Effects of Dietary Treatment, Gender, and Implantation on Calpain/Calpastatin Activity and Meat Tenderness in Skeletal Muscle of Korean Native Cattle

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ABSTRACT : The objectives of this study were to examine calpain activity and meat tenderness by three different feeding patterns in Korean native cattle (KNC). Total forty-five animals were assigned each fifteen in long term restriction feeding (LTFR), long-term restriction feeding and hormone treatment (LTFR-tH), and short term non-restriction feeding (STFNR), respectively. Concentrate was restricted based on body weight in exp 1 and 2. However, it was fed ad libitum in exp. 3. Hormonal implantation was made with M-PO^{TM} for bulls and with F-TO^{TM} for heifers at 18, 20, 22 months of age in exp. 2. Animals were purchased (3-5 month old) from local cattle market and managed in two local farms and university research unit at three different years. Animals were slaughtered at 24 months for long-term trial and at 18 month for short term-trial. Loin and tender loin muscle was used for calpain activity and meat quality. Calpain proteolytic system was not changed by treatment. However, calpastatin activity was low in short-term trial. The calpain and calpastatin activity is reciprocal relationship, therefore, the high calpain activity may effect on quality grade. The shear force value was decreased as the processing of aging after postmortem. On the other hand, the cooking loss was significantly higher in short-term than in long-term trial, and then gradually decreased by the aging. Hormone implants to increase meat yield influenced to calpastatin activity more powerfully than calpain activity to meat tenderness. In meat color-a*, there was not significant difference in loin. Meat color-b* was decreased as postmortem aging time increased in tenderloin. Western blots were done to learn whether these proteins are degraded during postmortem storage and whether this degradation temporally parallels the decrease of shear force value. Vinculin was detected at 0 day and 1 day and degraded after 3 day. In conclusion, Calpain activity was affected slightly on meat tenderness. But meat tenderness was influenced by calpastatin, more effectively. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1653-1658)***

Key Words : Calpain Activity, Meat Tenderness, Feeding Pattern, Korean Native Cattle

INTRODUCTION

As beef consumption increase, consumers want higher meat quality. Although there are many factors (color, tenderness, and flavor of meat), which influence on meat quality, the most considerate point of consumers is meat tenderness. Calpain I, II (calcium dependent protease) and calpastatin (calpain inhibitor) influence on the meat tenderness (Goll et al., 1989). Generally, castration improves meat tenderness in beef cattle. Cytoskeletal filaments are associated with the plasma membrane in areas of cell contact. Desmin is one of the five major groups of intermediate filaments and is found in predominantly in skeletal, cardiac, and smooth muscle. Vinculin is associated with the cytoplasmic aspect of contact areas close to the membrane. It has been suggested that vinculin is a possible link between ends of the bundles of actin filaments and the plasma membrane. It has also been proposed that vinculin may be involved in the transmembrane indution of actin bundle formation (Penny, 1980; Valin, 1985). Anabolic implants and adrenomintic components are used to improve growth rate and feed efficiency of cattle during finishing (Trenkle, 1987).

Calpastatin is an endogenous inhibitor of the calpain (EC 3.4.22.17, Ca^{2-}-dependent cystein proteinase) proteolytic system. In skeletal muscle, the calpain system has the potential to regulate growth through involvement in myogenic cell differentiation (Hong et al., 1996) and initiation of myofibrillar protein turnover (Goll et al., 1989). It is also well established that calpain are primarily responsible for postmortem proteolysis, which results in meat tenderization (Koohmaraie, 1992). Postrigor calpastatin activity, which is inversely proportional to postmortem tenderization, accounts for a greater proportion of the variation in beef tenderness (about 40%) than any other single measure (Shackelford et al., 1995).

Previous studies have been conducted to assess growth and meat characteristic differences between bulls and steers. In general bulls grow more rapid (15 to 17%), utilize feed more efficient (10 to 13%) at same age and produce higher yielding carcasses with less fat and more muscle than steers. Bulls produced leaner carcasses with lower quality grades than steers do. Meat from bulls had higher values than meat from steers (Morgan et al., 1989). In most cases, these researches have studied in shot-term feeding program beef cattle, but this study was conducted to examine correlation between bulls and steers through inspecting carcass related to long-term feeding program beef cattle. But the study for

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Korean Native Cattle (KNC) is not reported yet. Therefore, the purpose of this study is to understand the effect of different feeding (long and short) system and gender on carcass grade, calpain and calpastatin activity, and meat tenderness in KNC.

MATERIALS AND METHODS

Animals and management

Animal and managements were same as previous report (Choi et al., 2002). Forty-five KNC were randomly assigned each 5 in 3 (treatment)×3 (gender) factorial design during four years of experimental period. Three treatments were long-term (24 month) restriction feeding (LTFR), long-term restriction feeding- hormone treatment (LTFR-tH), and short-term (18 month) non-restriction feeding (STFNR). The trial included heifers as well as castrated and intact males. Korean native calves (about 4 month of age) were purchased from local farm each 15 calves per year during three years (1996-1998). In LTFR-tH, anabolic agent was implanted into ear subcutaneous at 18, 20, and 24 months of age. Bulls and steers were treated with M-PO™ implants (Progesterone 200 mg/dose, Oestradiol benzoate 20 mg/dose). Heifers were treated with F-TO™ implants (Testosterone 200 mg/dose, Oestradiol benzoate 20 mg/dose) (Upjohn, USA). The animals were slaughtered at the 24-month-old age (BW 550-650 kg) in local slaughterhouse. Five-gram samples were taken from the loin (L) and tenderloin (TL) in order to determine the levels of the calcium dependent proteases (calpain) and their inhibitor (calpastatin).

Calpain and calpastatin assay

Calpain and calpastatin were assayed by following the methods (Wheeler and Koohmaraie, 1991). Within 1 hour of slaughter, samples (5-6 g) were removed from the muscle of the loin (*Longissimus dorsi*) and tenderloin (*Psoas major*) of KNC. After muscle samples were homogenized in ice-cold homogenizing buffer (40mM Tris; 10 mM EDTA; 10 mM 2-mercaptoethanol; 0.2% Triton X-100; pH 7.5 at 4°C; 10 volumes), centrifuged at 30,000×g for 30 min at 4°C and filtered through glass wool (pre-washed with homogenizing buffer). For ion-exchange chromatography, sample was adjusted to pH 7.5 with 6 N HCl, diluted with ice-cold distilled deionized water to reduce its conductivity to below buffer A. The solution was loaded onto a 1.6 cm× 40 cm column of DEAE-sephacel at 24 ml/h. After loading, the column was washed with buffer-A overnight to remove unabsorbed proteins until A278 was closed to buffer-A. Each fraction was eluted with elution buffer. Fractions 1-7 for calpastatin activity, fractions 5-14 for calpain-I activity, and fractions 15-21 for calpain-II were screened by absorbance at 278 nm after pooling of fractions.

Warner-bratzler shear force and cooking loss

Three steaks (two 2.45 cm thick chops per sample) were removed from loin and tenderloin, vacuum packaged, and aged for 3, 9, 15, 21 days. The steaks were then cooked to 75°C in water bath. These steaks were cooled for 2 h before removal of six cores (1.24 cm diameter) paralleled to the longitudinal orientation of muscle fibers. Each core was sheared once with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine (Instron, Canton, MA) with a 50 kg load cell and crosshead speed of 5 cm/min.

Meat color

Meat surface color was measured with a chromameter (Minolta, CR 301). After chopped surface of each sample was exposed in air for 30 min, meat color was expressed by Commission International de Leclairage in L* (lightness), a* (red-green component), and b* (blue-yellow component) values.

Desmin and vinculin analysis

One gram of loin eye was homogenized using a ploytron homogenizer. The muscle homogenate (5ml) was diluted 1:1 with 2×protein denaturing buffer (PDB). For electrophoresis, 30 μg of protein/lane was loaded, electrophoresed, transferred to nylon membrane, and immunoblotted (Koohmaraie et al., 1995). Antibody incubations were carried out in 1% BSA-TTBS at room temperature and membranes were washed three times with blocking buffer after each incubation. Membranes were incubated for 90 min with primary antibody as follow: anti-vinculin (clone VIN-11-5; Sigma), anti-desmin (clone DE-U-10; Sigma). Secondary antibodies were alkaline phosphatase conjugates of anti-mouse IgG (1:1000, Sigma, A-3562). Antibody binding was visualized by exposure to BCIP/NBT (Sigma, B-5655). Image analyzer analyzed the band intensity.

Statistical analysis

SAS/STAT 6.03 package was used to analyze the association among bovine gender, feeding pattern, and economic trait in KNC. Statistical procedure was accomplished using General Liner Model (GLM) by least square procedure (Harvey, 1975). Tukey's Studentized Range Test analyzed the analysis of significance test for variable.

RESULTS AND DISCUSSION

Calpain and calpastatin activity

Calpastatin is a powerful regulator of calpain-mediated proteolysis during postmortem aging of meat (Koohmaraie, 1992). However, very little is known about the mechanism

or factors that control intracellular protein degradation in growing muscle. Calpastatin and calpain-I activity in LTFR-tH was higher than in LTFR and STFNR (Table 1). Treatment LTFR had a higher calpain-II activity than other treatment. In gender, calpastatin and calpain-II activity in bull was higher than in steer and heifer. Heifer had a higher calpain-I activity than bull and steer in all treatment. The ability to regulate muscle protein degradation could have a large effect on the rate of muscle growth (Goll et al., 1989). The proteolytic capacity of the calpain system may regulate muscle protein degradation during both muscle growth and postmortem storage of meat (Wheeler and Koohmaraie, 1992). In our study, the current data support this possibility. Calpain-I was higher in heifer than in bulls and steers. Calpain-II was higher in bulls than in steers and heifers. Calpastatin activity was higher in bulls than in steers ($p<0.05$), even if calpastatin activity in 24 month feeding treated with anabolic implants has no significantly difference. Therefore, meat quality is significantly affected by calpain-I and calpastatin ratio between bull and heifer in KNC. These results indicated that calpastatin activity is involved in the meat quality powerfully.

Shear force and cooking loss

As postmortem aging time increased, shear force value was decreased (Table 2). Shear force value in treatment LTFR was higher than another treatment through 15 days after postmortem. Thereafter, shear force value in treatment LTFR and STFNR was similar and higher than treatment LTFR-tH. In bull and heifer, shear force value of 3 days, 9 days, 21 days after postmortem were similar and higher than in steer. Bull had a higher shear force value than steer and heifer on 15 days after postmortem. Similarly, Kim et al. (2001) reported that aging in KNC influenced shear force.

Cooking loss was decreased as postmortem aging time increased (Table 3). In treatment STFNR, cooking loss on 3 days and 15 days after postmortem were higher than another treatment. There was not significantly different in each treatment on 9 days after postmortem. Cooking loss in treatment LTFR-tH and STFNR were similar and higher than treatment LTFR. In gender, there was no significant difference on 3 days and 9 days after postmortem. Bull had a higher cooking loss than steer and heifer on 15 days and 21 days after postmortem.

On meat tenderness, Field (1971) and Seideman et al. (1982) suggest that meat from bull carcasses was less tender than meat from steer carcasses, whereas, others have been unable to detect significant differences in tenderness of meat from young bulls and steers slaughtered at comparable ages. Although no differences in μ-calpain or m-calpain activities were observed between bulls and steers, the reduced proteolytic capacity of muscle due to increased calpastatin activity may serve as a regulator of myofibrillar protein degradation. Many reports link the growth advantages associated with intact males to greater amounts of androgens such as testosterone. And injected female rats with a synthetic androgen, trenbolone acetate increased muscle gain by the reduction of protein degradation (Hietzman, 1980).

Additionally, several reports have concluded that feeding b-adrenergic agonists to growing animals increased muscle mass and improved whole-body composition due at least in part to reduction in muscle degradation. These results have been observed in lambs (Bohorov et al., 1987), rats (Reeds et al., 1986), veal calves (Williams et al., 1987),

Table 1. Comparison of calpain and calpastatin activity (U/g) among treatments and genders (n=18, LS mean) in skeletal muscle of KNC

Items	Treatment			Gender		
	LTFR	LTFR-tH	STFNR	Bull	Steer	Heifer
Calpastatin	12.10^{b}	13.85^{a}	10.98^{b}	13.97^{a}	10.81^{b}	12.21^{b}
Calpain-I	0.43^{b}	0.75^{a}	0.45^{b}	0.52^{b}	0.33^{c}	0.79^{a}
Calpain-II	0.76^{a}	0.69^{b}	0.65^{b}	0.79^{a}	0.64^{b}	0.67^{b}

LTFR: Long-term feeding by restricted supply of diets.
LTFR-tH: Long-term feeding by restricted supply of diets with hormone treated.
STFNR: Short-term feeding by *ad libitum* of diets.
^{ab} Means in the same row with a common superscript do not differ ($p<0.05$).

Table 2. Comparison of shear force (kg) among treatments and genders (n=18, LS mean) in skeletal muscle of KNC

Day	Treatment			Gender		
	LTFR	LTFR-tH	STFNR	Bull	Steer	Heifer
3	10.95^{a}	8.69^{b}	8.36^{b}	9.76^{a}	8.60^{b}	9.64^{a}
9	9.94^{a}	7.69^{b}	7.92^{b}	8.70^{a}	7.80^{b}	9.05^{a}
15	8.44^{a}	7.40^{b}	7.59^{b}	8.61^{a}	7.24^{b}	7.57^{b}
21	7.76^{a}	6.91^{b}	7.80^{a}	7.85^{a}	6.84^{b}	7.79^{a}

LTFR: Long-term feeding by restricted supply of diets
LTFR-tH: Long-term feeding by restricted supply of diets with hormone treated.
STFNR: Short-term feeding by *ad libitum* of diets.
^{ab} Means in the same row with a common superscript do not differ ($p<0.05$).

Table 3. Comparison of cooking loss among treatments and genders (%, n=18, LS mean)* in skeletal muscle of KNC

Day	Treatment			Gender		
	LTFR	LTFR-tH	STFNR	Bull	Steer	Heifer
3	22.6^{ab}	21.70^{b}	26.64^{a}	24.79	22.78	23.4
9	20.25	20.03	23.04	21.48	20.79	21.06
15	16.88^{b}	19.46^{ab}	22.27^{a}	21.22^{a}	17.36^{b}	20.03^{ab}
21	10.94^{b}	18.50^{a}	18.68^{a}	18.14^{a}	13.94^{b}	16.03^{ab}

LTFR: Long-term feeding by restricted supply of diets
LTFR-tH: Long-term feeding by restricted supply of diets with hormone treated.
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* Means in the same row with a common superscript do not differ ($p<0.05$).

chickens (Morgan et al., 1989), rabbits (Forsberg et al., 1989), and cattle (Wheeler and Koohmaraie, 1992). Our results on meat tenderness by treated hormone indicated that calpastatin activity was higher meat from bull carcass than meat from steer carcass. Shear force values have significant difference by M-PO^{TM} and F-TO^{TM} implants. This result suggested that hormone implants increase meat yield influenced to calpastatin activity more powerfully than calpain activity to meat tenderness. The National Beef Quality Audit identified that reduced quality of beef, specifically lower marbling score and reduced beef tenderness is due to implants (Smith et al., 1992).

Meat color

Table 4 shows change in meat color-L*, a*, b* values of KNC beef (Loin and Tenderloin) pen fed with ad libitum feeding with a high concentrate diet for 18months. Meat color-L* was increased as postmortem aging time increased in loin. Bull had a lower meat color-L* than heifer and steer in loin. In meat color-a*, there was not significant difference in loin. Meat color-b was decreased as postmortem aging time increased in tenderloin. Bull had a lower meat color-b* than heifer and steer in tenderloin. Kang et al. (1997) has been reported similar result in KNC study.

Muscle protein (desmin, vinculin)

Western blots were done to learn whether these proteins are degraded during postmortem storage and whether this degradation temporally parallels the decrease of shear force value. Vinculin was detected at 0 day and 1 day and degraded suddenly after 3 day (Figure 1). The molecular weight of vinculin was about 90 KDa. As postmortem aging time increased, desmin was degraded. The molecular weight of desmin was 50 kDa.

There is ample evidence indicating proteolysis of key myofibrillar and associated proteins whose function is to maintain the structural integrity of the myofibrils is the cause of tenderization that occurs during storage of meat at 4°C. In our study, vinculin is very susceptible to degradation in postmortem muscle. Vinculin degradation begins during first 1 day postmortem. Almost half the vinculin in loin was degraded after 1 day postmortem, and most vinculin degradation occurs after 3 day, a period during which shear force value decreases. Western blots showed that little or no degradation of desmin occurs in loin of bull during the first 1 day postmortem. Over half the total desmin in the loin, however, was degraded between 1 day and 6 day after death (Figure 1), a period during which shear force value decreases dramatically. Taylor et al. (1995) reported a similar result in biceps femoris and semimembranosus muscle in bovine.

In conclusion, although meat quantity was high in short-term feeding, long-term feeding and hormone treatment increased meat quality grade than short-term feeding. Meat quality was influenced by genders. Calpain activity was affected slightly on meat quality. But meat quality was influenced by calpastatin, more effectively.

Table 4. Change in Meat color values of KNC beef in short-term feeding (n=3)*

DAY	Animal	L		a		b	
		Loin	Tenderloin	Loin	Tenderloin	Loin	Tenderloin
	bull	35.53^{b}±1.05	34.37±1.22	22.74±4.13	21.71^{b}±0.68	7.23±1.25	7.05^{b}±0.48
1	heifer	41.14^{a}±1.23	38.47±1.40	22.56±1.80	22.57^{b}±0.75	10.12±0.88	9.65^{ab}±0.56
	steer	36.67^{b}±0.94	33.84±1.19	20.52±0.60	28.13^{a}±0.98	8.46±0.71	10.16^{a}±0.88
	bull	32.92^{b}±1.11	31.78±1.34	15.72±0.92	18.63^{a}±0.93	5.98^{b}±0.63	6.83±0.37
3	heifer	36.84^{ab}±1.58	34.56±1.14	16.22±1.20	17.77^{a}±1.47	8.12^{a}±0.34	8.31±0.28
	steer	39.04^{a}±2.89	34.09±0.42	18.22±1.21	14.35^{b}±0.59	9.46^{a}±1.25	6.97±0.27
	bull	40.72±1.18	38.91^{b}±0.98	8.06±0.84	9.52±0.73	3.56±0.61	3.90±0.55
9	heifer	46.01±1.58	43.47^{a}±2.80	7.46±0.40	8.50±0.62	4.26±0.90	5.18±0.92
	steer	44.31±1.11	41.75^{ab}±3.92	9.90±1.19	8.30±1.26	5.79±1.13	5.18±1.44
	bull	39.64^{b}±1.12	38.15^{b}±1.68	10.31±1.07	11.59±0.40	1.99±0.88	3.17±0.56
15	heifer	44.96^{a}±2.35	43.77^{a}±1.33	9.83±1.05	12.43±1.34	3.26±1.30	4.63±0.39
	steer	44.16^{a}±1.30	42.59^{a}±1.82	9.54±0.79	9.93±1.31	3.30±0.69	4.03±1.30
	bull	30.84^{b}±8.80	40.82±1.02	9.74±1.04	10.08^{b}±1.56	3.34^{a}±1.11	2.37±0.81
25	heifer	43.36^{a}±1.91	43.82±2.79	12.12±1.62	14.68^{a}±1.01	1.84^{b}±0.68	3.17±1.42
	steer	44.72^{a}±1.51	40.32±1.64	10.64±0.64	10.64^{b}±0.84	4.09^{a}±0.88	3.27±3.29
	bull	40.32^{b}±2.11	40.18±1.91	9.74±1.49	15.48^{a}±2.04	2.43^{b}±1.09	0.62±0.58
28	heifer	48.12^{a}±5.26	40.25±1.76	10.49±1.52	13.13^{a}±1.22	3.58a±1.75	1.22±2.09
	steer	43.69^{b}±1.96	40.65±4.06	11.12±1.65	8.93^{b}±1.58	3.22a±1.48	1.12±2.68

^{ab} Means in the same column of same day with a common superscript do not differ ($p<0.05$).

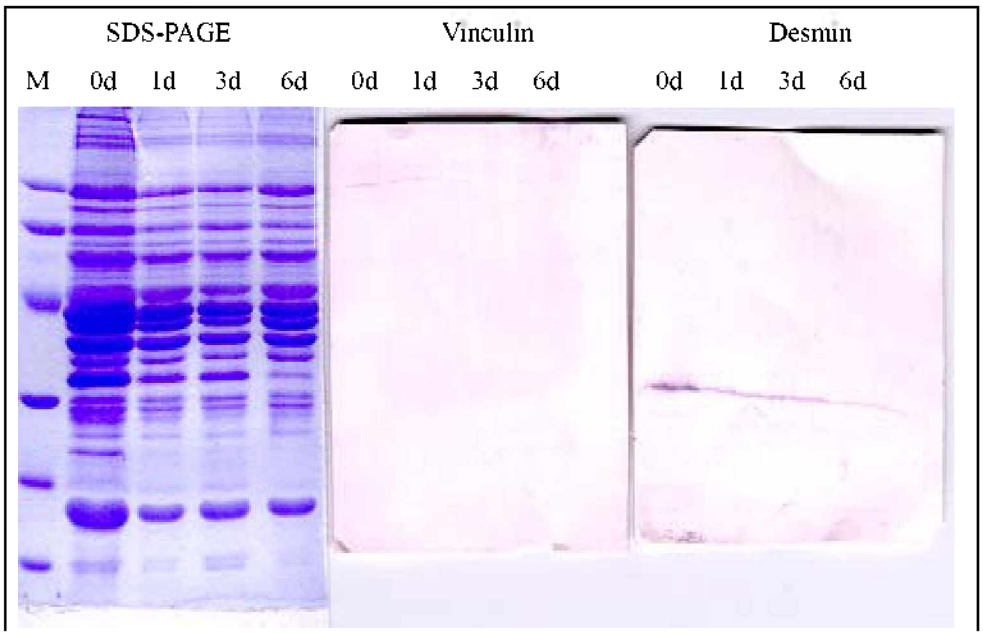

Figure 1. Western blot analysis of vinculin and desmin degradation at different postmortem times. Longissimus homogenates were prepared from samples taken at 0, 1, 3, and 6 day of postmortem storage at 4°C. Each lane was loaded with 30 μg of protein, electrophoresed, and blotted as described in Materials and Methods. Standard (M) lanees consist of molecular weight markers, including phosphorylase b (94 kDa), bovine serum albumin (83 kDa), ovalbumin (43 kDa), Carbonic Anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), a-lactalbumin (14.4 kDa).

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