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The Influence of *CAPN1* and *DGAT1* Genes Polymorphism and Meat Quality

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Abstract

This review was conducted with the objective of determining candidate gene (*CAPN1* and *DGAT1*) polymorphism potential on meat quality. Meat tenderness and marbling are the most economically important problem in the beef industry. Genetics and feed management are global problems that adversely affect animal meat quality and the economics of meat production in every country and cause huge financial losses. From the genetic perspective, some studies revealed that meat tenderness is highly associated with *CAPN1* gene polymorphism, bringing about an amino acid replacement glycine to alanine (Gly316Ala) and Isoleucine to Valine (Ile/Val) in the identified genetic marker CAPN1-316 and CAPN530 respectively and meat marbling associated with *DGAT1* gene polymorphism resulting in Lysine (K) to Alanine (A) amino acid change at location 232 K232A. Today meat tenderness and marbling attributes are determined only after an animal gets slaughter and also by using some phenotypic parameter estimations. However, there are many disadvantages associated with such beef animal selection, lengthy, costly, and labour-intensive. Therefore, alternative, live animal meat quality determination like marker-assisted selection methods is promising options. The development of molecular markers and techniques like PCR panel and sequencing will be used to identify the right live animal meat quality.

Keywords: Marbling; Tenderness; Candidate gene

Introduction

In several parts of the developed and developing world, livestock production is one of the principal means of achieving improved living standards. Livestock plays a critical role in both the national economies and the livelihoods of rural communities in sub-Saharan African countries [1,2]. In Africa, Ethiopia has the largest livestock and ranks 5th in the world. The agro-climate of the country is ideal for livestock production and the agricultural sample survey report (CSA, 2016/17) shows that about 59.5 million livestock, 60.90 million livestock, sheep and goats, 2.16 million horses, 8.44 donkeys, 0.41 million mules, and 1.21 million camels play a major role in the country's food and agriculture. In addition, livestock is the source of protein, fuel and, in general, animal products and by-products. Several urban and peri-urban farms are currently major suppliers to consumers of meat and milk products [3-6].

However, a number of factors restrict the productivity of this field, such as poor genetics, shortages of feed, water, treatment and prevention of diseases, and poor housing systems, resulting in low production and low reproductive efficiency. Determining the allelic and genotypic distribution of markers correlated with economically significant characteristics can be a powerful method from a genetic perspective to acquire immediate information to select the productive potential of breeds and populations of livestock [7]. Determination of functional differences such as missense variants and variants in indigenous cattle within upstream and downstream gene regions will enable these variants to be evaluated for their effects on complex traits [8-11].

Meat quality is the primary concern of the livestock industry and the needs of consumers, with appearance, juiciness, flavor, marbling, and tenderness being some of the most important sensory characteristics of meats [12]. Marbling and tenderness of meat are significant quality traits that are often used as a measure of beef products that are fresh and hygienic. Multiple genes are improved by meat tenderness and fatness, which are economically essential characteristics in breeding programs. For breeders and buyers, the selection of animals that have higher meat quality and composition is of great importance.

The Diacyl-Glycerol Acyltransferase-1 gene (*DGAT1*), has 17 exons and mapped on chromosome 14 encodes an enzyme which involved in the conversion of carbohydrates into fat and of maintaining them in fat depots and expressed in many tissues and mammary glands, especially in adipose and epithelial tissues, with the highest levels in the gut [13]. The *DGAT1* gene has also effects on the energy balance of the body and metabolic functions of the blood have also been reported. Previously researchers reported that the dinucleotide variant in the *DGAT1* gene exon 8 at the position 232 resulted in lysine to alanine (K232A) amino acid substitution significantly associated with higher muscle levels of Intra Muscular Fat (IMF). *DGAT1* genes influence the quality of carcasses and may be used as markers for improving the quality of carcasses in cattle [14-16].

The Calpain gene (*CAPN1*) mapped on chromosome 29 and has 24 exons encodes the protease μ-Calpain enzyme which degrades myofibrillar structural proteins and makes the meat tender. A number of reports show that Single Nucleotide Polymorphisms (SNPs) markers have been developed at exon 9 (CAPN316) and exon 14 (CAPN530) for the *CAPN1* gene are highly associated with meat tenderness [17,18].

Therefore, the purpose of this review is:

- To provide scientific information about how the possible candidate genes and their polymorphism effect on meat quality.

Materials and Methods

Trends of quality meat production in Ethiopia

Development of quality meat enables both a good export market and the domestic market of Ethiopia to be served. The total production of meat fluctuates every year because, even though the country has the highest livestock population, meat production is still poor, contributing to about 0.2 Percent of the total production of meat in the world, much of which is cattle, sheep, and goat meat. That ranked Ethiopia as the world's 55th largest meat-producing country. This is not commercially focused due to low off-take rates and meat production (inability to meet the minimum standard needed in the international processed meat market) and beef breeds not yet established, animals sold only in need of cash or when animals become too old after serving for the draft purpose [19].

The indigenous beef cattle breed development programs in the country remain too few, although demand for livestock meat products is increasing dramatically. The genetic improvement of meat animals depends primarily on phenotypic parameters such as body size, horn shape and size, coat colour, hump size and geographical distribution (i.e., characteristics such as weaning weight may be enhanced with little to no change in birth weight, based on genetic patterns in birth and weaning weight at the commercial level. However, young animals often do not have such characteristics assessed by their age, such as carcass characteristics and feed quality, so the genetic test is based on their parents' average). However, it has drawbacks associated with the selection of phenotypic races; it is labour-intensive, narrowing the genetic base of a population, expensive reduction of diversity, it can only be applied to characteristics that are easily tested, time-consuming procedure and it can also contribute to inbreeding, because phenotypic characteristics do not fully quantify genetic variation between populations [20-22].

The long-term research strategy (2015) of the Ethiopian livestock master plan focuses on the development of inside-breed selection programs for indigenous beef breeds; the crossbreeding of superior indigenous breeds; the development of synthetic indigenous breeds; the evaluation of indigenous breed potential for economically significant traits; the development of a strategy and program for genetic enhancement for each major breed of livestock. It is therefore necessary to characterize the phenotypic and genetic potential of Ethiopian indigenous cattle breeds in order to establish effective breeding strategies and to make sustainable use of genetic diversity.

History of Ethiopian beef industry

Cattle can be slaughtered in Ethiopia for the sale of meat within the nation. Often on special occasions and holidays, people have a cultural ceremony to slaughter cows or oxen, not any other animals, and to share the kircha party, which is a popular choice in rural areas where it is difficult to have access to meat. A group of 10 to 20 people typically buy a live animal for meat using phenotypic parameters without knowing the quality of the animal meat quality of the history live animal meat and divide the meat between them and known as kircha meat. This is a way of gathering people and exchanging meat with each other-slaughtering live animals and exchanging meat in groups. Ethiopians regard Kircha as their social capital. In some of the people of Ethiopia towns, the style is also popular, with the elders especially enjoying being involved in the activity [23].

Meat composition

Meat is a complex structure composed of water, proteins, fat and other minerals that are soluble. Depending on the species and breed of the animal, the animal's age at slaughter, the season, and the types of feed used, the components may be quite different. As well as being a source of calories, meat is a valuable source of essential amino acids, minerals, and vitamins. Red meat contains vitamin D and B and is an excellent source of micronutrients such as iron and zinc [24].

Bovine meat quality

The consistency of the composition (fat to lean ratio) and the palatability factors such as visual appearance, odour, firmness, juiciness, tenderness, and flavor are determined. It is a dynamic term and thus has a number of meanings, one of which is the market attractiveness of meat. Meat is a complex structure made up of water, proteins, fat and other minerals that are soluble. Depending on the species and race of the animal, the animal's age at slaughter, the season, and the types of feed used, the components may be quite different. Meat, besides being a source of calories, is an important source of essential amino acids, minerals, and vitamins. Red meat contains vitamin D and B and is an excellent source of micronutrients such as iron and zinc [25-28].

What is quality meat

Meat quality is crucial to the consumer; many issues arise as a result of meat quality to the beef industry as a whole like nutrition-related disease. Before improvement in the quality traits can commence it is important to define meat quality traits and gain an understanding of the factors influencing them. The overall assessment of meat quality, judged by several factors like; Tenderness, Juiciness, flavor, Fatness, Water Holding Capacity, Moisture, Firmness, Odor Flavor and PH.

Tenderness: It is an important palatability attribute, and consumers are willing to pay more for beef which is tender. The extensive body of research on beef tenderness has shown that many different factors influence beef tenderness. Tenderness has been associated with animal age, marbling, muscle location, and genetics. The older the animal, the tougher the meat in general. The higher tenderness quality of meat is achieved with cattle young age and the tougher meat associated with aged cattle.

Colour: It is the consumer's 1st measured element and is an indicator of freshness. Meat colour is based on species, age, and form of muscle, and variations are due to different muscle myoglobin content. The primary pigment in meat is myoglobin. The higher the myoglobin concentration, the darker the meat. The quality of myoglobin is influenced by factors such as exercise, animal diet, genetic and environmental factors. The bright red colour associated with high oxymyoglobin content is a positive quality determinant for red meat, whereas the myoglobin content of brown meat is a negative determinant.

PH: Every animal has a certain amount of energy stored in its muscles in the form of glycogen. Once it dies, glycogen is converted to lactic acid which causes the pH to reduce. This is shown as: muscle glycogen (stored energy) live animal pH 7.1 a conversion to lactic acid adequate levels will result in a pH level lowered to 5.3. The more glycogen, the more lactic acid is produced after it dies, the lower the pH, the darker the colour. The decrease in early post-mortem pH is an important factor affecting µ-Calpain autolysis and activation, with a rapid decrease in the favourable accelerated proteolysis of known calpain substrates. Slow decrease of pH reduction in the muscles, on the other hand, can also lead to an accelerated tenderization rate.

Meat juiciness: Juiciness refers to the quantity of apparent juice that comes from meat during chewing Juiciness and tenderness are closely connected and it is also observed that the more tender the meat, the more juices are released, and the more judgmental the meat is viewed.

Firmness: Meat should appear firm (i.e., neither soft nor tough should be intermediate). When handling it should give under pressure, but not soft and tough.

Marbling: In terms of eating consistency, fat in the beef is one essential property. Marbling or Intramuscular Fat (IMF) is called fat inside the muscle. In a loose network of perimysial connective tissues between the muscle bundles, intramuscular fat is stored within the muscle. Marbling has much stronger and more consistent effects than tenderness on juiciness and flavour. The majority of customers believe that beef that is well-marbled is both juicy and tasty. The fat content is somehow related to meat and tenderness.

Fat and meat flavour: T he most significant element in our food choices is taste.

- "Fat is one of the flavor sources of meat and is especially important for the characteristics of variations in species flavor."
- Fatty components are the consequence of differences in taste between beef, pork, chicken, turkey, and lamb.
- Fatty tissues give them unique profiles of flavor.

By mixing with amino acids from proteins and other ingredients when cooked, fat serves as one of the precursors of taste. It releases flavours as the fat melts and offers a sudden burst of these flavours. Fat consists of a molecule of glycerol attached to three fatty acids by ester bonds and is called triacylglycerol or triglyceride [29].

Results

Genetic influence and meat quality

Genetics significantly influence the consistency of beef. It is well known that in their phenotype feature, distinct genotype cattle breeds differ. Consequently, the nature of meat varies due to the genotype of animals. Meat, for instance, from B. Breeds with indices are less tender than B meat. Cattle Taurus and the magnitude of the B. The effect of indicus varies with the muscle. The lower tenderness is due to decreased B muscle proteolysis of myofibrillar proteins. Indicus linked to higher calcium-dependent protease inhibitor activity.

Candidate genes

For specific phenotypic characteristics such as development, essential trait genes have been adopted through breeding; meat quality and milk yield. These genes are correlated with phenotypic characteristics when candidate genes are identified. The phenotypic variation of interest is determined by candidate genes and are kindly defined on the basis of some prior knowledge of gene function and/or observed relation between phenotypic variance and variation of DNA (polymorphism) or differential gene expression. Candidate genes for the genetic control of phenotypic variations of interest, such as beef consistency, may have causative mutations that may influence gene expression, mRNA splicing or post-translational modifications of the encoded protein in coding sequences or non-coding sequences. DNA variations can affect the translation of all the proteins the body requires into amino acids. Nucleotide changes that alter the structure of the protein by differentiating between amino acids may alter the function of a protein and thus affect its behavior. It is considered a single nucleotide polymorphism if it is a single nucleotide variant. It is possible to determine the SNP genotypes of individual species, and to test hypothetical associations between SNP variants and phenotypic observations. Since the late 1900s, the use and interest of DNA tests as alternatives or enhancements to phenotypic evaluation has increased and the benefits are numerous. Beef animals with desired genotypes, for instance, can be selected for further breeding. Important research on genetic historical relationships can also be done with SNP analysis between cattle populations [30].

Calpain (*CAPN1)* gene

The calpain 1 gene that codes the Calpain Enzyme is mapped on chromosome 29 has 22 exons and the enzyme is reported as the most significant in the meat tenderization process. Shorshimoni reported an association between tenderness and polymorphism of the calpain 1 gene, resulting in a substitution of amino acids in the established markers CAPN1-316 and CAPN530 from glycine to alanine (Gly316Ala) and isoleucine to valine (Ile/Val). The variation in the amino acid has a great effect to the protein function due to amino acid chemical and physical property variation (i.e., Alanine is not much bigger but has a hydrophobic side chain, which glycine is lacking). The same SNP has also been denoted and is incorporated in commercial DNA-tests [31].

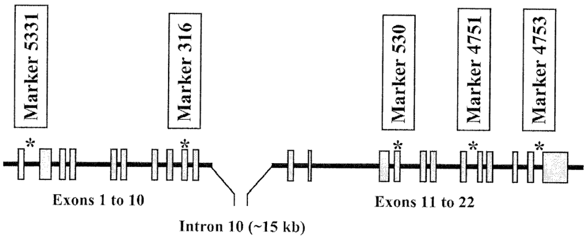

Figure 1: Proximate composition of sundried soybean milk residue.

Meat tenderness was closely related to the two markers (CAPN530 and CAPN316) based on sequences with GenBank accession numbers AF248054 and AF252504, respectively. The markers CAPN530 and CAPN316, named after the amino acid positions and mapped at exons 14 and 9, respectively, correspond to the nucleotide positions 4558G/A and 5709C/G , respectively. Single nucleotide polymorphism in the *CAPN1* marker-316 (alleles C/G) gene lies on exons 9 and marker-530 (alleles A/G) exon 14, both predict amino acid sequence changes.

CAPN1 gene polymorphism

At amino acid number 316 (marker-316), guanine (G-allele) to cytosine (C-allele) trans-version predicts either glycine or alanine, and at marker-530, an adenine (A-allele) to guanine (G-allele) transition predict either isoleucine or valine has shown that alleles C and G have a more significant effect on beef tenderness over the alternative G and A alleles [32]. Animals inheriting the CC genotype had more tender meat **(Tables 1 and 2).**

Table 1: *CAPN1* gene polymorphism associated with meat tenderness quality.

Gene	SNP name	Location	Position	Allele	aa Substitution	Associated trait	Reference
CAPN1	CAPN316	BTA 29 Exon 9	45221250	C/G	Gly/Al	Meat tenderness	(Groupand Group 2017)
	CAPN530	BTA 29	45237834	A/G	Ile/Val		
		Exon 14					

Calpain enzyme

Calpain enzymes in vertebrates are strongly conserved and have many roles, such as intracellular cysteine proteas. One type of the calpain enzymes that degrades structural proteins in the meat is Calpain 1 and thus makes the meat tender. Calpain 1 is calcium-activated and inhibited by the calpastatin enzyme. Two distinct forms of calpain 1 exist; m-calpain 1 and µ-calpain 1. M-calpain 1 is activated by micromolar calcium ions (requires 0.3 mm for half-maximum activity) while micromolar calcium ions activate µ-calpain 1 (requires approximately 50 µM for half-maximum activity). When required, both iso-enzymes of calpain can convert to the other variant.

Diacylglycerol Transferase 1 (*DGAT1)* gene

The *DGAT1* gene encodes an enzyme containing 489 amino acids that catalyses the final stage of triglyceride synthesis by covalently linking triacylglycerol to fatty acyl-CoA substrates. As the primary pharmacodynamics mediates the final stage in Tri-Glyceride (TG) synthetization during assay to direct structure-activity relationships, mostly expressed in the small intestine, liver, and adipose tissues and robust effect were seen [33]. The gene *DGAT1* improves the absorption of fat, with potential consequences for the control of gut hormones. Several studies have shown that the dinucleotide mutation resulting in the replacement of amino acids from Lysine (K) to Alanine (A) in the gene encoding *DGAT1* at position 232 (exon 8) is related to the amount of intramuscular fat in cattle. There is a positively charged side chain in lysine and there is a hydrophobic side chain in alanine, which is a smaller amino acid. Higher enzyme activity has been correlated with the suggested ancestral K allele. The A allele was found to be the most common allele in the majority of the studied beef breeds, according to Asmarasari.

DGAT1 K232A polymorphism

Mapping studies in bovine show that identification of an Adenine/Adenine to Guanine/Cytosine dinucleotide changes in exon 8, which leads Lysine (K) to Alanine (A) amino acid substitution at position 232 K232A .

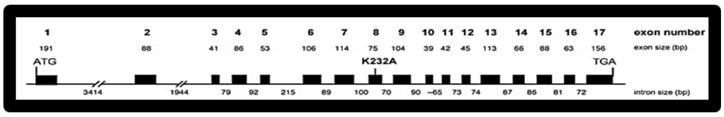

Figure 2: *DGAT1* gene exons location.

The examined SNPs are localized to nucleotide positions 10433 and 10434 in the *DGAT1* protein-coding region (exons 8). The simultaneous replacement by "A" at two positions leads to the disappearance of a restriction site for endonuclease CfrI and a replacement of lysine (K allele) by alanine (allele A) in the protein product causing lysine to alanine amino acid change.

DGAT1 polymorphism

Variant positions 10433 and 10434 are located in exon 8, GenBank no. AJ318490, position 1802265, and 1802266 on chromosome 14 causing a lysine-to-alanine exchange at position 232 of the predicted protein (K232A) [34].

Table 2: SNPs of *DGAT1* gene polymorphism associated with meat marbling quality.

Gene	SNP name	Location	Position	Allele	aa substitute	Associated trait	Reference
DGAT1	DGAT1 K23A	BTA14 Exon8	10433/ 10434	A/G and A/C	Lys/Ala	Intra muscular fat deposition	Shors himon and Cohen-zinder, 2017

K allele of *DGAT1* gene appears to be ancestral allele and the K232A substitution most likely occurred after the separation of the Bos indicus and Bos taurus lineages over 200000 years ago. The lysine allele (AA) variant of *DGAT1* has a positive effect on the milk fat content. The tendency holds for the intramuscular fat content. Thus, the enzyme encoded by the K allele (lysine allele) seems to be the more efficient version of the enzyme concerning triglyceride synthesis than the A allele (Alanine allele). The fact that the lysine variant is consistently associated with higher lipid content of different tissues argues for a causal involvement of this variant in fat content variation. The proposed ancestral “AA” allele has been associated with higher activity of the enzyme.

Diacylglycerol transferase 1 enzyme

It is a microsomal enzyme that uses diacylglycerol and fatty acyl CoA as raw-material to facilitate the final step of triacylglycerol biosynthesis. The triacylglycerol is needed to store energy in cells. The enzyme has also numerous advantages like physiological processes involving triacylglycerol metabolisms such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation, and the like. Triacylglycerol O-acyltransferase1 is one of the key enzymes involved in triglyceride metabolism. This enzyme catalyzes the final stage of triglyceride biosynthesis, using 1,2-diacylglycerol and acyl-CoA as substrates production resulting in an amino acid substitution from Lysine (K) to Alanine (A) at position 232 (exon 8) in the gene encoding *DGAT1* is related to the amount of intramuscular meat marbling in cattle.

Adipose tissue

The adipose tissue which is located within the muscle fiber bundles is termed as marbling in the livestock meat industry. The amount of marbling is generally distinct and is based on numerous factors, such as management, the maturity of the animal, gender, and genetics. The law of meat fats depots which includes inter and intramuscular, subcutaneous fat discovered around the interior organs represents financial importance to industries, however is not properly understood however may additionally be important for the enchantment of carcass fine. The adipose tissue is made of TGs in cattle and is recognized as TG storage tissue. TGs are entering into adipose tissue by way of the circulation by chylomicrons and very small density lipoproteins. The lipoprotein lipase interior the blood capillaries in adipose tissue hydrolyzes TGs maintained inside these lipoproteins. The un-esterified fatty acids are absorbed by way of adipocytes, re-esterified to TGs in most cases via the Kennedy pathway involving *DGAT* and stored in cytosolic lipid droplets. The adipose tissue is hydrolyzed to fatty acids and glycerol when needed by the cell. These compounds are launched into the circulation. Fatty acids are then transported in an albumin-bound shape to tissues such as muscle and liver the place they are oxidized to promote the synthesis of ATP [35].

Discussion

Beef cattle genetic improvement has been highly dependent on phenotypic characteristics like body size, horn shape and size, coat color, hump size, and their geographical distribution. But it has disadvantages associated with selection phenotypic feature breeding, it is labor-intensive, costly, population diversity reduction, time-consuming procedure and it may also lead inbreeding, why because phenotypic traits do not completely measure genetic variation between populations.

So, to develop intentional breeding strategies and sustainable use of genetic diversity, it is important to characterize the phenotype and genotype nature of all the indigenous cattle breed types. Animal genotyping and development of molecular markers and techniques have major importance for breed characterization in animals due to its speedy, simplicity, sensitivity, specificity, reproducibility, Accurate, and cost-effective methods. The development of molecular techniques like PCR, DNA sequencing, and hybridization-based methods provides a promising option for the rapid identification of traits within days. Furthermore, late expressed traits could also consider, so that avoids false-negative selection of what conventional methods do. Genetics greatly influences most livestock production that is why lack of genotypic information breeding limits the utilization of Ethiopian indigenous cattle breed.

The identification of molecular markers for genetic analysis has led to a great increment in our knowledge of livestock genetic features and our understanding of the structure and behavior of animal genomes. For example, Selection of breed for pork purpose is a very massive contributor to selections affecting genetic growth in contemporary pork cattle improvement with increasing knowledge of position and results of most important loci for quantitative variation, amendment of normal selection processes based only on phenotypes. Molecular data will help get rid of undesirable alleles and increase favorable alleles (genetic gain). In recent years, the pork cattle demonstration of genetic polymorphism at the DNA sequence stage has provided a large variety of marker strategies with a variety of applications [36,37].

However, the use of SNP marker-based data for meat quality genetic gain is the desire of a gorgeous marker system. A range of markers has been used in meat satisfactory testing. In recent years, SNP markers have been used, because of their high polymorphism records nature, significant distribution in the genome, the type of samples that can be used, the possibility to technique quite a few samples at the same time and the reality that the effects are effortless to interpret. MAS are starting to be implemented in nucleus breeding programs. Trait heritability is the most necessary issue influencing the effectiveness of MAS. MAS seem to be most promising for traits with low heritability. But trait heritability is additionally of primary importance for accuracy in the mapping of QTLs. Low heritability reduces the strength of detecting QTLs, which is primarily based on a correlation between phenotype and marker genotype. This could mean that for well-mapped QTLs MAS may additionally add little to phenotypic selection; whilst for characteristics with very low heritability, the underlying QTLs can't be identified. It is the region between these two intense cases that look most promising for the application of MAS. If QTLs can be mapped for a trait having a low heritability the accuracy of the QTL role may also not be very high, which is mirrored in a massive QTL support interval on the genetic map. It was also demonstrated that beef breeds were characterized by lower values for collagen content, compression and shear force in raw and cooked meat respectively, compared to dairy or dual-purpose breeds. Texture differences between animals and breeds decreased with ageing time. Differences in quality between breeds are often less than among animals within breeds.

Conclusion

Ethiopian livestock resources potential is not fully exploited and the production and productivity of this sector are still low and products being produced are of reduced quality no more value-added practice. Quality remains a key driving force within the beef industry. Tenderness and marbling are important palatability characteristics to evaluate the eating quality of beef. Marbling and tenderness are most often discussed as the fundamental determinant of a beef product's performance concerning eating quality. As a result, tender and marble meat yield, eating qualities attribute, and human nutritive value (proximate composition) are known as the most important quality determinant that requires attention for the future.

Tenderness is one of the most important meat palatability attributes and determined during chewing and can be measured by Warner-Bratzler Shear Force (WBSF), sensory analysis, and Slice Shear Force. Marbling represents the intramuscular fat deposited within the muscle in a loose network of perimysial connective tissues, between the muscle bundles. Most consumers agree that well-marbled beef is both juicy and tasty. Beef eating quality traits can be influenced by genetics and a wide range of management factors. In Ethiopia eating quality attributes standards concerning consumer safety and shelf life of meat have not been fully developed. The country mostly has beef standards measurements like chemical composition and microbiological content. Universities and research institutes capacitate beef cattle producers with knowledge and skills through training and they consider as priority research thematic area.

Conflict of Interest

The authors declare that there was no conflict of interests.

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