Journal of Agricultural Science and Food Technology
Vol. 2 (7), pp. 114-118, August, 2016
ISSN: 2465-7522
Full Length Research Paper
http://pearlresearchjournals.org/journals/jasft/index.html

Lowered Dietary Calcium and Its Effects on the Production Performance, Meat Quality and Adipogenic Marker Gene Expression of Korean Hanwoo Beef Cattle

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Accepted 11 August 2016

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ABSTRACT

Marbling is one of the most important characteristics in the production of high quality beef. In practice, nutritional manipulations are frequently performed in order to achieve better marbled and thus higher quality beef. The present study was designed in order to determine the effect of lowered dietary calcium on the meat quality of Korean Hanwoo beef cattle. Dietary calcium was reduced through the decrease of limestone content in the feed formulation. Sixty animals were divided into 3 groups "[20 animals each]", control (1.32% limestone), treatment 1 (1.02% limestone) and treatment 2 (0.72% limestone). After six months of feeding trial, the animals were slaughtered and the carcass characteristics and production performance were obtained. In addition, RNA samples were isolated from muscle samples collected from the cervical area and the expression of adipogenic marker genes (PPARγ, CEBP/α, aP2 and SREBPc) were measured through reverse transcriptase polymerase chain reaction. Results have shown that lowering the dietary calcium level causes no improvements or changes in the production performance, carcass quality, serum biochemical parameters, and mRNA expression of adipogenic marker genes. Also, no significant changes were seen in the calcium content of the serum suggesting a tight regulation for the mineral by the calcium homeostasis system of the animal. In conclusion, lowered dietary calcium at the level used in this study does not result in increased carcass characteristics in Hanwoo beef cattle.

Key words: Calcium, Marbling, Adipogenic genes, Micronutrient manipulation and Meat quality.

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INTRODUCTION

Calcium is the most abundant mineral (1 to 2%) in the body of vertebrates found mostly (99%) in the bones and teeth. Together with phosphorous, it constitutes a majority of the mineral content of the bone. Calcium is closely related to phosphorous and the deficiency or excess in one can lead to irregularities in the utilization of the other. Calcium is necessary for the normal function of a wide array of tissues and physiologic processes including bone formation, muscle contraction, nerve transmission, blood clotting, and as a second messenger that regulates the action of several hormones. As the most abundant mineral in an animal's body, it is involved and is essential in many processes such as vascular function (contraction and dilation), muscle function, transmission of nerve impulses, intracellular signaling and hormonal secretion (Mcdowell, 1992). Marbling is a characteristic used in meat production to describe the distribution and amount of intramuscular fat (Tong et al., 2015). It is already generally accepted that an increase in marbling characteristics of meat directly correlates to the

Table 1. Marbling characteristics of Hanwoo beef cattle with altered dietary calcium for 6 months.

	Control	T1	T2
Carcass percentage	59.56±1.45	59.12±1.75	59.46±1.59
Carcass grade	63.58±3.66	66.34±2.51	64.43±2.80
Marbling score	58.50±18.44	52.81±11.68	56.62±10.93

*Data are means ± SE (n=20).

increase in the palatability and acceptance of beef through its effects in meat tenderness and taste (Bolyston et al., 1995; Busboom et al., 1993).

In Japanese black steers, marbling begins at around 10 months of age, continues by 20 months of age and is completed by the 30th (MAFF, 2000). There are also some studies that show an increase in the proliferation and differentiation of intramuscular adipose tissue at 14 months of age (Hood and Allen, 1973), while others reporting an increase in the quantity of adipocytes in the longissimus muscle group at 13th to 19th months of age (Cianzio et al., 1985). The amount of adipose tissue present in between the muscle fibers relies on both the number and size of the constituent adipocytes. The increased rate of synthesis, storage and accumulation of lipid droplets into the cytoplasm results to an increase in the adipocyte volume (Walther and Farese, 2012). Numerous dietary factors which regulate the differentiation of adipocytes have already been clarified. Such is the premise of micronutrient manipulations wherein specific nutrients are altered in the diet of the animal to give rise to benefits on production parameters. It has already been established that several nutritional factors can markedly influence the development of intramuscular fat deposition in cattle ultimately leading to increased quality of beef produced (Kawachi, 2006). Previous studies in our lab demonstrated that low extracellular calcium levels in the extracellular environment results in increased adipogenesis *in vitro.* The current study was designed in order to determine the effects of dietary calcium on the marbling characteristics and production performance of growing Korean Hanwoo beef cattle.

MATERIALS AND METHODS

Animals and Experimental Design

Sixty Korean Hanwoo steers in their growing stage were randomly divided into 3 groups [20 animals each]; control, treatment 1 and treatment 2. Different groups were fed the same diet only differing in the dietary levels of calcium. Feeds were provided twice a day and water *ad libitum.* After six months of feeding trial, the animals were slaughtered and the carcass characteristics and production performance were obtained. Sampling of muscle and blood were also done for the analysis of adipogenic gene markers, calcium and phosphorous ratios, and serum biochemistry.

Production Performance

In order to gauge the production performance of the experimental animals various production values were measured. Body weights (initial and final) and daily feed intake were measured. Body weight gain, feed conversion ratio and average daily gain were computed using standard formulas.

Marbling Characteristics

At the end of the experiment all animals were brought to a commercial abattoir for slaughtering. Carcass percentage, carcass grade and marbling score were determined via a standardized protocol being used by the abattoir.

Plasma Calcium, Phosphorous and Biochemical Analysis

Prior to slaughter, blood was collected from the tail vein of the animals, stored in tubes with heparin as anticoagulant and transported to the laboratory on ice. Centrifugation (5000 rpm for 10 min) was done to obtain the plasma which was used for analysis of calcium, phosphorous and blood biochemistry. Calcium and phosphorous levels in the plasma were measured using an automatic analyzer (Beckman Coulter AU analyzer). Blood biochemistry profile was analyzed with the use of an Automatic Biochemical Analyzer (Spotchem EZ SP-4430, Arkray, USA).

Reverse Transcriptase – Polymerase Chain Reaction

Muscle tissue obtained from the *longissimus capitus et atlantis* area were processed for the expression of adipogenic gene markers (Table 1) (PPARγ, CEBP/α, SREBPc and FABP4). Trizol® reagent was used to obtain the total RNA of the muscle tissue. One microgram of RNA was used to obtain the complementary DNA (cDNA) using the protocol provided by M0MuLV reverse transcriptase (Fermentas, Lithuania). Primer sets specific

Table 2. Production performance of Hanwoo beef cattle following altered dietary calcium for 6 months.

Parameters[1]	Control	T1	T2
IBW (kg)	659.31±59.528	561.36±39.97	604±54.62
FBW (kg)	754.75±70.01	658.45±43.67	714.87±68.37
BWG (kg)	95.4375±37.00	97.09±27.14	110.87±37.00
ADG	0.52±0.21	0.45±0.13	0.51±0.16
ADFI (kg)	7.200±0.42	7.212±0.34	7.214±0.28
FCR	0.0726±0.02	0.0654±0.02	0.0739±0.02

*Data are means ± SE (n=20). [1]IBW- initial body weight; FBW – final body weight; BWG – body weight gain; ADFI – average daily feed intake; FCR – feed conversion ratio; ADG- average daily gain.

Table 3. Serum calcium and phosphorous levels of experimental animals with dietary manipulations of calcium.

	Control	T1	T2
Average Calcium (mmol/L)	2.53 ± 0.10	2.43 ± 0.08	2.43 ± 0.13
Average Phosphorous (mmol/L)	2.24 ± 0.14	2.55 ± 0.23	2.43 ± 0.21
Ca:P ratio	1.13:1	0.95:1	1:1

*Data are means ± SE.

Table 4. Serum biochemistry of experimental animals with diets having altered dietary calcium for six months.

Parameters[1]	Control	T1	T2
Glucose(mg/dL)	96±15.22	100±10.25	95±9.79
Total cholesterol(mg/dL)	143±12.8	146±28.4	134±20.79
BUN(mg/dL)	8.2±1.44	9.1±1.55	9.7±1.65
Total Bilirubin(mg/dL)	0.3±0.06	0.8±0.46	0.2±0.07
GOT(IU/L)	72.74±11.64	75.8±16.11	65.6±12.85
GPT(IU/L)	15.4±1.76	20.2±5.67	18.9±5.01

*Data are means ± SE (n=20). [1]Glucose = 40-100 mf/dL, Total cholesterol = 62-193 mg/dL, BUN = 10-25 mg/dL, Total Bilirubin = 0-0.1mg/dL, GOT = 60-125 IU/L, GPT – 6.9-35 IU/L. Normal values for the blood biochemistry parameters used in the study (The Merck Veterinary Manual, 10th ed, 2010).

for the genes of interest were used for the conventional PCR assay. Amplicons were separated via electrophoresis of 1.5% agarose stained with ethidium bromide. Visualization of bands was done using UV transillumination.

Statistical Analysis

The difference between means of different treatment groups were statistically analyzed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) ($P < 0.05$). Dependent and independent variables were the values obtained from each analytical parameter and treatment groups, respectively.

RESULTS

Marbling Characteristics and Production Performance

Dietary manipulation of calcium in the animals used in the study resulted in no significant changes in the carcass percentage, carcass grade and marbling score at slaughter (Table 1). These results signify that no improvements in the meat quality of the animals that were given a lower dietary calcium. Similarly, the same trend was seen in the production parameters at the end of the study (Table 2). No statistically significant changes ($P < 0.05$) were seen in the body weight gain, average daily gain, feed intake and feed conversion ratio were seen in the animals that were given lower dietary calcium levels.

Serum Calcium: Phosphorous Levels and Biochemistry

In order to assess if alterations will occur in the levels of calcium and phosphorous of the animals in relation to calcium homeostasis, the plasma levels of the two minerals were assessed. Results show that there were no significant changes ($P < 0.05$) in the calcium and phosphorous ratio of the treated animals as compared to the control (Table 3). In addition, no significant differences were seen in the blood biochemical parameters between the animals belonging to the treated and control groups (Table 4).

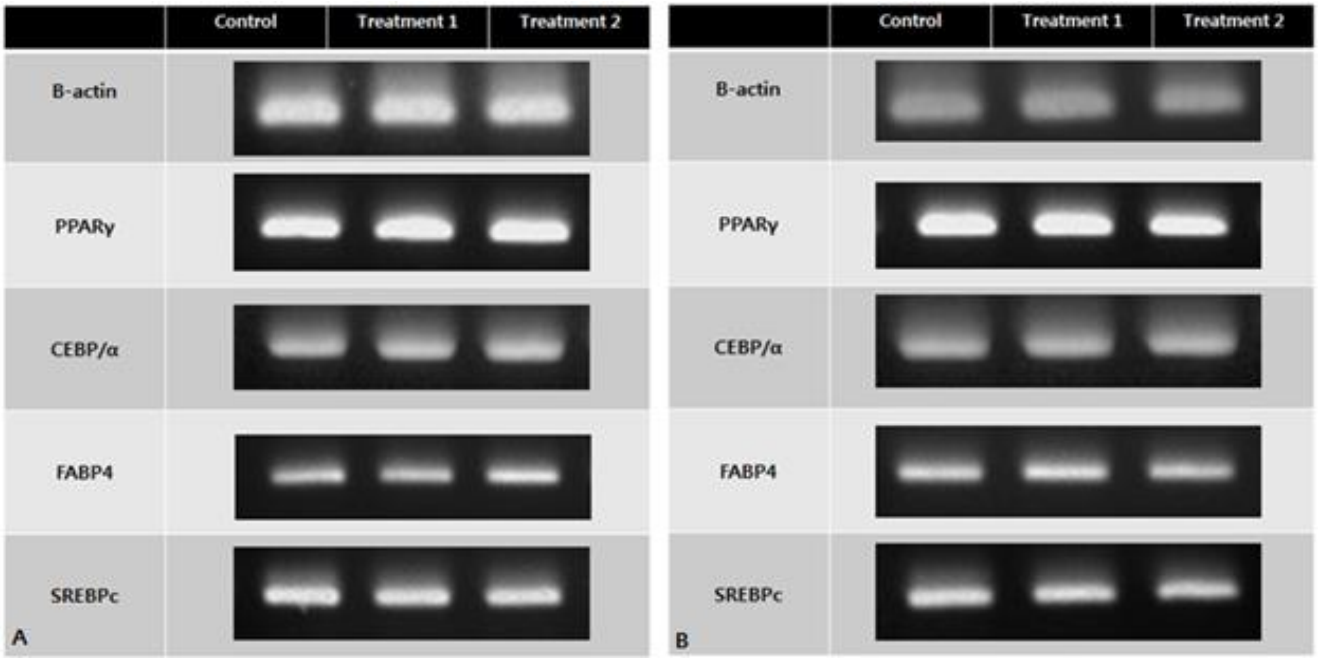

Figure 1. mRNA expression of adipogenesis-related genes of representative experimental animals from different treatment groups. (PPAR-peroxisome proliferator activated receptor gamma; CEBP/α – CCAAT/enhancer binding protein alpha; FABP4 – fatty acid binding protein 4; SREBPc – sterol regulatory element-binding protein).

PCR Assay for Adipogenic Genes Expression

In order to determine if changes in the mRNA expression of important adipogenic gene markers tissue were collected and PCR assay was done. Our results show that no changes were seen in the expression of the adipogenic genes (PPARγ, CEBP/α, SREBP1c, FABP4) used in the study (Figure 1).

DISCUSSION

Marbling is an important economic trait in beef production. In practice, better marbling characteristics correspond to better quality meat and thus increased value. As nutrient manipulations are already commonly practiced by some cattle raisers to obtain better quality meat, the study aims to determine the effect of lowered dietary calcium on the marbling characteristics and production performance of Hanwoo beef cattle. Results of the study show that dietary alterations in calcium at concentration used in the study do not result in improvements in the marbling characteristics of cattle. Moreover, there were no significant improvements in the production performance of the experimental animals (BWG, ADFI, ADG etc.). In addition, there was also no noticeable change in the mRNA expression of the different adipogenic genes used in this study. The absence of noticeable changes in the parameters used in the study demonstrates the multifactorial nature of the effects of micronutrients in improving meat quality. In the case of dietary restrictions of vitamin A, it has been shown that a diet deficient in the said vitamin results to a drop in plasma concentration of retinol from approximately 300 µg/L to 90 µg/L. From this, it can be inferred that the retinol present in the blood circulation may act as an inhibitor of adipocyte differentiation in normal fattening beef cattle, and a vitamin A deficient diet results in a partial cancellation of this anti-adipogenic action (Oka et al., 1998).

In addition, it was demonstrated that zinc concentrations reaching 30 µmol/L, from 12 to 18 µmol/L in normal cattle, after dietary supplementation, resulted in an increased beef marbling (Tanaka et al., 2001). In our study, however, it can be seen that there is no noticeable decrease in the levels of calcium in the blood of the animals across the different treatment groups ($P<0.05$). This implies that even if it has already been shown in previous studies that calcium has an inhibitory role in the adipogenic process *in vitro*, the phenomenon is hard to demonstrate in an *in vivo* setting due to the mineral's highly regulated nature. It is neither the calcium absorption nor excretion that plays a significant regulatory role in calcium homeostasis in animals; instead, the most significant factor regulating the levels of calcium in the circulation is the isoionic calcium uptake and release by bone surfaces (Bronner, 2001). Blood ionized calcium concentrations are highly stable in healthy animals because of the homeostatic system

involving the actions of the three calciotropic hormones on the target organs of bone, gut, and kidney, in addition to fluxes between the bone canalicular fluid and the extracellular fluid. The maintenance of normal levels of calcium within narrow ranges in different compartments of the body is primarily dependent on the interactions of PTH, active Vitamin D3, and calcitonin (Mundy and Guise, 1999). The serum biochemistry of experimental animals was evaluated to determine their general health. Data obtained showed no statistical difference ($P<0.05$) in the serum biochemical parameters across each group. Moreover, total cholesterol values obtained across all treatments had no significant differences. It was implied in some studies that the total cholesterol concentrations in blood is positively correlated with carcass traits in beef cattle (Matsuzaki et al., 1997; Ito and Hirooka, 2003; Kato et al., 2014). Results of the study reveal decreasing the dietary calcium causes no changes in marbling characteristics, production performance, blood calcium and phosphorous levels and mRNA expression of adipogenic marker genes. Results from the current study could serve as basis for the design of future experiments regarding dietary manipulations concerning calcium in relation to marbling characteristics.

ACKNOWLEDGEMENTS

The study is funded by the Research and Development Authority, Korea under project number PJ010023.

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