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Study the Effects of Pit-1 polymorphism on some blood Parameters in dairy cow in Karbala governorate

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Abstract

the aim of this study was to investigate the effect of Pit-1 polymorphism on the some blood parameters in dairy local Iraqi cattle, blood samples were collected from (120) healthy dairy cow breeds aged between (4-6 years), in peak stage (40-120) days of lactation, during period of time beginning from September 2017 till January 2018, in the northeast of rural areas in Karbala province. Amplification Refractory Mutation System (ARMS-PCR) techniques was used to detect Pit-1 polymorphism and to classified the genotyping in to three groups; wild group AA, recessive group BB and heterozygosity group AB.The frequencies of genotypes were recorded as 0.06 for AA, 0.70 for AB and 0.24 for BB on the other handthe frequencies of allele were recorded as 0.41 for Pit-1(A) and 0.59 – Pit-1(B).The current study showed no significant decrease ($p>0.05$) in the RBC, Hb&MCHC in AB&BB genotype groups compare with AAgenotype group ,also the PCV % was no significant difference in all groups. The conclusion our study showed that not found effects of pit 1 polymorphism on the some blood parameter in dairy cow .

Key words:-pit -1 polymorphism, blood parameter and dairy cow

Introduction:

Iraqi cows are spread in most areas and vary in appearance from each other and are believed to be mostly due to the origins of the Indian cows (Zibo) where they have similar characteristics of the species)Bosindicus), which live in the hot areas, and is different from European cows belonging to the genus (Bos Taurus) (1).

The most important types of Iraqi cows are the Janubi and Restaki, and these cows are considered two-purpose despite the lack of their milk and meat production (2).

Iraqi dairy cows did not receive any attention and did not take any selection process to make their production better so those who are in charge thought of providing animals with productive capacity to increase the production of milk the comparison with cattle.This idea of breed improvement was began in the year 1940s by importing some types of Friesian, where

the number of cattle were reached to Iraq about (35000 cows).the artificial insemination was spread throughout the country's provinces as well as the main center in (Abu Ghraib/Baghdad) and was supplied with the seminal frozen , which was imported from America, England, Holland, France and Germany (3).

Hematological parameters reflect the adaptability of cows to adverse environmental conditions, as well as other stressors. Hematological reflects a good health status and is highly correlated with milk production (4).

Erythropoiesis is the pathway that produces mature red blood cells from hematopoietic stem cells. This cellular process is characterized by commitment and differentiation steps that restrict the differentiation potential and the proliferative capacity of the cells as they go through the erythroid-specific program of gene expression (5).

Pit -1 gene consists of five introns and six exons and especially synthesized in the anterior pituitary and is limited expression level in sommatotropes ,Lactotropes and thyrotopes (6).

Pit-1 gene and signaling pathways are involved in many development of pituitary gland and mammary gland, growth traits , Milk protein expression and milk production of growth hormone (GH) , prolactin hormone (PR) and thyroid stimulant hormone B-subunit in mammals(7 and 8). Pit-1 polymorphism at exon 6 leads to defect in the GH, TSH and Prolactin mRNA expression and may lead to disturbance of lactotropic, somatotropic and thyropropics proliferation. Actully, Pit1 is transcription factor that binds to the promotor region of of the GH, PRL and TSH genes. Therefore GH, PRL and TSH are counted as direct target for Pit1 (9).There are too many studies were showed that this gene has a direct effect with hormones on the pituitary gland such as growth hormone (GH) , prolactin (PRL) and thyroid stimulant hormone subunit β (TSHβ) gene in the anterior pituitary gland, and this hormones are necessary for mammary gland development and milk yield, Sothe aim of this study to known that an association between the pit-1 polymorhism with some blood parameters in local Iraqi dairy cow breeding.

Materials and Methods:

 This study was conducted on 120cows was apparently healthy,5 ml of blood sample were taken from jugular vein by sterile disposable syringe and collected in to double tubes,3 ml divided intwo EDTA tubes used for blood analysis and DNA extraction, The age of these animals were ranged from 4- 6 years, and the mean age ±SD 5.5+4.24 years. Informed consents were gained from owners. All investigation was done in accordance with animalHealth local ethics committee.The hematological parameter were done in Laboratory of Research and Studies / Collage of Veterinary Medicine University of Karbala by using Hematological auto analyzer made in Genex company ,according the manufacture company.

The hematological parameters estimated by instrument were (RBC, PCV, Hb, MCV, MCHC).DNA from blood samples were extracted using the QIAamp DNA mini kit (Biocomapre, Iraq,) following the manufacturer's details. DNA was quantified using spectrophotometric nanodropemeasurements**.**Amplification Refractory Mutation System was performed using Taq polymerase (Qiagen). optimazation of PCR and nucleotide sequences of ARMS primers are taken from (10), and reactions were conducted in T3000 thermocyclers (Biometra, Goettingen, Germany).

The PCR product for Pit-1 gene was first denatured for 2 minutes at 95°C, followed by 35 cycles of denaturation at 95°C for 1 minute. Then the product was annealed at59°C for 1 minute and extension at 72°C for 1 min, and final extension at 72°C for 2 min. The amplification products were hold through 1.5% agarose gel stained with and ethidium bromide, the procedure involved all the fourdifferent primers in one reaction tube, the reaction tubehave detect the mutant-type B- allele (denoted by the letters C) and the wild-type A- allele denoted as(A).(10).

Statistical analysis

The Frequencies of the Pit-1 polymorphisms AA, BB and AB were expressed in numbers and percentages for wild-type, recessive and heterozygosity genotypes. $\chi 2$ test was used to evaluate consistency of genotype distributions with Hardy-Weinberg equilibrium as the following formula:

P=(2AA+AB)/2n

q =(2BB+AB)/2n

p+q=f(AA)+f(AB)+f(BB)=1

Results and Discussion:

One hundred twentylocal cattle were evaluated for the geneticpolymorphism of Pit-1 genethat results in an silent mutation (A ⟶ G) with the same amino acid change in thesignal peptide located within the exon 6 of the Pit-1 (11), the present study was developed an ARMS-PCR techniques for clinical testing of the pit-1 mutation. The original reactions were detailed in the figure 1 as a clear band of the mutant allele and wild type that barely detected in a blood extraction samples. This results were planned and completed a research project for (10) who was study on the Pit1 polymorphism and association it's with hormonal levels in the serum of local cattle Iraqi breed, because of an association of hormones with Pit-1 gene on dairy cow for milk production (12) and it was also found that the production and efficiency of cow's milk naturally depends on a number of pituitary hormones with protein and steroidal origin (13 and 14), our study have seen to show the new effectbetween the components of blood and Pit-1 gene, the genotype frequencies of SNP for this gene were studied among cattle .ARMS-primers nucleotides region, and size of the PCR fragments of these gene are shown in figure

1,Genotypes and allelic frequency of the PIT-1 gene are shown in the table (1), As can be seen in Table 2, the frequency of the PIT-1 AA genotype (0.06) was lower in cattle population than in AB and BB genotypeit was recorded as 0.7 and 0.24,respectivelly. It was disagreement with other studies, for example, the frequency of A allele was reported 0.347 and B allele as 0.626 in Brown Swiss cattle (8), on the other hand, A allele frequencies and B allele in Pit-1 were 0.42 and 0.58, respectively. Our study showed that the population was not in Hardy-Weinberg equilibrium because the probability that counts ($p < 0.01$) were drawn under Hardy-Weinberg equilibrium pattern, this may be due to the imbalance mating between cattle in the country, or increase frequent of local Iraqi breeds and limitation of pure selection cow breeds, the polymorphism of PIT-1 gene leads to replacement of nucleotides which may alter PIT-1 function. This change in biochemistry of the protein leads to the assumption that B allele may reduce the kinetics of repair, thus affecting the sensitivity to adverse health effects of milk and body weight of cattle (16).
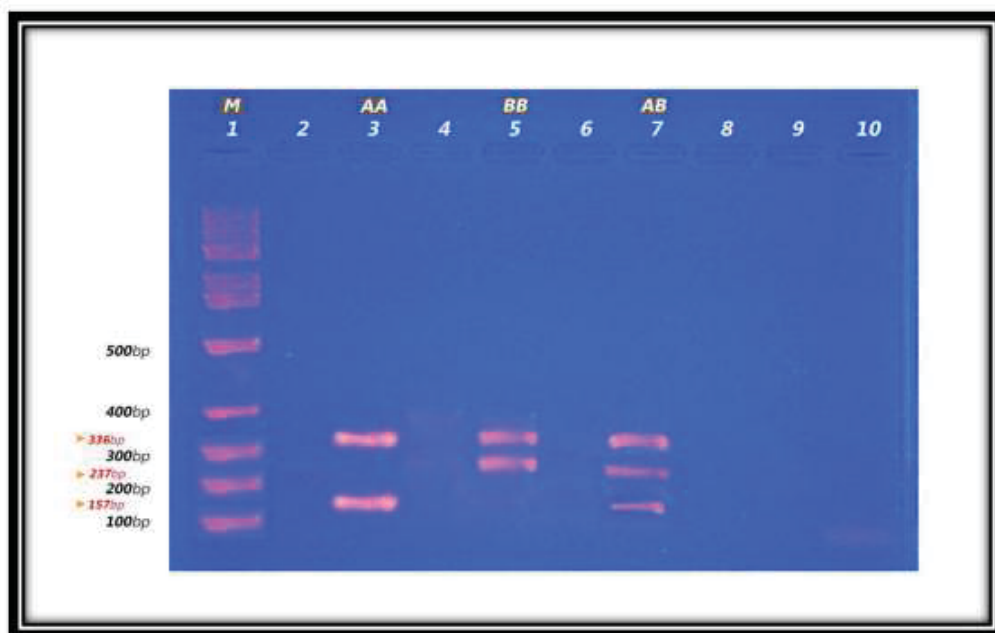

Figure 1: Genotype variation determined by Common primer (CP) fragment (336 bp) represented Lane 3; lane 5 and lane 7, two fragment of A allele (336 and 157bp) represnted lane 3, two fragment of B allele (336 and 237bp) represented lane 5, three fragment of heterozygosity A and B allele (336 , 157 and 237 bp) represented lane 7; M lane 1: DNA Ladder 100 - 2000 bp.were applied on (1%) agarose gel electrophoresis.

Table (1): Distribution of of pituitary transcription factor polymorphism (PIT1) alleles and genotypes in local Iraqi breeds.

PIT1alleles and genotypes	Allele and genotype frequencies of PIT-1 gene polymorphisms	Hardy-Weinberg equilibrium X^2
Allele frequency		17.26
Aallele	0.41	
B allele	0.59	
Genotype frequency		P < 0.01
AA	0.06	
AB	0.7	
BB	0.24	

Our result showed no significant decrease (p>0.05) of RBC&Hb in (BB&AB) genotype groups in comparison with, AA, while the (PCV %) have no significant difference in all groups, while the MCV was increase in(AB) group (30.19 ±2.91)in comparison with (AA&BB) groups it was recorded as (26.14 ±3.26 &29.77 ±4.81), respectively, but no significantly recording (p>0.05). The MCHC also was decreased in (AB&BB) groups it was found as (40.64 ±2.26&38.93 ±4.07), respectively, compare with AA group (43.00 ±3.38) but no significantly recording (p>0.05) between them table 2.

Table (2) Mean of some blood parameters for three pit-1 genotypes (AA,AB&BB) groups of dairy cattle breeds in Karbala province

Genotypes	RBC ×10⁶/µl	Hb g/dl	PCV %	MCV Fl	MCHC g/dl
AA	9.87 ±1.05 A	11.1 ±1.7 A	25.81 ±2.58 Aa	26.14 ±3.26 A	43.00 ±3.38 A
BB	8.60 ±1.57 A	10.05 ±1.8 A	25.61 ±3.67 A	29.77 ±4.81 A	38.93 ±4.07 A
AB	8.28 ±1.02 A	10.16 ±1.8 A	25.00 ±2.66 A	30.19 ±2.91 A	40.64 ±2.26 A

Mean ±SE , significant differences (p<0.01) ,.The different letters refer to significant difference between the difference groups .AA wild genotype ,AB heterozygote genotype, BB recessive genotype .RBC=red blood cell ,Hb=hemoglobin ,PCV=packed cell volume ,MCV =mean corpuscular hemoglobin ,MCHC= mean cellular hemoglobin concentration

The ruminant dietary requirements for energy and protein vary based on thephysical and physiological activities, reproduction status, parturition, and lactation stage (17), Hematological parameters in ruminants depend on many factors related to the animal's physiological status and management system, including housing hygiene and nutrition. Proper

management conditions are essential for the organism to function normally (18). Because of the important effects of hormonal pituitary gland in the growth and lactation and milk production in cow (data not shown), and depending on the previous studies say that there is strong relationship among the hormones and milk yield (19),So, the study highlight to know a potentially common effect in changing of blood parameters and pit-1 gene, unfortunately, we don't found any effect between this gene with blood parameters, hematological parameters of a cow is an important indicator of an udder's well being. Any changing from the normal state are definitely reflected through variation in the milk yield and composition (20). In conclusion.The current study was found that PIT-1 genotype was not risk for blood parameters of cattle breeding. However, more studies and large samples are required in the future.

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