6

[1]n = 10.

[2]L* = lightness; a* = redness; b* = yellowness.

†$P < 0.1$; *$P < 0.05$.

rumen microbes and a greater percentage of unsaturated fatty acids in the meat. Andrae et al. (2001) reported that supplementing finishing beef cattle diets with high-oil corn enhanced EFA deposition. Because no change were observed in either fatty acid composition or subcutaneous fat thickness in the present study, a large amount of fatty acids from the feed may not have accumulated directly.

During sanitary inspection, 2 heifers from the control group and 5 from the UDCA group showed liver defects, indicating that dietary supplementation with UDCA in the present study may damage the liver. The UDCA was originally used as a liver medicine and has milder effects when present in deoxycholic acids because of the small surface-active area. Although UDCA may have some beneficial effects in selected hepatic diseases (Beuers et al., 1992; Colombo et al., 1996; Poupon et al., 1999), excessive bile acids are known to cause damage to hepatocytes (Attili et al., 1986; Galle et al., 1990; Schmucker et al., 1990; Heuman et al., 1991; Sokol et al., 1995). Huang et al. (2006) reported that bile acids control liver regeneration and regrowth. Gong et

Table 5. Effects of supplemental ursodeoxycholic acid (UDCA) on fat quality of Wagyu heifers (mean ± SE)

Item	Control[1]	UDCA[1]
Intermuscular fat		
Melting point, °C	20.8 ± 0.6	21.5 ± 0.4
Refractive index	51.5 ± 0.6	50.7 ± 0.5
Fatty acid, %		
C14:0	2.18 ± 0.18	2.05 ± 0.13
C14:1	1.21 ± 0.13	1.08 ± 0.14
C15:0	0.47 ± 0.07	0.36 ± 0.02
C15:1	0.32 ± 0.09	0.20 ± 0.02
C16:0	20.84 ± 1.02	20.98 ± 0.60
C16:1	4.67 ± 0.39	4.70 ± 0.47
C17:0	0.85 ± 0.09	0.73 ± 0.03
C17:1	1.06 ± 0.07	0.88 ± 0.04
C18:0	9.69 ± 0.60	10.39 ± 0.64
C18:1	54.37 ± 1.15	54.42 ± 0.65
C18:2	2.33 ± 0.15	2.37 ± 0.16
C18:3	0.21 ± 0.04	0.12 ± 0.02
C20:0	0.31 ± 0.04	0.42 ± 0.08
C20:1	0.84 ± 0.10	0.79 ± 0.08
C20:2	0.19 ± 0.04	0.16 ± 0.05
C20:3	0.31 ± 0.06	0.16 ± 0.03
C20:4	0.17 ± 0.03	0.18 ± 0.06
SFA[2]	34.33 ± 1.26	34.93 ± 0.79
MUFA[3]	62.45 ± 1.15	62.07 ± 0.78
PUFA[4]	3.21 ± 0.26	3.00 ± 0.22
LM		
Fatty acid, %		
C14:0	2.30 ± 0.12	2.46 ± 0.15
C14:1	0.87 ± 0.05	0.90 ± 0.07
C15:0	0.36 ± 0.03	0.36 ± 0.02
C15:1	0.16 ± 0.02	0.21 ± 0.02
C16:0	24.32 ± 0.39	24.98 ± 0.57
C16:1	3.79 ± 0.14	3.86 ± 0.14
C17:0	0.58 ± 0.02	0.63 ± 0.03
C17:1	0.91 ± 0.05	0.89 ± 0.03
C18:0	10.96 ± 0.24	11.12 ± 0.26
C18:1	52.23 ± 0.65	51.22 ± 0.65
C18:2	2.44 ± 0.17	2.42 ± 0.14
C18:3	0.10 ± 0.01	0.09 ± 0.01
C20:0	0.16 ± 0.03	0.16 ± 0.03
C20:1	0.49 ± 0.08	0.47 ± 0.04
C20:2	0.08 ± 0.03	0.04 ± 0.01
C20:3	0.11 ± 0.02	0.09 ± 0.02
C20:4	0.12 ± 0.02	0.09 ± 0.01
SFA[2]	38.68 ± 0.51	39.71 ± 0.67
MUFA[3]	58.45 ± 0.60	57.55 ± 0.60
PUFA[4]	2.86 ± 0.19	2.74 ± 0.17

[1]n = 10.

[2]SFA = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0.

[3]MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1.

[4]PUFA = C18:2 + C18:3 + C20:2 + C20:3 + C20:4.

al. (2007) reported that UDCA did not improve liver histology. However, in the present study, the liver defects did not affect growth performance. Thus, the use of UDCA in beef production may not prove harmful.

Future studies with a larger data set are needed to investigate suitable methods of UDCA administration without negative effects and to investigate the mechanism of action of UDCA, including the relationship between marbling and serum concentrations of fatty acids.

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