Abstract: The present study was undertaken to characterize the genetic diversity of the aromatase cytochrome P450 (CYP19) gene in 34 cows (15 local, 14 Holstein, and 5 Crosses) in Iraq. The objectives of the present study are to detect SNPs (mutations) in promoter p1.1 of the CYP19 gene in cattle bred in Iraq using sequencing techniques. We identified five single-nucleotide polymorphisms (SNP) loci of the CYP19 gene that were detected, namely G933T, G994C, A1044G, A1062T, and C1468A. The results showed the presence of 3, 4, and 2 polymorphic sites leading to the construction of 4, 5, and 3 different haplotypes for Holstein, local, and crosses respectively. Haplotype diversity were 0.791, 0.752, and 0.700 respectively. While nucleotide diversity was 0.0017, 0.0022, and 0.0013 respectively. Besides, we carried out a phylogenetic analysis of these sequences to address the evolutionary relationship between the animal species. These fragments were assigned in the GenBank database under the accession numbers: LC490756, LC490757, LC491437, LC491438, LC491439, LC491588, and LC491589.

Keywords: CYP19 gene, Iraqi cattle, single nucleotide polymorphism, Genetic Diversity, Phylogenetic tree

Introduction

The tools of molecular genetics, which allow the detection of genes, have major effects on complex traits, such as reproductive performance. These tools can be used as selection criteria for reproductive traits for genetic improvement (Amitosh, 2018). A large number of genes affect the reproductive performance of cattle, one of them is CYP19. It is an important gene that is significantly associated with these important traits in cattle (El-Bayomi et al., 2018). CYP19 gene belongs to the cytochromes P450 genes. P450 genes are part of a multi-gene superfamily, which contains 27 distinct genetic families, ten of which are dedicated to mammals, one of which is the CYP19 gene (Jedrzejczak et al., 2011). In mammals, reproduction is mainly regulated by estrogen, which is synthesized in ovaries through the androgen's aromatization (Kowalewska- Luczak, 2010). The CYP19 gene encodes the cytochrome P450 aromatase enzyme, aromatase P450 catalyzes the last step in the steroidogenesis which converts the androgens (C19) to estrogens (C18) (Damiani & Damiani, 2007), which maps to band q2.6 on...
chromosomes 10 and has 11 exons in cattle (Aken et al., 2016). CYP19 utilizes different promoters in tissue-specific expression in the alternative splicing mechanism (Simpson & Davis, 2001). Different promoter regions correspond to different 5'-UTR transcripts but the coding region is identical for all tissues (Kalbe et al., 2000). The placental expression of the CYP19 gene is regulated by P1.1 promoter, and A> G mutation has been detected in this region (Keskin et al., 2015). As well as two SNPs in the P1.1 region of the CYP19 gene was identified, the first mutation was a G>A transition at position 1044 and the second one was an A>G transition at position 1179 (Mohamadnejad-Sangdehi et al., 2015). Several single nucleotide polymorphisms (SNPs) studies in CYP19 gene have been reported in different livestock species and breeds as; Slovak Simmental cattle (Trakovická et al., 2015), Jersey cattle (Kowalewska-Luczak et al., 2013), Rathi cattle (Amitosh et al., 2017), the crossbred cows in Egypt (Saber et al., 2017), Gyr dairy cows (Vega et al., 2018).

This study aimed at determining single nucleotide polymorphisms in the CYP19 gene using DNA sequencing methods and using bioinformatics tools to study this fragment in cattle bred in Iraq. As well as determining genetic diversity both within and between breed.

Materials & Methods

Animals and genomic DNA isolation
This study included the use of 34 cows (15 local, 14 Holstein, and 5 Crosses). Blood samples (10 ml.cow⁻¹) were collected from the jugular vein. Genomic DNA was extracted from whole blood using the gSYNC™ DNA Extraction Kit (Geneaid). A fragment (657bp) in the P.1.1 promoter region of the CYP19 gene in cattle was amplified by using our designed primer F: 5’-GGCAAGGGCCTCATATGGTT-3’ and R: 5’-TGTCAGGGAATGTGAGGTGC-3’. The PCR amplifications were conducted in a 50 μl volume containing 6 μl genomic DNA, 25 μl of Master Mix, 4 μl both primer, and 15 μl free water. The amplification conditions were as follows: initial denaturation at 94 C for 5 min followed by 35 cycles of denaturation at 94 C for 1 min, annealing at 56ºC for 40 Sec, and extension at 72ºC for 30 Sec., followed by the final extension at 72ºC for 5 min. The PCR product was detected by 2% agarose gel electrophoresis, stained with Ethedium bromide and visualized by ultraviolet light. For sequencing, the PCR product was sent to Yang ling Tianrun Aoka Biotechnology Company, China.

Data Analysis

The sequencing results of the CYP19 gene were compared with accession no. Z69241 at the NCBI by BioEdit 7.0 software (Hall, 1999). Haplototype diversity (HD) and nucleotide diversity (π) were analyzed using DnaSP v5. 10 software (Librado & Rozas, 2009). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt et al., 1999). The phylogenetic tree was drawn by using the MEGA X software (Kumar et al., 2018).

Results

The CYP19 haplotype sequences from cattle bred in Iraq have been assigned in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ) and the European Nucleotide Archive (ENA) under the accession
numbers (LC490756, LC490757, LC491437, LC491438, LC491439, LC491588, and LC491589).

**Genetic Diversity**

The total number of sequences (N) and haplotypes (H) were 34 and 7 respectively, resulted in 5 polymorphisms number (NH) distributed to 4 local, 2 crosses and 3 Holstein (Table 1). Holstein revealed the highest value of haplotype diversity (HD) (0.791), followed by the local breed (0.752) and the cross cattle (0.700). On the contrary, local cattle recorded the highest nucleotide diversity (\( \pi \)) followed by Holstein and cross cattle (0.0022, 0.0017 and 0.0013 respectively).

| Breeds      | (N) | (H) | (NH) | (HD) | (\( \pi \)) |
|-------------|-----|-----|------|------|-------------|
| Local       | 15  | 5   | 4    | 0.752| 0.0022      |
| Crosses     | 5   | 3   | 2    | 0.700| 0.0013      |
| Holstein    | 14  | 4   | 3    | 0.791| 0.0017      |

N: Number of Sequences; H: Haplotype; NH: Number of polymorphic; HD: Haplotype Diversity; \( \pi \): Nucleotide Diversity

**Haplotype Network**

A total number of haplotypes of the \( CYP19 \) gene showed by different breeds were seven (Fig. 1). Three haplotypes (H-2, H-3, and H-4) found in all breeds while the remaining four are divided into two for the local breed (H-6 and H-7) and two for Holstein (H-1 and H-5). Each pair of haplotypes (H-2, H-5 or H-3, H-4) differs from each other with a nitrogen base (592bp). The haplotypes (H-2, H-3 or H-4, H-5) differ from each other with a nitrogen base (563bp). The branches represented H-1 of Holstein cattle differed from H-3 by 28 and 89 bases. Whereas, the haplotypes H-6 and H-7 represented the local cattle differed from H-2 by 157 and 139 bases.

The results in fig. (2) observed the analysis of nucleotides in the P.1.1 promoter region of the \( CYP19 \) gene and they recorded five SNPs; guanine to thymine (G933T), guanine to cytosine (G994C), adenine to guanine (A1044G), adenine to thymine (A1062T) and cytosine to adenine (C1468A). All SNPs are recorded first time except (A1044G).

**Phylogenetic tree of \( CYP19 \) gene**

The evolutionary tree of the \( CYP19 \) gene showed that there were three main branches (Fig. 3). The first branch included the cattle bred in Iraq. The second branch included Holstein bred in Germany. While the third branch divided into two branches, the first branch was presented by the Australian cattle and the second Indian cattle.
Fig. (1): Haplotype network of *CYP19* gene among studied cattle.

Fig. (2): Sequencing of the *CYP19* gene in Iraqi Cattle (LC491589) vs. Reference Sequence (Z69241).
Fig. (3): The Phylogenetic tree of the CYP19 gene between some cattle bred in Iraq of different countries.

**Analysis of molecular variance AMOVA**

The results of the AMOVA of the CYP19 gene between and within cattle breeds showed that genetic variation between breeds was 0.30% and the variation within breeds was 99.70% from the total variance (Table 2). This finding illustrated by the fact that genetic variation within the breed is much greater than genetic variation among breeds.

**Table (2): -Molecular contrast analysis of gene CYP19 with strains in the world.**

| Source of variation | df | Sum Squares | Variance Components | Variation % |
|---------------------|----|-------------|---------------------|-------------|
| Between breed       | 2  | 1.650       | 0.0024              | 0.30        |
| Within breed        | 31 | 24.791      | 0.7997              | 99.70       |
| Total               | 33 | 26.441      | 0.8021              |             |

**Discussion**

Molecular markers could be powerful tools in the identification of SNPs and in revealing the current status of genetic diversity within and differentiation between livestock populations (Lenstra et al., 2012). There are a few numbers of studies carried on the CYP19 gene. The single-nucleotide polymorphisms can be used as a selection criterion for decreasing fertility problems. The present study was, therefore, aimed at determining both within and between breed genetic diversity of cattle bred in Iraq (local, Holstein, and crosses) using SNPs markers. Single-nucleotide polymorphisms (SNPs) markers are the most efficient molecular markers to evaluate genetic diversity, population differentiation, breed relationships, and
determine parentage in animal populations (Yang et al., 2013). The genetic diversity of cattle has also recently been studied using SNPs markers in Ethiopia (Kukučková et al., 2018) and South Africa (Makina et al., 2016). Single-nucleotide polymorphisms (SNPs) in position 1044 obtained from the analysis of the present study are consistent with the previous studies carried out for promoter p1.1 of the CYP19 gene in cattle (Keskin et al., 2015; Mohamadnejad-Sangdehi et al., 2015; Zaborski et al., 2014). However, in the present study, other SNPs were found in the position 933, 994, 1062 and 1468. These SNPs are novel to the studied Iraqi cattle. The reason behind that is that Iraq considers as an origin of sheep (Ayied & Zaqeer, 2019), camel (Ayied et al., 2018) and cattle (Faraj et al., 2019; Owaid et al., 2019).

The partitioning of the genetic variation from an AMOVA revealed that 0.30 % of the total genetic variation was between breeds. A similar pattern of variance partitioning was observed in similar studies (Edea et al., 2013; Ngono-Ema et al., 2014; Sanarana et al., 2016; Gororo et al., 2018), in which 90% or more of the variation is contained within breeds. In Ankole cattle breeds, Öner et al. (2019) reported within-population diversity to be 92.2 %.

**Conclusion**

Cattle bred in Iraq showed a high genetic diversity of the CYP19 gene. The tree of evolution has shown the presence of cattle bred in Iraq with a separate branch from other cattle breeds. Genetic variation within breed was greater than genetic variation among breeds. CYP19 gene of cattle bred in Iraq showed five mutations at the sites 933, 994, 1044, 1062 and 1468. Local cattle differ from Holstein cattle by two haplotypes (H1 and H5 for Holstein and H6 and H7 for local).

However, both share three haplotypes (H2, H3, and H4).

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**Conflicts of interest**

The authors declare that they have no conflict of interests.

**Ethical approval**

All applicable institutional, national and international guidelines for the care and use of animals were followed.

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تعدد أشكال النيوكليوتيدات الفردية (SNPs) في منظم جين CYP19 في الأبقار العراقية

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المستخلص
أجريت الدراسة الحالية لوصف التنوع الجيني لجين أروماتاز السيتوكروم (CYP19) في الأبقارmoid في العراق. أهداف هذه الدراسة هي الكشف عن تطورات في المنظم p1.1 للجين CYP19 في الأبقار المرباة في العراق باستخدام تقنيات التسلسل. اكتشفت خمسة شكلات متعددة للتشكلات أحادية النيوكليوتيد في المنظم. وأوضحت النتائج وجود 3 و 4 و 2 مناطق متعددة الأشكال مما أدى إلى بناء 4 و 5 و 3 أنماط احادية مختلفة لابقار الهولشتاين والمحلية والمضربة على التوالي. كان تنوع النمط الاحادي يساوي 0.791 و 0.752 و 0.700 و 0.752 و 0.700 و 0.752 و 0.700 للسلالات المدروسة على التوالي. بينما كان تنوع النوكليوتيدات كان 0.0017 و 0.0022 و 0.0013 و 0.0013 و 0.0013 و 0.0013 و 0.0013 و 0.0013 على التوالي. إلى جانب ذلك، اجري تحليل جيني للسلالات باستخدام هذه التسلسلات لحساب العلاقة التطورية بين الأنواع الحيوانية. تم تسجيل التسلسلات والتطورات التي تم الكشف عنها في قاعدة بيانات GenBank تحت LC491588 و LC491589 و LC491439 و LC491438 و LC490758 و LC490757 و LC490756 و LC490756 و LC490756 و LC490756.

الكلمات المفتاحية: جين CYP19، الأبقار العراقية، الشكل الوراثي للنيوكليوتيدات المفردة، التنوع الوراثي، شجرة التطور.