

Study of the relationship of genetic polymorphism of SLC27 gene, fatty acid transport protein, with some economic parameters and carcass characteristics of broilers

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Abstract

This study was conducted in the poultry field of the College of Agriculture and the Marshes - University of Dhi Qar for the period from 10/11/2021 to 26/12/2021 in cooperation with the Molecular Genetics Laboratories at the Marsh Research Center - College of Agriculture and Marshes for the purpose of the effect of multiple genotypes of the FATP gene on productive performance and some traits Physiology of broilers of ROSS 308 strain, 150 birds were used in this study. The results of this study showed the following. The results were analyzed by the sequence of nitrogenous bases of the gene studied outside Iraq by the South Korean company, MacroGen corporation-Korea. Carcass weight, dressing ratio, relative weight of the studied cuts Three genotypes were diagnosed, the first being GG, GA and AA, and there were significant differences ($P < 0.05$) in the distribution ratios of the genotypes of the FATP gene according to the mutation G237A, where the GA genotype recorded the highest percentage, followed by the GG genotype, and then followed by the AA genotype Its proportions were 0.49, 0.39 and 0.12, respectively, and the G allelic frequency is superior to the A allele, as it reached 0.64 and 0.36, respectively, and it was noted that there were no significant differences for the genotypes of the G237A mutation in the weekly body weights, as it was found that there are no significant differences between the genotypes GA, GG and AA gene FATP in the average live body weight on the first day of life of the bird and the second, third, fourth and fifth weeks, The results showed that there was a significant difference ($P < 0.05$) for the genotypes of the G237A mutation in the average carcass weight, as the AA genotype was superior to the GG and GA genotypes. As for the relative weight of the wings, the AA genotype was superior to the GA and GG genotypes, while the relative weight characteristic of the leg was superior to Structure AA on the genotypes GG and GA There was no significant effect of the relative weights of the thighs with the drum stick, wings, neck, back and belly fat



I. INTRODUCTION

The poultry industry at the present time is characterized by the increase in both production and the 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 the productive value (wiseman & Garnsworthy 2001). Some genetic selection, different variants represent a valuable source of protein, vitamins and minerals & cningham (Demby, 2002) as the percentage of protein in poultry meat is about (5%) (Insr et al., 2006). The next episode was completed in the next episode, and a member of body fat (Simopoulos et al., 1998) Fat deposition is the most important axis for broilers, which is an important aspect in the development of genetic maps, as it is one of the important characteristics that have a direct relationship in determining the quality and quality of meat produced in the world, but in recent years, abdominal fat deposition has become one of the main physiological problems facing the poultry industry as well as reduce The overall production efficiency because it reduces the quality of carcasses and their products and on the other hand affects the consumption of poultry meat as a result of the effect of fat deposition on the health of the consumer (Kahraman et al., 2004)

Therefore, the current study aimed to reveal the multiple genetic phenotypes of the FATP1 gene, the fatty acid transport protein, and its relationship to some economic criteria and carcass characteristics for broilers type ROSS 308) using the technique of deficient DNA sequencing.

II. MATERIALS AND METHODS

2-1- The process of drawing blood and the method of extracting the genetic material DNA.

Blood was drawn from the winged vein of birds, where 3 ml of blood was collected from each bird and the samples were kept in test tubes containing EDTA anticoagulant. Genetic DNA was extracted from blood samples according to the instructions of the diagnostic (Kit supplied by the Korean Geneaid Company) 30 µl of bird blood was added to the 1.5 ml tube, and 200 µl of PBS (Phosphate buffered saline) was added to the blood capping, then 20 µl of Proteinase K solution was added at a concentration of (20 mg/ml) the samples were agitated by (Vortex) the tube was incubated for 2 minutes at room temperature and 200 µl of GSB (Genomic lysis/Binding buffer) was added. At a temperature of (60°C), then the Elution Buffer substance was heated at a rate of 100 microliters for each sample at a temperature of 60°C, and 200 microliters of Ethanol Absolute were added at a concentration of 100%. From the mixture after removing the formed blood clot using a Micropipette, it is placed in a filter tube, then the samples are placed in a centrifuge 14000 r/min, and then the filter is disposed of and new filter tubes are used, and then 400 mikes are added Roller of Wash Buffer solution 1), then the samples are placed in the centrifuge 14000 cycles / minute for a minute, then the filtrate is disposed of, then the filtration membrane is returned to the collection tubes, and 600 microliters of



Wash Buffer solution were added 2), then the samples are placed in the centrifuge 14000 cycles / minute for 3 minutes, then the filtrate is disposed of, then the filtration membrane is returned to the collection tubes, and then a new Eppendorf tube with a capacity of 1.5 ml is placed in it. The filter tube is then added 100 microliters of the previously prepared Elution Buffer. Centrifuge at 14,000 rpm for 1.5 minutes for the purpose of extracting the purified DNA, so that the samples were ready for electrophoresis to verify the presence of the genetic material using 1% agarose gel.

2-2- Gel Electrophoresis

The migration process was carried out to separate the DNA segments through agarose gel to ensure the presence of the DNA. TAE buffer (Tris Acetate EDTA) solution of 50 X concentration was used, prepared by Bioneer Company. For every 10 ml of TAE buffer, 490 ml of distilled water was added. It was used to prepare solution 1. X To prepare the agarose gel and also to fill the tank of the electrophoresis device. Clean 30 ml of 1 X concentration of (TAE) solution into a glass conical flask (flask) that can withstand high temperature and add 0.3 g of agarose (1% agarose) and close the mouth of the flask with a piece of tin and put it on a hot plate device to heat the mixture to a boiling point and wait until the agarose minutes melt and get a transparent solution. Leave the solution to cool for several minutes and prepare the mold into which the agarose is poured by placing the special comb to make the pits of the agarose gel in the place designated for it at the end of the basin. We add the agarose mixture to cover the entire area of the basin and in order not to cause bubbles, leave the mixture until the agarose hardens and remove the comb carefully without creating A tear in the gel. Put the gel based on the plate in its designated place inside the electrophoresis device. Fill the electrophoresis basin with buffer solution to the extent that fills the loading holes and to the extent that the level of the buffer solution rises from the agarose gel by about 3 mm.

2-3- Loading samples of DNA

Mixed 5 μ l of DNA samples with 2 μ l of Loading Dye dye prepared from Promega in addition to 1.5 μ l of Diamond™ Nucleic Acid Dye dye also prepared from Promega and diluted by TE at a dilution ratio (1 μ l dye to 100 μ l). of TE solution) and mix the mixture well using a micropipette on a plastic tape (Laboratory Film). Then the samples were carried into the pits of the prepared agarose gel. After all the samples were loaded into the pits, the transparent plastic cap was placed and the electrodes were connected to the Power Splay using 70 volts for a period of 85 and Milliampere for half an hour and the agarose gel was examined after the end of the migration time using a UV Gel Documentation device, and transfer pictures were taken using the installed camera designated for this purpose.

2-5- Technique PCR

Table (2): Program for PCR technique of FATP gene using Touchdown method.

No.Cycle	Time	Temperature	Stages
1	5	95C °	Initial Denaturation
30	30	95C °	Denaturation
	30	60C °	Annealing
	1	72C °	Extension
1	10	72C °	Final Extension
1		4C °	Cooling

III. RESULTS AND DISCUSSION

3-1- Amplification of theFATP gene segment1-1 DNA extraction

Scheme of the piece of DNA to be studied, which is the required piece (799Pb), which is intron No. (12) of the FATP gene using primers, and the piece was multiplied by Polymerases chain reaction technique. Description of the PCR Thermo cycler program, fixing what was mentioned in the separation of materials and methods of work and using a piece of known size DNA It was placed in the first hole of Gel Agarose %2. The gel was placed in the hot atmosphere of electrolysis by electrolysis of the solution, and the voltage of the electric current and time was adjusted, then a picture of the electrolysis product was taken to make sure that the extraction process for the electrolysis gene in order to perform the extraction process for the gene. 3). When reading the sequence of nitrogenous bases, it was found that there are SNPs for five genotypes: GG-GA-AA-CT-CC

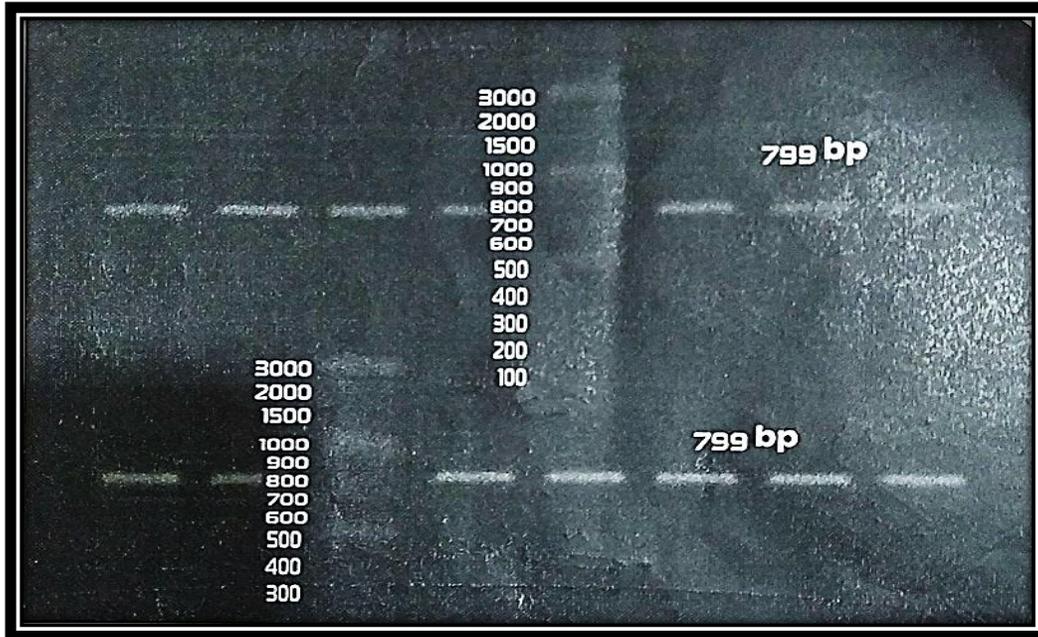


Figure (2)

The product demonstrates amplification of the segment (799) of the FATP gene

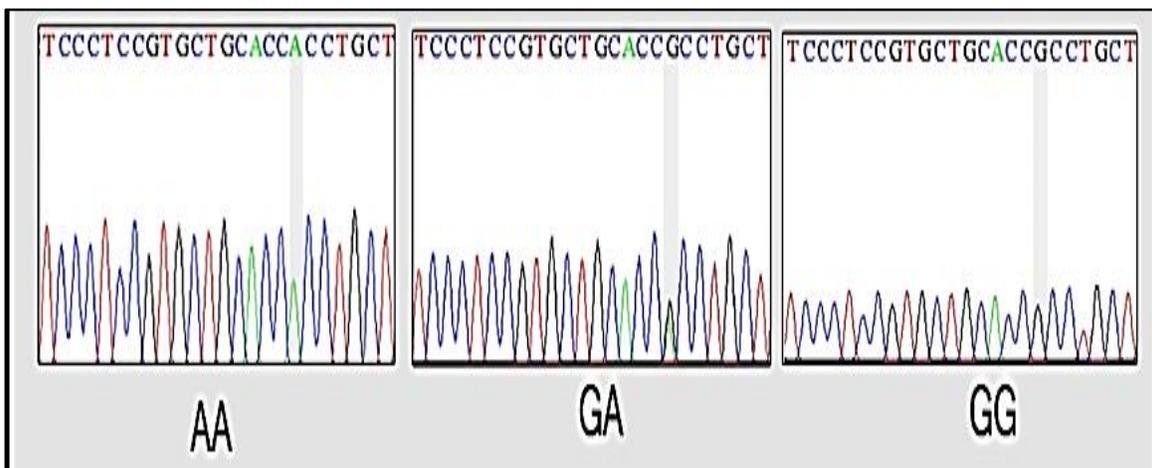


Figure (4)

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

4-4 Percentages and allelic frequency of FATP gene variation in 308ROSS broilers

Elucidation of the T C106 and G237A genotypes and the allelic repeats of the FATP gene in the ROSS 308 steak virus.

Frequency of genotypes and allele repeat value of the FATP gene				
Chi-square value x2	Repetition	Allele	Repetition of the genotype	genetic makeup
0,22	0,92	C	0,80	CC
	0,08	T	0,10	CT
0,07	0,64	G	0,39	GG
			0,49	GA
	0,36	A	0,12	AA

N.S: Not moral

**Highly significant differences (p<0.05)

4-1-5 Relationship of the polymorphism of the FATP G237A gene C106T with the live body weight of ROSS 308 broilers. The data shown in Table (9) indicate that there were no significant differences in the genotypes GG, GA, AA for the G237A genetic mutation of the FATP gene in intron No. (12), it is a silent mutation that does not change the amino acids, in the average live body weight during the 5 weeks) A significant decrease (P < 0.05)) was observed for the GG genotype, and the average date for the live weight trait was 1745.35 g for the GA and AA genotypes. During the fifth week of the experiment, whose averages were 1945.61 and 1958.23 g, respectively, due to the association of the FATP gene significantly with live body weight during the fifth week of the life of the birds. FATP for intron (2) to study the characteristic of live weight, it was observed that there is a significant increase (P<0.05)) in favor of the AA genotype, whose average during the fifth week of the experiment was 2253.85 gm on the AB and BB genotypes, whose rates were 1938.67 and 1919.20 g, respectively, due to the association of the A allele with the weight characteristic In vivo during the fifth week of life, there was no significant correlation between BB and AB genotypes, respectively. And in the third site of the genetic mutation of the FATP gene in exon (3) to study the character of live weight during the sixth week of the experiment, there was a significant increase (P<0.05)) in favor of the AA genotype, which averaged 1954.56 gm over the GA genotype, whose average was 1755.20 gm.



This gene contains many SNPs, some of which have been associated with vivo weight and skeletal growth, as it plays an important role in growth rates and metabolism. The results of the current study agree with what was found by Dihghing shu (2007).

When studying strains of Chinese chickens for the FATP gene in 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. The current in 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 there was a significant increase in favor of the CT genotype, which averaged 1521.35 g, while the CC genotype did not show any significant superiority, as its average was 1499.32 g The C allele had a positive effect on the vivo weight trait during the fifth week of life, and the genotype was not significant for the CC genotype in the vivo weight trait during the fifth week of life. The difference in the results of our current study with the results of previous studies may be due to the presence of more than two mutations of the same gene, as well as the difference in the breeds, the type of feed consumed for broilers during the (5) weeks of life, as well as the difference in the studied region of the FATP gene, as for the genetic mutation of the FATP C106T gene.) In intron No. (12), it is a silent mutation that does not change the amino acids. It was noted that there were no significant differences in favor of the CT and CC genotypes for the vivo weight characteristic of ROSS 308 broilers during the (5) weeks of the birds' life. The difference in the results of our current study with the results of previous studies may be due to the presence of more than two mutations of the same gene, as well as the difference in the breeds, the type of feed consumed for broilers during the (5) weeks of life, as well as the difference in the studied region of the FATP gene, as for the genetic mutation of the FATP C106T gene.) In intron No. (12), it is a silent mutation that does not change the amino acids. It was noted that there were no significant differences in favor of the CT and CC genotypes for the vivo weight characteristic of ROSS 308 broilers during the (5) weeks of the birds' life.

(المتوسط ± الخطأ القياسي)					Genetics
week Weight 5	week weight 4	week weight ٣	week weight ٢	week weight 1	
22.599± 155.95 A	68.593± 392.38 A	1771.52± 109.317 a	1290.62± 142.124 A	1816.62± 198765 a	CC
164.50± 12.036 A	73.161± 419.75 A	168.721± 745.75 a	229.847± 1268.50 A	299.456± 1808.50 a	CT

12.94± 151.78 A	72.55± 378.22 A	131.68± 74311 a	234.59± 1214.00 A	264.362± 1677.44 a	AA
26.73± 154.73 A	56.94± 399.73 A	99.62± 784.64 A	114.22± 1321.27	169.294± 1856.09 a	GG
16.77± 16295 A	82.11± 402.11 A	136.08± 758.95 A	144.97± 1282.11 a	220.282± 1833.42 a	GA
N.S	N.S	N.S	N.S	N.S	P≤0.05 morale level

4-1-7 Relationship of the genetic polymorphism of the FATP C106T-G237A gene

with carcass weight, dressing ratio and the relative weight of the cuts, the weight of the belly fat studied for broilers of ROSS 308 breed + standard error

It is noted from Table (11) that there are significant differences ($P < 0.05$) for the genetic mutation G237A in intron No. (12), as this silent mutation does not change the amino acids in the average carcass weight in favor of the AA genotype, as its average was 194.49 g for the two GG genotypes. AG, whose averages were 95.93 and 182.74 g, respectively, and the results of the current study indicated that there were no significant differences (the ratios of dressing with edible viscera) and (the ratios of dressing with uneaten viscera). For the genotypes GG, GA, and AA, respectively, and there were significant differences ($P < 0.05$) for the relative weight of the wings in favor of the AA genotype, which averaged 0.64 g on the genotypes GG, GA, whose rates were 0.69, 0.98 g, respectively, and the synthesis continues The AA genotype has a significant effect if it is superior to the relative weight of the stem if its average is 203.1 g over the other genotypes GG, GA, whose averages are 1.95 and 2.97 g, respectively.

As for the rest of the relative weights (thighs with drumstick, neck, chest, back and belly fat), no significant differences were observed between the genotypes GG, AA, and GA, respectively, and these results were in agreement with the study of Wang (and others, 2010). On broilers of strain ROSS 308 on the FATP gene in exon No. (4), a significant effect was found in the characteristic of average carcass weight, as the GG genotype decreased significantly by ($P < 0.05$), which reached an average of 1547.26 gm on the GA, AA genotypes, whose rates were 1749.98, 1746.38 g, respectively, and in the case of (purification ratios with edible viscera) (purification ratios without edible viscera) by

studying it on Ross 308 broilers. As there are no significant differences in favor of the genotypes G, GA, AA and in the characteristic of the weight of the chest muscles, it was noted that there are no significant differences for the genotypes GA, AA, and GG. As for the weight of the leg muscles, it was noted that there was a significant superiority of the genotype GA, which averaged 160.46 g during the week The sixth of the experiment was on the genotypes GG, AA, which averaged 153.66, 134.53 gm, respectively. As for the winged trait, the GA genotype significantly outperformed ($P<0.05$) and at a rate of 0.82 gm, on the genotypes GG, AA, whose rates reached 0.58,0.54 gm, respectively. While there were no significant differences in the relative weights (the weight of the thighs with the drumstick, the neck, the back, the chest, the weight of the belly fat), which found that the highest level of FATP gene expression for the average carcass weight and the level of expression in the calf muscle was the weight of the wings compared with the rest of the other cuts. The results were close to (Dihging shu, 2007) on ROSS broilers of type 308 in intron No. (5) of the FATP gene, as a significant effect ($P<0.01$) was observed in the sixth week of the experiment on the average carcass weight, as the TT genotype outperformed the genotype. CT of 1521.35, 1499.32 g, respectively, that the levels of gene expression are significantly related to the average carcass weight, and our study differed in the average carcass weight for intron (4) and exon (8), as there were no significant differences for the genotypes TT, CT during the sixth week of the experiment , as well as in the trait (clearance ratios with edible viscera) (and the ratios of dressing with unedible viscera) (for intron (4), the TT genotype significantly outperformed ($P<0.05$)), which averaged 1208.88 gm on the AA, TT genotypes, whose rates were 1156.16,1175.20 g StraightAs for the trait of chest muscle weight, the TT genotype significantly exceeded ($P<0.05$) during the sixth week of the experiment, which averaged 166.20 g on the genotypes AA, TT, whose rates were 176.56 and 171.92 g, respectively, while the back muscles trait was superior to the genotype Significantly, AA by $P<0.05$) and at an average of 0.75 g on genotypes TT, AT, whose rates were 0.55 and 0.25 g, respectively. As for wing and leg weight, there were no significant differences in favor of genotypes AA, AT, TT, respectively, as for fat weight trait. The abdomen of the FATP gene in intron (4) was significantly superior to the genotype AT by ($P<0.05$)), as its average reached 30.48 g over the genotypes AA, TT, whose rates during the sixth week of the experiment were 27.87, 21.13 g, respectively, one of the possible reasons is the researcher's use of different types of strains, the variable penetrance of mutations elsewhere in the gene, and cell-specific differences in gene expression, and that gene expression levels are significantly related to fat content and rate The weight of the carcass, the cut of the chest, the back and the neck, the weight of the belly fat, and therefore the FATP gene in chickens can be studied extensively. $P<0.05$ n the characteristic of abdominal fat weight in favor of the CT genotype, its average was 55.72 g on the TT and CC genotypes, whose averages were 40.84, 40.70 g, respectively due to the levels of gene expression significantly related to the weight of belly fat or the difference in the type of As for the genetic mutation C106T)) of the FATP gene in intron number (12), it is a silent mutation that does not change the amino acids. 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 the thighs with the drumstick, back, chest, wings, neck, leg, and belly fat weight) in favor of CT and CC genotypes, respectively.breeds, and also differed with Qwing



et al. (2006) when studying broilers of type ROSS 308, it was noticed that the AA genotype The differences in the results of these traits may be due to 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. This increase in the relative weight of the carcass and the relative weight of the wings and neck leads to a decrease in the average weight of other cuts, so when there is more than one genetic mutation for the same gene, it may be the reason for the decrease in the relative weights of other cuts, or because of the levels of gene expression of the FATP gene that was prevalent during that period, so it is reflected positively On the weights of other determinants, this is what is observed in our current study.decreased significantly by (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 reached 4.19 and 4.9 gm, respectively, due to Gene expression levels of FATP> gene, which is mRNA in weight of abdominal fat, compared with the rest of the tissues.

(mean ± standard error)					geneti
studied traits					
Relative weight of the wings (gm)	Relative weight of the thighs with the drumstick (gm)	Clearance Ratio 2	Clearance Ratio 1	carcass weight (gm)	
0.81 ± 104348 A	1.14± 28.476 A	2.01 ± 80.1612 A	2.60 ± 849245 A	148.42 ± 1457.55 a	CC
0.94± 10.1338 A	1.47± 28.2988 A	2.66± 790350 A	2.81± 83.9350 A	216.79 ± 1367.75 a	CT
٠,٦٤ ± ٩,٨٨ B	١,١٥ ± ٢٨,٤٩ A	٢,٠١ ± ٨٠,٦٦ A	٢,٠٨ ± ٨٥,٦٢ A	194.49± 1312.11 B	AA
٠,٦٩ ± ١٠,٤٠ A	١,١٠ ± ٢٨,٢٧ A	١,٩٨ ± ٧٩,٦٥ A	٢,١٥ ± ٨٤,٧٠ A	٩٥,٩٣ ± ١٤٦٧,٨٦ a	GG



0.98 ± 1.0, 6.0 A	1.32 ± 28, 62 A	1.84 ± 8.0, 0.3 A	1.70 ± 84, 43 A	182, 74 ± 1476, 68 a	GA
*	N.S	N.S	N.S	*	

(mean ± standard error)					Geneti
Belly Fat Weight (gm)	Relative chest weight (gm)	Relative weight of the back(gm)	Relativ weight of the stem (gm)	Neck Relative Weight (gm)	
1.18± 1.7540 A	1.90 ± 33.3929 A	0.87± 163424 A	2.49± 60.86 A	0.45± 2.6738 a	CC
1.28± 1.3012 A	1.86± 33.6663 A	1.05± 15.9212 A	59.88± 2, 88 A	0.18 ± 2.6625 a	CT
0.94 ± 0.93 A	1.00 ± 32, 72 A	0.80 ± 10, 8.0 A	2.31 ± 08, 89 B	0.20 ± 2, 6.0 a	AA
97.0. ± 1, 73 A	1, 70 ± 33, 42 A	0, 70 ± 16, 38 A	1, 90 ± 61, 0.0 A	0, 17 ± 2, 73 a	GG
1, 43 ± 1, 97 A	2, 36 ± 33, 79 A	1, 12 ± 16, 36 A	2, 97 ± 61, 21 A	0, 70 ± 2, 63 a	GA



A	A	A	A	a	
N.S	N.S	N.S	*	N.S	moral level P<0.05

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