

Effect of genotypes of calpastatin (CAST) gene for meat quality characteristics in cyprus and local male goats and their crosses

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Abstract

The study was conducted of the Ruminant Station belonging to the Directorate for Agricultural Research / Ministry of Agriculture Research Station. The aim of the study is to determine the genotype of the Calpastatin genes and the relationship between genotypes with a number of qualities characteristics of carcasses goats of local Cypriot and bred crosses. The study showed as regarding the genotypes that superiority of genotype MN on MM genotype in the studied traits : percentage of collagen solubility in LD muscle, percentage of myofibril and sarcoplasm proteins solubility, Sarcomere length, MFI of LD and SM muscles. While genotype MM superiority on genotype MN in the studied traits :the total collagen in LD , IS muscle , percentage of collagen solubility in SM muscle, shear strength in LD muscle, MFI in IS muscle .It can be concluded from this study of gene expression for each of the Calpastatin, that is possibility of adoption of in the development of plans for genetic improvement in goats and a snap election for the animals operations, depending on the genetic composition of the gene studied without resorting to animal husbandry for Reconstruction and wide and then resort to the elections, and this leads to get the economic return and reduce the costs of education. This study can be applied to quantitative animals feature using these same genes to be results more comprehensive as well as possible that experience female goats.

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تأثير التركيب الوراثي لجين الكالبستاتين على الصفات النوعية للحوم ذكور الماعز المحلي والقبرصي وتضريباتهما

المستخلص

أجريت الدراسة من محطة المجترات تابعة لهيئة البحوث الزراعية / وزارة الزراعة. والهدف من هذه الدراسة هو تحديد التركيب الوراثي لجين Calpastatin علاقته مع عدد من خصائص صفات ذبائح ذكور الماعز المحلي و القبرصية وتضريباتهما. وأظهرت الدراسة على النحو فيما يتعلق المورثات التي تفوق التركيب الوراثي MN على MM الوراثي في الصفات المدروسة: نسبة ذوبان الكولاجين في العضلات LD ، نسبة ذوبان بروتينات الميوفبيريل والبروتينات العضلية ، وطول الساركومير ، ودليل تكسر الليفات العضلية للعضلات LD و SM. في حين الوراثي MM التفوق على MN الوراثي في الصفات المدروسة: الكولاجين الكلي فيعضلة

LD، والنسبة المئوية للذوبان الكولاجين في SM ، قوة القص في العضلات LD ، دليل تكسر الليفات العضلية في عضلة IS. يمكن أن نستخلص من هذه الدراسة في التعبير الجيني لكل من Calpastatin ، وهذا هو إمكانية اعتماد في وضع خطط للتحسين الوراثي في الماعز واجراء انتخابات مبكرة لعمليات الحيوانات، اعتمادا على التكوين الجيني للجين المدروس دون اللجوء إلى تربية الحيوانات لفترات تربية طويلة ثم اللجوء إلى الانتخابات، وهذا يؤدي إلى الحصول على العائد الاقتصادي والحد من تكاليف التعليم. ويمكن تطبيق هذه الدراسة على الصفات الكمية للحوم الماعز وباستخدام نفس هذه الجينات لتكون النتائج أكثر شمولا وكذلك ممكن أن تجربة إناث الماعز.

Introduction

The traditional genetic improvement processes for farm animals in general and special goats, which relied on statistical methods and focused on the election for individuals with phenotypic structure better have made significant gains in the field of genetic improvement, but scientific acceleration and availability of information on the work of the genome has enabled the development of the election programs more accuracy and less time and cost Economic. The qualities are under the control of a number of genetic loci which are known Quantitative trait loci-(QTL) and to the identification of these sites and to identify markers associated predictable contrast phenotypic traits to be improved an early tipme and building election programs on the basis of these markers is mutations functional genes influencing the qualities (22).

The most important challenges facing specialists molecular genetics and genetic improvement is to identify markers that control the contrast of the phenotypic characteristics of the subject of optimization, these markers on two types associated with the first so-called markers (Linked marker).

The markers inherent gene on chromosome and passed on him regularly Examples are microsatellite, but of disadvantages that inherited varies from family to another and from one generation to another because of the re-installation of the DNA (Recombination of DNA). The second type of markers is the most important and the so-called direct which depends the Polymorphism in charge of character from which one can predict the impact of alleles on phenotypic variation of the trait for gene (8). In order to continue the improve meat of the goats, it requires to be updated methods of genetic improvement and the study of genotype of the animals the best ones and selection, through the study of genes that affect the qualities of growth, production and compared genotype of the goat with the world's breeds and knowledge of genetic mutations linked to polymorphism using the technology of PCR (polymerase chain reaction) and the multiplicity of manifestations of the lengths of the pieces constrained length RFLP (Restriction Fragment Length Polymorphism).

Therefore the objective of this study was :

Studying the effect of the genotype of the Calpastatin (CAST) gene in a number of specific qualities of carcasses for the different genetic groups of goats (local, Cyprus and their crosses). Identify good genetic group or one of their bred crosses depending on. Early election on the basis of a genetic test done by analyzing the blood of

animals and an early age for the purpose of reducing expenditures in the election process and the election of more efficient animals than if they relied on phenotypic traits only.

Materials and Methods:

A study was Conducted at the Ruminant Station belonging to the / Ministry of Agriculture, which is located in Akerkov / Abu Ghraib area West of Baghdad.

The study included 93 male goat age of (10) months of four genetic groups 45 Cypriot , 25 Local and 18 first-generation local cross with Cyprus as follows:

[Male (1/2 Local + 1/2 Cypriot) X female (1/2 Local +1/2 Cypriot)] and 5 local cross with Cypriot , as follows:

[Local Male X female Cypriot].

Blood samples collecting

Blood samples were collected from the jugular vein(3 ml) by using test tubes containing anticoagulant (K2EDTA) were Blood samples placed in a container tubes refrigerated and transported to the laboratory for safe keeping freeze degree -18 C° until the time of extraction of DNA from them.

DNA Extraction

1- Extraction kit

DNA extraction according to reference from the production of Korean Bioneer.

2- KIT extraction components are According (kit) DNA extraction

3- Electric deportation of nucleic acid gel:

4- Loading of DNA for electrophoresis

Precedes the process of downloading the DNA blending 10 microleter with 3 microleter of dye loading dye (bromophenol blue). Then carry all specimens of DNA in the single of the gel drilling, to begin after the electric deportation of samples on the voltage (70 volts) and a stream of 40 mM AMP and for one hour. After the end of this process transmits the gel to another place with a solution containing dye ethidium bromide (1%) for a period of 20 minutes .At the end is the introduction of the gel in the spectrometer UV machine (UV light transillmintor) for the purpose of show (bands) the colored DNA dye bromide ethidium depicting using (Photo documentation system).ethidium bromide 0.5mg was dissolved in 100ml of distilled water. kept in a dark place at 4°C.

5- Molecular characterization of genes studied:

A- choose Primers: The sequence of each gene primers used for the purpose of detection and molecular conformation and genetic mutations (if any) in the gene CAST (18).(Table 1)

primer sequencing which is used and processed by the relay

Table (1) Sequence of gene primers

Gene Name	Sequence	
CAST gene	Exon,6 565 p.b.	F: 5'- CCT TGT CAT CAG ACT TCA CC -3'
		R: 5'- ACT GAG CTT TTA AAG CCT CT -3'

B: Primers dilution: Prefixes supplied in powder dryer (Lyophilized product) of the company's processed, and therefore has been to prepare inventories solution (Stock solution) and the (Working solution), according to the company processed primers instructions. The solution was prepared inventories adding water removed ions (Deionized water) for a final concentration of stuck (100 Picomols / μ l). The working solution was prepared to withdraw from 10 microliter stockpiling solution and diluted with 90 micrometer ions removed from the water for a final concentration of the solution work, which is 10 Picomols / μ l.

6- PCR of studied genes

Can be seen from the Table (2) materials used in molecular detection using enzyme polymerase chain genes under study and interaction using several diagnostic Profi taq PCR PriMix Kit and size 25 microliter. Where these materials are placed together in a polymerase chain reaction device according to each piece of genetic exponentially reaction conditions are deported reaction product electrically to make sure the doubled piece required.

Table (2)The materials used in the enzyme polymerase chain reaction

Volumes μ l	Contents
14	Pre Mix Kit
7	DNA
1	Forward –Primer
1	Reverse –Primer
2	Deionized water
25	Total

7- Gradient

For the purpose of selecting the appropriate temperature for the detection of cutting the required genes, which gave the desired result, where it is selecting and installing the program and circulated to all samples.

8- PCR product loading & electrophoresis

Has been loaded 6 μ l of DNA ladder with 6 μ l of the output of the PCR (each gene) in agarose gel and a Concentration of 2% (1X TBE buffer), and was the deportation difference of effort of 70 volts and the current 40 AMP and for an hour and a half and then immersing the gel in dye methyl ethidium liquid (1%), then spotted recorded packets mediated by UV transiluminater photographed and saved to a Photo documentation system.

9- Revealing the studier genes polymorphism Using PCR-RFLP

The use of the restriction enzyme MSP1 from Bromiga Company and the size of 3000 units a Concentration of 10 units per microleter. To detect the presence or absence of a mutation in the piece genetic target gene MSP 1 and identified the interaction of previous polymerization. Digestion process included this enzymes prepare a combination of several attached components with the enzyme categorically as in the table (3).Incubate the reaction mixture at a temperature of 37 o C for a period of three hours in the incubator. During this period the enzyme cutter identification of a specific site within the genetic multiplying piece of cut from it. Then followed by the detection of these pieces sites using electrophoreses of enzymatically digested samples technique. As a result, leading to the identification of the genetic conformation (phenotypic diversity) genetic region of a gene duplication.

Table (3) Mix ingredients enzymatic digestion of genes MSP1

MSP1	
The ingredients	Volumes (µl)
Sterile , deionized water	4
RE 10 X Buffer	3
Acetylated BSA, 10 µg / µl	0.2
DNA , 1 µg / µl	10
Mix by Pipetting , then add ; Restriction Enzyme , 10 µ / µl	3
Final Volume	20

10- Enzymic digesting loading & electrophoresis

DNA has been loaded 7µl of ladder and 7µl of the output of enzymatic digestion of the target piece In agars gel and concentration of 2% (1X TBE buffer), and deported samples difference of effort of 70 volts and current 55 AM for one hour and thirty minutes and immersion gel dye bromide ethidium liquid (concentration of 1%). This was followed by Show packages (Bands) resulting from cutting enzyme cutter piece of genetic target using the UV transiluminater and photographed documenting photographic device and stored in the calculator.

11- Cutting enzymes are effective pieces - - RFLP - PCR

PCR- RFLP method was used to determine the genetic forms of the gene site Calpastatin. The method applied on the output of PCR and use the cutter enzyme MspI according to the (1).

12- Estimate of Collagen

Following all the ways using by the (11) and (16) and (14) in the collagen content of dissolved estimate with some modifications, and as follows: -

12-1 Materials used and lotions

Solution No. (1) Ringer Solution consists of sodium chloride concentration (0.15 M) , potassium chloride concentration (0.025 M) and magnesium chloride concentration (3 mM).

Solution No. (2), hydrochloric acid (6 mm).

Solution No. (3) of sodium hydroxide (6 mm).

Solution No. (4) acetate buffer (Acetate Buffer) concentration (0.2 M) with a pH (6).

Solution No. (5) Chloramine-T

Be prepared the day before use consists of: -

(1.41 g) of Chloramine-T, (20 ml) of distilled water (10 ml) of n-propanol blends are all rapidly and then slowly before adding (70 ml) of buffer solution acetate to avoid the formation precipitate White then mix is placed in a bottle glass dark and kept in the refrigerator until use.

Solution No. (6) Aldehyde / Acid

A P-DABA / Acid Solution (P-Dimethyl Amine Benzyl Aldehyde)

Prepared dissolving (15 g) of the P-DABA (25 ml) of the concentration of perchloric acid (60%) is stored in a bottle of dark color and stays for a few weeks in the refrigerator.

- B n-propanol

For the purpose of formation Aldehyde / Acid solution is mixed with the solution A B by 3: 1

Solution No. (7) acid hydroxyproline standard (10 mg / ml).

12-2 Preparation of the Sample

After removing the fat and connective tissue from the surface of the meat was Chopped and dried and then grinding it passed through a sieve (2 mm rib openings). Taking (4 g) of dried meat with (20 ml) of the solution (1), put in a water bath at a temperature (77 ° C) for (70 minutes), stirring constantly, leave for a period of (60 minutes) in the room temperature, then put in the central ostracism device quickly (xg5200 for 20 minutes). Then separate to two part precipitate (insoluble) and supernatant (soluble) , add each of the two parts soluble and insoluble , both in private (26 ml) of the solution (2) and put then in the electric furnace type Heraeus supplier of company Karlkolb in temperature (115 ° C) for 12 hours) after the nominated mixture using filtering No. paper (1), have been adjusted the pH of two parts to (7) using sodium hydroxide (6 N) and the filtrate dilution to (250 ml) after that determination of hydroxyproline acid concentration in the filtrate for each of the two parts is soluble and insoluble .

12-3 Measuring the concentration of hydroxyproline

1. Taken Pipette (0.25 ml) of the solution to be measuring the concentration of the acid in it blends well with the (1.75 ml) of distilled water in a test tube.

2. Added (1 mL) of solution No. (5) Blends well and leave for (15 minutes) at a temperature of The room.

3. Added (1 mL) of solution No. (6) To the previous mix blends well then test tube placed

Temperature water bath (60 ° C) for (20 minutes).

4. Cooled test tube under cool stream water and placed in a water bath at room temperature for a period of (5 minutes).

5. Measured by optical absorption at a wavelength (558 nm).

6. Calculated hydroxyproline acid concentration depending on the concentration of hydroxy Brolin standard Based on the equation of the straight line .

7. attended efficient solution (Blank) by following the above-mentioned steps with the exception is to add Distilled water instead of the protein solution.

12-4 Determination of collagen

To get the real value of the content collagen in each of the supernatant and the precipitate was multiplied by the concentration of hydroxyproline acid value obtained in the supernatant in (7.52) and the concentration of acid hydroxyproline obtained in the precipitate in (7.25) based on what advised by (5).

13- Measure Sarcomere length

Followed (13) way to estimate the length of Sarcomere by mixing 5 g of meat sample with a solution containing the 0.25 m sucrose and EDTA 1m (Ethylen Diamine Tetraacetic Acid), and put a drop of the solution on a glass slide, covered and examined microscopically existence filarmicrometer. It was measured the longest 10 sarcomeres of 10 fibrils chosen randomly under Object-oriented lens 100 x

14- Myofibril Fragmentation Index (MFI)

The manual measurement break the muscle fiber based on the way (6) after slaughter. After the removal of fat and connective tissue from the surface of the meat was taken from him Chop meat (4 g), mixing for two (30 seconds) with 10 times the weight of the sample Buffer consisting of (buffer potassium phosphate concentration of 20 mM, potassium chloride concentration of 100 mM, Na₂ - solution EDTA concentration of 1 mM Molar, magnesium chloride concentration of 1 mM sodium Azaid concentration of 1 mM) and used the generic type Tafesa model Ho4 supplier of company Hannover Germany then put the mixture naturalized in central ostracism device at a temperature (4 ° m) speed (xg 1000 for a period of 15 minutes) to separate the filtrate and re-naturalization of sludge with 10 times the weight of the buffer mentioned above for a period of (30 seconds) and after a central ostracism and the separation process filtrate sex sludge with (2.5 size) from the solution buffer itself then passed mixture naturalized across the rib openings refinery (250 Micrometer) to Detention a connective tissue was added (2.5 size) from the solution buffer to facilitate myofibril muscle Broken passage, then measured the protein concentration is stuck myofibril (Biuret) way with a standard curve for the two albums protein bovine serum Bovin Serum Albumin (BSA) (Figure 9), and based on the straight-line equation ($\hat{Y} = a + bx$). Protein concentration was measured in the mummy is the wavelength (540 nm) using a spectrophotometer equipped with a device type SP6-500 of Pye Unicam Company Ltd. English. The dilution stuck Myofibril with the same buffer solution if the concentration of the protein in which more than (0.5 ± 0.05) to arrive protein concentration (0.5 mg / ml) then divided the optical absorption value which appeared protein concentration (0.5 mg / ml) by a fixed is (200) to give the value of the Myofibril Fragmentation Index.

15- Statistical Analysis

Statistical Analysis System - (20) was used in the data to study the effect of different factors analysis (genetic group - Genotype - Maternal age and the type of birth) in the studied traits according to a random design full (CRD) and by mathematical model below, and compared the moral differences between the averages test (9) multi-ranges, as has been regression a number of economic

characteristics on the weight of the cold carcass for prediction equations coefficient was calculated. The statistical model was as follows :

$$Y_{ijkl} = \mu + B_i + G_j + A_k + T_l + e_{ijklm}$$

where :

yijkl: the value of viewing m

μ : the overall average for the recipe

B_i : the effect of genetic group (the study included four genetic groups.

G_j : the effect of the genotype of the gene (MM and MN).

A_k : the effect of maternal age (2-6 years).

T_l : the effect of birth type (1, 2 and 3).

e_{ij} : random error which is distributed naturally equal to zero and an average variation of σ^2_e .

Results and Disscutions

The DNA extraction was the focus between 50 to 75ng / μ l as a first step to extract A CAST and Myo G genes and then the samples were to migrate by 10 μ L of sample DNA 03 μ L of loading dye in agaross gel concentration 0.08 % and adjust voltages to 70 V and 40 mM AMP stream for one hour and photographing deportation output to ensure the success of the extraction process (Figure 1).

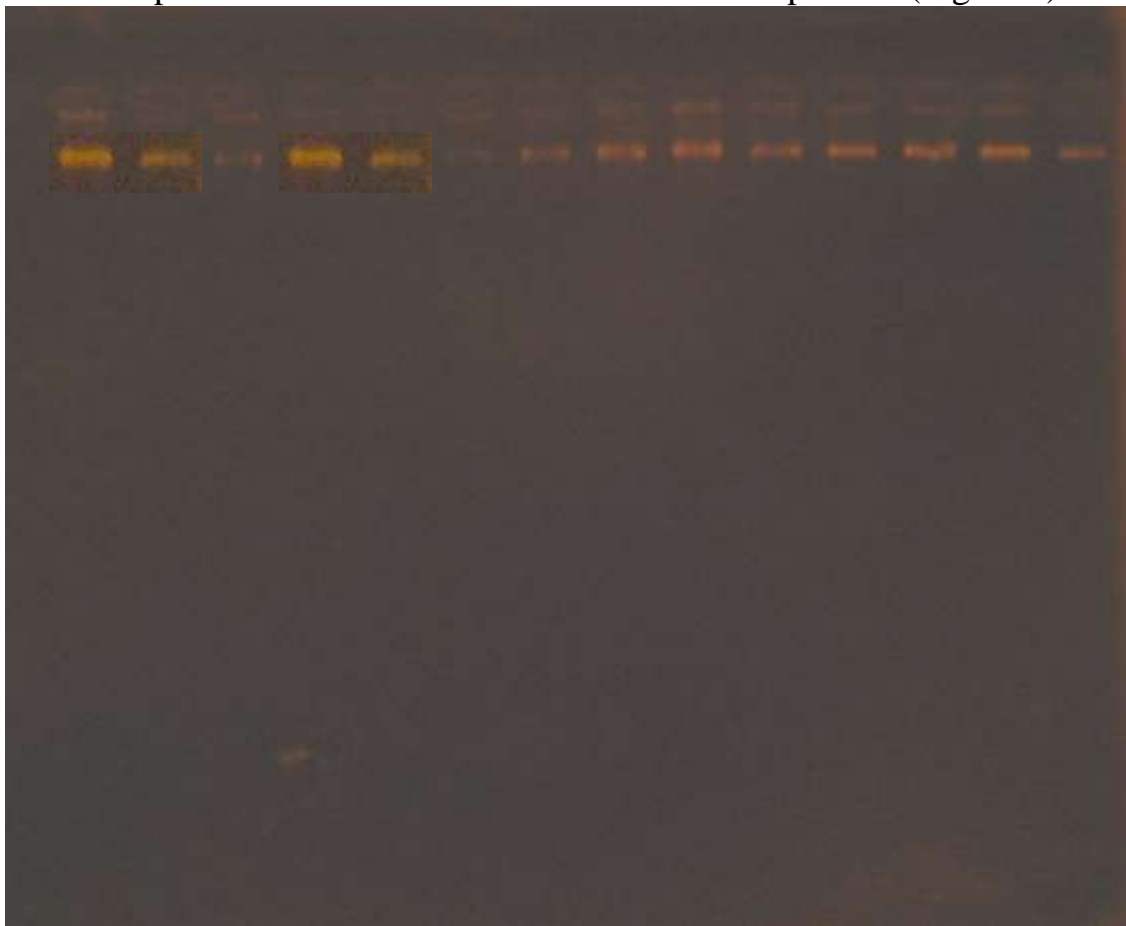


Figure (1) Agarose gel electrophoresis of Process of DNA Extracting

(CAST) 1 Calpastatin Gene

To amplify CAST gene by PCR technology and using PCR kit and the primer and samples of the total DNA and adjust the device thermal cycles as

mentioned in the chapter of materials and methods, and then sample was 6 μ L of each model to migrate in agarose gel concentration of 2% and adjust voltages to 70 V and stream 40 AMP for an hour and a half. Then photographing the result of deportation to ensure the success of the recovery process and get a piece the size of the required 565 bp (cut DNA was used information volumes (Marker 100-1000) (Figure 2).

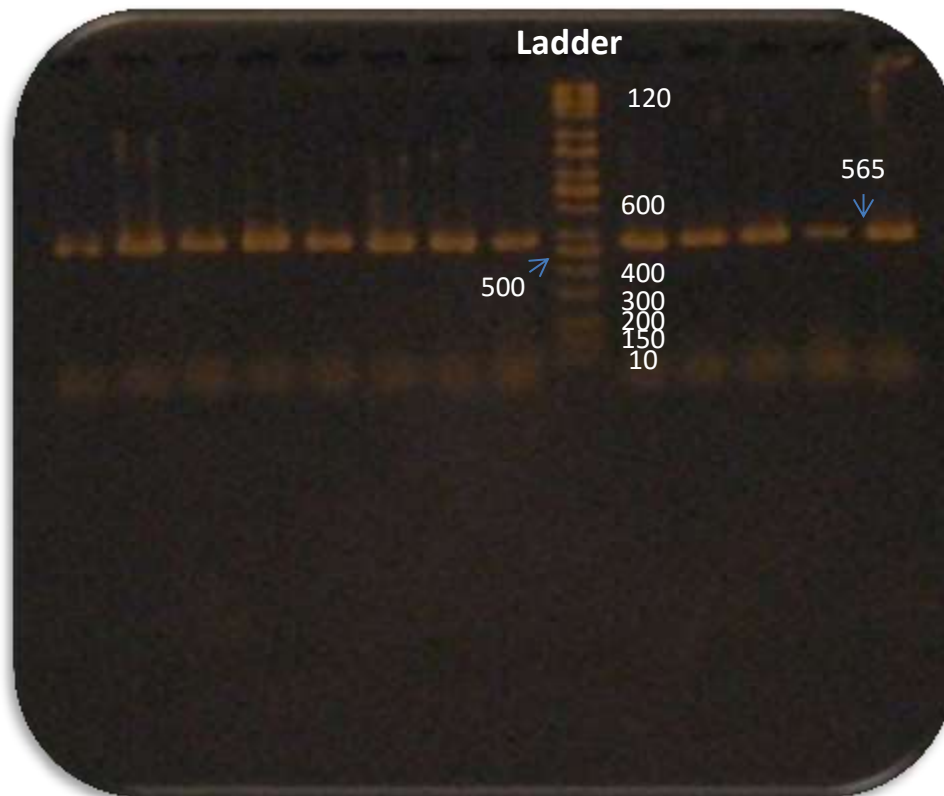
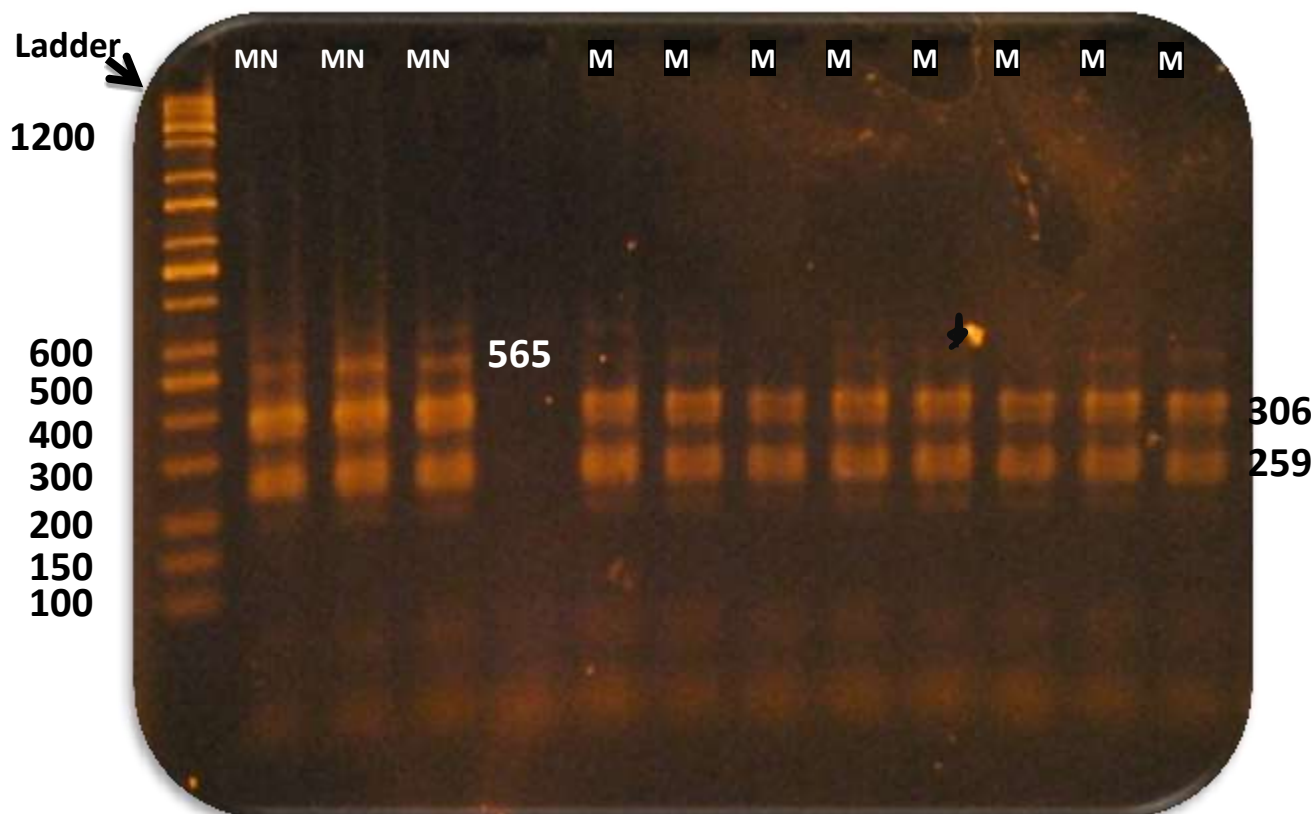


Figure (2) Agarose gel electrophoresis of The CAST Gene

It has been identified genotype of the test animals for CAST gene using technology RFLP and restriction enzyme MSP1 and by the way mentioned in the materials and methods and the deportation of 10 μ L in agarose gel 1.5% and adjust voltages to 70 V and stream 40 AMP for an hour and a half and photographing the result of deportation to learn about the distribution of genotypes within research animals (cut DNA was used information volumes (Marker 100-1000) . We can see from (figure 3) two genetic structure (MM , MN) which were responsible of two alleles (M and N), which determined by Calpastatin gene primer that used to goats samples . The aim of this research was to determine the polymorphism of Calpastatin gene which is located on fifth chromosome (2). And 565 of the piece and using PCR-RFLP method using restriction enzyme MSP1 according to (18). Two types of alleles were identified M and N and resulted in two types of genotypes homozygous MM and Heterozygous MN and groups genetic four as the MM consists of two bundles (306 and 259), while the genotype MN consists of three bundles (565, 306 and 259) this is consistent with what it says (17).

Figure (3) Result of the cutting CAST gene by restriction enzyme Msp1.



Allele frequency of CAST gene in goats

Table (4) showed allele frequencies M and N , which have been identified by the enzyme cutter MSP1. The allele M frequency was (0.81%) and the N allele (0.19%) . These results were approached with the results of a number of previous studies on the same area , there came mostly, similar to those found in the Blackheaded Mutton Sheep group, occurred in a Karakul population in Iran. The M allele frequency was 79%, and the N allele 21%. (10 and 12).

Table (4) Allele frequency of CAST gene in goats

Allele	Frequency
M	0.81
N	0.19
Total	1 (100%)

Gene calpastatin distribution in goats

In table (5) the number and percentage of gene calpastatin (CAST) in the studied sample, with the presence of highly significant shows ($P < 0.01$) between the different genotypes of different genetic groups of goats, which amounted to 70.97 and 29.03 and 0.00% genetic structures percentages MM and MN and NN sequentially, that there is clear and pure common carrier for individuals to install non-mutant MM

or hybrid MN with low mutant genotype NN percentages in the sample. These results were approached with the results of a number of previous studies to the same area of CAST gene . There came mostly genotype frequencies similar to those in the Berrichon du Cher sheep were identified in CA ST/MspI locus in populations of the Tsugai, Valachian, East Fresian, Lacaune breeds as well as in Tsigai and Lacaune crossbreds maintained in Slovakia. The NN genotype was not found either, and the MM and MN genotype frequencies were similar to those discovered in the authors' own research in Berrichon du Cher sheep, and they respectively equalled 87% and 13%. (10 and 12).

Table (5) Distribution of CAST gene polymorphism (No. & %)

Genotype	No.	Percentage (%)
MM	66	70.97
MN	27	29.03
NN	0	0.00
Total	93	100 %
Chi-square value (χ^2)	---	11.648 **
** (P<0.01).		

Effect of genotype on Total Collagen(mg/g meat) and Collagen solubility % for LD, SM and IS muscles

Table (6) Showed the impact of the genotype of an animal goats existence of significant differences (P <0.05), where animals carried MM genotype superior on the animals carried MN genotype in total collagen characteristic in LD muscle , and impact the genotype of an animal goats significant differences (P <0.01), where animals carrying for MM genotype superior on animals carrying MN genotype in total collagen characteristic in IS muscle. While, there was no significant differences between the animal carrying MM and MN genotype in SM muscle and in the same characteristic At the same table it turns out there was the effect of genotype in the characteristic in solubility of collagen solubility significant differences (P <0.05) in LD muscle recorded higher values in animals carrying MN genotype (12.60 ± 0.28%) on the animals carrying MM genotype(11.94 ± 0.28%) , In table (6) shown significant superiority(P <0.05)and recorded higher value (5.57±0.11%) for animals with MM genotype in SM muscle on animals with MN genotype. While, the same table mention that was no significant differences between animals carrying for both MM and MN genotypes in IS muscle. The reason is due to the presence of high genetic correlation between Calpastatin activity and carcass characteristics and which is consistent with the hypothesis that show that the activity of Calpastatin linked indirectly with carcass characteristics and tenderness (15).Also is due to genes responsible for reduced Calpastatin activity is result to improved tenderness and specifications of the carcass (19).

Table (6): Effect of genotype on Total Collagen(mg/g meat) and Collagen solubility % for LD, SM and IS muscles in goat(Mean \pm SE)

CAST Genotype	Mean \pm SE					
	LD Tota coll..	SM Tota coll..	IS Tota coll..	LD Sol. coll. %	SM Sol. coll. %	IS Sol. coll. %
MM	2.99 \pm 0.03 a	4.87 \pm 0.02 A	4.52 \pm 0.38 A	11.94 \pm 0.28 b	5.57 \pm 0.11 B	5.86 \pm 0.10 A
MN	2.91 \pm 0.02 b	4.84 \pm 0.02 A	4.42 \pm 0.05 B	12.60 \pm 0.28 a	5.93 \pm 0.11 A	5.98 \pm 0.07 A
Level of sig.	*	NS	**	*	*	NS

Means having different small later (a - b) in columns are significant difference.

* (P<0.05) ** (P<0.01).

Effect of genotype on Solubility of Sarcoplasma and Myofibril Protein for LD ,SM and IS muscles

It is clear from the table (7) shown the impact of genotypes in goats in the percentage of soluble protein sarcoplasma and myofibril in LD, SM, IS muscle where significant superiority (P <0.05) for the animals carrying MN genotype on animals that contain MM genotype ,and recorded the highest percentages as follows: sarcoplasma proteins (71.28, 69.73 and 68.26 mg/g meat) and myofibril proteins (75.05, 72.51 and 70.53 mg/g meat) and respectively.The MM genotype for Calpastatin gene (CAST) in Solubility of Sarcoplasma ,Myofibril Protein Characteristic explained in Inactive Calpastatin gene (CAST) that leading to Calpain activity and thus improve tenderness or improve traits (Chung et al, 2001 b). That is unlike of the results' the recent study which showed that the MN genotype for Calpastatin gene (CAST) in Solubility of Sarcoplasma and Myofibril Protein Characteristic explained in Inactive Calpastatin gene (CAST) that leading to Calpain activity and thus improve tenderness or improve traits.

Table (7)Effect of genotype on Solubility of Sarcoplasma , and Myofibril Protein for LD ,SM and IS muscles

CAST Genotype	Mean \pm SE					
	LD Sarco.	SM Sarco	IS Sarco.	LD Myof.	SM Myof.	IS Myof.
MM	66.02 \pm 0.81 b	60.97 \pm 1.25 b	59.83 \pm 1.13 b	70.16 \pm 0.74 b	68.13 \pm 0.71 b	65.78 \pm 0.76 b
MN	71.28 \pm 0.27 a	69.73 \pm 0.41 a	68.26 \pm 0.68 a	75.05 \pm 0.17 a	72.51 \pm 0.23 a	70.53 \pm 0.20 a
Level of sig.	*	*	*	*	*	*

Means having different small later (a - b) in columns are significant difference. * (P<0.05).

Effect of genotype on Sarcomere length and Myofibril Fragmentation Index (MFI) for LD, SM and IS muscles

Table (8) has shown in Warner–Bratzler shear force (WBSF) k.g trait in LD muscle, there is significant difference (P <0.05) for the animals which contain MM genotype with the highest value (3.59 k.g), on the animals that contain MN genotype which recorded lowest value (2.58 k.g) , while , from same table explained that sarcomere length has superiority significantly (P <0.05) in LD muscle of animals that contain MN genotype and recorded the highest value (1.96nm) on animals that contain MM genotype (1.82nm). Also in table (8) the effect of genotype is shown between the animals which carrying MM and MN genotypes in MFI trait , where the table recorded significant superiority (P < 0.05) for the animals carrying on MN genotype on animals carrying MM genotype in LD and SM muscles. While superior the animals carrying the MM genotype which recorded higher value (59.98%) on animals carrying MN genotype which recorded lowest value (62.77%) in IS muscle. The results were consistent with what it says (3) in a study of the impact of Calpastatin gene (CAST) in cattle meat, noting that there are significant differences in the shear force trait between the different genotypes for Calpastatin gene (CAST) and the output of the co-ordinat of different alleles. Schenkel et al.(2006) relationship between the genotype of Calpastatin gene(CAST) by using (RFLP),using restriction enzymes and tenderness of the meat. The results were compatible with in the case of MM and different in the case of MN with (4) , which contains the AA were less shear force. the result was agree with (7) in a study of the impact of genetic polymorphisim between pure breeds and bredcross of Nelore cattle. (21) explained that superiority the genotype MM with MN respect to the LD muscle tenderness in the shear force trait , this result do not agree with the result of recently research .the result of recently study do not agree with (13). In WBSF that founded no significant differences between animals that carry the polymorphism of CAST gene . The results in MFI trait were compatible with in the case of MN and different in case of MM with (4).

Table (8)Effect of genotype on Sarcomere length and Myofibril Fragmentation Index (MFI) for LD, SM and IS muscles

CAST Genotype	Mean \pm SE				
	LD Shear force	LD Sarcomere Length	LD, MFI	SM, MFI	IS, MFI
MM	3.59 \pm 0.18 A	1.82 \pm 0.02 B	62.34 \pm 0.37 B	57.51 \pm 0.25 b	59.98 \pm 0.30 A
MN	2.58 \pm 0.08 B	1.96 \pm 0.02 A	65.65 \pm 0.32 A	59.74 \pm 0.21 a	62.77 \pm 0.25 B
Level of sig.	*	*	*	*	*

Means having different small later (a - b) in columns are significant difference. * (P<0.05).

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