Effects of Deletion of Ca Supplement (limestone) on Growth and Beef Quality in Hanwoo Finishing Steers

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한우 비육후기 사료에 칼슘 첨가제(석회석) 제거가 성장 및 육질특성에 미치는 영향

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ABSTRACT

A study was conducted to determine the effects of feeding a diet containing no Ca supplement (limestone) during the late finishing period on growth, marbling and serum 1,25-dihydroxy vitamin D_3 level in Korean native cattle. Twenty-four steers (20~24 mo of age) were divided into two groups of 12 each: one group assigned to a control diet (concentrates containing 2.5% limestone) and the other to a diet containing no calcium supplement. They were allowed to have free access to diets (concentrates and orchard grass hay) and water during the entire feeding period (223 d). Serum Ca^{2+}, Ca and P concentrations were not influenced by diets, but serum 1,25-dihydroxy vitamin D_3 concentrations determined 2 or 6 mo after the beginning of feeding the experimental diets were higher ($P < 0.01$) in steers fed the diet without Ca supplement than in those fed the control diet (78.3 vs 51.7 and 80.3 vs 51.1 pg/mL, respectively). Steers fed the diet without Ca supplement tended to have a higher intake of concentrates, but a lower intake of hay, compared to those fed the control diet. Average daily gain was higher ($P < 0.05$) in steers fed the diet without Ca supplement than in those fed the control diet. Feeding the diet without Ca supplement remarkably ($P < 0.01$) increased the marbling score (5.1 vs 2.2) and the muscle (M. *longissimus dorsi*) fat content (10.2 vs 6.7%) with a concomitant decrease in moisture content (67.6 vs 70.4%), compared to feeding the control diet. Ribeye area was increased (77.2 vs 82.8 cm^2) with the diet without Ca supplement, compared to the control diet ($P < 0.05$). Meat color, pH and water-holding capacity in *longissimus* muscle were not different between the two groups. The Warner-Brazler Shear (WBS) force of the *longissimus* muscle was slightly ($P = 0.08$) lower in steers fed the diet without Ca supplement than in steers fed the control diet (2.9 vs 3.2 kg/1.27-cm diameter core). Sensory evaluation showed that feeding the diet without Ca supplement slightly ($P < 0.05$) improved tenderness (4.9 vs 4.5) and flavor (4.9 vs 4.6), compared to feeding the control diet, but juiciness was not affected by diets. Results showed that deletion of Ca supplement from finishing diets is beneficial, increasing growth and marbling partly through an increased energy intake and induced 1,25-dihydroxy vitamin D_3 synthesis that may increase intracellular Ca^{2+} concentration and in turn fat synthesis.

(**Key words** : Steers, Calcium, Growth, Carcass traits, 1,25-$(OH)_2$-D_3)

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Ⅰ. INTRODUCTION

Marbling score (or intramuscular fat content) is a major determinant of beef grading under the current system in Korea, and thus cattle farmers work hard toward increasing marbling of beef. To increase marbling, farmers and cattle nutritionists have developed various strategies, such as an increased ratio of concentrates/roughages and extended fattening period; however, these add in production costs that are already high in Korean beef production system.

Calcium has been known to be essential for not only growth and maintenance of bones and teeth, but also many metabolic and neurological functions, one of which is to enhance fatty acid synthesis in adipocytes. Ironically, a decreased intercellular Ca concentration in its deficiency increases the renal synthesis of 1□,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$-D_3), a hormone regulating homeostasis of blood Ca and P. The increased 1,25-$(OH)_2$-D_3 concentration increases Ca^{2+} influx into adipocytes and pancreatic β-cells, and consequently, the increased intracellular Ca^{2+} and the increased circulatory insulin concentrations induce fatty acid synthesis and inhibit lipolysis (Zemel et al., 2000). This role of Ca^{2+} has been shown in primary cultures of human adipocytes, and also in mice by demonstrating increased body weight gain and adipose tissue mass (Zemel et al., 2000). Recently, Heaney (2003) suggested that the prevalence of obesity (or overweight) can be reduced by 60~80% by increasing Ca intake in public. Studies (Montgomery et al., 2000; Montgomery et al., 2002) showed that supplemental dietary vitamin D_3 before slaughter improved beef tenderness by increasing muscle calcium content and suggested that 1,25-$(OH)_2$-D_3 may have increased intracellular Ca^{2+} antemortem and then calpain activity (Ca-dependant protease) postmortem. However, higher doses of dietary vitamin D_3 can cause reduction in feed intake (Wiegand et al., 2002).

On the basis of the above reports, we hypothesized that feeding a diet without Ca supplement during a late fattening period in steers might be beneficial, increasing intramuscular fat content or marbling. To examine the hypothesis, we conducted a study to determine the effects of feeding a diet without Ca supplement during the late fattening period on growth and beef quality using Korean native cattle.

Ⅱ. Materials and Methods

1. Animals and diets

Before experiment started, all Hanwoo steers (Korean native cattle castrated at 6 mo of age) had been under the same feeding regime at National Jeju Agri. Exp. Station, RDA; grazing without feeding supplementary concentrates for 7 mo, and then stable feeding with concentrates fed daily at 1% of their body weight and free access to hay. Twenty-four steers (20~24 mo of age) were divided into two groups of 12 each (three pens of four steers each) : one group assigned to a control diet (concentrates containing 2.5% limestone) and the other to a diet containing no calcium supplement (Table 1). Steers were allowed to have free access to diets (concentrates shown in Table 1 and orchard grass hay containing 10% crude protein, 37.2% ADF, 64.2% NDF, 0.21% Ca and 0.14% P on as-fed basis) and water during the experimental period (March 19 through October 28, 2002). Feed consumption and body weight were monitored monthly, and at the end of the feeding experiment, average daily gain (ADG), average daily feed intake (ADFI) and gain/feed over the 223-d feeding period were calculated.

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Table 1. Composition of experimental diets (% as-fed basis)

Ingredient	Ca supplemented	No Ca Supplemented
Corn	43.0	45.5
Soybean meal	17.3	17.3
Wheat	11.5	11.5
Wheat bran	11.0	11.0
Tapioca	8.0	8.0
Molasses	5.0	5.0
Tallow	0.2	0.2
Salt	0.6	0.6
Limestone	2.5	–
Premix[1)]	0.9	0.9
ME (calculated Mcal/kg)	2.59	2.66
CP (calculated %)	15.9	16.1
Ca (analyzed %)	0.98	0.27
P (analyzed %)	0.58	0.60

[1)] Provided the following per kg of diet: Fe, 50 mg; Cu, 7 mg; Mn, 24 mg; Zn, 30 mg; I, 0.6 mg; Se, 0.15 mg; vitamin A, 3,800 IU; vitamin D_3, 400 IU; vitamin E, 20 IU; vitamin B_2, 2.5 mg; vitamin B_6, 2.0 mg; pantothenic acid, 4 mg; niacin, 2.6 mg; biotin, 0.1 mg.

2. Collection of blood samples and determination of serum 1,25-$(OH)_2$-D_3, Ca^{2+}, Ca and P

Blood samples were taken into tubes without EDTA after a 16-h fasting period 2 and 6 mo after the beginning of the feeding experiment and centrifuged, and serum samples were collected and stored at −70℃ until analysis. Serum 1,25-$(OH)_2$-D_3 concentration was determined using a radioimmunoassay kit (Immuno Diagnostic Systems, Bolden, UK), and a γ-counter (COBRA Ⅱ 5005, Packard, Meriden, MD, USA), according to the manufacturer's instructions. Ionized Ca (Ca^{2+}) concentration in the serum was determined by the ionic selective electrode method using Na/K/Ca/pH Analyzer Solution Pack (Medica, Bedford, MA, USA), and an automatic analyzer (Easylyte Calcium, Nihonkoden, Japan). Total Ca and P contents were determined using commercial assay kits (WAKO Pure Chemical Ind., Tokyo, Japan) and an automatic chemistry analyzer (Hitachi 7150, Tokyo, Japan) in accordance with the manufacturer's instruction.

3. Evaluation of beef quality

At the end of the feeding experiment, steers were slaughtered after 16-h fasting. After a 24-h chilling period at 1℃, the left side of M. *longissimus dorsi* was cut between the last rib and the first lumbar vertebra to determine carcass characteristics (marbling score, ribeye area and back fat thickness) by a trained meat grader of the Animal Products Grading Service (APGS) on the basis of the Korean Beef Grading Standard.

Beef quality was evaluated in M. *longissimus dorsi* samples vacuum-packaged and aged at 2℃ for 7 d. At the end of the aging period, objective color (Hunter L*, a* and b*) was determined using a colorimeter (Model CR-301, Minolta, Osaka, Japan) after 30-min blooming at room temperature.

Water-holding capacity (WHC) was measured using the procedure of Laakkonen et al. (1970) with slight modification. Briefly, for the determination of free water (F) content in the tissue, 0.5 g of a *longissimus* sample, from which any adipose or connective tissue attached was removed, was boiled at 80℃ for 20 min in water bath, and cooled, centrifuged at 737 × g for 10 min and weighed. The weight loss during this process represented the free water (F). Total water (W) content of *longissimus* sample was determined using a different aliquot by the

AOAC (1996) method. Values for WHC were calculated by the formula: 100(W-F)/W. For the determination of cooking loss, muscle samples (3 cm thick) were boiled at 70℃ for 1 h, cooled under running water for 20 min and weighed. Water loss during boiling represented cooking loss, which was expressed as a percentage of total water. Cores (1.27 cm in diameter) were taken parallel to myofiber from the samples used for the determination of cooking loss using a Warner-Brazler Shear (WBS) meter (G-R Elec. Mfg. Co., Manhattan, USA) and values were expressed as kilograms per 1.27-cm core.

For sensory evaluation, steaks (2.5×4-cm cube, 2-mm thick) from *longissimus* samples were roasted at 250℃ of surface temperature to approximate medium doneness, and scored by 10 trained panels for juiciness, tenderness and flavor on the basis of the 6-point evaluation method (from 6; extremely juicy, tender and favorable beef flavor to 1; extremely dry, tough and unfavorable beef flavor). Moisture and ether extract contents in *longissimus* muscles were measured by the AOAC procedures (1996).

4. Statistical analysis

The student t-test was used to compare individual means of the two dietary groups using the SAS package (SAS, 1988).

Ⅲ. RESULTS

Serum Ca^{2+}, Ca and P concentrations were not influenced by diets, but serum 1,25-$(OH)_2$-D_3 concentrations determined either 2 or 6 mo after the beginning of feeding the experimental diets were higher ($P < 0.01$) in steers fed the diet without Ca supplement than in those fed the control diet (78.3 vs 51.7 and 80.3 vs 51.1 pg/mL, respectively) (Table 2). Average daily gain was higher ($P < 0.05$) in steers fed the diet without Ca supplement than in those fed the

Table 2. Effects of feeding diet containing no Ca supplement during the late finishing period on serum 1,25-$(OH)_2$-D_3, Ca^{2+}, Ca and P concentrations in steers[1)]

Item[2)]	Ca supplemented	No Ca supplemented	P
1,25-$(OH)_2$-D_3 (pg/mL)			
2 mo[3)]	51.7 ±4.9	78.3 ±5.4	0.001
6 mo[3)]	51.1 ±2.6	80.8 ±4.3	0.0001
Ca^{2+} (mmol/L)			
2 mo	1.08±0.02	1.07±0.01	
6 mo	1.09±0.02	1.11±0.01	
Ca (mg/dL)			
2 mo	9.1 ±0.22	9.4 ±0.08	
6 mo	9.5 ±0.11	9.7 ±0.08	
P (mg/dL)			
2 mo	6.5 ±0.19	6.7 ±0.16	
6 mo	6.7 ±0.25	6.8 ±0.16	

[1)] Values are means with SE of 12 steers.
[2)] Values were determined 2 or 6 mo after the initiation of feeding experimental diets.
[3)] Mean values differ between the two dietary groups according to the student t-test ($P < 0.01$).

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control diet (Table 3). Steers fed the diet without Ca supplement tended to show a higher intake of concentrates, but a lower intake of hay compared to those fed the control diet, although the differences were not significant ($P > 0.05$). Feeding the diet without Ca supplement remarkably ($P < 0.01$) increased the marbling score (5.1 vs 2.2) (Table 4), and the muscle (*longissimus dorsi*) fat content (10.2 vs 6.7%) with a concomitant decrease in moisture content (70.4 vs 67.6%) compared to feeding the control diet (Table 5). Ribeye area was slightly ($P < 0.05$) increased with the diet containing no Ca supplement compared that found with the control diet. Back fat thickness was not different between the two dietary groups (Table 4).

Meat color, pH and water-holding capacity in *longissimus* muscle were not different between the two groups. Cooking loss was lower ($P < 0.05$) in *longissimus* muscle of steers fed the diet without Ca supplement than the control. The Warner-Brazler Shear (WBS) force of the

Table 3. Effects of feeding diet containing no Ca supplement during the late finishing period on growth and feed efficiency in steers

Item	Ca supplemented	No Ca supplemented	P
Initial W. [1)] (kg)	373 ± 6.9	376 ± 7.3	
Final W. [1)] (kg)	577 ±11.2	592 ±10.9	
ADG[1,2,3)] (kg)	0.90 ±0.02	0.99 ±0.03	0.046
ADFI[2)] (kg)			
Concentrates	9.7 ±0.26	10.3 ±0.24	
Grass-hay	2.5 ±0.12	2.1 ±0.14	
Total	12.2 ±0.37	12.4 ±0.36	
F/G[2)]			
Concentrates	10.8 ±0.30	10.4 ±0.24	
Grass-hay	2.8 ±0.23	2.1 ±0.14	
Total	13.6 ±0.40	12.5 ±0.36	

[1)] Values are means with SE of 12 steers.
[2)] Values are means with SE of three pens of four steers each.
[3)] Mean values differ between two dietary groups according to the student t-test ($P < 0.05$).

Table 4. Effects of feeding a diet without Ca supplement during a late finishing period on carcass traits in steers[1)]

Item	Ca supplemented	No Ca supplemented	P
Hot carcass W. (kg)	312±9.1	322±7.8	
Backfat thickness (mm)	5.5±0.54	6.4±0.73	
Ribeye area[2] (cm^2)	77.2±1.6	82.8±1.8	0.034
Marbling score[2,3)]	2.2±0.29	5.1±0.55	0.003

[1)] Values are means with SE of 12 steers.
[2)] Mean values differ between two dietary groups according to the student t-test ($P < 0.01$).
[3)] Score ranges from 7 (extremely well marbled) to 1 (extremely poorly marbled).

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Table 5. Effects of feeding diet containing no Ca supplement during the late finishing period on beef quality in *longissimus* muscle of steers[1)]

Item	Ca supplemented	No Ca supplemented	P
Meat color, Hunter			
L	31.8 ±0.50	31.9 ±0.29	
a	17.5 ±0.41	17.9 ±0.44	
b	6.6 ±0.22	6.9 ±0.19	
Cooking loss[2)] (%)	27.4 ±1.0	23.4 ±1.2	0.019
WBS[3)] (kg/cm^2)	3.2 ±0.13	2.9 ±0.19	0.084
Water holding capacity (%)	52.4 ±0.58	51.8 ±0.90	
pH	5.6 ±0.02	5.6 ±0.01	
Moisture[2)] (%)	70.4 ±0.39	67.6 ±0.67	0.002
Ether extracts[2)] (%)	6.7 ±0.52	10.2 ±0.91	0.003
Sensory evaluation			
Juiciness	4.6 ±0.09	4.8 ±0.15	
Tenderness	4.5 ±0.17	4.9 ±0.16	0.054
Flavor[2)]	4.6 ±0.08	4.9 ±0.09	0.028

[1)] Values are means with SE of 12 steers.
[2)] Mean values differ between two dietary groups according to the student t-test ($P < 0.01$).
[3)] Warner-Brazler Shear (WBS) force in *longissimus* muscle cores (1.27 cm in diameter).

longissimus muscle was slightly ($P = 0.08$) lower in steers fed the diet without Ca supplement than in steers fed the control diet (3.2 vs 2.9 kg/1.27-cm diameter core). Sensory evaluation showed that feeding the diet without Ca supplement slightly ($P = 0.054$) improved tenderness and flavor compared to feeding the control diet, but juiciness was not affected by diets (Table 5).

Ⅳ. DISCUSSION

Our data demonstrated that feeding a diet without Ca supplement increases serum 1,25-$(OH)_2$-D_3 concentration compared to a control diet (Table 2), confirming that dietary low Ca increases the synthesis of 1,25-$(OH)_2$-D_3 to maintain the homeostasis of blood Ca and P. Serum Ca^{2+} and Ca contents were not influenced by dietary Ca (Table 2), indicating their homeostasis in the blood within the given dietary limit, probably through increased Ca absorption from the GI tract and bone mobilization. Recent studies have shown that higher dietary Ca intake is associated with the weight loss and the body fat reduction in humans (Zemel et al., 2000; Lin et al., 2000; Davies et al., 2000; Carruth and Skinner et al., 2001; Heaney et al., 2002). Zemel et al. (2000) indicated that extracellular 1,25-$(OH)_2$-D_3 increases Ca^{2+} influx into adipocytes, and the increased cellular Ca^{2+} inhibits lipolysis in a study done with primary cultures of human adipocytes. They also found that feeding a high-Ca (1.2% Ca) diet decreased weight gain and fat pad mass by inhibition of fatty acid synthase (FAS) gene expression and activity compared to a low-Ca (0.4% Ca) diet in mice. These results of Zemel et al. (2000) are

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in line with our data demonstrating that feeding a diet without Ca supplement during the late fattening period of cattle increases weight gain and intramuscular fat content.

Our data showed that steers fed the diet without Ca supplement tended to have higher concentrates intake and lower grass-hay intake, compared to steers fed the diet with Ca supplement (limestone) when they were allowed to have free access to diet. The decreased intake of concentrates in steers fed the diet with Ca supplement is not likely attributable to hypercalcemia because serum Ca contents were not different between two groups (Table 2), although hypercalcemia induced by a high dose of vitamin D_3 has been shown to decrease feed intake in pigs (Wiegand et al., 2002) and steers (Montgomery et al., 2002).

The increased concentrates intake and the higher energy content in the diet containing no Ca supplement (corn substituted for limestone) are considered to have partly contributed to the increased weight gain and intramuscular fat content in our study. However, we cannot exclude the possibility that the increased 1,25-$(OH)_2$-D_3 and in turn the increased intracellular Ca^{++} contributed to some extent to the weight gain and intramuscular fat synthesis in steers fed the diet containing no Ca supplement. For further clarification we are planning on another experiment, in which diets with or without Ca supplement are isocaloric.

Interestingly, Montgomery et al. (2000, 2002) found that tenderness was improved without a significant increase in plasma 1,25-$(OH)_2$-D_3 levels when steers were fed 5×10^6 IU of vitamin D_3 per day for 9 d before slaughter, while plasma concentrations of Ca^{2+}, and vitamin D_3 and its 25-hydroxy form were markedly increased, and also found that vitamin D_3 decreased feed intake. The increased intramuscular Ca^{2+} was assumed to activate calpain that increases the degradation of skeletal muscle postmortem and meat tenderness (Wiegand et al., 2002). By contrast, the effect of vitamin D_3 on meat tenderness was not shown in pigs (Wiegand et al., 2002) and lambs (Wiegand et al., 2001). Our data showed a slight decrease in Warner-Brazler Shear (WBS) force ($P = 0.08$) and increase in tenderness ($P = 0.054$) in *longissmus* muscle of steers fed the diet without Ca supplement compared to steers fed the control diet.

In conclusion, feeding steers diet without Ca supplement during the late fattening period is beneficial, increasing growth and marbling partly through an increased energy intake and also perhaps through induced 1,25-dihydroxy vitamin D_3 synthesis that may increase intracellular Ca^{2+} concentration and in turn fat synthesis.

Ⅴ. 요 약

이 연구는 소 비육후기에 칼슘제(석회석)를 첨가하지 않은 사료의 급여가 성장율, 근내지방도 및 혈청 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) 함량에 미치는 영향을 구명하기 위하여 수행되었다. 거세 한우 24두(20~24개월령)를 12두씩 대조구(석회석 2.5% 함유 농후사료)와 칼슘제 무첨가구(석회석 0%)로 배치하여 223일 동안 사료(농후사료 및 오차드그라스 건초)와 물을 무제한 급여하였고, 사양시험이 완료된 후 도축하여 육질을 평가하였다. 혈청 Ca^{2+}, Ca 및 P 함량에는 처리 간 차이가 없었으나 ($P > 0.05$), $1,25(OH)_2D_3$ 함량은 시험 시작 후 2 또는 6개월째 모두 칼슘제 무첨가구가 대조구보다 (각각 78.3 vs 51.7 또는 80.3 vs 51.1 pg/mL) 높았다 ($P < 0.01$). 칼슘제를 첨가하지 않은 사료를 급여한 비육우가 대조구보다 농후사료 섭취량은 증가하고 건초 섭취량은 감소하는 경향을 보였다. 일당증체량은 대조구보다 칼슘제 무첨가구에서 높았다 ($P < 0.01$). 등심단면적(82.8 vs 77.2 cm^2), 근내지방도(5.1 vs 2.2) 및 지방 함량(10.2 vs 6.7%)이 칼슘제 무첨가구

가 대조구보다 높았고 ($P < 0.05$), 수분 함량 (67.6 vs 70.4%)은 낮았다 ($P < 0.05$). 등심 육색, pH 및 보수력에서는 처리 간 차이가 없었으나 전단력에서는 칼슘제 무첨가구에서 (2.9 vs 3.2 kg/1.27-cm diameter core) 약간 낮게 ($P = 0.08$) 나타났다. 관능평가에서는 칼슘제 무첨가구가 대조구보다 연도 (4.9 vs 4.5) 및 향미 (4.9 vs 4.6)가 약간 개선되었으나 ($P < 0.05$) 다즙성에서는 처리간 차이가 없었다 ($P > 0.05$). 본 연구결과는 비육후기에 칼슘제(석회석)를 첨가하지 않은 사료의 급여는 에너지 섭취의 증가 또는 $1,25(OH)_2D_3$의 합성 촉진을 통하여, 근내지방합성이 증가되어 성장율 및 근내지방도를 개선한다는 것을 제시하였다.

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