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Thyroglobulin Gene Polymorphism in Fatty Acids for Back Area in Local and Imported Colombian Bull Calves

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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 Thyroglobulin Gene Polymorphism in the quality of fatty acids at back area for both species and impact of genetic makeup on studied criteria. The study includes taking samples of blood and meat from slaughtered animals. The Sequencing technology was adopted to determine mutations of Site 158 TG gene. Percentages of fatty acids in flesh were calculated using a device called (GC-MS) available at laboratories of the Ministry of Sciences and Technology. Study has shown that there was a substitute mutation, where the Nucleobases, of the same type, pyrimidine, had been changed, into pyrimidine. Also, the study has revealed that 158 SNP is of three genetic makeups (TT, CT & CC) for both breeds (local and imported). Besides, the study has come up with a fact that there's a correlation between genetic makeups for the Thyroglobulin Gene at a sequence 158 (C>T) significantly ($p<0.05$) with each of the palmitic fatty acids C16:0, SFA & PUFA, where the genetic makeup TT predominated significantly ($p<0.05$), and a breed mean for imported calves' species other genetic makeups CC & TC for both breeds, while the genetic makeup CC and the mean of species were significantly superior to species of local calves ($p>0.05$) at the concentration of mono-*unsaturated*- oleic acid and PUFA. Moreover, the mean of local calves' breed has recorded, as to UFA, IC 14 and IC 16 the highest predominance ($p<0.05$). Therefore, the genetic makeup of Thyroglobulin Gene can be relied on in the process of selection for meat quality purposes.
Keywords: Thyroglobulin Gene Polymorphism, Fatty Acids, Colombian Bull Calves

INTRODUCTION
Globally, meat manufacturing has experienced an intense change in response to the rise in demand for the product of best quality such as deposit of muscular fat in livestock at the expense of external fat, then getting known genes correlated with quality and traits of carcass for fat deposit. Genes are considered one of the basic patterns for the human orgasm, through which the body passes multiple stages. It enhances capability of the orgasm in different stages. Genes are responsible for vital functions in the body. Also, genes enable an orgasm to follow up internal chemical reactions in the body. Functions of body differs from one place to another in the body. These functions are largely responsible for driving body into crucial stages of its life because

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genetic mutations might cause lots of diseases, and a gene determines everything in an orgasm staring from formation of quality and quantity features, ending with the public health (Ibrahim *et al*, 2014).

Among these genes is Thyroglobulin Gene TG, which represents the gene of thyroid. It is well-known that hormones of thyroid play an important role in regulating metabolism. This gene might affect fat metabolism and growth of adipose cells (Carvalho, 2012).

The study aims at:

Getting known genetic appearances for TG genes using the technology of nucleotides DNA sequences, quality of fatty acids connected with muscles and correlation among different types of fatty acids and biochemical parameters of blood.

The region which precedes the first exon, known as Regulatory Region or Promoter, includes Thyroglobulin Gene TG. Mutations (SNP) were found among samples, and location 158 C>T in determining genetic makeups among samples because these locations are the most frequent in many samples registered in NCBI depending on results of Blast. Results of nucleotides DNA sequences' analysis have shown changes in Nitrogenous Bases specifically in location 158 C>T, and it is to be considered substitution mutation when there is a change occurred to the Nitrogenous Bases of the same type; pyrimidine to pyrimidine. The SNP 158 has revealed three genetic makeups (TT, CT & CC) for both species, local and imported ones.

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 back area. Isolation process was conducted in line with instructions accompanied the Kit provided by the Chinese TIANEN Kit Company. Concentration of DNA in (µl/ng) for each sample was measured, and pureness was measured as well through reading absorption at 260 and 280 nm by using a device called (Nanodrop spectrophotometer). After making sure of the availability of genome, samples were sent abroad, specifically Yang ling Tianrun aoka biotechnology, China in order to get actual sequencings for nitrogenous bases for the required Genes' nucleotides. Sequencing process was made for a single strip of DNA, that's Forward DNA. As per our request, for the purpose of determining genetic mutations, it's emphasized the importance of cultures selection for each of Reverse and Forward which are connected with the two strips of thyroglobulin gene nucleotide and strip (3 – 5) for amplifying the studied region of the gene.

Table (1) Selection of Cultures for Thyroglobulin Gene

Sequencing	Gene & Reference	#
F: 5'-GGGGGATGACTACGAGTATGAC-3'	TG5	1
R: 5'-AGCAGACCGAAGACCCATAG-3'	´ (Wu *et al*., 2005)	

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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.

Regression coefficient = B0, b1, b2

Also, correlation coefficients were calculated using Orgien Pro 92021) between blood criterions and fatty acids, and percentages of fat in nucleotides.

The total fatty acids were calculated at the back area of calves using an analysis via a device called Gas chromatography–mass spectrometry (GC-MS) in the Central Laboratory of the Ministry of Sciences and Technology. The method aimed at transferring normal free fatty acids into methyl ester. Later, the equation pertaining to figuring out concentration was applied;

Sample concentration = sample area /standard area X concentration of standard

RESULTS AND DISCUSSION

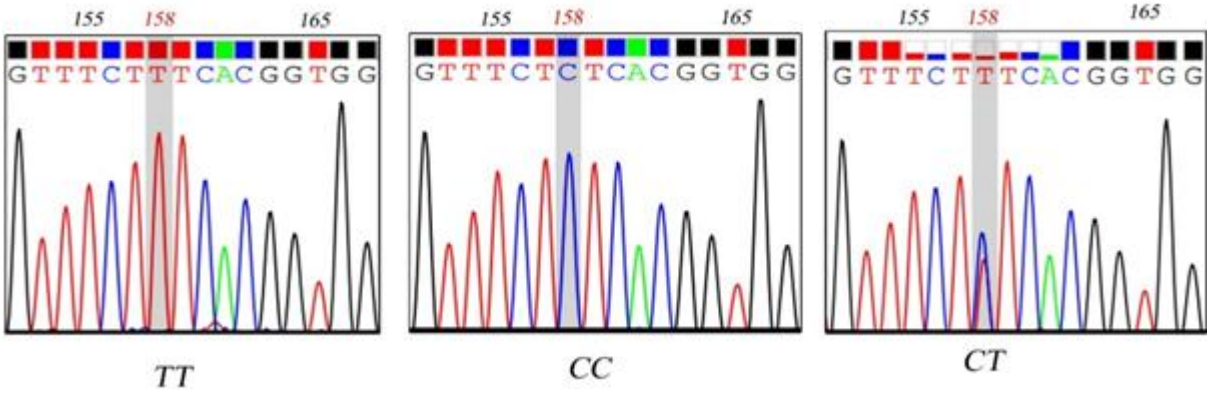

Figure 1: Locations of Change in Nitrogenous Bases for 158 Site TG in Genetic Sequence Reactions

The Impact of Breeds and Genetic Makeup for Percentage of Fatty Acids of Calf's Back Area

Genetic makeups of Thyroglobulin Gene, in the sequence 158 (C>T) were significantly ($p<0.05$) correlated with each of the fatty acids, palmitic C16:0 and oleic C18:1, which both account for the highest percentage among fatty acids in the fat of local and imported breeds of calves, table (2), where the genetic makeup ($p<0.05$) TT was superior to the two genetic makeups (CC & TC) for both species in the saturated fatty concentration, palmitic, as well as the mean of imported breeds was significantly superior to local one ($p<0.05$) in the concentration of saturated fatty concentration, palmitic.

While the genetic makeup CC of the local breeds of calves in the unsaturated mono fatty acid, oleic, was significantly ($p<0.05$) superior to other two genetic makeups TT and TC. As to the local species, it was superior to the imported species significantly at concentration of oleic fatty acid, table (2), and although stearic fatty acid recorded percentages not exceeding %20 for all genetic makeups, for both species, it was not affected significantly by the difference of genetic makeups for Thyroglobulin Gene.

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Also, imported species, in concentration of fatty acid, Linoleic (GLA), displayed significant superior to local breeds, where concentration for both breeds was (0.37 and 0.29) respectively. This fatty acid constitutes a small percentage in the products of ruminants, and studies made a reference to the fact that quality of GLA required for beneficiary impacts in the food system is much greater than that found in meat of beef (GEBAUR et al., 2011).

Generally, the local species was significantly superior to imported one in the concentration of unsaturated mono and whole fatty acids, while imported breed was significantly superior to local one at ($p<0.05$) in the concentration of saturated fatty acids and multiple unsaturated fatty acids. This indicates that flavor of meat of local species is better than imported species due to its large content of mono saturated fatty acids and less content of multiple unsaturated fatty acids. The rise of PUFAs in meat increases its vulnerability to oxidation and meat production of undesirable sense properties, and in return, a high concentration of mono unsaturated fatty acids enhances taste of meat (Crespo-Piazuelo et al., 2020).

On the other hand, the ratio of multiple unsaturated fatty acids to saturated fatty acids was (p: s), which was within the ratio adopted by the British Ministry of Health (1991). This ratio stresses that the ideal range for this ratio lies between (0.04-4.0). This ratio can be supported through modern studies and evidence discloses that substitution of SFA with PUFA is good for health (Lunn and Theobald, 2006 & Hooper et al. 2015). Studies indicated genetic effects on fatty acids formation have had a clear role on the genetic variation among livestock species and existence of differences in fatty acids formation and carcass's fats. This results from differences in the total of deposited fats. Besides, animals of double muscles have low levels of fats in muscles, which leads to the rise of BUFA, as a percentage of total fat, especially LA, in comparison with a natural genotype in the Belgian Blue cows' species (Wood et al., 2004). A comparative study involving four pure species found that White White had less concentrations than TL in muscles, and higher ratios than LA, and ratio of P: S was higher in muscles of Longissimu and Psoas than Berkshire and Duroc (Wang et al., 2012).

A study conducted on breeds of Holstein Friesians and Aberdeen Angus calves, values of Aberdeen Angus's carcasses were higher than TL in muscles, calves of Aberdeen Angus had lesser ratios than in PUFA, and fatty acids formation in muscles were less affected by the breed in comparison with the food system. And PUFA was deposited in higher quantities and ratios in Holstein Friesians, especially those calves feed on roughage than Aberdeen Angus calves (Wang et al., 2012).

This refers to a real genetic difference among breeds in metabolism for fats, as there was an increasing interest during the last ten years in developing genetic markers in order to form fatty acids. This includes identifying parts of the nucleic acid (locations of quantity markers, QTLs, multiple forms of single nucleotides and SNPs) correlated with fatty acids formation. Many filtered genes, organizing metabolism for fats, were identified, resulting in differences in fatty acids formation (García et al., 2012)

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Table (2): Impact of Breed and Genetic Makeup of Sequence 158 C>T) for Thyroglobulin Gene at Ratios of Fatty Acids in the Back Muscle

Breed								Fatty acids
Imported				Local				
Mean	TT	TC	CC	Mean	TT	TC	CC	
0.09	0.09	0.11	0.06	0.04	0.05	0.04	0.02	**C12:0(lauric)**
5.91	6.31	5.61	5.82	3.70	4.30	3.47	3.13	**C14:0(myristic)**
0.66	0.76	0.67	0.54	0.34	0.39	0.31	0.28	**C15:0(pentadecanoic)**
28.26^{A}	29.23^{a}	28.05^{b}	27.51^{c}	24.72^{B}	26.30^{d}	25.41^{e}	23.05^{f}	**C16:0(palmitic)**
0.82	0.90	0.78	0.78	0.68	0.73	0.71	0.63	**C17:0(margaric)**
17.45	17.87	17.41	17.06	16.14	16.41	16.32	15.83	**C18:0(stearic)**
0.63	0.60	0.64	0.64	0.86	0.78	0.84	0.94	**C14:1 c9(myristoleic)**
2.32	2.02	2.38	2.56	3.25	2.97	3.25	3.54	**C16:1 c9(palmitoleic)**
34.11^{B}	33.37^{d}	34.30^{c}	34.66^{c}	36.79^{A}	35.91^{b}	36.14^{b}	37.76^{a}	**C18:1 c9(oleic)**
3.36	3.59	3.45	3.03	2.48	2.69	2.54	2.27	**18:2c9,c12(linoleic)**
0.33	0.34	0.35	0.30	0.22	0.25	0.24	0.19	**18:3n-3(linolenic)**
0.37A	0.37	0.40	0.34	0.29B	0.32	0.31	0.26	**18:2c9,t11(GLA)**
0.24	0.26	0.26	0.20	0.14	0.16	0.15	0.12	**C20:3n-6(eicosatrienoic)**
0.79	0.84	0.77	0.74	0.63	0.68	0.64	0.58	**C20:4(arachadonic)**
0.31	0.35	0.31	0.27	0.22	0.25	0.24	0.19	**C20:5(timnodonic)**
0.42	0.48	0.43	0.36	0.29	0.32	0.30	0.25	**C22:5(docosapentaenoic)**
53.19^{A}	55.17^{a}	53.08^{b}	51.32^{c}	45.61^{B}	48.18^{d}	46.26^{e}	42.95^{f}	**SFA**
37.06^{B}	35.99^{e}	37.31^{d}	37.87^{d}	40.90^{A}	39.65^{c}	40.23^{b}	42.24^{a}	**MUFA**
5.82^{A}	6.24^{a}	5.98^{b}	5.24^{c}	4.28^{B}	4.68^{d}	4.42^{e}	3.86^{f}	**PUFA**
42.87^{B}	42.22	43.29	43.11	45.18^{A}	44.33	44.65	46.10	**UFA**
0.70	0.65	0.73	0.72	0.90	0.82	0.87	0.99	MUFA/SFA
0.81	0.77	0.84	0.82	1.00	0.92	0.97	1.08	UFA/SFA
0.11	0.11	0.11	0.10	0.09	0.10	0.10	0.09	PUFA/SFA
9.95^{B}	8.68	11.17	10.00	19.34^{A}	15.61	19.49	23.05	IC14
7.65^{B}	6.47	8.10	8.37	11.72^{A}	10.16	11.34	13.33	IC16
66.15	65.12	66.30	67.02	69.50	68.63	68.89	70.45	IC18

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

Relationship Between Bio-chemical Blood Parameters and Different Types of Fatty Acids for the Back Muscle

Figure (2) indicates correlation coefficients among different types of fatty acids for the back muscle and bio-chemical blood parameters. All correlation coefficients were significant and

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higher than 0.04. Also, saturated fatty acids showed positive correlation, of high significance at ($p<0.01$) with each of cholesterol, triglycerides, LDL, VLDL and fat ratio in the back on one hand, and a negative correlation, of high significance at ($p<0.01$) with each of unsaturated fatty acids (mono, multiple and whole). While each of cholesterol, triglycerides, LDL and VLDL revealed a significant negative correlation with each of unsaturated fatty acids (mono, multiple and whole).

These correlations pinpointed possibility of predicting types of fats in a carcass (the back) through estimating levels of each of cholesterol, its different types and triglycerides in blood. Therefore, it is possible to identify duration of fattening and quantity of fats deposited during the period of fattening through monitoring fat levels in blood. Or through selecting animals that would be fattened by checking level of fats in blood. This relationship has brought to light the nature of fat metabolism in the body. Besides, everything deposited or resulted in the body (such as milk) depends mainly on the content of blood of different items which come directly from food that animals consume.

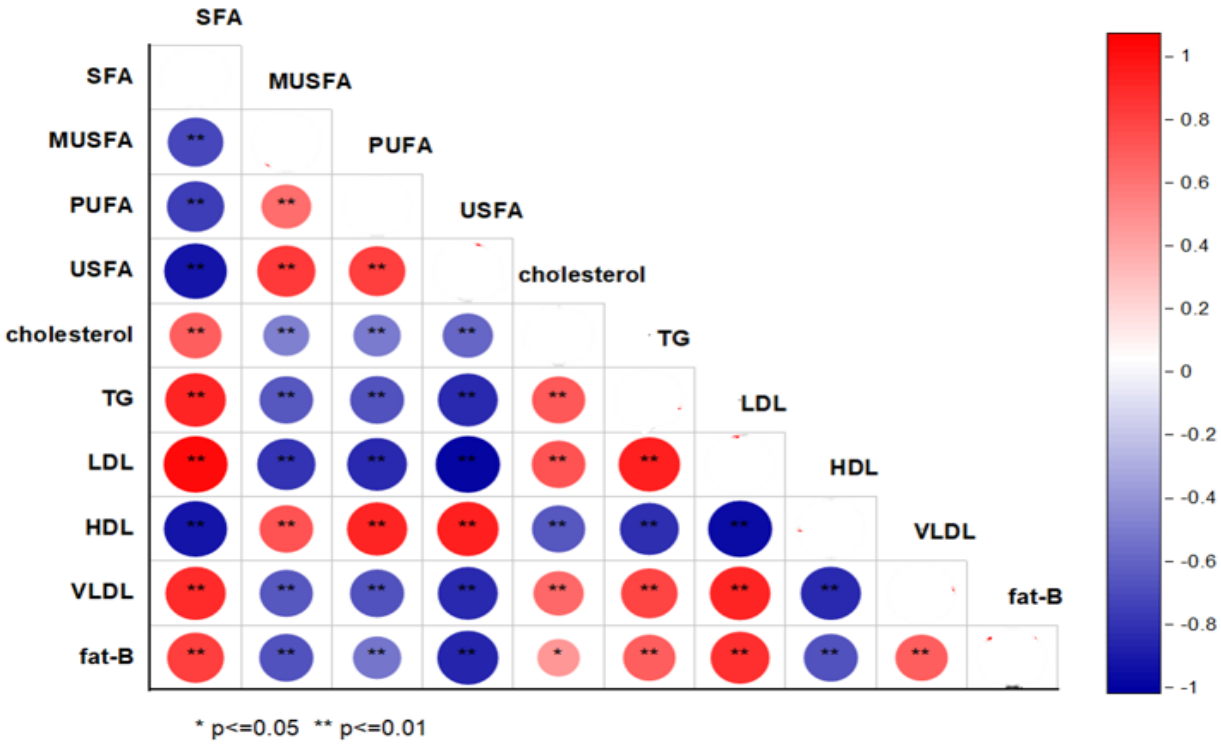

Figure (2) Correlation Coefficients among Bio-chemical Criteria for Blood Fats and Fatty Acids in the Back Muscle

This was consistent with what was revealed by studies carried out by Grundy et al. (2010) and Mattson and Grundy (185) which emphasized that saturated fatty acids (SFAs) heighten levels of cholesterol and a low-density fatty protein (LDL) in blood which results in the risk of

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cardiovascular disease. While unsaturated mono fatty acids (MUFAs) work on belittling harmful cholesterol (LDL), without affecting levels of a high-density protein (HDL) in the plasma, which has an anti- arteriosclerosis substance. Metabolic studies found that MUFA n-91:18 Oleic Acid has had low-cholesterol criteria. It is as effective as Linoleic Acid (LA) in lowering LDL cholesterol, but on the same time has no ability to lower HDL cholesterol (EFSA, 2010).

The rise in PUFAs level in the food system is correlated with the decrease in blood cholesterol levels and decrease of cardiovascular disease in the population (Sinclair and O'Dea, 1990). Linoleic Acid (LA) reduces harmful cholesterol (LDL), and also works through SREBPs on reducing cholesterol biosynthesis (Calder, 2015). Besides, studies disclosed that SFA containing 0:12 to 0:18 carbon atoms increase the cholesterol (LDL), and PUFA decrease it. Also, mono fatty acids have different impacts, and 0:14 has the highest impact on elevating cholesterol, and 0:18 has tiny impact Calder (2015). Moreover, it's found that SFA in phospholipid PL membranes has a crucial role in metabolism and sells' signals as well as modification of subscription factors such as SREBPs which regulate genes participating in cholesterol metabolizing (Calder, 2015).

However, not all blood-saturated fatty acids are correlated with cholesterol elevation in blood, which increases levels of harmful cholesterol (LDL). In line with French et al. (2003), myristic acid will be (C14:0), which represents only 3% of the total FA in meats (Freitas et al., 2006). But, main saturated fatty acids available in fats of beef meat are palmitic acids (C16:0) and fatty acids (C18:0), which constitute more than %50 of the total fats formation (Frank et al., 2006; Hwang et al., 2016 and Hwang et al., 2017). Also, availability of SFA in beef meat is the main cause for concern and correlation between health of humans and cardiovascular disease and obesity by affecting cholesterol levels in blood (Bingham et al., 2002). And palmitic acid has low impact on high cholesterol level in blood and the fatty acid citric (%43 of the total SFA in meats (Freitas et al., 2006). Beef meat contains a high total concentration of MUFA, especially oleic acid and multiple unsaturated fatty acids (PUFAs). Oleic acid may reduce harmful cholesterol concentration in blood, and are considered as healthy fats (Melton *et al*.,1982; Rudel *et al*.,1995; Smith *et al.*,1994; Kwon *et al*., 2015; Calder et al., 2009). High values of oleic acid are desirable due to availability of cholesterol shortage, along with a benefit of not decreasing cholesterol HDL (good cholesterol) and ability to protect against coronary heart disease (Freitas et al., 2006).

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