# EFFECT OF μ/m CALPAIN mRNA EXPRESSION ON TENDERNESS IN DUCK MEAT

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## Introduction

Tenderness is one of the most important factors contributing to overall meat quality. Meat tenderization involved complex changes, so tenderness varied according to breeding, husbandry, nutrition and slaughter regimes. Among the factors that have been identified as responsible for the postmortem meat tenderization prossess is the calpain proteolytic system. The calpain system comprises two ubiquitous μ- and m- isoforms, active in vitro at micro- and millimolar calcium ion concentration respectively (Ilian *et al*, 2001). The chicken μ/m calpain has been cloned but until now calpain gene in duck meat had not been reported. The objectives of the study were to explore the effect of breed and cutting position and μ/m calpain mRNA expression on tenderness in duck meat.

## Materials and Methods

Thirty animals were selected from muscovy duck, cherry-valley duck and Gaoyou duck respectively. The animals were raised according to NRC nutrition standard and dissected. 100 mg sample were removed from breast and thigh muscle respectively and then frozen in liquid nitrogen, transferred to lab and stored at -80℃ for total RNA isolation. 50g breast and thigh muscle were aging at 4℃ overnight for shear force measurement.

Total RNA was isolated from tissue (50~100mg) by guanidine isothiocyanate using Tripure kit (Sambrook *et al*., 1989). Total RNA was electropheroed using 1% agrose gel for quality validation. First stranded cDNA was synthesized with 24 units reverse transcriptase, 2.0 μg of muscle total RNA and 7.5 μg of oligo (dT) primers. Primers for the variable of calpain and β-actin were designed by comparison of the corresponding chicken sequences. Primers for calpain were as follows:

1058 CTGGAGATCTGCAACCTAACC1078 (forward),
2157 CTAGCCGCACATTGTCAGCAGCAG2132 (reverse).
Primers for β-actin were as follows:
895 AAACTACCTTCAACTCCATC914 (forward),
1679CCTTCATTCACATCTATCAC1660 (reverse) .

The single stranded cDNA served as the template in the PCR reactions. Conditions for PCR were 94℃ for 4 minutes, 95℃ for 15 seconds and 72℃ for 5 minutes for 32 cycles. The volume of PCR was 10μL and the final concentration of reagent was 1μL 10 × buffer， 0.8μL 25mmol/L $MgCl_2$, 0.8μL 10mmol/L dNTPs, 10μmol/L for each primer and 1 unit Taq DNA polymerase for each reaction. Newly amplified DNA fragment was treated with DNA polymerase at 94℃ for 4 minutes, 95℃ for 15 second and 72℃ for 5 minutes for 32 cycles to ensure the PCR fragments were blunt ended. Primary PCR products for calpain and β-actin were gel purified.

Statistical analyses were done using SPSS11.0. Significant differences between means were determined by the least significant difference ($P$<0.05).

## Results and Discussion

*Shear force analysis of duck meat.* The WBS values of thigh meat was significantly higher than breast meat among different duck breeds (45.03 ± 2.17 *vs* 32.02 ± 1.90, 48.71 ± 2.67 *vs* 37.78 ± 2.90, 49.88 ± 4.58 *vs* 39.87 ± 2.16, $P$<0.05), the result indicated that the tenderness of thigh meat was lower than breast meat. The WBS values of breast and thigh meat had no significant differences among three breeds, while the values of muscovy duck were lower than the other two breeds (Figure 1).

*Similarity analysis of calpain gene.* Duck calpain clones were sequenced to generate a consensus sequence by using BLAST analysis. The results showed that calpain had 90% and 79% identities with corresponding regions of chicken calpain and bovine μ-calpain, respectively. The cDNA sequence of 9~20 exon of calpain in duck meat was as follows:

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1   GCACCGTGTG CGTCCACAAG CTCCCGTGCA CCGCGGCCCC CTCGTGACGA CCCCCGACGT CCTTGGTGGG
71  TCGGTGGAAG ACCTAGTTGG GGGTCAAGTT CTAGTTCGAC GACCTCCTCC TGCTGCTGGG GCCCCTGCTG
141 CTCCACCGGA CGTCGAAGGA CCACCGGGAC TACGTCTTCG TGGCCGCCGC CCTCGCCGCG GACCCCCCGC
211 TGTACGTGTG GTAGCCGAAG CGCCAGATGC TCCAAGGACT CCTCCGGGTC CCGTCGGTCT TGCACGTGGA
281 CTTCTTCCTA AAGAAGGACG CGTTGGTCAG CGCCCGCGCG AGGCTCTGGA AGTAGTTGGA CGCCCTCCAC
351 TCGTTGGTCT AGGCCGACGG GGGGCCGCTC ATGTAGCACC ACGGGAGGTG GAAGCTCGGC GTGTTCCTCC
421 GGCTGAAGCA CGACGCCCAG AAGTGGCTCT TCGTCAGCCT GTGCCGCCTC GACCTGCTCC TCTAGAGGCG
491 TCTAGACCGG CTACTCCTCC TCTAGTGGCT CCTACTGTAA CTCCTACCGA AGTTCTTGTA CAAGGTCGTC
561 GACCGTCCCC TCCTGTACCT TTAGTCGCAG AAGCTCGAGT CCTGGTAGGA CTTGTCTCAG TAGCGCTCCG
631 TGTTTCTGGA CTTCTGCCTG CCCAAGTCGG ACCTGAGGAC GGCGTTGTAC CAGTTGGACT ACCTATTCCT
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701 GCCGTCGCGG GCGGACCCCG ACCACCTCAA GGTCTAGGAG ACCTTGTTCT AGGCGTCGAC CGACTGGTAG
771 AAGGCGGTCG TGCTGGACCT ATTCAGCCCG TGGTACTCGC GGATGCTCTA CGCGTACCGG GACCTCAGCC
841 GCCCGAAGTT CGACTTGTTG TTCGACGTGG TCCACCACCA CCGGGCGATG CGGCTGCGGC TCTACCCCCA
911 CCTGAAGCTG TTGAAGCAGA CGACGGACCA GTTCGACCTC CGGTACAAGT CCAAGAAGGC GCCGTACCTG
981 GGGCTCCCGT GCCCGTNCR
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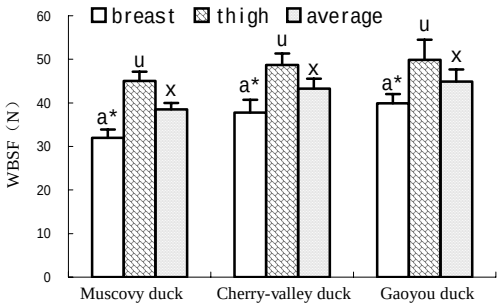
*Calpain gene mRNA expression level and meat tenderness.* There were significant differences between the breast and thigh for expression of calpain gene mRNA in different duck breeds ($25.43 \pm 1.85$ *vs* $24.1 \pm 1.59$, $24.95 \pm 1.92$ *vs* $23.65 \pm 1.43$, $24.57 \pm 1.82$ *vs* $23.66 \pm 1.30$, $P<0.05$). Regarding the quantity for expression of calpain gene mRNA, Gaoyou duck breast meat was lowest while muscovy duck was highest. However, the difference was not remarkable (Figure 2).



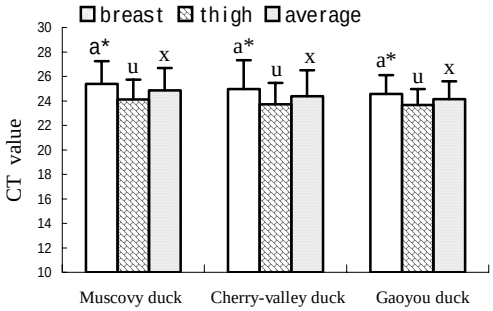




**Figure 1.** WBSF for Muscovy duck, Cherry Valley duck and Gaoyou duck

**Figure 2.** Expression of calpain gene mRNA between breast meat and thigh meat in different duck breeds

Note: Different abc, uvw, xyz separately express breast, thigh and average are significant differently; *expresses the same variety breast and thigh are significant differently.

The calpain family is a proenzyme that is regulated by $Ca^{2+}$ binding and autoproteolytic modification (Goll *et al*., 1992). The calcium dependent proteinases, calpains (EC 3.4.22.17), have been found in many different mammalian cells and tissues (Zhang *et al*., 1996). It showed that two ubiquitous calpains are present in chicken: 1) a μ-calpain having a greater calcium sensitivity and a lower electrophoretic mobility than the mammalian one, 2) a μ/m-calpain having a calcium sensitivity intermediate between that of the two mammalian μ-calpain and the m-calpain (Geesink *et al*., 2000). Tissue distribution of the two chicken isozymes vary and μ/m-calpain predominates, whereas μ-calpain levels are very low in some tissues, unlike in mammalian tissues. The characteristics of μ/m-calpain and its preponderance in all organs suggest that it may play a different role in chicken than in mammals (Lee *et al.*, 2006).

This study was to explore the quantity for expression of calpain gene mRNA for similar duck using real-time PCR analysis. The results showed that the expression of calpain gene was variable in different ducks, and significant differences between the expression of calpain gene and tenderness were observed. Our findings indicated that a significant positive correlation between the quantity for expression of calpain gene mRNA and tenderness.

## Conclusions

According to the sequence of chicken calpain and β-actin, two primers were designed to amply duck muscle mRNA, and calpain gene and β-actin gene were partially cloned. Then two primers were used to conduct real-time PCR analysis of calpain and β-actin with SYBR Green method. The result showed: (1) The similarity of calpain gene between duck and chicken was only 90%; (2) The expression level between breast and thigh meat differ dramatically, which indicated that calpain may play an important role in duck meat tenderization.

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