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ARTICLE

Comparison of Meat Quality Traits, Free Amino Acid and Fatty Acid on *Longissimus Lumborum* Muscles from Hanwoo, Holstein and Angus Steers, Fattened in Korea

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Abstract

This study was conducted to compare meat quality traits related to the tenderness of longissimus muscles obtained from domestic and imported steers. A total of 12 steers from three breeds were slaughtered, and were graded as quality grade 2. They were composed of 4 Hanwoo and 4 Holstein steers (domestic) as well as 4 Angus steers (imported from Australia and gained for six months in Korea until slaughtered). The *longissimus lumborum* muscles were separated and were stored at 4°C for 7 and 14 d. Sarcomere length of Hanwoo was significantly shorter than Holstein and Angus at storage day 14 ($p<0.05$). The myofibrillar index was significantly lower on Hanwoo than Angus at ageing day 7, and was significantly lower than Holstein and Angus steers at storage day 14 ($p<0.05$). Total collagen contents of Hanwoo and Angus steers were significantly higher than Holstein on storage day 7 ($p<0.05$), whereas soluble collagen contents of Holstein were significantly higher than Hanwoo and Angus on storage days 7 and 14 ($p<0.05$). There was no significant difference in the fatty acid composition of the three breeds ($p>0.05$). Glutamic acid contents of Hanwoo and Angus steers were higher than those of Holstein steers at ageing day 7 and 14 ($p<0.05$). The results of this study have shown that there were no dramatic differences between beef from the three breeds that were fattened for 6 months under equal conditions.

Key words: Hanwoo, Holstein, Angus, tenderness, meat quality

Introduction

In South Korea, per capita consumption of meat has increased from 19.9 kg (1980) to 36.8 kg (2009) with lifting gross income, per capita consumption of beef also had been greatly increased till 2000, if per capita consumption in now. Amount of beef consumption in South Korea has jumped by ten times from 37300 M/T to 395500 M/T since 1970. On the other hand, self-sufficiency ratio of beef was fell from 100 per cent to 36.6 per cent since 1970 (MIFAFF, 2010.8). However, recently it showed slightly increasing self-sufficiency ratio of beef, due to Bovine Spongiform Encephalopathy (BSE) outbreak in US, beef traceability in South Korea and enforcing indicate system of origin, but it was still less than other meats such as chicken and pork. Since 2001, South Korea opened customs for live beef cattle from overseas, total 6,860 heads of live beef cattle imported, 6,098 heads from Australia and 762 heads from United State, until May 2005. Then, the Rural Development Administration (RDA) declared a regulation that a meat from a cattle fed in Korea for above 6 months or over 6 months before slaughtered were indicated “Imported (Origin country)”or “Domestic (Origin country)”respectively for supervision to beef origin indication from importing live beef cattle (RDA regulation, 2001.9).

Several studies confirmed differences of meat quality between Hanwoo and imported beef; comparison of fatty acid composition between Hanwoo, Holstein and imported meat (Park and Yoo, 1994), comparison meat colour and physico-chemical traits (Kang *et al*., 1997; Kang *et al*., 1999; Kim *et al*., 1999; Lee *et al*, 2009a). Furthermore, only one case was reported by Park *et al*.

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(2002) that comparison meat physico-chemical properties of sirloin from Hereford, Angus, Murray Grey and Cross breed (Hereford×Angus). Meat tenderness was a major fact to percept taste of meat on consumers in South Korea (Cho *et al*., 2010) and also to be importantly considered when they purchased meat (Kim *et al*., 2009). The process of meat ageing was known that tough meat with rigor mortis became tender meat due to proteolysis in a meat conversed from muscle. In the same time, flavour was improved with tenderization (Lawrie, 1985). Park and Yoo (1994) studied to compare the palatability between Hanwoo, Cross breed and Holstein meat. According to their result, cross breed beef was tougher than Hanwoo and Holstein meat. Thus, this study was conducted to show objective information about meat tenderness, free amino acid contents and fatty acid contents of beef from Hanwoo, Holstein and Angus steers labelled the domestic on market condition.

Materials and Methods

Animals and experiment design

For this experiment, this research was designed to compare the meat tenderness between different three breeds (Hanwoo, Holstein and Angus) that consumers can closely and commonly eating in Korea. Both of Hanwoo and Holstein steers were born and grown in Korea (domestic), and Angus was born and grown in Australia until imported and/also gained for about 6 mon until slaughtered. Total 12 heads (4 heads per each breed) were slaughtered at a abattoir sited in Ochang-si and chilled for about 24 h, after then carcasses were graded by Korean carcass-grading system (NLCF, 2004). A meat quality grade of all carcasses was given 2 grade, and *longissimus lumborum* muscle was detached from carcass and moved to laboratory at NIAS (National Institute of Animal Science, RDA) for analysis, the muscle samples were stored at 4 ºC for 7 d and 14 d.

Warner-Bratzler shear force

For mechanical determination of meat tenderness, the sample (thickness, 2.5 cm) were put in a plastic bag, and heated in a 70ºC water bath until the temperature of middle part in the sample was reached at 70ºC, and cooled for 30 min in flowing water at approximately 20ºC. Warner-Bratzler shear force (WBsf) measurements were 1.27 cm circular core to determine sheared by cooked steaks (2.5 cm thick, cooked for determination of cooking loss) at 2 and 8 d of ageing. Eight cores were made for each sample, and peak force determined using an Instron (4465, Instron corp., UK) with a load cell 50 kg and head speed 200 mm/min.

Sarcomere length

Sarcomere length was measured on a thin block (1 mm) of frozen muscle sample, which was in the direction of the muscle fibres. The thin block was homogenized for 30 sec at low speed with glutaraldehyde, then fixed muscle fibres was drop on a microscope slide and covered using cover glass. Sarcomere length was measured using a Helium-Neon leaser (Lazerex Technologies PTY LTD., Australia) diffraction technique (Cross *et al*., 1980).

Myofibrilar Fragmentation Index

MFI was determined with 0.1 M potassium chloride, 1 mM EDTA, 1 mM sodium azid, 25 mM potassium phosphate buffer (pH 7.0) followed the method of Hopkins (2000) modified Culler (1978). Myofibrilar fragment suspension was concentrated using DC protein assay kit (BIO-RAD, USA) with Bovine Serum Albumin used for the standard curve. The suspension was diluted to 0.5± 0.05 mg/mL concentrations with potassium phosphate buffer, and then their absorbance was measured at 540 nm with a spectrophotometer (DU-800, Beckman, USA). It was multiplied the average of the triplicate absorbance by 150, and the result was considered Myofibrilar fragmentation index values.

Total Collagen and Heat Soluble Collagen contents

Total collagen was measured using colorimetric determination of followed Kurt (1990). Sample was hydrolysis with 7 N sulphuric acid, oxidation with Chloramine-T, and colour formation with 4-dimethylaminobenzaldehyde. It was read at 558 nm with a spectrophotometer (DU-800, Beckman Coulter, USA), and total collagen contents were calculated using hydroxyproline concentration (eight times of hydroxyproline contents). Heat soluble collagen contents was determined, followed a method of Silva (1999). Sample was homogenized with 0.1 M sodium phosphate buffer (pH 7.0), centrifuged at 10,000 rpm for 15 min, and then supernatant was collected and determined using the same colorimetric method with total collagen contents. Also soluble collagen was calculated with 7.5 times absorbance.

Fatty acid composition

Fatty acid was extracted and evaporated following Folch's (1957) method, and methylated with a method of Morri-

son and Smith (1964). Sample was analysed using gas chromatography (Varian 3600, Varian, USA). 1 μL methylated sample was injected to Silica capillary column (Supelco-omegawax 320, 30 m, 0.32 mm ID, 0.25 μm) and set 254ºC injection temperature, 250ºC detector temperature and 200ºC oven temperature, and carry gas was N_2.

Free Amino Acid

According to a method of Aristoy and Toldra (1991), free amino acid was extracted with 0.01 N HCl. 300 μL extracted sample was mixed with 10 μL internal standard (L-Citrulline) and 690 μL Acetonitril, and mixed sample was incubated for 30min at 4ºC, after then, centrifuged at 10,000×g for 15 min kept at 4ºC. The supernatant was filtered through 0.45 μL membrane filter. Filtered sample and external standard (amino acid standard: 0.25 nM, Agilent Technologies, USA; glutamine, Sigma) was analysed with OPA (O-phthalaldehyde) and FMOC (9-fluorenylmethyl chloroformate) derivatization using HPLC (Agilent, USA) of Herbert *et al.* (2000). Analysis condition was following a method (DAD detector, 262nm, 338 nm; Column, Zorbax Eclipse AAA, 4.6×60 mm, 5 μm; column temp, 40ºC; Mobile phase A, 40 mM sodium phosphate buffer, pH 7.8; Mobile phase B, Acetonitril: Methanol:Water, 45:45:10, v:v:v) of Henderson *et al*. (2000).

Sensory test

The muscle samples were trimmed to a block (40×50 mm) with parallel to the muscle fibre. Then the block was sliced into 5 mm thickness to be rectangular with the muscle fibre, and then arranged randomly. Sensory properties were evaluated for tenderness (Extremely tough=1, Extremely tender=8), juiciness (Extremely dry=1, Extremely juicy=8), flavour intensity (Extremely bland=1, Extremely intense=8) and overall acceptability (Extremely bad=1, Extremely good=8) using eight-point descriptive scales by eight panels trained professionally.

Statistical analysis

Variance analysis and signification verify of this experiment was analyzed by using PROC GLM of SAS (2000) system.

Results and Discussion

Table 1 showed a result Warner-Bratzler shear force (WBsf), sarcomere length, and MFI of meat from Hanwoo, Holstein and Angus. WBsf of Hanwoo, Holstein and Angus meats stored for day 7 were no significantly different on both of storage day 7 and 14 ($p>0.05$), however WBsf of Hanwoo meat was shown to tend to lower WBsf with storage duration. Also WBsf of Hanwoo and Holstein meat were stored for 14 d was determined under 4.0 kg that can make consumers a pleasing meat tenderness. We expected that the higher WBsf on Hanwoo meat than others due to various age of each breed from different growing characters with different age when they was slaughtered. Universally, Hanwoo steers are killed age over 27 mon as slow growing breeds, however Holstein and Angus steers, which are fast growing breeds, are slaughtered at about 18-20 mon age.

Table 1. Warner-Bratlzer shear force, sarcomere length and Myofibril Fragmentation Index of *M. longissimus lumborum* from Hanwoo*, Holstein*, and Angus steers stored at 4ºC for 7 and 14 d**

Items	Storage days	Hanwoo	Holstein	Angus	SEM***
Shear force (kg/0.5 inch^2)	7	4.53	4.39	3.62	0.42
	14	3.74	3.53	3.19	0.39
Sarcomere length (μm)	7	1.83	1.91	1.94	0.05
	14	1.81^b	1.93^a	1.98^a	0.02
Myofibrillar index	7	89.21^b	93.39^{ab}	101.91^a	2.69
	14	109.43^b	117.37^a	116.73^a	1.74

^{a,b}Means having different letters in the same row were significantly different ($p<0.05$).
*Hanwoo and *Holstein, Born and raised in Korea
**Angus, Imported from Australia and gained for 6 mon in Korea
***SEM, Standard error means

Sarcomere length of Hanwoo beef at 7 d storage was shorter than Holstein and Angus, and there was no significant difference. At storage day 14, sarcomere length of Hanwoo beef was significantly shorter than Holstein and Angus ($p<0.05$). This result was contrast with study of Kim (1999) that Hanwoo meat was shown longer sarcomere length than imported Australian beef. Myofibrillar index (MFI) was increasing with ageing (Warriss, 2010). Similarly, in our study showed that higher MFI longer storage day on all of three breeds. When compare MFI of three breeds on same storage days, MFI of Hanwoo beef stored for 7 d was determined to be significantly lower than Angus ($p<0.05$), although these meat was stored for 14 d, MFI of Hanwoo meat was lower than Holstein and Angus significantly ($p<0.05$). Culler *et al.* (1978) reported that MFI is a good indicator to distinguish meat tenderness on beef. In this result, Angus meat showed the highest MFI among three different breeds. Therefore, Angus was evaluated great tender meat than other two breeds,

Hanwoo and Holstein, meats. In contrast, Hanwoo meat was indicated lower tenderness than Holstein and Angus according to comparison of WBsf, sarcomere and MFI.

According to the study of McClain *et al*. (1970), tenderization of a meat closely concerned intramuscular connective tissue was decreased during storage, and Etherington (1987) reported meat tenderization was progressed due to weakening intramuscular connective tissue whilst a meat had storage duration, also collagen is major connective tissue among intramuscular connective tissue related meat tenderness. Collagen contents in intramuscular tissue had traits that animals were getting the more age, gradually getting the more toughness, firmness and unfixable on a meat of the animal (Jacques, 2008), also Bosselmann *et al.* (1995) suggested that there was strict relevance between collagen contents and meat tenderness. As studies of many authors, WBsf and tenderness related sensory trait was shown differences between different breeds or sex (Boles and Swan, 2002; Lee *et al*., 2009b; Mandell *et al*., 1997), whereas Chambaz *et al.* (2003) announced no significantly different shear force between breeds, which were Simmental, Charolaise and Limousin.

Fig. 1 showed total collagen contents and soluble collagen contents in Hanwoo, Holstein and Angus beef at ageing day 7 and 14. Total collagen contents of Hanwoo and Angus at day 7 were significantly higher than Holstein (p<0.05), and at day 14, Holstein beef was obtained slightly lower total collagen percentage on the meat, however it was no significant difference of them. Recently, many researchers have been arguing whether negative relationship or else not between collagen contents and tenderness on meat (Moon *et al*., 2003; Purslow, 2005), Bosselmann *et al.* (1995) asserted that collagen contents in a meat has very close relevance with meat tenderness, whereas Lee *et al* (2009b) presented that instrumental tenderness on beef with different meat quality grades (1++, 1and 2; Korean meat quality grading system) were measured clearly dissimilar, contrastively total collagen and soluble collagen contents of the meats were not any significant difference. Similarly our result was shown that WBsf and MFI related meat tenderness was not responded total collagen contents.

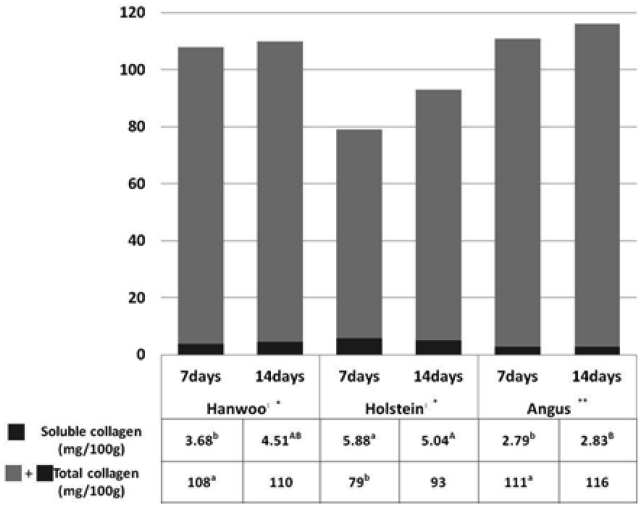

Fig. 1. Soluble and total collagen contents of *M. longissimus lumborum* from Hanwoo, Holstein and Angus steers stored at 4°C for 7 and 14 d. SEM of total collagen, 0.06; SEM of soluble collagen 0.45; [a,b]Means having different letters in the same row are significantly different on storage day 7 (p<0.05). [A-B]Means having different letters in the same row are significantly different on storage day 14 (p<0.05). *Hanwoo and *Holstein, Born and raised in Korea. **Angus, Imported from Australia and then gained for about 6 mon in Korea).

Soluble collagen contents in a meat at ageing day 7 of Holstein meat was significantly higher than Hanwoo and Angus (p<0.05), also at ageing day 14, Holstein meat soluble collagen contents was show significant different with Hanwoo and Angus (p<0.05). The result of this experiment was demonstrated less relationship between collagen contents and tenderness contrasted with a result reported by Hill (1966) that is the more soluble collagen amount, the more tender on beef, otherwise our result was coincided with that Pierson and Fox (1976) proved soluble collagen contents did not affect tenderness of a meat because of that collagen solubility is not changed by ageing. Although Sañudo *et al.* (2004) reported a result of comparison meat quality by live weight, breeds and meat aging on beef, total collagen amount was not significant difference by live weight, nevertheless breeds was a acknowledged to influence total collagen contents by their study.

Fatty acid composition had an important role to develop meat quality on beef (Wood *et al*., 2008). Fatty acid composition of LL muscle from Hanwoo, Holstein and Angus was shown in the Table 2. SFA (Saturated Fatty Acid), myristic acid, palmatic acid and stearic acid, SFA of Angus was tended to higher than Hanwoo and Holstein at storage day 7. As a report of Hwang *et al.* (2004), flavour score was decreased with steric acid contents on beef, steric acid of the three breeds was that Angus was slightly higher than Hanwoo and Holstein. However steric acid of LL muscle from Hanwoo and Holstein were similar between them. Palmitoleic acid, which was found in animal fat and compounded from oleic acid (De Smet *et al*., 2004), was no significant difference between Hanwoo, Holstein and Angus on each

Table 2. Fatty acid composition of *M. longissimus lumborum* from Hanwoo*, Holstein*, and Angus steers stored for day 7 at 4°C**

Fatty acids (%)	Hanwoo	Holstein	Angus	SEM***
Myristic acid	0.91[b]	3.26[a]	2.23[ab]	0.37
Palmitic acid	26.76	27.35	29.12	0.76
Palmitoleic acid	4.58	4.87	4.47	0.29
Stearic acid	11.67	11.73	12.24	0.60
Oleic acid	52.86	48.98	49.11	0.92
Vaccenic acid	0.21[b]	0.06[c]	0.27[a]	0.01
Linoleic acid	1.86[b]	2.77[a]	1.60[b]	0.10
γ-Linoleic acid	0.09	0.00	0.11	0.17
Linolenic acid	0.15[b]	0.20[a]	0.14[b]	0.01
Eicosenoic acid	0.47[a]	0.13[b]	0.41[a]	0.02
Eicosadienoic acid	0.08[b]	0.31[a]	0.05[b]	0.01
Eicosatrienoic acid	0.12	0.14	0.10	0.01
Arachidonic acid	0.26	0.29	0.19	0.03
SFA	39.34	42.34	43.60	1.00
USFA	60.67	57.67	56.41	1.00
MUFA/SFA	1.49	1.28	1.25	0.06
PUFA/SFA	0.07[b]	0.09[a]	0.05[c]	0.00

[a-c]Means having different letters in the same row are significantly different (p<0.05).
*Hanwoo and *Holstein, Born and raised in Korea
**Angus, Imported from Australia and raised for 6 months in Korea.

day 7 and 14. Oleic acid what is contained large amount of bovine adipose tissue, oleic acid contents in beef were various depended on breeds because of stearyl coenzyme A de-saturated gene activity related accumulation of oleic acid (De Smet *et al*., 2004). However in our study, oleic acid of Hanwoo beef was slightly higher than Holstein and Angus, but there was no significant difference. De Smet *et al*. (2004) studied that poly- and mono- saturated fatty acid ration was significantly different between grass feeding and grain feeding, especially oleic acid was greatly differed. On the contrast, in this study, there was not different on each saturated fatty acid contents between three breeds. Smith *et al.* (2009) presented that fatty acid composition of beef adipose tissue was depended by feeding, sex, breed, and age. The reason why the result showed no significant difference on fatty acid composition between the three breeds, the beef samples were collected from commercial market base. Previously numerous studies investigated differences of fatty acid composition between Hanwoo and imported beef (Cho *et al*., 2011; Hwang *et al*., 2004; Park and Yoo, 1994). However, as our result,

Table 3. Free amino acid contents of *M. longissimus lumborum* from Hanwoo*, Holstein*, and Angus steers stored for 7 d and 14 d at 4°C**

Free amino acids (%)	Day 7				Day 14			
	Hanwoo	Holstein	Angus	SEM***	Hanwoo	Holstein	Angus	SEM***
Aspartic acid	Traces	Traces	Traces	-	Traces	Traces	Traces	-
Glutamic acid	3.08[a]	1.26[b]	3.63[a]	0.33	4.04[a]	1.83[b]	4.24[a]	0.31
Serine	1.54[ab]	1.23[b]	1.84[a]	0.13	2.01[b]	1.84[b]	2.80[a]	0.13
Glutamine	14.67[b]	32.91[a]	10.58[b]	1.72	12.13[b]	30.56[a]	9.29[b]	0.32
Histidine	3.49[a]	1.76[b]	2.92[a]	0.21	3.16	2.39	2.80	0.19
Glycine	2.16[b]	1.72[c]	2.77[a]	0.12	2.21[b]	2.01[b]	2.77[a]	0.09
Threonine	2.12[a]	1.23[b]	2.25[a]	0.06	2.32[b]	1.89[c]	2.67[a]	0.04
Arginine	5.36[ab]	3.35[b]	6.46[a]	0.61	5.98[a]	4.06[b]	6.10[a]	0.36
Alanine	45.88	46.38	48.90	2.29	42.69	40.89	40.51	1.32
Tyrosine	2.54[a]	0.84[c]	1.68[b]	0.19	1.92[b]	1.63[b]	2.88[a]	0.17
Cystine	3.18	0.77	2.71	0.15	2.35[b]	0.87[c]	3.32[a]	0.12
Valine	1.88[a]	1.10[b]	2.23[a]	0.15	2.60[b]	1.81[c]	3.59[a]	0.13
Methionine	6.54[a]	2.36[b]	3.89[ab]	0.81	6.16[a]	1.19[b]	4.56[a]	0.51
Phenylalanine	1.84[a]	0.96[b]	1.59[a]	0.15	2.22[b]	1.80[b]	2.85[a]	0.20
Isoleucine	1.28	0.89	1.30	0.11	1.44[b]	1.42[b]	2.18[a]	0.11
Leucine	2.00[b]	1.61[b]	3.03[a]	0.24	3.03[b]	2.77[b]	4.38[a]	0.34
Lysine	3.08[a]	1.47[b]	3.62[a]	0.18	3.82[b]	2.45[c]	4.97[a]	0.14
Proline	1.15	0.76	1.99	0.30	1.95[a]	0.63[b]	2.46[a]	0.20
Umami[1)]	3.08[a]	1.26[b]	3.63[a]	0.24	4.04[a]	1.83[b]	4.24[a]	0.31
Sweet[2)]	51.69	50.55	55.75	2.22	49.22	46.64	48.74	1.07
Bitter[3)]	20.64[a]	12.03[b]	21.40[a]	1.22	24.57[a]	15.41[b]	25.76[a]	0.95
Others[4)]	24.60[b]	36.17[a]	19.21[b]	2.23	22.17[b]	36.12[a]	21.26[b]	1.52

[a-c]Means having different letters in the same row are significantly different at the same stored days (p<0.05).
*Hanwoo and *Holstein, Born and raised in Korea
**Angus, Imported from Australia and raised for 6 months in Korea
***SEM, Standard error mean
[1)]Umami, Aspartic acid and glutamic acid
[2)]Sweet, Threonine, serine, glycine and alanine
[3)]Bitter, Valine, methionine, isoleucine, leucine, phenylalanine, histidine and arginine
[4)]Others, Glutamine, tyrosine, cysteine, lysine and proline

when consumers purchase domestic beef, the domestic beef in the market had no differences of fatty acid composition.

Apart from nutritional aspect, free amino acid is very important non-volatile materials for meat taste and flavour (Gardner and Sterwart, 1966; Davey and Gilbert, 1966), free amino acids from heated meat improve meat flavour (Ockerman and Cresopo, 1982). Tseng *et al*. (2005) classified free amino acid in food to umami (aspartic acid, glutamic acid), sweet (serine, glycine, threonine, alanine), bitter (valine, methionine, isoleucine, leucine, phenylalanine, histidine, arginine), and others (Glutamine, tyrosine, systine, lysine and proline). In our study, glutamic acid, umami taste, of Hanwoo and Angus had significantly higher percentage than Holstein beef at storage day 7 and 14 ($p<0.05$). Angus beef had significantly higher percentage of serine, glycine, and threonine those belong to sweet taste group than Holstein at day 7. Hanwoo and Angus beef contained more bitter taste free amino acids than Holstein significantly ($p<0.05$) at day 7 and 14. Free amino acid contents were increased with acid lipase and protease that released from lysosome during ageing (Geromel and Montgomery, 1980), meat taste and flavour were affected these variations (Koutsidis *et al*., 2007; Koutsidis *et al*., 2008; Tseng *et al*., 2005). Feidt *et al.* (1996) reported that glycine, threonine, alanine, methionine, isoleucine, and leucine were increased with ageing period on longissimus dorsi muscle, in this study, similarly glycine, threonine, and leucine percentage at day 14 on all of three breeds was higher than day 7.

In general terms, the results of this study have showed that no distinctive differences found between Hanwoo, Holstein and Angus beef that were fattened for 6 months in market base. However this study was conducted on real market base in Korea, thus it can be consumed to reflect a condition for consumer-meat market. Moreover, this study has provided basic information about meat quality and precursor of taste of the three breed fed in Korea.

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