Poster exhibition parallel session 2: Protein oxidation

PE2.01 Meat tenderness and the calpastatin degradation of Thai native and crossbred cattle 54.00

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Abstract—**the effect of calpastatin protein degradation during ageing period of Thai native and crossbred cattle on beef tenderness were determined by Warner-Bratzler shear force (WBS) measurement and ELISA method. Three groups of cattle were used: 1) Thai native bulls (n = 10) or TN, 2) two steers and three heifers of Ponyangkham (2/8 Simmental x Brahman (B), Thai native (N)) or BxN (2/8 SBN) (n = 5) and 3) three steers and five heifers of Ponyangkham (5/8 Simmental x B, N) or BxN (5/8 SBN) (n = 8). After slaughtered, longissimus muscle samples were excised from carcasses at 6th-12th interface, cut into 5 sections and aged at 2-4^{o}C for 2, 7, 14, 21 or 30 d post-mortem. Shear force and calpastatin protein were measured at each ageing period. The result revealed that while post-mortem ageing time increased, the shear force value and calpastatin protein decreased (P<0.01). According to meat tenderness, 5/8 SBN was most tender even though calpastatin level was highest (P<0.01). There was no significant interaction between ageing period and breed cross effects for shear force and calpastatin degradation.**

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I. INTRODUCTION

CONSUMERS consider tenderness to be the single most important component of the meat quality. The majority of the tenderness problems are to improve the consistency of meat tenderness by post-mortem ageing and mechanical tenderization. The calcium-dependent proteolytic systems, calpains, are responsible for proteolysis of the myofibrilla proteins and meat tenderness. One of the primary factors that influence calpain activity in post-mortem muscle is calpastatin activity [3]. Calpastatin, endogenous inhibitors of the calpains, are a principal regulator of the calpains in post-mortem proteolysis [1]. Variation in calpastatin activity has been shown to be associated with the variation in the rate and extent of post-mortem proteolysis and the tenderization in beef. Post-rigor calpastatin activity, which inversely proportional to post-mortem tenderization, accounts for a greater proportion of the variation in tenderness of aged beef longissimus muscle (~40%) than any other single measure [8]. The decrease in tenderness associated with the *Bos indicus* breed seems to be highly related to the increase in the calpastatin activity. Furthermore, higher level of calpastatin activity has been associated with higher percentage of the Brahman beef breed [6][10].

Due to, most Thai normally consume grass fed Thai native cattle (which have high percentage of *Bos indicus*) more than finished steers crossbred and the important role of the calpastatin on meat tenderness, therefore, investigation of calpastatin protein and beef tenderness during ageing period of Thai native and crossbred cattle were determined.

II. MATERIALS AND METHODS

Three groups of cattle were used: 1) Thai native bulls (n = 10) or TN, 2) two steers and three heifers of Ponyangkham (2/8 Simmental x Brahman (B), Thai native (N)) or BxN (2/8 SBN) (n = 5) and 3) three steers and five heifers of Ponyangkham (5/8 Simmental x B, N) or BxN (5/8 SBN) (n = 8). The Thai native cattle were grazed in the field until 200-250 kg of live weight while Ponyangkham cattle were fed grass and concentrate finished for 12 to 18-mo until 600-700 kg

of live weight. Cattle were selected randomly and slaughtered. Longissimus muscle samples were excised from carcasses at 6^{th}-12^{th} interface, cut into 5 sections and aged at 2-4^{o}C for 2, 7, 14, 21 or 30 d post-mortem. At the end of each ageing period, two steaks were cut, one for Warner-Bratzler shear force (WBS) and another for determination of calpastatin protein. Calpastatin extraction was performed using the method of Geesink *et al*. [2]. Recombinant bovine calpastatin corresponding to domain I was expressed and purified as previously described by Tavitchasri *et al*. [9]. Polyclonal antibodies raised in rabbit against recombinant bovine skeletal muscle calpastatin were used to assay the degradation level of calpastatin protein by absorbance ELISA method. The data were analyzed by variance analysis using General Linear Models procedures of SAS [7] for completely randomized design of factorial experiments comparing of 3 cattle groups (TN, 2/8 SBN and 5/8 SBN), 4 periods of ageing (2, 7, 14 and 21 d) for shear force and 5 periods of ageing (2, 7, 14, 21 and 30 d) for calpastatin determination. Least squares means were statistically compared using the PDIFF option and considered significantly differ if $P<0.01$.

III. RESULTS AND DISCUSSION

Tenderness evaluation and calpastatin degradation of the longissimus muscle of cattle during ageing period are shown in Table 1 and Table 2. WBS values and calpastatin protein differed ($P<0.01$) among ageing periods. Post-mortem WBS values declined from 2d to 21d of ageing corresponded with decreasing of calpastatin protein. These results indicated that meat tenderness increased over ageing period. Moreover, these data shown that degree of tenderization and decreasing of calpastatin at d14 and d21 was non-significant different.

In this present study, breed groups had a highly significant ($P<0.01$) effect on shear force and calpastatin degradation. Tenderness was highest for 5/8 SBN, intermediate for 2/8 SBN, and lowest for TN, vice versa to calpastatin protein.

The confliction of results suggested that the 5/8 SBN crosses had higher beef tenderness despite higher values for calpastatin protein than the others. These could be because of 5/8 SBN crosses were mixed gender and fed with high concentrate finishing diet, resulted in higher marbling score. Whereas, TN were fed with grass only. Similar to O'Conner *et al*. [5] reported that heifer not only had higher marbling scores but also had higher values for calpastatin activity.

In addition, the 5/8 SBN or 2/8 SBN crosses were older than TN resulted in higher marbling scores and tenderness of beef. Corresponded with McBee and Wiles [4] reported that increased animal age was associated with decreased tenderness, and increased animal age was associated with higher marbling scores, while Wulf *et al*. [11] found that animal age was positively correlated with marbling scores but was not correlated with calpastatin activity.

Furthermore, we found no significant of the interaction between ageing period and breed cross effects for shear force and calpastatin degradation.

IV. CONCLUSION

Ageing improves tenderness of meat and decreases calpastatin in all groups. Degree of tenderization at d14 were similar to d21 therefore, extended beef ageing longer was unnecessary. The contradict result of beef from crossbred in more tender but higher in calpastatin protein, need to be further investigated for the influence of animal age, gender difference and production system.

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REFERENCES

[1] Doumit, M.E., S.M. Lonergan, J.R. Arbona, J. Killefer and M. Koohmaraie. (1996). Development of an enzyme-linked immunosorbant assay (ELISA) for quantitative of skeletal muscle calpastatin. J. Anim. Sci**.** 74: 2679-2686.

[2] Geesink G.H., J.G.P. van der Palen, M.P. Kent, E. Veiseth, G. Hemke and M. Koohmaraie. (2005). Quantification of calpastatin using an optical surface plasmon resonance biosensor. Meat Sci. 71: 537-541.

[3] Koohmaraie, M. (1990). Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. J. Anim. Sci. 68: 659-665.

[4] McBee, J. L., Jr., and J. A. Wiles. (1967). Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Anim. Sci. 26:701.

[5] O'Conner, S.F., J.D. Tatum, D.M. Wulf, R.D. Green and G.C. Smith. (1997). Genetic effects on beef tenderness in *Bos*

indicus composite and *Bos Taurus* cattle. J. Anim. Sci. 75: 1822-1830.

[6] Pringle, T.D., S.E. Williams, B.S. Lamb, D.D. Johnson and R.L. West. (1997). Carcass characteristics, the calpain proteinase system and aged tenderness of Angus and Brahman cross bred steers. J. Anim. Sci**.** 75: 2955-2961.

[7] SAS®. (2004). SAS User's Guide. Release 9.1. SAS Institute, Inc., North Carolina, USA.

[8] Shackelford, S.D., M. Koohmaraie, L.V. Cundiff, K.E. Gregory, G.A. Rohrer and J.W. Savell. (1994). Heritabilities and phenotypic and genetic correlations for bovine post-rigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. J. Anim. Sci**.** 72: 857-863.

[9] Tavitchasri, P., C. Kanthapanit, W. Wajjwalku and J. Sethakul. (2007). Production of polyclonal antibodies against calpastatin gene domain I from skeletal muscle of the Kamphaeng Saen beef breed, Kasetsart Journal Natural Science, 41: 493-501.

[10] Wheeler, T.L., J.W. Savell, H.R. Cross, D.K. Lunt and S.B. Smith. (1990). Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. J. Anim. Sci. 68:4206-4220.

[11] Wulf, D.M., J.B. Morgan, J.D. Tatum and G.C. Smith. (1996). Effects of animal age, marling score, calpastatin activity, subprimal cut, calcium injection, and degree of doneness on the palatability of steaks from Limousin steers. J. Anim. Sci. 74: 569-576.

Table 1. Least Squares Means (±SE) for Warner-Bratzler shear value.

Cattle	Ageing periods (day)				Means
	2	7	14	21	
TN	7.77±0.35	6.97±0.35	5.67±0.35	4.44±0.35	6.21±0.17[a]
2/8 SBN	7.20±0.50	6.02±0.50	5.06±0.50	4.87±0.50	5.79±0.25[a]
5/8 SBN	6.36±0.39	4.87±0.39	4.72±0.39	4.61±0.39	5.14±0.20[b]
Means	7.11±0.24[x]	5.95±0.24[y]	5.15±0.24[z]	4.64±0.24[z]	

[a,b]Means within a column without a common superscript letter differ (P<0.01).
[x,y,z]Means within a row without a common superscript letter differ (P<0.01).

Table 2. Least Squares Means (±SE) for $O.D._{650\ nm}$ of calpastatin protein.

Cattle	Ageing periods (day)					Means
	2	7	14	21	30	
TN	0.30±0.02	0.19±0.02	0.16±0.02	0.13±0.02	0.12±0.02	0.18±0.01[a]
2/8 SBN	0.36±0.03	0.24±0.03	0.22±0.03	0.20±0.03	0.17±0.03	0.24±0.01[b]
5/8 SBN	0.42±0.02	0.31±0.02	0.25±0.02	0.25±0.02	0.20±0.02	0.29±0.01[c]
Means	0.36±0.01[w]	0.25±0.01[x]	0.21±0.01[y]	0.19±0.01[yz]	0.16±0.01[z]	

[a,b,c]Means within a column without a common superscript letter differ (P<0.01).
[w,x,y,z]Means within a row without a common superscript letter differ (P<0.01).