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Effectiveness of Calpastatin and Calpain Genes on Meat Quality and Tenderness in Iranian Livestock Breeds

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ABSTRACT

Today, meat tenderness is one of the issues challenging international commercial companies to find a proper solution to enhance its quality. The factors affecting meat quality and tenderness before slaughter include nutrition, stress, genetics, gender, management, and post slaughter factors include aging time after rigor mortis, electrical excitability of muscle, pH, muscle tremors during slaughter, the nervation, frequent freezing and thawing of meat and finally cooking method. Small genes with great effects on genetic changes are called major genes, that are able to be transferred together to form a unit. Calpastatin and calpain genes influence growth and quality of carcass. Most of the researches have represented the relation between polymorphism of calpastatin and calpain genes and quality of carcass. Components of calpastatin and calpain system determine the speed of mead tenderness after slaughter, and acts as private and internal preventive. Hence, one of the methods to evaluate tenderness of the meat is determining level of calpastatin and calpain activity.

KEYWORDS: Gene, Calpastatin and Calpain, Meat Quality.

1. INTRODUCTION

Iran, with more than 50 million sheep with 25 different breeds, is one of the significant countries in breeding sheep up in the world; which this diversity of breed is due to its climatic variations and wide area [1, 2]. Various breeds of sheep in Iran include Bakhtiyari Lori, Shal, Moghani, Sangsari, Ghezel, Arab, etc., that are adapted to a special region of Iran [3].

The importance of sheep-bearing in Iran is not only due to breeding diversity, population and its place in the world, but for its role in economics of agriculture and animal husbandry. Sheep bearing industry has significant role in national economy of the country; hence, the share of this industry in meat production is 50% of total meat production of the country. On the other hand, sheep bearing in most of the countries, especially in Iran, depends on grasslands. According to the statistics and reports on the pressure of the number of animal units on the grasslands of the country, one of the appropriate solutions to reduce the pressure of livestock to grassland is decreasing animal unit in grasslands [4, 5, 6]. Since reduction of livestock decreases income of the ranchers, and the ranchers are less likely to reduce the number of animals, therefore, the solution for this problem is to reduce the number of productive livestock without decreasing income of the rancher. To achieve this goal, productive livestock with high potential is used [5, 6].

One of the main international marketing issues on meat sales and export is quality of produced meat according to diverse tastes of consumers. In recent century, the industry of meat animals' production and growth has enhanced toward producing low fat meats which results in increasing pleasant and tender meats. Today, tenderness of the meat is one of the issues that struggle universal commercial companies to find appropriate solutions to increase quality of this parameter.

Factors affecting tenderness and toughness of the meat

Parameters influencing meat quality before slaughter include nutrition, stress, genetic, gender and management. The factors affecting tenderness and toughness of the meat after slaughter include aging time after rigor mortis, electrical excitability of muscle, pH, muscle tremors during slaughter, the nervation, frequent freezing and thawing of meat and finally cooking method. Comer [7] reported in a special race, 30 percent of phenotype diversion of meat tenderness related to the increasing-genetic and the rest (70%) relates to environmental and non-increasing genetic effects. in recent century, ideas about improving characteristics of the meat include studying and investigating biochemical mechanism of molecular analysis of muscle [8].

The breed of livestock is one of the determinant factors in tenderness and toughness of the meat. Age of the livestock is also another important factor, since muscle fibers become thicker over the time; meanwhile, increasing connective tissue decreases tenderness of the meat, in another word, it decreases water in meat tissue. Younger livestock have less connective tissue and muscle fibers are thinner, so the meat is tenderer and more water can be found in its tissue. Gender is another significant factor; male livestock have soft and delicate tissue than female ones.

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Post slaughter changes finish after strengthening of mortis. Storing 10-14 days of the meat in temperature above zero degree causes chemical interactions and makes the meat to be tender. Tenderness or toughness of the meat relates somehow to nutrition of the livestock. In some countries, some specific calves are fed exclusively with milk. Their meat has special tenderness and a very good taste. However, Position of muscles in the body is also important. For instance, the meat of shoulder or hip has a thick texture since most of the body pressure is on them and makes the livestock to move. But muscles on the back to the spine, also known as sirloin and filet, bear less pressure, so it has not thick texture and is tenderer.

As we know, quality traits are morphological traits that can be evaluated visually. Quality of the meat is recognizable by taste, appearance, and its texture. Using new innovative methods along with traditional and usual methods of anticipating tenderness and classifying the meat, can influence improving quality and tenderness of the meat.

Consumers believe tenderness of the meat is one of the main traits of meat quality and its toughness is the problem in its quality [9]. Since consumers pay much money for tenderness of the meat, it requires developing meat tenderness evaluation methods and complete quality classifying of meat to grow meat animals more economic and qualitative [10]. If causes of changes in meat tenderness are recognized, by improving effective function in tenderness process, the objectives can be achieved. Although meat texture, especially its tenderness, is different in various animals, similar structures and chemical features are observed in all vertebrates. All of them are formed of 75% of water, and 25% of protein, lipid, carbohydrate, and dissolved organic components. In recent decades, few researches study mechanisms to improve meat quality, especially about different livestock and various breeds. In general, results of the studies represent that small but considerable changes occur in muscle to tender the meat. For instance, Koohmaraie et al. [11] believe the most important factor in meat tenderness diversity is difference in protein breakdown of main muscle fibers after slaughter. Results of the study represent among all internal proteolysis system of the skeleton muscle, only calpain enzyme participate in tenderness of the meat.

Gene

Genes are units of heredity. Genetic order of a living organism (composition of its genes) determines its traits such as eye color or smell of a flower or plant. Most of the genes contain information about protein formation and usually are stored in DNA molecule sequences. For a gene to represent its effect, first it should be translated to a protein. Gene translation is done through another macromolecule (RNA). Gene adjustment allow cell to control its structure and function, which is basic for cell differences, evolution and adaptation skill of organism [13, 14].

Gene expression is a process in which inter gene information is used to produce an applicable production. Production of genes is mostly proteins. In genetic science, gene expression is one of the fundamental issues that help genotype to appear as phenotype. In fact, genetic codes are saved in DNA, and interpreted by gene expression. Features and expression type of gene creates phenotype in organism. In genetic improvement field, having knowledge about genetic structure of populations, significantly help planning eugenic plans and saving genetic resources [15]. Molecular methods and using molecular indicators is the best option, since high amount of information it provides can prove and complete or reject results takes from analyzing records using statistic methods. Eugenic plans typically try to improve livestock production status, which depends on genetic variety of herds. Regardless of the number of alleles, genetic variation within or outside population is considered as genetic, mutation, selection, migration and reproduction methods, and is used as a valuable tool in eugenic plans.

Genetic system related to increase and improvement of meat

Quantitative traits are those controlled by many genes and each one of these genes has negligible effect on quantitative traits. Some small genes with great impact on genetic changes are called main genes. These genes can have close relation with genetic markers, since they are able to be transferred as a unit. Calpain and Calpastatin are examples of these genes related to growth and quality of the carcass [16, 17].

Many researches have represented relation between polymorphism of Calpain and Calpastatin genes and quality traits of carcass, while some reports investigate relationship between polymorphism of these genes and growth traits of cow and sheep. Development of skeletal muscle depends on three main factors: protein synthesis of muscle, decomposition of muscle proteins, and muscle cells. Increasing skeletal muscle growth can be due to decreasing protein decomposition of muscle, caused by calpain system activity and increasing activity of calpastatin.

Based on these notions, Calpain and Calpastatin expressing genes are introduced as effective candidate genes in meat production [18, 19].

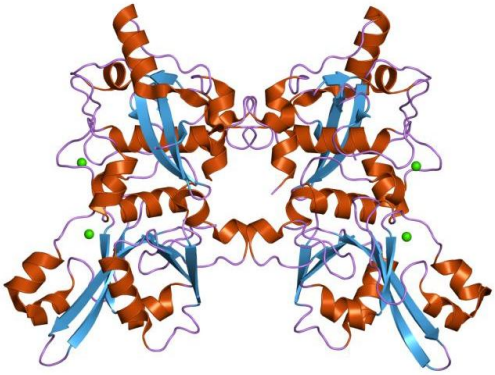
2. DISCUSSION

Calpain

In 1976, the first protein of calpain family was recognized, that play key role in muscle and meat decomposition after slaughter. Calpain system is a proteolytic and cytosolic protein complex. This system

includes natural protease related to calcium, playing significant role in muscle growth and tenderness of the slaughtered meat [4]. Calpains are considered as initiating factor in myophibril muscle decomposition. In general, calpain enzymes control muscle growth by controlling myophibril decomposition during livestock life. After slaughter, it causes tenderness of the meat by decomposing Z-Disks in skeletal muscles and weakening bounds between myophibrils.

Figure 1. Crystal structure of the peptidase core of Calpain II



Calpain activity depends on Ca+2, and as it was mentioned, proteolysis of myophibrils by this system plays key role in tenderness of slaughtered meat [4]. In general, two types of calpains have been recognized; both of which are heterodimer and contain common sub-units of K30 and big different sub-unit of k80. Calpain A, known as µ-Calpain, have maximum activation with 50-100 micromole calcium ion, and calpain B, known as M-Calpain, shows maximum activity with 1-2 millimol of calcium ion [20]. Activity of calpain B is more than calpain A, and need millimolar concentration of Calcium for activity, both of them are normally a heterodimer [15]. Most of the researchers believe genetic methods can solve the problem of meat tenderness and calpain enzyme has the most important role in this regard [7, 21]. Therefore, the best method of anticipating meat tenderness should be based on recognizing indicators that are able to measure this system. Recent studied approved calpain protease is one of the main factors in improving tenderness and quality of the meat, by decomposing titin and tubulin proteins. So far, many researches have been done on sequence of regulatory subunit of calpain II gene, all of which emphasize on polymorphism of this area.

Moreover, other studied are done to investigate the effect of this polymorphism on quality of after slaughter meat and growth traits. Chung et al. [17] amplified parts of the Calpain III gene of the sheep genome and studied point mutations in this region using PCR-SSCP method. Furthermore, Chang et al. [22] found there is a meaningful difference between fat around the hips, kidneys and heart of under study genotypes. Chang et al also studied this area in Angus cattle breeding and their relation with meat tenderness and carcass traits. Zhang et al. [23] reported allelic polymorphism of regulatory subunit of Calpain in cattle, using PCR-RFLP and HhaI enzyme. In this study digestion of 1800 bp fragment of three genotypes of AA, AB and BB were identified. Sorimmachi et al. [24] represented that along with introduced proteases, calpastatin enzyme is another member of calpain family which play different role than others and acts as a specific inhibitor of calcium related proteases, and recently its role on growth, wasting and consequent loss after slaughter has been introduced. Therefore, meat tenderness rate is highly influenced by calpastatin enzyme function [24].

Calpastatin

Calpastatin is specific inhibitor of calcium related protein-degrading enzymes, m and µ of calpains in mammalian tissues. The components in the calpain-calpastatin system determines meat tenderness rate after slaughter. Therefore, one of the methods to determine meat tenderness is calpain-calpastatin activity.

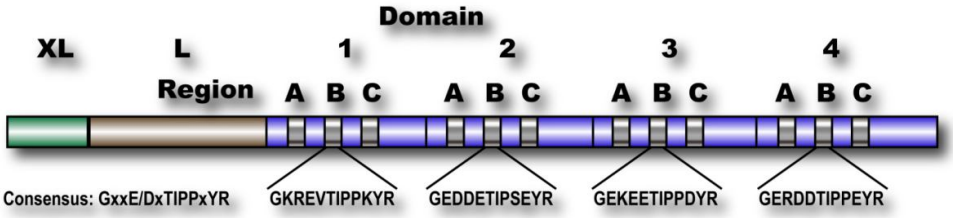


Figure 2. Schematic structure of human calpastatin: Calpastatin has four repetitive inhibitory domains (1~4), which can inhibit one molecule of heterodimer calpains, although their inhibitory activities vary. At the N-terminus, there are two extended domains, XL and L, whose functions are unknown. Each inhibitory domain has three regions, A, B, and C, which binds to domain IV of catalytic subunit, the active site of domain II, and domain VI of regulatory subunit (see Fig. 2), respectively. Peptide corresponding to only the Region B has inhibitor activity, which is less than full-length domain. The Region B has highly conserved sequences at the center, whose consensus is GxxE/DxTIPPxYR.

In recent years, increasing and improving quality of meat have been emphasized, in a way that various reports confirm relationship between calpastatin level of muscle and meat tenderness [25]. Tahmourethpour et al. [25] introduced the best genotype, AC, in increasing weight of the sheep. Palmer et al. [26] reported AC genotype (123g/day) increases the weight 18% more than AA genotype in the crossbred Dorset and Coopworth sheep. However, polymorphism in calpastatin gene of the sheep can be studied as a productive marker related to weight increase and quality of meat.

Results of the study showed that livestock with lower calpastatin activity will increase meat tenderness [8, 27, 28, 29, 30]. Calpastatin gene is located in chromosome no.5 of the sheep and its length is 100 kb. It includes 4 exons; in exon I two alleles were recognized [27]. Calpastatin gene is producer of an enzyme system, and any changes in enzymes of the system will cause various illnesses [21]. Calpain-calpastatin system influence different processes, for instance, this system in skeletal muscle have significant role in regulation of protein degradation and rebuilding, development and breakdown of muscle, organogenesis, cell cycle, cataract formation, movement of muscle fibers, and cell death. Studies show the fact that recognizing inactivation mechanisms of calpastatin in skeletal muscles after slaughter are important. For the first time in the sheep, polymorphism of this gene was carried out by Palmer et al. [27] using PCR-RFLP method, which measures alleles m and n of Dorset Horn sheep with frequency of 0.77 and 0.23, respectively.

Recently, Garcia et al. [31] reported a meaningful relationship between polymorphism of calpastatin and reproductive traits and length of productive life of dairy cattle. Morris et al. [32] and Casas et al. [33] reported relationship between calpastatin and calpain genotypes of some breeding of cattle with carcass features. In general, both calpain and calpastatin exist in all tissues, but their ratio in various tissues and cells differs significantly [20]. Moreover, all skeletal muscles of domestic animals contain about the same amount of m-calpain and µ-calpain, and activity of calpastatin is more than activity of both calpains [34].

In addition to the two introduced proteases, calpastatin enzyme is another member of calpain family which plays a different role and acts as special inhibitor of calcium related proteases [34]. However, rate and amount of meat tenderness, softness and juiciness mostly is influenced by calpastatin enzyme function and there is meaningful relationship between them [35].

Calpastatin is special inhibitor of calcium related protein decomposing enzymes, i.e. m-calpain and µ-calpain of mammal's tissue. Components of calpain-calpastatin system determine tenderness rate of the meat after slaughter. Calpastatin inhibits rate and amount of protein decomposition after slaughter and play significant role in increasing quality and quantity of the meat [19]. Application of molecular genetic to study meat tenderness of the sheep, introduces calpastatin gene as indicator genes for this trait [14].

Calpastatin activity has high heritability (h2= 0.65 ±0.19). Therefore, it's possible to achieve rapid genetic response in selecting activity against calpastatin, and this selection can improve livestock meat tenderness [15]. Currently, there is interest in using oral synthetic agonists to re-decompose nutrients to produce lean meat and fat reduction. These compounds are derivatives of catecholamine with some characteristics same as epinephrine. Now, it's clearly considered that beta-agonists change mRNA of calpains and calpastatins, since injection of these compounds causes hypotrophy of muscles and prevents muscle decomposition. Therefore, the hypothesis is emphasized that these compounds influence promoter area of calpastatin gene and changes appearance of this gene.

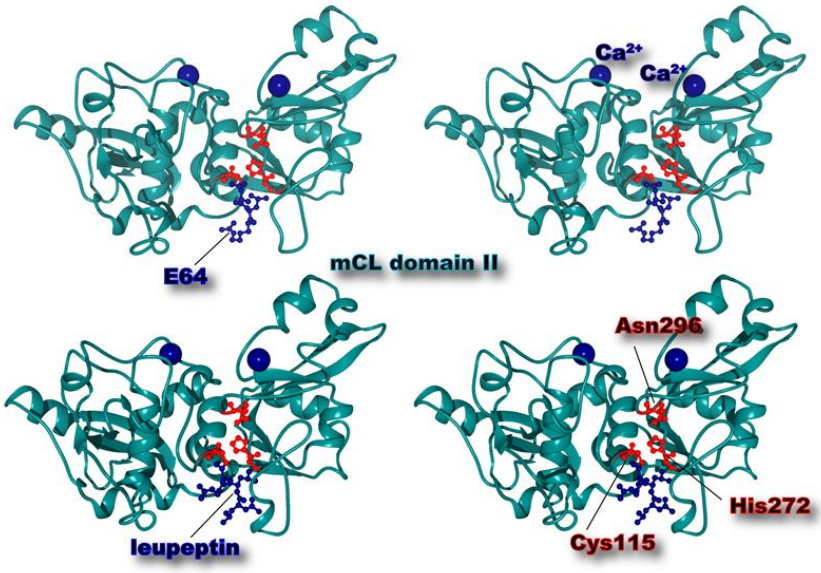


Figure 3. Three-dimensional structure of µ-calpain protease domain (domain II) with Ca^{2+} and inhibitor molecules Ca^{2+} (dark blue ball) binds to the upper side of the active site cleft, which consists of Cys115, His 282, and Asn 296 (red ball-and-stick). E64 (upper) and leupeptin (lower) covalently bind to the sulfur atom of the active site Cys115. While E64 is an irreversible inhibitor, leupeptin dissociates from calpain when Ca^{2+} is depleted.

Results of the studies on Dorset Down and Coopworth sheep to determine relationship between meat tenderness and genetic diversity of calpastatin indicate that calpastatin accelerate growth and quality of the meat [26]. In another study, the effect of genotype on increasing weight of Ghezel lambs from birth to 6-month is meaningful [3]. The latest research findings show allele C of calpastatin gene meaningfully increases lamb growth and increases muscle mass. Investigating polymorphism of calpastatin gene in exon 1 of this gene to determine its relation with daily growth of Kurdish sheep using PCR-SSCP cause recognition of aa, ab and ac genotypes with frequency of 0.55, 0.32 and 0.13, respectively. Least square means of ab genotype (215.22 g) is meaningfully more ($p<0.05$) than aa (24.88 g) and ac genotype (17.62 g) [1].

Polymorphism of calpastatin gene in exon 1 of this gene in Karakul sheep using PCR-RFLP method and restriction enzyme of MspI was studied and alleles M and N with frequency of 0.79 and 0.21 were recognized; mean heterozygosis of Karakul sheep is 33% [2]. Relationship between some forms of alleles of this gene with weight increase and carcass traits of the sheep was recognized; resulted from simple substitution of nucleotides 1 in exon I of calpastatin gene using RELP technique and restriction enzyme of MspI or NcoI, and can be used as indicator of eugenics. Activity of calpastatin gene among various breeding of cattle was studied. In these tests, resulted calpastatin gene of Bos Taurus and Bos Indicus cattle genome were studied, and results of the study show the diversity of calpastatin gene activity is the main difference factor in meat tenderness; the meat of Bos Taurus breed is tenderer than Bos Indicus [7].

To study polymorphism of calpastatin gene of Systani cattle, random blood sampling was done in Zahak Systani research station. MM, MN, NN genotype with frequency of 51.86, 32.27 and 4.87 pf calpastatin gene of the cattle was reported. M and N allele frequency was 76.4 and 23.6%, respectively. Low heterozygosis of calpastatin gene of the cattle was studied and it was caused of closed herd. Although, based on statistical analysis, mean weight increase in age 0-3 and 9-12 month was considered, and NN genotype had the best function. Duncan test represented that there is meaningful difference between weight of MM, MN and NN genotypes in 0-3 and 9-12 months ($p < 0.05$), which represents the effect of calpastatin on growth. X2 and G tests show Hardy - Weinberg equilibrium of the population. As regards, there is no selective advantage or disadvantage of a particular genotype in this population, and Hardy - Weinberg equilibrium is established in this cattle [16].

Polymorphism of calpastatin gene in Angus cattle and their relation with growth traits is also studied. In this study, genotypes of calpastatin gene had meaningful effect on weights of 28, 42, 56 and 140 days but no effect on birth weight. In general, this enzyme acts as modifiactor of calpain activity. Calpastatin gene of cattle is placed on chromosome 7 and is considered as housekeeping genes. End of 5 promoters of this gene is rich of GC with no TATA case [21]. Reports of different researches indicate that nearly 40% of diversity in meat tenderness is related to calpastatin enzyme activity within first 24 hours after slaughter. All in all, two main mechanisms are related to calpastatin activity: first turnover reduction (synthesis and decomposition) of muscle and increasing skeletal muscle growth, and second, decreasing calpain activity of muscle after slaughter and decreasing meat tenderness and quality [18, 20, 34, 35].

2. CONCLUSION

In recent decades, using methods based on population genetic and statistic develops efficiency of livestock, and composition of these methods with new sciences such as DNA indicators create significant developments in eugenic. Existing polymorphism in production related genes can be applied as genetic indicators, and calpastatin and calpain genes are considered as the main economic genes due to their relation with productive traits, especially meat tenderness and carcass quality. Molecular genetic introduces these two genes as indicator gene of this trait. Using molecular technologies explores mutations with main effect on production efficiency of sheep, lamb and cow.

Due to the close relation of these main genes with genetic markers, they are able to be transmitted as a unit. Calpain activity is related to calcium ion and by proteolysis of myofibrils play key role in meat tenderness after slaughter. The importance of studying these genes is that they create an enzyme system which causes various illnesses in the case of changing enzyme ration of this system.

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