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Comparative studies on calpain activity of different muscles of cattle, camel, sheep and goat

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Summary

Tenderness is the single most important factor influencing consumer acceptance of meat. The calpain proteolytic system is known to be responsible for the post-mortem tenderization of meat. The purpose of this study was to determine and compare the tensile strength and total calpain activities in different muscles of camel, cattle, sheep and goat. In camels, the effect of age and sex of animal was also studied. Twenty-four animals (camel, cattle, sheep and goat) were sampled randomly after slaughtering. Samples from *biceps femoris*, *longissimus dorsi* and *triceps brachii* and heart were obtained from each animal. The tensile strength was calculated using an Instron Universal testing machine. After homogenization of samples in 0.1 M NaCl and centrifugation, total calpain activity was determined by fluorometric method. Despite significant differences in tensile strength, no significant difference ($P>0.05$) was observed among calpain activities of different muscles in each species. Inter-species differences however, were significant ($P<0.05$). In all muscles, the highest calpain activity was found in camel (3.08–5.36 RFU/mg protein) followed by cattle (3.65–4.43 RFU/mg protein), sheep (1.17–2.82 RFU/mg protein) and goat (1.24–2.23 RFU/mg protein). No significant differences were observed between camel and cattle and also between sheep and goat in tensile strength ($P>0.05$). In camel, adult animals had higher calpain activity and tensile strength than youngs; sex had no significant effects. Correlation coefficients of calpain activity and tensile strength were negative and not significant in all species. In conclusion, meats from different species might show different degrees of tenderness, partly due to the difference in their calpain activity.

Key words: Calpain, Cattle, Camel, Sheep, Goat

Introduction

In recent years, livestock producers have been putting more emphasis on improving the meat quality. Many factors contribute to the quality of meat and the perception of taste, with tenderness being considered as one of the most important attributes (Perez *et al*., 1998; Morton *et al*., 1999; Lametsch *et al*., 2004). The tenderness of meat varies considerably among species, animals within a species, and different muscles held for different times post-mortem. The ability to optimize the production of a tender meat depends on in-depth understanding of the mechanisms involving in the tenderization process. It is well-known that storage of meat improves tenderness and that numerous variables including the amount of intra-muscular fat, sarcomer length, collagen content, ultimate pH, size and type of muscle fibers and enzymatic activities involved in post-mortem aging, are important factors (Koohmaraie *et al*., 1991; Koohmaraie, 1992; Redmond *et al*., 2001; Veiseth *et al*., 2004).

It is generally accepted that proteolysis of the myofibrillar structure is an important factor in meat tenderness and that more than 90% of the proteolytic tenderization that occurs during the first 7–10 days of post-mortem storage at 2–4°C can be attributed to the calpains (Sazili *et al*., 2003; Sazili *et al*., 2005).

The calpain system comprises two ubiquitous μ- and m-isoforms. They are

active *in vitro* at low and high calcium ion concentration, respectively. They have a growing number of tissue-specific variants, all of which are products of various genes. They have also a specific endogenous inhibitor, calpastatin (Lee *et al*., 2000; Ilian *et al*., 2003). Meats from different muscles of the same species and meat from different species exhibit significant variation in tenderness which is supposed to be a result of variation in the calpains activity (Whipple *et al*., 1990; Koohmaraie *et al*., 1991; Shackelford *et al*., 1991; Dransfield, 1994; Gill *et al*., 1998).

Despite numerous reports on different animals, no information on calpain activity in muscles of camel is available. The objective of this study was to determine and compare the total calpain activities in different muscles of camel, cattle, sheep and goat by fluorometric assay. The effect of sex and age on enzyme activity was also studied in camel. Tensile strength test was used for evaluation of the tenderness; the possible correlation between calpain activity and tenderness of muscles was also studied.

Materials and Methods

Animals and muscle samples

Camel, sheep, cattle and goat muscles were obtained from local slaughterhouse. Twelve one-humped Iranian-breed camels were divided into four groups; six male and female adults (~5 years old) and six male and female young (one year old), and 12 adult Holstein cattle, native breeds of sheep and goat were used for this study. Carcasses were allowed to chill for 24 hrs at +3ºC for completion of rigor mortis. Samples from *biceps femoris*, *longissimus dorsi*, *triceps brachii* and heart muscles were removed and their external fat and epimysial connective tissues were separated.

Calpain activity assay

Twenty grams of each muscle was homogenized in the presence of 20 ml of 0.1 M NaCl using a laboratory homogenizer at a constant speed for one min. The suspension was then centrifuged at 25°C for 10 min in 1000 × g. The supernatant was used as crude extract. Total calpain activity was measured using a calpain activity assay kit (Biovision, Mountain view, USA) according to the manufacturer's instructions. The fluorometric assay is based on the detection of cleavage of calpain substrate (Ac-LLY-AFC). Ac-LLY-AFC emits blue light (λ_{max} = 400 nm); upon cleavage of the substrate by calpain, free AFC emits a yellow-green fluorescence (λ_{max} = 505 nm), which can be quantified fluorometrically (Lee *et al*., 2005). Equal volume extraction buffer provided by the manufacturer was added to 100 µl of meat extract. The mixture was incubated on ice for 20 min and samples were mixed gently by tapping several times during incubation. Mixture was then centrifuged for one min in a microfuge (10,000 × g, 4°C). Protein content in supernatant was determined by the method of Lowry *et al*. (1951). The supernatant was diluted to 50–200 µg protein in 85 µl extraction buffer. For positive control, 1–2 µl active calpain solution (provided by the manufacturer) was added to 85 µl of reaction mixture. For the negative control, one µl of calpain inhibitor was added. Ten µL of the 10x reaction buffer and five µl of calpain substrate were added to each assay mixture. The samples were incubated at 37°C for one hr in the dark. The samples were read in a fluorometer equipped with 400 nm extinction and 505 nm emission filters. Calpain activity was reported as the relative fluorescent unit (RFU) per mg protein.

Texture evaluation

Tensile strength was calculated from the maximum load during a tension test carried out to rupture the specimen (Honikel, 1998) by using an Instron Universal testing machine (Instron Co, Model 1140, California, USA). Muscles were cut perpendicular to the muscle fiber orientation to produce one-cm thick slices. Slices were then hooked to the testing machine and the resistance to tearing (tensile stress) was determined at tensile velocity of 2 cm/min.

Statistical analysis

The means of calpain activities and shear forces in different muscles of different species were analysed using independent-

samples Student's t-test, one-way ANOVA and Duncan's multiple range tests. The correlation coefficient between the two variables was calculated using SAS software, version 6.1.

Results

Calpain activities in different muscles of domestic animals studied are shown in Table 1. In each species, no significant difference ($P>0.05$) was observed among different muscles. Inter-species differences, however were significant ($P<0.05$). The highest calpain activity was found in camel *triceps brachii* and *biceps femoris* muscles. In cattle *longissimus dorsi* and heart muscles showed higher calpain activity than other muscles. In all muscle types, sheep and goat had significantly lower calpain activity than camel and cattle. The sex of camel did not have any significant effects on calpain activity (Table 2), however, adults had higher calpain activity than youngs ($P\leq0.05$ for *biceps femoris*, *longissimus dorsi* and heart muscles). The difference was not significant ($P = 0.09$) for *triceps brachii* muscles.

Table 1: Mean ± SD calpain activity in different muscles of adult male animals (RFU/mg protein)

Animal species	Muscle type			
	Triceps brachii	*Longissimus dorsi*	*Biceps femoris*	Heart
Cattle	4.04 ± 0.25^{ac}	4.42 ± 0.49^{a}	4.43 ± 0.50^{a}	3.65 ± 0.61^{a}
Sheep	2.79 ± 1.26^{ab}	2.75 ± 1.51^{b}	2.82 ± 1.15^{ab}	1.17 ± 0.33^{b}
Goat	2 ± 0.49^{b}	2.23 ± 0.5^{b}	1.60 ± 0.39^{b}	1.24 ± 0.49^{b}
Camel	5.36 ± 1.85^{c}	3.7 ± 0.57^{ab}	5.08 ± 2.74^{a}	3.08 ± 0.95^{a}

Means in the same column with different superscripts are significantly different ($P<0.05$)

Table 2: Mean ± SD calpain activity in different muscles of young and adult camels (RFU/mg protein)

Age and sex	Muscle type			
	Triceps brachii	*Longissimus dorsi*	*Biceps femoris*	Heart
Young male	1.98 ± 1.40	2.11 ± 1.18^{A}	3.18 ± 0.55	1.54 ± 0.33
Young Female	2.2 ± 0.43^{ab}	$2.17 \pm 0.51^{ab, A}$	3.55 ± 1.33^{b}	1.32 ± 0.27^{a}
Adult Male	5.36 ± 1.85	3.7 ± 0.57^{B}	5.08 ± 2.74	3.08 ± 0.95
Adult Female	3.38 ± 2.67	3.71 ± 0.55^{B}	4.85 ± 1.21	2.48 ± 0.99

Means in the same row with different lowercase superscripts are significantly different ($P<0.05$). Means in the same column with different uppercase superscripts are significantly different ($P<0.05$)

Table 3: Mean ± SD tensile strengths of different muscles of adult male animals (kgF)

Animal species	Muscle type			
	Triceps brachii	*Longissimus dorsi*	*Biceps femoris*	Heart
Cattle	$9.03 \pm 0.87^{a, A}$	$7.13 \pm 0.42^{b, A}$	$8.87 \pm 1.01^{a, A}$	$6.57 \pm 1.12^{b, A}$
Sheep	$5.67 \pm 0.42^{a, B}$	$4.57 \pm 0.40^{bc, B}$	$5.37 \pm 0.55^{ac, B}$	$4.13 \pm 0.76^{b, B}$
Goat	$6.37 \pm 0.31^{a, B}$	$5.23 \pm 0.50^{b, B}$	$6.07 \pm 0.40^{a, B}$	$4.53 \pm 0.32^{b, B}$
Camel	$8.53 \pm 0.90^{a, A}$	$7.27 \pm 0.31^{ab, A}$	$8.27 \pm 0.85^{a, A}$	$6.73 \pm 0.67^{b, A}$

Means in the same row with different lowercase superscripts are significantly different ($P<0.05$). Means in the same column with different uppercase superscripts are significantly different ($P<0.05$)

Table 4: Mean ± SD shear forces of different muscles of young and adult camels (kgF)

Age and sex	Muscle types			
	Triceps brachii	*Longissimus dorsi*	*Biceps femoris*	Heart
Young male	$6.70 \pm 0.46^{a, A}$	$6.03 \pm 0.35^{ab, A}$	$6.27 \pm 0.81^{a, A}$	$5.03 \pm 0.60^{b, A}$
Young Female	$7.10 \pm 0.50^{a, A}$	$6.23 \pm 0.57^{ab, A}$	$6.77 \pm 0.40^{a, AC}$	$5.60 \pm 0.72^{b, A}$
Adult Male	$8.53 \pm 0.90^{a, B}$	$7.27 \pm 0.31^{ab, B}$	$8.27 \pm 0.85^{a, B}$	$6.73 \pm 0.67^{b, B}$
Adult Female	$8.57 \pm 0.75^{a, B}$	$7.20 \pm 0.66^{bc, B}$	$8.03 \pm 0.76^{ac, BC}$	$6.27 \pm 0.51^{b, AB}$

Means in the same row with different lowercase superscripts are significantly different ($P<0.05$). Means in the same column with different uppercase superscripts are significantly different ($P<0.05$)

Shear force data are shown in Tables 3 and 4. In all species, significant differences ($P<0.05$) were observed among different muscles. The values in decreasing order belonged to *triceps brachii*, *biceps femoris*, *longissimus dorsi* and heart muscles. No significant differences were observed between camel and cattle and also between sheep and goat ($P>0.05$). In all muscle types, sheep and goat had significantly lower shear forces than camel and cattle. The sex of camel did not have any significant effects on shear force and except for *biceps femoris* and heart muscles of female camels, adults had higher shear forces than youngs ($P<0.05$).

Calpain activity and shear force were negatively correlated. The correlation coefficients were non-significant in all species ($r = -0.62, -0.73, -0.56, -0.81$ for camel, cattle, sheep and goat, respectively; $P>0.05$).

Discussion

In this study, we reported on a simple method for assaying total calpain activity in tissue homogenates. The method allows measurement of calpain activity in large number of samples for comparative studies without the need for long and tedious procedures which involve specific extraction solutions as well as chromatographic steps for separating different calpains.

Our results showed different calpain activities among species. In most muscle types, camel and cattle exhibited more calpain activity than sheep and goat. One might expect that the differences in calpain activity result in difference in meat tenderness observed between species studied. Sarcomer length, connective tissue contents, and proteolysis of myofibrillar proteins account for most, if not all, of the explainable variation observed in tenderness of aged meat. However, the relative contribution of each of the above components to tenderness is muscle dependent. For example, in lamb, while sarcomer length is the major determinant of *psoas major* tenderness, proteolysis is the major determinant of *longissimus* tenderness and connective tissue contents is a major contributor to tenderness of muscles such as *biceps femoris* and *semimembranosus* (Koohmaraie *et al*., 2002). In our study, it was shown that calpain activity was not significantly different in various muscles of all species studied. Geesink *et al*. (1992) reported no difference in μ-calpain activity between six bovine muscles. Although limited data is available on μ-calpain activity between ovine muscle types, it has been demonstrated that no difference in μ-calpain activity exists between ovine *M. longissimus dorsi* and *M. vastus intermedius*. Both the *M. longissimus dorsi* and *M. biceps femoris* are similar in their metabolic and contractile muscle type, so it is possible that the activity of the calpain system would be similar in both muscles (Mcdonagh *et al*., 1999).

Several researchers have suggested that calpain activity in muscle is the cause of many changes observed in the structural proteins and this, in turn, is related to tenderization (Koohmaraie, 1992; Dransfield, 1994; Hwang *et al*., 2003). They attributed these differences to the significant calpastatin activity which might the reason for differences observed in rates of post-mortem tenderization in the beef, lamb and pork carcasses. Additional evidence supporting this conclusion is as follows: 1) infusion of lamb carcasses with $ZnCl_2$, which prevents post-mortem proteolysis and tenderization, also prevents the loss in calpastatin activity that occurs in non-infused carcasses (Koohmaraie, 1990; Koohmaraie *et al*., 1991), 2) feeding a β-adrenergic agonist to lambs results in a reduced rate of myofibrillar protein degradation during post-mortem storage and also significantly elevates the activity of calpastatin (Kretchmar *et al*., 1990) and 3) a reduced rate of myofibrillar protein degradation during post-mortem storage is reported to be one major reason for reduced tenderness of meat from *Bos indicus* compared to *Bos taurus* breeds of beef (Wheeler *et al*., 1990). The elevated calpastatin activity probably was the cause of reduced proteolysis and tenderness of the *Bos indicus* breed.

Nevertheless, there is now considerable evidence linking the calpains to tenderization in beef, lamb and pork. Correlation coefficients have shown that the different tenderization rates between species (beef<lamb<pork) relate inversely to the

ratio of calpastatin:calpain (beef>lamb> pork) (Rhee and Kim, 2001).

We observed that adult animals had higher calpain activity than youngs ($P<0.05$) in camel and that sex had no significant effects. Because calpains may play an important role in myofibrillar protein degradation, we propose that the variation of calpain specific activity underlies or contributes to age-related changes in fractional rates of muscle protein degradation.

Sheep muscle μ-calpain, m-calpain and calpastatin specific activities declined ($P<0.05$) between birth and weaning. Losses were caused, in part, by accumulation of muscle proteins. Age-dependent attenuation of muscle calpain and calpastatin activities may underlie or contribute to age-related changes in muscle protein turnover and muscle growth. No significant ($P>0.05$) differences in activities of muscle calpains or calpastatin were detected between two groups at weaning and market weight (Ou *et al*., 1991).

An objective measure of tenderness is the force required to shear a standardized piece of meat with low shear values being desirable. In this experiment, tensile strength had negative and non-significant correlation with calpain activity in each species. The reason for each correlation between calpain activity and tenderness was not investigated. It is possible that other factors, including other proteolytic enzymes or the level of calpastatin, are responsible. Significant relationship between calpastatin activity and shear force has been reported in beef carcass (Shackelford *et al*., 1991). The ratio of μ-calpain to calpastatin is negatively correlated ($P<0.01$) with the rate of decrease in shear force during post-mortem meat storage in the *M. longissimus dorsi* in lamb (Mcdonag *et al*., 1999).

Taken together, data obtained in this study clearly showed that calpain activity is different among selected domestic animal species. Calpains presumably play a major role in post-mortem meat tenderization but numerous variables have been related to tenderness. It remains to be studied if other factors can be related to difference in the tenderization of meat obtained from these animals.

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