THE CORRELATION OF SLIGHT, MEDIUM AND EXTREME RIGOR MORTIS CARCASS TO HALOTHANE GENOTYPE, SARCOPLASMIC CALCIUM AND PROTEIN FUNCTIONAL CHARACTERISTICS IN ABATTOIR SLAUGHTERED PIGS

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Background

Halothane gene (HAL gene) is a genetic defect that can be triggered by stress during hog's handling treatments . The clinical symptoms of Halothane gene are gross muscular rigidity, high temperature, and arrhythmia. After the exsanguination of the animals, the blood circulation stops, and consequently, the hogs muscle couldn't obtain energy by respiration. Then, the energy is depleted which results in the development of carcass's rigor mortis. Glycogen transfers to ATP as an energy resource during rigor mortis and consequently affects the rigor development, because of the accelerated rigor mortis caused by the lack of calcium ion recovery activity during rigor mortis. After the hogs have been hanged and have been dressed, shaking hand method (Swatland, 1995) might give information about softening and tendering of the meat in which softening and tendering are in relation to muscle protein functional characteristics.
There have been shown three kinds of rigor mortis degrees (extreme, medium and slight) in abattoir slaughtered pigs in Taiwan. The different kinds of developed rigor mortis carcasses are measured by shaking hand method in early abattoir slaughtered pigs. The sarcoplasmic calcium concentration and glycolysis may be involved in extreme, medium and slight rigor mortis carcasses. The sarcoplasmic calcium concentration and glycolysis at postmortem carcasses play important roles in the formation of normal or pale, soft and exudative meat. The rate of glycolysis is controlled by sarcoplasmic calcuim levels; the carcass's rigor mortis might be affected by sarcoplasmic calcium concentration and rate of glycolysis. Rapid glycolysis within the first hour post mortem results in a low muscle pH value and high body temperature, leading to denaturation of muscle protein and development of inferior meat quality.

Objectives

The objective of this study was to determine the correlation of slight, medium and extreme rigor mortis carcass to halothane genotype, sarcoplasmic calcium and protein functional characteristics in abattoir slaughtered pigs.

Materials and methods

Materials

The live weight of the hogs were approximately 100-110 kg. They were slaughtered at local abattoir and stunned electrically. Within 45 min after exsanguination, the three groups of rigor mortis degree carcasses (slight, medium and extreme) were determined by tension load tester (Fig. 1). Longissimus dorsi (LD, from 4th to 5th rib) samples were subsequently excised within 45 min and 24 h after post mortem from 10 carcasses of three groups of rigor mortis degree, respectively, and immediately stored at -70℃ until analysis.

Methods

Halothane genotype identified

Halothane genotype of three kinds of rigor mortis degree carcasses were identified by mutagenically separated polymerase chain reaction (MS-PCR) (Liaw *et al.*, 2000).

Measurements of sarcoplasmic calcium concentration and the products of glycolytic metabolic response

Sarcoplasmic Ca^{2+} concentration in LD muscle was determined by using 10 g sample in 10ml of 150mM KCl (Cheach *et al.*, 1984). The Ca^{2+} was analyzed by an atomic absorption spectrophotometer at 422.7 nm. The concentration of glycogen was analyzed by a method of Hartschun *et al.* (2002), and glucose, glucose-6-phosphate, adenosine triphosphate（ATP), and creatine phosphate（CP）concentration were analyzed by a enzyme analyze method (Yang，1993). The R value was analyzed by a method of Thompson *et al.* (1987).

Measurements of protein functional characteristics

The pH, lightness value, protein solubility, myofibrillar ATPase activity, water-holding capacity(WHC), and myofibril fragmentation index (MFI) were determined at 45 min and 24 h post mortem, respectively. The protein solubility and WHC were measured by Joo *et al.* (1999). The assayed method of myofibrillar ATPase activity was modified from Lin *et al*. (1999). MFI was determined by Hopkins and Thompson (2002).

Statistical analysis

The data from the three kinds of rigor mortis degree carcasses were compared by analysis of variance (ANOVA) using the General Linear Model (GLM) of SAS（1988).

Results and discussion

The results showed that there was one heterozygote in the samples of extreme rigor and medium rigor mortis carcasses; however, the Halothane gene did not exist in the samples of the slight rigor mortis carcasses. Within 45 min of post mortem, both sarcoplasmic Ca^{2+} and R value in the extreme rigor mortis carcasses were significantly higher than those in the others ($p<0.05$); on the other hand, the adenosine triphosphate (ATP) and the creatine phosphate (CP) content in the extreme rigor mortis carcasses were lower than those in the slight rigor mortis carcasses ($p<0.05$), and the sarcoplasmic Ca^{2+} concentration did not have difference among the three groups at 24 h of post mortem. The results were similar to Cheah *et al.*(1984).

The increased level of sarcoplasmic Ca^{2+} in extreme rigor mortis carcasses was due to a disturbed regulation of calcium release channel in sarcoplasmic reticulum, and Ca^{2+} was fast releasing in sarcoplasmic reticulum. Cheah *et al*.(1986) indicated that Ca^{2+} release of sarcoplasmic reticulum might be caused by the mechamism of Ca^{2+}-induced Ca^{2+} released. Monin *et al*.(1980) indicated that the rigor mortis score was higher in Halothane positive pigs at 1 hour post mortem, for explaining that the extreme rigor mortis might to have a mutational Halothane gene. The faster rate of glycolysis in extreme rigor mortis carcasses is caused by the higher sarcoplasmic Ca^{2+} concentration they have. The rate of glycolysis was controlled by Ca^{2+} concentration which stimulated the degradation of glycogen. This study showed that the extreme rigor mortis carcasses were higher in free sarcoplasmic Ca^{2+} concentration and the ATP as well as the CP content to be lower. The results also indicated that the rate of glycolysis was faster within 45 min post mortem due to the extreme rigor mortis carcasses which had a higher Ca^{2+} concentration in sarcoplasmic.

Additionally, the solubility of sarcoplasmic protein, total protein, myofibrillar ATPase activity and WHC in the extreme rigor mortis carcasses were lower than those in the slight rigor mortis carcasses ($p<0.05$), but the extreme rigor mortis carcasses had significantly higher lightness value, drip loss and MFI than the other rigor mortis carcasses ($p<0.05$). Protein denaturation was quantified by myofibrillar ATPase activity and protein solubility. Within 45 min post mortem the extreme rigor mortis carcasses had a faster rate of glycolysis and it would result in lactate accumulation, pH value decrease and protein denaturation in muscle. Our study showed that extreme rigor carcasses had lower protein solubility and ATPase activity, in which it supported that extreme rigor carcasses had lower protein function characteristics than the others.

Conclusions

The results showed that although the relationship between hog carcasses and halothane genotypes did not reach to a significant level, extreme rigor mortis carcass had significantly higher sarcoplasmic Ca^{2+} level and caused rapid glycolysis and decreased pH value in pork muscle. Thus, the meat qualities were seriously affected.

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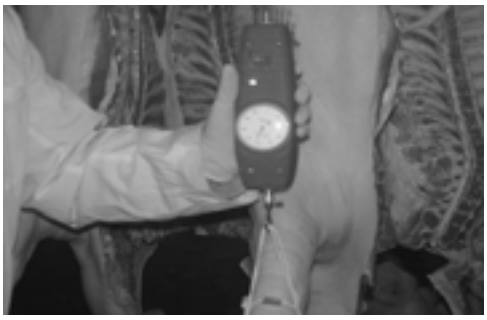

Fig. 1. The degree of rigor mortis carcass was measured by tension load tester.

Table 1. Sarcoplasmic Ca^{2+}, glycolytic metablic response concentration and R value in LD muscle at 45 min post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
sarcoplasmic Ca^{2+} (μg/g)	12.41±1.45^{a}	12.35±1.88^{a}	8.75±2.34^{b}
Glycogen(mg/g)	109.58±11.28	110.27±11.90	111.83±11.44
Glucose (mg/g)	7.293±2.41	6.690±2.24	8.111±3.50
G-6-p (mg/g)	0.1213±0.044^{a}	0.0549±0.013^{b}	0.0363±0.020^{b}
ATP (μmol/g)	0.1368±0.039^{b}	0.1673±0.031^{a}	0.1717±0.025^{a}
CP (μmol /g)	4.126±1.41^{b}	6.591±2.7^{a}	6.206±2.28^{a}
R value	1.2943±0.035^{a}	1.2862±0.027^{ab}	1.2687±0.017^{b}

Different superscripts(a, b)indicate significant differences between means.($p<0.05$)

Table 2. Protein solubility, pH_1, WHC, MFI, myofibrilar ATPase activity, lightness in LD muscle at 45 min post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
pH_1	6.19±0.33^{b}	6.37±0.10^{a}	6.43±0.06^{a}
protein solubility(mg/g)			
sarcoplasmic protein(mg/g)	71.47±7.03	74.39±9.07	74.06±6.14
total protein (mg/g)	212.94±23.12	220.29±15.57	216.36±11.75
WHC(%)	69.66±1.95^{b}	71.57±0.78^{a}	71.71±1.22^{a}
MFI	0.138±0.012^{a}	0.116±0.008^{b}	0.115±0.008^{b}
myofibrillar ATPase activity (μmol/min/mg protein)	0.2958±0.077^{c}	0.3680±0.064^{b}	0.4286±0.099^{a}
lightness value	34.36±0.97^{a}	33.17±1.73^{b}	32.96±0.98^{b}

Different superscripts(a, b, c)indicate significant differences between means.($p<0.05$)

Table 3. Sarcoplasmic Ca^{2+}, pHu, protein solubility, WHC, drip loss, myofibril fragmentation index, myofibrilar ATPase activity and lightness in LD muscle at 24 h post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
pHu	5.84±0.20^{a}	6.10 ±0.21^{b}	6.11±0.36^{b}
sarcoplasmic Ca^{2+} (μ g/g)	8.25±1.74	8.83±2.90	7.81±1.38
protein solubility			
Sarcoplasmic protein (mg/g)	64.64±1.74^{b}	67.27±2.90^{ab}	70.73±1.38^{a}
total protein (mg/g)	169.70±15.87^{b}	174.39±10.70^{ab}	181.27±13.04^{a}
WHC(%)	66.17 ±0.82	68.37 ±0.90	68.93 ±1.06
drip loss	3.16±0.83^{a}	1.78±0.35^{b}	1.21±0.19^{b}
MFI	0.187 ±0.010^{a}	0.166 ±0.008^{b}	0.145 ±0.004^{c}
myofibrillar ATPase activity(μ mol/min/mg protein)	0.281±0.11^{b}	0.362±0.08^{ab}	0.425±0.139^{a}
lightness value	42.75 ±1.54^{a}	40.73 ±0.87^{b}	37.79 ±2.04^{c}

Different superscripts(a, b, c)indicate significant differences between means.($p<0.05$)