POSTMORTEM CALPAIN AND DESMIN CHANGES IN EXCISED LANYU PIG *LONGISSIMUS* MUSCLE

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Abstract – The objective of this study was to examine the postmortem calpain and desmin changes in excised *longissimus* (LM) muscles of Lanyu pigs. The LM muscle was removed from the carcasses after 30 min postmortem, cut into seven equal portions, vacuum-packed individually, and stored at 5 ℃ for 0- (30 min postmortem), 3-, 6-, 12-, 24-, 48- and 72-h. At the end of each storage period, muscle was sampled and analyzed the pH value, calpain activity, the contents of 80 kDa μ-calpain subunit and desmin. The results showed that the ultimate pH was reached by 24-h postmortem. While a mild reduction in m-calpain activity was found, μ-calpain activity decreased rapidly during the entire 72-h postmortem storage period. Western blot showed that the decrease in the contents of μ-calpain 80 kDa subunit and desmin was parallel to the reduction of μ-calpain activity. Therefore, these results confirmed that μ-calpain activation and autolysis may explain the variation of desmin degradation in postmortem muscle.

Key Words –Porcine muscle, Postmortem proteolysis, Calpain.

I. INTRODUCTION

The Lanyu pig is one of the native miniature breeds in Taiwan, which existed on Orchid islands, also called Lanyu in Chinese. The characteristics of the Lanyu pigs are small in size with small erect ears and black coat color [1]. The pigs grow slowly [2] but have an excellent ability with heat tolerance and disease resistant compared to those commercial breeds [3]. Lanyu pig also has a potential to meet the demand of the profitability of porcine production [3, 4, 5, 6]. However, the information regarding the postmortem calpain changes in Lanyu pig muscle is very limited. The purpose of this study, therefore, was to investigate the postmortem calpain changes in excised *longissimus* muscles of Lanyu pigs.

II. MATERIALS AND METHODS

One side of the *longissimus* (LM) muscle, which was excised from 5/13th rib in approximately 30 min postmortem, of Lanyu pigs (n = 6, gilt, 12 months old with 60-70 kg of live weight) was purchased in a government-regulated abattoir. The LM muscle from each pig was divided into 7 equal sections, vacuum-packed individually and stored at 5℃ for 0- (approximately 30 min postmortem), 3-, 6-, 12-, 24-, 48-, and 72-h. At the end of each storage period, muscle was sampled, finely cut with scalpel, snap-frozen in liquid nitrogen and stored at -80 ℃ for subsequent analysis. After crushing in liquid nitrogen, two grams of each sample were used for the pH measurement of Farouk & Swan [7]. The protocol used for calpains extraction was done by the method of Veiseth, *et al*. [8]. The protein concentration of the supernatant was determined [9] after centrifugation. The procedure used for casein zymography was performed by the method of Chang *et al*. [10]. Samples for μ-calpain Western Blot were based on the method of Helman *et al*. [11]. Myofibrils were isolated at 2 ℃ by using differential centrifugation according to the method of Goll *et al*. [12] with the modifications by Huff-Lonergan *et al*. [13]. The SDS-PAGE was done with 10% tris-glycine slab gels. Proteins were transferred from the gel to a nitrocellulose membrane. A monoclonal antibody to desmin or to μ-calpain was used as a primary antibody. The image analysis was performed by the method of Chang *et al*. [10]. Each blot and gel included a pooled 0-h LM sample as a reference standard to normalize the band intensities. All results were analyzed using the Mixed model procedure of SAS (PROC Mixed). A Tukey's test was used to separate multiple means at a 5% significant level.

III. RESULTS AND DISCUSSION

Previous studies have shown that the ultimate pH is an important factor for affecting the quality of meat attributes such as meat color [14], drip loss [15] and water-holding capacity [16]. In Lanyu pig LM muscle (Fig. 1), the mean pH of 0-h samples was 6.50±0.16, and the pH decreased ($P < 0.05$) rapidly to 6.38±0.11, 6.23±0.13 and 5.90±0.19 in 3-h, 6-h and 24-h samples, respectively. However, the pH remained unchanged in 24-h, 48-h (5.90±0.18) and 72-h (5.91±0.18) postmortem samples. These results suggested that the ultimate pH of Lanyu pig LM muscle was approximately 5.9 after 24-h postmortem, consistent with the findings of Bidner et al. [17].

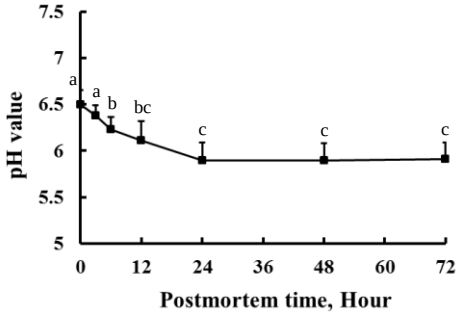

Figure 1. Postmortem changes in pH of Lanyu pig *longissimus* muscle stored at 5 ℃. Vertical bars show the standard deviation of the means. Means in each sampling time labelled with different letters (a-c) are significantly different ($P < 0.05$).

Casein zymography is a sensitive method to detect calpain activity [9]. In good agreement with the previous studies [18, 19], results (Fig. 2) shows that the upper and the bottom rows of bands were μ- and m-calpain bands in the presence of 4 mM Ca^{2+}, respectively. The results indicated that μ-calpain and m-calpain bands in Lanyu pig LM samples decreased as postmortem time proceeded (Fig. 2). While the m-calpain band remained clearly visible at the 72-h postmortem samples, the μ-calpain band nearly disappeared.

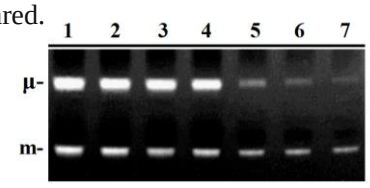

Figure 2. Casein gel showing postmortem changes in μ- and m-calpain activity of Lanyu pig *longissimus* muscle stored at 5°C. μ = μ-calpain; m = m-calpain. Lane 1 = 0-h; lane 2 = 3-h; lane 3 = 6-h; lane 4 = 12-h; lane 5 = 24-h; lane 6 = 48-h; lane 7 = 72-h.

Image analysis results (Fig. 3) shows that the μ-calpain activity in the 0-h postmortem LM samples, which was taken as 100%, changed insignificantly to 95±11% in 3-h samples. But the level of the μ-calpain activity reduced ($P < 0.05$) to 81± 9%, 60±6% and 28±12% in 6-h, 12-h and 24-h postmortem samples respectively. Further significant decrease of the activity was not found among 24-h, 48-h (15±5%) and 72-h (10±3%) postmortem samples. This implied that μ-calpain activation and autolysis in Lanyu pig LM muscle was extensive between 6-h and 24-h postmortem.

However, the m-calpain activity in the 0-h postmortem LM samples, which was taken as 100%, changed insignificantly among 0-h, 3-h (92±8%) and 6-h (78±11%) postmortem samples (Fig. 3). Although the m-calpain activity was not different between 6-h and 12-h postmortem samples, the activity in 12-h samples (78±14%) is lower than that in 0-h and 3-h samples. Further reduction in m-calpain activity was not observed among 12-h, 24-h (77±14%), 48-h (64±19%), and 72-h (58±12%) samples. The results indicated that a mild reduction of m-calpain activity during 72-h postmortem storage period was found in Lanyu pig LM muscle, similar to the findings in porcine [18] and bovine [21] muscle. Although Pomponio *et al*. [18] observed the appearance of autolyzed m-calpain activity in postmortem porcine muscle by 72-h postmortem on casein gels, the activity was not found in present studies. Additionally, Camou *et al*. [21] also did not observed the autolyzed m-calpain activity in postmortem bovnie muscle.

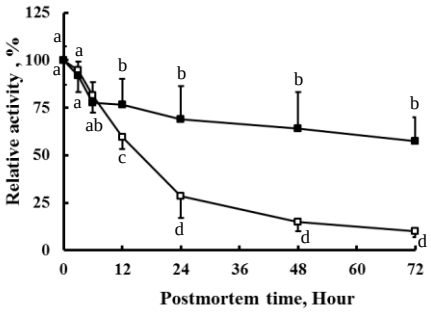

Figure 3. Postmortem changes in relative μ- and m-calpain activity of Lanyu pig *longissimus* muscle stored at 5°C. The activity of the μ- and m-calpain are expressed as percentage of the 0-h samples, which is taken as 100%, respectively. □: μ-calpain; ■: m-calpain. Vertical bars show the standard deviation of the means. Means in each sampling time within a calpain labelled with different letters (a-d) are significantly different ($P < 0.05$).

Recent studies [20] in postmortem bovine muscle showed that the content of 80 kDa μ-calpain subunit was autolyzed first to a 78 kDa intermediate product, from which NH_2-terminal 14 amino acids of the 80 kDa subunit were removed [21, 22], and completely autolyzed to produce a 76 kDa form, from which an additional NH_2-terminal 12 amino acids were removed [21, 22]. Camou *et al*. [21] concluded that the 76/28 kDa μ-calpain was proteolytically inactive and that this accounted for the loss of μ-calpain activity during postmortem storage. As shown in Figure 4, the content of 80 kDa μ-calpain subunit in 0-h (100±6%) and 3-h (90±9%) samples was not different, but the content decreased significantly ($P < 0.05$) to 79±6%, 63±12%, 32±7% and 10±9%, in 6-h 12-h, 24-h, 48-h postmortem samples, respectively. Further reduction in the content of 80 kDa μ-calpain subunit was not found between 48-h and 72-h (6±5%) samples. On the other hand, the 78 and 76 kDa degradation products were increased significantly ($P < 0.05$) from 0-h to 72-h samples (data not shown). The results indicated that the decrease in content of 80 kDa μ-calpain subunit in Lanyu pig LM muscle significantly occurred after 6-h postmortem with time postmortem, consistent with the loss of μ-calpain activity (Fig. 3).

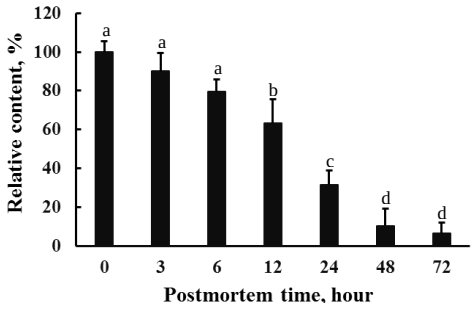

Figure 4. Postmortem changes in relative content of 80 kDa μ-calpain subunit of Lanyu pig *longissimus* muscle stored at 5℃. Vertical bars show the standard deviation of the means. Means in each sampling time labelled with different letters (a-e) are significantly different ($P < 0.05$).

Figure 5 shows that the desmin content in the 0-h postmortem LM samples, which was taken as 100%, changed insignificantly to 97±7% in 3-h samples and to 92±6% in 6-h samples. But the desmin content reduced ($P < 0.05$) to 78±4%, 70±4% and 68±4% in 12-h, 24-h and 48-h postmortem samples, respectively. Further decrease in the desmin content was observed in 72-h (55±5%) postmortem samples. These results indicated that significant desmin degradation occurred after 12-h postmortem in Lanyu pig LM muscle, parallel the decrease in μ-calpain activity (Fig. 3) and in the content of 80 kDa μ-calpain subunit (Fig. 4). Accordingly, these results were consistent with the previous studies [23].

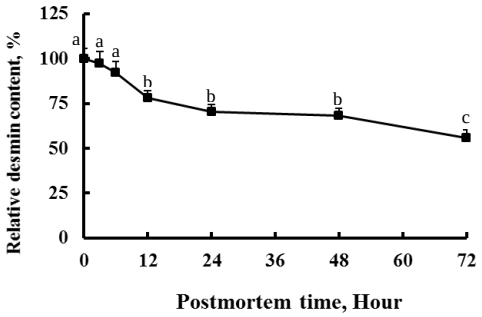

Figure 5. Postmortem changes in relative desmin content of Lanyu pig *longissimus* muscle stored at 5℃. Vertical bars show the standard deviation of the means. Means in each sampling time labelled with different letters (a-c) are significantly different ($P < 0.05$).

IV. CONCLUSION

The present results showed that while a mild decrease in the m-calpain activity was observed, the μ-calpain activity reduced very rapidly during 72-h postmortem storage period in Lanyu pig LM muscle stored at 5℃. The loss of μ-calpain activity and content of 80 kDa μ-calpain subunit was associated with the decrease in desmin content found in postmortem Lanyu pig LM samples. Therefore, these results confirmed that μ-calpain activation and autolysis may explain the variation of desmin degradation in postmortem muscle.

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