EFFECT OF SLAUGHTERING PROCESS ON MUSCULAR PROTEOLYTIC ENZYME, MEAT QUALITY AND TENDERNESS DURING PRE-RIGOR MORTIS STAGE

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Abstract – This paper aimed to examine changes in various factors involving the slaughtering process on meat quality, tenderness and muscular proteolytic enzymes during pre-rigor mortis stage. Ninety barrows with similar genetic line and similar feed composition were raised separately in 2 farms located in Nakhon Ratchasima (60 pigs, groups A and C of 30 pigs each) and Khon Kaen (30 pigs in group B). At commercial live weight, all 90 pigs were transferred and processed at 2 abattoirs in Khon Kaen (groups A and B) and Lopburi (group C) which had different slaughtering facility. The results showed that serum cortisol of pigs in group A were significantly higher than other two groups. This was clearly defined that distance of transportation and slaughtering method had influence on hormone that was responsive to stress. The slaughtering process of large abattoir affected directly on the meat quality i.e. lower drip loss and slower decrease in muscular pH at different interval. Moreover, the shear-force of muscle was also superior in group C to group A, although these pigs came from the same farm. The higher levels of proteolytic enzymes i.e. calpain and cathepsin in muscle of group C pig may be a candidate bioindicator for meat tenderness as it had correlation with lower shear-force.

Key Words – Proteolytic enzyme, Cortisol, Meat tenderness

I. INTRODUCTION

Pig production in Thailand is still growing to serve higher consumption demand in various sectors i.e. household, food services, modern trade market, butcher shop etc. as well as local manufacturer who produce further pork products e.g. sausage, sour pork and dried shredded pork etc. Quality and tenderness are required differently among these end-user groups. There are many factors affecting the quality of pork meat from genetic, feed, transportation and slaughtering procedures. The biochemical changes of pork meat during pre-rigor mortis within the first 24 hr. after slaughtering is of interest especially the level of proteolytic enzymes, calpain and cathepsin [1]. There are few studies investigating the effect of changes in pork meat during pre-rigor mortis stage that may influence the quality and tenderness of meat before delivering to the end-users. The objective of this study was to examine various factors involving slaughtering processes on meat quality, tenderness and muscular proteolytic enzymes during pre-rigor mortis stage.

II. MATERIALS AND METHODS

The study was performed using ninety commercial crossbred barrows with similar genetic line. Sixties were raised in Nakhon Ratchasima (NR, Farm 1) and other thirties were raised in Khon Kaen (KK, Farm2). Feed used in both farms was similar in nutritional specification at all stages of production. When the body weight of pigs reached 110±10 kg, 30 pigs from Farm 1 were transported to the large abattoir (processed 2,000 pigs/day) in Lopburi (group C) while other 30 pigs were sent to medium abattoir (600 pigs/day) in Khon Kaen (group A) (Figure 1). The distance from Farm 1 to large and medium abattoirs was equal (approx. 200 km). Pigs in Farm 2 (group B) were sent to the same slaughter plants as group A on the same date and time. The pre-slaughter procedures were complied with both plants. The stunning method in medium abattoir was conventional electrical stunning and large abattoir was auto electrical stunning. Serum cortisol concentrations were analyzed from blood drained at the bleeding points using ELISA technique.

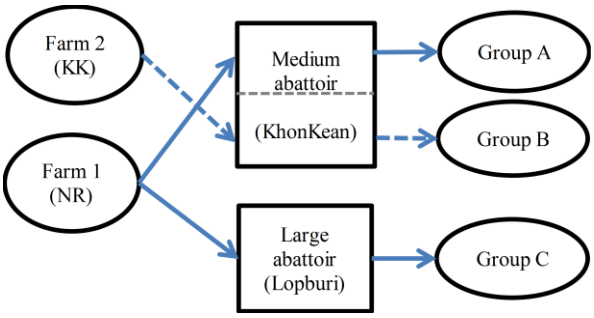

Figure 1 Source of experimental pig and slaughtering plants, distance from farm 1 to Medium and large abattoirs were approximately 200 km while Farm 2 to Medium abattoir was approximately 60 km.

The carcass was processed and kept in chiller according to the plant's regulation. The pH was measured at *Longissimus dorsi* on 1st, 6th and 22nd hr after bleeding. Samples of *Longissimus dorsi* (10-15 g) were collected and kept frozen in liquid nitrogen and moved to deep refrigerator (–80 °C) until analysis of proteolytic enzymes. Calpain and cathepsin in muscle were analyzed using ELISA kits (Porcine calpain 1 ELISA kits (Cloud-Clone Corp. China) and Porcine cathepsin ELISA kits (Wuhan, China). Drip loss was determined in muscle samples kept overnight in a refrigerator at 4°C. Shear force was determined in pork meat using Warner-Brazler shear force measurement. Data were analyzed using one-way ANOVA with significant difference set at $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 summarized parameters measurement in various groups of the trial. The body weights of all pigs were not different (110 kg). The pH of muscle in group A and B dropped to approximately 6.0 within 6 hr after slaughter while pH of muscle in group C slightly decreased. The drop of pH was due to the production of lactic acid and the rate of pH decline to more acidic may cease the energy metabolism and decrease proteolytic enzymes activity in the meat [2]. Both pH of muscle in groups A and B at 6 and 22 hr were significantly lower than group C ($P<0.05$). The different in slaughtering process in both abattoirs influenced the pH of meat. The changes in pH of meat were similar to change in drip loss which meat in group C had the lowest drip loss and this may be relevant to the higher pH at 22 hr. The lower drip loss in group C compared to groups A & B was also relevant to the significantly lower shear force, indicating the more tenderness of meat. The serum cortisol concentrations of pigs in group B were lower ($P< 0.05$) than group A and were not different ($P< 0.05$) from group C. This may be resulted from slaughtering process not only the distance of transportation and rearing pen in lairage prior to abattoirs but also the different stunning process. Previous work demonstrated that at the beginning of transportation to the abattoir the cortisol rose immediately and decreased during lairage but still higher than pre journey period [3]. However, the highest values of cortisol were found at the time of slaughter [4]. In this study, the cortisol level was in accordance with Hambrecht *et. al*. (2004) [5] that pigs slaughtered with auto electrical stunning had lower cortisol level than conventional stunning. Moreover, in the medium abattoir (Khon Kean), the lower stressed pig (by short distance transport) had equal level of cortisol as in the large abattoir (Lopburi) which was transported from longer distance.

Table 1 Parameters measured in different groups of the experiment.

	Group A	Group B	Group C
Live weights (kg)	110.5 ± 7.4[a]	104.5 ± 3.8[a]	110.1 ± 6.5[a]
pH 1 hr	6.38 ± 0.21[a]	6.31 ± 0.14[a]	6.64 ± 0.21[a]
pH 6 hr	6.03 ± 0.19[b]	6.01 ± 0.20[b]	6.40 ± 0.17[a]
pH 22 hr	5.63 ± 0.14[b]	5.52 ± 0.08[b]	6.04 ± 0.12[a]
% Drip Loss	12.58 ± 3.85[a]	11.37 ± 3.19[a]	7.28 ± 0.71[b]
Serum cortisol(µg/dL)	9.48 ± 0.53[a]	6.59 ± 0.54[b]	6.61 ± 0.40[b]
Shear force (Newton)	25.76 ± 1.08[a]	27.40 ± 1.04[a]	21.45 ± 0.93[b]

[a b] Mean ± SE, Means in the same row with different superscripts are significantly different ($P<0.05$).

The proteolytic enzymes, calpain and cathepsin were measured at various times as shown in Table 2. The calpain levels (ng/mg protein) were higher than cathepsin at each time. The calpain levels (1 hr) were significantly lower in group B and C compared to group A. Meat in group C also contained lower calpain at 6 hr but increased to higher levels than other groups at 22 hr. Dransfield (1994) [6] discussed that calpain 1 was Ca^{2+} dependent protease enzyme responsible

for myofibrillar degradation during post-mortem ageing and its activity was decreased overtime after slaughter. The lower pH in both groups A and B may influence on the low activity of calpain in this study. Similar changes were found in the other protease, cathepsin, which decreased when time passed. In addition, cathepsin concentrations in group C started to markedly increase in 6 hr compared to other groups. The sustained bioindicator concentrations in the period of pre-rigor mortis may relate to the lower shear force indicating the tenderness of meat.

Table 2 Muscular concentrations of enzyme calpain and cathepsin in various groups at different times after slaughter.

	Group A	Group B	Group C
Calpain (ng/mg protein)			
1 hr	100.87±12.22[a]	48.31±4.29[b]	27.10±2.45[c]
6 hr	11.20±0.94[a]	11.74±1.35[a]	8.44±1.29[b]
22 hr	7.32±0.58[ab]	5.75±0.77[b]	16.19±3.27[a]
Cathepsin (ng/mg protein)			
1 hr	0.648±0.119[a]	0.641±0.099[a]	0.307±0.054[b]
6 hr	0.112±0.027[b]	0.094±0.027[b]	3.957±0.969[a]
22 hr	0.129±0.021[b]	0.135±0.082[b]	0.281±0.026[a]

[a b c] Mean ± SE, Means in the same row with different superscripts are significantly different ($P<0.05$).

IV. CONCLUSION

In conclusion, the result of this study showed that the stress hormone, cortisol, may be influenced by the slaughtering handling for instances the distance of transportation and stunning method. Higher stress reactivity was detected in a long distance transportation and conventional electrical stunning (group A). The rise of proteolytic enzymes, calpain and cathepsin, at 6 and 22 hr may be used as candidate bioindicator since they were related with the lower shear force, indicating the tenderness of the pork meat.

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