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# Postmortem calpain changes in ostrich skeletal muscle

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ABSTRACT

The objective of this study was to study the postmortem calpain change in ostrich muscle. *Iliotibialis cranialis* and *Obturatorius medialis* muscles were removed from the both sides of carcasses (n = 8). The muscles from the left side were sampled after 0, 1, 2, 3, and 7 days of storage at 5 °C, while the right-side muscles were taken at 1-, 3-, and 7-day postmortem for shear force measurements. The results showed that the calpain-1 activity was not detected in ostrich muscle during the entire 7-day postmortem storage period, while the calpain-11 was. The unautolyzed calpain-11 activity decreased and the autolyzed calpain-11 activity increased with time postmortem. Desmin content and shear force did not change during postmortem storage although a minor degradation of desmin was observed. Therefore, our results suggest that limited postmortem proteolysis (as suggested by the limited degradation of desmin) and tenderization might be due to the lack of calpain-1 and/or insufficient calpain-11 activity present in ostrich muscle.



## 1. Introduction

It has been suggested that postmortem tenderization is as a result of degradation of myofibrillar/cytoskeletal proteins by endogenous proteases present in skeletal muscle (Huff-Lonergan, Zhang, & Lonergan, 2010). Although this proteolysis may involve a multi-enzymatic process (Ouali et al., 2006), considerable evidence has shown that the calpain proteases may be responsible for postmortem proteolysis and tenderization (Koohmaraie & Geesink, 2006).

It has been reported that two ubiquitous calpain isoforms, calpain-1 (μ-calpain) and calpain-11 (the nomenclature for μ/m-calpain as reported by Macqueen, Delbridge, Manthri, and Johnston (2010) and Sorimachi, Hata, and Ono (2011)), are present in avian skeletal muscle (Sorimachi et al., 1995). Both calpain-1 and calpain-11 are active at low $Ca^{2+}$ levels (10 μM and 30 μM, respectively) (Lee, Santé-Lhoutellier, Vigouroux, Briand, & Briand, 2007). In ostrich muscle, however, the presence of ubiquitous calpain isoforms is uncertain. Some researchers (van Jaarsveld, Naudé, & Oelofsen, 1997) observed one isolated calpain in postmortem ostrich muscle by using a phenyl Sepharose column, and others (Velotto, Varricchio, Vitale, & Crasto, 2010) found more than one calpain by using immunoprecipitation with monoclonal antibodies against human calpain-1 and bovine calpain-2 (m-calpain).

Casein zymography is a single assay that can separate the unautolyzed and autolyzed forms of calpain-1 and calpain-2 activity in bovine muscle (Camou et al., 2007). Because the calcium concentration required for calpain activation is different between isoforms, previous studies (Chang, Stromer, & Chou, 2013; Lee et al., 2007) have further demonstrated that calpain isoforms in avian muscle can be identified by increasing the concentration of $Ca^{2+}$ present in the incubation buffer of casein gels. The purpose of this study, therefore, was to examine the presence and the change of calpain activity by using zymography in postmortem ostrich muscle stored at 5 °C.

## 2. Materials and methods

### 2.1. Sample preparation

The use of animals in this study was reviewed and approved by the Institutional Animal Care and Use Committee, National Chiayi University. Female ostriches (*Struthio camelus* var. *domesticus*, 12–14 months old, 90–100 kg live weight, n = 8) were harvested on the same day in a government-regulated abattoir according to a standard commercial practice (Hoffman, 2012). Briefly, the birds were electrically stunned (90–110 V, 400–600 mA, 4–6 s) and bled for 10–15 min followed by plucking, skinning, evisceration and a health inspection. Right after inspection, both sides of top loin (*M. iliotibialis cranialis*, IC) and tender loin (*M. obturatorius medialis*, OM) were excised from each carcass at approximately 1 h postmortem. The IC and OM muscles from left side of the carcass were cut into five equal portions and randomly allocated to five sampling times. The portions were then vacuum-packed individually and stored at 5 °C for 0 (approximately 1 h postmortem), 1, 2, 3, and 7 days. At the end of each storage period, the samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C until required for subsequent analysis. The IC and OM muscles from right side of the carcass were cut into three equal portions,

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**Table 1**
Postmortem changes in pH and shear force of ostrich *M. iliotibialis cranialis* (IC) and *M. obturatorius medialis* (OM) samples stored at 5 °C.

| | Postmortem time (day) | IC | OM |
|---|---|---|---|
| pH value | 0 | 6.08 ± 0.04 | 6.08 ± 0.06 |
| | 1 | 6.12 ± 0.05 | 6.10 ± 0.05 |
| | 2 | 6.10 ± 0.05 | 6.13 ± 0.04 |
| | 3 | 6.13 ± 0.05 | 6.12 ± 0.06 |
| | 7 | 6.14 ± 0.02 | 6.13 ± 0.07 |
| Shear force (N) | 1 | 84.54 ± 6.65$^{x*}$ | 59.16 ± 3.74$^{y}$ |
| | 3 | 82.61 ± 5.29$^{x}$ | 59.98 ± 5.00$^{y}$ |
| | 7 | 83.90 ± 6.76$^{x}$ | 58.57 ± 4.17$^{y}$ |

*x,yWithin a row, means without a common superscript differ ($P < 0.05$).

vacuum-packed, and stored at 5 °C for the shear force measurements at 1 day, 3 days, and 7 days postmortem.

### 2.2. Measurements of pH

After crushing in liquid nitrogen, 2 g of each sample was used for the pH measurement (Farouk & Swan, 1997). The pH was determined by a pH meter (Model 470, Suntex Instruments Co., Taiwan) equipped with a glass electrode, which was calibrated with pH 7.00 and with pH 4.01 buffer solutions at 25 °C before measuring.

### 2.3. Casein zymography

The protocol used for protein extraction was done by the method of Veiseth, Shackelford, Wheeler, and Koohmaraie (2001). The protein concentration of the supernatant was determined (Robson, Goll, & Temple, 1968) after centrifugation (22,000 × *g* for 25 min at 5 °C). The procedure used for zymography was performed by the method of Chang et al. (2013) except that 300 μg protein from each sample and the SE 400 casein gels (16 × 18 × 0.75 mm slab gel, Hoefer Scientific Instruments, San Francisco, CA, USA) were used in this study. The casein gels of the 0-day samples were incubated in the presence of 0.01 mM, 0.03 mM or 4 mM $Ca^{2+}$ for identifying the ostrich calpain activity. The casein gels of the 0–7 day postmortem samples incubated in the presence of 4 mM $Ca^{2+}$ were used for analyzing the changes in calpain activity during postmortem storage of ostrich muscle.

### 2.4. Western blot analysis

The preparation of ostrich myofibrils was performed according to the methods of Huff-Lonergan, Parrish, and Robson (1995). The procedures of SDS-PAGE of myofibrils and Western blotting with a monoclonal antibody to desmin (Clone DE-U-10, 1:500 dilution) were performed following the method of Liao and Chou (2014).

### 2.5. Image analysis

The image analysis was performed by the method of Chang et al. (2013). The bands in both blots and casein gels incubated in the presence of 4 mM $Ca^{2+}$ were digitized with an Epson scanner (Model J131B) using Photoshop software and quantified using ImageJ (US National Institutes of Health). Each blot and gel included a pooled 0-day IC sample as a reference standard to normalize the band intensities. The unautolyzed and autolyzed calpain-11 activity and the desmin contents in 0-, 1-, 2-, 3-, and 7-day postmortem IC and OM samples were also determined, respectively. Results were expressed as percentages of the unautolyzed calpain-11 activity of 0-day IC samples.

### 2.6. Shear force measurement

The shear force of 1-day, 3-day, and 7-day postmortem samples of each bird was measured by the method of Vieira and Fernández (2014) with slight modifications. The samples were placed in open polyethylene bags and cooked in a water bath at 85 °C until they reached an internal temperature of 71 °C, measured with a digital thermometer with a temperature probe (Hanna Instruments, Woonsocket, RI, US) in the geometric centre of the sample. The cooked samples were allowed to cool under running water for 30 min and then refrigerated overnight at 5 °C. After overnight cooling, ten cubes (1 $cm^3$) from each sample were cut. The shear force was measured by a Texture Analyzer (Model TA-XT-plus, Stable Micro Systems Ltd., Godalming, UK) equipped with a Warner-Bratzler blade (code HDP/BS). The samples were sheared perpendicular to the fiber, at a crosshead speed of 5 mm $s^{-1}$. The value reported for each sample was the average of the ten cubes evaluated.

### 2.7. Statistical analysis

A split-plot design was used in this study. Whole units were the IC and OM muscles from each ostrich, and subunits were the muscle samples taken at each sampling time. All results were analyzed using the Mixed model procedure of SAS (PROC Mixed). The fixed effects included muscle, time postmortem and their interaction (muscle location × time postmortem), and the random effects were birds. A Tukey's test was used to separate multiple means at a 5% significant level.

## 3. Results and discussion

### 3.1. Postmortem changes in pH

The mean pH in 0-day (approximately 1 h postmortem) ostrich IC and OM samples was 6.08 ± 0.04 and 6.08 ± 0.06 (Table 1) respectively, consistent with previous studies (Morris et al., 1995) who noted that the ostrich muscles could reach 6.0–6.2 within 30 min postmortem.

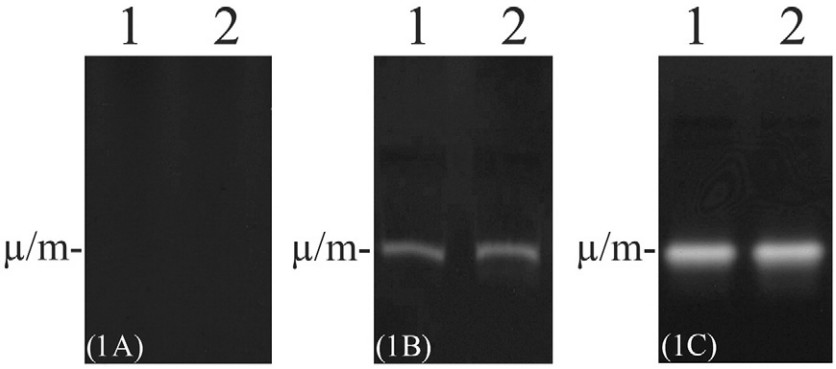


**Fig. 1.** Casein gels showing calpain-11 activity in 0-day ostrich *M. iliotibialis cranialis* (IC) and *M. obturatorius medialis* (OM) samples. The gels were incubated in the presence of 10 μM (1 A), 30 μM (1B) and 4 mM $Ca^{2+}$ (1C). Lane 1 = IC; lane 2 = OM. μ/m- = calpain-11.