Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 163-171
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(14)
Hussein & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

ASSOCIATION OF THE C2077G SNP IN GDF-9 GENE WITH THE PRODUCTIVE PERFORMANCE OF LOCAL AND SHAMI GOAT

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Received 14/ 6/ 2023, Accepted 15/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

The study was conducted on a sample of 44 local goats and 52 Sham goat at the Ruminant Research Station of the Agricultural Research Department / Ministry of Agriculture. Nucleotide sequencing technology was used to detect the SNP (C2077G) in the studied segment of the GDF-9 gene, in order to determine the relationship of the genotypes resulting from the mentioned mutation to the growth traits, milk yield and fertility. The genetic polymorphism are wild (CC), Heterozygous (CG), and mutant (GG). The percentage of individuals carrying the wild genotype was superior to each of the individuals carrying the mutant and hybrid genotype (52.27, 45.45, 2.27)%, respectively, in the local sample, while the percentage of individuals was superior The carriers of the wild genotype over each of the individuals carrying the heterozygous and mutant genotype were (65.38, 32.69 and 1.92)%, respectively, in the Shami goat sample, and the allelic frequency of the wild allele C in both Goat was (0.53, 0.82%), compared to the mutant allele G (0.47, 0.18). The results showed that there was a significant effect of the polymorphism of the mutation (C2077G) in the characteristics of the weaning weight and the current weight in the local goat and in favor of individuals with hybrid genotype (CG) (20.70, 38.21) kg, respectively, but in the sample of the Shami goats, an effect was found significant of the genetic variation caused by the mutation (C2077G) in birth weight and weaning weight in favor of individuals carrying the hybrid genotype (3.04, 22.20) kg, respectively, The results showed a significant effect of the polymorphism of the C2077G SNP on litter size in local and Shami goats in favor of individuals carrying the hybrid genotype CG (1.49, 1.76) births per litter. The mutation (C2077G) in the GDF-9 gene was recently discovered and is not registered in NCBI, so it has no identification number(rs) .

Keywords: GDF-9 gene , local goats, Shami goats, productive traits.

علاقة تعدد المظاهر الوراثية للطفرة (C2077G) في جين GDF-9 في الاداء الانتاجي للماعز المحلي والشامي

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الخلاصة

تم اجراء الدراسة على عينة مؤلفة من 44 معزة محلية و 52 معزة شامية في محطة ابحاث المجترات العائدة لدائرة البحوث الزراعية/ وزارة الزراعة. استخدمت تقانة التتابع النيوكليوتيدي في الكشف عن الطفرة (C2077G) في القطعة المدروسة من جين (GDF-9) ليتم تحديد علاقة التراكيب الوراثية الناتجة من الطفرة المذكورة بصفات النمو وانتاج الحليب والخصب، اظهرت نتائج الدراسة ظهور الطفرة في كل من سلالتي الماعز المحلي والشامي، اذ ظهرت بثلاث مظاهر وراثية هي البري (CC) والهجين (CG) والطافر (GG) وتفوقت نسبة الافراد الحاملة للتركيب الوراثي البري على كل من الافراد الحاملة للتركيب الوراثي الطافر والهجين (52.27،45.45، 2.27)% على التوالي في عينة المحلي، في حين تفوقت نسبة الافراد الحاملة للتركيب الوراثي البري على كل من الافراد الحاملة للتركيب الوراثي الهجين والطافر (65.38،32.69 و 1.92)% على التوالي في عينة الماعز الشامي، وتفوق التكرار الاليلي للاليل البري C في كلا السلالتين (0.53،0.82)% موازنة بالاليل الطافرG (0.47،0.18)%.وبينت النتائج وجود تأثير معنوي لتعدد المظاهر الوراثية للطفرة (C2077G) في صفتي وزن الفطام والوزن الحالي في عينة المحلي ولصالح الافراد الحاملة للتركيب الوراثي الهجين (CG) (20.70 ، 38.21) كغم على التوالي، اما في عينة الماعز الشامي فقد وجد تأثيراً معنوياً للتغاير الوراثي الناجم عن الطفرة (C2077G) في الوزن عند الميلاد ووزن الفطام لصالح الافراد الحاملة للتركيب الوراثي الهجين (3.04 ، 22.20) كغم على التوالي، وبينت النتائج تأثير معنوي لتعدد المظاهر الوراثية للطفرة (C2077G) في عدد المواليد في البطن الواحدة في الماعز المحلي والشامي لصالح الافراد الحاملة للتركيب الوراثي الهجين CG (1.49 ، 1.76) مولود/البطن الواحدة. لقد تم اكتشاف الطفرة (C2077G) في جين GDF-9 مؤخرًا وهي غير مسجلة في NCBI، لذا ليس لها رقم تعريف (rs).

الكلمات المفتاحية : جين GDF-9، الماعز المحلي ، الماعز الشامي، الاداء الانتاجي.

INTTRODUCTION

Goat are among the first animals that were domesticated by humans since the Neolithic era, that is, approximately more than 10,500 years ago, by humans in the Fertile Crescent region (Mesopotamia and the Levant), and its breeding spread after that to all parts of the world **(Amills *et al*., 2017; Gipson, 2019).** Goats are also considered the most adapted animals to harsh environmental conditions, which can maintain their productivity despite the lack of fodder and poor pasture compared to sheep and cattle **(Ibtisham *et al*., 2017)**, in addition to that goat are more resistant to epidemic disease and parasites compared to other farm animals **(Al-Jorani *et al.*, 2020)**, its management is simple and does not require much investment **(Al-Samarai *et al*., 2016).**

Due to the lack of natural pastures and the spread of diseases and infection with diseases, including viruses, in sheep and goats **(Mohammed & Yousif ,2022)** and exposure to conditions of heat stress resulting from high or low temperature, which led to a decrease in the reproductive performance of agricultural animals **(Ishaq & Ajeel ,2013)**. The breeder always seeks to increase the herd of animals by increasing the number of births, which is linked to the high fertility rate of the herd **(Al- Khazraji *et al,* 2014)**. This requires improving reproductive performance through an extensive study of the most important genes related to reproduction, including those associated with the ovulation process and the number of births in one litter, as it was found that there are many Among the genes that have a role in improving fertility **(Abdullah & Al-Khazraji, 2021)**, one of these genes is growth differentiation factor 9 (GDF-9), which is one of the genes that affects ovulation or sterility in agricultural animals **(Demars *et al.,* 2013; Al-Barzinji, 2022)**. **Al-Jubori *et al.* (2019)** indicated a significant effect of the GDF-9 gene on the number of births per litter in Awassi sheep. Therefore, the research aims: To identify the multiple manifestations of the C2077G mutation in the GDF-9 gene in a sample of local and Shami goats, and to determine their relationship to productive performance (growth, milk production, and fertility).

MATERNALS AND METHODS

The research was conducted on total 96 does belonging to two breeds (Local and Shami goat), with the same management and feeding conditions. Animals used were 2–5 years old, multiparous, lactating and in their first to fourth lactation.

Sampling, data collection and DNA extraction: A jugular vein blood sample was collected from each female goat using vacuum tubes with EDTA k2 as an anticoagulant. Birth weight was measured by using a small scale after birth directly. Kids were weaned at 120 days/ age, scale capacity 150 kg was used for weaning weight and body live weight. Total milk yield was calculated according to equation mentioned below.

Total milk production = (one milking / day* 2) × No. of milking days.

fecundity was calculated from equation mentioned below according to **Amnate *et al* (2016)**:

$$\text{litter size} = \frac{\text{Total no. of kids}}{\text{No. of parturition ewes}}$$

DNA extraction Genomic DNA: was isolated from blood sample according to the protocol ReliaPrep™ Blood g DNA Miniprep System, Promega.

Primers designed: The primer were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/ìl as a stock solution. A working solution of the primer was prepared by adding 10µl of primer stock solution (stored at freezer -20C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl (Table 1).

Table (1): Primer sequence.

Primer Name	**Seq.**	**Annealing Temp. (°C)**	**Product size (bp)**
GDF9-F	5’-CTCTCACCCTCCAAATCAAC-3’	60	987
GDF9-R	5’-TTCTTCCCTCCACCCATTA-3’		

Reaction setup and thermal cycling protocol:

Materials and PCR reaction condition showed in Tables 2 and 3.

Table (2): Master Mix components and volumes.

Master mix components		**Volume**
Master mix		12.5 µl
Primer	Forward	1 µl
	Reverse	1 µl
Nuclease free water		7.5 µl
DNA		3 µl
Total volume		25 µl

Table (3): Thermal cycling protocol.

Steps	**°C**	**m: s**	**Cycle**
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Standard sequencing: PCR product were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software.

Statistical analysis: The data was analyzed by used Statistical Analysis System **(SAS, 2012)** to study of GDF-9 gene polymorphism **(C2077G)** (CC, CG, GG) according to the mathematical model, significant differences was compared by used Least square means method.

$Yijk = \mu + Gi + eijk$

Yijk: Observed value, μ: Overall means, Gi: Effect of C2077G SNP polymorphism (CC, CG, GG), eijk: Random error which distributed normally with mean = 0 and variation σ^2e. Chi-square- χ 2 test were used to compare between the percentages of GDF-9 gene polymorphisms. Calculator of allele frequency of GDF-9 gene according to Hardy Weinberger's equilibrium **(Falconer &Mackay, 1996).**

$$P_A = \frac{2^{*}\text{ No. of Homozygous} + \text{No. of Heterozygous}}{2^{*}\text{ Total number of sample}}$$

$p+q = 1$

$qa = 1 - PA$

RESULTS AND DISCUSSION

It appears from the results of reading the sequence of the nitrogenous bases of the studied second exon region of the GDF-9 gene (Fig 1), that there is a point mutation in the base (2077) from cytosine to guanine, and it resulted in the change of the allele amino acid to glycine. As a result of the mutation (C2077G) in the studied local goat sample, three genotype appeared Hereditary (wild CC 52.27%, hybrid CG 2.27% and mutant GG 45.45%) with a highly significant difference among. As for the allelic recurrence, the wild allele C (53%) was distinguished over the mutant allele (47%), with a highly significant difference. The results of the Shami goats are in the same direction, with the emergence of three genetic manifestations as a result of the mentioned mutation (CC 65.38%, the hybrid CG 32.69% and the GG mutant 1.92%). The allelic frequency was in favor of the wild allele with a highly significant difference (C 82%) over the mutant allele (G 18%). (Table 4).

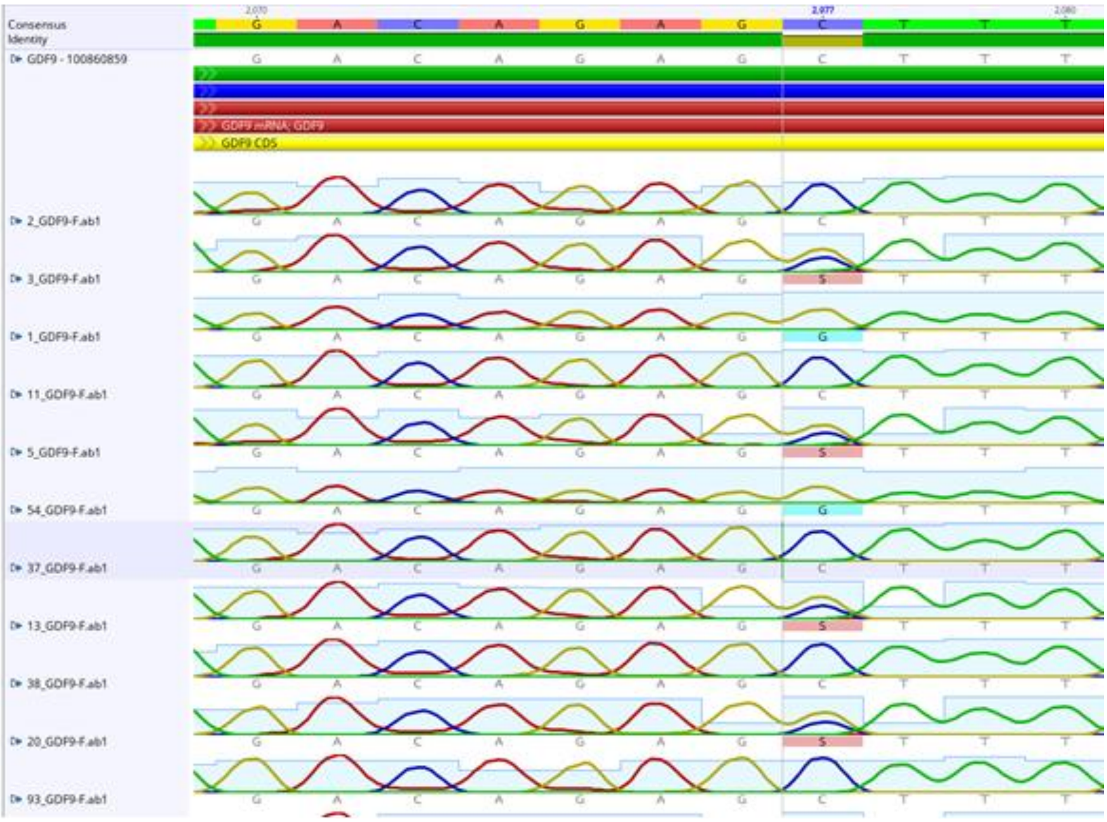

Figure (1): Sequence chromatograph of C2077G SNP of GDF-9 goat gene.

Table (4): Distribution and allele frequency for the mutation (C2077G) in the GDF-9 gene in local and Shami goat.

Genotype (Local goat)	**No.**	**(%)**	χ^2	**Allele frequency**	
CC	23	52.27	19.609**	C	G
CG	1	2.27		0.53	0.47
GG	20	45.45			
Total	44	100%	p-value **		
Genotype (Shami goat)	**No.**	**(%)**	χ^2	**Allele frequency**	
CC	34	65.38	37.230**	C	G
CG	17	32.69		0.82	0.18
GG	1	1.92			
Total	52	100%	p-value**		

** :($p \leq 0.01$)

It is noted from the results of Table (5) that there is a highly significant effect of the genotypes of the mutation (C2077G) in the local goat sample at weaning and current weight, in favor of the mutant genotype in both traits (20.70, 34.08) k:g. No significant effect was observed for the mentioned mutation in the birth weight trait.

In the Shami goat sample, both birth weight and weaning weight were significantly affected in the genetic variation resulting from the mentioned mutation and in favor of the hybrid genotype (3.04, 22.20) kg over individuals carrying the wild genotype (2.65, 18.12) kg, while the current weight was not affected with the genetic variation mentioned in Table (5). These results were consistent with what **Abbas (2018); Ali (2022)** mentioned in terms of the relationship and importance of the GDF-9 gene in growth traits.

Table (5): Relationship of C2077G mutation polymorphism in GDF-9 gene on the growth traits of local and Shami goat.

Genotype (Local goat)	**Mean ±SE**		
	BWT(Kg)	**WWT(Kg)**	**LBWT(Kg)**
CC	2.64±0.31	15.99±0.24 b	34.08±1.64 b
GG	2.65±0.29	20.70±0.30 a	38.21±1.13 a
p-value	N.S	**	**
Genotype (Shami goat)	Averages ±SE		
	BWT(Kg)	WWT(Kg)	LBWT(Kg)
CC	2.65±0.15 b	18.12±0.33 b	38.59±1.44
CG	3.04 ±0.19 a	22.20 ± 0.45 a	37.34±1.16
p-value	*	*	N.S

**($p \leq 0.01$) ،-*($p \leq 0.05$), (N.S) non-significant.

-Values in columns with different letters differ significantly.

It is noted from Table (6) a significant effect of the genetic variation resulting from the mutation (C2077G), as it was found that there were significant differences in litter size, as individuals carrying the mutant genotype(1.49) Kids/litter, were superior to individuals carrying the wild genotype (1.29 Kids/litter) This result is consistent with what was mentioned by **Al-Jubouri *et al* .(2019)** regarding the importance of the GDF-9 gene in fertility, while no significant effect of the aforementioned mutation appeared on the rest of the milk production traits studied (total milk production, daily milk production, season length.

As for the Shami goat sample, there were significant differences in the litter size and in favor of individuals carrying the hybrid genotype (1.76 Kids/litter) over individuals bearing the wild genotype (1.24Kids/litter), and no effect was shown. Significant in the rest of the traits (Table 6). The current results agreed with the results of the study of **Ali (2022)** that there was no significant effect on milk production traits.

Table (6): The relationship of genetic polymer phism of the mutation (C2077G) in the GDF-9 gene withe the total and daily milk production, length of milk season, and Litter size in Local and Shami goats.

Genotype (Local goat)	**Averages ±SE**			
	TMP(Kg)	**DMP(Kg)**	**SL(Day)**	**Litter size (Kids/litter)**
CC	444.15± 5.19	2.14 ± 0.17	207.46± 7.14	1.29±0.07 b
GG	451.35± 5.82	2.10± 0.29	215.14±7.06	1.49±0.09 a
p-value	N.S	N.S	N.S	*
Genotype (Shami goat)	**Averages ±SE**			
	TMP(Kg)	**DMP(Kg)**	**SL(Day)**	**Litter size (Kids/litter)**
CC	488.30±14.07	1.90 ±0.19	257.00 ±9.29	1.24 ±0.17 b
CG	464.18± 19.26	1.93±0.21	240.51±8.13	1.76±0.20 a
p-value	N.S	N.S	N.S	*

-*($p \leq 0.05$), (N.S) non-significant.

-Values in columns with different letters differ significantly.

There are many genes that affect litter size in local goats, including LHX3 and CYP17 gene (**Salam *et al.*, 2022; Abdullah *et al.*, 2023**).

CONCLUSION

By studying (C2077G) SNP in the GDF-9 gene, we can say that there are broad prospects for the future selection program as well as improvement strategies for farm animals to increase economic return and select individuals that have the best productive performance.

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