# LPL Genotypes in Meats' Chemical Content and Physiological Traits for Local and Imported Colombian Bull Calves' Carcasses

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## ABSRACT

This study was conducted using a sample of (30) bull calves partitioned into (15) local calves and (15) imported, Colombian calves, of close ages, from different areas in Basra. This study aims at getting known the impact and LPL Gene Polymorphism in the chemical content for the thigh region and physiological traits in both breeds, and disclosing the impact of gene polymorphism on studied traits.

The study includes taking samples of blood and meat from slaughtered animals. The Sequencing technology was adopted to determine mutations of Site 158 LPL gene. Study has shown that there were changes in in nitrogenous bases, especially in location C>T110 of LPL gene, which is considered a substitution mutation.

Also, the study has revealed that SNP at location 110 is of three genetic makeups (TT, CT & CC) for both breeds (local and imported). This was not changed by amino acid, Glutamine (E). study didn't reveal any significant differences as to the two traits: moisture and ash percentage for all genetic makeups in both breeds, while genetic makeup (TT) recorded the highest

significant value for the breed of imported calves ($p<0.05$) to traits of percentage of protein and fat.

Also, the study showed a significant predominance ($p<0.05$) for genetic makeups of the breed of imported calves as to the level of cholesterol, triglycerides, LDL and VLDL, while genetic makeups in local calves recorded the highest significant predominance ($p<0.05$) as to HDL level in blood serum.

## INTRODUCTION

The recent development that the science of molecular biology and DNA analytical methods witnessed during the last two decades has opened the door widely to a new era, where selection of farm domesticated animals is conducted in a precise and fast manner (Milan et al., 2017. Besides, the rise of molecular technologies has made available possibilities to use procedures of genetic enhancement though direct selection of a domesticated animal depending on specialized genes or specific regions of an individual genome.

The LPL is considered one of the genes that influences quality of meat, and a gene contains chemical markers at the level of DNA necessary to produce lipase enzyme primarily available at on cell's surfaces lining blood vessels inside muscles and fatty tissues (Okazaki et al., 2002). In a study conducted by Sevane et al. (2013), it was found that diversity of LPL genes in some livestock breeds is correlated with an increase in fatty acids (*Gamma*–Linolenic acid and Arachidonic acid). This gene lies on chromosome 2 in sheep (www.animalgenome.org/cgi-bin/QTLdb/OA). As to pigs, it lies on chromosome 14 (Gu et al. (1992), while in cows, it lies on chromosome 8, and its codon consists of (10) exons, that can be expressed largely in skeletal muscle and fatty tissues (Holmes et al., 2011).

Also, in a 2012's study carried out by Wang et al. disclosed that polymorphism of (T355420C and A355427T) for LPL gene is correlated with growth traits in livestock Xiangxi. Besides, Oh et al. (2013) stated availability of three new SNPs (G322A, A329T and G1591A) in the LPL cow's gene, which can affect formation of fatty acids and traits of a carcass in Korean livestock. In Bos grunniens, the difference of C19913T is correlated with the mean of a daily weight increase of a carcass' weight (Ding et al., 2012). And due to the importance of LPL gene in the use of fatty acids, the activity of LPL in tissues may have significant impacts on energy metabolism and repartition of fatty tissues, which could affect content of muscular fats and sensual quality for meats in meat animals (Saez et al., 2009).

## MATERIALS & METHODOLOGY

This study was carried out on (30) male bull calves, local and imported (Columbian) at different places in Basra city. Blood was withdrawn from calves via vein jugular before slaughtering, and meat was taken from the thigh area.

## STUDIED TRAITS

1. Meat Chemical Analysis

Moisture, protein, fat and ash were estimated pursuant to the prescribed method A. O. A. C. (2008).

2. Biochemical standards

The concentration of Triglycerides, Cholesterol and DHL were measured suing the Kit device furnished by the French Biolabo SA Company. Also, low-density fatty proteins were measured in the blood serum using the following equation:

LDL = Cholesterol – (HDL +VLDL

Where VLDL = Triglycerides/5 (Friedewald et al., 1972).

## DNA ISOLATION PROCESS

Isolation process was carried out according to markers accompanied the Kit device supplied by the Chinses TIANEN Kit Co. The DNA concentration µl/ng was measured for each sample. Also, purity was measured through reading absorption at 260 degrees and 280 nm using Nanodrop Spectrophotometer. After making sure of the genome availability, samples were sent to Yang ling Tianrun aoka biotechnology, China, in order to get actual sequences of nitrogenous bases for genes' required nucleotides. It is important to mention that the sequencing process was conducted on a single DNA strip, that is Forward, and as per our request, and in order to identify genetic mutations, the said isolation process made use of starters selection such as (Forward and Reverse), which are connected with two strips of a gene segment LPL and strip (3-5) for enlarging the studied region of a gene.

**Table 1: Selection of Starters of Gene LPL**

| Sequence | Gene and Reference | # |
|---|---|---|
| F: 5− TTAACGAACCCGACTAGCATCC−3 | LPL (Wang *et al*. 2016) | 1 |
| R: 5− CACCACAGCCACAGCAACTC−3 | | |

**STATISTICAL ANALYSIS**

The ready-made statistical program (SPSS), version 27 (SPSS 2020) was used. Significance of differences among studied means was tested at a probability level of (0.05). Before conducting a statistical analysis. Data was corrected for each of age and management through considering it as deviation at the mean of the age group.

**RESULTS AND DISCUSSION**

First of all, variations were seen in nitrogenous bases, especially at location C>T110 of LPL gene, which is considered as a substitution mutation. Three genetic makeups of SNP (TT, CT & CC) appeared at location 110 for both breads (local and imported, which did not affect amino acid, Glutamine. A study conducted, on Korean livestock, by OH et al. (2012) revealed that there are five types of genetic mutations, three of which are correlated with a carcass weight, one correlated with sweat fat and other with fat thickness. On the other hand, Gui et al. (2015) found three types of genetic makeups; two are available in intron 5 (C18306T & C18341T), one in exon 6 (G18362A).

Besides, a mutation leads to a change in glycine 325 to serine. While, seen types of mutation in LPL gene on Qinchuan livestock, most of which are substitution mutations. One of the mutations was a transfer mutation, where the nitrogenous base changed from purine into pyrimidine, as a result of a study conducted by Gui et al. (2016). The study came up with prominent changes occurred to growth and quality of a carcass. This was evidence that LPL gene can be used as a molecular marker to enhance growth quality traits for a livestock carcass through more use of MAS.

Table (2): Changes occurred to Nucleotides and Genetic Codes for LPL Gene

| Mutation type | Amino acids | Codes | Nucleotides | Location | # |
|---|---|---|---|---|---|
| Synonymous | E>E | ACG>ATG | C>T | 110 | 1 |

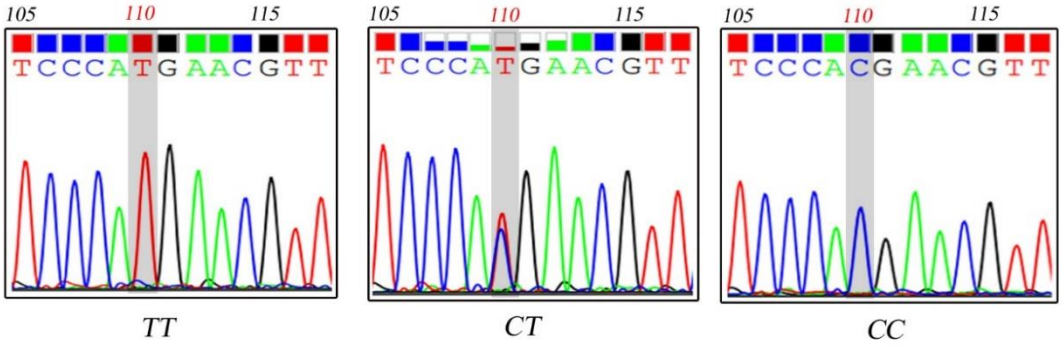


*Figure 1: Locations of Change in Nitrogenous Bases for 158 Site TG in Genetic Sequence Reactions*

**Chemical Analysis for Thigh Region**

First of all, table (3) indicates that there is no significant impact of the LPL multiple genetic manifestations for a thigh region to local and imported breeds in moisture percentage. These results were consistent with Jukna et al. (2017) in their study on the impact of a breed on a meet chemical analysis. As to the quantity of moisture, the study found that there were no differences among breeds. Also, table (3) made a reference to a significant superiority of the genetic makeup TT (21.52) for LPL gene in location 110 of thigh region in imported calves at ($p<0.05$) to other genetic makeups as to a trait of protein percentage in meat. Besides, a study carried out by Xie et al. in 2012 showed impact of a group of livestock breeds on the quality of meat. The study revealed that the Limousin breed contains less content of a dried substance and high content of protein.

Moreover, Ardicli et al. (2017) stated that the functional importance of genes along with the substitution of amino acids produced by multiple manifestations for a mono nitrogenous base (SNPs) might get resulted protein structures varied, which results in a change in the mRNA interpretation and mechanisms of a

protein synthesis. Then, this molecular genetic marker has a main role in the process of calves' selection on the basis of difference in the level of DNA. Besides, reliance on direct genetic markers already identified, which correlated with fat deposition and weight increase in the program of calf breeding in Iraq would lead to a faster genetic progress for the purpose of obtaining livestock of good weights.

As table (3) made it clear, the LPL gene, of the imported breed, significantly predominated over local breed as to percentage of fat in thigh region. Also, table (3) disclosed no significant differences as to ash percentage in thigh region in the breeds of local and imported calves.

**Table (3): Impact of a Breed and Genetic Makeup for Gene 110 under Study on Meats in Different Regions of a Carcass**

| Thigh | | | | Number | Genetic makeup | Breed | SNP | Gene |
|---|---|---|---|---|---|---|---|---|
| Ash | Fat | Protein | Moisture | | | | | |
| 1.80 | 2.06b | $20.43^{d}$ | 71.40 | 4 | CC | Local | Sequence 110 (C>T) | (LPL) |
| 1.50 | 2.47b | $20.71^{c}$ | 72.50 | 9 | CT | | | |
| 1.32 | 2.84b | $20.81^{c}$ | 73.12 | 2 | TT | | | |
| 1.25 | 3.09ab | $20.87^{c}$ | 73.42 | 3 | CC | Imported | | |
| 1.17 | 3.34a | $21.13^{b}$ | 74.16 | 5 | CT | | | |
| 1.11 | 3.43a | $21.52^{a}$ | 74.56 | 7 | TT | | | |

Means that hold different letters for the single sequence within a gene for each trait are significantly differs at ($p<0.05$).

**BIOCHEMICAL TRAITS**

As mentioned in figure (2), the level of cholesterol in blood serum of a calf that carries LPL gene reached at a mean of 213.67, 224.74, 230.46 mg/100 ml in bold of local calves, which hold the 110 location for genetic makeups CC, TC and TT respectively. And mean of cholesterol reached at 238.84, 239.20 and 244.4 mg/ml for same genetic makeups in imported calves respectively. As revealed in figure

(2), there was a decrease in the level of cholesterol for the genotype CC in comparison with the two genotypes (TC & TT). Besides, original genotypes of calf breed have adapted to dry and hot environments, shortage of food and disease resistance, and therefore it would belittle of fat in meat and cholesterol.

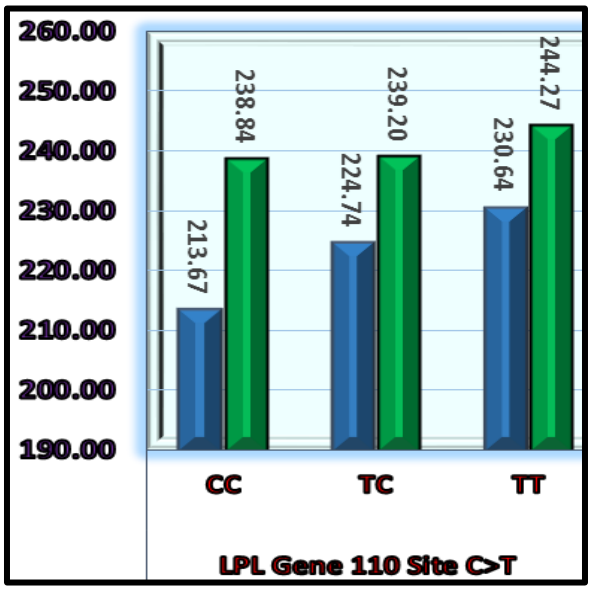


**Figure (2): Impact of Breed and Genotype for LPL (110) Gene in Level of Blood Serum's Cholesterol**

As to figure (3), results indicated that the highest mean of Triglycerides was in the blood of imported calves, of the genotype (TT), carrying LPL gene, where mean was 189.12 mg/100 ml Site T<C 110 for the same gene. The LPL gene plays a crucial role in chemical interactions and metabolic pathways that lead to synthesizing fatty acids. These fatty acids can be released through hydrolysis that occurs in fats and oils. The activity of this gene can be enhanced in fatty tissues and a subsequent increase in deposition of Triglycerides by insulin (Wang et al., 2009).

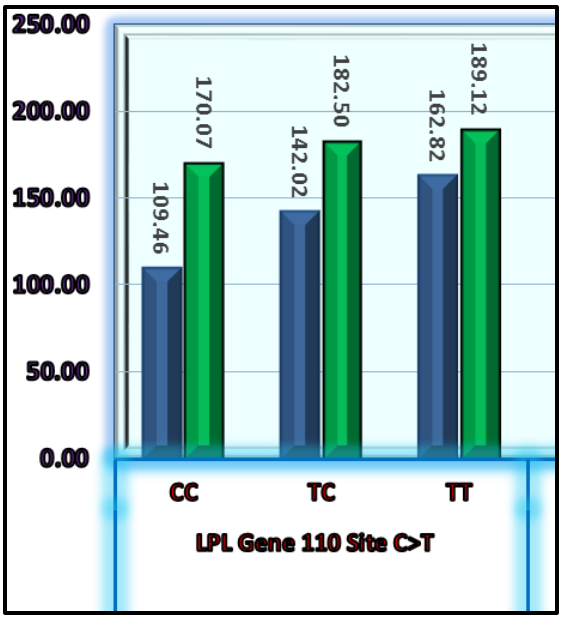


**Figure (3): Impact of Breed and Genotype for LPL (110) Gene in Level of Blood Serum's Triglycerides**

Moreover, results pinpointed in figure (4) disclosed that the level of LDL in bold serum of imported calves, carrying LPL gene, has the highest significance ($p<0.05$) compared to local calves for all genotypes at location C>T Site 110. The highest mean of LDL was 94.11 mg/100 ml for location 110, genotype TT, while its lowest mean recorded 33.41 mg/100 ml for the genotype CC. Okazaki et al. (2002) came up with a result stating that an LPL gene is one of the genes that influences the quality of meat. The LPL gene contains chemical markers on the level of the DNA necessary for a lipase enzyme production primarily available on cells' surfaces lining blood vessels inside muscles and fatty tissues.

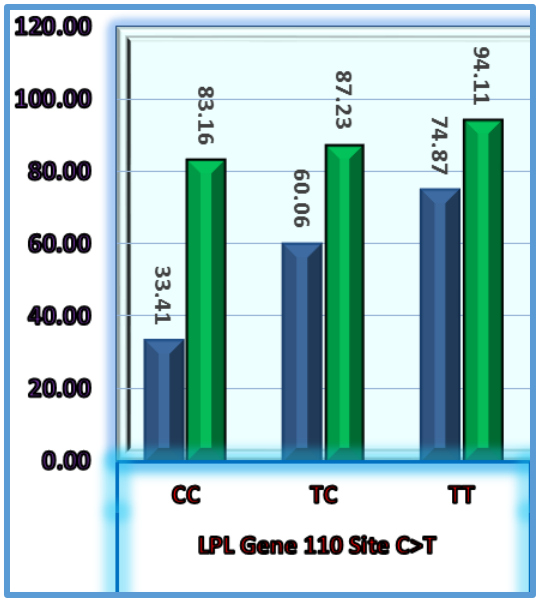


**Figure (4): Impact of Breed and Genotype for LPL (110) in LDL's Blood Serum**

Regarding results shown in figure (5), it was indicated that the lowest level of HDL was 112.33 mg/100 ml in calves' blood, of the genotype TT for LPL gene, Site 110. This was attributed to the importance of LPL gene in the usage of fatty acids. The LPL activity in tissues may have significant impacts on an energy metabolism fatty tissues repartitioning, which could affect the content of muscular fats and sense quality of meats in animal meats (Saez et al. (2009).

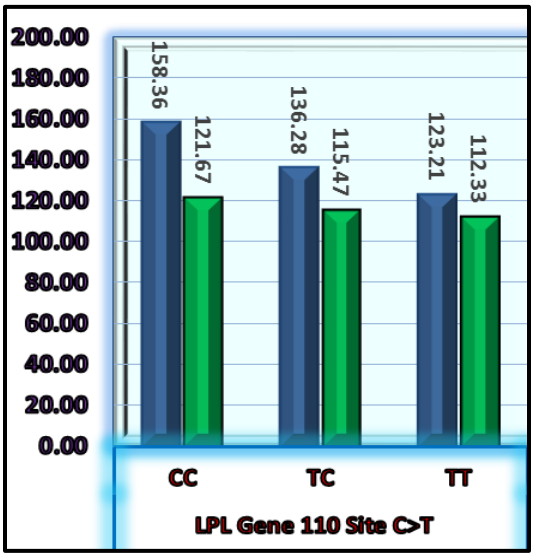


**Figure (5): Impact of Breed and Genotype for LPL (110) in HDL's Blood Serum**

First, the results disclosed in figure (6) brought to light a significance increase ($p<0.05$) in the VLDL level in imported calves' blood compared with local calves. Second, the said figure made it clear that the genotype (TT) VLDL was 29.10 mg/100 ml in imported calves, on the other hand, there was a significant decrease in the same level for genotype (CC) at 16.84 mg/100 ml in local calves' serum. The reason may be attributed to the role played by LPL in the breakdown of fatty proteins rich in Triglycerides for providing muscles, the skeleton, heart, fatty tissues with fatty acids for energy or storage. The LPL gene is correlated with fatty proteins (LDL & VLDL) independently of its enzymatic activity, and mediated its absorption through LDL and VLDL receptors (Goldberg et al., 2002).

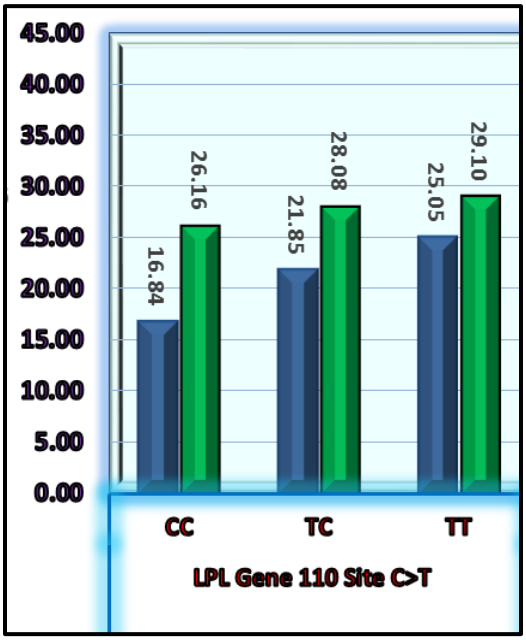


**Figure (6): Impact of Breed and Genotype for LPL (110) in VLDL's Blood Serum**

**REFERENCES**

**A.O.A.C. (2008). Official Method of Analysis.17 th ed.** Association of official Analytical Chemists. International Arlington Virginia, U.S.A

**Ardicli, S., Dincel, D., Samli, H., & Balci, F. (2017).** Effects of polymorphisms at LEP, CAST, CAPN1, GHR, FABP4 and DGAT1 genes on fattening

performance and carcass traits in Simmental bulls. Archives Animal Breeding, 60(2), 61-70.

**Ding, X. Z., Liang, C. N., Guo, X., Xing, C. F., Bao, P. J., Chu, M., ... & Yan, P. (2012).** A novel single nucleotide polymorphism in exon 7 of LPL gene and its association with carcass traits and visceral fat deposition in yak (Bos grunniens) steers. *Molecular biology reports*, *39*(1), 669-673.

**Friedewald , W. ; Levy , R. and Fredrickson , D.** (1972) . Estimation of concentration of low density lipoprotein cholesterol in plasma without use the preparative ultra centrifuge . Clin. Chem. 18 : 499-509.

**Gu, F., Harbitz, I., Chowdhary, B. P., Davies, W., & Gustavsson, I. (1992).** Mapping of the porcine lipoprotein lipase (LPL) gene to chromosome 14q12→ q14 by in situ hybridization. *Cytogenetic and Genome Research*, *59*(1), 63-64.

**Gui, L. S., Huang, Y. M., Hong, J. Y., & Zan, L. S. (2016).** Polymorphisms in the LPL gene and their association with growth traits in Jiaxian cattle. *Genet. Mol. Res*, *15*(2).

**Gui, L., Jia, C., Zhang, Y., Zhao, C., & Zan, L. (2016) a.** Association studies on the bovine lipoprotein lipase gene polymorphism with growth and carcass quality traits in Qinchuan cattle. *Molecular and cellular probes*, *30*(2), 61-65.

**Holmes, R. S., Vandeberg, J. L., & Cox, L. A. (2011).** Comparative studies of vertebrate lipoprotein lipase: a key enzyme of very low density lipoprotein

metabolism. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *6*(2), 224-234.

**Jukna, V., Jukna, C., Prusevičius, V., ME KINYTĖ-KAU ILIENĖ, E., & Pečiulaitienė, N. (2017).** Meat quality of different beef cattle breeds fed high energy forage. *Zemdirbyste-Agriculture*, *104*(3).

**Merkel, M., Eckel, R. H., & Goldberg, I. J. (2002).** Lipoprotein lipase. *Journal of lipid research*, *43*(12), 1997-2006.

**Milan P. P. MuslicD.R. Petrovic,V.C. Maksimovic,N. Cekic,B. Y. A. Yuldashbaev,A and Selionova,M.(2017).** Trends And Challenges In The Genetic Improvement Of Farm Animals. Review paper. Proceedings of the 11th International Symposium Modern Trends in Livestock Production. BELGRADE – SERBIA.

**Oh, D., La, B., Lee, Y., Byun, Y., Lee, J., Yeo, G., & Yeo, J. (2013).** Identification of novel single nucleotide polymorphisms (SNPs) of the lipoprotein lipase (LPL) gene associated with fatty acid composition in Korean cattle. *Molecular biology reports*, *40*(4), 3155-3163.

**Okazaki, H., Osuga, J. I., Tamura, Y., Yahagi, N., Tomita, S., Shionoiri, F., ... & Ishibashi, S. (2002).** Lipolysis in the absence of hormone-sensitive lipase: evidence for a common mechanism regulating distinct lipases. *Diabetes*, *51*(12), 3368-3375.

**Saez, G., Davail, S., Gentes, G., Hocquette, J. F., Jourdan, T., Degrace, P., & Baéza, E. (2009).** Gene expression and protein content in relation to

intramuscular fat content in Muscovy and Pekin ducks. *Poultry Science*, *88*(11), 2382-2391.

**Sevane, N., Armstrong, E., Cortés, O., Wiener, P., Wong, R. P., Dunner, S., & Gemqual Consortium. (2013).** Association of bovine meat quality traits with genes included in the PPARG and PPARGC1A networks. *Meat Science*, *94*(3), 328-335.

**Wang, H., & Eckel, R. H. (2009).** Lipoprotein lipase: from gene to obesity. *American Journal of Physiology-Endocrinology and Metabolism*, *297*(2), E271-E288.

**Wang, W., Xue, W., Xu, X., Jin, B., & Zhang, X. (2016).** Correlations of genes expression in PPAR signalling pathway with porcine meat quality traits. *Czech Journal of Animal Science*, *61*(7), 333-339.

**Wang, X. P., Luoreng, Z. M., Li, F., Wang, J. R., Li, N., & Li, S. H. (2012).** Genetic polymorphisms of lipoprotein lipase gene and their associations with growth traits in Xiangxi cattle. *Molecular biology reports*, *39*(12), 10331-10338.

**Xie, X., Meng, Q., Cui, Z., & Ren, L. (2012).** Effect of cattle breed on meat quality, muscle fiber characteristics, lipid oxidation and fatty acids in China. *Asian-Australasian journal of animal sciences*, *25*(6), 824.