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Relationship of genetic Polymorphisms of FADS2 gene in some productive traits of Holstein cows in Iraq

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ABSTRACT

The research was conducted at the Al-Khalis Al-Kubra cattle station in Diyala Governorate and the central laboratory of the College of Agriculture - Tikrit University on a sample of 63 Holstein cows (dairy) for 748 days, with the aim of extracting the genetic material and identifying the genotypes polymorphism of the gene (FADS2) and the relationship of these genotypes to the characteristics of milk production and its components, as well as the study of the distribution ratios of its structures in the herd, the frequency of the obtained alleles, and the calculation of the chi-square value ($\chi 2$). Where the distribution percentage of genotypes of the FADS2 gene in the studied bovine sample was 25.40, 60.32 and 14.28 for genotypes AA, AG and GG respectively, the frequency of allele A was 0.56, while the frequency of allele G was 0.44 according to the analysis of the FADS2 gene in the study stream. The effect of FADS2 genotypes on the percentage of milk composition was significant ($P<0.05$), where cows with AG hybrid genotype achieved the highest rates in (non-fat solids, protein, lactose) (7.79 ± 0.15), (2.85 ± 0.06) and (4.19 ± 0.09) respectively, while cows with other genotypes gave the lowest averages for the mentioned traits. It can be concluded from the study of genetic variation of the FADS2 gene that it is possible to develop plans for genetic improvement in milk-producing cows.

علاقة المظاهر الوراثية لجين FADS2 في بعض الصفات الانتاجية لأبقار الهولشتاين في العراق

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الخلاصة

أجري البحث في محطة أبقار الخالص الكبرى في قضاء الخالص – محافظة ديالى والمختبر المركزي لكلية الزراعة – جامعة تكريت على عينة مكونة من 63 بقرة هولشتاين (حلوب) لمدة 748 يوماً, بهدف فصل المادة الوراثية وتحديد التراكيب الوراثية لجين (FADS2) وعلاقة هذه التراكيب بصفات انتاج الحليب ومكوناته, فضلاً عن دراسة نسب توزيع تراكيبها في القطيع وتكرار الاليلات المتحصل عليها وحساب قيمة مربع كاي (χ^2) . حيث بلغت نسبة توزيع التراكيب الوراثية لجين FADS2 في عينة الابقار التي تمت دراستها 25.40 و 60.32 و 14.28 للتراكيب الوراثية AA و AG و GG على التوالي, بلغ تكرار الاليل A هو 0.56 في حين كان تكرار الاليل G هو 0.44 على وفق تحليل جين FADS2 في الدراسة الحالية. كان تأثير التراكيب الوراثية لجين FADS2 في النسبة المئوية لمكونات الحليب معنوي ($P<0.05$) , إذ حققت الابقار ذات التركيب الوراثي الهجين AG اعلى متوسطات في النسب (المواد الصلبة غير الدهنية, البروتين, اللاكتوز) اذ بلغت (7.79 ±0.15) و (2.85 ±0.06) و (4.19±0.09)% على التوالي بينما اعطت الابقار ذات التراكيب الوراثية الاخرى اقل المتوسطات للصفات المذكورة. يمكن الاستنتاج من خلال دراسة المظاهر الجينية لجين FADS2 إمكانية وضع خطط للتحسين الوراثي لدى الابقار المنتجة للحليب, كما نوصي تطبيق الدراسة على عينة اكبر ولعدة مواسم ومواقع مختلفة من الجين مع دراسة عدد اكثر من الصفات الاقتصادية لإعطاء نتائج اكثر دقة لتطبيق استراتيجية الانتخاب والاستبعاد.

الكلمات المفتاحية: جين FADS2, انتاج الحليب, ابقار الهولشتاين.

INTRODUCTION

Livestock contributes 40% of global agricultural production (FAO, 2015). Cows play an important role in the agricultural economy, contributing about 85% of global milk production (Fox, 2003). Milk and its products occupied leading positions in the world as one of the most important foods that constitute the vital center in the human diet at all stages of life because it contains many important elements such as fatty acids, proteins and calcium, which play an important role in maintaining a balanced health level (García-Montoya *et al.,* 2012). Milk and its components are affected by several genes, so genes have been used and the genetic selection of individuals to obtain a quality of milk that is in line with the desire of the consumer. Several candidate genes or polymorphisms have been identified within these genes that have a positive relationship with the characteristics of milk production in dairy cows (Chamberlain *et al.,* 2012, Li *et al.,* 2018, Khan *et al.,* 2019).

One such candidate gene is the Fatty acid desaturase2 (FADS2) gene that affects the fatty acid composition of milk (Li *et al.,* 2019, Li *et al.,* 2020, Ma *et al.,* 2021). The FADS2 gene is located on bovine chromosome 29 (Witold *et al.,* 2019, Li *et al.,* 2020). The FADS2 gene is located on the long arm of the chromosome within the region 29q17- 29q18. It encodes 359 amino acid sequences (Li *et al.,* 2020), and consists of 12 exons (Takahashi et al. , 2016 and Li *et al.,* 2020). The FADS2 gene encodes an enzyme (FADS2) that is important in the biosynthesis of long-chain

polyunsaturated fatty acids and is able to catalyze the insertion of double bonds at the sixth carbon atom in a large number of fatty acids (Li *et al.,* 2019). It was also noted that there is a statistically significant relationship between FADS2 and fatty acids, as it converts alpha-linolenic acid and linoleic acid into stearidonic acid and gamma-linolenic acid (Proskura *et al.,* 2019). Soyeurt et al. (2006) reported that FADS2 plays an important role in raising the nutritional quality of milk. An enzyme known as FADS2 plays an important role in regulating physiological and pathogenic processes such as immune and inflammatory responses including asthma and arthritis (Calder, 2013).

Ibeagha-Awemu *et al.,* (2014) demonstrated through his study on several SNP on different regions of the gene that there is a relationship of gene polymorphism with monounsaturated and polyunsaturated fatty acids. Xu et al. (2016) indicated through his study that there are three SNP in the 3′UTR region and it was found that the mutations c.1571 A>G and c.2776 A>G significantly affected the fatty acid content of milk, while the third mutation did not affect. Proskura *et al.,* (2019) did not find significant correlation between location (rs209202414) resulting in A-to-G substitution and milk production in cows. The study aim of extracting the genetic material and identifying the genotypes polymorphism of the gene (FADS2) and the relationship of these genotypes to the characteristics of milk production and its components.

MATERIALS AND METHODS

The field part was conducted at Al-Khalis Al-Ahgar station in Diyala Governorate and molecular analyzes were conducted in the central laboratory of the Faculty of Agriculture - University of Tikrit for a period of 748 days on 63 cows with a feeding system consisting of providing green fodder represented by alfalfa plant in the form of bales on two meals after coarse fodder and by 20-25 kg per cow, while coarse fodder represented by hay is provided on two morning and evening meals after concentrated feed and by 4-5 kg per cow. The concentrated feed is provided after the morning and evening fenugreek at the rate of 10-12 kg per cow. The study aims to identify the genotypes of the FADS2 gene and the relationship of these genotypes to the characteristics of milk production and its components. Milk production was calculated by taking samples of milk for the morning and evening milking daily (according to the station's program), as well as analyzing the components of milk by taking samples of milk for the morning milking every 30 days and examining them using a special device for analyzing the components of milk from the company (FUNKE GERBER) of German origin. For the purpose of extracting genetic material and conducting molecular analyzes, 4 ml of blood was drawn by sterile syringes of 5 ml of jugular vein of 63 Holstein cows, and the blood was discharged into tubes containing the anticoagulant K2EDTA in a volume of 2 ml.

DNA extraction

The DNA was extracted using the chemical extraction method (buffered solutions) in which chemicals (sucrose, Tris HCL, MgCl2, Triton X-10, Na2EDTA, Sodium Dodecyl Sulfate (SDS), sodium citrate, NaCl, Ammonium acetate, absolute ethanol and 70% ethanol) were used. The extracted samples were electrophoresed in an agarose gel at a concentration of 1% with a voltage difference (80 V - 70 mA - 60 minutes) as in (Figure 1).

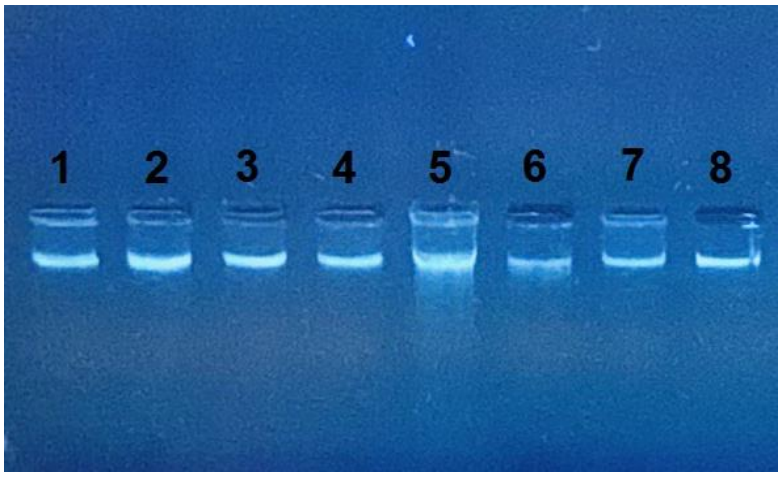

Figure 1. The extracted genetic material

PCR and RFLP

Genotypes were determined from the extracted genetic material with amplifying the target region in the Exon3 region of the FADS2 gene by PCR using a mixture of Master Mix manufactured by the American Promega company and the primer prepared by the Korean company Macrogen (F- 5`-TCCCAGATCACCGAGGACTT-3`) and (R- 5`-TTCAGAGCGTTGGCACCTAG-3`) according to the following program: the Initial Denaturation stage at a temperature of 94 ° C for 5 minutes for one cycle, then comes the stage of Denaturation at a temperature of 94 ° C for 45 seconds and Annealing at a temperature of 60 ° C for a period of 30 seconds and Extension at a temperature of 72 ° C for a period of 45 seconds 35 A cycle, then the final Extension phase at 72°C and the Final incubation (hold) at 10°C for one cycle. After that, The amplified samples were electrophoresed in an agarose gel at a concentration of 2.5% with a voltage difference (80 V - 70 mA - 60 min) and the PCR output was a size of (292 bp) as in (Figure 2).

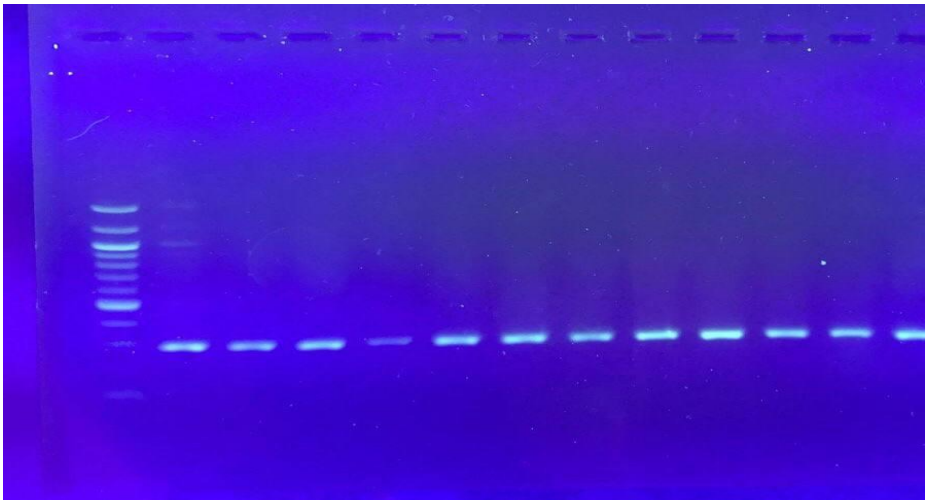

Figure 2. Samples on an agarose gel after electrophoresis

After that, RFLP technology was used by cutting the PCR product by the restriction enzyme TseFI (prepared by the Russian company Sib Enzyme) to cut within a sequence (*GTSAC) and this was done by taking 8 µl of PCR output, then taking 2 microliters of TseFI diluted by the buffer solution (10X Buffer) at a concentration of 1 IU, then adding to the mixture so that the total mixture is 10 microliters, then incubating the mixture at a temperature of 65 °C for 16 hours, after which it

cutted Samples were electrophoresed in agarose gel at a concentration of 3% at a voltage difference (80V-70mA-60min) with the use of DNA Ladder (1500-100)bp.

Statistical analysis

The data were analyzed statistically using the SAS (2018) statistical analysis system software to study the effect of genetic polymorphism of the FADS2 gene on the traits and components of milk production in Holstein cows, significant differences between the averages were extracted using the Duncan (1955) polynomial test by applying the (Least Square Means) Method.
Mathematical model: The relationship of the genotypes of the *FADS2* gene in the studied traits:

$Y_{ijklm} = \mu + FADS2_i + P_j + S_k + X_l + e_{ijklm}$

Yijklm: observed value m for genotype i, Parity j, birth season k, and sex newborn l
μ: the overall mean of the trait.
FADS2i: influence of Genotypes of the FADS2 gene (AA, AG, and GG).
P_j: influence of Parity (second, third and fourth).
S_k: influence of birth season (winter, spring, summer and autumn).
X_l: the influence of the sex of the newborn (male, female).
eijklm: the random error that is normally distributed with a mean of zero and a variance of 2eσ.

RESULTS AND DISCUSSION

The RFLP technique revealed the existence of an SNP by substituting the nitrogenous base A to G in the Exon3 region of the gene, and after using the TseFI enzyme, the genotypes appeared on the basis of bundles in the agarose gel, and the result was: AA(205, 87 bp), AG(205, 87, 72, 15). bp), GG(205, 72, 15 bp) as in (Figure 3).

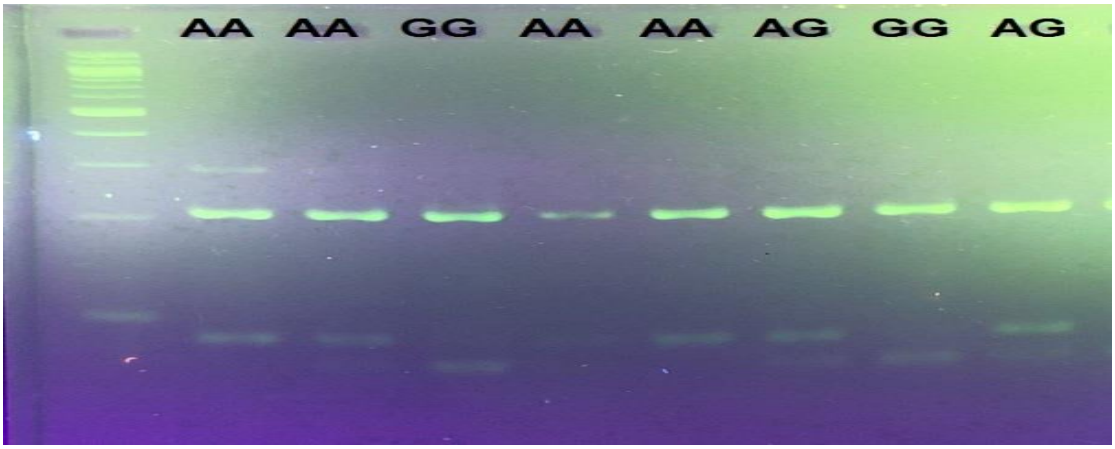

Figure 3. Polymorphism of Genotypes of the FADS2 gene in Holstein cows..

Table (1), there were no significant differences between the observed and expected numbers by calculating chi-square, as the allele frequency A (0.56) and G (0.44) were calculated. Unlike a study (Proskura et al., 2019) which discovered a significant difference in allele frequency distribution ratio, where the G allele prevailed in the Polish Holstein-Friesian cows.

Table 1: The number, percentages and allelic frequency of the FADS2 gene for Holstein cows.

Genotype (FADS2)	Number	Percentage %
AA	16	25.40
AG	38	60.32
GG	9	14.28
Total	63	% 100
chi-square value ($\chi 2$)	----	4.238 NS
Allele	Allelic frequency	
A	0.56	
G	0.44	

It was shown through the results of the analysis of the current study in Table (2), which showed that there were no significant differences between the genotypes of the FADS2 gene in lactation period, daily milk yield and 305-day milk yield .

Table 2: Relationship of genotypes of FADS2 gene to milk production in Holstein cows.

Genotype	N	Least Squares Means		
		Lactation period (day)	daily milk yield (kg)	305-day milk yield (kg)
AA	16	280.52 ± 26.25	15.68 ± 1.62	4782.90 ± 494.31
AG	38	286.64 ± 19.43	14.78 ± 1.20	4508.98 ± 365.98
GG	9	299.86 ± 27.06	14.52 ± 1.67	4430.81 ± 509.59
Significant		NS	NS	NS

N: number, NS: Non significant

Table (3) showed that there were no significant differences (P<0.05) between the three genotypes AA, AG, and GG in the fat percentage trait. She supported a study (Proskura et al., 2019) that found no discernible variation in SCD1 genotypes between Holstein-Friesian Polish cows. While it was observed in the percentage of solids non-fat, the cows with the genotype AG gave the highest average and reached 7.79% ± 0.15, and It was significantly superior (P<0.05) to the cows bearing the genotype GG, which gave the lowest mean and reached 7.34 ± 0.22, with a significant difference of 0.45, while the cows showed Those carrying the AA genotype had an average value of 7.74% ± 0.21, As for the percentage of protein, the cows of the genotype AG were characterized by the highest average, which amounted to 2.85% ± 0.06, and were significantly superior (P<0.05) to the genotype GG, which amounted to 2.67% ± 0.08, with a difference of (0.18), while there was no significant difference with the cows of the genotype AA which gave an average of 2.80 ± 0.08. The results also showed a significant superiority (P<0.05) of lactose in milk for cows of the AG genotype, which gave an average of 4.19% ± 0.09, a difference of (0.25) on cows with a genetic structure GG, which averaged 3.94% ± 0.13, and there were no significant differences in cows with AA genotype, which averaged 4.13 ± 0.12.

Table 3: Relationship of genotypes of FADS2 gene with milk components of Holstein cows.

Genotype	N	Least Squares Means			
		Fat %	S.N.F %	Protein %	Lactose %
AA	16	3.71 ± 0.38	7.74 ± 0.21 ab	2.80 ± 0.08 ab	4.13 ± 0.12 ab
AG	38	3.71 ± 0.28	7.79 ± 0.15 a	2.85 ± 0.06 a	4.19 ± 0.09 a
GG	9	3.53 ± 0.40	7.34 ± 0.22 b	2.67 ± 0.08 b	3.94 ± 0.13 b
Significant		NS	*	*	*

The means that carry different letters within the same column differ significantly among themselves

S.N.F: Solids non fat *: (P<0.05), NS: Non significant

From Table (3) the results of The study's findings, which were in agreement, showed that there was no connection between the FADS2 genetic polymorphism and the percentage of fat in milk (Proskura et al., 2019). There are no published reports on the relationships between FADS2 polymorphism and solids non-fat percentage (SNF%), protein percentage (PP%) and lactose percentage (LP%) in milk, This study was the first investigation associated with the FADS2 gene polymorphism and solids non-fat percentage (SNF%), protein percentage (PP%) and lactose percentage (LP%) in milk traits in Holstein cattle. At present, the precise molecular mechanisms of how FADS2 could affect Milk composition traits.

CONCLUSION

Amplification of the target region in Exon 3 from the FADS2 gene and the use of the TseFI enzyme resulted in the emergence of alleles (A, G) and three genotypes (AA, AG, GG) and subject to Hardy-Weinberg equilibrium. The genotype (AG) showed an increase in the proportion of non-fat solids, the percentage of protein, and the percentage of lactose in milk compared to other genotypes and with a significant difference (P<0.05).

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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