Comparative Studies on Metabolic Rate and Calpain/Calpastatin Activity between *Hanwoo* and *Holstein* Beef

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ABSTRACT : The objectives of this study were to examine the effect of early short-term temperature conditioning on metabolic rate and calpain/calpastatin system and to compare variations in metabolic rate and calpain/calpastatin system between *Hanwoo* and *Holstein* beef. *Longissimus thoracis et lumborum* of the right carcass from 3 Hanwoo and 3 Holstein bulls were removed within 30 min of exsanguinations, cut into three pieces, and then temperature conditioned until 3 h postmortem (PM) at 2, 16, and 30°C, respectively. Rigor values (R-values; R_{248}, R_{250}, and R_{258}), pH, muscle temperature, glycogen content, μ- and m-calpain activities, and calpastatin acitivity were measured at 1, 3, 9, and 24 h PM, respectively. *Hanwoo* beef had higher muscle temperature, faster metabolic rate at early PM stage in R-values, and lower μ-calpain activity than *Holstein* beef ($p<0.05$). The 30°C treatment maintained muscle temperature of 30°C until 3 h PM and resulted in faster pH decline at 3 and 9 h PM ($p<0.05$) than other treatments. The 16°C had higher ($p<0.05$) muscle temperature at 3 h PM than the 2°C, but no difference in all other traits was observed between the 2°C and the 16°C. Early short-term temperature treatment used in this study was not sufficient to effectively activate calpain/calpastatin system. Correlations among all traits except m-calpain and muscle temperature were generally high ($r>0.60$; $p<0.001$). Among R-values, R_{258} had higher correlations with other metabolic traits than those of R_{248} and R_{250}. These data suggest that early PM metabolic rate, μ-calpain activity, and calpastatin activity may be closely related to each other. Variations in metabolic rate and μ-calpain activity at early PM stage between *Hanwoo* and *Holstein* beef may imply variations in meat quality between both breeds. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 12 : 1747-1753)***

Key Words : *Hanwoo* Beef, *Holstein* Beef, Temperature Conditioning, Metabolic Rate, Calpain, Calpastatin

INTRODUCTION

Consumers consider tenderness is one of the single most important components of meat quality. Moreover, inadequate tenderness is one of the top 10 quality concerns of the beef industry (Boleman et al., 1998). It is generally accepted that extent of postmortem (PM) proteolysis, extent of rigor shortening, and the amount and the solubility of connective tissue are the sources of variation in meat tenderness (Seideman et al., 1988; Koohmaraie et al., 1996; Wheeler and Koohmaraie, 1999). Recently, Rhee et al. (2002) found that most of variation in tenderness of beef *longissimus* at 14 d PM resulted from the variation in the extent of PM proteolysis among above factors.

The rate of PM glycolysis influences on several meat qualities (Marsh, 1993; Kim et al., 2001) through its effect on cold shortening and proteolytic enzyme activity. It depends primarily on cooling rate and use of electrical stimulation. The glycolytic process varies among carcasses in its extent and can be made to vary widely in its rate (Marsh, 1993). Additionally, the rate of glycolysis varies widely according to breeds (Wheeler et al., 1990), muscle type, and stress (Bickerstaffe et al., 1996; Morton et al., 1997).

It is well established that beef *longissimus* tenderness decreases as the percentage of *Bos indicus* inheritance increases (McKeith et al., 1985; Wheeler et al., 1994; Shackelford et al., 1995). In addition to genetics, an array of other factors including time-on-feed, age-at-slaughter, PM processing condition (electrical stimulation, carcass suspension, temperature conditioning) and length of PM aging time may contribute to variation in tenderness.

Beef production in Korea has been shared mostly with *Hanwoo* and *Holstein* beef. *Hanwoo* were originated from a hybrid of *Bos taurus*×*Bos zebu* which was transmitted and settled in the Korean peninsula in BC 4,000 (Han, 1996). *Hanwoo* has been regarded as a premium beef in Korea. It is partly due to its high palatability and desirable chewiness. On the contrary, the black animals and white cows of the *Batavians* and *Friesians* were bred by migrant European tribes settled the Rhine Delta region about 2,000 years ago. These animals genetically evolved into the efficient, high producing black-and-white dairy cow, known as the *Holstein-Friesian* (Yoon, 1990). Although sensory and physico-chemical properties have been compared between *Hanwoo* and *Holstein* (Oh, 1990; Kim et al., 1993), little is known about the changes of metabolic rate and

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Received April 23, 2002; Accepted July 2, 2002

calpain/calpastatin system between *Hanwoo* and *Holstein* beef during PM storage.

The objectives of this study were to examine the effect of early short-term temperature conditioning on metabolic rate and calpain/calpastatin system and to compare variations in metabolic rate and calpain/calpastatin system between *Hanwoo* and *Holstein* beef.

MATERIALS AND METHODS

Animals and treatments

Three *Hanwoo* and three *Holstein* bulls were humanely slaughtered at 24 to 25 months of age and processed at a commercial packing plant in three groups spanning 21 d. To create variation in metabolic rate and calpain/calpastatin activity, early short-term temperature conditioning was applied. In the previous study (Kim et al., 2001), we obtained that early short-term temperature treatment did not produce a negative effect on rancidity and microbiological safety.

Sample preparation

Longissimus thoracis et lumborum of the right carcass were removed within 30 min of exsanguinations and then cut into three pieces. In order to avoid positional bias, these pieces were assigned in equal numbers to one of three-temperature conditioning (2, 16, and 30°C) and then temperature conditioned until 3 h PM (approximately 2 h 15 min) at 2, 16 and 30°C, respectively. After conditioning, all samples were stored at 2°C throughout the experiment. Two 1.5 thick slices were cut from both the costal end and the caudal end at 1, 3, 9, and 24 h, respectively, and pH and muscle temperature were measured immediately. Samples for R-values, glycogen content, calpastatin activity, and μ- and m-calpain activity were removed, stored in liquid nitrogen, and transported to the meat laboratory at Korea University. Glycogen content and R-values were carried out within a week. Calpastatin activity and μ- and m-calpain activity were determined within 3 months.

pH and muscle temperature

The pH was measured in the lateral, central, and medial locations using a spear type electrode (Model 290A, Orion Research Inc., USA). Muscle temperature was measured in the center of the muscle using a portable thermometer (Model TES-1300, TES Electrical Electronic Co., Taiwan).

Glycogen content

Muscle glycogen was determined using the iodine binding assay as described by Dreiling et al. (1987). Approximately 2 g of powdered muscles in 10 ml of 9% perchloric acid were centrifuged at 15,000×g for 20 min at 2°C. The supernatant was mixed with an iodine color reagent (0.26 g of potassium iodide in 10 ml distilled water and 100 ml of saturated $CaCl_2$) and absorbance at 460 nm was measured. Bovine liver glycogen (1 mg/ml, Sigma Chemical Co., USA) was used as a standard.

R-values

Samples were prepared as described by Koh et al. (1993). Samples were placed in 6% perchloric acid were homogenized (Ace Homogenizer AM-8, Nissei Co., Japan) at 5,000 rpm for 90 s and then centrifuged (Centrikon T-124, Kontron instruments Co., Switzerland) at 3,000×g for 10 min at 2°C. R_{248}, R_{250}, and R_{258} were defined as the ratios of A_{248}/A_{260}, A_{250}/A_{260}, and A_{258}/A_{250}, respectively, and the absorbances were measured using a calibrated spectrophotometer (Model Du-64, Beckman Co., USA).

Calpain/calpastatin system

μ-Calpain, m-calpain, and calpastatin were extracted from the samples according to the procedure of Koohmaraie (1990) with slight modifications. The powdered samples in three volumes (w/v) of extraction buffer (50 mM Tris-HCl, pH 7.8, 10 mM EDTA, 10 mM 2-mercaptoethanol) were homogenized twice for 30 s at low and high speed with a 30s interval. After homogenization, the homogenate was centrifuged at 15,000×g for 40 min at 2°C and the supernatant was filtered through four layers of cheesecloth and glass wool. The pH of filtered supernatant was adjusted to pH 7.5 using 0.1 N NaOH. After clarification, samples were dialyzed against four volumes of equilibrating buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM 2-mercaptoethanol) for 20 h and were ultra-centrifuged at 105,000×g for 1 h at 2°C. The extracts were applied to DEAE-sephacel column (ion-exchange column chromatography, 2.5×25 cm, flow rate 24 ml/h) and washed with 5 column volumes of equilibrating buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM 2-mercaptoethanol) to remove unbound proteins. The bound proteins were eluted with a continuous gradient buffer (NaCl gradient from 0 to 400 mM, 350 mM of each in equilibrating buffer).

Activities of μ- and m-calpain were assayed described by Koohmaraie (1990) and were expressed as the amount of calpain caseinolytic activity per gram of muscle. Fractions were incubated with assay media 1 (100 mM Tris, 10 mM EDTA, 1 mM NaN_3, 5 mg/ml casein, 10 mM 2-mercaptoethanol, pH 7.5) for 60 min at 25°C. After incubation, the reaction was stopped by addition of an equal volume of 5% trichloroacetic acid. The resultants were centrifuged at 2,000×g for 30 min, and absorbances at 278 nm were measured. One unit of μ- and m-calpain activities were defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm. The activity of the calpastatin was determined according to the procedure of Homma et al. (1995). For standard, inhibitor

fraction (calpastatin) was replaced by equal volume of elution buffer. The reaction was stopped by addition of the equal volume of 5% trichloroacetic acid. Inhibitor activity was calculated by A (standard)-A (sample)/A (standard)× 100.

Statistical analysis

Data from a completely randomized design were analyzed with mixed model analysis of variance using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) for a split-plot treatment arrangement. The whole-plot treatments were animal breeds. Subplot treatment was temperature conditioning and sub-, subplot treatment was PM time. The effect of breed was tested with animal×breed as an error term. The effect of temperature treatment was tested with animal×breed×temperature treatment as an error term. The effect of PM time was tested with the overall error term. Least-squares means were separated using the PDIFF option (a pair-wise t-test). Pearson's correlation was used for simple correlations among all traits.

RESULTS

There was no three-way interaction (breed × temperature treatment×PM time; $p>0.05$) for all measurement traits in this study.

pH and muscle temperature

The two-way interaction ($p<0.01$) was found in both muscle temperature (Table 1) and pH (Table 2) between temperature treatment and PM time. Temperature treatment resulted in different decline pattern of muscle temperature at 3 h PM among treatments ($p<0.05$). The 30°C treatment maintained muscle temperature of 30°C until 3 h PM and the 16°C had higher ($p<0.05$) muscle temperature at 3 h PM than the 2°C. However, no significant differences in muscle temperature were observed among treatments at 9 and 24 h PM. In the main effect (Table 3), *Hanwoo* beef had higher muscle temperature than *Holstein* beef ($p<0.05$).

Table 1. Changes in muscle temperature by early short-term temperature conditioning during postmortem time[a]

Postmortem time (h)	Temperature conditioning (°C)[b]		
	2°C	16°C	30°C
1	36.1[c]	34.1[c]	34.6[c]
3	18.1[d]	21.8[e]	30.1[f]
9	10.6[g]	9.7[g]	9.9[g]
24	6.5[h]	6.1[h]	6.4[h]

[a] Pooled standard error mean is 0.7.
[b] Conditioning was done at given temperature until 3 h postmortem (approximately 2 h 15 min).
[c,d,e,f,g,h] Least-squares means lacking a common superscript differ ($p<0.05$).

Table 2. Changes in pH by early short-term temperature conditioning during postmortem time[a]

Postmortem time (h)	Temperature conditioning (°C)[b]		
	2°C	16°C	30°C
1	6.89[c]	6.86[c]	6.89[c]
3	6.51[d]	6.43[d]	6.19[e]
9	6.10[e]	6.09[e]	5.76[f]
24	5.63[g]	5.61[g]	5.55[g]

[a] Pooled standard error mean is 0.04.
[b] Conditioning was done at given temperature until 3 h postmortem (approximately 2 h 15 min).
[c,d,e,f,g] Least-squares means lacking a common superscript differ ($p<0.05$).

The higher muscle temperature in the 30°C treatment reflected in the result of muscle pH. The 30°C had faster ($p<0.05$) pH decline at 3 and 9 h PM than other treatments (Table 2). Especially, the 3 h pH by 30°C treatment was not different ($p>0.05$) to the 9 h pH by 2 and 16°C. Although the 16°C showed slower decline pattern of muscle temperature until 3 h PM than the 2°C, no difference in pH was observed between the 2°C and the 16°C at the same measurement times. Also, the higher muscle temperature in *Hanwoo* beef did not occur difference ($p>0.05$) in pH between *Hanwoo* and *Holstein* beef (Table 3).

Glycogen content and R-values

There was no significant difference ($p>0.05$) in glycogen content for the main effect of breed and temperature treatment (Table 3). Only difference in postmortem time on glycogen content was detected ($p<0.01$) and the glycogen content was significantly decreased ($p<0.05$) during the PM course.

A significant ($p<0.01$) interaction was found in all R-values between breed and PM time. *Holstein* beef had slower metabolic rate in R_{248} at early PM stage than that of *Hanwoo* beef (Table 4). R_{248} of *Hanwoo* beef at 1 and 3 h PM was similar to R_{248} of *Holstein* beef at 9 h PM, but there was no significant difference ($p>0.05$) in R_{248} between breeds at 9 and 24 h PM. The breakdown of energy source by R_{248} was accelerated above 3 h PM in *Holstein* beef, whilst no significant difference ($p>0.05$) between 3 and 9 h PM in *Hanwoo* beef was found. Changes in R_{258} showed the similar trends to those of R_{248}, but the mean segregation for R_{258} was not consistent with that of R_{248} (Table 5). Differences in R_{258} between *Hanwoo* and *Holstein* beef were detected only at 1 h PM and the breakdown of energy source was accelerated above 3 h PM in both breeds. Although mean values of R_{250} were different to those of R_{248}, the trend of PM changes and the mean segregation of R_{250} were similar to those of R_{248} except the significant difference at 9 h PM between breeds (Table 6). In the main effect, clear differences in R-values by temperature treatment did not occur ($p>0.05$). Only in R_{258} by temperature treatment had a trend ($p=0.06$) as having faster glycolysis for the 30°C (data not shown).

Table 3. Least-squares means for effects of breed, temperature treatment, and postmortem time on pH, muscle temperature, and glycogen content

Main effect	n	pH	SEM	Temperature (°C)	SEM	Glycogen (mg/g tissue)	SEM
Breed							
Hanwoo	36	6.17	0.02	19.6[b]	0.3	3.1	0.1
Holstein	36	6.25		17.8[c]		2.1	
P>F		0.23		0.04		0.12	
Treatment[a]							
2°C	24	6.28[b]	0.02	17.8[b]	0.4	2.9	0.1
16°C	24	6.25[b]		17.9[b]		2.7	
30°C	24	6.10[c]		20.3[c]		2.2	
P>F		0.01		0.01		0.18	
Postmortem time							
1 h	18	6.88[b]	0.02	34.9[b]	0.4	4.1[b]	0.1
3 h	18	6.38[c]		23.3[c]		3.3[c]	
9 h	18	5.98[d]		10.0[d]		2.4[d]	
24 h	18	5.60[e]		6.3[e]		0.7[e]	
P>F		0.01		0.01		0.01	

[a] Conditioning was done at given temperature until 3 h postmortem (approximately 2 h 15 min).
[b,c,d,e] Least-squares means in the same column lacking a common superscript differ ($p<0.05$).

Table 4. Changes in R_{248} between breeds during postmortem time[a]

Postmortem time (h)	Breed	
	Hanwoo	Holstein
1	0.864[b]	0.695[d]
3	0.882[bc]	0.731[d]
9	0.980[c]	0.886[bc]
24	1.211[e]	1.298[e]

[a] Pooled standard error mean is 0.039.
[b,c,d,e] Least-squares means lacking a common superscript differ ($p<0.05$).

Table 5. Changes in R_{258} between breeds and postmortem time[a]

Postmortem time (h)	Breed	
	Hanwoo	Holstein
1	1.191[bc]	1.297[d]
3	1.180[bc]	1.265[bd]
9	1.073[e]	1.149[ce]
24	0.901[f]	0.829[f]

[a] Pooled standard error mean is 0.033.
[b,c,d,e,f] Least-squares means lacking a common superscript differ ($p<0.05$).

Table 6. Changes in R_{250} between breeds during postmortem time[a]

Postmortem time (h)	Breed	
	Hanwoo	Holstein
1	0.918[b]	0.771[d]
3	0.935[bc]	0.793[d]
9	1.032[c]	0.915[b]
24	1.251[e]	1.311[e]

[a] Pooled standard error mean is 0.037.
[b,c,d,e] Least-square means lacking a common superscript differ ($p<0.05$).

Calpain/calpastatin activity

Differences in μ-calpain activity were detected between breeds ($p<0.05$) and during PM course ($p<0.01$), respectively (Table 7). *Hanwoo* beef had lower μ-calpain activity than *Holstein* beef and μ-calpain activity decreased consistently ($p<0.05$) during PM course. However, no significant difference in μ-calpain ($p=0.08$) by temperature treatment was observed, although the mean value of 30°C treatment had the lowest μ-calpain activity. In terms of m-calpain and calpastatin activity, temperature treatment did not affect ($p>0.05$) the activity of m-calpain and calpastatin, and no difference ($p>0.05$) between breeds was found (Table 7). Difference in m-calpain and calpastatin activity was detected only by PM time ($p<0.01$). Both enzyme activities decreased significantly ($p<0.05$) after 3 h PM, but the extent of decline in m-calpain activity was small compared to that of μ-calpain and calpastatin activity.

Correlation among traits

Correlations among all traits were highly significant ($p<0.01$) except the correlation between m-calpain and glycogen content (Table 8). pH was highly correlated ($r>0.60$; $p<0.001$) with all other traits except m-calpain. Calpastatin was also highly correlated ($r>0.65$; $p<0.001$) with all other traits. The high correlations between μ-calpain and metabolic traits were observed, but μ-calpain was moderately correlated with glycogen content. m-Calpain was moderately to lowly correlated with metabolic traits but highly correlated with μ-calpain and calpastatin. Among R-values, R_{258} had higher correlations with other metabolic traits than those of R_{248} and R_{250}. The strongest correlation between R_{248} and R_{250} indicates that both R_{248} and R_{250} could provide similar information.

DISCUSSION

The conversion from muscle to meat is accompanied by quantitative changes in several metabolites (glycogen, ATP, lactic acid, phosphate) and physical properties (pH, ionic

Table 7. Least-squares means for effects of breed, temperature treatment, and postmortem time on μ- and m-calpain activity and calpastatin activity

Main effect	n	μ-Calpain[a]	SEM	m-Calpain[a]	SEM	Calpastatin[b]	SEM
Breed							
Hanwoo	36	34.8[d]	0.3	41.9	0.2	44.7	0.3
Holstein	36	42.6[e]		47.6		47.9	
P>F		0.04		0.18		0.36	
Treatment[c]							
2°C	24	39.2	0.4	44.8	0.2	46.3	0.4
16°C	24	39.2		45.1		46.7	
30°C	24	37.7		44.4		45.9	
P>F		0.08		0.32		0.43	
Postmortem time							
1 h	18	46.2[d]	0.4	47.4[d]	0.2	53.4[d]	0.5
3 h	18	43.2[e]		46.7[d]		51.7[d]	
9 h	18	37.0[f]		44.0[e]		43.8[e]	
24 h	18	28.2[g]		40.9[f]		36.3[f]	
P>F		0.01		0.01		0.01	

[a] Unit/50 g muscle.
[b] % Inhibition.
[c] Conditioning was done at given temperature until 3 h postmortem (approximately 2 h 15 min).
[d,e,f,g] Least-squares means in the same column lacking a common superscript differ ($p<0.05$).

Table 8. Simple correlation coefficients for metabolic and calpain/calpastatin traits

Trait	Temperature	R248	R258	R250	Glycogen	μ-Calpain	m-Calpain	Calpastatin
pH	0.87***	-0.64***	0.75***	-0.65***	0.82***	0.80***	0.45***	0.77***
Temperature		-0.57***	0.64***	-0.56***	0.74***	0.66***	0.37**	0.75***
R248			-0.92***	0.99***	-0.65***	-0.62***	-0.65***	-0.82***
R258				-0.93***	-0.73***	0.68***	0.54***	0.76***
R250					-0.65***	-0.63***	-0.66***	-0.81***
Glycogen						0.49***	0.17	0.66***
μ-Calpain							0.75***	0.82***
m-Calpain								0.79***

** $p<0.01$, *** $p<0.001$.

strength, extensibility, contractility; Marsh, 1993). Among the traits, measurements of pH and muscle temperature have been used implemental tool to predict meat tenderness (Marsh et al., 1987; Pike et al., 1993; Jones and Tatum, 1994). The rate of glycolysis also could be estimated by the measurement of ATP depletion (Bendall, 1978). Our data showed that R-values were highly correlated with other metabolic traits. The rate of dephosphorylation and deamination of ATP was more rapid in *Hanwoo* beef than *Holstein* beef at early PM stage. The faster metabolic rate in *Hanwoo* beef at early PM stage can be partially explained by higher muscle temperature than Holstein beef. Although *Hanwoo* carcasses were lighter and had smaller ribeyes than Holstein carcasses, muscle temperature of *Hanwoo* remained higher than that of *Holstein*. However, this phenomenon cannot be explained on the basis of our results.

Inconsistent findings have been reported regarding the rate of pH decline for the best beef *longissimus* tenderness. For the best tenderness, O'Halloran et al. (1997) insisted the faster glycolysis (3 h pH of 5.94 and 6 h pH of 5.61), whereas Marsh et al. (1987) and Smulders et al. (1990) argued the intermediate rate (3 h pH of 6.1) and the fast glycolysis (3 h pH < 5.9) adversely affected beef tenderness. When compared to those reports, the pH results obtained from this study showed slower pH decline at early PM stage irrespective of breed. In terms of temperature treatment, the 30°C only hastened metabolic process, and reach pH 6.2 at 3 h PM. These results suggest that application of PM processing that can accelerate PM metabolic rate is needed for the improvement of *Hanwoo* and *Holstein* beef.

It is well established that μ-calpain and calpastatin levels play an important role in PM tenderization (Taylor et al., 1995; Koohmaraie, 1996; Goll et al., 1999). The present study demonstrates that *Hanwoo* beef have lower μ-calpain activity than *Holstein* beef. Additionally, the dramatic decrease in μ-calpain activity takes place within the first 24 h PM. These indicate early activation and subsequent loss of activity through autolysis, and faster degradation of key myofibrillar and cytoskeletal proteins (Koohmaraie, 1996; Goll et al., 1999; Rhee et al., 2000) in *Hanwoo* beef may be expected during further aging process than in *Holstein* beef. However, reduced μ-calpain activity *in vitro*

is not meant a dramatic reduction in μ-calpain activity *in situ* (Geesink and Koohmaraie, 2000) and calpain-induced proteolysis at 1 d PM is limited (Rhee et al., 2000).

A rapid pH decline and high muscle temperature interactively causes early reduction in μ-calpain and calpastatin levels (Rhee and Kim, 2001). Early pH drop promotes the early activation of μ-calpain and consequent tenderizing process through its effect on early increases in intracellular calcium concentration. Furthermore, high correlations between metabolic traits and calpain/calpastatin system emphasize the importance of early PM metabolic rate. However, even though early-short term temperature treatment induced different phase of muscle temperature at early PM stage, the clear differences in activities of μ-calpain and calpastatin were not detected by temperature treatment. On the contrary, Whipple et al. (1990) observed that high-temperature conditioning (22°C for 6 h) affected the activities of μ-calpain and calpastatin. These indicated that early short-term temperature treatment used in this study still was not sufficient to effectively activate calpain/calpastatin system. Therefore, other techniques besides early short-term temperature treatment must be utilized to have enough effect on calpain/calpastatin system.

IMPLICATIONS

Variations in metabolic rate and μ-calpain activity at early postmortem stage between *Hanwoo* and *Holstein* beef may be related to variations in meat quality between both breeds. However, early short-term high-temperature conditioning until 3 h postmortem, which resulted in higher muscle temperature and faster pH decline in both breeds, does not guarantee to effectively activate calpain/calpastatin system. High correlations between metabolic traits and calpain/calpastatin system indicate that early PM metabolic rate may be an important factor determining the time of commencing proteolysis.

ACKNOWLEDGEMENTS

This work was supported by a Korea university grant program. The authors also express their gratitude to Hannaeng Co. for experimental assistance.

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