The Relationship Between The PIT-1 Gene at The Rate of Daily Milk Production and Some of Its Components in Buffaloes

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Abstract

This study was conducted at the College of Agriculture / University of Al-Muthanna for the period between 1/10/2020 until 30/6/2021, 60 buffaloes were used in the experiment with the aim of determining the genotypes and their distribution ratios for the PIT-1 gene. The genotypes were altered (P<0.01), which amounted to 18.52 and 81.48 respectively, and the allelic frequency was 0.10 and 0.90 for each of the two alleles A, B, respectively. Daily milk production and fat percentage were not significantly affected, milk protein percentage was significantly affected (P<0.05) in the eleventh week, reaching a maximum of 3.88±0.28 (BB), as well. Significant differences (P<0.05) were found in the percentage of genetic non-fatty solids in the first week of the study, with a maximum of 23.28 (BB). BB genotype.

Keywords: Milk, Buffaloes, Animals.

1.Introduction

As a result of the actual increase in the number of the world’s population on the one hand and the shortage of food on the other hand, it has become necessary for developing countries to increase the production of their indigenous animals to ensure a permanent source of animal food, the most important of these animals being buffalo [1]. The world in 2017 was about 200,967,747 heads, of which 97.4% were in Asia, and 1.7% in Africa [2]. Buffalo plays an important role in the animal economy in many countries of the world as it is a producer of milk and meat, which is one of the important projects in developing countries. It is less important than domesticated animals. However, this animal is of special importance to many societies that lived in central and southern Iraq in the marshes for thousands of years, as this animal is a main source of their livelihood [3]. The Iraqi buffalo specializes in milk production and then meat production and has the ability to convert poor feeds such as reeds and sedge into high-quality products such as milk and meat. Iraq comes after Egypt in the Middle East in terms of buffalo numbers and ranks twelfth in the world [4]. Candidate genes can be identified according to the interrelationship between phenotype and genotype, and then studied further to ensure the validity of their functions [5].

Pit_1 consists of 129 amino acids linked on one chromosome in bovine[6] It consists of six exons and five introns [7] The Pit-1 gene has been identified as a pituitary-specific transcription factor that regulates the expression of growth hormone genes, (GH) and prolactin (PRL) in the anterior pituitary gland [8]. As the development and functions of the mammary gland are regulated mainly by growth hormone and the prolactin gene, which is formed by the anterior lobe of the pituitary gland, and its secretion is controlled by a factor gene The pit-1 versions [9]. Inhibition of PIT-1 synthesis leads to a marked decrease in the expression of growth hormone and luteinizing hormone and to a decrease in the proliferation of cell lines that produce these two hormones. Because they are essential for udder development and milk production, the PIT_1 gene can be an indicator of genetic variation in production traits (the pit_1 polymorphism has been associated with reproductive and reproductive traits). In livestock [10-14].

2.Materials and Methods

The study was conducted at the University of Al-Muthanna/College of Agriculture/Department of Animal Production for the period from 1/10/2020 to 30/ 6/2021 for one productive season, as 60 buffaloes were used in the experiment. From 30 female buffaloes, their ages ranged between (3-15) years, with their offspring, with 30 males and females born. Some preliminary data on the animals were determined with the help of the owners of the fields. The experiment was in two phases: the first stage was field work for the period from, and included the drawing of blood samples and taking samples. of milk and
measuring body dimensions for experimental animals. As for the second stage, the laboratory work continued until the end of (May) and included analyzes for the analysis of milk components (protein %, fat %, glucose %, non-fat solids %), which were carried out in the laboratories of the Faculty of Agriculture, Production Department Animal and civil laboratories and genetic analyzes that were carried out in private laboratories in Al-Muthanna Governorate.

2.1 Blood sample collection

10 ml of blood was collected from the jugular vein for each animal, using a 15 ml sterile syringe. The blood was placed in a 10 ml EDTA anticoagulant tube and divided into two tubes numbered with the buffalo number.

2.2 Molecular genetics

The stages of molecular analysis of genes included the following:

DNA extraction, extraction of the required piece of the PIT-1 gene, its amplification and the use of a special trimer enzyme to determine the genotypes.DNA was extracted from buffalo blood samples using a kit supplied by the Korean Geneaid Company, according to the following steps:

- 200 microliters of blood were taken from each sample and placed in a sterile 1.5 ml microcentrifuge tube.
- 20μl of (Proteinase K) solution was added and 500 μl of Binding Buffer 3 solution was added in then the tube was shaken with the vibrator (Vortex).
- The tube was incubated for 10 minutes at room temperature for 10 minutes.
- The centrifuge is placed for a short period of time.
- Transfer the mixture for each sample to special tubes equipped with an extraction kit called the spin column, which in turn was placed inside larger tubes called collection tubes with a capacity of 2 ml, then these tubes were placed in a centrifuge at a speed of 12000 rpm. ) for one minute, and then the filtered solution was disposed of by neglecting the solution in the collection tubes, and the holding column tubes were placed in new collection tubes for the purpose of the washing process.
- Add 500 microliters of Clean Buffer 3 solution (making sure to add ethanol to the solution) to the existing connecting column tubes in the new collection tubes for each sample, then put these tubes in a centrifuge at a speed of 12,000 rpm for 30 seconds and then The sludge collected in the collection tubes was discarded,
- Adding 500 microliters of 3 Wash Buffer solution (making sure to add ethanol to the solution) to the existing connecting column tubes in the new collection tubes for each sample, then placing these tubes in a centrifuge at a speed of 12,000 rpm for 30 seconds and then Disposal of the sludge collected in the collection tubes, then 500 microliter Wash Buffer (WB3) was added, then these tubes were placed in a centrifuge at 12000 rpm for 30 seconds, then the sludge collected in the collection tubes was disposed of, and then the centrifugation process was restarted. Centrifuge again at 1200 rpm for 2 minutes to remove any residue from the WB3 solution, discard the collected leachate in the collection tubes, and then dry the spin column in air at room temperature for several minutes.
- The spin column tubes containing the DNA for each sample were transferred to sterile 1.5 ml Eppendorf tubes, and then 70 μl of heated Elution Buffer solution was added in a water bath at a temperature of 60 ° C, and centrifuged at a speed of 12000 One cycle/minute for one minute in order to dissolve the DNA inside the connecting column tubes and descend into the sterile tubes. Then the extracted DNA was preserved by freezing at a temperature of 20-C in the freezer until the molecular examination was performed.

2.3 Electrical relay materials

- Agarose
- TBE Buffer Solution (10 X TBE Buffer Solution).
- Bromophenol blue dye.
- Ethidium bromide stain.
- Size parameters (100- 1500bp) DNA ladder Marker

2.4 Preparation of agarose jell

- Add 1g of acarose in 100ml of 1X TBE in a flask, and heat the mixture to boiling point using the microwave until all the gel minutes are dissolved until the mixture looks clear without any suspended particles of powder.
- The agarose liquid was stirred in the flask to mix and to avoid bubbles, and left to cool at a temperature of 50-65°C, after which the 2% ethidium bromide dye was added.
Pour the mixture into the backing plate and after dipping the Comb near one end of the plate.

Leave the mixture to solidify at room temperature.

Softly remove the comb as well as the plate supports.

The plate was placed in its support in the electric relay unit and then covered with 1XTBE relay buffer, as the gel was covered.

### 2.5 Sample migration

The extracted DNA samples were migrated into the holes by adding 5 microliters to each hole, then the samples were migrated at an electrical power of 70 volts and a current of 40 mA for an hour. Use the ultraviolet spectrophotometer (UV light transilluminator), the image for the purpose of viewing the DNA bundles, the colored bundles with ethidium bromide fluorescence were of a bright orange or pink color, indicating the presence of DNA, and they were photographed using a photographic documentation device (Photo documentation system).

### 2.6 Molecular characterization of the studied gene segments

The primer was selected, as shown in the table (1), for the purpose of conducting molecular detection and knowing the phenotypic polymorphism of the PIT-1 gene, resulting from the presence of the SNP of the pti_1 gene [15].

| Gene | Sequencing |
|------|------------|
| PIT-1 | Forward. AAA CCA TCA TCT CCC TTC TT  
|       | Reverse. AAT GTA CAA TGT GCC TTC TGA G |

### 2.7 Polymerase chain reaction (PCR) for the PIT-1 gene

Molecular detection of the (PIT-1) gene was carried out using polymerase chain reaction technology and using the GoTaq Green Master Mix Kit supplied by Promega, USA. It is clear from Table (2) the materials used in the molecular detection using the polymerase chain reaction of the studied genes, with a volume of 25 microliters.

| Component | Reaction size 25 reaction (µl) |
|-----------|--------------------------------|
| Hinf I    | 1                              |
| DDH2O     | 9                              |
| Product PCR | 10                           |
| 1X NEBuffer 4 | 5                            |
| Total     | 25                             |
2.10 Loading of the product of enzymatic digestion and electrophoresis

1.8 μl of DNA ladder and 5 μl of PCR-RFLP products were loaded into 4% agarose gel, migrated at a voltage of 70 V/cm and at a current of 100 mV for 1.5 h. Then the gel was immersed in liquid ethidium bromide dye at a concentration of 2%. The beams were viewed by UV transiluminater It was photographed using the Photo documentation system.

2.11 Calculating the daily milk production rate and measuring the components of milk

The rate of milk production was measured based on the daily production and throughout the production season, as the total amount of milk taken during the experiment period was divided by the number of days in which the milk was collected. Milk samples were taken every two weeks for each buffalo throughout the experiment period by manual milking method, at six o'clock in the morning. The milk sample was taken after mixing the milk produced from the buffalo well from the morning ring to make the sample more homogeneous with an amount of about (50 ml) and was transferred directly after that to examine the milk components to preserve the samples and not be exposed to sunlight or high temperatures, and then the components were calculated. Milk each of fat and protein every two weeks for the duration of the experiment using the Dutch EKO MiK laboratory milk analyzer in the laboratory of the College of Agriculture / University of Al-Muthanna after collecting samples during the milking process.

The data were statistically analyzed using the program Statistical Analysis System [16] to study the effect of the genetic phenotypes of the PIT1 gene on the studied traits according to the three mathematical models below, and the significant differences between the means were compared using [17] polynomial test by applying the least squares averages method (Least square means).

The second mathematical model: the relationship of the genetic phenotypes of the PIT1 gene to the studied traits:

\[ Y_{ijklm} = \mu + PIT1i + Aj + Sk + e_{ijkl} \]

Since:

PT1i: influence of genetic phenotypes of the PIT1 gene (AB, BB).

Aj: The effect of maternal age (3, 4, 5, 6, 7 and over years.)

SJ: influence of the gender of the newborn (male, female.)

eijkl: the naturally distributed random error with a mean of zero and a variance of \( \sigma^2e \).

3. Results and Discussion

Using RFLP-PCR technology to determine the genotypes of buffaloes by cutting the studied gene two genotypes of the PIT_1 gene were determined for the buffalo samples included in the experiment by means of the studied gene segment (451bp) using the Hinf1 restriction enzyme by applying RFLP-PCR technology. Size information (DNA Ladder100bp-1500) as shown in Figure (1).

![Figure 1. The digestion products of the PIT_1 gene segment studied using the restriction enzyme Hinf 1.](image-url)
The slicing process was carried out with the Hinf1 trimer enzyme after identifying the sensitive position within the specific sequence from the severing site of the gene segment, so either one or two bundles of each sample were formed from the slicing process, which can be compared with the guide bundles or the molecular ladder (Ladder), and the structures were identified. The genotype of the PIT-1 gene in the samples studied in this way is as follows:

- If the cutting enzyme does not occur in both alleles of the studied plot, one bundle of the same size will appear from both alleles (i.e. the same studied plot), then the genetic structure of this model is homozygous, which represents the wild genotype (AA) That is, the normal or the original phenotype (ie, without the presence of a SNP) which is not present in the studied buffalo sample.
- If the splitting occurs in one of the two alleles without the other, three bands will appear, and this means that the genotype of this model is the heterozygous structure, i.e. the presence of a SNP in one of the two bands (i.e. the change of base C to base T) and it represents the AB genotype.
- If the cut-off occurs by the enzyme cutting in both alleles of the studied segment, two segments of each allele will appear as two bundles, because every two bundles of the same size from both alleles overlap in one bundle. BB, i.e. the presence of a SNP in both alleles (i.e., the change of the C base to the T base).

3.1 Ratios of the distribution of genotypes and allelic frequency of the PIT-1 gene

Table (3) shows the number of genotypes, their percentages, and the allelic frequency of the PIT-1 gene, as there were two genotypes for this plot, AB and BB, and individuals carrying the pure wild genotype (Wild-AA) did not appear in the studied sample, and the numbers carrying these two Structures 5 and 22, respectively, with a percentage of 18.52% and 81.48%, respectively, meaning that the frequency of the two alleles A and B were 0.10 and 0.90, respectively, and there were highly significant differences (P<0.01) between the distribution ratios of the genotypes of the sample.

| Number | Genotype   | Percentages% |
|--------|------------|--------------|
| 0      | AA: Wild   | 0.00         |
| 5      | AB: Hetero.| 18.52        |
| 22     | BB: Mutant.| 81.48        |
| 27     | total      | % 100        |

**(χ²)** **48.037**

The appearance of the AA genotype with a higher percentage of the BB genotype is similar to what was found by [18]and similar to that of[19] when studied on Basudan cattle by obtaining two genotypes and also noted by[20] the absence of the AA genotype in Indonesian cattle , while the result was different from what was reached [21] which indicated the presence of three genotypes AA, AB and BB in his study on Holstein cows.

3.2 The relationship of the genotypes of the pit_1 gene to the rate of daily milk production, milk fat and milk protein

The results of the study in Table (4) showed that there were no significant differences between the AB and BB genotypes in the daily milk production rate, which amounted to 7.81 and 8.60, respectively. These results were different from what was found[5,20] (Brym et al., 2005, Othman et al., 2011), as they noted a decrease Milk production for the BB structure, while [22] found the superiority of the A allele in milk production. It is noted from Table (5) that there were no significant differences between the AA and BB genotypes of the PIT-1 gene in the percentage of fat in different weeks, as the percentage of fat was in the first week 5.50 and 5.32 for the mentioned genotypes, respectively, [5,20] noted the superiority of the BB synthesis in the percentage of fat, while [23,24] the superiority of the AB synthesis in the percentage of fat, the absence of Significant differences between the AA and BB genotypes of the PIT-1 gene in the protein percentage in the first, third, fifth, seventh and ninth weeks, as the percentage of protein in the first week was 3.37, 3.55 for AB and BB genotypes, respectively, and there was significant (P<0.05) for the PIT gene genotypes -1 in protein In the eleventh week, there was a superiority in the percentage of the genotype BB over the animals carrying the genotype AB, as the percentage of protein in the eleventh
week was 3.06, 3.88 for the genotypes AB, BB, these results were similar to what was reached [25] If the composition exceeds BB by milk protein.

**Table 4.** The relationship of the genotype of the PIT-1 gene to the daily milk production rate of female buffaloes.

| Genotype | number of buffalo | Daily milk production (kg) mean ± standard error |
|----------|-------------------|--------------------------------------------------|
| AB       | 22                | 7.81 ± 0.35 a                                    |
| BB       | 5                 | 8.60 ± 0.50 a                                    |
| Significant level | Total number 27 | N.S                                              |

Averages that do not have the same letters within the same column do not differ significantly among themselves N.S insignificant

**Table 5.** The relationship of the genotypes of the PIT-1 gene in the proportion of milk fat and milk protein for mothers.

| Milk components | Genotype | first week | third week | fifth week | Seventh week | ninth week | eleventh week |
|-----------------|----------|------------|------------|------------|--------------|------------|---------------|
| %fat            | AB       | 5.50 ± 0.61 | 4.05 ± 0.48 | 0.65       | 3.92 ± 0.54  | 4.40 ± 0.65 | 3.83 ± 0.51   |
|                 | BB       | ± 0.64     | ± 0.36     | ± 0.32     | 4.63 ± 0.92  | 4.12 ± 0.55 | 3.78 ± 0.52   |
| Significant level | N.S    | N.S        | N.S        | N.S        | N.S          | N.S        | N.S           |
| %protein        | AB       | 3.37 ± 0.07 | 3.55 ± 0.09 | 0.12       | 2.88 ± 0.14  | 0.12 ± 3.37 | 3.06 ± 0.09   |
|                 | BB       | ± 0.24     | ± 0.21     | ± 0.21     | 3.10 ± 0.26  | 3.47 ± 0.28 | 3.88 ± 0.28   |
| Significant level | N.S    | N.S        | N.S        | N.S        | N.S          | N.S        | *             |

### 3.3 Relationship of the genotypes of the PIT-1 gene in the proportion of lactose and non-fat solids in mothers' milk.

It is noted from the table (6) that there are no significant differences between the AA and BB genotypes of the PIT-1 gene in the percentage of non-fatty solids in the third, fifth, ninth and eleventh weeks, and there are significant (P<0.05) differences in the genotypes of the PIT-1 gene in the first week, as there was a superiority in The percentage of the BB genotype on animals carrying the AB genotype, the percentage of non-fat solids in the first week was 8.96, 23.28 for the AB and BB genotypes, respectively, and the percentages of non-fatty solids in the eleventh week ranged from 7.99, 8.90 for the AB and BB genotypes, respectively, no There were significant differences between the AA and BB genotypes of the PIT-1 gene in the percentage of lactose in the first, third, fifth, seventh and ninth weeks.

There is a superiority in the percentage of lactose for genotype BB over animals carrying genotype AB, as the percentage of lactose in the eleventh week was 4.50 and 5.31 for genotypes AB, BB, respectively. It was found by[4] that there were no significant differences between the genetic polymorphism of the PIT-1 gene in the percentage of non-fat solids and the percentage of lactose in Brown Swiss cattle.
Table 6. The relationship of the genotypes (Genotype) of the PIT_1 gene in the ratio of non-fat solids and lactose in buffalo milk.

| Milk components | Genotype | first week | second week | third week | fourth week | fifth week | sixth week | seventh week | eighth week | ninth week | tenth week |
|-----------------|----------|------------|-------------|------------|-------------|------------|------------|--------------|-------------|------------|------------|
| %S.N.F          | AB       | 0.19± 8.96 | 0.32 ± 9.33 | ± 8.83     | ± 3.5       | ± 7.58     | ± 0.38     | ± 8.87       | ± 0.31      | 0.27 ± 7.99 |
|                 | BB       | ± 23.28    | 14.18 ± 0.53| ± 10.03    | ± 9.12      | ± 0.57     | ± 8.02     | ± 0.75       | ± 0.48      | 0.72 ± 8.90 |

Significant level: a: P < 0.05; b: P < 0.01; N.S: Not Significant

References

[1] Abdel-Salam SA, Sayed AI, Elsayed M, Abou-Bakr S. (2010). Genetic gain in open nucleus breeding scheme to improve milk production in Egyptian Buffalo. Livest Sciences (131), 162-167.
[2] Alipanah, M., Kalashnikova, L., and Rodionov, G. (2007). Association of PRL gene variants with milk production traits in Russian Red Pied cattle. Iranian Journal Biotechnology (5), 158-161.
[3] Al-Zahery, N.; Pala, M.; Battaglia, V.; Grugni, V.; Hamod, A.; Kashani, B. H. and Semino, O. (2011). In search of the genetic footprints of Sumerians: a survey of Y-chromosome and mtDNA variation in the Marsh Arabs of Iraq. BMC evolutionary biology, 11(1), 288-289.
[4] Aytekin, I., & Boztepe, S. (2013). Associations of Pit-1 gene polymorphism with milk yield and composition traits in brown swiss cattle. Journal Animal, Plant Sciences, 23(5), 1281-1289.
[5] Brym, P., Kaminski, S. and Wojcik, E.( 2005). Polymorphism within the bovine prolactin receptor gene (PRLR). Animal Sciences. Papers Reports, (23), 61-66.
[6] Buny, A. and Portetelle, D.( 1997). Pit-1 Gene Polymorphism, Milk Yield, and Conformation Traits for Italian Holstein-Friesian Bulls. Journal. Dairy Sciences., (80), 3431-3438.
[7] Carsai TC, Balteanu VA, Vlaic A, Cosier V,(2012). The polymorphism of pituitary factor 1 (POU1F1) in cattle. Journal J Animal Sciences Biotechnol(45), 142-146.
[8] Corrales-Alvarez JD, Ceron-Mu-noz MF,Ca-nas-Alvarez JJ, Acevedo-Valladarez Z, Sepulveda-Restrepo JC, Calvo-Cardona SJ, Moreno-Ochoa M. (2010). Study of Hinf I polymorphisms of the Pit-1 gene and their associations with type traits, milk yield and days open in Holstein cattle from Antioquia, Colombia. Actu Biotecnology (132), 139-145.
[9] Cosier V, Vlaic A, Carsai C, Claudia S,Constantinescu R, (2008). Associations between polymorphism at Pit1 locus and milk yield in Romanian Simmental and Muramares brown breed cattle, BulletinUSVM-CN. Journal Animal Sciences Biotechnol65,471.
[10] Duncan, D.B.( 1955). Multiple Rang and Multiple F-test. Biometrics. 11.
[11] Dybus, A., Szatkowska, I., Czerniawska-Pitkowska, E., Grzesiak, W., Wojcik, J., Rzewucka, E. and Zych, S. (2004). Pit-1/Hinf I gene polymorphism and its associations with milk production traits in polish Black-And-White cattle. Archiv Tierzucht , 47 (6): 557-63.
[12] Edriss, V., Edriss, M. A., Rahmani, H. R., & Sayed-Tabatabaei, B. E. (2008). Pit-1 gene polymorphism of Holstein cows in Isfahan Province. Biotechnology, 7(2), 209-212.
[13] Falconer, D. S. and T. F. C.Mackay. (1996). Introduction to quantitative genetics (4th ed.). Longman, Harlow, UK.
[14] FAO (2003). Food and Agriculture Organization of the United Nations. FAOSTAT online statistical service. FAO. http://www.fao.org.
[15] FAO (2019). Live Animals data. Rome, Italy
[16] Jakaria and R. R. Noor,(2015). Identification of a single nucleotide polymorphism at Hinf-1 enzyme restriction site of Pit-1 gene on Indonesian Bali population. Media Peternakan. 38(2):104-109
[17] Drebee, H.A. (2017). The Impact of Cultivated Area and Price on Production of Rice in AL-Qadisiyah -Iraq During the Period (1990-2014) by Using VECM. Al-Qadisiyah Journal for agriculture science, Vol,7,No,1:123-135.
[18] Mistranti, R., Sumantri, C., & Farajallah, A. (2010). Polymorphism identification of Pit1 gene in Indonesian buffaloes (Bubalus bubalis) and Holstein-Friesian cows. Media Peternakan, 33(3), 131-131.
[19] Moody, D. E., Pomp, D., & Barendse, W. (1995). Restriction fragment length polymorphism in amplification products of the bovine PIT1 gene and assignment of PIT1 to bovine chromosome 1. Animal genetics, 26(1), 45-47.
[20] Othman, O. E., Zayed, F. A., El Gawead, A. A., & El-Rahman, M. R. (2011). Genetic polymorphism of three genes associated with milk trait in Egyptian buffalo. Journal of Genetic Engineering and Biotechnology, 9(2), 97-102.

[21] Putra, W. P. B., Agung, P. P., & Said, S. (2019). The polymorphism in g. 1256G>A of bovine pituitary specific transcription factor-1 (bPIT-1) gene and its association with body weight of Pasundan cattle. Journal of the Indonesian Tropical Animal Agriculture, 44(1), 19-27.

[22] Renaville, R., Gengler, N., Vrech, E., Prandi, A., Massart, S., Corradini, C., ... & Portetelle, D. (1997). Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. Journal of Dairy Science, 80(12), 3431-3438.

[23] SAS. (2012). Statistical Analysis System, User's Guide. Statistical Version 9.1. ed. SAS Inst. Inc. Cary. N.C. USA.

[24] Tuggle, C. K., Yu, T. P., Helm, J., & Rothschild, M. F. (1993). Cloning and restriction fragment length polymorphism analysis of a cDNA for swine PIT-1, a gene controlling growth hormone expression. Animal genetics, 24(1), 17-21.

[25] Woollard, J., Schmitz, C. B., Freeman, A. E., & Tuggle, C. K. (1994). Rapid communication: Hinfl polymorphism at the bovine Pit-1 locus. Journal of animal science, 72(12), 3267-3267.