# Calpain isoforms in goose skeletal muscle

Ya-Shiou Chang [1], Rong-Ghi R. Chou [2]

*[1] Chinese Culture University, [2] Naional Chiayi University, Taiwan*

**Objectives:** Although calpain-1 (μ-calpain, micromolar calcium-requiring $Ca^{2+}$-dependent neutral proteinase) and calpain-2 (m-cal- pain, millimolar calcium-requiring $Ca^{2+}$-dependent neutral proteinase) are ubiquitously expressed in mammalian tissues, calpain-11 (μ/m-calpain, an ortholog of mammalian calpain- 11) is mainly found in avian skeletal muscles and in some in placental mammals (Macqueen et al., 2010). In chicken skeletal muscles, calpain-1 and calpain-11 can be activated in the presence of 10 μM $Ca^{2+}$ and 30 μM $Ca^{2+}$ by casein zymography, respectively, and are clearly detected on casein gels (Lee et al., 2007; Chang and Chou, 2010). In goose gizzard smooth muscle, in addition to the presence of calpain-1 and - 11, a third calpain, high calcium-requiring calpain, which might be putative calpain-2, could also be found by casein gels (Chang et al., 2013). However, this putative calpain-2 is not found in chicken and duck skeletal muscles (Chang and Chou, 2010; 2012). Therefore, the objective of this study was to identify the presence of calpain isoforms in goose breast muscle.

**Materials and Methods:** Breast muscles (*Pectoralis major*) were sampled from each White Roman goose carcass (n = 10) in 15 min postmortem. Calpain activities were analyzed by casein zymography (Raser et al., 1995). Image analysis of each casein gel was done by the method of Chang et al. (2013). The resulting signals of casein gels were quantified by Image J (Schneider et al., 2012). The total calpain activity (summation of calpain-1, calpain-11 and putative calpain-2) in breast samples was taken as 100%.

**Results and Discussion:** Casein zymography results showed that, in the presence of 10 μM $Ca^{2+}$, only the calpain-1 (or μ-calpain) band was present in breast samples. As the $Ca^{2+}$ concentration was increased to 30 μM, the calpain-11 (or μ/m-calpain) band began to appear. While $Ca^{2+}$ concentration was kept increasing to 4 mM, an extra minor band was present and migrated between calpain-1 and calpain-11 bands, suggesting that a calpain isoform requiring a higher $Ca^{2+}$ concentration for activation, compared with calpain-1 and -11, was present in goose breast muscles. When total calpain activity (summation of calpain-1, calpain-11 and putative calpain-2 activities) in breast samples was taken as 100%, the activity of calpain-1, calpain -11 and putative calpain-2 was 32.5%, 65.6% and 1.9%, respectively.

**Conclusions:** Our results showed that goose breast muscle has three calpain isoforms, two requiring micromolar and one requiring millimolar (a possibly putative m-calpain) $Ca^{2+}$ concentration for activation. Among them, calpain-1 and calpain-11 are the two ma- jor isoforms in goose skeletal muscle.

**References:**

1. Macqueen, D. J., Delbridge, M. L., Manthri, S., & Johnston, I. A. 2010. A newly classified vertebrate calpain protease, directly an- cestral to CAPN1 and 2, episodically evolved a restricted physiological function in placental mammals. Molecular biology and evolution, 27: 1886-1902.
2. Lee, H. L., Santé-Lhoutellier, V., Vigouroux, S., Briand, Y., & Briand, M. 2007. Calpain specificity and expression in chicken tis- sues. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 146: 88-93.
3. Chang, Y. S., & Chou, R. G. R. 2010. Postmortem degradation of desmin and calpain in breast and leg and thigh muscles from Tai- wan black-feathered country chickens. Journal of the Science of Food and Agriculture, 90: 2664-2668.
4. Chang, Y. S., & Chou, R. G. R. 2012. Postmortem role of calpains in Pekin duck skeletal muscles. Journal of the Science of Food and Agriculture, 92: 1620-1626.
5. Chang, Y. S., Stromer, M. H., & Chou, R. G. R. 2013. μ-Calpain is involved in the postmortem proteolysis of gizzard smooth mus- cle. Food Chemistry, 139: 384-388.
6. Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9: 671-675.
7. Raser, K. J., Posner, A., & Wang, K. K. 1995. Casein zymography: a method to study μ-calpain, mcalpain, and their inhibitory agents. Archives of Biochemistry and Biophysics, 319: 211-216.

**Key words:** Calpain-1, Calpain-11, Putative Calpain-2, Goose Skeletal Muscle, Casein zymography.