



POLYMORPHISM OF MTRN1A GENE AND ITS ASSOCIATION WITH SOME PRODUCTIVE TRAITS IN IRAQI BUFFALOES (*BUBALUS BUBALIS*)

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Abstract

This investigate dunder taken in two location for breeding Buffaloes in Baghdad (White Gold Village/Abu Ghhriub AL-Fadhalya) and Laboratories for Biotechnology and Molecular Genetics Analysis for the period from 1/11/2017 to 1/12/2018. The objective to identify the genotypes for MTRN1A gene and the relationship between these genotypes with some productive for a sample from 50 female buffalo. Genotype differed for the target MTRN1A gene-encoding region due to different genetic bundles resulting from enzymatic digestion. Which were three genotype TT (Wild) TC (Heterozygous) and CC (Mutant) and their distribution ratios were 34.00, 28.00 and 38.00% respectively, and that the differences between these percentages were highly significant ($P < 0.01$), and allele frequency were 0.51 and 0.49 for both alleles A and B respectively. The effect of MTRN1A genotypes in the daily milk production was significant ($P < 0.05$) while the cows with TC genotype gave the highest daily milk production (9.41 ± 0.47 kg). The results of this study showed that the components of the studied milk (protein, fat, lactose, non-fatty soluble solids and ash%) were influenced highly significantly ($P < 0.01$) by differences between the genotype of MTRN1A gene. Was can concluded from the study of the MTRN1A genotype that the use of these markers to put a strategically method of genetically improvement for the buffaloes in of Iraq to increase of economical income from these buffaloes farms by selection and genotypes crossing which gave a good productive and performance.

Key words: Iraqi Buffaloes, MTRN1A gene, productive traits

Introduction

Buffaloes play an important role in the livestock economy in many countries of the world, especially in developing countries. This animal has particular importance for a lot of families living in the south of Iraq, specifically in the Marsh lands for thousands of years, Thus it is considered a major source of their livelihood (Al-Zahery *et al.*, 2011).

The contribution of buffalo in the production of milk in the world is estimated at 12.8% The formula of buffalo milk is unique in its composition compared with the other sources of milk for its fat percent gives milk a special nature in the manufacture of special sorts of cheese and cream and it has more the highest calcium percent than other sources of milk (Catozzi *et al.*, 2019). Buffalo is also characterized by its high efficiency in converting low-quality feed and fiber to protein and products. (Singh,

2014).

In the mammalian melatonin is synthesized in the pineal gland and released in a periodical manner consistent with light and darkness. It interacts with its receptors to perform many biologic functions such as the regulation of the biological clock and sleep (Reiter, 2003). Pars tuberalis of the pituitary gland (PT) concedes the most important part of the melatonin receptors expression. (Majidinia *et al.*, 2018) (Hong *et al.*, 2014).

In seasonal animals, the response of receptors expression varied according to the season. The MTNR1A receptor gene is located on chromosome 1 in the Buffalo and contains 2 exonins, encodes the receptor that affects melatonin stimulation in periods of darkness. Thus, MTNR1A has a close relation to the production and reproductive traits. The present study aimed at investigating polymorphism of the MTNR1A gene of an Iraqi buffalo sample in Baghdad. This study also extracts polymorphism distribution ratios and their allelic

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frequencies and clarifies the relationship between the polymorphism of this gene and buffalo milk production and synthesis.

Materials and Methods

The research was conducted in Baghdad (White Gold Village / Abu Ghraib - 20 km west of Baghdad City and Al-Fadhiliya location - 20 km northeast of Baghdad) for the period from 1/12/2017 until 1/2/2018 on a sample of 50 female Buffalo. This study aimed at determining the genotypes of the melatonin receptor gene on the buffalo sample (the extraction of the distribution ratios of these manifestations and their allelic frequencies) by the means of RFLP-PCR technique and its relationship between genotypes and buffalo milk production and composition.

Blood collected by a medical syringe from the jugular vein in a 15 ml sterile polypropylene tubes containing 0.5 ml of EDTA (0.5M) as an anticoagulant by the phenol chloroform extraction by the veterinarian at the station, The blood samples were then transferred by a cool box then stored in freezer at -200C temperature till transferred to the lab to extracting DNA, for the calves blood also collected by medical syringe from the jugular vein in a 10 ml tubes, the DNA samples were checked for their quality, purity and concentration, the quality of the genomic DNA was checked by using agarose gel electrophoresis, DNA samples of good quality, purity and concentration were used for further analysis. The polymerase chain reaction (PCR) technique for MTRN1A typing is based upon the extensive polymorphism that is present in Exon 2 of the MTRN1A gene under consideration depending on the size of the pieces and type of primers used, the 825 bp fragment consisting (Barbosa *et al.*, 2016) present in the genomic DNA of cattle was amplified by employing the corresponding primer pairs (forward and reverse). The details of the primer sequences are as follows:

F : 5'TGTGTTTGTGGTGAGCCTGG3'

R : 5' ATG GAG AGG GTT TGC GTTAA3'

After the polymerase reaction was completed, the polymorphism of MTRN1A gene were identified in blood samples from the cows by used sequence technique through the program Blast software from NCBI (National Center For Biotechnology Information) and information program. And the genotype's of MTRN1A identified by the different between the sequences in nitrogen bases for the studied cows and compared it with the wild sequence of the gene in NCBI.

The daily milk production of each buffalo sample was measured by using a graduated cylinder twice a month for the first three months of the production season (6 tests for each female buffalo). Calves are isolated in the evening and then milking them early in the morning. Milk components was analyzed twice for each female buffalo for three months of milk production. Milk samples were taken after weighing and mixing them well in 50 ml clean plastic containers with sealed caps and carried them refrigerated to a laboratory of Abu Ghraib Dairy to examine the samples of milk by analysis device named Julie-Z7.

The data was analyzed by used Statistical analysis system (SAS, 2012) to study the polymorphism of MTRN1A gene according the mathematical model, significant differences was compared by used Least square means method.

$$Y_{ijk} = \mu + G_i + L_j + e_{ijk}$$

Y_{ijk} : observed value k, which belong to phenotype i and Location j.

μ : Overall means.

G_i : Effect of MTRN1A polymorphism (TT, TC, CC).

L_j : Effect of Location (White Gold Village/Abu Ghriub AL-Fadhalya).

e_{ijk} : Random error which distributed normally with mean = 0 and variation σ^2_e .

Chi-square- χ^2 test were used to compare between the percentages of polymorphisms.

Results and Discussion

The genotypes of the animals which have been studied are determined by the application of RFLP-PCR and the HpaI restriction enzyme to identify the distribution of the studied animal genotypes (Table 1) according to the number and the size of the formed bands.

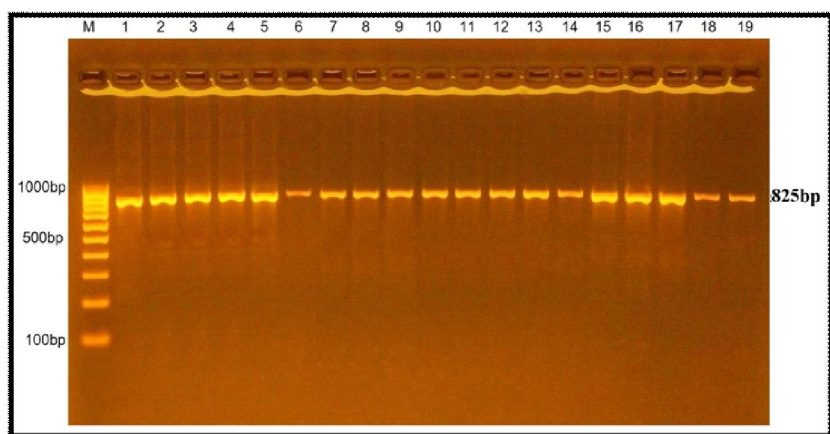


Fig. 1: Extraction fragment (1000bp) of the MTRN1A gene by PCR technique

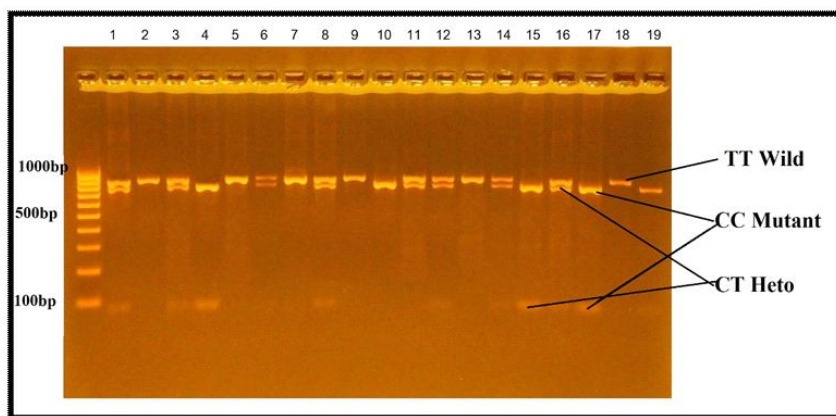


Fig. 2: Genotypes of the MTRN1A gene studied using the restriction enzyme-*HpaI*.

The size of the DNA fragments was used as a marker 1500-100 bp DNA ladder., as it appears in Fig. 2.

The *HpaI* was cut off after identifying the sensitive position within the specific sequence (GTT / AAC) of the target gene segment, thus forming the cutting process forms one band, two or three bands which can be compared with the ladder. The enzyme cuts in the site 824bp base pair sequence of the target gene. The genotype of MTRN1A has been identified as three genetic structures (TT, TC, and CC)

The genotypes of the MTRN1A gene in buffalo sample

Table 1 shows the number and percentage of

Table 1: Number and percentages of genotypes and allele frequency MTRN1A.

Genotype	Numbers	Percentages%
TT: Wild	17	34
TC: Hetro	14	28
CC: Mutant	19	38
Total	50	100
Chi-square(χ^2)	---	**9.840
Allele		Frequency
T		0.51
C		0.49
** (P<0.01).		

Table 2: Relationship of the MTRN1A gene genotypes with the production of daily milk.

Genotype	Numbers	Daily milk production rate
TT: Wild	17	0.46±8.41b
CT: Hetro	14	0.47±9.41 a
CC: Mutant	19	0.39±8.80 ab
Significant	---	
The different letters within a row indicate a significant difference; *(P<0.05).		

MTRN1A genotypes in the studied sample. There are significant differences (P<0.01) between different buffalo genotypes, which reached 34.00, 28.00 and 38.00% for the TT, TC and CC sequences respectively, That is, the ratio of individuals with a mutant type is higher than those of the TC hybrid with a low rate of the same wild genotype in the MTRN1 gene .The law for the allele frequency was applied according to the Hardy and Weinberg equilibrium rule. The frequency of the allele T was 0.51% while allele C was 0.49%. In previous studies, of the water buffalo indicated that

allelic frequency of C and T was 0.41 and 0.59%, respectively. The distribution genotype for TT, TC and CC was 16.58, 34.76 and 48.66% respectively (Gunwant *et al.*, 2018). In another study, there were three genotypes of MTRN1A, CC, AA, and CA The frequencies of them were 0.45, 0.41 and 0.14 and their allele frequency were 0.65 and 0.35% for C and A respectively, (Kianpoor *et al.*, 2018).

Relationship of the MTRN1A gene genotypes with the production of daily milk

The results of the present study showed the daily milk production variance significantly (P <0.05) according to the difference of genetic type of MTRN1A. females of hybrid genotype TC achieved the maximum milk production rate of (9.41±0.47 kg) while the production rate in TT genotypes was the lowest of (8.41±0.46 kg) whereas females with pure structure CC was between the structures TC and TT with a daily production rate of (8.80 ± 0.39 kg), this agree with Zetoumi *et al.*, (2014), through their study of buffalo and the future relationship of MTRN1A in milk production as well as some reproductive traits. That it illustrates the effect of multiple MTRN1A genotypes in the second Exone, who observed the hybrid genotypes surpass in the highest rate of milk production, The rapport between the future of melatonin and milk production is the high concentration of the receptor in the pars tuberalis-PT region, which is closely associated with the secretion of prolactin hormone and its relationship with milk production (Dupre *et al.*, 2008).

Relationship of the MTRN1A gene genotypes with the milk composition

It is clear from table 3 that the percentage of milk components studied, (protein, fat, lactose, non-fatty solids, as well as ash), were all significantly affected (P<0.01) with polymorphism of MTRN1A gene. The highest

Table 3: Relationship of the MTRN1A gene genotypes with the milk composition.

Genotype	Numbers animals	mean \pm standard error				
		Ash%	NSF%	Glucose%	Fat%	Protein%
TT: Wild	(34) 17	0.595 \pm 0.04a	7.41 \pm 0.25c	4.23 \pm 0.07c	6.14 \pm 0.16a	3.45 \pm 0.09c
TC: Hetro	(28) 14	0.227 \pm 0.04	9.19 \pm 0.20b	5.16 \pm 0.07a	5.20 \pm 0.17b	4.45 \pm 0.10a
CC: Mutant	(38) 19	0.378 \pm 0.03	10.24 \pm 0.21a	4.64 \pm 0.06b	6.07 \pm 0.14a	3.99 \pm 0.08b
Significant	---	**	**	**	**	**

The different letters within a row indicate a significant difference; *(P<0.01).

protein content was found in buffalo milk 4.45% TC, while in TT, it was 3.45%, also fat in milk was 6.14, 5.20 and 6.07% for the genotypes TT, TC, CC respectively the high-fat ratio of buffalo females which have TT genotype and reduction in their counterparts TC was correspondent with rate of daily milk production as it mentioned above.

The increase in the amount of milk produced is usually accompanied by a decrease in milk fat and obverse of it. Lactose ratio were 4.23 \pm 0.07 and 5.16 \pm 0.07 and 4.64 \pm 0.06% in the milk of buffalo females with TT, TC and CC mutants of MTRN1A sequentially, viz., the highly significant of lactose ratio was for mothers with hybrid structure TC genotypes, That agrees with Zetouni *et al.*, (2014), through his study of buffalo and the relationship of the multiple genotypes second Exxon of MTRN1A with milk production and he refers that the highest level of milk production and highest lactose ratio were an accompaniment of the Individuals with hybrid genotype. Table 3 show the difference of MTRN1A genotype has significantly affected (P < 0.01) on non-fat solids ratio that it was higher in mutant genotype CC (10.24 \pm 0.21%) than the hyperid type TC (9.19 \pm 0.25%) In turn, it was higher than that of mothers with TT (7.41 \pm 0.25%). The results of the present study showed that the percentage of ash in milk was 0.593 \pm 0.04, 0.277 \pm 0.04 and 0.378 \pm 0.03% for the TT, TC, and CC sequentially and the differences were highly significant, as well as Carcangiu *et al.*, (2011) who indicated in his study about Italy buffalo that was the mutant type surpasses the wild type TT.

This study was concluded that in local buffalo females the highest rate of daily milk production showed in individuals which have hypired type of MTRN1A gene, and there was a significant statistical relationship between the polymorphism of MTRN1A gene and all milk components studied.

Therefore, it is possible to develop the selective programs to improve the milk components based on the results of the polymorphism of the MTRN1A gene, especially if the aim is to exploit the milk in different manufacturing processes.

Conclusion

The study found that the MTRN1A showed that the highest rate of daily milk production was found in local buffalo females. There was a significant statistical relationship between genotypes of MTRN1A and all milk components studied. Depending on the genotypes of MTRN1A, especially if the aim is to exploit the milk in different manufacturing processes.

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