Research Article

The Relationship Between the Genetic Marker DIK20 and A Range of Physiological and Productive Traits in Holstein Cattle

Hadi Awad Hassooni[1], Safaa Sabbar Atiyah[2]*, Alaa Saleh Jassim[1]

[1]University of Al-Muthanna, Faculty of Agriculture, Department of Animal Production, Al-Muthanna, Iraq; [2]Al-Furat Al-Awsat Technical University, Technical Institute of Kufa, Department of Animal Production Techniques, Kufa, Iraq.

Abstract | The use of gene markers in livestock breeding plays a crucial role in enhancing productivity. Specific genetic markers can be identified, facilitating the task of breeders in selecting desired traits, such as milk production, disease resistance, and growth monitoring, which in turn leads to improving the overall productivity of animals. This research aims to study the linkage among the genetic marker DIK20 and several physiological and productive features in Holstein cattles, which includes milk production, mastitis, and percentages of fat, lactose, and protein. The research was conducted in one milk production season, starting from October 2022 to April 2023, using 60 Holstein cows aged between 4-6 years. The results showed that cows had two genotypes of DIK20, with sizes of 190/181 and 180/170 base pairs, with distribution rates of 36.21 and 63.79%, respectively. Results obzerved highly significant differences (P≤0.01) between the genetic variants. The study also found significant differences (P≤0.05) between the genetic polymorphisms 180/170 and 190/181 in the California mastitis test. However, no statistically significant differences noticed in the genetic polymorphisms related to fat, protein, lactose, and solid non-fat in milk elements, with percentages of 3.60% and 3.48% for fat, 2.90% and 3.1% for protein, 4.18% and 4.31% for lactose, and 7.94% and 8.26% for solid non-fat for the two genetic polymorphisms 180/170 and 190/181, respectively. It is concluded that there is a correlation between the genetic marker DIK20 and various physiological and productive traits in Holstein cows.

Keywords | Cattle, DIK20 marker, Genetic polymorphisms, Milk production, Physiological traits

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***Correspondence** | Safaa Sabbar Atiyah, Department of Animal Production Techniques, Technical Institute of Kufa, Al-Furat Al-Awsat Technical University, Iraq; **Email:** safaa.sabbar.iku@atu.edu.iq
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INTRODUCTION

A large number of genes have an influence on the quantitative traits of milk production (Zghair and Hassooni, 2021). The decision on which of these potential genes to select, which have a significant impact on traits such as growth and milk production and are affected by genetic mutations in the nucleotide sequence that makes up the selected genes, is a crucial tool for improving the performance of cattle and as a substitute for traditional selection methods. Identifying genes associated with milk production traits has the benefit of reducing generation time and increasing the genetic advancement of economically important features (Ma et al., 2021). Mastitis disease may consider one of the most common problems in dairy cattle farms, which can be defined as an increasing in somatic cell (SCC). SCC is the number of white blood cells counted in milk, which are one of the most important natural defenses in animal body (Kul et al., 2019). In order to detect mastitis, California test (CMT) is used to diagnose mastitis disease in by to ensure udder health , detection milk abnormalties, as well as identifying the infected quarters. CMT is consider

it a quick, simple, and economically inexpensive process that anyone can do, even if they have little experience. Also, CMT is not considered an indicator. SCC which is considered an important marker for detecting mastitis in the early stages of lactation (Kandeel et al., 2017). The discovery of PCR technology has had a significant impact on biological sciences, with implications for various sub-disciplines in biology (Liang et al., 2014a). Studies have utilized this technology as genetic markers for selection, particularly for traits with low heritability controlled by multiple genetic sites known as quantitative traits loci (QTL), as well as economic traits (Alsaffar, 2022). One of the techniques dependent on the polymerase chain reaction is microsatellites, commonly used in genetic research and molecular marker studies, extending beyond genetic fields (Liang et al., 2014b). Microsatellites are employed in animals to assess genetic variance within breeds (Kunene et al., 2014). There is also a link between the genetic marker DIK20 and mastitis, as it is a sign of the presence of mastitis in dairy cattle (Gupta et al., 2016). Ajafar et al.(2022) reviewed the association of bone morphogenetic protein 15 and growth differentiation factor 9 with litter size in livestock, indicating the potential of these genetic markers in improving fertility and productivity traits in livestock, suggesting that genetic polymorphisms related to fertility traits may have implications for overall productivity in sheep and goats. Furthermore, the genetic diversity and population structure of indigenous cattle breeds indicated moderate genetic differentiation among ecotypes (Bora et al., 2023). Therefore, this study aims to explore the relationship amongest the genetic marker DIK20 and production traits such as total milk yield and mastitis, as well as physiological traits including protein, fat, and lactose ratio in Holstein cows.

MATERIALS AND METHODS

The present study was carried out at Al-Nahrain Farm for cattle production in Al-Qadisiyah province, in addition to the postgraduate laboratory of the Agriculture College – University of Al-Muthanna, for the period from October 2021 to April 2022 for only one production period. Before the study, institutional approval was obtained for ethical considerations in experiment (Ethical approval No. 152LBI05003). The number of cows used in the experiment was sixty Holstein cows from Germany, whose ages ranged between 4-6 years.

Blood samples collection

Eight milliliters of jugular vein blood were collected using a ten-milliliter medical syringe post cleaning and sterilization vein, for one sample per cow. The study samples separate into two parts. The first, with a volume of three milliliters, was placed in a tube containing anticoagulant and cryopreserved at -40°C till DNA extraction method. The second part, with a volume of five milliliters, was used for conducting blood tests for physiological and productive traits.

Coefficient of heat tolerance

The Coefficient of Heat Tolerance for cows (HTC) was estimated according to the next equation by Rhoad (1944) as follows: HTC = 100-10(ART-38.3) Since: (ART) is the measured rectal temperature, (10-100): Constants, (38.3 C): the perfect temperature of a cow's rectum.

California mastitis test

The mastitis test of California was performed each two weeks through study period to examine the SCC count. The milk from each udder quarter was collected and placed on the CMT test device in four separate parts of the dish. Then, reagent drops were added to each part and the dish was gently swirled in a circular motion for a period of time not exceeding ten seconds. After that, it was left for twenty seconds to notice the changes in the milk. Table 1 show he average somatic cell count and a description of each case according to Nickerson and Heald (1981).

Measurement of milk flow rate

The method of Griffin and Dodd (1962) was adopted in measuring the milk flow rate, as it was measured for each cow using a stopwatch, calculating the time from the beginning of milking till the end for the morning and evening milkings, and through knowing the quantity of milk per each cow. Then, collection process the quantity of the morning and evening milkings and divided by the total milking time for the cow for both morning and evening times. The method can be summarized according to the following equation: Milk flow rate (liters/minute) = (The amount of milk produced per cow/day)/(Total morning and evening milking times/cow).

Table 1: The average somatic cell count and a description of each score.

Score	Average somatic cell count (cell per ml)	Score discretion
Negative	200,000	Homogeneous (No thickening)
Trace	200,000 – 400,000	A little thicken
1	400,000 – 1,200,000	Clear and distinct thickening without gel formation
2	1,200,000 – 5,000,000	Immediately thickening and jelly begins to form at the bottom of the dish
3	More than 5,000,000	Gel formation on the surface of the dish

Measurement of milk components and calculating milk production rate

Samples of milk were immediately transferred to the laboratory for analysis after collection to preserve them from direct sunlight and high temperatures. The milk components, including lactose, protein, total solids, and fat, were analyzed using the EKO milk apparatus in the Agriculture lab. In this study, the rate of milk production during the production season was determined based on the daily production. The total amount of milk produced over the production season was equivalent to the daily production rate. Milk samples were collected every two weeks per cow during the study period using a manual method in the morning at Six O`clock, with a quantity of 50 ml of milk.

DNA extraction

Some previous papers were relied upon to conduct the proccecs, either mainly or partially (Naccache et al., 2013). The genetic material extraction from blood samples was conducted using a kit provided by Trans-Gen Biotech Co., LTD. Initially, 200 µl of each blood sample were placed in a 1.5 ml micro-centrifuge sterile tube. Proteinase solution (20 µl) and binding buffer solution (500 µl) were added to the tube under shaking process using the Vortex apparatus. The next step was incubation tubes at room temperature till ten minutes and placed in a centrifuge for a short time. The resulting mixture was transferred to a specific tube containing the extraction kit (spin column) and placed into large 2 ml tubes (collection tubes) for centrifugation at 12000 rpm for one minute. After discarding process, the holding column tubes underwent a washing process. Clean buffer was added to the column tubes, followed by centrifugation at 12,000 rpm for 30 seconds, and the precipitated solution was then discarded into the collection tubes. Subsequently, wash buffer was added to the column tubes, followed by centrifugation at 12,000 rpm for 30 seconds, and the precipitated solution was then discarded into the collection tubes. This step was repeated, and the centrifugation was done at 1200 rpm for two minutes to discarding any leftover residue found in WB3 solution. The filtered solution obtained in the collect tubes was then discarded, and the spin column was allowed to dry in the air for a few minutes at ambient temperature. The DNA from each sample was transferred from the spin column tubes to a 1.5 ml sterile Eppendorf tube. Then, added 70 microliters of heated elution buffer solution in bath at 60°C, followed by centrifugation at 1200 rpm for one minute to melt the DNA inside the tubes. The isolated DNA was stored at -20°C for molecular examination.

DNA electrophoresis

After the DNA extraction, the samples underwent electrophoresis. 5 microliters were added to each well, and the samples were then run at 110 volts for 40 minutes using an electric power source. The DNA bands were visualized using a UV light transilluminator, and those stained with ethidium bromide fluorescence appeared orange or bright pink, obzerved the presence of DNA. A photo documentation system was utilized to photograph the bands.

Primers selection

Table 2 shows the chosen primers for molecular detection and genetic polymorphism analysis of the marker DIK20, related to productive and physiological traits. The temperature grading process was used to determine the binding degree of the initiator to the complement sequence in the template DNA of the marker (Ranjan et al., 2017).

Table 2: Primer's sequence supplied from Alpha DNA investment management, LLC, USA.

Marker	Product size (base pair)	Sequence
DIK20	160-200	Forward 5- AAC CAG TAA TCG TGA GAG GA -3 Reverse 5- AAG AAA GTC CCT ACC ATG AG -3

Table 3: PCR assay protocol.

Component	25µl reaction
10 µM Forward Primer	0.5 µl
10 µM Reverse Primer	0.5 µl
Template DNA	1.5 µl
One Taq quick-load 2X Master Mix with Standard Buffer	12.5 µl
Nuclease-free water	10 µl
Total	25µl

Polymerase chain reaction (PCR)

Molecular detection of the DIK20 marker was conducted through polymerase chain reaction. Table 3 outlines the necessary supplies for molecular identification of the marker using the PCR method with 25 µl. Following the recommended reaction parameters for the marker, the samples were introduced into the polymerization reaction apparatus. After the completion of the reaction, the polymerization reaction product was transferred to ensure the desired doubling outcome. The program of marker DIK20 are showen in Table 4.

Table 4: Stages of PCR cycling process

No.	Steps	Temperature	Time	Cycles number
1	Primary denaturation	94	15 seconds	1
2	Denaturation	94	15-30 second	30
3	Annealing	58	15-60 second	
4	Elongation	68	1 minute	
5	Final elongation phase	68	5 minute	1

Statistical analysis

The data underwent statistical analysis using analysis of variance (ANOVA) and Tukey test through the utilization of SAS software version 14.3 (SAS Institute Inc. 2017) to identify variations across different variables. Cross Tabulation (Chi-square test) accombined with Fisher excat test were used to analyze the different rates of genetic polymorphisms. A significance level of $p<0.05$ was employed for the analysis.The significant differences between the means were compared using the Duncan (1955) multinomial test by applying the least square means method..Mathematical model of the relationship of genetic conformations of the Marker DIK20 to the studied traits:$Yil=\mu + Gi + eil$ Since: Yil: View value 1, μ: the general average of the trait, Gi: effect of genetic variants of the gene marker, eil: Random error that is normally distributed with a mean of zero and a variance of e2.

RESULTS AND DISCUSSION

Genetic polymorphisms

The number of genetic polymorphisms for the marker DIK20 is presented in Table 5. The study samples exhibit two genetic polymorphisms, 270/260 and 280/271. The percentages indicating highly significant differences ($P\leq0.01$) for the two genetic polymorphisms, 270/260 and 280/271, were 63.79 and 36.21, respectively. It was shown that cows with genetic polymorphisms 180/170 outperformed those with 190/181, and there were a total of 116 alleles in the genetic polymorphisms, with 74 found in cows with 180/170 and 42 in those with 190/181.

Milk components

The study's findings revealed no significant variation in the overall milk production between cows with genetic polymorphisms 180/170 and 190/181. The total quantity produced was 2511.51 kg and 2419.10 kg, respectively. Additionally, there were no significant differences in milk constituents (protein, fat, lactose, and solid non-fat) between the two genetic polymorphisms. The percentages were 3.60% and 3.48% for fat, 2.90% and 3.1% for protein, 4.18% and 4.31% for lactose, and 7.94% and 8.26% for solid non-fat for 180/170 and 190/181, respectively (Table 6).

Table 5: Genetic polymorphisms expressed as percentages and numbers, as well as the frequency of DIK20 marker alleles.

Genetic polymorphisms	No.	%
160-180	37	63.79[a]
181-190	21	36.21[b]
Total	58	100 %
Chi -square value		28.031

Different superscripts in the same column are significantly different at $p\leq0.01$.

Coefficient of heat tolerance, milk flow rate and mastitis

The study found no significant differences in heat tolerance coefficient between cows with genetic polymorphisms 181/180 and 190/190, which were 103.01 and 102.06, respectively. For the genotype 180/181, the heat coefficient value was 102.06. There were also no significant differences in milk flow rate between the two genetic polymorphisms, which were 3.61 and 3.51 for 181/180 and 190/190, respectively. However, significant differences ($P\leq0.05$) were observed in the CMT among the genetic polymorphisms 180/170 and 190/181, with cows bearing the genetic polymorphisms 280/271 showing superiority over those with 180/170, at 2.37 and 1.30, respectively (Table 7).

Genetic markers, which encompass any observable or testable phenotype, as well as the genetic basis for assessing the observed phenotypic variability, are collectively known as. There are three categories for genetic markers: morphological and productive features, which are visually determined; biochemical markers, which are based on gene products; and molecular markers, which are based on DNA analysis (Sudan et al., 2016; Tan et al., 2023). The study samples revealed two genetic polymorphisms, 270/260 and 280/271, with significantly different percentages of 63.79

Table 6: Relationship among genetic polymorphism of the DIK20 genotype with milk production and milk components.

Size	Number of cows	Mean ±standard error				
		Total milk production/liter	Fat %	Protein %	Lactose %	Solids non-fat %
260-270	37	2511.51±86.72	3.60 ±0.19	2.90 ±0.01	4.18 ±0.03	7.94 ±0.05
271-280	21	2419.10±72.20	3.48 ±0.22	3.01 ±0.03	4.31 ±0.09	8.26 ±0.08
P	Total 58	N.S	N.S	N.S	N.S	N.S

NS: Non Significant.

Table 7: Relationship among genetic polymorphisms for the genotype DIK20 with coefficient of heat tolerance, milk flow rate and mastitis.

Size	Cow (No.)	Mean ±Standard error		
		Heat tolerance coefficient	Milk flow rate	Mastitis
160-270	35	103.01±0.40	3.61±0.07	2.37±0.25[a]
171-180	23	102.06±0.58	3.51±0.06	1.30±0.18[b]
P NS NS ≤0.05				

NS: Non Significant; Different superscripts in the same column are significantly different at p<0.05.

and 36.21, respectively (Table 5). The relationship between genetic polymorphisms for marker DIK20 and productivity in sheep and goats has been a subject of interest in livestock research as Abu Al-Hassan et al. (2021) pointed out in research studied genetic polymorphisms of the GDF9 gene in Egyptian goats and sheep, who found polymorphic types in each of them, which indicates that genetic polymorphisms in the GDF9 gene may have an impact on quantitative traits in goats and sheep. As for Vitale et al. (2016) conducted a study on small ruminants in Sicily using PRNP analysis, which indicates the possibility of a relationship between genetic polymorphisms and disease resistance, which may affect productivity in one way or another.

Onzema et al. (2018) in his research indicated that distribution of genetic homology (ROH) paths in cows, sheep, and goats is important in the genetic diversity of these mentioned species, which indicates that genetic diversity is affected by genetic polymorphisms, which in turn affects productive and physiological traits. Hoffmann (2010) pointed out the high productivity potential of using techniques for estimating and preserving genetic material, as well as emphasizing that genetic markers have a role in preserving animal breeds. Some studies obzerved the ability of a relashinship between the number of births per litter and genetic conformation of bone morphogenetic protein 15 and growth factor Y, therfore, affects productive traits (Ajafar et al., 2022). In our study, a relationship between genetic marker DIK20 and milk production and its a relationship was found between it and the production of milk and its elements in dairy cows, which has been extensively carried out in goats and sheep, and this applies on the DGAT1 gene, which has been conducted in cows, buffalo, goats and sheep which is linked to components of milk and production (Khan et al., 2021).

Kaplan (2018) explained that genetic variation in fertility rates Holstein calves could affects affect milk production. In sheep, it was found that genetic polymorphisms for both caseins and whey proteins showed varying results compared to cows and goats due to genetic influences on milk production (Ropp et al., 2015). From the genetice side, the production of milk and its componants is basically have important role depending on their ability to development of the strategies of breeding, therefore, may reflect with increasing the qualiy of milk through the techniques of improving the productivity of animals. Some studies indicated that thermal tolerance in dairy cows is affected by some genes which acts by thier possibility to gain the value of accurate genomic breeding which in turn affect on the production of milk Nguyen et al. (2016). Polvero et al. (2021) observed that there is coorelation among mastitis and various genetic patterns by isolation of staphylococcus from goat milk, which lead to persistent. The study of genetic patterns of markers is considered as a potential signs of resistance to mastitis and as a tool of selection against intestinal diseases in goat breeds (Ili et al., 2018). As well as the structure of population and considerable among livestock is moderate is according to different environments of animals lives.The evaluation of correlation among phenotypic and genotypes traits for sheep, goat and cows by highlightings both population structure and genetic patterns (Bora et al., 2023).

Results of the current study agreed with findings reported Essa et al. (2023), who found that allergy of udder in cows and fertility traits was identified by identified single nucleotide polymorphisms. In the same side, some authers confirms the importance of genetic diversity in dairy cows (Hartanto et al., 2023), which proceding researchs in ruminants have confirmed that age, physiological condition and breed has important role by effecting on biochemical measurements of blood when assessd physiological traits and productive performance.Thus, this marker can be relied upon selection programs because of its importance to livestock breeders in reducing the effort and time required to diagnosis mastitis disease, as well as milk production and its components (Sharma, 2007). The results of the current study indicated no significant genetic correlation amongst the size of the different genetic markers and milk production and its elements. On the other hand, significant correlation was found between the size of the marker and mastitis disease, therefore this may enables the genetic marker to be applied to detect the possibility of infection with mastitis and the possibility of excluding animals that carry the genetic marker at an early age for Holstein cows. These results can be applied to clarify the relationship amongst genetic markers and productive traits and exploited by cattle's breeders in early selection programs for features that have a positive correlation within genetic markers. Also, the findings of the present experiment could be applied in genetic improvement programs through various breeders by taking advantage of the better time to select or exclude agricultural animal.

CONCLUSIONS AND RECOMMENDATIONS

Based on the present findings, it can be concluded that the genetic marker DIK20 detected in this research has been associated with various productive and physiological properties in Holstein Friesian cows. This marker also shows pleiotropic effects on several pairs of traits, indicating its influence on different physiological aspects. These results emphasizes the vital role of DIK20 in formulating the productive and physiological traits of Holstein Fresian cattle. This makes it a valuable genetic marker for breeding and selection of different programmes. We did not find significant differences between animals carrying different genotypes in milk production and components, thermal tolerance factor, milk flow rate, however, there correlation was found between the size of the marker and infection with mastitis disease.

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NOVELTY STATEMENT

The objective of this research was to link the relationship among the genetic marker DIK20 and physiological characteristics such as the heat tolerance coefficient and quantitative characteristics which include the percent of protein, lactose, fat, and non-fat solids, as well as the rate of milk flow by the size of the genetic marker. We consider this research is one of a few studies that study the direct relationship between quantitative and physiological traits with the size of the genetic marker in our study and the trait, which is one of the methods of early selection using genetic markers, called MAS (Marker Assisted Selection).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR'S CONTRIBUTION

The experiments were conceived and planned by HAH and SSA, while HAH, SSA, ASJM carried them out. Simulations were planned and conducted by HAH and sample preparation was contributed to by HAH, SSA, ASJM. The interpretation of the results was contributed to by HAH and SSA. The manuscript was primarily written by HAH, SSA, ASJM. All authors provided critical feedback and contributed to the research, analysis, and manuscript development.

REFERENCES

Aboelhassan D, Darwish A, Ali N, Ghaly I, Farag I (2021). A study on mutation points of gdf9 gene and their association with prolificacy in egyptian small ruminants. J. Genet. Eng. Biotechnol., 19(1). https://doi.org/10.1186/s43141-021-00181-8

Ajafar M, Kadhim A, Al-Shuhaib M, Hussein T (2022). Association of bone morphogenetic protein 15 and growth differentiation factor 9 with litter size in livestock: A review study. Acta Sci. Anim. Sci., 45: e57927. https://doi.org/10.4025/actascianimsci.v45i1.57927

Alekish MO (2015). The association between the somatic cell count and isolated microorganisms during subclinical mastitis in heifers in Jordan. Vet. Med., 60(2): 71-76. https://doi.org/10.17221/7980-VETMED

Alsaffar AA (2022). Analysis a number of Quantitative Traits and Genetic Variation of Different Generation of Wheat (*Triticum aestivum*) by using RAPD-PCR. Bionatura,

Bora S, Tessema T, Girmay G (2023). Genetic diversity and population structure of selected ethiopian indigenous cattle breeds using microsatellite markers. Genet. Res., pp. 1-12. https://doi.org/10.1155/2023/1106755

Chu MX, Guo LZ, Hai-Guo J, Wan-Hai S, Fu-Cun C, Li F, Su-Cheng Y, Yan Z (2005). Study on relationships between seven microsatellite loci and somatic cell score in Beijing Holstein Cows. J. Genet. Genom., 32: 471-475.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11: 1. https://doi.org/10.2307/3001478

Essa B, Al-Sharif M, Abdo M, Fericean L, Ateya A (2023). New insights on nucleotide sequence variants and mrna levels of candidate genes assessing resistance/susceptibility to mastitis in holstein and montbéliarde dairy cows. Vet. Sci., 10(1): 35. https://doi.org/10.3390/vetsci10010035

Eusebi P, Martínez A, Cortés O (2019). Genomic tools for effective conservation of livestock breed diversity. Diversity, 12(1): 8. https://doi.org/10.3390/d12010008

Griffin TK, Dodd FH (1962). A note on an indirect measure of machine milking rate. J. Dairy Res., 29(2): 207-210. https://doi.org/10.1017/S0022029900017829

Gupta JP, Bhushan B, Panigrahi M, Ranjan S, Asaf VNM, Kumar A, Sulabh S, Kumar A, Kumar P, Sharma D (2016). Study on genetic variation of short tandem repeats (STR) markers and their association with somatic cell scores (SCS) in crossbred cows. Indian J. Anim. Res., 50: 450-454. https://doi.org/10.18805/ijar.6709

Hartanto S, Budiyanto A, Widayanti R, Setyawan E, Prasetya I (2023). Characterization of polymorphisms in the follicle-stimulating hormone receptor and insulin-like growth factor-1 genes and their association with fertility traits in jawa-brebes cows. Vet. World, pp. 711-716. https://doi.org/10.14202/vetworld.2023.711-716

Hoffmann I (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. Anim.

Genet., 41: 32-46. https://doi.org/10.1111/j.1365-2052.2010.02043.x

Ilie D, Kusza S, Sauer M, Gavojdian D (2018). Genetic characterization of indigenous goat breeds in romania and hungary with a special focus on genetic resistance to mastitis and gastrointestinal parasitism based on 40 snps. PLoS One, 13(5): e0197051. https://doi.org/10.1371/journal.pone.0197051

Kandeel M, El-Ashker M, El-Seedy F, El-Gohary F (2017). Association of California mastitis test scores with intramammary infection status in lactating dairy cows admitted to a veterinary teaching hospital. J. Vet. Intern. Med., https://doi.org/10.1111/jvim.14876

Kaplan S (2018). Characterization of bubaline leptin gene polymorphism in anatolian buffaloes by using pcr-rflp method. Alınteri Zirai Bilimler Dergisi, 33(1): 93-97. https://doi.org/10.28955/alinterizbd.402760

Khan M, Ma Y, Ma J, Xiao J, Liu Y, Liu S, Khan A, Khan IM, Cao Z (2021). Association of dgat 1 with cattle, buffalo, goat, and sheep milk and meat production traits. Front. Vet. Sci., 8. https://doi.org/10.3389/fvets.2021.712470

Kul E, Şahin A, Atasever S, Uğurlutepe E, Soydaner M (2019). The effects of somatic cell count on milk yield and milk composition in Holstein cows. Vet. Arhiv, 89(2): 143-154. https://doi.org/10.24099/vet.arhiv.0168

Kunene NW, Ceccobelli S, Lorenzo PD, Hlophe SR, Bezuidenhout CC, Lasagna E (2014). Genetic diversity in four populations of Nguni (Zulu) sheep assessed by microsatellite analysis. Ital. J. Anim. Sci., 13(1): 3083. https://doi.org/10.4081/ijas.2014.3083

Liang Q, Wen D, Xie J, Liu L, Wei Y, Wang Y (2014a). A rapid and effective method for silver staining of pcr products separated in polyacrylamide gels. Electrophoresis, 35(17): 2520-2523. https://doi.org/10.1002/elps.201400182

Liang Z, Zhang Q, Wang Y (2014b). Regulatory science accelerates the development of biotechnology drugs and vaccines by NIFDC. Emerg. Microbes Infect., 66. https://doi.org/10.1038/emi.2014.66

Ma Y, Khan MZ, Xiao J, Alugongo GM, Chen X, Chen T, Liu S, He Z, Wang J, Shah MK, Cao Z (2021). Genetic markers associated with milk production traits in dairy cattle. 11(10): 1018. https://doi.org/10.3390/agriculture11101018

Naccache S, Greninger A, Lee D, Coffey L, Phan T, Rein-Weston A, Aronsohn A, Hackett J, Delwart E, Chiu C (2013). The perils of pathogen discovery: Origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. J. Virol., 87(22): 11966-11977. https://doi.org/10.1128/JVI.02323-13

Nguyen T, Bowman P, Haile-Mariam M, Pryce J, Hayes B (2016). Genomic selection for tolerance to heat stress in australian dairy cattle. J. Dairy Sci., 99(4): 2849-2862. https://doi.org/10.3168/jds.2015-9685

Nickerson SC, and Heald CW (1981). Histopathologic response of the bovine mammary gland to experimentally induced Staphylococcus aureus infection. Am. J. Vet. Res., 42: 1351-1355.

Onzima R, Upadhyay M, Doekes H, Brito L, Bosse M, Kanis E, Groenen M, Crooijmans R (2018). Genome-wide characterization of selection signatures and runs of homozygosity in ugandan goat breeds. Front. Genet., 9. https://doi.org/10.3389/fgene.2018.00318

Polveiro R, Granja M, Roldão T, Lopes I, Vidigal P, Lima M, Moreira M (2021). Multilocus sequence analysis reveals genetic diversity in staphylococcus aureus isolate of goat with mastitis persistent after treatment with enrofloxacin. Sci. Rep., 11(1). https://doi.org/10.1038/s41598-021-96764-z

Ranjan S, Bhushan B, Gupta JP, Asaf VNM, Panigrahi M, Kumar A, Raveendran R (2017). Study on genetic variation of microsatellite and their association with mastitis occurrence in crossbred cattle. J. Anim. Res., 7(1): 127-132. https://doi.org/10.5958/2277-940X.2017.00018.3

Rhoad AG (1944). Physiological responses of cattle to the environment. J. Dairy Sci., 27(8): 613-628.

Rupp R, Senin P, Sarry J, Allain C, Tasca C, Ligat L, Portes D, Woloszyn F, Bouchez O, Tabouret G, Lebastard M, Caubet C, Foucras G, Tosser-Klopp G (2015). A point mutation in suppressor of cytokine signalling 2 (socs2) increases the susceptibility to inflammation of the mammary gland while associated with higher body weight and size and higher milk production in a sheep model. PLoS Genet., 11(12): e1005629. https://doi.org/10.1371/journal.pgen.1005629

SAS, Institute Inc. (2017). SAS/STAT 14.3 User's Guide. SAS Institute Inc.

Sharma N (2007). Alternative approach to control intramammary infection in dairy cows. A review. Asian J. Anim. Vet. Adv., 2: 50-62. https://doi.org/10.3923/ajava.2007.50.62

Sudan J, Khajuria P, Gupta SK, Singh R (2016). Analysis of molecular diversity in indian and exotic genotypes ofbrassica junceausing ssr markers. Indian J. Genet. Plant Breed., 76(3): 361. https://doi.org/10.5958/0975-6906.2016.00054.7

Tan C, Zhang H, Chen H, Guan M, Zhu Z, Cao X, Ge X, Zhu B, Chen D (2023). First report on development of genome-wide microsatellite markers for stock (*Matthiola incana* L.). Plants, 12(4): 748. https://doi.org/10.3390/plants12040748

Thompson-Crispi K, Atalla H, Miglior F, Mallard BA (2014). Bovine mastitis: Frontiers in immunogenetics. Front. Immunol., 5. https://doi.org/10.3389/fimmu.2014.00493

Vitale M, Migliore S, Giglia M, Alberti P, Presti V, Langeveld J (2016). Scrapie incidence and prnp polymorphisms: Rare small ruminant breeds of sicily with tse protecting genetic reservoirs. BMC Vet. Res., 12(1). https://doi.org/10.1186/s12917-016-0766-9

Zghairand ZS, Hassooni HA (2021). The relationship between the PIT-1 gene at the rate of daily milk production and some of its components in buffaloes. In: IOP conference series: Earth and environmental science, IOP Publishing. 923(1): 012029. https://doi.org/10.1088/1755-1315/923/1/012029