

The Effect of polymorphism of a Promoter region in the CYP19 gene on body weight, milk production and its components in Iraqi buffaloes

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Abstract: *This study was conducted on a sample of Iraqi buffaloes to study the effect of genetic mutation in the Promoter region of the CYP 19 gene and its relationship to weight, total milk production and change in the proportions of milk components. It was also found that there was no significant effect of the mutation on body weight and total milk production. The mutation did not affect the proportions of milk components, except for the proportion of protein. The effect of the mutation on the proportion of protein without the rest of the studied traits shows the possibility of improving the proportion of protein in buffalo milk depending on the mutation recorded in the Promoter region of the CYP19 gene within the genetic selection programs and programs to improve the ability of local buffaloes to produce.*

Keywords: *polymorphism, Promoter region, CYP19 gene, body weight, milk production*

1. INTRODUCTION

The buffalo is characterized by its great adaptability in different environmental conditions, as well as its high efficiency in making use of poor quality feed such as reeds and sedge available in its areas of existence, which contributed to protecting it from the threat of extinction in Iraq (Baghdasar et al., 2012), in addition to the high percentage of fat in milk compared to other agricultural animals (Idris et al., 2006), which made it one of the animals with high economic returns, especially if the necessary requirements for its development and development are available, such as improving management, production, reproduction, shelter and nutrition systems and solving some intractable problems that most buffalo breeders suffer from. It is the problem of the lack of agricultural lands they own for the purpose of cultivation or grazing and raising their herds there. Its upbringing is important because it is linked to the livelihoods of a large group of Iraqi society, as well as its economic importance and the consumer's desire for its products, especially the local custard. It serves as an insurance policy for the breeder and his family in the face of the urgent needs of the family (Ali et al., 2007).

Despite all the above, the buffalo remains in need of further studies in order to genetically improve the herds in order to increase their productivity through selection programs, which require the presence of markers associated with productive traits as a means for early selection. One of these markers is the CYP19 gene, which is one of the candidate genes to be a marker in selection programmes. The gene (CYP 19) is located on chromosome (11) in river buffaloes and it has (12) exons (NCBI 2021). In most mammals, CYP19 gene

expression regulates the functioning of specialized tissues by reciprocal transcription using more than one promoter. In humans, it possesses (8) promoters (promoters), one of which has its own 5-UTR (Simpson et al. 1994), four of them have been extensively studied. The gene expression (P450) in the ovaries uses the promoter close to the translation site, while the Promoter (promoter) 1.1 in adipose tissue as well as in the liver (promoter) loses 15 K.B from the beginning of the translation region (Mahendtoo 1993).

Most of the gene expression of (CYP 19) in the glands appears in different forms to those that function in the brain in both the ovaries and testes that glandular stimuli (LH, FSH) are effective by increasing the (AMP) conversion of AMP to the aromatase P450 and conversely In the brain (Roselli and Resko. 1993)

Aromatase is one of the cytochrome P450 subfamilies that has a major role in the biosynthesis of estrogen. This enzyme contains iron within its structure and is found in many vertebrates and has expression in both sexes, mainly from the glands as well as in tissues such as the brain and placenta (Blakemor and Naftolin, 2016).

The manufacturing process of steroid hormones is a critical step in the reproductive ability of animal species. The biosynthesis of steroid hormones begins first with cholesterol, which is converted into several types of steroid hormones through a series of enzyme-catalyzed chemical reactions (Fatima, and colleagues, 2020). The main step in estrogen synthesis and production is the final conversion of Androstenedione and testosterone into estrogen through a three-step, six-electron process that is catalyzed by the cytochrome P450 enzyme called aromatase (Conley AJ, 2001 and Lange and Meyer, 2003). From the foregoing, it becomes clear how important the study of the CYP19 gene and the relationship of genetic diversity of this gene to the productivity of buffaloes, so this research aims to study the effect of genetic mutation in the second coding region on milk production in buffaloes.

2. MATERIALS AND WORKING METHODS:

The study was conducted in the village of Al-Dahab Al-Abyad, about 15 km west of Baghdad, on a sample of 65 adult and productive buffaloes. The daily and weekly milk production was calculated for the 2019-2020 season, and milk samples were taken in summer and winter. 10 mL of blood was collected from the jugular vein, 2 mL was placed in each tube supplemented with K2 EDTA anticoagulant and transported in a refrigerated container to the laboratory for cryopreservation at -20°C and proceed to DNA extraction. DNA was extracted from buffalo (maternal) blood samples for the purpose of molecular assay for CYP 19 gene. Select the initiator for Promoter coding region amplification and select the PCR program as defined by Yazdani et al. (2010).

promoter	F : 5'- CAAGGGCCTCATATGGTTCA -3'
	R : 5'- CCAGATCAGAACCACCTTTGT – -3'

The components of the polymerization reaction are as shown in the following table

Component	Reaction size 25 reaction (µl)
Template DNA	5
Primers	F 1, R 1
DNase Free Water	13
Total	25

The doubling program in PCR technology was as in the following table
 Promoter Multiplication Program (Yazdani et al. 2010)

No.	Steps	Temperature (°C)	Time
1.	First mutant phase	94	2 minutes
2.	mutant	94	30 seconds
3.	fusion	60	30 seconds
4.	elongation	72	45 seconds
5.	Final elongation phase	72	10 minute

The data were statistically analyzed using the program Statistical Analysis System–SAS (2012) to study the effect of the genetic phenotypes of Cyp19 / EXON2 (the first mathematical model) and Cyp19 / Promoter (the second mathematical model) on different traits on a sample of local buffaloes. The significant differences between the means were compared using the Duncan (1955) polynomial test by applying the Least square means method.

3. RESULTS AND DISCUSSION

The polymerase chain reaction was carried out to determine and multiply the promoter region of the CYP19 gene, and the success of this process was confirmed by conducting electrophoresis of the PCR product and checking the sizes of the resulting pieces as in (Fig. 1).

Figure (1) The studied segment of the Promoter region of the CYP19 gene (419 bp) was extracted at 2% across concentration at a voltage of 120 amps and 90 for an hour and a half, $m = 100$

Nitrogenous base sequencing technology was used to determine the polymorphism of the genetic phenotypes in the studied region, and it was found that the same mutation recorded globally in this region KT596715.1). (KT596714.1) which is G ----- A ((NCBI.2021)) as in Figure (2)



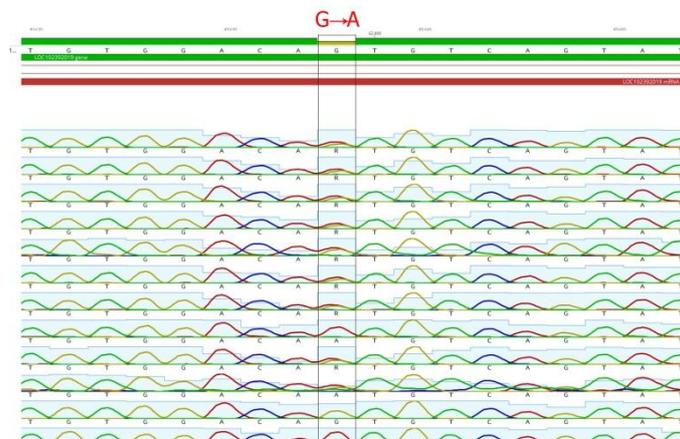


Figure 2 shows the sequence of nitrogenous bases for the studied region from the Promoter region of the CYP 19 . gene

(R = Pyrin)

wild genotype (GG)

Hybrid genotype (R=GA)

After conducting nitrogenous base sequence technology, it was found that there are three genotypes in the Promoter region (promoter GG, GA and AA as a result of changing the nitrogenous base from G to A in position 197 of the plot (G197A). The hybrid structure came first, then the pure wild structure, then the pure structure The mutant, the frequency for the structures was GG, GA and AA (Table 1).

25.53, 51.06 and 23.40, respectively. The frequency of the wild G allele was close to its mutant counterpart A, which was 0.51 and 0.49, respectively. The results of this study did not agree with the study of Aboelenin et al., (2017) on the Egyptian buffalo, as all the sample he studied had the pure wild composition. In similar studies conducted on Holstein cattle (Szatkowska et al., 2011 and Wierzbicki et al., 2014) the preference for the A allele was greater than 0.92 and this may be due to differences between species despite the great closeness between cows and buffaloes. The superiority of the percentage of the hybrid structure over the other two structures indicates that the mating was random in the herd, as it predicts that the presence or absence of mutation in this site does not have a clear phenotypic effect (whether positive or negative) on the traits of interest to the breeders, which in turn leads to the breeders' tendency to Indirect selection for or against mutation as a natural consequence of excluding low-production animals.

Table (1) Number and Percentages of Genotypes and the allelic frequency of the Cyp19/Promoter gene in a local buffalo sample

Genotype	No.	percentage(%)
GG	13	25.53
GA	25	51.06
AA	12	23.40
Sum	50	% 100
(χ^2)	----	NS 0.0638
(Allele)	(Frequency) التكرار	

G	0.51
A	0.49
No Sig.	

The presence of a mutation in the promoter region of the CYP19 gene did not affect the average body weight or daily milk production (Table 2). This may be attributed to two reasons, the first is that the Promoter region is responsible for binding mRNA polymaras (Vanja et al., 2019), the enzyme responsible for creating copies of this gene and completing the process of gene expression

The occurrence of a defect in this area would directly affect and may have a negative effect on production, and since the production of milk as well as the weight of the animals are the focus of the breeders' attention, therefore, the lean and low-production animals are excluded from the herd, meaning that there is a phenotypic selection against harmful mutations. The second reason is that this gene does not directly affect weight, but through its effect on estrogen, a sex hormone that is affected by the physiological state of the animal (Sana et al., 2018).

Table (2): The relationship of the genotype of Cyp19/Promoter gene to body weight and daily milk production of a local buffalo sample.

Genotype	No.	Mean \pm Standard Error	
		animal weight (kg)	Daily milk production (kg)
GG	12	83.60 \pm 756.08	0.93 \pm 10.41
GA	24	42.68 \pm 726.00	0.48 \pm 10.87
AA	11	58.34 \pm 729.27	1.24 \pm 12.03
		NS	NS
No Sig.			

The wild genotype in the Promoter region was significantly ($P < 0.05$) superior to the hybrid and mutant genotypes in the percentage of protein in milk (6.76, 5.77 and 4.66 for AA, AG and GG structures, respectively) (Table 3). As for the rest of the components (non-fat solids, lactose and fat) they were not significantly affected by the presence or absence of the mutation in the Promoter region (promoter). Milk production is affected by changing environmental matters such as nutrition and temperature, as well as the physiological state of the animal (Kizito et al., 2005), which makes it difficult for a single gene to have a significant impact on this trait (Khatkar et al., 2004). Also, buffalo breeders always focus on the amount of milk produced as well as the percentage of fat in the milk, which leads to their preference for certain animals and their exclusion of animals with low values for this trait. As for the protein content, they are not taken into account as it does not affect the nature of buffalo milk products.

Milk production is a quantitative trait affected by a large number of genes (Noorisadegh and colleagues, 2018), and the CYP19 gene affects this trait indirectly through its effect on the physiological state of the animal as a result of its effect on the estrogen hormone Sana and colleagues, 2018, which weakens its direct quantitative effect on this trait.

In addition to the phenotypic selection carried out by the breeders (although it occurs in an unstudied and accurate manner), which tends to prefer specific specifications of the bred animals, which as a result makes a great similarity between them, and this similarity may be due to the intense internal upbringing in these herds.

Table (3) The Genotype Relationship of Cyp19 Gene /Promoter with the studied milk components of the local buffalo sample.

Genotype	No.	Mean \pm Standard Error			
		Protein (%)	Solids (%) not fat	Lactose (%)	Fat (%)
GG	12	a 0.50 \pm 6.76	1.51 \pm 13.47	0.14 \pm 4.25	0.19 \pm 6.72
GA	24	ab 0.44 \pm 5.77	0.65 \pm 12.98	0.15 \pm 4.50	0.19 \pm 6.90
AA	11	b 0.51 \pm 4.66	0.99 \pm 11.28	0.25 \pm 4.48	0.43 \pm 7.12
Sig. Level	---	*	NS	NS	NS
Not Sig (P<0.05)					

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