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Article

# Relationship between the SLC27 gene's polymorphism and some broiler carcass and economic factors

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## Abstract

This study was conducted at the poultry field, College of Agriculture and the Marshes, University of Thi-Qar from 10/11/2021 to 26/12/2021, and the Molecular Genetics Laboratories at the Marsh Research Center to determine the FATP gene polymorphism on some productive and physiological traits of broilers of ROSS 308. A total of 150 birds were used. The results of this study showed the following. Three genotypes were identified in the sequence of nitrogenous bases in the presence of the mutation G237A: GG, GA, and AA. It was noted that there were significant differences in the distribution ratios of the genotypes of the FATP gene according to the mutation G237A, where the genotype GA recorded the highest percentage, followed by genotype GG and then genotype AA. The G allele frequency is superior to the A. It was noted that there were no significant differences for the genotypes of the G237A mutation on the body weight and no significant differences between the GA, GG and AA genotypes of the FATP gene on the body weight, a significant difference in the genotypes of the G237A mutation on carcass weight, AA genotype outperformed the GG and GA genotypes, the AA genotype was superior to the GA and GG genotypes on wings relative weight.

**Keywords:** Polymorphism SLC27 gene, economic, carcass, broiler.

## Introduction

The poultry industry at present is characterized by an increase in both production and high efficiency of birds in converting the consumed feed into meat and eggs, as the main direction for the development of this industry now and in the future is always to improve productivity[1], in addition to obtaining a healthy product from eggs. Poultry meat has outperformed other meat of other agricultural animals, with a high percentage of purification that reaches more than (70%) on average. In comparison, it ranges in cows and sheep from (50-60%). It is considered An economical source of meat as it is characterized by being easy to digest and absorb, reaching a digestibility rate of 97%. It is a valuable source of protein, vitamins and minerals[2].

The protein percentage in poultry meat is about 5%, while it ranges in beef and sheep meat 5.4-7.9%[3]. The content of broiler meat is usually low in fat, cholesterol and saturated fatty acids, and this is achieved by increasing the feed mixture with some additives such as copper and others[4], as well as adding some types of oils and fats rich in omega-3 fatty acids[5].

The genetic and morphological developments of domestic birds over time due to environmental changes led to the emergence of natural changes, as they produced communities of birds adapted to these changes, whose physiological and phenotypic characteristics emerged and are inherited and continuous within the members of the same species[6]. Genetic selection has been used during the past decades. To improve the growth and food conversion efficiency of broilers and change the productive performance of growth by (85-90%) by shortening the period required for the marketing life as a result of cross-breeding and producing lines to obtain specialized lines, the best hybrids at the commercial level[7].

As a result of the increase in the intensity of selection for growth traits, several effects emerged, including concerning lipid pathways and the use of selection based on genetic markers in tracking the genetic locations of quantitative traits (QTL), which is difficult to measure directly, as well as eliminating unwanted genetic associations between some traits when selecting to increase growth Carcass yield and fat content between muscle tissue[8].

In conjunction with the advances in quantitative genetics, molecular genetic techniques have provided a tremendous possibility to reveal the genetic locations of many genes responsible for the inheritance of quantitative traits and to search through the genome for these locations and know their allelic frequencies[9].

Molecular genetics applications have been implemented to detect chromosomal loci that contain genes affecting economic traits. It was possible to apply for genetic improvement programs in herds based on direct selection methods, which employ genes carried on chromosomes and the multiple manifestations of those genes, which were called genetic markers, identifying quantitative traits and knowing the nature of their association with those markers[7].

## Materials and Methods

Experience Site

This experiment was conducted in the poultry field of the Department of Animal Production at the College of Agriculture and the Marshes, Thi Qar University, and the fieldwork lasted for (35) days from 10/11/2021 to 29/12/2021. The experiment included raising 150 unsexed broilers of the Ross308 at one day old. Blood samples were taken from the birds to separate the genetic material (DNA) at the end of the breeding period, to determine the genotypes of the FATP1 gene and study the association of genetic phenotypes with the productive and physiological performance of birds, laboratory analyses were carried out for genetic material separation (DNA), electrophoresis and PCR polymerase chain reaction in the laboratory of the Marsh Research Center for the period from 12/29/2021 to 2/3/2022.

*Blood samples*

Blood samples were drawn at the age of (35) days. The number of birds was (100 using medical syringes with a capacity of (5) ml and the appropriate size for the size of the vein, drawing blood through the pterygoid vein. It was placed in tubes using ice cubes and kept at a temperature of (20 -) C◦ until the molecular examinations.

*Extraction of genetic material*

1. Take 30 microliters of blood and put it in a 1.5ml Eppendorf tube
2. Add 200 µl of PBS (phosphate-buffered saline) solution.
3. Add 20 µl of Proteinase K (20ml/mg)
4. The samples were mixed using the Vortex mixer and incubated at room temperature for two minutes.
5. Add 200 µl of Binding buffer/Genomic lysis (GSB) solution and shake with the

mixing device for one minute.
6. Incubation for 10 minutes in a water bath at 60°C.
7. Add 200 microliters of 100% Ethanol Absolute, then shake with the mixer device for one minute.
8. Take a filter tube with the collection tube and add 600 microliters of the mixture after removing the formed blood clot using the Micropipette, then put the solution in the filter tube and then put it in a centrifuge 14000 r/min and the filtrate was disposed of.
9. 400 microliters of Wash Buffer solution were added with centrifugation at 14000 rpm, and the filtrate was disposed of.
10. 600 µl of 2 Wash Buffer solution was added and centrifuged at 14,000 rpm for three minutes, and the filtrate was discarded.
11. A new 1.5ml Eppendorf tube was taken, and a filter tube was placed in it. Then, 100 µl of Elution Buffer was added and centrifuged at 14,000 rpm for 1.5 minutes.
12. Then, the extracted DNA samples were examined using electrophoresis, where 1% agarose gel was used.

*DNA electrophoresis*
1. EDTA Tris Acetate buffer solution with a concentration of 50 X was supplied by Bioneer company, where 490 ml of TAE buffer was added for every 10 ml of distilled water. It was used to prepare 1X solution to prepare the agarose gel and to fill the electrophoresis device's basin.
2. Clean 30 ml of TAE solution with a concentration of 1X glass conical flask (flask) that can withstand high temperature and clean it with 0.3 g of acarose (the percentage of acarose is 1%) until the agarose particles dissolve until it produces a transparent solution.
3. Leave the solution to cool slightly and prepare the mold into which the agarose is poured by placing the special comb for drilling the agarose jelly in the designated place at the end of the basin.
4. Clean the agarose mixture to cover the entire area of the tank in order not to cause bubbles.
5. Leave the mixture until the agarose hardens, and carefully remove the comb without causing any tear in the gel.
6. Put the gel based on the plate in the place designated for it inside the relay device for the electrician.
7. Filling the basin of the electric relay device with the buffer solution to the extent that it fills the loading pits and to the extent that the level of the buffer solution rises from the agarose gel by about 3 m

Primers processing
The primers of the FATP1 gene were prepared by the Korean company Macrogene in the form of a lyophilized powder of two primers separated from each other, each of which is placed in a unique tube with a label showing the sequence of nitrogenous bases. Stock Solution and 10 microliters were taken from it, and 90 microliters of water were added again. The starting concentration was 10 pikamol, the concentration required for the PCR reaction.

| Gen | Primers | The segment size |
|---|---|---|
| **FATP1** | 5-AACTGGAGCCACTTGGTTGG-3 | 799 base pairs |
| | 5-GGCATTCATCCAACGGAACG-3 | |

**Table 1. The primers used in the experiment.**

| chemical | Master Mix | Template DNA Concentration (100 ng) | Primers | | Distilled water | final size |
|---|---|---|---|---|---|---|
| | | | Forward | Reverse | | |
| Volume µl | 18 | 2-5 | 1.5 | 1.5 | 9-12 | 35 |

**Table 2. Substances used in the PCR technique and their quantities.**

| Initial program | Stages | Temperatures (C◦) | Time (min) | Cycle No. |
|---|---|---|---|---|
| | Initial metamorphosis | 95 | 5.00 | 1 |
| | Metamorphosis | 95 | 0.30 | 20 |
| | Adhesion | 54-64 | 0.45 | 20 |
| | Elongation | 72 | 1.00 | 20 |
| | Final elongation | 72 | 10.00 | 1 |
| Specialized Amplification Program | Initial metamorphosis | 95 | 5.00 | 1 |
| | Metamorphosis | 95 | 0.30 | 20 |
| | Adhesion | 54 | 0.45 | 20 |
| | Elongation | 72 | 1.00 | 20 |
| | Final elongation | 72 | 10.00 | 1 |

**Table 3. The PCR program for the FATP1 gene using the Touchdown method.**

*Migration of PCR product samples*

Agarose gel was prepared with the same previous steps in which the DNA of the samples was removed. However, the concentration of agarose prepared for the transfer of samples of PCR product was 1.5%, where 0.45 g of agarose was added to 30 ml of TAE solution of X1 concentration, load 5 µL of PCR product with 2 µL of Dilute Diamond Nucleic Acid Dye dye into each hole of the agarose gel and use a marked hole to load 5 µL of DNA Ladder with the dye. The electrical relay program was set using 70 volts and 85 mA for 45 minutes, and after the migration process of the PCR product, images were taken using a UVGel Documentation device.

*Nitrogenous base sequence analysis*

After confirming the size of the specialized PCR product for the studied gene and comparing it with the standard DNA strand DNA Ladder with a size of (100 base pairs), 20 microliters were taken for each sample of the PCR product. It was sent to the Korean Macro gene company, where the samples were purified, and then the nitrogen base sequence analysis was carried out using the Sanger Sequencing technology. The base sequencing results were received and analyzed using BLAST tools at the NCBI International Gene Bank website using some bioinformatics programs.

Statistical analysis

The data were statistically analyzed using the statistical program SPSS[11] to study the effect of polymorphism of the FATP gene on the productive traits of broilers 308 ROSS. The significant differences among the means were compared using the Duncan[12] multiple range test using the Least Square Means method.

## Results

*DNA extraction*

The DNA extraction process was an essential step in studying the FATP gene in exon, as shown in Figure (1), which shows the process of DNA transfer on agarose gel, mentioned in the chapter on materials and work methods. The DNA concentration and purity were estimated at a wavelength of 260-280 nm, its concentration was 1% at voltages of 70 volts and 85 mA for 20 minutes, and its purity was 75-98

ng/µl, which means that the purity and concentration of the DNA are within the levels that guarantee a successful amplification process by PCR technique.

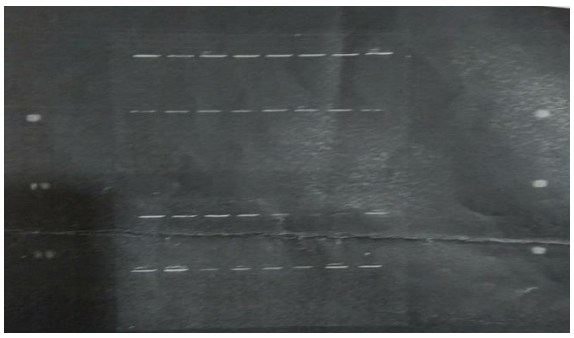

**Figure 1. The DNA extraction process.**

*Amplification of FATP gene cut-off*

The desired piece of DNA was used, the required piece (799Pb), which is the exon of the FATP gene. Using primers, the piece is doubled using polymerase chain reaction technology.

The program of the PCR Thermocycler, according to what was mentioned in the separation of materials and methods of work and using a piece of known size DNA, was placed in the first hole of the 2% agarose gel. The gel was placed in the designated basin inside the electrophoresis device filled with buffer solution, and the voltage and time of the electric current were adjusted. A picture of the electrolysis product was taken to ensure the success of the extraction process for the gene and to obtain the required pieces, as shown in Figure (2); when reading the sequence of nitrogenous bases, it was found that there are SNPs for five genotypes: GG-GA-AA-CT-CC.

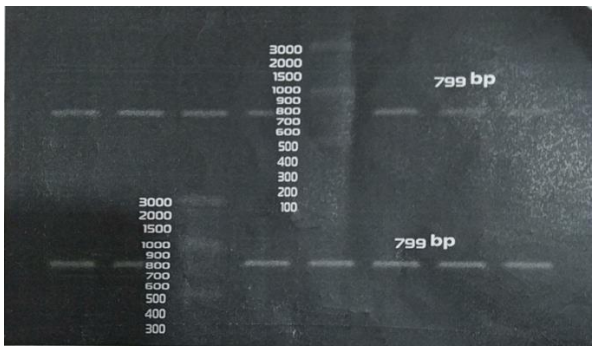


**Figure 2. The product of the amplification process for the segment (799) of the FATP gene.**

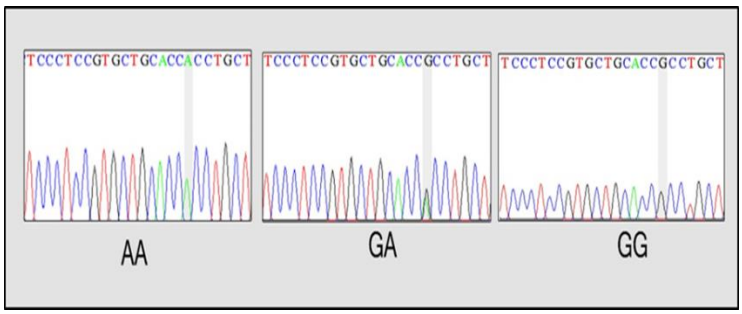


**Figure 3. The location of the mutations ((237)) in the studied segment of the FATP gene in ROSS 308 broilers.**

Percentages and allelic frequency of FATP gene variation in ROSS 308 broilers when aligning the sequence of samples under study to detect changes or mutations that occur and the genetic structures that are the cause. A change was found in two different sites of the studied segment in the FATP gene. The first site is the number

(106) in which the base changed C to T. This resulted in two homozygous genotypes, CC and TC, with allele repeats of 0.92 and 0.08 for C and T alleles, respectively, as for the second change, it was found at site 273), in which the base G changed to A, and this resulted in three genotypes: GG, GA, and AA with allelic frequency of 0.64 and 0.36 for the G and A alleles, respectively. Table 4 shows the distribution of the different genetic phenotypes of the FATP gene for genetic variation (106) in broilers. It was found that there are highly significant differences (P<0.05) in the percentages of genetic phenotypes, a clear commonality of individuals carrying the CC genotype, which obtained the highest percentage (92%), followed by genotype CT (8%), as for the second site of the mutation (237), the distribution of genetic phenotypes of the FATP gene, there were highly significant differences (P<0.05)) in the percentages of genetic phenotypes, individuals carrying the genotype GG, GA got the highest percentage (64%), followed by the AA genotype (36%), according to the Hardy Weinberg equilibrium law.

| Genotypes | Genotype frequency | Allel | Frequency | X2 Value |
|---|---|---|---|---|
| **CC** | 0.85 | C | 0.92 | 0.22 |
| **CT** | 0.15 | T | 0.08 | |
| **GG** | 0.39 | G | 0.640.36 | 0.07 |
| **GA** | 0.49 | | | |
| **AA** | 0.12 | A | | |

**Table 4. The genotypes of the T sites C106 and G237A and the allelic frequency of the FATP gene in ROSS 308 broilers.**

Relationship of the polymorphism of the FATP G237A gene (C106T) with the body weight of ROSS 308 broilers.

Table 5 indicates that there were no significant differences between the genotypes GG, GA, and AA of the FATP gene in the average live body weight during the (5) weeks of bird life A significant decrease (P<0.05) was observed for the GG genotype, which averaged for live weight trait was 1745.35 gm on GA and AA genotypes during the fifth week of the experiment, whose rates reached 1945.61 and 1958.23 g, respectively, in the second site of the genetic mutation of the FATP gene for intron (2) to study the trait of live weight, there was a significant increase (P<0.05) in favor of the AA genotype, which averaged during the fifth week of the experiment was 2253.85 gm on the AB and BB genotypes, whose averages were 1938.67 and 1919.20 g, respectively. At the third site of the genetic mutation of the FATP gene in exon (3) to study the characteristic of live weight during the sixth week of the experiment, it was noticed that there was a significant increase (P<0.05) in favor of the AA genotype, which averaged 1954.56 gm on GA genotype, which averaged 1755.20 gm. It was noticed that there were no significant differences in favor of the genotypes GG, GA and AA, respectively. In the third region of the gene for exon (8), it was noted that there were no significant differences for the study of live weight traits during the sixth week of the experiment in favor of the TT and CT genotypes, respectively. The current study does not match the second site of the genetic mutation of the FATP gene in intron (4) to study the characteristic of live weight during the fifth week of the experiment. As for the genetic mutation of the FATP C106T gene, it was noticed that there was a signif-

icant increase in favor of the genotype CT, which averaged 1521.35 gm. However, the genotype CC showed no significant superiority, reaching an average of 1499.32 gm. It was noted that there were no significant differences in favor of the CT and CC genotypes for the live weight trait of ROSS 308 broilers during the (5) weeks of the birds' life.

| Genotypes | Age (Week) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| **CC** | 1816.62±198765 A | 1816.62±198765 A | 1771.52±109.317 A | 68.593±392.38 A | 22.599± 155.95 A |
| **CT** | 299.456±1808.50 A | 299.456±1808.50 A | 168.721± 745.75 A | 73.161±419.75 A | 164.50± 12.036 A |
| **AA** | 264.362±1677.44 A | 264.362±1677.44 A | 131.68± 74311 A | 72.55± 378.22 A | 12.94± 151.78 A |
| **GG** | 169.294±1856.09 A | 169.294±1856.09 A | 99.62± 784.64 A | 56.94± 399.73 A | 26.73± 154.73 A |
| **GA** | 220.282±1833.42 A | 220.282±1833.42 A | 136.08± 758.95 A | 82.11± 402.11 a | 16.77± 16295 A |
| **Sig.** | N | | | S NS NS NS | NS |

**Table 5. Relationship of the polymorphism of the FATP(C 106 T) gene (G237 A) with the body weights of 308ROSS broilers (mean ± standard error).**

*Relationship of the polymorphism of FATP C106T-G237A gene with carcass weight, dressing ratio and relative weight of cuts. Weight of abdominal fat studied for broilers of ROSS 308 breed + standard error*

Table 6 shows that there are significant differences (P<0.05) in the average carcass weight in favor of the AA genotype, as its average was 194.49 g for the two genotypes, GG and AG, whose averages were 95.93 and 182.74 g, respectively. The current study's results indicate no significant differences (the dressing with giblet) and (the dressing with unedible viscera). Also, there were significant differences (P<0.05) for the relative weight of the two wings in favor of the AA genotype, which averaged 0.64 g on the genotypes GG and GA, whose rates were 0.69 and 0.98 g, respectively. The AA genotype continues to have a significant effect if it exceeds the relative weight of the stem if its average is 203.1 g over the other genotypes, GG GA, whose rates were 1.95 and 2.97 g, respectively.

A significant effect was found in the characteristic of the average carcass weight, as the genotype GG decreased significantly by (P<0.05), whose average amounted to 1547.26 gm on the genotypes GA, AA, whose rates reached 1749.98 and 1746.38 g, respectively. In the dressing percentage with giblet and without giblet by studying it on Ross 308 broilers, there were no significant differences in favor of the genotypes GG, GA, AA and in the characteristic of the weight of the breast muscles, as it was noted that there are no significant differences for the genotypes GA, AA, GG, as for the stem muscle weight, it was noted that there was a significant superiority of the genotype GA, which averaged 160.46 gm during the sixth week

of the experiment, over the genotypes GG and AA, whose averages reached 153.66 and 134.53 g, respectively.

As for the wings, the GA genotype was significantly superior to the GG and AA genotypes (P<0.05) with an average of 0.82 gm, whose rates were 0.58 and 0.54 g, respectively, while there were no significant differences in the relative weights (thighs, drumstick, neck, back, breast and abdominal fat). While the TT genotype outperformed the CT genotype by 1521.35 and 1499.32 g, respectively, our study differed in the average carcass weight for the intron (4) and exon (8), as there were no significant differences for the TT and CT genotypes during the sixth week of the experiment, as well as in the attribute (dressing percentage with and without giblet for intron (4). The genotype TT significantly outperformed (P<0.05), whose average amounted to 1208.88 gm, over the genotypes AT and TT, which averaged 1156.16 and 1175.20 g, respectively.

As for the weight of the breast muscles, the TT genotype significantly exceeded (P<0.05) during the sixth week of the experiment, which averaged 166.20 g on the AA and TT genotypes, whose averages were 176.56 and 171.92 g, respectively, as for the back muscles trait, the AA genotype significantly outperformed (P<0.05) and at a rate of 0.75 g, over the TT and AT genotypes, which had rates of 0.55 and 0.25 g, respectively, as for the wing and leg weight, there were no significant differences in favor of the genotypes AA, AT, TT, respectively. As for the weight of abdominal fat for the FATP gene in intron (4), the AT genotype significantly outperformed by (P<0.05), as its average reached 30.48 gm over the AA and TT genotypes, whose averages during the sixth week of the experiment were 27.87 and 21.13 g, respectively.

| **Genotypes** | **Traits** | | | | |
|---|---|---|---|---|---|
| | Carcass weight | Dressing with giblet | Dressing without giblet | Thigh with drum | Wings |
| **CC** | 148.42 ± 1457.55 A | 2.60 ± 849245 A | 2.01 ± 80.1612 A | 1.14± 28.476 a | 0.81 ± 104348 A |
| **CT** | 216.79 ± 1367.75 a | 2.81± 83.9350 A | 2.66± 790350 A | 1.47± 28.2988 a | 0.94± 10.1338 A |
| **AA** | 194.49± 1312.11 b | 3.08 ± 85.62 A | 3.01 ± 80.66 A | 1.15 ± 28.49 a | 0.64 ± 9.88 B |
| **GG** | 95.93 ± 1467.86 a | 2.15 ± 84.70 A | 1.98 ± 79.65 A | 1.10 ± 28.27 a | 0.69 ± 10.40 A |
| **GA** | 182.74 ± 1476.68 a | 1.75± 84.43 A | 1.84 ± 80.03 A | 1.32 ± 28.62 a | 0.98 ± 10.60 A |
| **Sig.** | * | N S | NS | NS | * |
| **Genotype** | Traits | | | | |
| | Neck | Stem | Back | Breast | Abdominal fat |

| | | | | | |
|---|---|---|---|---|---|
| **CC** | 0.45± 2.6738 a | 2.49± 60.86 A | 0.87± 163424 A | 1.90 ± 33.3929 a | 1.18± 1.7540 A |
| **CT** | 0.18 ± 2.6625 a | 59.88± 2.88 A | 1.05± 15.9212 A | 1.86± 33.6663 a | 1.28± 1.3012 A |
| **AA** | 0.20 ± 2.60 a | 2.31 ± 58.89 B | 0.85 ± 15.80 A | 1.05 ± 32.72 a | 0.94 ± 0.93 A |
| **GG** | 0.17 ± 2.73 a | 1.95 ± 61.00 A | 0.65 ± 16.38 A | 1.60 ± 33.42 a | 960. ± 1.73 A |
| **GA** | 0.65 ± 2.63 a | 2.97 ± 61.21 A | 1.12 ± 16.36 A | 2.36 ± 33.79 a | 1.43 ± 1.97 A |
| **Sig.** | NS | * | N S | NS | NS |

**Table 6. Relationship of polymorphism of FATP gene (G237A) (C106T) with carcass weight, dressing ratio and relative weight of the studied cuts. Weight of belly fat for broilers type ROSS 308 (mean ± standard error).**

## Discussion

The relationship of the polymorphism of the FATP G237A gene (C106T) with the body weight of ROSS 308 broilers. Table 5 indicates that there were no significant differences between the genotypes GG, GA, and AA of the FATP gene in the average live body weight during the (5) weeks of bird life, which are inconsistent results with Wang et al.[13] when studied on broilers of type ROSS 30. The results of the current study agree with the findings of [14]. When studying strains of Chinese chickens for the FATP gene in an intron (4) to study the characteristic of live weight during the sixth week of the experiment, it was noticed that there were no significant differences in favor of the genotypes GG, GA and AA, respectively. However, the difference between the results of our current study and previous studies may be due to more than two mutations of the same gene. As well as the difference in the breeds, the unbalanced feeding of broilers during the (5) weeks of life, and the difference in the studied region of the FATP gene. On the other hand, Table 6 shows significant differences ($P<0.05$) in the average carcass weight and the relative weight of the two wings in favor of the AA genotype compared to GG and genotypes. In contrast, no significant differences were observed between the different genotypes in the rest of the relative weights (thighs with drumsticks, neck, breast, back, and abdominal fat). These results were close to the study of [13] on the FATP gene in exon No. 4. Similarly, The results were close to [14] on ROSS broilers of type 308 in intron No. (5) of the FATP gene, as there was a significant effect ($P<0.01$) in the sixth week of the experiment on the average carcass weight[16,17.]

## Conclusions

The results of our current study also differed [from 15] when studying three strains of Chinese chicken for the FATP gene in exon No. (1). A significant superiority ($P<0.05$) was observed in the characteristic of abdominal fat weight in favor of the CT genotype, with an average of 55.72 g On genotypes TT and CC, whose rates

were 40.84 and 40.70 g, respectively, it also differed with.[16] when studying it on ROSS 308 broilers. It was noticed that the AA genotype decreased significantly ($P<0.05$) in the characteristic of belly fat weight and at an average of 3.72 gm on the BB and AB genotypes, whose rates were 4.19 and 4.9 straight clouds, as for the C106T genetic mutation of the FATP gene, it was observed through the results of our study that there is no significant effect on the characteristics of carcass weight, net ratio and relative weights (relative weight of thighs with drumstick, back, breast, wings, neck, leg, and abdominal fat) in favor of The genotypes CT and CC, respectively, have differences in the results of these traits, it may be caused by the difference in the studied region of the FATP gene, as well as to the different strains on which the research was conducted, or the presence of more than one genetic mutation within the same gene, so the mutation is beneficial, as there was an increase in the relative weight of the edible cuts and viscera, and this increase could be accompanied by the relative weight of the carcass and the relative weight of the wings and the neck to a decrease in the increase in the average weights of other cuts, therefore, when there is more than one genetic mutation for the same gene, it may be the reason for the low relative weights of other cuts, or because of feeding chickens on diets that are low in fats, oils, food additives, vitamins and mineral elements that cause an increase in the rate of carcass weight and other cuts.

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